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Research Paper

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FORMULATION AND OPTIMIZATION OF VITAMIN-C RICH BEVERAGE PREPARED FROM ZIZIPHUS JUJUBE

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ABSTRACT

Zizyphus jujube is a fruit contains richest source of vitamin C ranges upto 76mg/100g. They are traditionally used for antifungal, antibacterial, antiulcer, anti-inflammatory, sedative, antispastic, antifertility/contraception, hypotensive and antinephritic, cardiotonic, antioxidant, immunostimulant, and wound healing properties. The present study was carried out to develop a suitable formulation for preparation of zizyphus – orange beverage. The Response Surface Methodology was used to determine the optimum level of vitamin C enrichment and was evaluated by a panel for sensory attributes. The optimization result shows that 75g of zizyphus, 45g of orange and 2.24g of spices whereas the titrable acidity 1.138, pH 4.359, ascorbic acid 54.596 and overall acceptability 8.408. The sensory attribution result shows that variation 8 was highly acceptable compared to the other variations. Hence it is concluded that RSM was used successfully to optimize the level of zizyphus jujube, orange and spices for the development of the developed beverages.

Key words: Ziziphus Jujube, vitamin –C, orange juice.

INTRODUCTION

Fruits beverages are well relished by all age groups of the society. Fruit beverages is produced and consumed all over the can help to reduce high cholesterol levels in our blood. They are considered as healthy food supplement because they contain high quantity water, carbohydrates, protein, vitamin, A,B₁,B₂,C,D and E; and minerals such as Ca, Mg, K, Zinc and Fe (Enienike et al., 2006). The ascorbic acid content of fresh orange fruits ranges up to 30mg/100g. Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic, compound and pectin's that are important to human nutrition (Fernandezlopez et al., 2005). Fresh fruits and fresh juices from vegetables and fruits are very good sources of valuable nutrients and completed our diet with essential vitamins (Especially hydro soluble vitamins) and minerals, dietary fibers, small quantities of lipids and proteins, being in the same time good sources of carbohydrate. In the juice from fresh fruits respect the rules from guide to good hygienic agricultural and manufacturing practices for the primary production, conditioning, packing, storage and transportation of fresh fruits, proposed by FAO (Lopez camelo, 2004).

Ascorbic acid is a hydro soluble vitamin, very important for growth, which helps in healing different wounds, kipping the gums and teeth healthy, and very important in capillary fragility treatment. Ascorbic acid is an essential nutrient for humans and because of its high antioxidant power it provides protection against the presence of free radicals participating in the prevention of many diseases, currently, there is much biomedical interest in citrus fruits because consumption of them appears to be associated with lower risk of colorectal (Hen *et al.*,2002).

The jujube fruit from (Rhamnaceae) family is one of the ancient fruits crops which is popular due to its wider adaptability under adverse soil and climatic conditions. As one of the true native species of Arabia, Zizyphusspinachristi and Z.numulaia are growing in Arab states as native plant along with the exotic plants (Ashkanani, 2008). Physio-chemical analysis shows that both fruits are good source of nutrients in summary, jujube may be exploited for development of various food products jam jelly and pickle. New therapeutic diseases are known to be treated with herbal medicines throughout the human civilization. Even today plant materials continue to play major role in primary health care and higher plant have been shown to be potential sources for the new anti-microbial agents (Drake *et al.*, 2009).



MATERIALS AND METHODS

Present study was conducted in the year 2013 at the Department of Food Science, Periyar University, Salem. The fresh orange and zizypus, sugar and spices were also procured from local market. The glass bottle was used for the bottling of juice.

MORPHOLOGY AND EXTRACTION OF SELECTED FRESH FRUITS

Fruit characters such as shape, colour, skin surface and pulp, weight, length and diameter of selected fresh Fruits were described. Fresh orange and zizyphus were used for extraction of juice. Each fruits were cleaned thoroughly

Washed, blanched and blended in a blender to a pulp and the juice was extracted by filtering through muslin cloth and stored separately.

