Application of Sensory Evaluation in Flavor Analysis of Pomegranate Juice

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***Abstract*—Pomegranate juice has become a popular fruit juice not only because of its pleasant flavor but also health promoting benefits. This research is aimed to establish consumer acceptance, to investigate flavor descriptors and to identify flavor compounds of pomegranate juice. Five pomegranate juice samples (Fresh-1, Fresh-2, Commercial-1, Commercial-2 and Commercial-3) were evaluated by consumer acceptance test. Fresh-1 and Commercial-1 obtained the highest liking score for all attributes ($p<0.05$), except sour and sweet tastes. However, Commercial-1 received higher liking score for aroma and sour taste than Fresh-1 ($p\leq0.05$). Therefore, it was selected to evaluate flavor profile using descriptive test. Seven aromas by nose, four aromas by mouth, along with three tastes, one trigeminal and two aftertastes were detected by trained panel. Moreover, major volatile aroma compounds in Commercial-1 were identified using headspace solid phase micro-extraction (HS-SPME) and gas chromatography–time-of-flight mass spectrometer (GC-TOFMS). Taste compounds were also determined by high performance liquid chromatography (HPLC).**

***Index Terms*—pomegranate juice, sensory, flavor, volatile aroma compound, taste compound**

I. Introduction

Pomegranate (Punica granatum L.) is considered as an ancient fruit. It is originated from Persia, and widely distributed around the world. Pomegranate has more than 3,000 cultivars and it is commonly grown in subtropical and tropical regions. Aril of pomegranate fruit is consumed fresh or processed into juice. In addition, it can be used to produce jam, jelly, wine, sauce, medicine, food supplement [1], food coloring and food flavoring [2]. There are many beverages claiming to contain high amount of antioxidants. Pomegranate juice has been reported to provide the highest amount of phenolic compounds and antioxidant capacities compared to red wine, berry fruit juice, açaí juice, tea, orange juice and apple juice [3]. These benefits cause increase in demand of pomegranate. A famous brand of pomegranate juice in California increased their production from 35,000 tons in 2004 to 283,000 tons in 2013 [4]. Moreover, Pomegranate juice has become the popular fruit juice with pleasant flavor. Therefore, the consumer acceptance, flavor descriptors and flavor compounds of pomegranate juice were investigated in this study.

II. Materials and Methods

A. Materials

1) Freshly-squeezed pomegranate juice

Pomegranate fruits (Nampeung cultivar), imported from China, were peeled and the pericarp covering seeds were removed. The arils were extracted by hydraulic press (Sakaya, Thailand) to obtain the juice (Fresh-1). Fresh-2 sample was prepared from the whole fruits in the same manner.

2) Commercial pomegranate juice

Three brands of Commercial-1, Commercial-2 and Commercial-3 pomegranate juices were purchased from the local supermarkets. Two former samples were the products of Thailand, while the later sample was a product of Turkey. All producers claimed 100% pomegranate juice without any additives.

B. Methods

1) Consumer acceptance

Forty-five untrained panelists (female:male=25:20, aged between 20-35 years) volunteered for this study. The participants were selected based on regular purchase and consumption of pomegranate juice. White bread and drinking water were used to clean the panelists' mouths. Five pomegranate juices (Fresh-1, Fresh-2, Commercial-1, Commercial-2 and Commercial-3) were prepared in covered plastic cup with 3-digit codes and kept in the refrigerator before tasting. Samples were served monadic sequential and counterbalance orders. The consumer acceptance was evaluated on 9-point hedonic scale (9=like extremely; 5=neither like nor dislike; 1=dislike extremely). Each participant was asked to rate how much he/she liked or disliked the overall, color, aroma, flavor, sour, sweet and astringent of the product.

2) Flavor analysis

a) Descriptive analysis

Commercial-1 was described for its aroma by nose, aroma by mouth, taste, trigeminal and aftertaste using descriptive sensory analysis. The procedure for selecting and training 12 panelists (female:male=9:3, aged between 20-30 years) was followed the Ref. [5]. Practice sessions were carried out afterwards to get familiar with the scaling intensity of the references (Table I) and various samples.

