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Research Article

Characterization and optimization of *Casimiroa* (*Casimiroa Edulis*) fruit juice using Response Surface Methodology (RSM)

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Abstract

Background: *Casimiroa edulis* fruit is an underutilized seasonal fruit of Ethiopia, is a rich source of many medicinal and nutritional properties. The fruit is found in different parts of the country, Ethiopia. However, there is no clear research evidences of the fruits on post-harvest handling, preservation, processing, and value addition. Purpose of the study: The present study is aimed at the characterization, optimization of enzyme assisted casimiroa fruit juice clarification process based on selected quality parameters of the final product.

Methodology: D-Optimal Mixture Design and RSM were used to investigate level of ingredients and the effects of independent variables on physicochemical composition, functional properties, mineral values and acceptability tests of casimiroa fruit juice. Graphical and numerical optimization were conducted to find the best variables conditions. Casimiroa fruit was treated at various enzyme concentrations (0.01-0.2%), incubation temperature (30-55°C) and incubation time (30-100 mini.) of treatment. A second order Central composite (CCD) was employed to identify the effect of independent variables on the final product.

Results: In this study, a statistical significant difference ($p < 0.05$) was observed within the process variables on nutritional profiles of casimiroa fruit juice. Furthermore, the enzyme treatment reduced the cloudy appearance of casimiroa fruit juice and improved the clarity of juice. The coefficient of determination, R^2 values for the dependent variables were almost greater than 0.8. This study revealed that, clarity, viscosity, TSS, TA, pH, phenolic compounds, flavonoids, β-carotene, ascorbic acid, Na, K, Ca, Fe, Zn, P and overall acceptability were significantly ($p < 0.05$) correlated to incubation temperature, incubation time and enzyme concentration. Incubation time and enzyme concentration were the most important factors affecting the characteristics of the casimiroa fruit juice as it exerted a highly significant influence ($p < 0.05$) on the dependent variables.

Conclusion: In conclusion, enzyme-treatment improves qualities of fruit juice and the overall optimization suggests that enzymatic-treated juice made with 75% fruit pure, 21.25% water, 2.63% sugar and 1.13% citric acid with process variables of incubation temperature (44.01°C), incubation time (62.74 min.) and enzyme concentration (0.2%), respectively achieved the best formulation for this combination of variables with a desirable of 0.9029.

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Abbreviations

RSM: Response Surface Method; TSS: Total Soluble Solids; TA: Titratable Acidity; R2: Regression Coefficient; AOAC: Association of Official Analytical Chemists (AOAC).

Introduction

Casimiroa (*casimiroa edulis L. Iave*) is a climacteric fruit with potential for commercialization due to its organoleptical quality. The fruit is native to the Mexican and the Central American highlands [1,2]. This species is popularly known as "zapoteblanco", "matasano", "cochitz´apotl", "abache" and "zapotedormil´on" [2]. The plant is well adapted to tropical and sub-tropical environment [2] and it is an evergreen plant because of its deep rooted system which enable it to tap water and minerals from deep ground table water resources [3]. The leaves are alternate, palmately compound with 3 to 5 leaflets and the leaflets are 6 to 13 cm long and 2.5 to 5 cm broad with an entire margin and the leaf petiole are 10 to 15 cm long [3]. Casimiroa is usually found growing naturally at elevations between 600 and 2,700 masl [2]. The plant is known to be easily managed and established with a minimum agricultural practices and it is also known for its high biomass production [4,5] and [2].

Casimiroa fruits are edible and having nutritional food value, which provides the minerals like sodium, potassium, magnesium, iron, calcium, phosphorous etc. The fruit is rich in vitamins A and C (180 and 800 mg/kg, wet weight respectively) and it possesses a high content of carbohydrates (160 g/kg) [2]. It provides fibers which prevent constipation. High amount of the carbohydrate was reported in white sapote than all the fruits, it is a good indication that these are good source of ready energy. The fruits were reported the energy higher than apple, Banana, Mango, Guava and the high amount of ash than the apple. The high ash content of the fruit indicates the good mineral source which is very important for the metabolism. Calcium content of 9.9 mg/100 g of white sapote was reported which is higher than banana and apple and equals to mango. The phosphorous content of 20.4 mg/100gm was reported in casimiroa fruit which is higher than apple, mango and almost all equals to banana. Iron content (0.33 mg/100g) was also reported grater in casimiroa fruit than the apple, Mango, Banana and Guava. In vitamins, Thiamine content is greater in the casmiroa fruit than Apple, Banana and Mango [2]. Riboflavin content of 0.043 mg/100g casimiroa fruit was reported. It is higher than the riboflavin content of apple, Mango and Guava fruits and Niacin content is higher than the apple. In addition to its fresh consumption; it is known to possess medicinal properties. They are immune to many diseases and often used in different formulation of Folk- medicine [2].

Casimiroa fruit with common name of white sapote is locally known in Ethiopia as Amba in Afan Oromo and casimire in Amharic languages and belongs to the kingdom Plantae, division Tracheophyta, class Mognoliopsida, order Sapindales, genus Casimiroa and species Casmiroa [2]. Different researchers reported the presence of casimiroa (*Casimiroa edulis* L.) in the different parts of the country (Ethiopia); Hawasa, Yirgachefe, Dilla, Wolayta, Arbaminch, Dire Dawa, Konso, Benishangul gumuze, Hassosa, Gambela, Tigray, Amhara and Oromiya regions are the most plantation areas of white sapote fruits [2]. There were no much data which has been reported on the processing, preservation, and value addition of the casimiroa fruits [2]. It is consider that special attention should be paid in order to maintain and improve this important source of food supply. In order to remedy, a wider and sustained acceptance of wild fruits as important dietary components must be stimulated [3].

Response Surface Methodology (RSM) is a useful statistical technique to select optimum levels of parameters in a process by conducting considerably fewer number of experiments compared to full factorial experimentation. It is more efficient since it provides minimum time and cost of experimentation required to arrive at the optimum conditions. The RSM technique gives the effect of an individual parameter and interactive effect of the parameters on the response. This has been successfully used to optimize a process and to develop a product. Polynomial regression model could be used to decide optimum operating conditions based on individual response or multiple responses [2]. *Casimiroa edulis* fruit is an underutilized seasonal fruit of Ethiopia, is a rich source of many medicinal and nutritional properties [2,6]. However, there is no clear research evidences of the fruits on post-harvest handling, preservation, processing, and value addition. The present study is aimed at the characterization of casimiroa fruit pulp, optimization of enzyme assisted casimiroa fruit juice clarification process based on selected quality parameters of the final product using RSM.

