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Article

Effects of Albedo Addition on Pomegranate Juice Physicochemical, Volatile and Chemical Markers

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Abstract: Five commercial juices, representing the five clusters of this juice, were characterized before and after maceration with 10% pomegranate albedo (control- and albedo treated (AT)-juices, respectively). Commercial juices were macerated with albedo homogenate for 24 h, and then the albedo was removed. Total soluble solids, titratable acidity, maturity index (MI), total phenolic content (TPC), volatile composition, and flavor profile were evaluate in control- and AT-juices. From all physico-chemical characteristics, only the TPC was significantly affected by the treatment and ranged from 846 to 3784 mg gallic acid L^{-1} and from 2163 to 5072 mg gallic acid L^{-1} in control- and AT-juices, respectively; the increment in TPC was more than 1.3-fold in all AT-juices. No clear pattern was found when studying the volatile composition; only significant increases were observed in the contents of hexanal, 2-hexenal, and 3-hexenal in all AT-samples. The flavor profile study indicated that three of the five samples increased their bitterness and/or astringency. In addition, new attributes, which were not present in the control juices, appeared after maceration with albedo in some samples: green-bean, brown-sweet, and green-viney. This information will be useful in developing and promoting new "healthy" products based on pomegranate.

Keywords: *Punica granatum* L.; flavor; phenolic content; SPME-GCMS; volatile compounds

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1. Introduction

Pomegranate and its derivatives, such as pomegranate juice, have become very popular in recent years. Pomegranate has been reported as being capable of addressing different health diseases, or at least having a significant effect over them. This fruit, and especially its juice, seems to have, among others, antiatherogenic, antioxidant, and antihypertensive effects [1–3]. As reported by Johanningsmeier and Harris [4], the sales of pomegranate juice increased from over $84,500 in 2001 to $66 million in 2005 in the USA, probably due to the wide promotion of its healthy effects.

The antioxidant activity, AA, of pomegranate juice is positively correlated with its total phenolic content, TPC [5–7]. The phenolic compounds are more abundant in the non-edible portions of pomegranate, especially the rind and carpelar membranes [8,9]. Consequently, it is reasonable to assume that depending on the extraction method used to obtain the juice, the final product will have different antioxidant activity. In this way, processing steps extending the maceration of the juice with rind will lead to juices with high values of TPC. The TPC has been studied previously by Gil *et al.* [8] and Tezcan *et al.* [9] in commercial juices, and large differences were found. Recently, other authors [10,11] have determined that the cultivar used to elaborate the juice has also a significant effect on TPC and the associated AA.

Ibrahim [12] reported that pomegranate rind extract had strong antimicrobial effect, high AA, and also enhanced liver and kidney functions in animal models. Therefore, this type of rind extract can be used as food preservative or even as a nutraceutical ingredient for new enriched foods [13]. However, there are no scientific studies describing the effects of the addition of pomegranate extracts on the chemical and/or sensory quality of pomegranate juices.

Koppel and Chambers [14] studied 33 commercial pomegranate juices and developed a sensory lexicon to describe the main sensory attributes of these products. As a result of their study, pomegranate juices were grouped into five different clusters characterized by the following sensory attributes: *cluster 1* berry, dark-fruity flavors, and toothetch; *cluster 2* grape, cranberry, and wine-like flavor; *cluster 3* fermented flavor and toothetch; *cluster 4* brown color, and a characteristic musty/earthy flavor; and *cluster 5* candy-like and sweet overall flavors.

The aim of this study was to determine the influence of macerating pomegranate albedo with pomegranate juice on the main chemical and sensory characteristics of the juice. To achieve this goal, the main physico-chemical (total soluble solid content, titratable acidity, maturity index, and total phenolic content), aromatic (volatile composition), and flavor (sensory profile) characteristics of five commercial juices, which represented each one of the aforementioned clusters, were studied before and after macerating them with a 10 % of pomegranate albedo.

