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Research Article

Effects of L-Ascorbic Acid, Bentonite and Gelatin on Clarification of Apple Concentrate and Optimization with Desirability Function

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Abstract: The main aim of this study is using of L-ascorbic acid to improve quality of apple concentrate. Generally, the bentonite-gelatin mix is used to improve a quality of fruit juice and concentrate, but in this study, L-ascorbic acid as a new clarificant matter was used to improve apple concentrate quality (clarity and color enhancement and turbidity decrease) by flocculation and precipitation. So two mix, including 1- bentonite-gelatin mix (as a blank mix) and 2- L-ascorbic acid-gelatin-bentonite mix was used. The mix solution was added to apple concentrate in the four separate stages including; 1: to the mash enzyme tank product, 2: to the crude apple juice, 3- to the fining tank product (in single-stage) and 4- to the fining tank product (in two-stage). The ratio of bentonite, gelatin and L-ascorbic acid in the mix solution and adding stage were studied by General Full Factorial Design (GFFD). Bentonite, gelatin and L-ascorbic acid concentration in two levels as a numeric factor and adding stage in four levels as text factor were considered. The linear regression method was used to study the relation between bentonite, gelatin and L-ascorbic acid concentration and adding stage of the clarificant mix. The desirability function (D) was used to find the optimum condition for analysis. The results showed that L-ascorbic acid had a significant effect on the clarification process and adding of clarificant mix to the fining tank product (in single-stage) is the best way to adding the clarificant mix.

Keywords: Apple concentrate, clarification, desirability function, General Full Factorial Design (GFFD), L-ascorbic acid

INTRODUCTION

Apple is one of the most nutritious and popular among all the fruits. Apples are one of the most consumed fruits worldwide and are consumed fresh or in processed forms such as jam, juice or dried. Apples contain over 84% water, a variety of vitamins, minerals (K, Mg, Ca and Na), trace elements (Zn, Mn, Cu, Fe, B, Se, Mo) and have high fiber content (Benitez and Lozano, 2007; Diamante *et al*., 2013). Over one million tons of apple pomace is produced in the process of apple juice concentrate production [1 and 2]. Transparent materials such as bentonite, gelatin, silica sol and Chitosan were used to study variations of antioxidant activity, phenolic compounds and the color of some variety apple juice during the classical clarification procedure (Santini *et al.,* 2014; Babsky *et al*., 1986).

Gelatin is a heterogeneous mixture of water-soluble proteins of high molecular weight. On a dry weight basis, gelatin consists of 98 to 99% protein. The molecular weight of these large protein structures typically ranges between 20000 and 250000, with some aggregates weighing in the millions. Gelatin is not a nutritionally complete protein. It contains no tryptophan and is deficient in Isoleucine, threonine and methionine (Devi and Kakati, 2013; Stocke, 1998). Gelatin has a considerable number of applications and uses. The petitioned use is in foods as a beverage clarifier, also used as a fining agent for white wine, as a beer clarifier and to clarify fruit and vegetable juice, especially for clarified apple juice and pear juice. Gelatin is used in desserts in 8 to 10% of the dry weight, in yogurt at 0.3 to 0.5% as a thickener, in ham coatings at 2 to 3% and in confectionery and capsules (vitamin supplements) at 1.5 to 2.5%. Further uses include fruit toppings for pastry, instant gravy, as a stabilizer in ice cream, cream cheese and cottage cheese as well as in food foams and fruit salads (Versari*et al.*, 1998; Stocke, 1998).

Bentonite is an absorbent aluminum phyllosilicate clay consisting mostly of montmorillonite. It was named by Wilbur C. Knight in 1898 after the

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Cretaceous Benton Shale near Rock River, Wyoming. The different types of bentonite are each named after the respective dominant element, such as potassium (K), sodium (Na), calcium (Ca) and aluminum (Al). Bentonite usually forms from weathering of volcanic ash, most often in the presence of water (Didi *et al*., 2009; Koyuncu *et al*., 2007). The absorbing feature of bentonite is an important factor in purification of food oils including soy oil, date oil, canola and beverages like beer, wine and mineral water as well as products like sugar and honey. The application of gelatin and bentonite for absorption of phenol materials from apple skin and core as well as mango juice and skin and also using these two combinations before ultrafiltration in clarification of apple juice has been described in many investigations (Li *et al*., 2010; Mekhemer *et al*., 2008).

Ascorbic acid is a natural organic compound with antioxidant properties. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. Ascorbic acid is one form of vitamin C (Abbasi and Niakousari, 2007; Ahmad *et al*., 2011). The ascorbate ion is the predominant species at typical biological pH values. It is a mild reducing agent and antioxidant. It is oxidized with the loss of one electron to form a radical cation and then with the loss of a second electron to form dehydroascorbic acid. It typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical. Ascorbic acid and its sodium, potassium and calcium salts are commonly used as antioxidant food additives. These compounds are water-soluble and, thus, cannot protect fats from oxidation: For this purpose, the fat-soluble esters of ascorbic acid with long-chain fatty acids can be used as food antioxidants (Carr and Frei, 1999; Farhang *et al*., 2012; Jang and Lee, 2008; Khalid *et al*., 2013).

