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# Cryoconcentration of Sour Cherry and Orange Juices with Novel Clarification Method; Comparison of Thermal Concentration with Freeze Concentration in liquid Foods

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## ABSTRACT

**Sour cherry and orange juice were successfully cryoconcentrated. Novel clarification (Electro-Flotation and Ultra-Filtration) improved cryconcentration efficiency. EF-UF clarified sour cherry and orange juices were cryoconcentrated in three stages up to 34.52±0.14, 44.42±0.19, 52.44±0.13 and 28.43±0.16, 40.51±0.15, and 45.42±0.19° Brix at -10˚C respectively. Duncan's multiple range test was used to compare mean values of various parameters. At similar total soluble solid, cryoconcentrated samples showed significantly (P< 0.05) higher retention of aroma number, ascorbic acid, and TAA compared to those thermally concentrated. Thermal concentration induced formation of hydroxymethylfurfural more than cryoconcentration process used for concentration of orange juice.**

**Keywords** : Aroma number, Ascorbic acid, Electro-flotation, Hydroxylmethylfurfural, Total antioxidant activity.

## INTRODUCTION

Production of fruit juice concentrate is based on different technologies that include thermal concentration, membrane concentration, and cryoconcentration. Fruit juices are important sources of nutrients and energy, and play an important part in human nutrition. Also, it has been observed that consumption of fruit juices can prevent certain diseases such as cancer and cardio-vascular diseases as fruit juices are rich in antioxidant vitamins including vitamin C and E, phenolic compounds, and carotene (Block *et al.*, 2001; Burns *et al.*, 2003; Gardner *et al.*, 2000; John *et al.*, 2002; McCall and Frei, 1999). Thus, thermal concentration is not a suitable method for fruit juice concentration, because prophylactic and nutraceutical components of fruit juices are adversely affected. Cryoconcentration is a promising method in fruit juice concentration in which water is removed as ice and not vapor. It can be an alternative to thermal concentration, but the achievable concentration is lower (about 40 g TSS 100 $g^{-1}$) than values obtained by thermal concentration (60 g TSS 100 $g^{-1}$) (Aider and de Halleux, 2008). Cherry and apricot juices were successfully cryoconcentrated to 45 and 35 g TSS 100 $g^{-1}$, respectively (Aider and de Halleux, 2008).

Clarification can remove suspended solids and colloidal materials of the single strength fruit juice and improve the efficiency of cryoconcentration. Juice clarification is a typical step where ultra-filtration process has

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been successfully utilized for different fruit juices (Cassano et al., 2003; Cassano and Drioli, 2006 ; Cassano et al., 2007 ; He, Ji, and Li, 2007). Electro-flotation is a convenient pre-clarification process for fruit juices. It is a solid/liquid separation process that is significantly based on the suspension of particles by gas bubbles generated at the surface of electrodes, which are immersed in the fruit juice by the application of a current (Burns *et al.*, 1997). Apple juice was successfully clarified by electro-flotation and enzymatic treatment better than traditional clarification process (Araya-Farias *et al.*, 2008). The objective of this study was to assess the effect of integrated clarification process (electro-flotation and ultra-filtration) on the efficiency of cryoconcentration technology for obtaining higher total soluble solids with better nutritional properties.

## MATERIALS AND METHODS

### Preparation of Fruit Juices

Orange and sour cherry fruits were purchased from a local market in Amol and washed with tap water in order to remove foreign material from the skin. Then, the juice was extracted by FMC juice extractors with a 2-mm-diameter perforated plate and placed in a tank. Extracted juices were 100 and 120 L from 120 and 160 kg sour cherry and orange fruits, respectively. 4 gr $kg^{-1}$ $Na_2So_3$ was added to single strength juice to avoid browning reactions. Preparation of fruits juices is shown in Figure 1.

### Electro-flotation Unit

An electro-flotation cell was made for pre-clarification of fruit juices. The flotation cell was square (20 cm by 22 cm tall) and made of Plexiglas (figure is not shown). A sampling valve was fixed 11 cm above the cell bottom. The cathode was a stainless steel screen (wire diameter of 2 mm), and was positioned horizontally on top of graphite rods forming the anode. The distance between the two electrodes was 10 mm. The anode was fixed at 1.2 cm above the cell bottom. The anode's area was 144 $cm^2$ and the current density was calculated by using this area. Electrical wires were attached to the electrodes with conductive resin and then connected to an external power supply. Electro-flotation cell was made by a design suggested by Araya-Ferias *et al*. (2008).