OPTIMIZATION OF VITAMIN-C RICH BEVERAGE USING RESPONSE SURFACE METHODOLOGY (RSM)

Response surface methodology (RSM) is an effective statistical technique of process optimization (Myera *et al.*, 2009). Central composite experimental design (CCD) (Box & Wilson, 1951) with quadratic model is employed to study the combined effect of three independent variables which influence highly the vitamins content of fruit beverage namely zizyphus Jujube fruit juice (X₁) range from 55 to 75ml, Orange juice (X₂) range from 25 to 45ml and Spices essence (X₃) range from 2 to 3 will be selected for optimization. The dependent variables (Y) to be measured are Titrable acidity (Y₁), pH (Y₂), Ascorbic acid (Y₃) and Overall acceptability (Y₄) of the

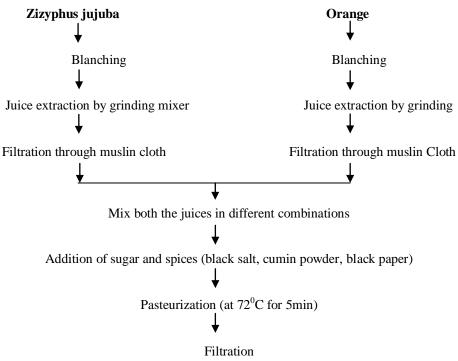
developed Vitamin- C rich beverage. In CCD, the range and the levels of the variables investigated as a preliminary step in this study is given in the Table - 1. A 2³-factorial CCD, with six axial points ($a = \sqrt{3}$) and six replications at the centre points ($n_0 = 6$) leading to a total number of 20 experiments for the optimization of the fruit beverage.

The variance for each factor will assess partitioned into linear, quadratic and interactive components and were represented using the second order polynomial function as follows

 $\begin{array}{l} Y=\!\beta_0\!+\!\beta_1X_1\!+\!\beta_2X_2\!+\!\beta_3X_3\!+\!\!\beta_{11}X_2\!+\!\beta_{22}X_2\!+\!\beta_{33}X_2\!+\!\!\beta_{12}\\ X_1X_2\!+\!\beta_{13}X_1X_3\!+\!\beta_{23}X_2X_3 \end{array}$

Where Y is the predicted response, X_1 , X_2 , X_3 are independent variables, β_0 is the offset term, β_1 , β_2 and β_3 are linear effects, β_{11} , β_{22} and β_{33} are squared effects and β_{12} , β_{23} and β_{13} are interaction terms. The response surface and contour plots will be generated for different interactions of any two independent variables, while holding the value of the third variable as constant. Such three dimensional surfaces (3D) plots will give you an accurate geometrical representation and provide useful information about the behavior of the system within the experimental design. The optimization of the fruit beverage is aimed at finding the levels of independent variables, viz. zizyphus jujuba fruit juice (X1), Orange juice (X_2) and Spices mix (X_3) , which would give maximum Titrable acidity, pH, ascorbic acid and overall acceptability of the developed beverage. The final prepared juices were stored in glass bottles at room temperature for the period of 60 days.

Flow chart for the preparation of Vitamin C rich beverage



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Filled in glass bottles and capping

Stored in refrigerated temperature

TITRATABLE ACIDITY, pH AND ASCORBIC ACID

These juices were analyzed for its titrable acidity and pH according to Ranganna (1986). Sample solution equivalent to 0.2mg ascorbic acid ML^{-1} was prepared in water containing 3% (W/V) metaphosphoric acid. It was titrated against standard 2.6 dichlorophenol indophenols (2.6 DCIP) solution of 0.5 mg ML^{-1} concentration until the pink colour developed completely. The operation was repeated with a blank (Indian pharmacopoeia, 1996).

Plate I - Development of vitamin C rich beverage



SENSORY ANALYSIS

To carry out the initial optimization of ingredients, the prepared formulation were judged by a

trained panel of 15-member using a 9 point Hedonic rating (9-Like extremely and 1-dislike extremely) (murrary *et al.*, 2001) for colour, texture, flavor and overall acceptability.

STATISTICAL ANALYSIS

The means of collected data were analysed by AVOVA using SAS 9.1 statistical analysis software. The differences between the mean values were calculated using Duncan's multiple comparison tests at 95% confidence level [p > 0.05].

RESULTS AND DISCUSSION

MORPHOLOGICAL PROPERTIES OF SELECTED FRESH FRUITS

The morphological property of fresh zizyphus weight was 66.13g, length 43.06cm and diameter 51.65cm while the weight of the orange was 8.66g, length 5.36cm and diameter 5cm respectively.