2. Materials and Methods

2.1. Samples

Three different pomegranate juices were purchased from different parts of the US and shipped to the Sensory Analysis Center (Kansas State University), in Manhattan, KS, USA. The fourth and fifth juices were purchased in Estonia and Spain, respectively, and shipped in the same way to Kansas. These five samples had been previously studied by Koppel and Chambers [14] and were chosen in the present study for representing the five different aforementioned clusters: 618 (cluster 1), 324 (cluster 2), 707 (cluster 3), 612 (cluster 4), and 981 (cluster 5).

At the same time, 15 pomegranates, cultivar *Wonderful*, were purchased from a local grocery store in Manhattan, KS. After discarding damaged fruits, the arils from all the fruits were manually removed, and the albedo and carpelar membranes of each pomegranate rind were grated, blended in a food processor, and frozen (−20 °C) until preparation of albedo-treated (AT-juice) samples.

Initially, the physico-chemical parameters and the volatile composition were analyzed in control juices. Later, 10% of homogenized pomegranate albedo was added to the control juices to prepare AT-juices. Samples were left macerating at 4 °C during 24 h; then, the juices were filtered using a strainer (mesh size < 1 mm) to remove all solid particles. Three batches of AT-juices were prepared, and all samples were analyzed in triplicate.

2.1. Physico-Chemical Analysis

2.1.1. Total Soluble Solids, Titratable Acidity, and pH

Total soluble solids (TSS) were measured with a digital refractometer (Model PR-101a; Atago, Bellevue, WA, USA) at ~20 °C, with values being expressed as °Brix. Titratable acidity (TA) was determined by titrating 1 mL of each sample (diluted to 20 mL final volume with deionized water) with 0.1 mol L^{-1} NaOH. Results were expressed as g citric acid 100 mL^{-1}. pH was measured with a pH-meter (Accumet Basic AB15, Thermo Fisher Scientific, Waltham, MA, USA). All analyses were run in triplicate, with each replication corresponding to a different bottle of juice. Finally, the maturity index (MI: ratio of TSS to TA) was calculated for each sample.

2.1.2. Total Phenolic Content

Total phenolic content (TPC) was measured as indicator of the antioxidant activity in the juices. TPC was determined by using the Folin-Ciocalteau method with some modifications [15]. Results were expressed as mg gallic acid equivalent L^{-1}. Analyses were run in triplicate.

2.2. Analysis of Volatile Composition

2.2.1. Extraction Procedure

Two mL of each sample were placed in a 10 mL vial with a polypropylene hole cap PTFE/silicone septa. The compound 1,2-dimethoxybenzene was used as internal standard to semi-quantify the volatile compounds. The vials were equilibrated during 10 min at 60 °C in the autosampler (Pal system, model CombiPal, CTC Analytics AG, Zwingen, Switzerland). After this equilibration time, a 50/30 μm DVB/CAR/PDMS fiber was exposed to the sample headspace for 30 min at 60 °C. The desorption of the volatile compounds from the fiber coating was made in the injection port of GC at 250 °C during 5 min in splitless mode. Experiments were run in triplicate.

2.2.2. Chromatographic Analyses

The isolation, identification, and quantification of the volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian, Inc., Walnut Creek, CA, USA), coupled with a Varian mass spectrometer detector (Saturn 2200), and operated with the MS Workstation software. The GC-MS system was equipped with a VF-5MS column (Varian, Inc., Walnut Creek, CA; 30 m × 0.25 mm × 1.0 μm film thickness). The temperature of the column began at 40 °C, was held for 10 min, increased 8 °C min^{-1} to 180 °C, and, finally increased 10 °C min^{-1} to 280 °C, and held for 10 min. The column flow was 1 mL min^{-1}, using helium as the carrier gas.

Most compounds were identified using two different analytical methods: (1) retention indexes, and (2) mass spectra (authentic chemicals and Wiley spectral library collection).

2.3. Sensory Evaluation with Trained Panel

Six highly trained panelists from the Sensory Analysis Center participated in this study. Each panelist had more than 120 h of training in sensory testing and more than 1000 h of testing experience with a variety of foods.

All samples were poured into odor-free, disposable 90 mL covered plastic cups (Sweetheart Cup Co., Inc., Owings Mills, MD, USA) for evaluation. Each panelist received ~60 mL of each product. The samples were served from the refrigerator around 30 min before testing.