Clarification of apple juice with gelatin and bentonite is a common industrial practice. These fining agents work either by sticking to the particles or by using charged ions to cause particles to stick to each other, in any case, making them heavy enough to sink to the bottom by the action of gravity. What is left is a transparent though not a clear juice. Subsequent filtration operations are needed to obtain a crystal clear product. Differences in the nature of ionic charges of protein, polyphenols and the fining agents, induce flocculation and sedimentation and result in the removal of these potential haze precursors from solution (Onsekizoglu *et al*., 2010; Oszmianski and Wojdylo, 2007).

Determination of appropriate doses of clarifying apple juice agents (bentonite, gelatin) is usually made at the industry by trial and error: basically, in a matrix of test tubes filled with enzymatically treated juice, increasing quantities of bentonite and gelatin are added in rows and columns, respectively. The dose that in a shorter time gives the most compact flocks and the transparent supernatant is selected for the bulk treatment of juice (Onsekizoglu *et al*., 2010; Oszmianski and Wojdylo, 2007; Rai *et al*., 2007; Benitez and Lozano, 2006).

A full factorial experiment is an experiment whose design consists of two or more factors, each with discrete possible values or "levels" and whose experimental units take on all possible combinations of these levels across all such factors. A full factorial design may also be called a fully crossed design. Such an experiment allows the investigator to study the effect of each factor on the response variable, as well as the effects of interactions between factors on the response variable [23 and 24]. For the vast majority of factorial experiments, each factor has only two levels. A factorial experiment can be analyzed using ANOVA or regression analysis. It is relatively easy to estimate the main effect of a factor. Other useful exploratory analysis tools for factorial experiments include main effects plots, interaction plots and a normal probability plot of the estimated effects (Davies, 1993; Gawande and Patkar, 1999; Carrillo *et al*., 2011).

In this study GFFD and response surface methodology were used to study and optimization of L-Ascorbic acid, Bentonite and Gelatin concentration and clarificant mix adding stage on the clarification of apple concentrate. According to this study, L-ascorbic acid has a significant role in the clarificant mix to clarify and improve the quality of apple concentrate.

MATERIALS AND METHODS

Reagents and Chemicals: L-ascorbic acid (purchased from china), gelatin and bentonite (Iran Barite) were used. Apple concentrates products (in a different stage of the process) were provided in Azarkam Company in Urmia, Iran. Amylase and pectinase enzymes (DMS) were used.

Apparatus: Clarity, color and turbidity of apple concentrate samples was determined by turbidimeter (N2100, HACH, United State) and spectrophotometer (DR2800, HACH, United State)

L-Ascorbic acid-Bentonite-Gelatin mix adding process: To study L-ascorbic acid effects on the apple concentrate character, two mixes including 1- bentonite-gelatin mix (as a blank mix) and 2- the bentonite-gelatin-L-ascorbic acid mix was used. The mix solutions were added to apple concentrate (200 mL) in the four separate stages including; 1: to the mash enzyme tank product (S1), 2: to the crude apple juice (S2), 3- to the fining tank product (in single-stage) (S3) and 4- to the fining tank product (in two-stage) (S4). In the all mixing process after flocculation and precipitation of added mix, the apple concentrate filtered and analyzed (Clarity, color and turbidity) by spectrophotometer and turbidimeter instruments.

Color determination: The color of apple concentrates samples was determined by spectrophotometer at the wavelength of 440 nanometers. Firstly the spectrophotometer instrument was calibrated in the 100 degrees (100% transmission) by deionized water, then apple concentrates sample was put in the cell and the shown number as the color was recorded.

Clarity determination: The clarity of apple concentrates samples was determined by spectrophotometer at the wavelength of 640 nanometers. Firstly the spectrophotometer instrument was calibrated in the 100 degrees (100% transmission) by deionized water then apple concentrate sample was put in the cell and the shown number as clarity was recorded.

Turbidity determination: The turbidity of apple concentrates samples was determined by turbidimeter. Firstly the turbidimeter instrument was calibrated in the 0 degrees (100% transmission) by deionized water, then apple concentrate sample was put in the cell and the shown number as turbidity was recorded. In the apple concentrate samples, high color and clarity (near to 100) and low turbidity (near to 0) show the high quality of samples.