### UF Process

Orange and sour cherry juices were clarified by ultra-filtration. The plant, with a 30 l feed tank was equipped with a Kock tubular membrane module. Its specifications were: type series-Cor HFM-251, PVDF, nominal molecular weight cut-off 15 k Da, surface membrane area 0.23 $m^2$, average diameter of pores 59 A˚, pressure operating range 0.8-5.5 bar, temperature operating range ( 0–55°C ) and pH operating range of 2-11. Ultra-filtration unit was supplied by Gela food factory (producing white Iranian cheese by UF).

### Cryoconcentration Process

Fruit juices clarified by electro-flotation and ultra-filtration were introduced into a cylindrical container and put in the freezer with circulation of icy air at -10±1°C. A thermocouple was inserted in the center of the cylinder to record the final temperature of the frozen juices. Thawing procedure was carried out at room temperature by means of simple gravitational thawing. Fruit juice samples (10 L) were cryoconcentrated. The frozen juices were kept at room temperature under positive pressure to avoid probable contamination due to bacterial growth. A little part of the sample was kept for experiment and the remaining was used as feed solution for the second step of cryoconcentration. Concentrated fruit juices obtained in the second step were used as





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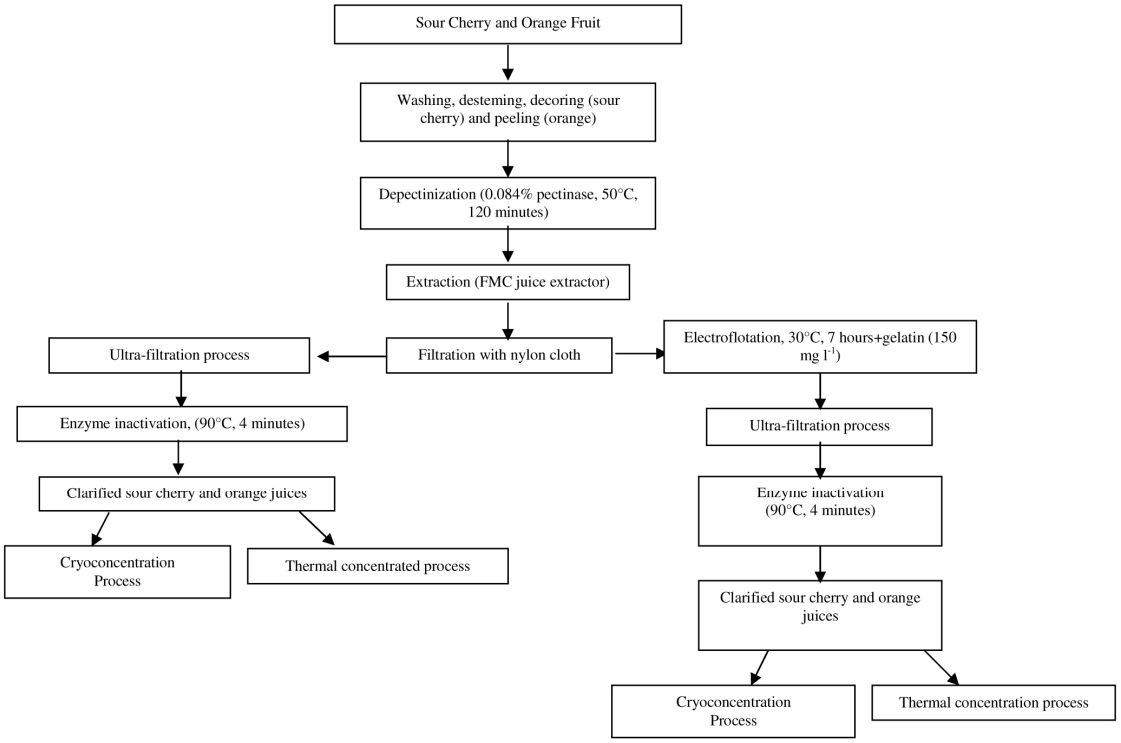


**Figure 1**. Processing flow chart of clarified sour cherry and orange juices into concentrated ones.

feed solution for the third step of cryoconcentration process. After each step, the juices were stored for analysis at 1-0°C in a dark glass bottle.

