INTERNATIONAL JOURNAL OF FOOD AND NUTRITION SCIENCE VOL3 NO1 APRIL 2014
ISSN 2165-5308 (PRINT) ISSN 2165-5316 (ONLINE) http://www.researchpub.org/journal/ijfns/ijfns.html

Variability of Sorbitol/Xylitol Content in Pomegranate (*Punica Granatum*) Juice As Affected by Processing Conditions (April 2014)

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***Abstract*— In this study, effects of factors including decaying of material, heating, enzymation, fermentation and pressing with or without peel/husk on the amount of sorbitol/xylitol in pomegranate juice were examined. In comply with findings, while decaying of the fruit and fermentation of the juice increased sorbitol/xylitol content in pomegranate juice, heating and enzymation process had no significant effect.Pressing of fruit with peel also increased sorbitol/xylitol content in pomegranate juice due to the fact that sorbitol/xylitol content of peel is more than that of aril. The results reveal that while sorbitol/xylitol content in the juice samples obtained from arils of five different pomegranate varieties ranged between 61-190 mg/L, the amount of sorbitol/xylitol in juice samples obtained from the peel of fruits was between 408 and 864 mg/L.**

***Keywords* — polyols, pomegranate, pomegranate juice, sorbitol, xylitol.**

I. Introduction

Sorbitol and xylitol are compounds belong to the group referred as sugar alcohols or polyols. They are naturally found in several types of fruits and vegetables, are formed by hydrogenation of glucose and xylose respectively and well dissolved in water. The heat of solution for sorbitol is -111 kJ/mol, therefore it causes a refreshing effect in the mouth. Since sorbitol does not participate in Maillard reaction, interferes with blood at a slow rate and has a cariostatic effect, it can be considered as a multi-purpose food additive.[1]

Another feature of sorbitol is that its amount may vary depending on the type of fruit and vegetable. According to Washültt *et al.*[2], pear and peach have high sorbitol content, while strawberry and raspberry contain xylitol. According to Kauko and Soderling[3], fruits containing highest amount of sorbitol are apple, rowan berry and wild oleaster. On the other hand, sorbitol contents of some of the fruits are very low or even none.

According to Eksi and Karav[4], fruit juices obtained from white grape and orange do not naturally include sorbitol. Sour cherry, pear and apricot juices contain high levels of sorbitol (20.2-44.2 g/L, 20.1-46.6 g/L and 11.9-44.0 g/L respectively) whereas quince and peach juices contain medium levels of sorbitol (6.7-24.8 g/L and 2.0-18.7 g/L respectively) and apple, pomegranate and red grape juices contain low levels of sorbitol (2.0-5.1 g/L, 0.06-0.2 g/L and 0.005-0.064 g/L respectively). According to "Code of practice for evaluation of fruit and vegetable juices" of AIJN [5], sorbitol content of apple, pear, aronia and sour cherry juice should be between 2-7, 10-25, 65-100 and 10-35g/L, respectively. Such variations among different types of fruits make sorbitol content a criterion to detect adulteration in fruit juice.

In recent years, the interest in pomegranate juice has increased. The high antioxidant content of pomegranate juice [6-9] is the main reason for this case. The increasing interest in pomegranate juice is also the primary reason for adulteration.[10]

Sorbitol is one of the compounds discussed as control criteria for authenticity of pomegranate juice. Fischer-Zorn and Ara [11] suggest that pomegranate juice does not contain sorbitol, it may occur in pomegranate juice due to microbial decay of raw material. Jones and Silveira[12] stated the potential of yeasts such as *Zymomonas, Candida*, *Saccharomyces* to convert fructose into sorbitol.

Sorbitol/xylitol content of the fruit is influenced by not only genetic but also ecological factors. Bianco *et al.*[13] declared that drought stress resulted in sorbitol accumulation in the fruit leaf. Moreover, an increase in sorbitol content of the fruit due to water deficiency was reported.[14]

According to the provisional reference guideline for pomegranate juice prepared by AIJN[15] sorbitol content of pomegranate juice can be maximum 250 mg/L. It is stated that amount of sorbitol exceeding maximum value may arise from addition of other fruit juices or from processing rotten raw material. However, according to Türkmen and Ekşi[16], pomegranate juice naturally contains sorbitol ranging between 16 and 423 mg/l.

On the other hand, it was also discussed that some other factors (heating, mash enzymation due to side activity and fermentation) might lead to an increase in sorbitol content of pomegranate juice. Besides, it was stated that pressing pomegranate together with peels was another factor that increased sorbitol amount in pomegranate juice. [17-18]

Because the sorbitol/xylitol is the key compound to detect adulteration in pomegranate juice, factors that may change the level of sorbitol/xylitol content should be investigated. The

objective of this study was to figure out the effects of raw material, heating, mash enzymation, fermentation processes in sorbitol/xylitol content of pomegranate juice. In fruit juice industry, pomegranates are pressed with peels together. Therefore, the sorbitol/xylitol level of pomegranates that was processed with peel was also determined to find out any sorbitol/xylitol migration from peel to juice.

