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Effect of Electric Field Strength and Applied Temperature on Vitamin C, Total Phenolic Content and Total Carotenoids Content of Ohmic Heated Orange Juice

Manish Kumar[1]* and Aradhita Ray[1]
Department of Food Technology,
Guru Jambheshwar University of Science and Technology,
Hisar, Haryana 125001
*Corresponding address: +919896164879, gjumanish@gmail.com

Abstract

The present study was carried out to evaluate the impact of electric field strength (5.87-16.33 V/cm) and temperature during ohmic heating on vitamin C and total phenol content and total carotenoids content of orange juice. The experiments of ohmic heating of orange juice (250 ml) were performed in glass assembly. Further cooling of ohmic heated orange juice was carried out by transferring the juice in a beaker and placing the beaker in ice tray till further analysis. The central composite rotatable design CCRD (Design expert trial version) was used to optimize the variables; involved 13 experiments with 5 replications of the central points. Maximum retention vitamin C, carotenoid content and total phenol content was found at the combination of electric field strength and temperature at (+1, -1) (+1, -1) and (+1, +1).The least deviation in actual values (carotenoids: 0.52 mg/100 g, vitamin C content: 20.17 mg/100 g, total phenol content: 32.57 mg/100 g and heating rate 5.10 °C/min) of response against predictedvalues (carotenoids: 0.54 mg/100 g, vitamin C content: 19.72 mg/100 g and total phenol content: 31.08 mg/100 g) was found in condition at electric field strength of 6.8 V/cm and temperature of 60 °C. Therefore, it was found best among respective 3 optimum conditions having less than 5% deviation.

Keywords: *Ohmic Heating, Orange Juice, Electric field strength*

Introduction

Ohmic heating is a novel technique of food processing in which an alternating current is passed through food products to heat them. It is also known as electrical heating, electro conductive heating, direct heating, electrical resistance heating and joule heating[4]. This technique is based on the principle. When an alternating current is passed through any conductive material, there is heat generation due to I^2R losses within it. When electricity is passed through food product, the movement of ions takes place. The moving ions collide with molecules and transfer their kinetic energy to molecules which results in heating of food product. Ohmic heating can either be achieved by direct current (DC) or alternating current (AC) but in most of food applications alternating current is used because of easy availability of AC supply and occurrence of electrode polarization in DC system. However, AC power supply of various frequencies different from the local power supply can be used. The rate of ohmic heating is directly proportional to the square of electric field strength and electrical conductivity. The most important parameter in ohmic heating is electrical conductivity[7], which depends on temperature, frequency and product composition; it can be increased by addition of ionic compounds (salt and acid) and decreased by addition of nonpolar components e.g. emulsified lipids. Material must be capable of conducting an electrical current for ohmic heating processing. Moreover, electrical conductivity of liquid food increases with increase in temperature while in case of solid food it is also dependent on electric field strength.

To perform ohmic heating, food is placed in between two electrodes and current is passed. In comparison to conventional heating, ohmic heating is a better technique of thermal processing for food due to better retention of nutrients and oraganoleptic characteristics. Rapid and uniform heating is a unique feature of this technique, volumetric heating takes place due to internal heat generation during ohmic heating, while heat is transferred from outside to inside by conduction, convection and radiation in conventional heating.Oranges are consumed all over the world as it is an excellent source of vitamin C, which is said to be a potent natural antioxidant. Traditionally, this fruit has been used to treat diseases such as constipation, cramps, colic, diarrhoea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, depression and stress. There are many processed products in market developed from oranges. Heating is the most common method to arrest the growth of micro-organisms, but in case of citrus fruit it commonly achieved by refrigeration. Ohmic heating of orange juice was carried out to develop pasteurized product due to its rapid uniform nature of heating. Orange juice quality parameters: physical characteristics (colour, appearance and fluidity), heat sensitive compound and inactivation of microbes and enzymes are important from industry point of view. Vitamin C degradation kinetics and microbial inactivation in ohmic heated orange juice was studied by many others[5,8]. Ohmic heating has been observed as a good technique to preserve nutrient in orange juice. In the present work effect of electric field strength and applied temperature at 50 hz frequency on vitamin C, total phenolic content and total carotenoids content of ohmic heated orange juice was studied.