## Thermal Concentration

A three-stage column evaporator (Type PAF 53 S, Zürich and Switezerland) concentrated orange and sour cherry juices at 70-90°C applying vacuum of 450 mbar and a feed rate of 70 L $h^{-1}$. Concentrated fruit juices were kept refrigerated at 2°C for further analysis.

## Sample Analyses

Total soluble solid measurement was carried out using hand refractometers (Atago Co., Ltd., Tokyo, Japan) with scale range of 0-32, 28-62, and 58-90° Brix. For sample analyses, concentrated fruit juices were diluted to single strength fruit juice by distilled water. Vitamin C was analyzed by oxid-reduction reaction using 2,6- dichlorophenol indophenol for concentrated orange juice. Measurement of ascorbic acid was not applicable by oxid-reduction reaction for concentrated sour cherry juice due to coincidence of pink color (termination of titration) with the color of sour cherry juice. We used spectrophotometer method (Pepkowitz, 1943; Robinson and Stotz, 1945) for determination of ascorbic acid in concentrated sour cherry juice. The measurements were performed by a spectrophotometer at wavelength of 500 nm against xylene. Aroma number was determined by the method reported by Kovalenko (1997). Hydroxymethylfurfural (HMF) was determined by a method proposed by IFFJP (1984). This method is based on HMF reaction with barbituric acid and p-toluidin, forming a red compound. This reaction had a maximum rate at 3 - 4 minutes and afterwards, Hydroxymethylfurfural (HMF) content of samples were measured at 550 nm absorbance by spectrophotometer. Total





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antioxidant activity (TAA) was determined by the improved version of 2,2́ azinobis diammonium salt assay in which the radical cation is generated by reaction with potassium persulphate before addition of antioxidant that was reported by Re (1999). Forty-five ml of sample (clarified and non-clarified) was prepared to determine suspended solid content in relation to total juice (w/w %) by centrifuging at 200 rpm (g= 670.27) for 20 minutes. The weight of the settled solids was determined after removing the supernatant.

### Statistical Analysis

Measurements were replicated (n= 3) for each parameter and reported as mean value ± standard error. Statistics was performed on a completely randomized design with the analysis of variance (ANOVA) procedure in SAS software. Duncan's multiple range test ($P< 0.05$) was used to detect differences among mean values of various parameters.

## RESULTS AND DISCUSSION

### Effect of Electroflotation on UF

Analytical measurements on fresh sour cherry and orange juices and in samples coming from ultra-filtration and integrated electro-flotation and ultra-filtration are shown in Table 1. Permeate fluxes of orange juice and sour cherry juices were 37.42 and 39.58 L $m^{-2}$ $h^{-1}$ during ultra-filtration process, respectively. Orange and sour cherry juices permeate flux increased from 37.42 and 39.58 to 47.32 and 49.57 L $m^{-2}$ $h^{-1}$, respectively, which were pre-clarified by electro-flotation before UF. As a technological point of view, electro-flotation process increased permeate fluxes by 9.90 and 9.99 L $m^{-2}$ $h^{-1}$ for orange juice and sour cherry juice, respectively. As a conclusion, ultra-filtration efficiency increased by pretreatment of fruit juices with electro-flotation. However, the practicality and cost effectiveness of using electroflotation-ultrafiltration process may be questioned. Solid content in flocculation as a function of electro-flotation of sour cherry and orange juice is shown in Figure 2. No suspended solids in fruit juices coming from integrated electro-flotation and ultra-filtration were detected in our experiment. Ultra-filtration was used to remove suspended solids in fruit juices so as to obtain concentrated fruit juices with higher total soluble solid during cryoconcentration.