After two days of orientation, all samples were evaluated by the panelists in two different days: day one the control juices, and 24 h later the AT-juices. All juices were evaluated in triplicate. The order of product evaluation was randomized, and samples were coded with three-digit random numbers. The descriptive attributes, their definitions, and the list of references used for this study corresponded to the ones reported by Koppel and Chambers [14]. The testing room was at 21 ± 1 °C and 55% ± 5% of relative humidity; the illumination was a combination of natural and non-natural (fluorescent) light.

A modified consensus flavor description method, which uses a panel to determine flavor intensities on a numerical scale from 0 (representing “none”) to 15 (representing “extremely strong”) was used in this study [16–18].

2.4. Statistical Analysis

Physico-chemical data was subjected to statistical analysis using SAS® (version 9.2; SAS Institute, Cary, NC, USA) used for analysis of variance and Fisher’s Least Significant Differences test (LSD) for post-hoc mean separation. In addition, Partial least square regression (PLS regression map) was conducted using the Unscrambler version 9.7 (Camo Software, Oslo, Norway) with the objective of relating sensory and instrumental data [19].

3. Results and Discussion

3.1. Physico-Chemical Analyses

Total soluble solids (TSS), titratable acidity (TA), and maturity indexes (MI) were significantly different ($p \leq 0.05$) among the control juices; however, treatment of pomegranate juices with albedo extract had no significant effects on any of these physico-chemical parameters in any sample ($p > 0.05$ between each control and its corresponding AT-juice) (Table 1).

Table 1. Physico-chemical characteristics (total soluble solids content: TSS, titratable acidity: TA, maturity index: MI, and total polyphenol content: TPC) of original and AT-juices.

		Physico-chemical characteristics †			
Sample	**Cluster ‡**	**TSS (°Brix)**	**TA (g citric acid L^{-1})**	**MI**	**TPC (mg gallic acid eq. L^{-1})**
		Before albedo extract addition			
324	2	16.6 ± 0.3 bcd	13.2 ± 0.1 de	12.6 ± 0.4 c	2577 ± 50 cd
981	5	18.2 ± 0.5 a	10.0 ± 0.2 def	19.6 ± 0.8 b	997 ± 77 f
618	1	13.7 ± 0.4 f	5.13 ± 0.1 f	27.3 ± 2.4 a	2003 ± 70 e
612	4	15.4 ± 0.3 de	16.0 ± 0.3 bc	10.2 ± 1.5 c	846 ± 4 f
707	3	17.6 ± 0.9 abc	20.9 ± 0.2 a	8.5 ± 3.3 c	3784 ± 5 b
		After 24 h of maceration with albedo extract			
324	2	16.2 ± 0.1 cde	14.0 ± 0.1 cd	11.7 ± 0.3 c	3732 ± 116 b
981	5	18.0 ± 0.1 ab	9.5 ± 0.1 ef	19.4 ± 0.1 b	2163 ± 113 de
618	1	13.7 ± 0.1 f	5.13 ± 0.1 f	27.1 ± 2.1 a	2937 ± 113 c
612	4	14.9 ± 0.2 ef	13.5 ± 0.2 cd	11.3 ± 0.2 c	2367 ± 114 de
707	3	16.6 ± 1.1 bcd	19.4 ± 0.1 ab	8.5 ± 0.7 c	5072 ± 233 a

† Mean of 3 replications. Values followed by the different letter, in the same column, were significantly different ($p < 0.05$), Fisher's Least Significant Difference (LSD). ‡ According to Koppel and Chambers [14].

Vázquez-Araújo *et al.* [15] reported that the MI of some blended juices, which main ingredient was pomegranate juice, was related to consumer overall liking. Calín-Sanchez *et al.* [11] reported a similar relationship between MI and consumer liking, when studying pure fresh pomegranate juice. In addition, MI and has been commonly used as an index of sensory acceptability in different juices [20–22]. Consequently and because treatment with albedo extract did not change TSS, it should not influence the acceptability of the juices under study.

Considering the MIs of the different juices, 618 might be the juice with the highest acceptability of the studied samples, because it had the highest MI ($p \leq 0.05$). However, and although this could be a reasonable assumption, consumer studies should be conducted to prove it. This statement can be affected by other factors, such as the content of citric acid. Hasnaoui *et al.* [23] reported that citric acid content controls pomegranate sourness and a low content of this organic sugars leads to intense sweetness perception.