Materials and Methods

Manual peeling of oranges was carried out prior to juice extraction by mechanical juice extractor. The experiments of ohmic heating of orange juice (250 ml) were performed in assembly number 5. The process was stopped immediately after attainment of desired temperature. Further cooling of ohmic heated orange juice was carried out by transferring the juice in a beaker and placing the beaker in ice tray till further analysis.Electric field strength (x_1) and temperature (x_2) were used as the independent variables. The experimental range and the levels of the experiment variables used in the coded and uncoded form are given in **Table -1**.The CCRD was used to optimize the independent variables (electric field strength and temperature) based on dependent variables (total carotenoids, vitamin C, total phenol content and heating rate). The experimental designs involved 13 experiments with 5 replications of the central point and presented in **Table -2** in coded and uncoded levels.

Table 1 Values of independent variables at five levels of the Central Composite Rotatable Design (CCRD) for ohmic heating of orange juice

Independent variables	Code	Levels in coded form				
		-1.41	-1	0	+1	+1.41
Electric field strength	x_1	3.6	4.5	6.8	9.1	10
Temperature	x_2	67.93	70	75	80	82.07

Table 2 The Central Composite Rotatable Design (CCRD) in coded and uncoded levels of variables employed for ohmic heating of orange juice

Experiment No.	Coded variables		Uncoded variables	
	x_1	x_2	x_1	x_2
1	-1.00	-1.00	4.5	70
2	1.00	-1.00	9.1	70
3	-1.00	1.00	4.5	80
4	1.00	1.00	9.1	80
5	-1.41	0.00	3.6	75
6	1.41	0.00	10	75
7	0.00	-1.41	6.8	67.93
8	0.00	1.41	6.8	82.07
9	0.00	0.00	6.8	75
10	0.00	0.00	6.8	75
11	0.00	0.00	6.8	75
12	0.00	0.00	6.8	75
13	0.00	0.00	6.8	75

Estimation of total carotenoid content

A known weight of sample (5 g) was accurately weighed and extracted with a solvent mixture containing 40 mL acetone and 60 mL hexane by using a pestle and mortar, 0.1 g of magnesium carbonate was added during extraction. The residue was allowed to settle and decanted slowly in a separating funnel. It was then washed twice with 25 mL portion of acetone and 25 mL hexane and the repeated washing with water was also done. The upper layer was transferred to a 100 mL volumetric flask containing 9 mL acetone and volume was made up with hexane. The total carotenoid content was calculated by measuring OD at 450 nm using spectrophotometer as given below. Mixture of hexane and acetone in 9:1 mL ratio was used as blank. Total carotenoids content was calculated as following:

$$\text{Total carotenoid (mg/100g)} = \frac{\text{OD at 450 mm} \times \text{volume make up}}{250 \times \text{weight of sample}}$$

Estimation of total phenol content

5 g sample was homogenized with cold 80 % ethanol using a pestle and mortar. The extract was centrifuged for 15 min10,000 rpm. The residue was extracted again and centrifuged. The clear supernatant was cooled and volume was made up to 25 mL with 80 % ethanol, 0.5 mL of ethanolic extract, 8.5 mL of water and 0.5 mL of phenol reagent (Folin-Ciocalteau, 1:2 diluted) was added and the contents allowed to stand at room temperature for 3 min. Saturated sodium carbonate **(**1 ml) was added and incubated for 1 h at room temperature. The

absorbance was recorded at 675 nm. Gallic acid in 80% ethanol was used as standard.