OPTIMIZATION OF THE PHYSICAL AND SENSORY PROPERTIES OF VITAMIN- C RICH BEVERAGE VITAMIN C RICH BEVERAGES

The beverage prepared with the help of Zizyphus (X_1) , Orange (X_2) and Spices (X_3) for its Physical and Organoleptic characteristics. The Titrable acidity (Y_1) , pH (Y_2) , Ascorbic acid (Y_3) and Over all acceptability (Y_4) were measured for response variables. The Physical and Sensory properties of Vitamin- c rich beverage was given in Table – 1.

S.No	Zizyphus	Orange	Spices	Titrable	pН	Ascorbic	Over All
5.110	(\mathbf{X}_1)	(\mathbf{X}_2)	(X ₃)	Acidity	(\mathbf{Y}_2)	Acid (Y ₃)	Acceptability
				(Y ₁)	/		(Y ₄)
1	55	25	1	0.7	4.12	89.6	8
2	75	25	1	0.8	4.1	67.2	9
3	55	45	1	0.7	4.35	56	9
4	75	45	1	0.8	4.28	23.2	9
5	55	25	3	0.7	4.39	44.8	9
6	75	25	3	0.6	4.37	44.8	9
7	55	45	3	0.5	4.2	89.6	9
8	75	45	3	0.8	4.1	67.2	8
9	48	35	2	1.2	4.13	78.4	9
10	81.82	35	2	0.8	4.1	67.2	9
11	65	18.18	2	1.4	4.25	67.2	7
12	65	51.82	2	0.8	4.1	67.2	7
13	65	35	0.32	0.8	4.1	67.2	7
14	65	35	3.68	0.4	4.39	56	7
15	65	35	2	0.8	4.1	67.2	6
16	65	35	2	0.9	4.44	44.8	8
17	65	35	2	0.9	4.18	56	8

Table – 1- Physical and Sensory properties of Vitamin C rich Beverage



Spices

 $x_3 + 1.8100 x_2 x_3$

figure 1 to figure 12.

y₄ (Overall

x₂+1.555 x₁ x₃+2.158 x₂ x₃

of squares, X_1 – Zizyphus, X_2 – Orange, X_3 –

 y_1 (Titrable Acidity) = 1.109+0.011 x_1 -0.0476

 $x_2+0.582x_3-2.388$ $x_1^2+1.146$ $x_2^2-0.165$ $x_3^2+5.000$ x_1

 $y_2(pH) = 0.324 + 0.079 x_1 + 0.055 x_2 + 0.501 x_3 - 5.734 x_1^2$

 y_3 (Ascorbic acid) = 510.09-8.155 x_1 -3.987 x_2 -99.59

 $x_3+0.062 x_1^2+0.042 x_2^2+2.243 x_3^2-0.041 x_1 x_2+0.4100 x_1$

 $x_1+0.29697$ $x_2+2.98271$ $x_3+5.8643x_1^2-1.20677$ $x_2^2-1.20677$

indicates that the positive and negative contribution between the Zizyphus, Orange and Spices. The linear terms of orange have negative effect; quadratic terms of orange have positive effect and the interactive terms of all the variables have the positive effect for titrable acidity. The linear terms of all the variance have the positive effect while the quadratic and interactive terms have the negative effect for the pH. The linear terms have negative effect while the quadratic terms have the positive effect and the interactive terms of zizyphus and orange have the negative effect for ascorbic acid. For overall acceptability, the linear term of zizyphus have the negative effect, the quadratic terms of zizyphus have the positive effect and the interactive terms of all the variance have the negative effect respectively. The 3D plots of the vitamin C rich beverages discussed from

The magnitude of P and F value in table 2

 $0.12068 x_3^2$ -2.5000 x₁ x₂-0.0250 x₁ x₃-0.02500 x₂ x₃

Acceptability) = 20.0952-0.6248

3.613 x_2^2 -0.011 x_3^2 -1.625 $x_1 x_2$ -3.750 $x_1 x_3$ -0.010 $x_2 x_3$

18	65	35	2	0.7	4.36	23.2	8		
19	65	35	2	0.9	4.41	67.2	9		
20	65	35	2	1.5	4.42	44.8	9		
The titrable acidity may ranges from 0.4 to df –Degree of							of freedom	SS – Sun	n

The titrable acidity may ranges from 0.4 to 1.5%, pH 4.1 to 4.44, ascorbic acid 23.2 to 89.6mg and overall acceptability 6 to 9 respectively for the developed vitamin C rich beverages.