Dafny-Yalin *et al.* [24] studied the main differences among juices prepared from arils and from pomegranate peel homogenates and found that the later exhibited lower levels of TSS, TA, soluble sugars and organic acids than aril juices. Different manufacturing processes [25] and/or different pomegranate cultivars [10,11], brought different TSS, TAs, and MIs to the original samples in the present study; however, the addition of only a 10% of albedo was not enough to have a significant effect in these parameters.

Total phenolic content (TPC) and antioxidant activity (AA) have been studied by different authors in pomegranate and pomegranate-based products from different countries: e.g., Spain [11], and Iran [10]. As can be seen in Table 1, control samples had different differences in their TPC values.

No information about the pomegranate cultivar was provided in the samples labeling, but these differences may be due to geographical origin, cultivar, and/or different manufacturing procedures. Adding albedo homogenate increased TPC in the juices 1.3–2.7 times. The juices which had an initial lower TPC (samples 981 and 612) were the samples which experimented higher increases, rising values over 2100 mg gallic acid equivalents L^{-1}. Consequently, maceration of juices with albedo extract could be an interesting option to develop more competitive and healthy products. Sample 707, which had already the highest TPC (~3700 mg gallic acid equivalents L^{-1}), rise to more than 5000 mg gallic acid equivalents L^{-1} after treatment. Vrhovsek *et al.* [26] stated a recommended daily intake (RDI) of polyphenols of 1 g day^{-1}, so the consumption of only 200 mL of this AT-juice will meet the RDI for polyphenols.

3.2. Volatile Composition and Sensory Analyses

Table 2 shows the main differences in the volatile profile among all studied samples. Up to 69 compounds belonging to 9 chemical families were detected in the juices: alcohols, aldehydes, ketones, acids, furans, esters, benzene derivatives, terpene derivatives, and lactones. All these groups, but lactones, had been reported in commercial pomegranate juices by Vázquez-Araújo *et al.* [27]. Sample 981 was the juice with the highest concentration of volatile compounds (Table 2); esters, benzene derivatives, and terpenes predominated in this sample. Esters are significant aromatic compounds for fruits, synthesized only by intact cells during the β-oxidation of fatty acids or from amino acid metabolism [28], but have been reported previously in pomegranate fresh juices [11,27,29], but in much lower contents, especially in the headspace of the juices. Due to the high concentration of these compounds and benzene derivatives, it could be assumed that were used as flavorings to increase the overall aroma of the juice. Pomegranate juice has low concentration of volatile compounds, which leads to low intensities in odor and aroma [11]. Sample 981 was the only concentrate, so during its production there was a concentration stage in which the volatile compounds would be lost and replaced, or collected and after that added back to the juice.

Table 2. Aromatic volatile compounds found in pomegranate juices.