Materials and methods

Raw materials source

Fresh and fully ripened casmiroa edulis fruits (*Casimiroa edulis* L.) variety was collected from local communities of Kombolcha, Dire Dawa and Areka Agricultural Research Center. The collected fruit samples were immediately transported to the food science and Nutrition laboratory, Ethiopian Public Health Institute by using a cold storage (-18°C). Sound and the defect free fruits were carefully selected and washed thoroughly with running water and sodium hypochlorite solution (2%) to remove surface dirt and microbial loads. Cleaned and dried whole fruits were packed in the polyethylene zip-lock bags and stored in the deep freezer (8°C) to avoid any further damage to the fruit sample for further analysis.

Enzyme and chemicals

All required chemicals and reagents for this study were purchased from the local chemical suppliers in Addis Ababa. Alpha-amylase 1, 4-Glucan 4-glucanohydrolase from Aspergillus Oryzae, was purchased from local chemical suppliers (Alkeam chemicals and reagents supplier Ltd) in Addis Ababa.

Sample extraction

The cleaned fruits were peeled, deseeded, trimmed using stainless steel (SS) knife, cut into slices and crushed by using

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screw type juice extractor machine (Gallia, Henribialead, France, 2006), seeds were automatically removed from the pulp during extraction process. The deseeded fruit pure was filtered out through muslin cloth to separate the pulp from the impurities and homogenized using a homogenizer (Stuart SS30, UK) at 350 rpm for 10 minutes.

Physicochemical and functional properties

The physicochemical properties (moisture content, crude protein, crude fat, crude fiber, total ash, carbohydrate and gross energy) of the casmiroa fruit pure and enzymatic treated casimiroa fruit juice were determined as described in [7]. Ascorbic acid content was determined according to the method in [8] and Β-carotene was determined according to the methods described by (Carvalho, et al. 2014). The functional properties (pH and titratable acidity) were determined according to the procedure in [7].

Phytochemical properties

The phytochemical properties (phytate and tannin) were determined according to the methods described by [9-13]. Total phenolic compound was estimated by the method stated by [14-16]. Flavonoid content was also determined according to the procedure described by [16].

Elemental analysis

Mineral analysis was conducted on both fruit pure and enzymatic-treated fruit juices. The mineral (Ca, Na, K, Fe and Zn) contents of the fruit pure and enzymatic-treated fruit juice were determined by dry ashing according to the method in [8,17]. The phosphorous content was determined according to the procedures described by [18,19].

Selection criteria for process variable optimization among the three samples

Based on the physicochemical composition, functional properties, mineral values, vitamins and phytochemical capacity, fruit pure obtained from Dire Dawa was selected for juice preparation and process variables optimization. Among the three fruit samples analyzed, relatively high values (protein, crude fat, energy, vit-C, β-carotene, Fe, Zn, Ca, flavonoids and phenolic compounds) were obtained from Dire Dawa fruit sample and taken as a selection criteria for ingredients formulation and enzymatic-clarification.

Microbial analysis

The microbial loads (aerobic count, plate count, fecal colony count, yeast and mould) of Enzymatic-treated fruit juice was done according to the method stated by [20,21].

Organoleptic characteristics

Sensory analysis of the enzymatic- treated fruit juice in terms of color, taste, aroma, flavor, appearance, mouth feel and overall acceptability) was done on a nine-point hedonic scale by 15 trained panelists following the recommendations described in [8]. All panelists have given their informed consent to participate in the study. The sensory evaluation was conducted in food science and nutrition research sensory booths at Ethiopian Public health Institute (EPHI).

Experimental design and statistical analysis

D-Optimal Mixture design was used to formulate level of ingredients (water, fruit pure, sugar and citric acid) for casimiroa fruit juice preparation. In the blending of the component, the ingredients were constrained as fruit pure (60-80%), water (15-35%), sugar (2-4%) and citric acid (0.5-3%). For enzymatic-treatment RSM was used for optimization of independent variables (incubation time, incubation temperature and enzyme concertation), which is coded as X1, X2 and X3, respectively. The Response Surface Method, Central Composite (CCD) design set-up was taken to analyze on the combined impact of these three process variables. Total of 15 experiments with 5 replications at the center point were designed through software. Independent variables were also constrained as incubation temperature (X1) 30-55°C, incubation time (X2) 30-100 min. and enzyme concentration (X3) 0.01-0.2%. The effect of variables in terms of linear, quadratic, and interaction terms can be explained by mathematical model as shown below:

$$Y = b_0 + \sum b1X1 + \sum b11X1^2 + \sum b12X1X2$$

Where Y the experimental response; X1 and X2 the levels of variables; b_o the constant; b1 the linear coefficient; b11 the quadratic term; and b12 the coefficient of the interaction terms. Analysis of variance (ANOVA) was considered to validate the model. The contour and interaction plots obtained by changing the variables (i.e. one variable constant at the center point and changing the other two variables within the experimental range). The model equations with the help of contour plots are used to describe the individual and cumulative effects on the responses.

Enzymatic treatment

The fruit pure was treated with alpha-amylase enzyme at its natural pH 4.6. Two hundred (200 g) gram of homogenized fruit pure was treated with enzyme. The independent variables, such as incubation temperature X1 (30-55 oC), incubation time X2 (30-100 min.) and enzyme concentration X3 (0.01-0.2%) were considered. An incubator orbital shaker at 120 rpm was used for vigorous mixing of enzymatic treated fruit pure at particular temperature and time. Then the suspension was kept at 90°C for 5 minutes for inactivation of the enzyme (Molinari and Silva, 1997; Sandri, et al. 2011). A muslin cloth was used to filter the juice. The filtrate was taken for further analysis of different physicochemical properties. Samples without enzymatic treatment was kept as a control sample for comparison purpose.

Results and discussions

Physicochemical and functional properties of casmiroa fruit pure (non-enzyme added)

The physical measurements (number of seeds per

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fruit pods, fruit seed weight, pulp weight and fruit yield) of *casimiroa edulis* fruits collected from Areka Agricultural Research Center, Dire Dawa and Kombolcha were (3.67+0.034, 3.56+0.029 & 3.334+0.017); (12.058+0.055, 9.219+0.018 & 11.400+0.094); (75.063+0.04, 88.225+0.107 & 65.671+0.04) and (73.00, 76 & 72%), respectively. The results found in this study are in agreement with the reported values by [22,23]. The physicochemical composition, functional properties and antioxidant capacity results of untreated *casimiroa edulis* fruit pure was presented in (Tables 1-3) and in Figure 1, as shown below. Statistical significant difference was observed within the three fruit samples (Tables 1-3) and in Figure 1. Accordingly the results obtained, relatively higher nutritional profiles (i.e. fiber, protein, ash, clarity, viscosity, TSS, pH, TA, phenolics, flavonoids, β –carotene, Vit-C, Na, K, Ca, Fe, Zn and P) were generated from Dire Dawa fruit sample. On the other hand, lower nutritional value was obtained from Kombolcha fruit sample. Results obtained in this study was in comparable with the nutritional values of white sapote fruit reported by [2,22].

Thus, because of the results obtained, casimiroa fruit sample obtained from Dire Dawa was chosen for ingredients formulation and independent variables optimization with the support of alpha amylase enzyme by using RSM techniques.