### Cryoconcentration of Samples

Clarified orange and sour cherry juices submitted to electro-flotation and ultra-

**Table 1**. Analytical measurements during ultra-filtration of electroflotated sour cherry and orange juice and enzymatically treated single strength sour cherry and orange juice.

| Samples | TSS[a] (Brix) | Suspended solids(w/w%) | Ascorbic acid (mg $l^{-1}$) | TAA[b] (mM trolox) |
|---|---|---|---|---|
| Sour cherry | 14.20±1.10 | 4.18±0.74 | 8.02±1.36 | 4.60±0.44 |
| UF permeate (sour cherry) | 14.17±1.22 | 0.82±0.84 | 8.15±0.98 | 3.27±0.11 |
| UF retentate (sour cherry) | 15.08±1.40 | 48±7.78 | 5.24±0.88 | 3.82±0.24 |
| Orange | 10.24±1.13 | 5.27±0.48 | 34.22±5.94 | 8.72±0.76 |
| UF permeate (orange) | 10.11±0.70 | 0.97 ± 0.66 | 34.18±4.36 | 7.53±0.84 |
| UF retentate (orange) | 11.14±0.88 | 53±4.74 | 29.17±3.58 | 7.77±1.04 |
| UF permeate (EF[d]-orange) | 10.07±0.91 | ND[c] | 34.20±7.88 | 7.92±1.12 |

[a] Total soluble solid; [b] Total antioxidant activity; [c] Not detected, [d] Electro-flotation.





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**Table 2**. Total soluble solid of orange and sour cherry juice during cryoconcentration [a].

| | EF-UF sour cherry juice | | EN-UF sour cherry juice | |
|---|---|---|---|---|
| | -10°C | -20°C | -10°C | -20°C |
| $NO_1$ | 34.52±0.14[e] | 34.42±0.15[e] | 33.01±0.32[f] | 32.23±0.7[f] |
| $NO_2$ | 44.42±0.19[c] | 44.32±0.33[c] | 41.8±0.26[d] | 40.98±.03[d] |
| $NO_3$ | 52.44±0.13[a] | 51.5±0.36[a] | 46.65 ±.02[b] | 46.14 ±0.33[b] |
| | EF-UF orange juice | | EN-UF orange juice | |
| $NO_1$ | 28.43±0.16 [g] | 27.55±0.13 [h] | 26.90±0.22 [i] | 25.08±.20 [i] |
| $NO_2$ | 40.51±0.15[c] | 40.54±0.18 [c] | 38.63±0.18 [e] | 35.80±0.14 [f] |
| $NO_3$ | 45.42±0.19[a] | 44.63±0.13 [b] | 39.86±0.20 [d] | 38.75±0.22 [e] |

[a] NO 1, 2, 3 are representative of cryoconcentration levels. Mean values of ° Brix with different letter are significantly different (P< 0.05) by Duncan's multiple range test. EF-UF and EN-UF are representative of Electroflotation-Ultrafiltration and Enzymatic-Ultrafiltration treatment respectively.

filtration (EF-UF) were cryoconcentrated to higher total soluble solids rather than fruit juices which were only clarified by ultra-filtration and enzymatic treatment. The cryoconcentration process took nearly 10 hours. In our experiment, cryoconcentration of integrated clarified fruit juices (EF-UF) increased efficiency of this process so that higher total soluble solids were obtained. Final total soluble solids of fruit juices obtained during cryoconcentration are shown in Table 2. All of the main effects, as well as the associated interactions, were significant at the *P*< 0.05 level for Brix (df= 2, 59; F= 8,045.588), temperature (df= 1, 59; F= 145.0193), method (df= 1, 59; F= 1283.2449), temperature×method (df= 1, 59; F= 44.3551), temperature×Brix (df= 2, 59; F= 1.8444), and method×Brix (df= 2, 59; F= 112.5248) in cryoconcentration of orange juice. All of the main effects, as well as the associated interactions, were significant at the *P*< 0.05 level for Brix (df= 2, 59; F= 2,495.5672), method (df= 1, 59,;F= 364.1589), temperature×method (df = 1, 59; F= 20.935 ), temperature×Brix (df= 2, 59; F= 38.574), and method×Brix (df = 2, 59; F= 36.8118) in cryoconcentration of sour cherry juice; but, there was no significant difference between different temperatures (df= 1, 59; F= 0.7939 ).