RESULTS AND DISCUSSION

General full factorial design: General Full Factorial Design (GFFD) was used to design the experiments. The general full factorial design determines an acceptable amount of information for testing well fitting and does not require an unusually large number of design points, thereby reduces the overall cost associated with the experiment. The desirability function plots obtained through statistical process describes the design and the modeled GFFD data. In order to obtain the optimum conditions, the effect of various parameters such as L-ascorbic acid, bentonite, gelatin concentration and mix adding stage were taken account and the optimum conditions were chosen. Three variables, namely, L-ascorbic acid amount (A), gelatine amount (B), bentonite amount (C) at two levels and mix adding stage (D) in four levels were investigated. Polynomial equations, main effects plots, interaction plots and a normal probability plot of the estimated effects were created using Minitab 17. The performance of the system was defined by the following Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \qquad (1)$$

where, Y is the response; β_0, β_i, β_{ii} and β_{ij} are constant coefficients and xi the coded independent variables. For three numerical variables, high (coded value: 2) and low (coded: 1) set points were chosen to construct an orthogonal design as tabulated in Table 1. Also, Table 2lists the coded values of designed experiments based on GFFD and responses.

Study interaction between variables and determine main effects: The purposes of this GFFD design were:

- Investigation of the influence of L-ascorbic acid amount (mg), gelatin amount (mg), bentonite 10% amount (g) and mix adding stage (four stages) on

Table 1: General full factorial design used to evaluate the effects of four factors

	Factors						
	A: L-ascorbic acid (mg)		B: Gelatin (mg)		C: Bentonite 10% (g)		
Run	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	D: mix adding stage
1	1	5	2	7.5	2	13	S2
2	2	7.5	2	7.5	2	13	S4
3	1	5	2	7.5	1	8	S4
4	2	7.5	2	7.5	1	8	S4
5	1	5	1	5.5	1	8	S3
6	1	5	1	5.5	1	8	S4
7	2	7.5	1	5.5	1	8	S4
8	2	7.5	1	5.5	1	8	S1
9	2	7.5	1	5.5	2	13	S2
10	2	7.5	2	7.5	1	8	S3
11	1	5	1	5.5	2	13	S3
12	2	7.5	2	7.5	1	8	S2
13	1	5	2	7.5	1	8	S1
14	1	5	1	5.5	2	13	S2
15	2	7.5	1	5.5	1	8	S2
16	2	7.5	1	5.5	2	13	S4
17	2	7.5	2	7.5	1	8	S1
18	1	5	1	5.5	2	13	S1
19	1	5	2	7.5	1	8	S2
20	1	5	2	7.5	1	8	S3
21	2	7.5	2	7.5	2	13	S3
22	1	5	1	5.5	1	8	S1
23	1	5	2	7.5	2	13	S3
24	2	7.5	2	7.5	2	13	S2
25	2	7.5	1	5.5	2	13	S1

Table 1: Continue

26	2	7.5	2	7.5	2	13	S1
27	1	5	2	7.5	2	13	S1
28	1	5	1	5.5	2	13	S4
29	2	7.5	1	5.5	2	13	S3
30	1	5	2	7.5	2	13	S4
31	2	7.5	1	5.5	1	8	S3
32	1	5	1	5.5	1	8	S2

Table 2: List of experiments in the GFFD and the responses of each run

	Factors				Responses		
Run order	A: L-ascorbic acid (mg)	B: Gelatin (mg)	C: Bentonite 10% (g)	D: mix adding stage	NTU	Colour	Clarity
1	1	2	2	S2	0.93	66	96.4
2	2	2	2	S4	0.96	56	80
3	1	2	1	S4	0.88	62	86
4	2	2	1	S4	0.92	66	91
5	1	1	1	S3	0.85	75	98.2
6	1	1	1	S4	0.85	70	97.1
7	2	1	1	S4	0.89	61	87
8	2	1	1	S1	0.96	56	64
9	2	1	2	S2	0.98	63	90
10	2	2	1	S3	0.88	64	87
11	1	1	2	S3	0.87	68	92
12	2	2	1	S2	0.97	64	91
13	1	2	1	S1	0.96	58	66
14	1	1	2	S2	0.94	64	89
15	2	1	1	S2	0.97	62	89
16	2	1	2	S4	0.94	61	81
17	2	2	1	S1	0.96	60	86
18	1	1	2	S1	0.96	57	65
19	1	2	1	S2	0.95	62	85
20	1	2	1	S3	0.87	64	89
21	2	2	2	S3	0.97	60	86
22	1	1	1	S1	0.98	54	51
23	1	2	2	S3	0.94	66	89
24	2	2	2	S2	0.96	64	89
25	2	1	2	S1	0.94	60	85
26	2	2	2	S1	0.92	66	96
27	1	2	2	S1	0.95	59	87
28	1	1	2	S4	0.87	68	91
29	2	1	2	S3	0.93	65	90
30	1	2	2	S4	0.93	64	89
31	2	1	1	S3	0.87	70	91
32	1	1	1	S2	0.99	58	84