Code	Compound	RI (Lit.) *	RI (Exp.)	Volatile compounds (mg kg^{-1}) #									
				324	324 AT	981	981 AT	618	618 AT	612	612 AT	707	707 AT
				Alcohols									
A1	2-Butanol	605	608	n.d.	n.d.	0.004	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A2	1-Pentanol	746	759	n.d.	n.d.	0.037	0.030	0.002	0.002	n.d.	n.d.	n.d.	n.d.
A3	3-Hexen-1-ol ¥	860	859	n.d.	n.d.	0.065	n.d.	0.047	n.d.	n.d.	n.d.	n.d.	n.d.
A4	4-Methyl-1-pentanol	872	860	n.d.	0.007	0.050	0.044	0.024	0.020	n.d.	0.005	0.028	0.028
A5	3-Octanol ¥	997	990	0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A6	2-Ethyl-1-hexanol	1030	1032	0.067	0.029	0.027	0.035	0.051	0.040	0.040	0.032	0.044	0.028
A7	Linalool	1101	1098	0.015	n.d.	n.d.	n.d.	0.024	0.034	n.d.	n.d.	n.d.	n.d.
A8	Viridiflorol ‡	1617	1569	n.d.	n.d.	0.042	0.019	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total			0.087	0.036	0.226	0.128	0.149	0.097	0.040	0.037	0.071	0.056
				Aldehydes									
A9	2-Pentenal	750	721	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.004	0.003	n.d.	n.d.
A10	2-Methyl-2-butenal	750	741	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.007	0.003	n.d.	n.d.
A11	3-Hexenal ¥	800	800	n.d.	0.007	n.d.	n.d.	n.d.	0.016	n.d.	n.d.	n.d.	n.d.
A12	Hexanal ¥	802	802	n.d.	0.060	n.d.	0.047	0.008	0.053	n.d.	0.034	n.d.	0.089
A13	2-Hexenal ¥	860	865	0.006	0.193	n.d.	0.213	n.d.	0.197	n.d.	0.100	0.116	0.219
A14	Octanal ¥	1005	1001	0.007	n.d.	n.d.	n.d.	0.006	0.006	0.003	0.004	n.d.	n.d.
A15	Nonanal ¥	1108	1108	0.017	0.014	n.d.	n.d.	0.011	0.018	0.012	0.019	n.d.	0.010
	Total			0.029	0.274	n.d.	0.260	0.025	0.289	0.026	0.164	0.116	0.318
				Ketones									
A16	3-Methyl-2-pentanone	751	750	n.d.	n.d.	0.007	0.006	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A17	2-Methyl-2-hepten-6-one ¥	987	986	n.d.	n.d.	n.d.	n.d.	n.d.	0.003	n.d.	n.d.	n.d.	n.d.
A18	β-Damascenone	1400	1384	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.008	n.d.	n.d.	n.d.
A19	β-Ionone	1499	1503	n.d.	n.d.	0.026	0.008	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total			n.d.	n.d.	0.033	0.014	n.d.	0.003	0.008	n.d.	n.d.	n.d.
				Acids									
A20	Acetic acid †	602	600	0.014	0.003	n.d.	0.010	0.029	0.012	0.134	0.237	0.013	0.006
A21	4-Butoxy butanoic acid ‡	856	856	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.104	0.038
	Total			0.014	0.003	n.d.	0.010	0.029	0.012	0.134	0.237	0.117	0.044

Values are mean of 3 replications. * [30]. ¥ Aroma compounds found in fresh pomegranate juices [11,27,29]. † Semi-quantification relative to the internal standard concentration. ‡ Tentatively identified: only mass spectral data (retention index, RI, was not found in the literature [30]), the experimental RI differs in more than 20 units from the literature RI, or no standard was available. Standard error was ≤0.01 for all mean values.