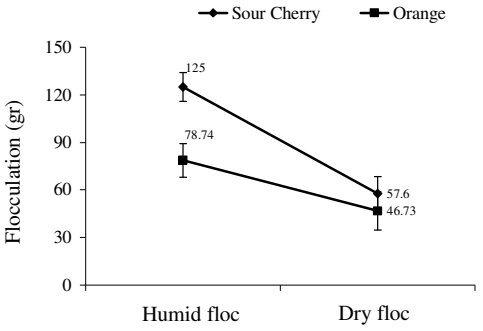


**Figure 2**. Humid and dry flocculation as efficiency of electro flotation in removing of suspended solids from single strength sour cherry and orange juice during electro-flotation.

## Mechanism of Cryoconcentration Method

Water molecules exist in two forms in foodstuff: free water and bounded water. In low temperature of cryoconcentration, free water is easily frozen and can be removed from feed solution being concentrated. However, bounded water does not freeze and this phenomenon causes lower total soluble solid in cryoconcentrated fruit juices compared to thermal concentration (Aider and de Halleux, 2008). Water in the form of bound water can bind to any molecule that has -OH or $-NH_2$ groups, thereby reducing their mobility (because water molecules surround them). Consequently, the tendency of these molecules to form crystals decreases and they can resist freezing at low temperatures. After thawing, this bounded water changes into liquid and decrease the total soluble solid of the juices. Suspended solids including pectin, protein, and tannin can surround water molecules and increase percentage of bounded





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water. Clarification removes suspended solids of fruit juices. Integrated electro-flotation and ultra-filtration (EF–UF) was more effective in removing suspended solids compared to enzymatic and ultra-filtration process. According to Table 1, after ultra-filtration, suspended solids measured 0.97, 0.82 and 0 (w/w %) for enzymatic clarified orange and sour cherry juices and UF-EF clarified orange and sour cherry, respectively. It means that the UF-EF treatment could completely remove suspended solids of fruit juices and this led to higher efficiency of cryoconcentration by obtaining higher total soluble solid .

## Mechanism of Electro-flotation

The higher total soluble solid during cryoconcentration of EF-UF clarified fruit juices is due to better removal of suspended solids. In our experiment, fruit juices treated with pectinase enzyme and gelatin were submitted to electro-flotation at 30° C. Polyphenol oxidase enzyme is active in single strength fruit juices in this range of temperature. In the presence of oxygen produced at the surface of electrodes, polyphenol oxidas enzyme oxidizes tannin more effectively, producing oliyomers which are not soluble in fruit juices and can be brought at the surface by the gas bubbles. This phenomenon was reported by Mayer and Harel (1979). This oligomer not only can be brought to the surface of the liquid but can also absorb better other colloidal matters such as proteins and pectin. As a conclusion, combination of electro-flotation and ultra-filtration processes clarified juices and eliminated suspended solids better than ultra-filtration process alone, and led to obtaining higher total soluble solid during cryoconcentration.

## Effect of Process on Vitamin C and Aroma Number

Ascorbic acid content of orange juice concentrated by cryoconcentration (-10±1°C) and thermal concentration at 27.55±0.13, 40.54±0.18, 44.63±0.13° Brix was 336.72±0.17, 340.52±0.24, 343.66±0.23 and 290.50±0.23, 225.57±0.21, 116.51±0.26 mg $l^{-1}$, respectively (Figure 3a). All of the main effects as well as the associated interactions were significant at the $P< 0.05$ level for Brix (df= 2, 23; F= 70,090.6439), type of concentration process (df = 1, 23; F= 493,231.0246) and Brix×type of concentration process (df= 2, 23; F= 81,829.1197). According to Figure 3b,