EFFECT OF VARIABLES ON VITAMIN-C BEVERAGES

Effects of coefficient for Vitamin C rich Beverage were analyzed for the effect analysis of dependent variables. Regression analysis indicated that the fitted quadratic model accounts that about 44% ($R_2>0.44$) of titrable acidity, 60% of pH ($R_2>0.60$), 70% of ascorbic acid ($R_2>0.70$) and 42% of overall acceptability ($R_2>0.42$).

Table – 2- Effect of coefficient for Vitamin C rich

Beverage								
Source	Titrable	pН	Ascorbic	Overall				
	Acidity		Acid	acceptability				
Model	0.96	4.32	50.79	7.96				
X ₁	-0.020	-0.019	-7.06	0.000				
X ₂	-0.074	-0.022	-0.76	0.000				
X ₃	-0.079	0.051	-0.62	0.000				
X1 ²	-0.024	-0.057	6.20	0.26				
X_{2}^{2}	0.011	0.036	4.22	0.26				
X_{3}^{2}	-0.17	-0.011	2.24	0.26				
X ₁ X ₂	0.020	-0.016	-4.10	0.35				
X ₁ X ₃	0.000	-3.750	4.10	0.35				
X_2X_3	0.000	-0.11	18.10	0.35				
R ²	0.4391	0.6029	0.7043	0.4250				
Adj	-0.0656	0.2454	0.4382	-0.0924				
R ²								
Pred	-1.4717	-	0.1442	-1.2085				
R ²		0.1680						
Adeq	3.894	3.980	6.282	2.935				
Prec								

Figure-1 Effect of Zizyphus and Orange on Titrable acidity

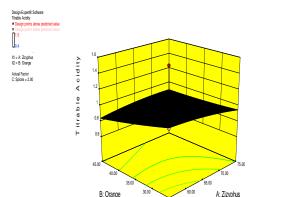


Figure-2 Effect of Orange and Spices on Titrable acidity

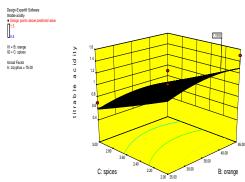




Figure-3 Effect of Zizyphus and Spices on Titrable acidity

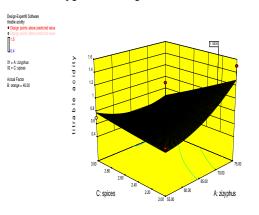


Figure-5 Effect of Zizyphus and Orange on pH

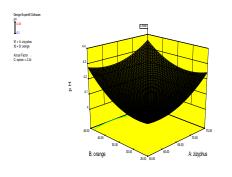


Figure-4 Effect of Zizyphus and Spices on pH

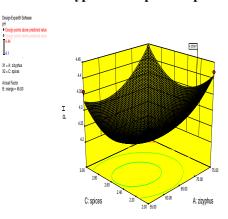


Figure-6 Effect of Orange and Spices on pH

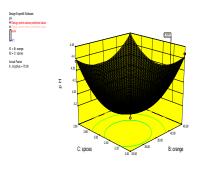


Figure-7 Effect of Orange and Spices on Ascorbic acid

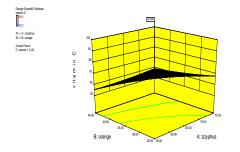


Figure-9 Effect of Orange and Spices on Ascorbic acid

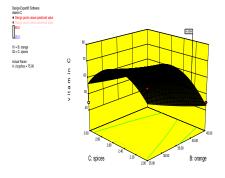


Figure-8 Effect of Zizyphus and Spices on Ascorbic acid

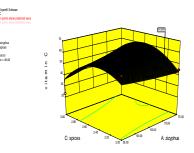
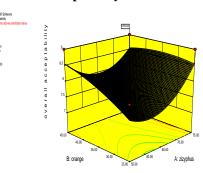
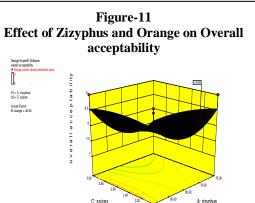