Table 2. *Cont.*

Code	Compound	RI (Lit.) *	RI (Exp.)	Volatile compounds (mg kg^{-1}) # 324	324 AT	981	981 AT	618	618 AT	612	612 AT	707	707 AT
				Esters									
A22	Ethyl acetate	614	628	n.d.	n.d.	0.018	0.002	0.171	0.088	n.d.	0.010	0.485	0.286
A23	Ethyl butanoate	801	804	n.d.	n.d.	0.094	0.047	n.d.	n.d.	n.d.	n.d.	0.317	0.075
A24	Butyl acetate	816	816	n.d.	0.009	n.d.	0.004	n.d.	0.006	n.d.	n.d.	n.d.	0.006
A25	Ethyl 2-methyl butanoate	852	849	n.d.	n.d.	0.180	0.082	n.d.	n.d.	n.d.	n.d.	0.198	0.078
A26	3-Methyl-1-butanol acetate	878	875	n.d.	n.d.	0.212	0.091	n.d.	n.d.	n.d.	n.d.	0.088	0.033
A27	2-Methyl-1-butanol acetate	879	877	n.d.	n.d.	0.031	0.015	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A28	Ethyl hexanoate	996	1001	n.d.	n.d.	0.302	0.123	n.d.	n.d.	n.d.	n.d.	0.014	0.003
A29	3-Hexen-1-ol acetate	1004	1005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.082	0.026
A30	Hexyl acetate ¥	1010	1014	n.d.	0.015	0.131	0.057	n.d.	0.008	n.d.	n.d.	0.243	0.087
A31	Methyl 2,4-hexadienoate	1022	1021	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.012	0.006	n.d.	n.d.
A32	2-Methyl-3-methylbutyl propanoate	1056	1056	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.009	0.003
A33	2-Propenyl hexanoate ‡	1079	1071	n.d.	n.d.	n.d.	n.d.	0.014	0.008	n.d.	n.d.	0.186	0.065
A34	Ethyl 2,4-hexadienoate	1100	1111	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.075	0.060	n.d.	n.d.
A35	*n*-Amyl isovalerate	1104	1108	n.d.	n.d.	3.660	1.58	n.d.	n.d.	n.d.	n.d.	0.020	n.d.
A36	Ethyl benzoate	1190	1187	n.d.	0.010	1.320	0.687	n.d.	n.d.	n.d.	0.014	n.d.	0.005
A37	β-Phenylethyl acetate	1231	1260	n.d.	n.d.	n.d.	n.d.	0.026	0.015	n.d.	n.d.	n.d.	n.d.
A38	Neomenthol acetate ‡	1246	1300	n.d.	n.d.	0.028	0.009	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total			n.d.	0.034	5.976	2.697	0.210	0.124	0.087	0.090	1.641	0.669
				Furans									
A39	Furfural	839	829	0.087	0.064	0.620	0.527	0.086	0.067	0.197	0.150	0.377	0.290
A40	2-Acetylfuran	915	911	n.d.	n.d.	0.014	0.016	0.004	0.004	0.004	0.005	0.016	0.015
	Total			0.087	0.064	0.634	0.542	0.090	0.070	0.201	0.155	0.393	0.305
				Benzene derivatives									
A41	Benzaldehyde	936	936	0.025	0.030	3.68	2.65	0.027	0.022	0.020	0.041	0.392	0.265
A42	1-Methy-3-(1methylethyl)-benzene ‡	1037	1021	n.d.	n.d.	0.019	0.008	0.005	0.004	n.d.	n.d.	0.002	0.001
A43	3-Methyl phenol	1077	1075	0.003	0.004	0.006	0.006	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A44	4-Methyl benzaldehyde	1101	1080	n.d.	0.036	2.448	1.310	n.d.	n.d.	n.d.	0.037	n.d.	0.054
A46	Mequinol ‡	1197	-	0.153	0.127	0.107	0.114	0.167	0.129	0.133	0.126	0.133	0.117
A47	*p*-Cymen-8-ol	1200	1183	n.d.	n.d.	0.109	0.103	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A48	1,2-Dimethoxy-3-methylbenzene ‡	1288	-	n.d.	n.d.	n.d.	n.d.	0.024	0.018	0.018	0.014	n.d.	n.d.
A50	2,4-di-tert-butyl phenol ‡	1512	1512	n.d.	n.d.	0.028	0.008	n.d.	n.d.	n.d.	n.d.	0.005	n.d.
	Total			0.181	0.197	6.644	4.361	0.235	0.185	0.192	0.242	0.612	0.501

Values are mean of 3 replications. * [30]. ¥Aroma compounds found in fresh pomegranate juices [11,27,29]. ‡ Tentatively identified: only mass spectral data (retention index, RI, was not found in the literature [30]), the experimental RI differs in more than 20 units from the literature RI, or no standard was available. Standard error was ≤0.01 for all mean values.