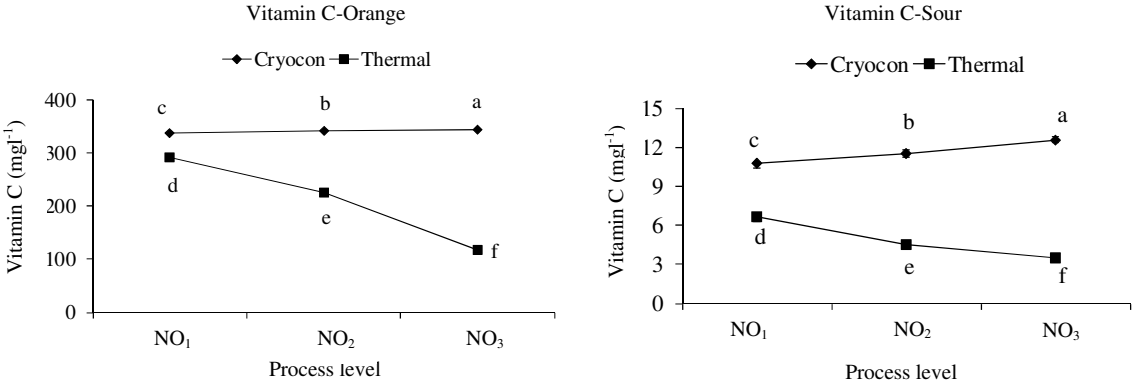


**Figure 3**. Ascorbic acid content in concentrated orange juice during cryoconcentration and thermal concentration. Means with different letter are significantly different (P< 0.05) by Duncan's multiple range test. $NO_1$, $NO_2$, $NO_3$ are steps of concentration produced by cryoconcentration at –10°C and thermal concentration process. (Orange juice: 28.43±0.16, 40.51±0.15, 45.42±0.19° Brix and Sour cherry juice: 34.52±0.14, 44.42±0.19, 52.44±0.13° Brix).





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vitamin C content of sour cherry juices concentrated at 34.42±0.15, 44.32±0.33, 51.50±0.36° Brix by cryoconcentration (-10±1°C) and thermal concentration was 10.75±0.14, 11.50±0.25, 12.56±0.23 and, 6.62±0.34, 4.50±0.23, 3.52±0.24 mg $l^{-1}$, respectively. All of the main effects as well as the associated interactions were significant at the $P< 0.05$ level for Brix (df= 2, 23; $F$= 4.8607), type of concentration process (df= 1, 23; F= 1,108.6441), and Brix×type of concentration process (df= 2, 23; $F$= 49.4835). The significant decrease in vitamin C content of the samples concentrated by thermal concentration is due to sensitivity of ascorbic acid to heat. Our results were in agreement with Qiu *et al.* (1998) who reported loss of vitamin C was 7-15% during heat pasteurization of orange juice, while this loss was less than the other non-thermal treatments (5%) such as pulsed electric field. Aroma number was better retained in cryoconcentrated fruit juices than thermally concentrated ones (Figure 4-a&b). All of the main effects as well as the associated interactions were significant at the $P< 0.05$ level for the type of concentration process (df= 1, 23; F= 4,831.5121) and type of concentration process×Brix (df= 2, 23; F= 391.9436) for aroma number of concentrated orange juice, but there was no significant difference between Brix (df= 2, 23; F= 0.1099). All of the main effects, as well as the associated interactions, were significant at the $P< 0.05$ level for Brix (df = 2, 23; F= 6.7175), type of concentration process (df= 1, 23; F= 2,619.8344) and Brix×type of concentration process (df= 2, 23; F= 311.4736) for aroma number of the concentrated sour cherry juice.

## Hydroxymethylfurfural Content of Orange Sample (HMF)

No significant HMF increase was observed during cryoconcentration of orange juice (Figure 5). Thermally concentrated orange juice had higher HMF content than cryoconcentrated orange juice. However, HMF content was lower than critical limit (< 20 mg $l^{-1}$) in thermally concentrated orange juice. The Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (AIJN, 1996) has included the amount of HMF among the absolute parameters of quality (< 20 mg $l^{-1}$) in the code of practice for the evaluation of fruits and vegetables juices. According to results, cryoconcentration did not lead to degradation of ascorbic acid to intermediate reactive products due to low temperature