Figure-10 Effect of Zizyphus and Orange on Overall acceptability



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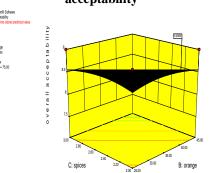


The response surface plots for the titrable acidity figure 1 showed that the titrable acidity of zizypus and orange neither decreased nor increased, figure 2 shows that the effect of titrable acidity of spice may slightly increased while the orange neither increased nor decreased. The effect of zizypus and spices increased on titrable acidity where shown in figure 3. The effect of zizypus, spices and orange increase the pH of the beverages where shown in figure 4, 5 and 6. Figure 7 shows that the effect of orange and spices on ascorbic acid neither increased nor decreased while the effect of spices on ascorbic acid slightly increased to a certain points and then gets decreased gradually, where as zizypus neither increased nor decreased on the ascorbic acid (figure 8). Figure 9 shows that the effect of spices on amino acid neither increased nor decreased while orange showed a slightly decreased on spices. Figure 10 shows that the effect of orange increased while figure 12 shows that zizypus decreased on overall acceptability while the effect of Zizyphus and Orange neither increased nor decreased on overall acceptability.

PHYSIOCHEMICAL PARAMETERS OF PROCESSED MIXED JUICES

The physio- chemical composition of studied zizyphus jujuba and orange juice were presented in the reveals that the acidity 3.8 and 1.01%. Vitamin-C or Ascorbic acid content of optimized mixed juice was observed to be high. Vitamin-C or ascorbic acid was reduced from 134 to 54.9mg/100g in mixed juice (Mishra *et al.*, 2011). Acidity was calculated on the basis of titrable acidity as citric acid. Acidity for the optimized formulation was calculated. The polynomial model and estimated coefficient for titrable acidity to vitamin-C rich

Figure-12 Effect of Zizyphus and Orange on Overall acceptability



beverage. Which is gradually decreased and this is the main reason for lowering the value of acidity. The pH of the mixed beverage 4.359.

OPTIMIZATION OF INDEPENDENT VARIABLES

For the optimization variables, the responses (ie) titrable acidity, pH, ascorbic acid and overall acceptability were selected on the basis that these responses had direct effect on the acceptability and quality of vitamin C rich beverage.

Table -3- Optimum value for the responses

Process variable	Optimum value	Response	Optimum value
Zizyphus	75	Titrable acidity	1.138
Orange	45	рН	4.359
Spice	2.24	Ascorbic acid	54.596
		Overall	8.408
		acceptability	

To consider all the responses simultaneously for optimization, the multiple regressions was used to get compromise optimum conditions and it as found that the scores were 1.138, 4.359, 54.596 and 8.408 for titrable acidity, pH, ascorbic acid and overall acceptability respectively. The optimum condition of Zizyphus 75gm, orange 45gm and spices 2.24gm.

ORGANOLEPTIC EVALUATION OF VITAMIN C RICH BEVERAGE

The organoleptic evaluation of vitamin C rich beverage using Duncans multiple range test was given below.

Table-4-Mean	Organoleptic	Evaluation of	Vitamin	C Rich Beverage	e Using Duncar	Multiple Range Test

Туре	Appearance	Colour	Flavour	Texture	Taste	Over all acceptability
V1	$6.90\pm0.87^{\rm a}$	7.00 ± 0.816^{b}	$7.00\pm0.81^{\rm a}$	6.90 ± 0.71^{b}	6.60 ± 0.96^{a}	$6.80\pm0.78^{\rm c}$
V2	6.60±0.84 ^a	5.30±0.67 ^a	8.10±0.67 ^b	8.20±0.56 ^b	7.90±0.73 ^a	7.20±0.15 ^a
V3	7.10±1.197 ^a	6.90±0.99 ^b	7.50±0.67 ^a	5.90±0.16 ^b	6.00±1.05 ^a	6.20±1.03 ^b
V4	6.30±1.05 ^a	7.00±0.94 ^b	5.40±0.99 ^a	6.70±0.29 ^b	5.90±0.87 ^a	5.20±0.63 ^a
V5	$6.60{\pm}1.05^{a}$	5.30±0.67 ^a	5.60 ± 0.68^{a}	6.50 ± 0.57^{a}	6.20±1.03 ^a	5.70 ± 0.82^{a}