Estimation of vitamin C

2, 6-dichlorophenol indophenols dye method of ascorbic acid estimation was used for ascorbic acid analysis. Dye factor determination by using formula**:** Dye Factor = 0.5/titre value

Procedure

3% HPO_3 was used as a dilatant to increase the volume of sample from 10 mL to 100 ml. Diluted samples were then subjected to centrifugation and filtered to obtain a clear solution. 5 mL of 3 % HPO_3 extracted sample was titrated with dye solution and results were calculated as follows:

Ascorbic acid content of the sample (mg) =

$$\frac{\text{(Titrate × dye factor × volume make up)}}{\text{(Aliquot of extract for estimation × wt. or volume of sample taken)}}$$

Results and Discussion

Effect of ohmic heating on total carotenoid content of orange juice

Total carotenoids of orange juice varied from 0.31 to 0.86 mg/100 g **(Table - 3)**. Maximum carotenoid content was found at the combination of electric field strength and temperature at (+1, -1) coded points level and it was 2.78 times higher than the minimum value of total carotenoids in orange juice. The coefficients of model and other statistics are given in **Table 4**. The model's F-value of 26.03 for total carotenoids indicates that model is significant ($P<0.05$). R^2 value (0.839), adjusted R^2 value (0.807) and adequate precision (14.828) indicates that the model can be used for prediction purposes. It could be observed from the **Table- 4** that rise in temperature causes higher degradation of the total carotenoids of orange juice as, the coefficient of $x_1$is negative. The electric field strength has shown no significant effect on the total carotenoids in ohmic heated orange juice (Fig.- 1). Bozkir*et al.* (2015) studied carotenoid content of orange juice processed by conventional method. It was reported that due to oxidation, isomerization takes place which can be further accelerated by presence of oxygen, heat, light, enzymes, metals and co-oxidation with lipid hydroperoxides[2], this leads to reduction in carotenoid content. But a little a variation in carotenoid content was observed in present study; moreover, there was no significant effect of electric field strength. The method of estimation also results in variation of total carotenoids content in orange. Vitamin C content was also not much affected in this study; it could also be a possible reason for minor variation of total carotenoid in treated samples, as vitamin C has a protective role on degradation carotenoids.

Table 3 The CCRD matrix used for ohmic heating of orange juice and experimental value of responses

Sr. No.	Electric field strength	Temperature	Total Carotenoids	Vitamin C	Total phenol content
1	-1.00	-1.00	0.615	18	32
2	1.00	-1.00	0.864	25	38
3	-1.00	1.00	0.31	16	33
4	1.00	1.00	0.364	22	57.23
5	-1.41	0.00	0.511	10	26
6	1.41	0.00	0.451	23	56.5

7	0.00	-1.41	0.786	21	26.2
8	0.00	1.41	0.385	17	40.12
9	0.00	0.00	0.536	21.3	32.15
10	0.00	0.00	0.545	19	31
11	0.00	0.00	0.526	21.4	32.15
12	0.00	0.00	0.532	21.1	28.2
13	0.00	0.00	0.533	21.5	32

Table 4 Analysis of variance for carotenoids

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob>F
Constant	2.272					
x_1	0.606^{ns}	0.006	1	0.006	1.320	0.277
x_2	-0.172^{***}	0.235	1	0.235	50.740	0.000
Complete model						
Regression		0.241	2	0.121	26.030	0.000
Lack of Fit		0.046	6	0.008	159.375	0.000
Pure Error		0.000	4	0.000		
Residual		0.046	10	0.005		
Total		0.288	12			
R^2		0.839		Adeq. Precision		14.828
Adjusted R^2		0.807				

Level of significance: *P<0.1, **P<0.05, ***P<0.01, ns: non significant, df: degree of freedom

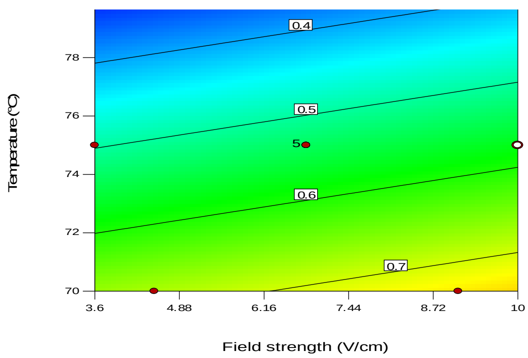

Fig. 1 Effect of temperature and electric field strength on carotenoids content of orange juice