Table 3: The characteristics of the abstracted model for all responses

		NTU		Clarity		Color	
Source	DF	Adj SS	Adj MS	Adj SS	Adj MS	Adj SS	Adj MS
Model	31	0.053987	0.001742	3315.82	106.962	656.719	21.1845
Linear	6	0.031175	0.005196	1401.79	233.632	257.188	42.8646
A	1	0.002812	0.002812	25.03	25.028	9.031	9.0313
B	1	0.0008	0.0008	75.34	75.338	3.781	3.7812
C	1	0.0018	0.0018	88.11	88.113	0.031	0.0312
D	3	0.025762	0.008587	1213.31	404.437	244.344	81.4479
2-Way Interactions	12	0.020325	0.001694	1748.47	145.706	312.125	26.0104
A*B	1	0.00005	0.00005	2.48	2.475	7.031	7.0312
A*C	1	0.00045	0.00045	30.23	30.225	9.031	9.0312
A*D	3	0.004262	0.001421	555.92	185.308	91.094	30.3646
B*C	1	0.000312	0.000312	2.94	2.94	0.031	0.0312
B*D	3	0.005025	0.001675	611.96	203.988	128.344	42.7812
C*D	3	0.010225	0.003408	544.94	181.646	76.594	25.5313
3-Way Interactions	10	0.002425	0.000242	155.47	15.547	61.812	6.1812
A*B*C	1	0.000613	0.000613	71.1	71.103	9.031	9.0313
A*B*D	3	0.000475	0.000158	40.98	13.659	14.594	4.8646
A*C*D	3	0.000775	0.000258	35.98	11.992	20.094	6.6979
B*C*D	3	0.000562	0.000187	7.41	2.47	18.094	6.0313
4-Way Interactions	3	0.000063	0.000021	10.1	3.366	25.594	8.5313
A*B*C*D	3	0.000063	0.000021	10.1	3.366	25.594	8.5313
R^2		100		100		100	

the quality (NTU, color and clarity) of apple concentrate.

- Recognition of the variables with highest effect on the NTU, color and clarity (note that, in the apple concentrate it is desirable NTU be the least, color and clarity be the highest).
- Finally displaying interactions between the variables. In order to find out the significant factors that affect responses (NTU, color and clarity) and build a model to optimize the procedure, a full linear model including all terms of Eq. (1) (for all responses) was constructed at the first step. Then, in order to obtain a simple and yet a realistic model, the insignificant terms were eliminated from the model through 'backward elimination' procedure. By the elimination of nonessential terms of Eq. (1) From the constructed model, calibration R^2 was calculated for all the responses. The characteristics of the abstract model, including R^2 values, adjusted sum of squares and mean square are shown in Table 3. To gain further insight about the influence of each variable, the interaction plots and main effect plots for the predicted responses were formed based on the model function. Figure 1 to 3 show interaction and main effect plots of clarity, color and NTU versus pairs of variables.