Ingredients formulation and optimization

The optimization criteria was applied for (proximate composition, energy, vit-C, β –carotene, phenolics, flavonoids, Fe, Zn, Ca, Na, K and P) values. The ingredients were constrained in upper and lower limits. The desired goal for each of the dependent variables and ingredients were chosen. The optimization criteria were applied for both graphical as well as numerical optimization techniques were employed using the Design Expert Software (version 7 Stat-Ease, Inc.). D-optimal Mixture Design was conducted for ingredients (fruit pure, water, sugar and citric acid) formulation.

Physicochemical and phytochemical properties of casmiroa fruit juice (enzymatic-treated)

The quadratic model was generated for every responses (physicochemical composition, functional properties, phytochemicals and antioxidant capacity and mineral values) under different treatment conditions with the linear, quadratic and interaction terms of dependent variables. The regression coefficients of linear, quadratic and interaction terms for responses are given in (Tables 4-7). The quadratic and interaction terms have shown a significant effect, whereas linear terms have given the least effect on the responses. The values of R2 and adjusted R2 were found to be within acceptable limits for every response. The R2 values for clarity, viscosity, TSS, pH, TA, tannin, phaytate, phenolic compounds, flavonoids, β -carotene, ascorbic acid, Na, K, Ca, Fe, Zn, P and overall acceptability test were 0.780, 0.834, 0.330, 0.6085, 0.791, 0.944, 0.744, 0.927, 0.963, 0.781, 0.770, 0.677, 0.519, 0.822, 0.954, 0.818, 0.656 and 0.931, respectively. The result obtained on this study was in agreement with the values reported by [1] and comparable with the values reported by [24] on Mesoamerican Fruits. However, some results like (fat, Iron, & P) are not in agreement with the values reported by [1,2,4] on casimiroa fruit samples not in juice form. The difference between adjusted R^2 and predicted R^2 was found to be less than 0.2 for all responses, and this indicated the reasonable agreement between adjusted R^2 and predicted R^2. The closer the value of R2 to the unity, the better the empirical model fits the actual data. The smaller the value of R2 the less relevant the dependent variables in the model have to explain of the behavior variation [25-27]. The analysis of variance (ANOVA)

Table 1: Physicochemical Analysis Results (Wet-Basis) of Untreated Fruit Pure.

Regions	Clarity (Abs.)	TSS (oBrix)	Viscosity (mPa.s)	pH	Titer table Acidity (%)	β -carotene (µg/g)	Ascorbic acid (mcg/100g)
Areka A. R. Center	2.82±0.2^a	20.97±0.5^b	300.72±0.8^c	4.97±0.1^b	0.08±0.1^b	14.37±0.2^c	34.82±0.9^c
Dire Dawa	2.81±0.1^b	21.06±0.1^a	311.16±0.4^a	4.77±0.3^c	0.09±0.0^c	16.53±0.5^a	38.26±1.2^a
Kombolcha	2.83±0.3^a	20.96±0.2^b	309.41±0.6^b	5.08±0.2^a	0.08±0.1^a	15.26±0.3^b	35.76±1.1^b

All Values are means of duplicates ±Standard Deviation, $^{a-d}$ Means value having different superscript within the same column are significantly different (p<0.05).

Table 2: Proximate Analysis Results (Dry-Basis) of Untreated Fruit Pure.

Regions	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy (KCal.)
Areka A. R. center	79.61±0.02^c	1.45±0.21^b	0.45±0.01^b	1.35±0.01^a	1.56±0.02^b	15.51±0.03^a	72.17±0.015^b
Dire Dawa	79.62±0.05^b	1.458±0.02^a	0.49±0.02^a	1.36±0.04^a	1.56±0.03^a	15.51±0.05^b	72.27±0.01^a
Kombolcha	79.89±0.15^a	1.40±0.32^c	0.42±0.1^c	1.36±0.02^a	1.45±0.02^c	15.47±0.01^c	71.30±0.02^c

All Values are means of duplicates ±Standard Deviation, $^{a-d}$ Means value having different superscript within the same column are significantly different (p<0.05).

Table 3: Mineral Analysis Results (mg/100g, dry basis).

Regions	Na	K	Ca	Fe	Zn	P
Areka A. Research Center	24.50±0.1^c	176.09±1.4^b	17.70±0.2^c	2.87±0.1^c	0.15±0.0^c	22.43±0.3^c
Dire Dawa	24.99±1.2^a	178.99±0.2^a	19.65±0.1^a	2.98±0.4^a	0.33±0.1^a	23.87±0.5^a
Kombolcha	24.74±0.3^b	173.97±0.6^c	19.14±0.3^b	2.93±0.3^b	0.28±0.0^b	23.46±0.7^b

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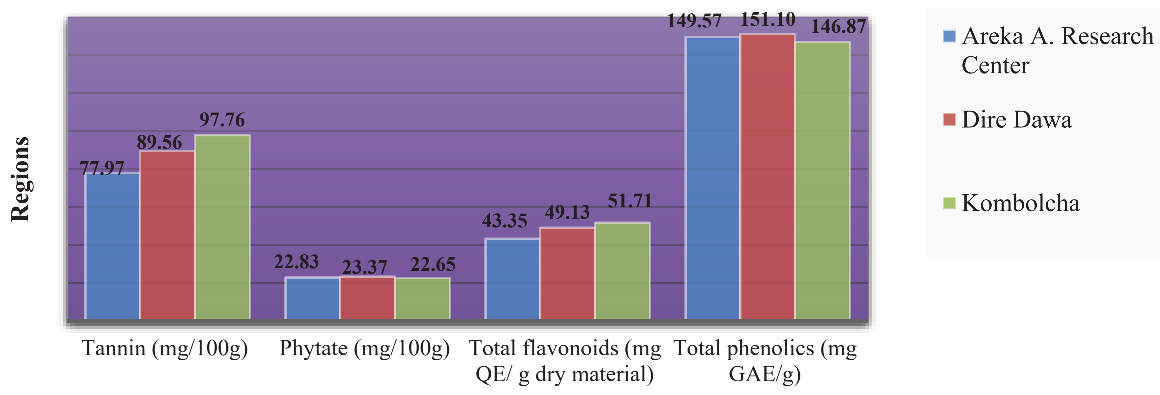

Figure 1: Phytochemical Analysis Results (Non-enzyme added) (Wet-Basis).

indicates the significance of the developed model for every response. The F-values of developed models except TSS, pH, Na, K, flavor and mouth fee have shown that all models are significant with the lack-of-fit as a not significant ($P > 0.05$), which is desirable.