Effect of ohmic heating on vitamin Ccontent of orange juice

The vitamin C content of orange juice varied from 10 to 25 mg/100 g. Maximum vitamin C content was found at the combination of electric field strength and temperature at (+1, -1) coded point level and it was 2.5 times higher than the minimum value. The models F-value of 17.98 for vitamin C content indicates that model was significant (P<0.05).R^2 value (0.782), adjusted R^2 value (0.739) and adequate precision (11.722) indicates that the model can be used for prediction purposes. The coefficients of model and other statistics are given in **Table - 5**. It could be noticed that increase in electric field strength (x_1) increases the vitamin C content as the coefficient of x_1 is positive (**Fig.- 2**). The temperature (x_2) also had significant (P<0.1) effect on the vitamin C content. Higher losses of vitamin C were observed at higher temperature. Retention of vitamin C was better with applied field strength which indicates the suitability of process. It could be correlated to faster rate of heating at higher field strength. It was expected that degradation of vitamin C will be higher with temperature but results had not given such indication, it may be due to shorter heating time. Ohmic heating of orange juice at 90°C had resulted 21% degradation in ascorbic acid content. Vitamin C degradation (15%) in orange juice during ohmic heating (90, 120 and 150°C for different time interval) was observed by many others[5, 8].

Table 5 Analysis of variance for vitamin C content

Source	Coefficient of model terms	Sum of Squares	Df	Mean Square	F Value	Prob>F
Constant	265.60					
x_1	85.85***	122.77	1	122.773	32.234	0.000
x_2	-1.33*	14.20	1	14.196	3.727	0.082
Complete model						
Regression		136.97	2	68.484	17.980	0.000
Lack of Fit		33.68	6	5.613	5.089	0.069
Pure Error		4.41	4	1.103		
Residual		38.09	10	3.809		
Total		175.06	12			
R^2		0.782		Adeq. Precision		11.722
Adjusted R^2		0.739				

Level of significance: *P<0.1, **P<0.05, ***P<0.01, ns: non significant, df: degree of freedom

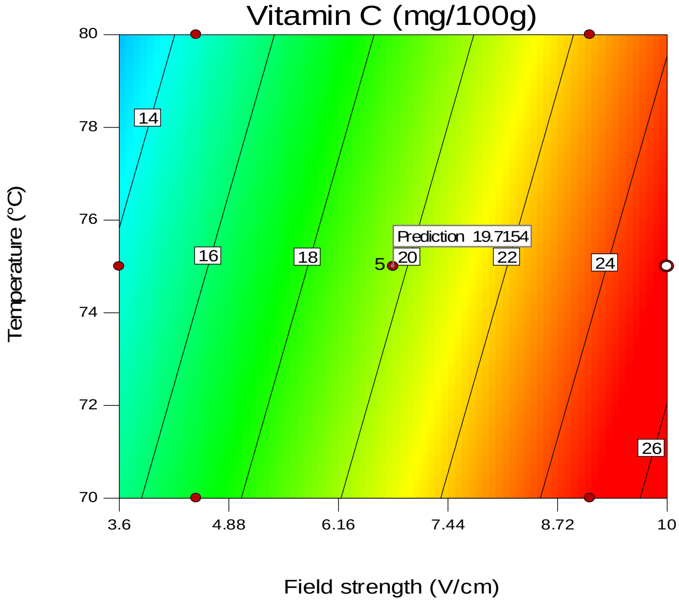

Fig. 2Effect of temperature and electric field strength on vitamin C content of orange juice