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TABLE I. FLAVOR ATTRIBUTES, DEFINITIONS AND REFERENCES USED FOR DESCRIPTIVE ANALYSIS OF COMMERCIAL-1 POMEGRANATE JUICE

Attribute	Definition	Reference	Intensity
Aroma by nose			
Tomato	Aromatic associated with tomato	Tomato juice with mixed fruits juice (Kagome, Thailand)	11
Green	Aromatic associated with green vegetable	Bitter melon	7
Fermented	Aromatic associated with overripe fruit	Apple cider with 30% pomegranate juice (Tipco, Thailand)	7
Acid	Aromatic associated with acetic acid	Acetic acid (Kewpie, Thailand)	11
Apple	Aromatic associated with apple	Apple juice (Malee, Thailand)	6
Plum	Aromatic associated with plum	Sweet cured plum (Tuo Shui Lee, China)	5
Berry	Aromatic associated with beery fruits	Blackcurrent juice with mix fruits and omega 3 (Malee, Thailand)	4
Aroma by mouth			
Green	Aromatic associated with green vegetable	Bitter melon	12
Tamarind	Aromatic associated with tamarind	Tamarind juice (IF, Thailand)	6
Alcohol	Aromatic associated with alcohol from fermented grape	Sparkling wine (Spy wine cooler, Thailand)	5
Fermented	Aromatic associated with overripe fruit	Apple cider with 30% pomegranate juice (Tipco, Thailand)	8
Taste			
Sour	Fundamental taste factor associated with citric acid	Citric acid solution (Best Odor, Thailand)	4
Sweet	Fundamental taste factor associated with sucrose	Sucrose solution (Mitr phol, Thailand)	5
Bitter	Fundamental taste factor associated with caffeine	Coffee (Nescafe red cup, Thailand)	11
Trigeminal			
Astringent	Dry puckering mouthfeel associate with tea leaves	Tea leaves (Lipton, Indonesia)	11
Aftertaste			
Sour-astringent	Sour and dry sensations recorded at the end of tasting in the back of throat	Concentrated orange flavored drink (Sunquick, Thailand)	10
Bitter	Bitter sensation recorded at the end of tasting in the back of throat	Coffee (Nescafe red cup, Thailand)	14

b) Identification of flavor compounds

Volatile aroma compounds were extracted using headspace-solid phase micro extraction (HS-SPME). Five grams of Commercial-1 pomegranate juice and 1 g of NaCl (Ajax Finechem, New Zealand) were placed into a 20-mL screw-cap vial. Twenty five microliters of 2-methyl-3-heptanone solution (0.11 mg/mL methanol) was used as an internal standard. Sample was equilibrated with magnetic stirring (250 rpm) at 40 °C for 20 min. After that, a SPME fiber (50/30 µm divinyl-benzene/carboxen/polydimethylsiloxane;Supelco, United States) was exposed to the volatile compounds at the headspace for 30 min. The fiber was desorbed at 240 °C for 4 min in the splitless injection port of gas chromatography (GC) (GC7890A; Agilent technologies, United States) coupled with time-of-flight mass spectrometer (TOFMS) (Pegasus 4D LECO®, United States). Helium was used as carrier gas at constant flow of 1 mL/min. The GC-TOFMS was equipped with a Rxi®-5 column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek®, United States). The initial temperature of oven was 35 °C and held for 5 min. The temperature was then increased at 4 °C/min to 250 °C and held for 10 min. The pomegranate juice was analyzed in duplicate and identified the aromas based on their mass spectra (MS) and retention indices (RIs) [6].

Taste compounds were investigated by high performance liquid chromatography (HPLC) (Waters 600, United States) paired with a diode array detector (DAD) (Waters 2998, United States). Citric acid along with caffeic acid, gallic acid and p-coumalic acid were analyzed at 280 nm and 320 nm, respectively. The column used was symmetry C18 (250 nm × 4.6 nm × 5 µm; Waters, United States). The mobile phase was the mixture of 1% formic acid (A) and acetonitrile (B) at flow rate 1 mL/min. The gradient program was started with 95% of A and then decreased to 90%, 85%, 75% and 50% within 10 min of each gradient. After that, it was increased to 95% within 15 min. Sugars were detected using HPLC (Agilent 1100, United States) equipped with a refractive index detector (RI) (Agilent 11200, United States) at 350 nm. All taste compounds were identified by comparing with the authentic standards.

III. RESULTS AND DISCUSSION

A. Consumer Acceptance

Pomegranate juices used in this study were divided into two groups, freshly-squeezed and commercial samples. Fresh-1 and Fresh-2 were obtained from arils and whole fruits, respectively. Commercial juices (Commercial-1, Commercial-2 and Commercial-3) were purchased from different manufacturers. All samples were examined consumer acceptance of overall, color, aroma, flavor, sour, sweet and astringent attributes. Fresh-1 and Commercial-1 were obtained highest liking scores of all attributes ($p<0.05$), except sweet and sour tastes (Table II).