II. Methods

A. Material

The experiment was designed so as to determine the effects of rotten fruit, heating, enzymation, fermentation and pressing on sorbitol/xylitol content of pomegranate juice.

Decaying of fruit

Each pomegranate fruit was divided into two and one part was immediately pressed, while the other was pressed after it was kept in ambient conditions for one and half month. In each juice samples the amount of sorbitol/xylitol was determined and compared.

Heating process

Fresh pomegranate juice was heated up to boiling point (approximately 97ºC) and sorbitol/xylitol content of juice was determined just before heating and at 10^{th}, 20^{th} and 40^{th} minutes of the boiling.

Mash enzymation

After fresh pomegranate juice was heated up to 90 ºC, it was cooled up to 50°C and mash enzymation was applied for 1 hour at this temperature. The sorbitol/xylitol content was determined before and after enzymation.

For this purpose, 3 different commercially available preparates (PANZYM XLL, NOVOFERM G1 and FRUCTOZYM P) were used and each of mash was applied with the dose of 0.125 g/250 gram. Experiment was repeated twice for each preparation using two different samples.

Fermentative deterioration

This is an undesired process, which may be sometimes experienced in fruit juice factories. In order to determine the potential effects of the fermentation, sorbitol/xylitol content of fresh squeezed pomegranate juice was determined and juice samples that were stored at room temperature in closed container for 4, 9 and 13 days. The experiment was repeated using two different fruit samples.

Pressing with peel

While pomegranate is process into pomegranate juice at industrial scale, first the arils are separated from husk and then pressed. However, depending on processing system, some peel pieces may be left, which are pressed together with arils. In order to determine whether this process increases sorbitol/xylitol content of pomegranate juice, sorbitol/xylitol contents of pomegranate juice were determined in juice samples obtaining through pressing from aril, aril-peel (80%+20%) mixture and peel. The experiment was repeated using five different fruits juices.

B. Method

The soluble solid content (brix degree) of the samples was determined by refractometric method.[19]

D-sorbitol/xylitol analysis was carried out by using enzymatic method.[20-21] The enzyme kit for the analysıs was supplied by R-BIOPHARM AG (Darmstadt). Before the analysis, samples were diluted 5-10 times according to color intensity. At the beginning of analyses, the possible presence of interfering substances was checked for each sample and they were eliminated by treating the sample with KOH solution and H_2O_2 and than incubating for 10 minutes at 70°C according to the IFU method.[20]

According to enzymatic method, if the sample contains only one of the polyols, the result reflects the amount of that polyol. Otherwise, if both polyols exist in the sample, then the resulting amount corresponds to the total amount of these polyols. Therefore, the findings of analyses were given in terms of "sorbitol/xylitol".Analyzes were performed in three technical replicates.

III. Results

The potential factors to affect sorbitol/xylitol content in pomegranate juice, experimental conditions and amount of sorbitol/xylitol determined in samples are summarized in Table I.

TABLE I
FACTORS POTENTIALLY AFFECTING SORBITOL CONTENT IN POMEGRANATE JUICE

Potential Factor	Experimental Condition	Sorbitol mg/L
Rotten fruit Storage at ambient for six weeks, N=1	Juice from fresh half of the fruit	99±9
	Juice from rotten half of the fruit	484±12
Heating up to 97°C N=1	Control	116±4
	10^{th} minute	126±4
	20^{th} minute	127±5
	40^{th} minute	132±0.1
Enzymation at 50 ºC for 1 hour N=2 for each enzyme	Control	154±1.7
	Preparate 1	194±1.9
	Preparate 2	191±3.2
	Preparate 3	186±2.9
Fermentation held in closed jar and at room temperature N=2	Control	130±3.7
	4^{th} day	151±1.7
	9^{th} day	298±2.3
	13^{th} day	312±3.8
Pressing with aril N=5	100 % aril	121±2.4
	80 % aril + 20% peel	172±1.9

According to findings presented in Table I, sorbitol/xylitol content of the pomegranate juice obtained from fresh half of the fruit is substantially different than that of other half subjected to decay process. Amount of sorbitol/xylitol in fruit juice obtained from rotten part of the fruit was about 5 times higher than that of fruit juice obtained from healthy half (Fig. 1).

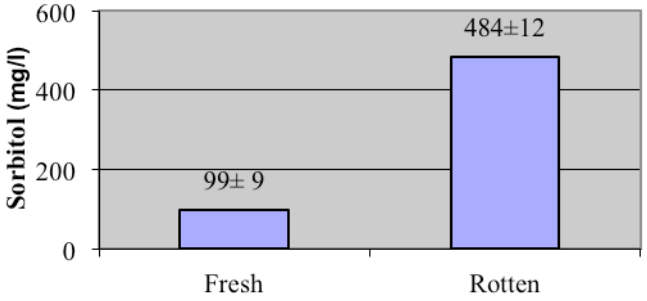

Figure 1: Amount of sorbitol in pomegranate juice obtained from fresh and rotten parts of the fruit.