Effect of ohmic heating on Total phenol content of orange juice

Total phenol content of orange juice varied from 26 to 57.23 mg/100 g. Maximum total phenol content was found at maximum level of electric field strength and temperature and it was 2.2 times higher than the minimum value. The coefficients of model and other statistics are given in **Table – 6**.The models F-value of 34.55 for total phenol content indicates that model was significant (P<0.05). R^2 value (0.961), adjusted R^2 value (0.933) and adequate precision (16.841) indicates that the model can be used for prediction purposes. The electric field strength (x_1) and temperature (x_2) had shown a significant (P<0.1) linear effect on the total phenol content in orange juice (Fig. - 3). Interaction effect of both the variables were also found significant (P<0.01). The quadratic effect of electric field strength was also significant (P<0.01) but temperature had a non-significant effect on total phenol content also found similar trend during ohmic heating of water melon juice. The change in total phenolic content and colour of bottle guard juice processed by conventional and ohmic blanching was reported earlier[1]. They reported that ohmic treated samples shows better colour retention and higher phenolic content. Higher phenol content might be due to release of bound phenols and breakdown or salting of cellular material[5].

Table 6 Analysis of variance for total phenol content

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob>F
Constant	23697.23					
x_1	16325.96***	241.04	1	241.043	34.842	0.001
x_2	288.74***	86.02	1	86.020	12.434	0.010
$x_1.x_2$	99.08***	83.08	1	83.083	12.009	0.010
x_1^2	2815.17***	235.17	1	235.167	33.992	0.001
x_2^2	1.70ns	20.11	1	20.112	2.907	0.132
Complete model						
Regression		1195.12	5	239.024	34.550	0.000
Lack of Fit		36.99	3	12.331	4.313	0.096
Pure Error		11.44	4	2.859		
Residual		48.43	7	6.918		
Total		1243.55	12			
R^2		0.961		Adeq. Precision		16.841
Adjusted R^2		0.933				

Level of significance: *P<0.1, **P<0.05, ***P<0.01, ns: non significant, df: degree of freedom

Optimization

The numerical multi-response optimization technique with desirability function was used to estimate the optimum level of electric field strength and temperature. Three optimum conditions having desirability of 1 were determined for ohmic heating of orange juice. These optimum conditions (in the range constraint) were used to process the orange juice by ohmic heating and validated the predicted and actual values of responses at the optimized condition of experiments. The least deviation in actual values (carotenoids: 0.52 mg/100 g, vitamin C content: 20.17 mg/100 g, total phenol content: 32.57 mg/100 g and heating rate 5.10 °C/min) of response against predicted values (carotenoids: 0.54 mg/100 g, vitamin C content: 19.72 mg/100 g, total phenol content: 31.08 mg/100 g and heating rate: 5.15 °C/min) was found in condition at electric field strength of 6.8 V/cm and temperature of 60 °C. Therefore, it was found best among respective 3 optimum conditions having less than 5% deviation. Hence the optimized conditions were considered for further study.