Effect of temperature, time and enzyme concentration on the quality of casmiroa fruit juice

The effect of different treatment conditions on physicochemical composition and functional properties, phytochemical capacity, and mineral values are presented in (Table 4-6). As shown in Figure 2a-2p, clarity, pH and TSS were positively related to the quadratic effect of incubation temperature (P<0.05), incubation time ($P < 0.05$) and enzyme concentration and the linear terms of these variables were not found to be significant. Whereas, viscosity and TA were positively related to the linear effect of incubation temperature ($P < 0.05$), incubation time ($P < 0.05$) and enzyme concertation and the quadratic terms of these variables were not found to be significant. The response surface contour plots representation of clarity, pH, viscosity, TSS, pH, TA, tannins, phenolics, β-carotene, ascorbic acid, Na, K, Ca, Fe, P and an overall acceptability are presented in Figure below (2a-2p), respectively. Findings in this study was in lined with the values of sapodilla fruit juices reported by [2,24,28-30]. There is an interaction effect between temperature, time and enzyme concentration on the results of clarity, viscosity, TSS, pH and TA. The clarity (Abs.) in this study ranged from 1.61+0.01 to 1.96+0.01 (Table 4). There was statistical significant difference (p<0.05) among different formulations. The highest absorbance (1.96) and lower absorbance (1.61) were obtained at F1 and F15, respectively.

In this study enzyme concentration and incubation time showed a positive effect on clarity of casimiroa fruit juice. As enzyme concentration increased then the absorbance of the sample was found to be decreased. There was an interaction effect between enzyme concentration and incubation time on this study. Similarly, the result shows, there was an interaction effect between incubation temperature and enzyme concentration. The lesser the absorbance the more the clarity of the juice samples were observed [31]. The clarity depends on the enzyme concentration and incubation temperature where it's quadratic ($p < 0.05$) effects were significantly affected by alpha amylase concentration and incubation time. It was evident that the absorbance value decreased with an increase in enzyme concentration (Figure 3a). Low absorbance values indicates a clear juice is being produced [32]. Clarity is an important index of clarified juice [32]. Clarified juice is a natural juice that is purples and clear in appearance [33]. Presence of starch gives the thickness and viscosity which contribute to the turbidity and cloudy of juice. Amylase is a starch degrading enzyme which breaks down starch into amylose and amylopectin [34]. As shown in (Table 4) the clarity of juice ranged from 1.61 to 1.96 and the lowest absorbance was found to in formulation F15. The regression model interms of coded factors showing the effect of process parameters on the clarity of juice is given below.

Clarity = 1.67-0.028*A-0.011* B-0.049*C-8.750E-003*A*B-8.750E-003*A*C-0.014*B*C+0.084*A2+0.089*B2+0.079*C2

The R^2 value for this model was found to be 0.78, which means this regrassion model can explain 78% of the variability of the data. It ca be clearly observed that at higher enzyme concentration, midium temperature and incubation time, there is an improvement in the clarity of the juice.

Fruit juice with high viscosity may lead to a few problem during the filtration process [35]. To get a better filtration performance, it is recommended that fruit juices be enzymatically treated before filtration for the purpose of hydrolyzing soluble polysaccharides responsible for its high viscosity [36,37]. The viscosity was positively related to the linear effect of encubation time and enzyme concentration (0.11) at a constant incubation temperature (42.50C)(Figure 3a). The viscosity was found to increased with increasing of incubation time. As shown in (Table 4) the viscosity was found to decreased from 219.018 mPas to 192.433 mPas at (42.50C) with a fixed enzyme concentration (0.11%) and at elevated

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Table 4: Physicochemical and Functional Properties of Enzymatic-Treated Juice (Wet-Basis).

Formulation	Real Variables			Experimental Responses				
	Incubation Temp (oC)	Incubation time (min.)	Enzyme Conc. (%)	Clarity (Abs.)	Viscosity (mPa.S)	TSS (°Brix)	pH	TA (%)
C	0	0	0	2.64±0.21	263.35±0.21	13.51±0.20	5.91±0.01	0.075±0.1
F1	30	30	0.01	1.96±0.13	220.31±0.41	16.16±0.80	4.84±0.12	0.076±0.0
F2	55	30	0.01	1.91±0.12	208.35±0.71	17.68±0.12	4.77±0.02	0.079±0.2
F3	30	100	0.01	1.96±0.30	192.46±0.21	14.80±0.03	4.76±0.12	0.078±0.0
F4	55	100	0.01	1.96±0.12	196.55±0.81	14.61±0.40	4.74±0.32	0.079±0.1
F5	30	30	0.20	1.86±0.01	241.55±0.31	17.31±0.30	4.69±0.41	0.082±0.3
F6	55	30	0.20	1.86±0.02	217.26±0.31	14.79±0.51	4.73±0.11	0.085±0.1
F7	30	100	0.20	1.89±0.05	168.41±0.51	13.63±0.22	4.83±0.12	0.084±0.1
F8	55	100	0.20	1.77±0.02	174.71±0.01	17.61±0.73	4.75±0.17	0.079±0.3
F9	30	65	0.11	1.89±0.04	239.75±0.51	14.53±0.11	4.73±0.12	0.078±0.1
F10	55	65	0.11	1.78±0.21	186.35±0.60	14.83±0.51	4.79±0.10	0.082±0.2
F11	42.5	30	0.11	1.89±0.04	209.95+0.09	14.41±0.70	4.76±0.35	0.081±0.1
F12	42.5	100	0.11	1.79±0.01	187.95±0.32	14.60±0.31	4.75±0.21	0.075±0.1
F13	42.5	65	0.01	1.87±0.01	207.56±0.71	14.59±0.23	4.73±0.02	0.073±0.3
F14	42.5	65	0.20	1.79±0.06	201.25±0.01	15.98±0.32	4.77±0.19	0.079±0.1
F15	42.5	65	0.11	1.61±0.01	210.35±0.47	17.70±0.09	4.72±0.13	0.079±0.1

All Values are means of duplicates ±Standard Deviation, [a-d] Means value having different superscript within the same column are significantly different (p<0.05).
F1-70.63 % pulp, 25.62% water, 2% sugar and 1.75% citric acid; F2-62.5% pulp, 35% water, 2% sugar and 0.5% citric acid; F3- 70.0% pulp, 25.0% water,4.5% sugar and 0.5% citric acid; F4-80.0% pulp, 16.25% water, 3.25% sugar and 0.5% citric acid; F5- 78.75% pulp, 15.0% water,4.5% sugar and 1.75% citric acid; F6- 75.0% pulp, 21.25% water, 2.63% sugar and 1.13% citric acid; F7-60.0% pulp, 35.0% water, 3.25% sugar and 1.75% citric acid; F8- 60.0% pulp, 32.5% water,4.5% sugar and 3.0% citric acid; F9- 60.0% pulp, 35.0% water, 2.0% sugar and 3.0% citric acid; F10- 65.0% pulp, 28.75% water, 3.88% sugar and 2.37% citric acid; F11- 74.38% pulp, 20.0% water, 3.88% sugar and 1.75% citric acid; F12- 80.0% pulp, 15.0% water, 2.0% sugar and 3.0% citric acid; F13-60.0% pulp, 35.0% water,4.5% sugar and 0.5% citric acid; F14- 66.25% pulp, 30.0% water, 2.63% sugar and 1.13% citric acid and F15- 69.38% pulp, 24.38% water, 3.25% sugar and 3.0% citric acid.