The sorbitol/xylitol content of fresh samples differs slightly from those of samples heated up to boiling point (approximately 97°C). But the sorbitol/xylitol amount of the pomegranate juice samples did not increase clearly depending on boiling time (Fig. 2).

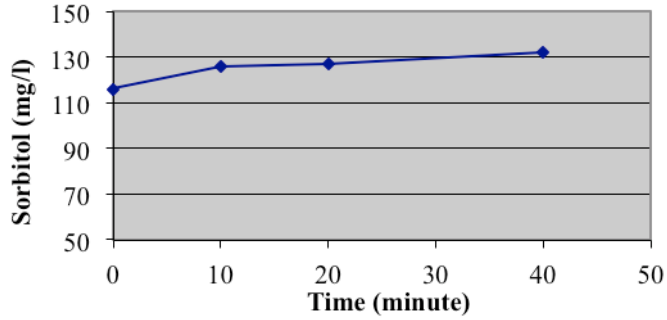

Figure 2: Amount of sorbitol in pomegranate juice depending on heating time

There was a slight difference between samples subjected to enzymation and the control for the sorbitol/xylitol levels; however, no difference was found between the commercial enzyme preparates (Fig. 3).

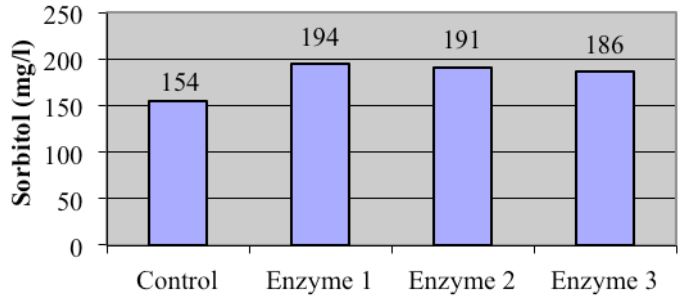

Figure 3: Amount of sorbitol in pomegranate juice with and without enzymation

The effect of fermentation on sorbitol/xylitol content of pomegranate juice was found to be significant. Sorbitol/xylitol concentration was 130 mg/L initially, and then it reached 312 mg/L after 13 days (Fig. 4).

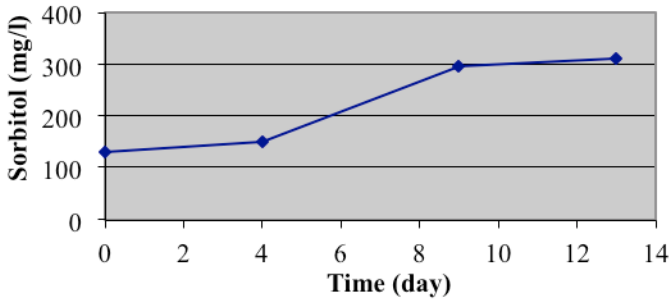

Figure 4: Increase in sorbitol content of pomegranate juice depending on fermentation time

The difference in sorbitol/xylitol content between pomegranate juice obtained from aril and juice obtained from aril and peel (80% and 20%, respectively) mixture is highly significant. The results revealed that sorbitol/xylitol may diffuse from peel/husk to the juice. In order to verify this result, sorbitol/xylitol content was determined in juices obtained from only arils of 5 different types of pomegranate and in pomegranate juice obtained from peels of same fruits, and the results are given in Table II.

TABLE II
D-SORBITOL CONTENT OF JUICE FROM ARIL AND PEEL

Pomegranate variety	Sorbitol (mg/l)	
	Juice from aril	Juice from peel
Hicaz	61± 2[a]	562±25[e]
Devedisi	89±12[b]	408±12[f]
X	98±8[b]	864±11[g]
Ernar	190±8[c]	586±13[e]
Izmir 1265	166±3[d]	789±8[h]
Mean	121	642

X: unknown

Values (within columns), which have been assigned the same letters, are not significantly different (Tukey Test, $P < 0.05$).

Results in Table II clearly shows that amount of sorbitol/xylitol in juices from aril were significantly higher than that of juices from husk.

IV. Discussion

Rotten fruit and fermentative deterioration leads to increase in sorbitol/xylitol content of pomegranate juice. Possible reason for the increase could be the formation of sorbitol/xylitol from fructose or xylose via microbial conversion.[11-12]

On the other hand, heating process and enzymation have no significant effect on sorbitol/xylitol content of pomegranate juice.

Pressing with peel leads to increase of sorbitol/xylitol amount in pomegranate juice. It can be an indication for sorbitol and/or xylitol diffusion from peel to the juice during pressing, and this view is strongly supported by the finding that juice from husk contains approximately 5 times more sorbitol/xylitol than juice from aril.

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