Pasteurized orange juice was prepared by conventional and ohmic heating processing methods. Xanthophyll and tannic acid were not in traceable amount in orange juice therefore only vitamin C, total phenol, total carotenoid, viscosity of juice sample and sensory analysis of processed juice sample were carried out for physicochemical examination. As expected vitamin C contents of both type of processed juice was found lower than fresh juice of orange. A significant difference between vitamin C content of raw and both type processed juice samples was found. There was no significant difference between vitamin C contents of conventional heated and ohmic heated juice. HTST pasteurization (80°C, 15 Sec) of juice was carried out for conventional heating and lower applied field strength for ohmic heating which required more time to achieve the desired temperature resulting in higher loss of vitamin C contents as shown in **Table – 7**. Total phenol content of conventional processed juice (39.73 mg/100g) was higher than ohmic (32.57mg/100g) heated and fresh orange juice. A significant difference was observed between total phenol content of ohmic heated orange juice and fresh orange juice. Higher applied temperature might be resulted in more extraction of total phenol in conventional heated juice. Though, temperature of ohmic heating was also higher so the possible electrochemical change had contributed for degradation of phenols in ohmic heating. Total carotenoids were found to be decreased in both type of processing without any significant difference between total carotenoid content of conventional (0.73 mg/100 g) and ohmic heated (0.52 mg/100 g) samples. Isomerization of carotenoids at applied condition could be the possible reason of degradation of carotenoids. There was no significant difference in viscosity of all three types of juice samples. Heating either by conventional or ohmic methods resulted in very poor sensory score of fresh juice samples. Rather, a gummy flavor was observed in ohmic treated juice sample. The sensory characteristics of conventional processed juice (7.33) were better than ohmic processed juice (5.00). It might be due to leaching of rubber material from rubber closer and dissolution of Teflon tape gum inside the product. Leaching of metal ion can also be another possible reason of poor sensory score of ohmic processed juice sample. From microbiological safety aspect both conventional and ohmic heated juices has shown sound results. Total plate count was found to be decreased in both processing and a complete removal of yeast and mold count and coliform count was observed. Despite of better retention of nutrient and microbiological safety (**Table–8**), ohmic heating was not found suitable for processing of orange juice due to poor sensory score at applied processing condition.

Table 7 Physicochemical and sensory properties of raw and treated orange juice

	Vitamin C	Total phenol content	Total Carotenoids	Viscosity	Sensory OAA (n=12)
Raw	29.33±3.06[a]	38.19±2.46[a]	1.18±0.19[a]	102.33±10.02[a]	9.25±0.45[a]
Conventional	18.17±1.04[b]	39.73±2.67[a]	0.73±0.16[b]	105.67±8.50[a]	7.33±0.49[b]
Ohmic	20.17±1.60[b]	32.57±2.46[b]	0.52±0.09[b]	97.33±6.03[a]	5.00±0.43[c]

(n=3, mean ± SD); OAA- overall acceptability
Means with different superscripts within a column for a particular parameter differ significantly ($P<0.05$)

Table 8 Microbiological quality of raw and treated orange juice

	Total plate count (log cfu/g)	Total coliform count(log cfu/g)	Yeast and mold count(log cfu/g)
Raw	4.82±0.37	1.9±0.34	3.68±0.29
Conventional	2.52±0.22	ND	ND
Ohmic	1.78±0.31	ND	ND

(n=3, mean ± SD); ND- not detected

Conclusion

A lot of literature is available on ohmic heating of orange juice but still the finding could not be truly justified. Beside the nutrient retention, sensory quality was found adversely altered in both conventional and ohmic processing. So, it was concluded that heating must be replaced by another suitable technique for preservation of orange juice to obtain a sensory acceptable product. Manual peeling of oranges was carried out prior to juice extraction by mechanical juice extractor. Thirteen experiments were performed for orange juice (250 ml) in assembly number five by using different combinations by applied field strength (3.6, 4.5, 6.8, 9.1 and 10 V/cm) and temperature (67.9, 70, 75, 80 and 82^{o}C) as suggested by Design expert software (CCRD). Optimized result of ohmic heating of orange juice expressed that there was least deviation in actual values (carotenoids: 0.52 mg/100 g, vitamin C content: 20.17 mg/100 g, total phenol content: 32.57 mg/100 g and heating rate 5.10 °C/min) of response against predicted values (carotenoids: 0.54 mg/100 g, vitamin C content: 19.72 mg/100 g, total phenol content: 31.08 mg/100 g and heating rate: 5.15 °C/min) was found in condition at electric field strength of 6.8 V/cm and temperature of 60 °C. Therefore, it was found best among respective 3 optimum conditions having less than 5% deviation. Hence the optimized conditions were considered for further study. A lot of literature is available on ohmic heating of orange juice but still the finding could not be truly justified. Beside the nutrient retention, sensory quality was found adversely altered in both conventional and ohmic processing. So, it was concluded that heating must be replaced by another suitable technique for preservation of orange juice to obtain a sensory acceptable product.

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