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V6	7.00±0.81 ^a	5.60 ± 0.69^{a}	5.30±0.78 ^a	6.60±0.66 ^a	5.90±0.87 ^a	$5.80{\pm}0.78^{a}$
V7	$6.90 \pm 0.87^{\rm bc}$	7.00 ± 0.81^{a}	7.00 ± 0.66^{a}	6.80 ± 0.918^{b}	6.60 ± 0.96^{ab}	$6.80\pm0.78^{\rm c}$
V8	$7.50 \pm 0.52^{\rm c}$	5.20 ± 0.63^a	6.90 ± 0.87^{a}	7.20 ± 0.78^{a}	$7.00\pm0.81^{\text{b}}$	6.60 ± 0.69^{ab}
V9	6.90 ± 0.73^{b}	5.70 ± 0.67^{a}	6.90±0.73 ^a	$6.20{\pm}0.78^{a}$	6.40 ± 1.07^{ab}	7.60 ± 0.91^{ab}
V10	6.80±0.78 ^b	7.60±0.51 ^b	6.80±0.91 ^a	6.30±1.05 ^{ab}	6.00±0.942 ^a	6.80±1.27 ^{ab}
V11	6.90±0.73 ^b	7.20±0.63 ^b	$7.20{\pm}0.788^{a}$	$6.50{\pm}0.70^{ab}$	6.80±0.788 ^{ab}	7.50±0.52 ^b
V12	5.80±0.788 ^b	5.70 ± 0.82^{a}	6.60 ± 0.84^{a}	6.90±0.73 ^a	7.10±0.73 ^b	6.30±0.82 ^a
V13	$6.90 \pm 0.87^{\rm bc}$	$7.00\pm0.81^{\rm a}$	7.00 ± 0.66^{a}	$6.80\pm0.918^{\text{b}}$	6.60 ± 0.96^{ab}	$6.80 \pm 0.78^{\circ}$
V14	$7.50 \pm 0.52^{\circ}$	5.20 ± 0.63^{a}	$6.90\pm0.87^{\rm a}$	$7.20\pm0.78^{\rm a}$	$7.00\pm0.81^{\text{b}}$	6.60 ± 0.69^{ab}
V15	6.90 ± 0.73^{b}	5.70 ± 0.67^{a}	6.90±0.73 ^a	$6.20{\pm}0.78^{a}$	6.40 ± 1.07^{ab}	7.00 ± 0.91^{ab}
V16	6.80 ± 0.78^{b}	7.60 ± 0.51^{b}	6.80±0.91 ^a	6.30±1.05 ^{ab}	6.00±0.942 ^a	6.80±1.27 ^{ab}
V17	6.90±0.73 ^b	7.20 ± 0.63^{b}	$7.20{\pm}0.788^{a}$	$6.50{\pm}0.70^{ab}$	6.80 ± 0.788^{ab}	7.50 ± 0.52^{b}
V18	5.80 ± 0.788^{b}	5.70 ± 0.82^{a}	$6.60{\pm}0.84^{a}$	6.90±0.73 ^a	7.10±0.73 ^b	6.30±0.82 ^a
V19	6.00 ± 0.66^{a}	6.10±0.73 ^{ab}	6.10±0.87 ^b	6.30±1.05 ^b	6.50±0.84 ^c	6.20±0.91 ^a
V20	6.60 ± 8.84^{ab}	6.90±1.10 ^{bc}	$6.00{\pm}0.94^{a}$	6.00 ± 0.66^{ab}	5.70±0.82 ^{ab}	$6.20{\pm}0.78^{a}$
S	6.30±.48a ^b	5350±0.52 ^a	5.70±0.48 ^c	$5.90{\pm}0.87^{ab}$	5.70±0.67 ^{ab}	$7.00{\pm}0.66^{b}$

Values with difference superscripts different with each other an application of Duncan Multiple range test

The above table explained the inter relationship within the samples. Compared with the standard sample variation 8 sample showed the best than other variations.

CONCLUSION

The developed Zizyphus jujube fruits juices are good nutritive products, being natural products with important concentration of some micro nutrients and water. It the sanitation condition is complied, then fresh juices should be a part of every one's diet as healthy diet habits. The titrable acidity may ranges from 0.4 to 1.5%, pH 4.1 to 4.44, ascorbic acid 23.2 to 89.6mg and overall acceptability 6 to 9 respectively for the developed vitamin-C rich beverages.

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