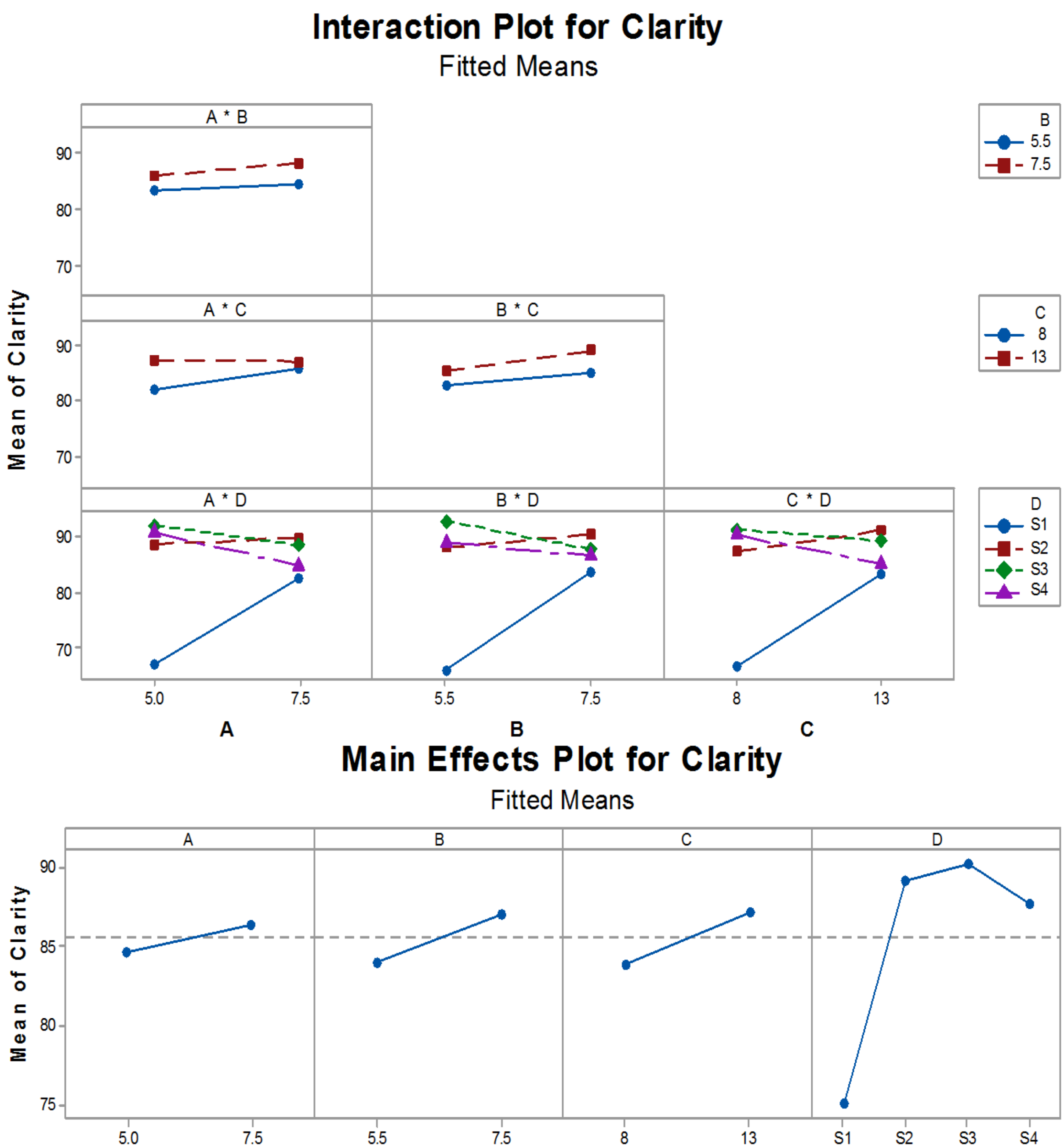


Fig. 1: Interaction and main effect plots of clarity versus variables

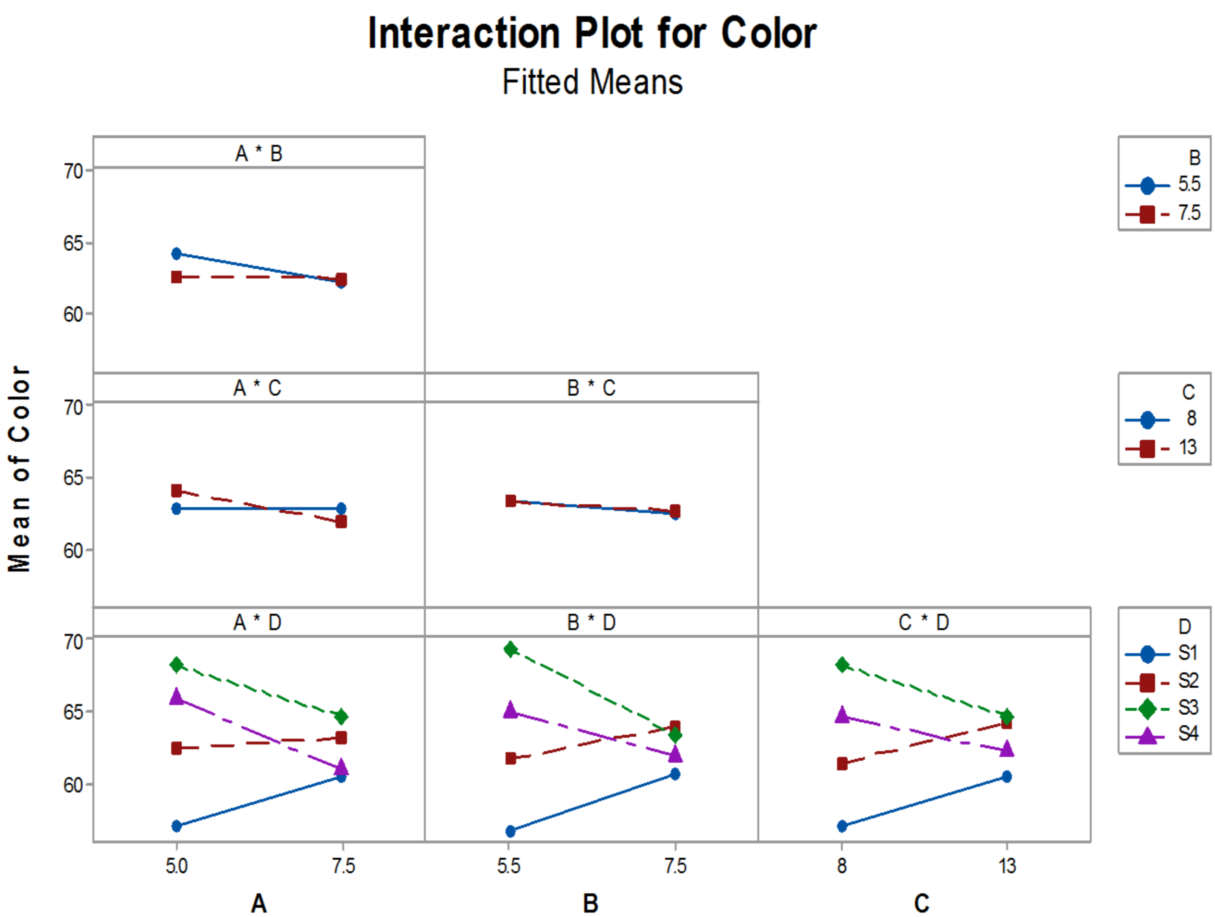
In Fig. 1, increasing of L-ascorbic acid, gelatin and bentonite amount (in the mix) cause to increase clarity of apple concentrate and adding mix in stage 3 (fining tank product), the highest clarity is obtained. Figure 2 shows that increasing of L-ascorbic acid and gelatin amount cause to decrease color, but increasing of the bentonite amount cause of the slight increase color of samples.