To compare the tastes between fresh-squeezed products, Fresh-1 obtained higher liking score of astringent than Fresh-2 ($p<0.05$). This might be because of different preparation process. Tannins which presented in the peel and pericarp [7] were negative affected to astringent acceptance of Fresh-2. In term of visual color, both Fresh-1 and Fresh-2 were red which related to anthocyanin pigments. Anthocyanins in arils were in glycoside forms of delphinidin, cyanidin and pelargonidin [8] and the total contents were range from 50.5 to 490.4 mg/L [9]. pH was importance factor affected product color. The flavylium cation (red) changed into quinonoidal (blue), pseudobases (colorless) and chalcones (colorless) when pH was increased [10]. Nonetheless, Fresh-2 was received lower color liking scores than Fresh-1 ($p<0.05$). This could be because of pigments from peel. Peel consisted of N-methyl granatonine which reported as yellow and brown alkaloid [11].

TABLE II. LIKING SCORES OF FRESHLY-SQUEEZED AND COMMERCIAL POMEGRANATE JUICES

Attribute	Fresh-1	Fresh-2	Commercial-1	Commercial-2	Commercial-3
Overall	6.16±1.94a	5.02±1.8b	6.07±1.78a	5.27±1.70b	4.93±2.26b
Color	7.27±1.29a	4.93±1.71c	6.73±1.30ab	5.33±1.87c	6.60±1.72b
Aroma	5.69±1.60b	4.76±1.76c	6.56±1.52a	5.78±2.07b	4.18±2.08c
Flavor	5.87±1.85a	4.76±1.96bc	6.04±1.62a	5.09±1.83b	4.20±2.15c
Sour	5.09±1.73b	4.69±1.73b	5.89±1.85a	5.27±1.79ab	5.16±2.03ab
Sweet	6.04±2.13a	5.58±1.82a	5.82±1.60a	5.62±1.59a	4.80±1.70b
Astringent	6.20±1.85a	5.24±1.98c	6.16±1.26ab	5.47±1.56bc	4.40±2.08d

Note: The liking was scored on a 9-point scale from 9=like extremely to 1=dislike extremely (n=45).
Mean ±standard deviation followed by different letters in the same roll are significantly difference ($p\leq0.05$).

The color acceptability was also different among commercial brands. This might be because anthocyanins were not stable resulting in color changes during process and storage. The degradation and polymerization of anthocyanin were observed in pasteurized pomegranate juice [12]. In addition, Commercial-1 was higher liking scores of overall, aroma and flavor in comparison to Commercial-2 and Commercial-3 ($p<0.05$). Different raw materials and processes might be the reasons. Commercial-1 was made from concentrated pomegranate juice. Freeze concentration is normally used to retaine the quality of fresh juice. However, the degradation and/or formation of aroma compounds occure during thermal process, such as pasteurization or ultra high temperature (UHT). Ref. [13] found that the concentrations of fourteen aroma compounds including hexanal, ethanol, 2-methypropanol, cis-3-hexanol, linalool, octanol, 2-pinene, subinene, myrcene, limonene, γ-terpinene, ethyl acetate, ethyl butanoate and ethyl hexanoate were decreased in pasteurized Valencia orange juice. Ref. [14] suggested that the non-heated carrot juice using high pressure process was obtained liking scores of overall, aroma and taste more than juice using high temperature short time (HTST) pasteurization.

It should be noted that the overall acceptability and color acceptability of all samples were not related. The overall liking of Fresh-2 was rated at high score, while the color liking score was low. Therefore, color did not the significant factor of pomegranate juice acceptability. On the other hand, overall acceptability and flavor acceptability were highly correlated. Commercial-1 and Fresh-1 showed the highest overall liking scores among all pomegranate juices. However, Commercial-1 had higher liking score of sour and aroma than Fresh-1 ($p<0.05$). Accordingly, Commercial-1 was chosen as a representative for flavor characterization by descriptive sensory and instrumental analysis.

B. Flavor Analysis

a) Descriptive analysis

Seven attributes of aroma by nose including tomato, apple, green, fermented, acid, plum and berry were found in Commercial-1 (Fig. 1). Green, fermented, alcohol and tamarind were also perceived as aromas by mouth (Fig. 1). Other flavor descriptors consisted of three basic tastes (sour, sweet and bitter) along with one trigeminal (astringent) and two aftertastes (sour-astringent and bitter) (Fig. 1). It was similar to the previous research as pointed out by Ref. [15]. They reported that apple, berry, fermented, sweet, sour, bitter and astringent were flavor characteristics of Wonderful pomegranate juice.