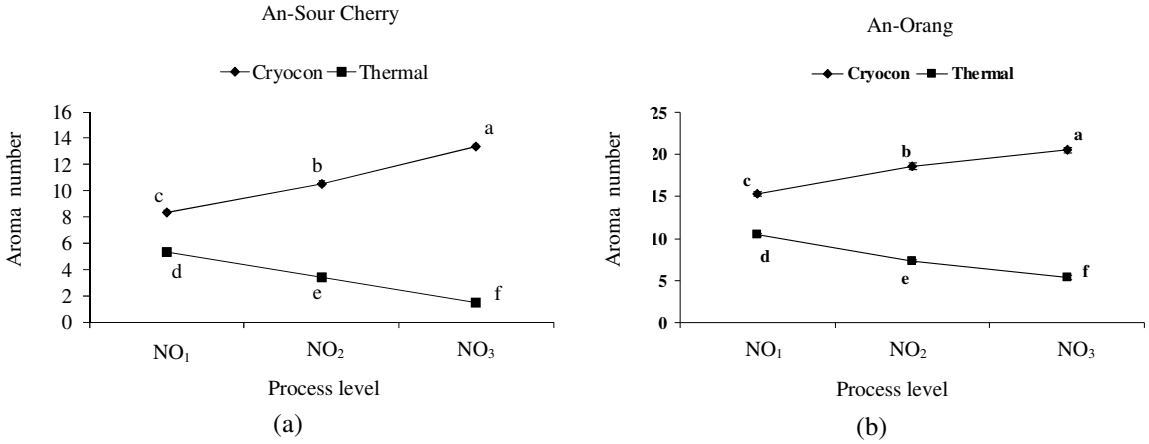


**Figure 4**. Aroma number (An) in concentrated orange and sour cherry juice during thermal concentration and cryoconcentration process. Mean values with different letter are significantly different (P< 0.05) by Duncan's multiple range test. $NO_1$, $NO_2$, $NO_3$ are steps of concentration which are produced by cryoconcentration at – 10 ° C and thermal concentration process. (Orange juice: 28.43±0.16, 40.51±0.15, 45.42±0.19° Brix and Sour cherry juice: 34.52±0.14, 44.42±0.19, 52.44±0.13° Brix).





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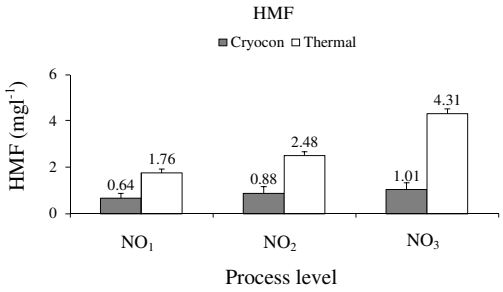


**Figure 5**. Hydroxymethylfurfural formation (HMF) in concentrated orange juice during cryoconcentration and thermal concentration process. $NO_1$, $NO_2$, $NO_3$ are steps of concentration which are produced by cryoconcentration at –10°C and thermal concentration process. (Orange juice: 28.43±0.16, 40.51±0.15, 45.42±0.19° Brix and Sour cherry juice: 34.52±0.14, 44.42±0.19, 52.44±0.13° Brix).

applied during concentration. It has been reported that several reactive products of decomposition occur via the degradation of vitamin C (Eskin, 1990; Hulein *et al.*, 1971) and these compounds may combine with amino acids and result in the formation of brown pigments (Clegg, 1964; Larisch *et al.*, 1998).

## Total Antioxidant Activity of Orange and Sour cherry Samples

Total antioxidant activity (TAA) of cryoconcentrated sour cherry and orange juice did not decrease significantly (Figure 6), whereas TAA of thermally concentrated sour cherry and orange juices significantly decreased. All of the main effects as well as associated interactions were significant at the *P*< 0.05 level for Brix (df= 2, 23; F= 100.4650), type of concentration process (df= 1, 23; F= 2,114.2903), and Brix×type of concentration process (df= 2, 23; F= 67.0057) in total antioxidant activity of concentrated orange juice. All of the main effects as well as the associated interactions were significant at the *P*< 0.05 level for Brix (df= 2, 23; F= 23.2495), type of concentration process (df= 1, 23; F= 1,461.2041), and Brix×type of