In the similar study, Benitez and Lozano (2007) reported that increasing of gelatin increase the clarity of apple juice that confirmed this work results. Jalali *et al.* (2014) evaluated bentonite and gelatin effects on clarification of variety of date fruit Kaluteh juice with response surface methodology. According to the their results bentonite and gelatin was used as important materials to improve date syrup quality (Jalali *et al.*, 2014).

The highest color is obtained by adding to the mix in stage 3 (fining tank product). According to the Fig. 3, increasing of L-ascorbic acid, gelatin and bentonite amount (in the mix) cause to decrease turbidity of apple concentrate and adding mix in stage 3 (fining tank product), the lowest turbidity is obtained. It is totally clear that increasing of L-ascorbic acid, gelatin and bentonite amount (in the mix) increases apple concentrate quality and the best condition is obtained in stage 3 (fining tank product).

Desirability function and selection of optimal condition: Several approaches have been used to multi-objective optimization. One approach uses a constrained optimization procedure, the second is to superimpose the contour diagrams of the different response variables and the third approach is to solve the problem of multiple responses through the use of a desirability function that combines all the responses into one measurement and its value changes between 0 and 1. The desirability functions as an approach to use optimization procedure have some advantages:

- It is possible to compare responses with different scaling
- It is very fast and simple to transform different responses to one measurement
- It is possible to use qualitative and quantitative responses. All values of desirability for each response variable are computed. The overall