Table 5: Phytochemicals and Antioxidant Properties of Enzymatic-Treated Juices (Wet-Basis).

Formulation	Incubation Temperature (°C)	Incubation Time (min.)	Enzyme Concentration (%)	Tannin (mg/100g)	Phytate (mg/100g)	Phenolic (mg GAE/g)	Flavonoids (mg Of QE/g Dry Mater)	β -carotene (µg/g)	Ascorbic Acid (mg/100g)
C	0	0	0	5.49±0.20	8.08±0.04	19.35±0.10	13.79±0.10	12.67±0.30	20.63±0.32
F1	30	30	0.01	2.88±0.11	5.82±0.06	19.35±0.50	13.79±0.21	10.61±0.13	17.51±0.01
F2	55	30	0.01	1.78±0.21	2.55±0.11	18.74±0.31	13.56±0.50	8.63±0.01	16.52±0.03
F3	30	100	0.01	2.17±0.01	3.95±0.02	20.14±0.70	14.34±0.12	8.78±0.30	16.47±0.04
F4	55	100	0.01	2.21±0.03	3.32±0.06	19.91±0.21	14.19±0.01	6.54±0.80	12.37±0.06
F5	30	30	0.20	2.09±0.05	3.14±0.09	20.25±0.90	13.65±0.11	8.15±0.20	16.74±0.07
F6	55	30	0.20	7.08±0.21	7.03±0.09	28.07±0.21	16.12±0.90	9.83±0.11	15.19±0.08
F7	30	100	0.20	2.36±0.12	2.23±0.01	24.89±0.32	17.17±0.11	9.91±0.40	16.12±0.01
F8	55	100	0.20	2.47±0.10	2.53±0.01	46.55±0.10	18.80±0.40	6.81±0.10	12.85±0.03
F9	30	65	0.11	1.77±0.03	4.37±0.03	30.42±0.30	17.06±0.70	11.20±0.31	16.52±0.02
F10	55	65	0.11	4.19±0.04	4.28±0.04	32.24±0.50	17.64±0.80	7.35±0.10	13.05±0.01
F11	42.5	30	0.11	9.78±0.10	8.35±0.06	43.23±0.22	20.06±0.12	10.45±0.70	16.97±0.09
F12	42.5	100	0.11	11.31±0.12	5.22±0.11	39.30±0.01	19.73±0.33	7.58±0.81	12.10±0.06
F13	42.5	65	0.01	3.34±0.01	2.03±0.12	23.68±0.12	16.16±0.31	8.96±0.10	18.44±0.03
F14	42.5	65	0.2	6.95±0.02	8.71±0.10	47.32±0.10	21.16±0.20	11.79±0.20	19.33±0.04
F15	42.5	65	0.11	7.99±0.05	8.12±0.10	47.43±0.63	21.49±0.70	11.82±0.50	21.07±0.02

All Values are means of duplicates ±Standard Deviation, [a-d]Means value having different superscript within the same column are significantly different (p<0.05).
F1-70.63% pulp, 25.62% water, 2% sugar and 1.75% citric acid; F2-62.5% pulp, 35% water, 2% sugar and 0.5% citric acid; F3- 70.0% pulp, 25.0% water,4.5% sugar and 0.5% citric acid; F4-80.0% pulp, 16.25% water, 3.25% sugar and 0.5% citric acid; F5- 78.75% pulp, 15.0% water,4.5% sugar and 1.75% citric acid; F6- 75.0% pulp, 21.25% water, 2.63% sugar and 1.13% citric acid; F7-60.0% pulp, 35.0% water, 3.25% sugar and 1.75% citric acid; F8- 60.0% pulp, 32.5% water,4.5% sugar and 3.0% citric acid; F9- 60.0% pulp, 35.0% water, 2.0% sugar and 3.0% citric acid; F10- 65.0% pulp, 28.75% water, 3.88% sugar and 2.37% citric acid; F11- 74.38% pulp, 20.0% water, 3.88% sugar and 1.75% citric acid; F12- 80.0% pulp, 15.0% water, 2.0% sugar and 3.0% citric acid; F13-60.0% pulp, 35.0% water,4.5% sugar and 0.5% citric acid; F14- 66.25% pulp, 30.0% water, 2.63% sugar and 1.13% citric acid and F15- 69.38% pulp, 24.38% water, 3.25% sugar and 3.0% citric acid.

incubation time. Different reports were revealed that the effect of enzymes on viscosity of different fruit juices [33,38]. The interaction effect between incubation time and enzyme concentration was positive (p<0.05) towards the viscosity. Similar reports were revelead the effect of enzymes on viscosity of different fruit juices [39].

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Table 6: Mineral Analysis Results (mg/100g, in dry-basis) of Enzymatic-Treated Juice.

Formulation	Incubation Temp. (°C)	Incubation Time (min.)	Enzyme Conc. .(%)	Na	K	Ca	Fe	Zn	P
C	0	0	0	18.021±0.12	178.968±0.11	17.469±0.12	0.483±0.01	0.242±0.01	18.538±0.03
F1	30	30	0.01	24.524±0.30	195.014±0.21	19.741±1.01	1.015±0.02	0.652±0.11	21.861±0.16
F2	55	30	0.01	24.986±0.21	190.642±0.03	20.993±0.34	1.061±0.07	0.405±0.01	22.971±0.04
F3	30	100	0.01	25.271±0.09	189.895±0.10	21.359±0.54	1.311±0.01	0.232±0.00	22.078±0.02
F4	55	100	0.01	24.281±0.11	187.719±0.23	19.786±0.01	1.197±0.00	0.368±0.02	20.532±0.31
F5	30	30	0.2	23.948±0.21	187.098±0.11	23.103±0.11	1.096±0.03	0.282±0.04	24.869±1.03
F6	55	30	0.2	24.602±0.02	191.584±0.03	20.844±0.03	1.620±0.00	0.278±0.09	23.674±1.11
F7	30	100	0.2	24.393±0.04	189.762±0.06	20.976±0.32	2.062±0.02	0.281±0.01	20.668±0.05
F8	55	100	0.2	23.783±0.01	192.023±0.26	20.116±0.01	2.028±0.03	0.315±0.03	23.288±0.02
F9	30	65	0.11	22.308±0.02	187.292±0.12	23.873±0.11	1.893±0.02	0.298±0.01	23.416±1.02
F10	55	65	0.11	24.661±0.04	189.589±0.13	22.719±1.01	1.255±0.07	0.449±0.05	22.313±1.02
F11	42.5	30	0.11	25.695±1.10	186.893±1.21	19.214±0.02	2.683±0.01	0.467±0.02	21.438±0.02
F12	42.5	100	0.11	24.652±0.9	189.901±1.03	20.642±0.09	2.661±0.04	0.518±0.05	23.187±0.05
F13	42.5	65	0.01	25.011±0.13	189.746±0.32	21.372±1.23	2.609±0.01	0.506±0.04	23.826±0.06
F14	42.5	65	0.2	26.196±1.01	192.305±0.1	21.905±0.16	2.666±0.01	0.665±0.05	24.405±1.02
F15	42.5	65	0.11	26.252±0.41	194.437±1.36	23.634±0.02	2.964±0.05	0.732±0.02	25.384±0.09