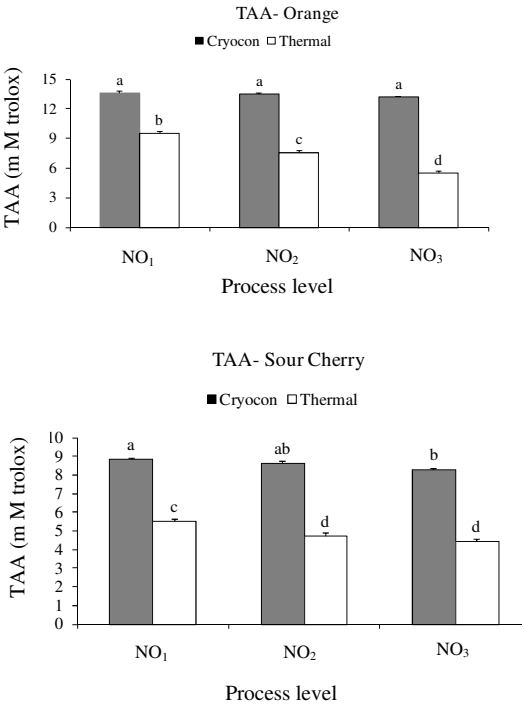


**Figure 6.** Total antioxidant activity (TAA) in concentrated orange and sour cherry during cryoconcentration and thermal concentration process. Means with the same letter are not significantly different (P< 0.05) by Duncan's multiple range tests. $NO_1$, $NO_2$, $NO_3$ are similar ° Brix of concentrated produced by cryoconcentration at –10°C and thermal concentration process. (Orange juice: 28.43±0.16, 40.51±0.15, 45.42±0.19° Brix and Sour cherry juice: 34.52±0.14, 44.42±0.19, 52.44±0.13° Brix).

concentration process (df= 2, 23; F= 3.5963) in total antioxidant activity of concentrated sour cherry juice. Results obtained from comparison of the mean values by Duncan's multiple range test showed that cryoconcentrated orange and sour cherry juices had significantly (P< 0.05) higher total antioxidant activity than those thermally concentrated.

## CONCLUSIONS

Juices clarified by electro-flotation and ultra-filtration were better cryoconcentrated





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than those clarified by enzymatic process and ultra-filtration process. Efficiency of cryoconcentration was improved due to obtaining higher total soluble solid (TSS) in orange and sour cherry juices. Ascorbic acid, aroma number, and total antioxidant activity retention were better preserved in concentrates produced by cryoconcentration compared to concentrates produced by thermal concentration. Besides, hydroxymethyfurfural content of thermally concentrated sample (orange juice) was significantly more than cryoconcentrated sample. To our knowledge, we are the first group investigating effect of improved clarification process on efficiency of cryoconcentration process for obtaining concentrated fruit juices with higher total soluble solids.

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## تغلیظ انجمادی عصاره ی آلبالو و پرتقال با استفاده از روش شفاف سازی جدید: مقایسه ی تغلیظ حرارتی با تغلیظ انجمادی مواد غذایی مایع

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## چکیده

عصاره پرتقال و آلبالو به طور موفقیت آمیز مورد تغلیظ انجمادی قرار گرفتند. شفاف سازی جدید ( الکتروفلوتیشن – اولترافیلتراسیون ) کارآیی تغلیظ انجمادی را بهبود بخشید. عصاره شفاف شده آلبالو و پرتقال به روش الکتروفلوتیشن – اولترافیلتراسیون به بالاتر از۰.۱۴± ۳۴.۵۲ ، ۰.۱۹± ۴۴.۴۲ ،۰.۱۳ ± ۵۲.۴۴ و ۰.۱۶ ± ۲۸.۴۳ ،۰.۱۵± ۴۰.۵۱ ،۰.۱۹ ± ۴۵.۴۲ درجه بریکس در $C^{\circ}$ ۱۰- تغلیظ انجمادی شدند. در درجه بریکس مشابه، نمونه های تغلیظ شده انجمادی به طور معنی داری ($p < 0.05$) باعث ابقای بالاتر آروما نامبر، اسید اسکوربیک و مقادیر آنتی اکسیدانی کلی نسبت به نمونه های تغلیظ شده به روش حرارتی گردید. تغلیظ حرارتی منجر به تولید بیشتر هیدروکسی متیل فورفورال در عصاره تغلیظ شده پرتقال نسبت به فرآیند تغلیظ انجمادی گردید.





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