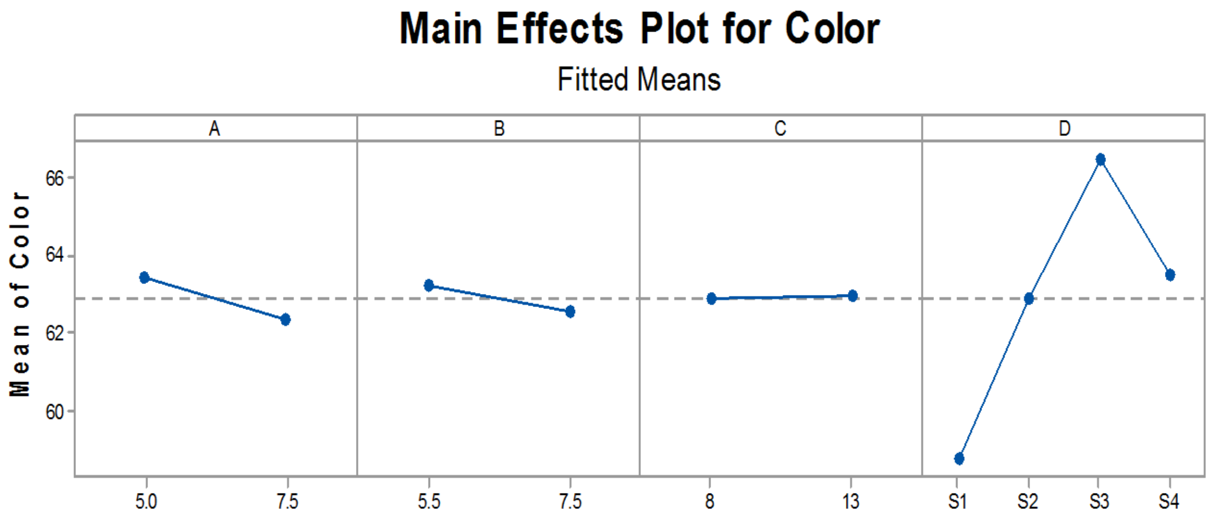
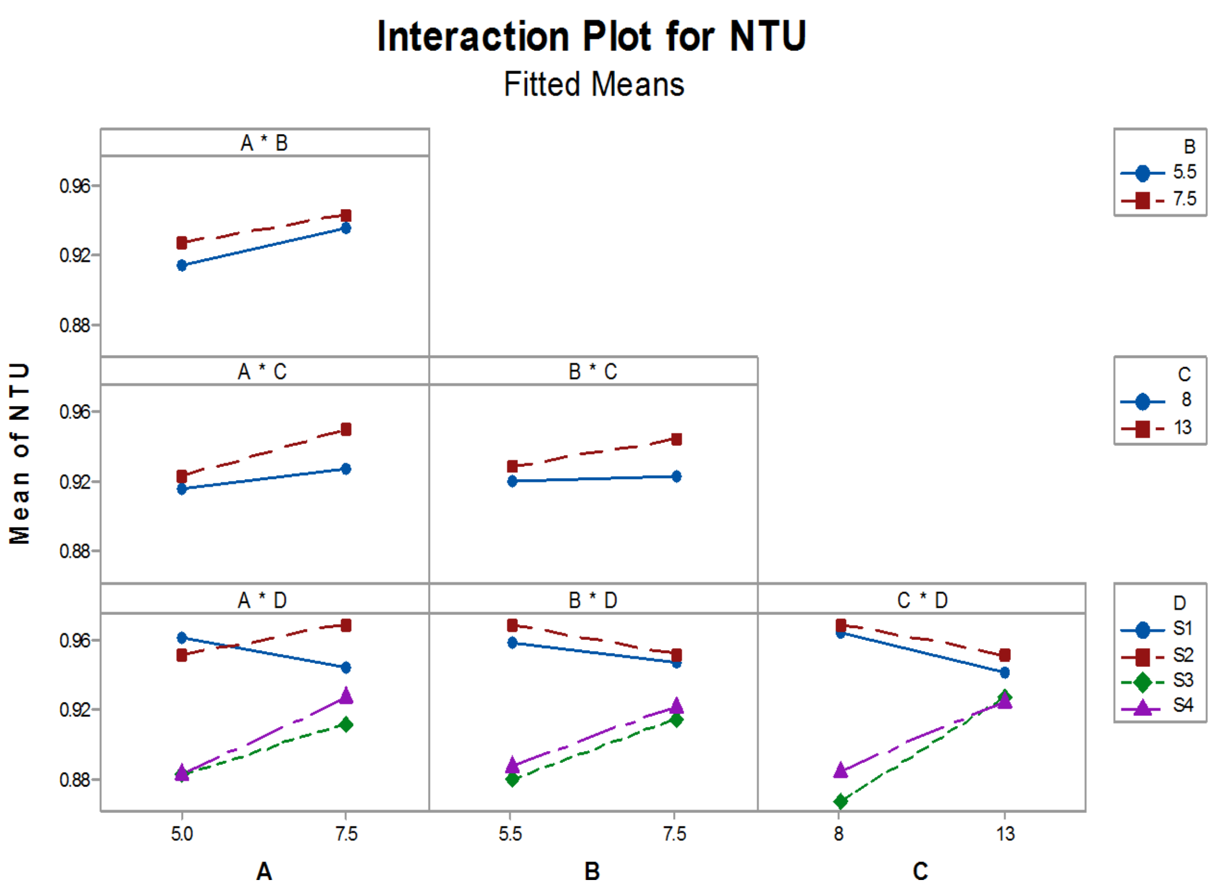


Fig. 2: Interaction and main effect plots of color versus variables

desirability function (D) is calculated by geometric mean. Then desirability values combined into an overall desirability function. In the overall desirability function D, each response can be assigned an importance relative to the other responses. The following equation is used to compute the overall desirability function (Pirsa *et al.*, 2016):

$$D = (d_1^{r1} \times d_2^{r2} \times \ldots d_n^{rn})^{\frac{1}{\sum ri}} = \left(\prod_{i=1}^{n} d_i^{ri}\right)^{\frac{1}{\sum ri}} \quad (2)$$

where, n is the number of responses in the measure.