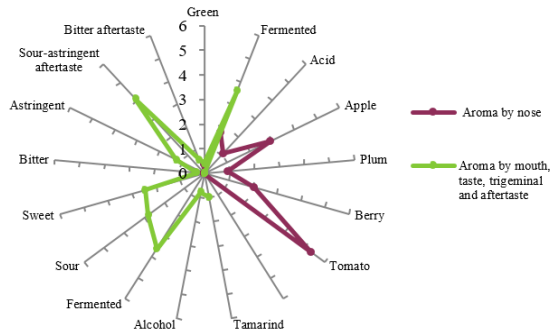

Figure 1. Descriptive flavor profile of Commercial-1 pomegranate juice (n=12)

b) Identification of flavor compounds

Aromas by nose and aromas by mouth of pomegranate juice were related to the volatile aroma compounds. Top ten major volatiles in Commercial-1 pomegranate juice were shown in Table III.

TABLE III. MAJOR VOLATILE AROMA COMPOUNDS IN COMMERCIAL-1 POMEGRANATE JUICE

Volatile aroma	RI[a]	Concentration[b] (ng/g)	Description [16]	Threshold[c] (ng/g)
Ethyl acetate	<800	337.77	Ethereal, sharp, wine-brandy	5 [17]
3-Methylbutanal	<800	7.18	Cheesey-sweaty-fruity	0.2 [18]
2-Methylbutanal	<800	6.02	Cocoa, weak fruity	3 [18]
Isobutyl acetate	<800	328.42	Fruity, banana-pear	65 [17]
Furfural	840	169.67	Sweet, cereal, bread, yeasty	3,000 [18]
Ethyl 2-methylbutanoate	849	4.15	Fruity, apple-strawberry	0.3 [17]
3-Hexen-1-ol	863	52.40	Green, grass, leafy	70 [19]
Benzaldehyde	954	5,003.29	Bitter almond	350 [18]
1,8-Cineole	1023	32.46	Camphoraceous, cool, fresh	12 [20]
α-Terpineol	1191	5.60	Sweet, floral, lime	350 [19]

[a] Retention index obtained from Rxi®-5 column.
[b] Relative concentration.
[c] Odor detection threshold in water obtained from public literature [17]-[20].

Among top ten volatile aroma compounds in Commercial-1, benzaldehyde and 3-methylbutanal demonstrated to be as impact aromas with highest concentration and lowest detection threshold, respectively. The aromas of fruit were generated during ripening [21]. Ethyl acetate, isobutyl acetate and ethyl 2-methylbutanoate were aroma compounds in ester group which might contribute to fermented, berry and apple notes, respectively (Fig. 1). Esters normally present in ripe fruits from biosynthetic pathways by two enzymatic systems including esterase and alcohol acyltransferase [22]. 3-Methylbutanal, 2-methylbutanal and benzaldehyde are in aldehyde group which can be created from two metabolic pathways. The aromas are degradation products of straight chain fatty acids in fruit and vegetable by β-oxidation pathway. The unsaturated fatty acids are changed into aromas in lipoxygenase pathway [23, 24] as well. On the other hand, 1,8-cineole and α-tepineol which belong in terpene group can be derived from carbohydrate through mevalonic pathway and acetyl-CoA with enzymatic catalyse of terpene synthases [25]. The other volatile compound, furfural, is generated from degradation of pentoses [26]. This compound could be originated from fructose which presented in the sample.

Sweet taste of juice was contributed by sugars which were generated from starch degradation during fruits maturation. As suggested by Ref. [27], unripe fruits contained starch and starch content decreased as ripening proceed. Commerical-1 was rich in fructose and glucose. Ref. [28] reported that these monosaccharides, fructose and glucose, were in aril of pomegranate fruits and they were major sugars in Spanish pomegranate juices [28]. The juice sample also contained a little amount of sucrose, while lactose and maltose were not detected. In addition, sour taste related to organic acids in fruits. Citric was the major organic acid presented in Commercial-1 sample. This acid along with oxalic, malic, lactic, tartric [29], quinic and succinic [28] acids were reported in various pomegranate juices.

Bitter taste generally associates with phenolic compounds, such as caffeic acid and 5-o-caffeoyl quinic acid in roasted coffee [30] and coutaric acid, procyanidin B1, catechin and epicatechin in red wine [31]. Thus, caffeic acid found in Commercial-1 could result in bitterness of the sample. Furthermore, gallic and p-coumaric acid in the sample might affect to astringent of Commercial-1. Ref. [32] found these two phenolic compounds in pericarp and peel of pomegranate fruit. Other compounds have been reported as astringent compounds in various juices, such as quercetin-3-o-β-D-glucoside, quercetin-3-o-β-D-galactoside, kaempferol-3-o-β-D-glucoside and kaempferol-3-o-β-D-rutinoside in red currant juice [33].

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