All Values are means of duplicates ±Standard Deviation, [a-d]Means value having different superscript within the same column are significantly different (p<0.05).
F1-70.63 % pulp, 25.62% water, 2% sugar and 1.75% citric acid; F2-62.5% pulp, 35% water, 2% sugar and 0.5% citric acid; F3- 70.0% pulp, 25.0% water,4.5% sugar and 0.5% citric acid; F4-80.0% pulp, 16.25% water, 3.25% sugar and 0.5% citric acid; F5- 78.75% pulp, 15.0% water,4.5% sugar and 1.75% citric acid; F6- 75.0% pulp, 21.25% water, 2.63% sugar and 1.13% citric acid; F7-60.0% pulp, 35.0% water, 3.25% sugar and 1.75% citric acid; F8- 60.0% pulp, 32.5% water,4.5% sugar and 3.0% citric acid; F9- 60.0% pulp, 35.0% water, 2.0% sugar and 3.0% citric acid; F10- 65.0% pulp, 28.75% water, 3.88% sugar and 2.37% citric acid; F11- 74.38% pulp, 20.0% water, 3.88% sugar and 1.75% citric acid; F12- 80.0% pulp, 15.0% water, 2.0% sugar and 3.0% citric acid; F13-60.0% pulp, 35.0% water,4.5% sugar and 0.5% citric acid; F14- 66.25% pulp, 30.0% water, 2.63% sugar and 1.13% citric acid and F15- 69.38% pulp, 24.38% water, 3.25% sugar and 3.0% citric acid.

Table 7: Optimized model solution.

Sr. No.	Incubation temperature (44.01), Incubation time (62.74) and Enzyme concentration (0.2)				
1	Clarity	1.692	12	Na	26.086
2	Viscosity	203.763	13	K	193.583
3	TSS	16.929	14	Ca	22.693
4	pH	4.727	15	Fe	2.939
5	TA	0.082	16	Zn	0.630
6	Tannin	6.329	17	P	25.574
7	Phaytate	6.990	18	Color	8.962
8	Phenolic Compounds	45.086	19	Taste	8.219
9	Flavonoids	20.691	20	Flavor	8.571
10	β -Carotene	11.401	21	Appearance	8.646
11	Ascorbic Acid	20.625	22	Mouth feel	8.185
			23	Overall acceptance	8.954
				Desirability	0.903
				Model	Selected

The quadratic effect of incubation temperature and enzyme concentration showed a positive effect on the TSS content of the treated juice at (p<0.05). The TSS content increased with incubation temperature at a slightly increasesing enzyme concentration (Figure 3c). The increased in TSS content may be due to the release of solids content due to the enzyme breakdown of cell wall of the fruit pulp [37]. The regression model developed for TSS for coded factors is given below.

TSS =16.72+0.31*A-0.51* B+0.15* C+0.60*A*B+0.016*A*C+0.45*B*C-0.56*A2 -0.74*B2+0.041*C2

The pH value in this study ranged from 4.69 to 4.84 and differ statistically ($p < 0.05$) compared to the untreated fruit juice (Table 4). However, the pH value obtained in this study was in agreement with the ported value by [3].

Titratable acidity deals with the measurment of total acid concentration present in the juice . The TA provides a better prediction of the impact of acid on the flavor of the food than pH [40]. Incubation temperature, time and enzyme concentartion showed a significant effect on the titratable acidity of casimiroa fruit juice. The increase in the TA may be due to the decrease in the pH this results in the breakdown of the cell wall releases organic acids. Similar results were showed for enzymatically extracted Sohiong juice [36,41]. The following regression model is explaining the relation between acidity and process parameters in its coded form.

TA=0.075993+4.80000E-005*IncubationTemperature-2.28571E-005*IncubationTime+0.025263*Enzyme Concentration

As shown in (Table 5) of the present study, phenolic compounds level (mg of GAE/g) ranged from 13.65 to 21.49. All independent variables revealed a positive result on the values on phytochemicals and antioxidant capacity of enzymatic treated fruit juice formulations. This study confirmed that

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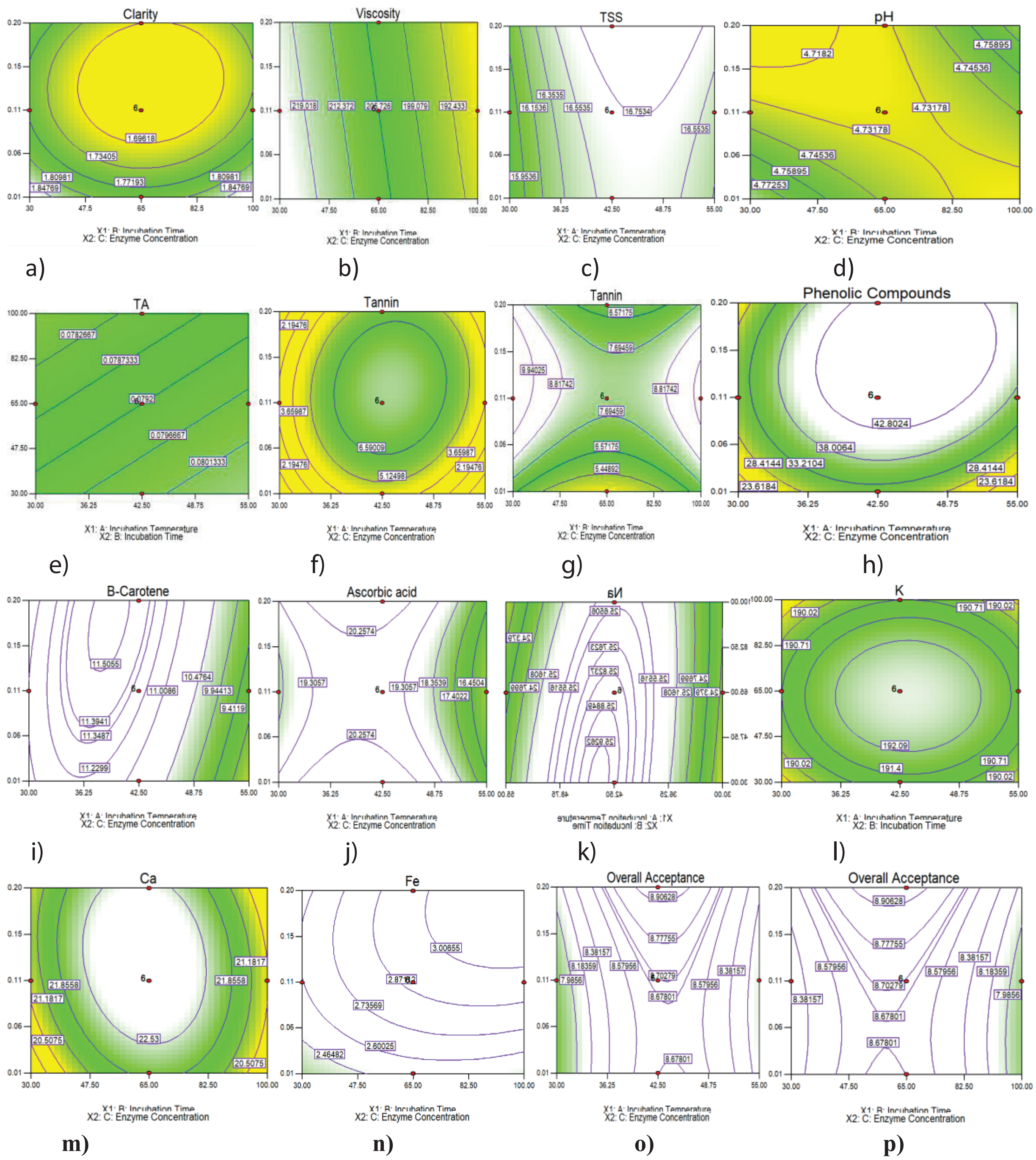