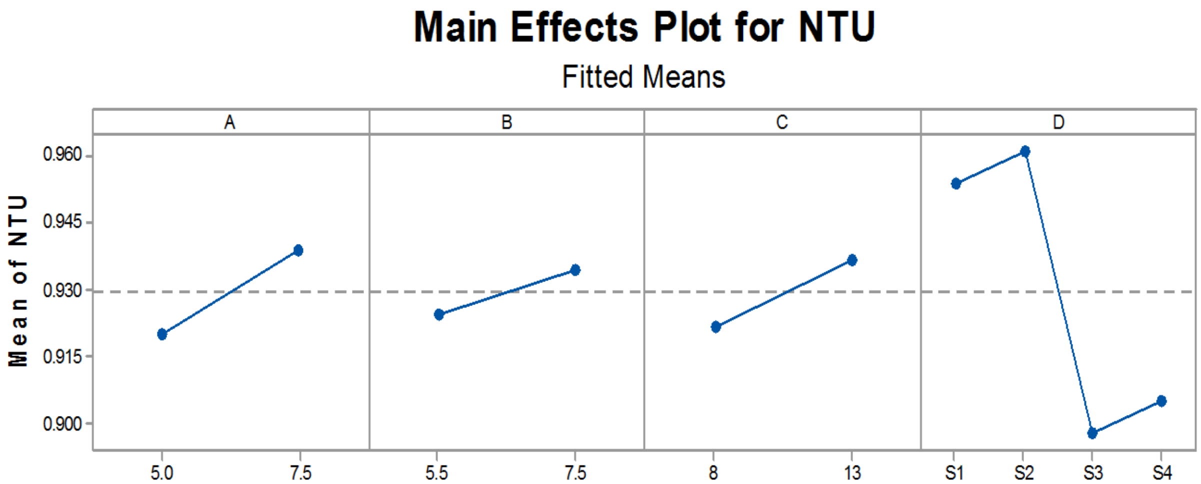


Fig. 3: Interaction and main effect plots of NTU versus variables

Table 4: Optimum amount of mix in the best condition

Mix adding stage	Vitamin C (mg)	Gelatin (mg)	Bentonite 10% (g)	NTU	Color	Clarity
*Blank	0	7.5	13.3	1	60	95.1
Mash enzyme	7.5	7.5	13	0.92	66	96
Crude juice tank	5	7.5	13	0.93	66	96.4
Fining tank (single-stage)	5	5	8	0.85	75	98.2
Fining tank (two-stage)	5	5.5	8	0.85	70	97.1

*Blank: The mix (without L-ascorbic acid) was added to the apple concentrate in the fining tank (single-stage)

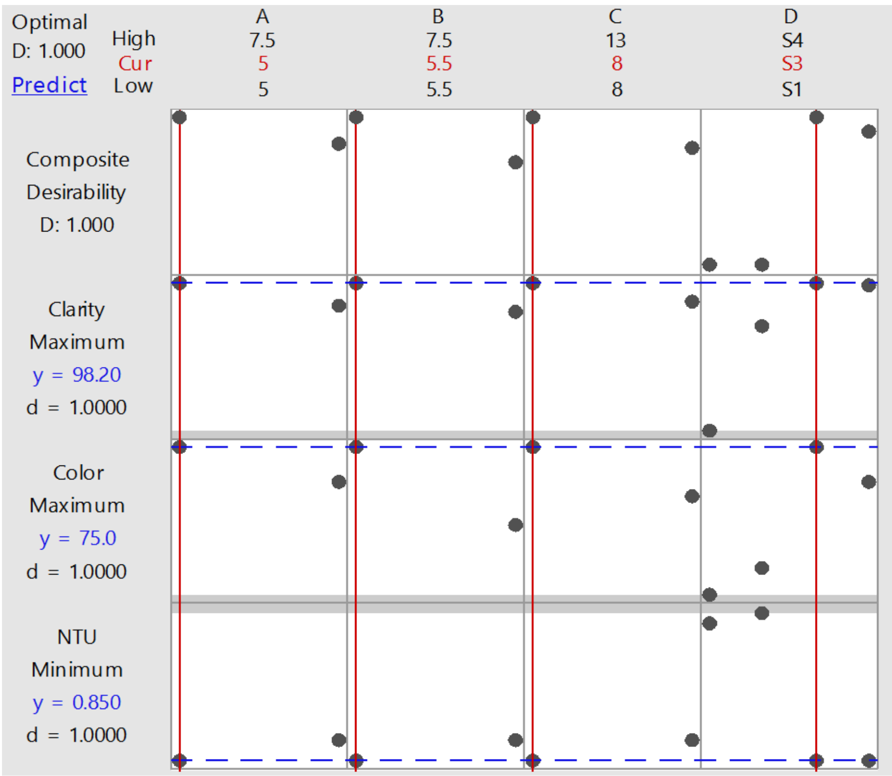


Fig. 4: Desirability and composite desirability of responses based on variables

Desirability functions for each stage of mix adding were done and the best condition (highest color and clarity and the lowest NTU) was calculated for a mix adding the stage and listed in Table 4. Figure 4 shows desirability and composite desirability of responses based on four variables. According to the results in the compared with the blank (mix without L-ascorbic acid) adding of L-ascorbic acid in the clarificant mix in any stage of apple concentrate production cause to enhance the quality of samples. Adding of the L-ascorbic acid mix in the fining stage has the best results.

CONCLUSION

L-ascorbic acid as an additive in flocculation agents (gelatin-bentonite) was used for clarification of apple concentrate samples in flocculation and precipitation process. L-ascorbic acid was added to apple concentrate in different products of apple concentrate in four stages of production. The General full factorial design was used to design experiments that study effects of four variables (L-ascorbic acid amount, gelatin amount, bentonite 10% amount and mix adding stage) on the apple concentrate quality (Turbidity, color and clarity). Desirability function was used to predict and calculate optimum condition of analysis. Results showed that adding of L-ascorbic acid in mash enzyme tank and crude apple juice had no significant effects on the quality of apple concentrate, but adding of it on the fining tank product significantly enhance sample quality.

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