Figure 2: The Contour Plots of clarity, viscosity, TSS, pH, TA, tannin, Phytate, Phenolics, flavonoids, B-carotene, Ascorbic acid, Na, K, Ca, Fe, Zn, P and overall acceptability of enzymatic treated casimiroa fruit juice as a function of enzyme concentration, incubation time and incubation temperature.

casimiroa edulis fruit juice has a considerable value of ascorbic acid and ranged from 12.37 (mcg/100g) to 21.07 (mcg/100g) (Table 5). Incubation temperature, incubation time and enzyme concentration significantly ($p<0.05$) affected the phytochemicals (tannin, phytate) and antioxidant capacity (phenolics, flavonoids, β -carotene and ascorbic acid) in quadratic terms.

As shown in (Table 5), comparing to the untraeted fruit juice the ascorbic acid content was gradually decresed with increasing the enzyme concentration at higher incubation temperature and incubation time. According to this study there was an interaction effect between incubation time, incubation temperature and enzyme concentration on the phytochemical and antioxidant quality of casimiroa fruit juice. The ascorbic acid value ranged from (12.37 mcg/100g to 21.07 mcg/100 g) at incubation temperature (55°C) and incubation time (100 min.). This study revealed that, the concentration of β-carotene affects negatively at higher incubation temperature, whereas relatively high value of β-carotene (11.82 mcg/100g) was obtained at incubation temperature (42.5 °C) and incubation time (65 min.).

The coefficient of determination values for phenolics,

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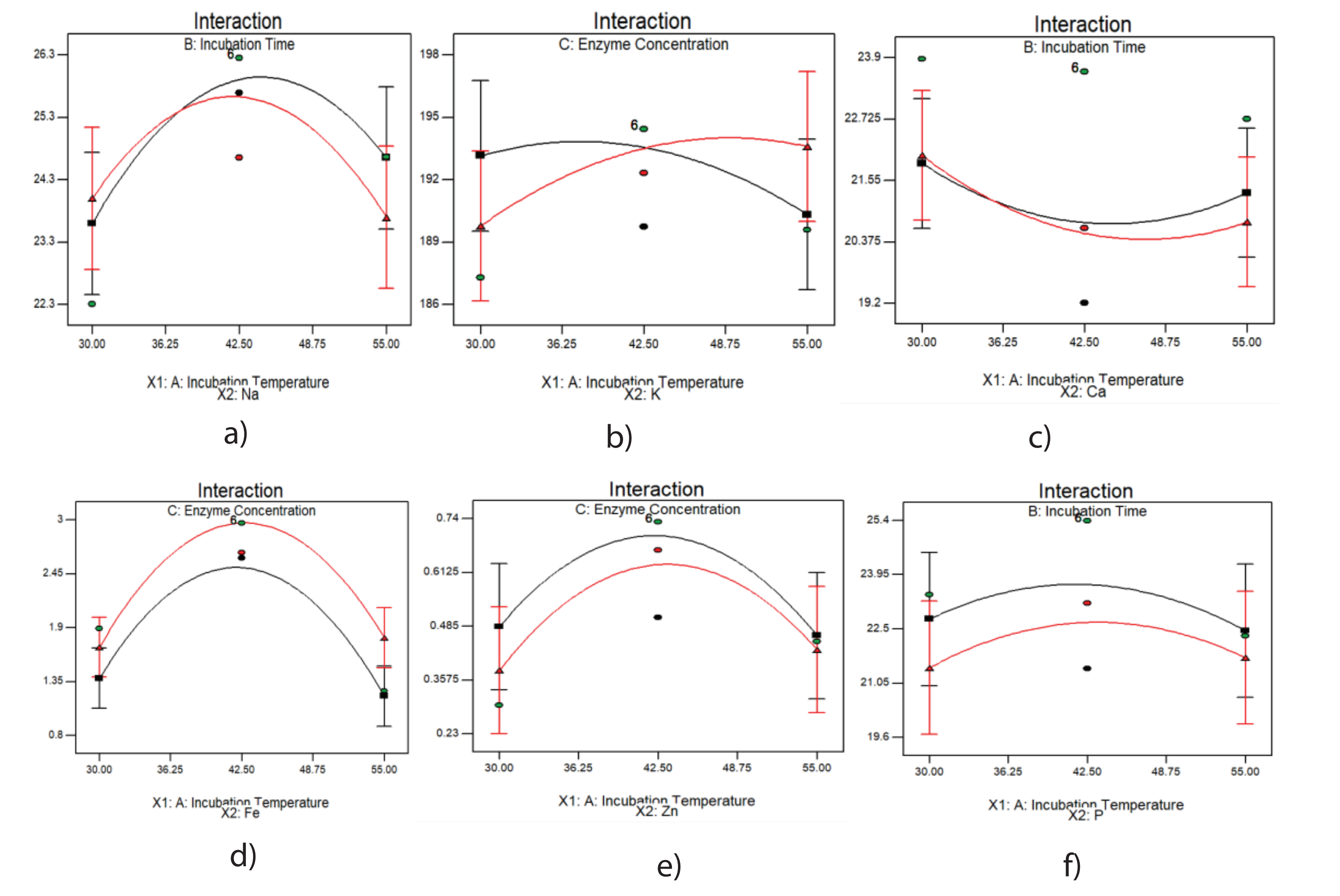

Figure 3a-f: Interaction plots for Na, K, Ca, Fe, Zn and P as a function of temperature, time and enzyme concentration.

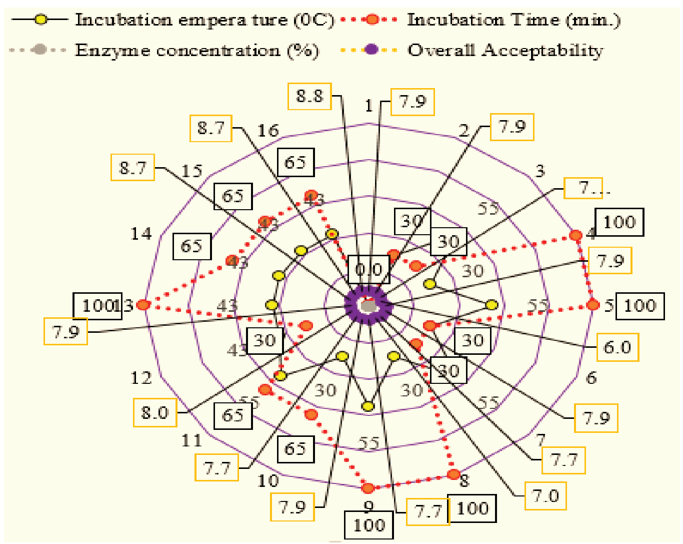

Figure 4: Sensory Evaluation of Enzymatic-Treated Casimiroa Fruit Juice.

flavonoids B-carotene and ascorbic acid were 0.93, 0.96, 0.78 and 0.77, respectively, which results in better accuracy of the model. The lack of fit was found insignificant that is desirable. The regression model for β -Carotene was presented as shown below.

$\beta - Carotene = 11.29-0.95*A-0.81*B+0.30*C-0.63*A*B+0.35*A*C+0.33*B*C-1.22*A2-1.48*B2-0.12*C2$

The regression model interms of coded factors showing the effect of process parameters on the Fe, Zn, Ca and Na of juice is given;

$Fe = 2.90-0.022*A+0.18*B+0.23*C-0.090*A*B+0.070*A*C+0.12*B*C-1.22*A2-0.12*-0.16*C2$

$Zn=0.68+7.000E-003*A-0.037*B-0.034*C+0.053*A*B+0.018*A*C+0.062*B*C-0.23*A2-0.11*B2-0.014*C2$

$Ca=23.16-0.46*A-0.10*B+0.37*C-0.18*A*B-0.35*A*C-0.41*B*C+0.85*A2-2.52*B2-0.81*C2$

$P = 24.81-0.011*A-0.51*B+0.56*C+0.14*A*B+0.23*A*C-0.30*B*C-1.08*A2-1.63*B2+0.17*C2$

The analyzed results presented in (Table 6) shows the effect of independent variables (enzyme concentration, incubation temperature and incubation time) on the mineral profiles of enzymatic-treated fruit juice. Minera values in dry weight basis of Na, K, Ca, Fe, Zn and P in this study ranged from 23.783 to 26.252, 186.893 to 195.014, 19.214 to 23.634, 1.015 to 2.964, 0.232 to 0.732 and 21.438 to 25.384 (mg/100g), respectively and there was statistical differences ($p < 0.05$) among the

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formulations (Table 6). The result of this study shows that the mineral content differ significantly ($p < 0.05$) due to the interaction effect of independent variables.

Figure 3a-f showed the interaction effect of the independent variables on the mineral profiles of fruit juice formulations. The Na and Ca value depends on the incubation temperature and incubation time and affects positively on the mineral value of enzyme treated fruit juice. Enzyme concentration and incubation temperature shows a positive effect on Zn and K concentrations. Whereas incubation temperature and enzyme concentration showed a negative interaction effect on Fe and P concentrations (Figure 3d & f). According to this study, the mineral values were mainly a function of incubation time, incubation temperature and enzyme concentration. The values Fe, Ca, Zn, p and Na increased gradually as enzyme concentration and incubation time was elevated. The interaction of enzyme concentration and incubation temperature were also significant ($p < 0.05$) (Figure 3a-f).

Sensory evaluation

The sensory evaluation results are presented as shown in (Figure 4), it can be seen that the most accepted juice formulations were F15 and F14, F14, F13 and F11, respectively. The less accepted formulations was F3 with average sensory scores lower than the others. As indicated in (Figure 4), in this study the sensory evaluation score ranged from 6.009 to 8.769. The maximum sensory score (8.769) observed in formulation F15. This study revealed that the sensory evaluation scores were a function of enzyme concentration and incubation time. As enzyme concentration increased the sensory score was also gradually increased as shown in (Figure 4). Enzyme concentration and incubation time had a positive effect on the overall acceptability of treated fruit juices.

According to the current study findings, independent process variables (enzyme concentration, incubation time and incubation temperature) affects the sensory evaluation results positively. Enzymatic treatment improves the clarity of casimiroa fruit juice through degradation of starch granules.

Optimization

The physicochemical composition, functional properties, phytochemicals and antioxidant capacity, mineral values and sensory evaluation scores were considered for optimization of enzymatic-treated *casimiroa edulis* fruit juice. The overall optimization suggests that enzymatic-treated juice made with 75% fruit pure, 21.25% water, 2.63% sugar and 1.13% citric acid with process variables of incubation temperature (44.01°C), incubation time (62.74 min.) and enzyme concentration (0.2 %), respectively achieved the best formulation for this combination of variables with a desirable of 0.9029 (Table 7).

Conclusions

In conclusion, the physicochemical composition, functional properties, antioxidant capacity, mineral values and sensorial acceptability test were conducted on both *casimiroa edulis* fruit pulp and enzymatic treated fruit juices.

Design Expert Software (D-Optimal Mixture Design and Response Surface Methodology) were used to formulate level of ingredients and to investigate the optimum conditions of the independent variables that affect clarification of *casimiroa edulis* fruit juice using alpha amylase enzyme. The optimum conditions were obtained both numerically and graphically in order to obtain the desirable levels of properties (clarity, viscosity, TSS, pH, TA, phenolic compounds, flavonoids, B-carotene, ascorbic acid, Na, K, Ca, Fe, Zn, P and overall acceptability) of fruit juice. The process variables (incubation temperature, incubation time and enzyme concentration) showed a significant effect on the clarification of *casimiroa edulis* fruit juice. It is also improved the cloudy appearance of casimiroa fruit juice by degrading of the starch granules. The overall optimization suggests that enzymatic-treated juice made with 75% fruit pure, 21.25% water, 2.63% sugar and 1.13% citric acid with process variables of incubation temperature (44.01°C), incubation time (62.74 min.) and enzyme concentration (0.2%), respectively achieved the best formulation for this combination of variables with a desirable of 0.9029.

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