Table 2. *Cont.*

Code	Compound	RI (Lit.) *	RI (Exp.)	Volatile compounds (mg kg^{-1}) # 324	324 AT	981	981 AT	618	618 AT	612	612 AT	707	707 AT
							Terpenes						
A51	β-Pinene ¥	991	980	n.d.	n.d.	0.009	0.004	0.002	0.003	n.d.	n.d.	n.d.	n.d.
A52	Limonene ¥	1041	1031	0.021	0.006	0.378	0.155	0.136	0.079	n.d.	n.d.	0.005	0.004
A53	Eucalyptol	1048	1029	n.d.	n.d.	0.021	0.009	n.d.	n.d.	n.d.	n.d.	0.004	0.003
A54	γ-Terpinene ¥	1070	1062	n.d.	n.d.	n.d.	n.d.	0.009	0.006	n.d.	n.d.	n.d.	n.d.
A55	β-Terpineol	1166	1188	n.d.	n.d.	0.030	0.028	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A56	Pulegone	1181	1176	0.027	0.025	n.d.	n.d.	0.038	0.033	n.d.	n.d.	n.d.	n.d.
A57	α-Terpineol ¥	1207	1189	0.035	0.030	1.402	1.150	0.106	0.078	n.d.	0.010	0.060	0.052
A58	β-Elemene ‡	1411	1392	n.d.	n.d.	0.019	0.013	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A59	*Z*-α-Bergamotene ¥	1430	1402	n.d.	n.d.	n.d.	n.d.	0.010	0.006	n.d.	n.d.	n.d.	n.d.
A60	*E*-α-Bergamotene ¥	1448	1435	n.d.	n.d.	0.036	0.013	0.062	0.043	n.d.	n.d.	n.d.	n.d.
A61	β-Caryophyllene ¥	1451	1466	n.d.	n.d.	0.042	0.019	0.045	0.032	n.d.	n.d.	n.d.	n.d.
A62	γ-Himachalene ‡	1491	1460	n.d.	n.d.	n.d.	n.d.	0.054	0.038	0.372	0.261	n.d.	n.d.
A63	Valencene	1521	1490	n.d.	n.d.	0.049	0.026	0.129	0.092	n.d.	n.d.	n.d.	n.d.
A64	β-Himachalene ‡	1530	1497	n.d.	n.d.	n.d.	n.d.	0.084	0.056	n.d.	n.d.	n.d.	n.d.
A65	γ-Cadinene	1536	1524	n.d.	n.d.	0.049	0.033	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A66	4,5,9,10-Dehydro longiflorene ‡	1537	-	n.d.	n.d.	n.d.	n.d.	0.103	0.065	n.d.	n.d.	n.d.	n.d.
A67	Unknown	1739	-	0.053	0.031	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A68	Unknown	1772	-	0.019	0.009	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total			0.156	0.101	2.036	1.449	0.779	0.531	0.372	0.271	0.068	0.059
							Lactones						
A69	γ-*n*-Heptylbutyrolactone ‡	1585	1547	n.d.	n.d.	1.82	0.888	n.d.	n.d.	n.d.	0.021	0.010	0.021
	Total			n.d.	n.d.	1.824	0.888	n.d.	n.d.	n.d.	0.021	0.010	0.021
	Total Concentration			0.55	0.71	17.4	10.4	1.52	1.31	1.06	1.22	3.03	1.97

Values are mean of 3 replications. * [30]. ¥ Aroma compounds found in fresh pomegranate juices [11,27,29]. ‡ Tentatively identified: only mass spectral data (retention index, RI, was not found in the literature [30]), the experimental RI differs in more than 20 units from the literature RI, or no standard was available. Standard error was ≤0.01 for all mean values.

Just by looking at the differences in total volatiles between control and AT-juices, no general trend could be deduced, because two of the samples (324 and 612) had higher concentration of total volatile compounds after the albedo homogenate treatment, but the other three samples had the opposite behavior (981, 618, and 612). Only studying chemical families some tendency can be seen: a decrease in the total concentration of alcohols and an increase in the total concentration of aldehydes. Mainly hexanal, 2-hexenal, and 3-hexenal were the compounds which rise seemed to be directly related with the albedo, because they were absent in the original/control juices, but were present in all AT-juices (Table 2). These compounds have sensory descriptors such as fatty, green, apple, floral, or fruity.

Figure 1 shows the main relationships among instrumental and sensory attributes in the different juices. When taking into account the first two dimensions of the PLSR biplot (PLS1 and PLS2), 63% variation in instrumental data explained 58% variation in the sensory data. As can be seeing in the map, most of the differences were related with the samples, and not with treatment. Each one of the AT-sample was close to the original sample and had similar sensory characteristics. Figures 2–6 illustrate the different sensory attributes detected in each juice, and how these attributes changed after the maceration with pomegranate albedo. These radar graphs represent the detection of all flavors and mouth-sensations of the juices sequentially: starting from the top of the graph and reading it clockwise. Attributes "sour2", "bitter2", or "astringent2" represented the sourness, bitterness, or astringency of the samples detected on a second time during the testing.

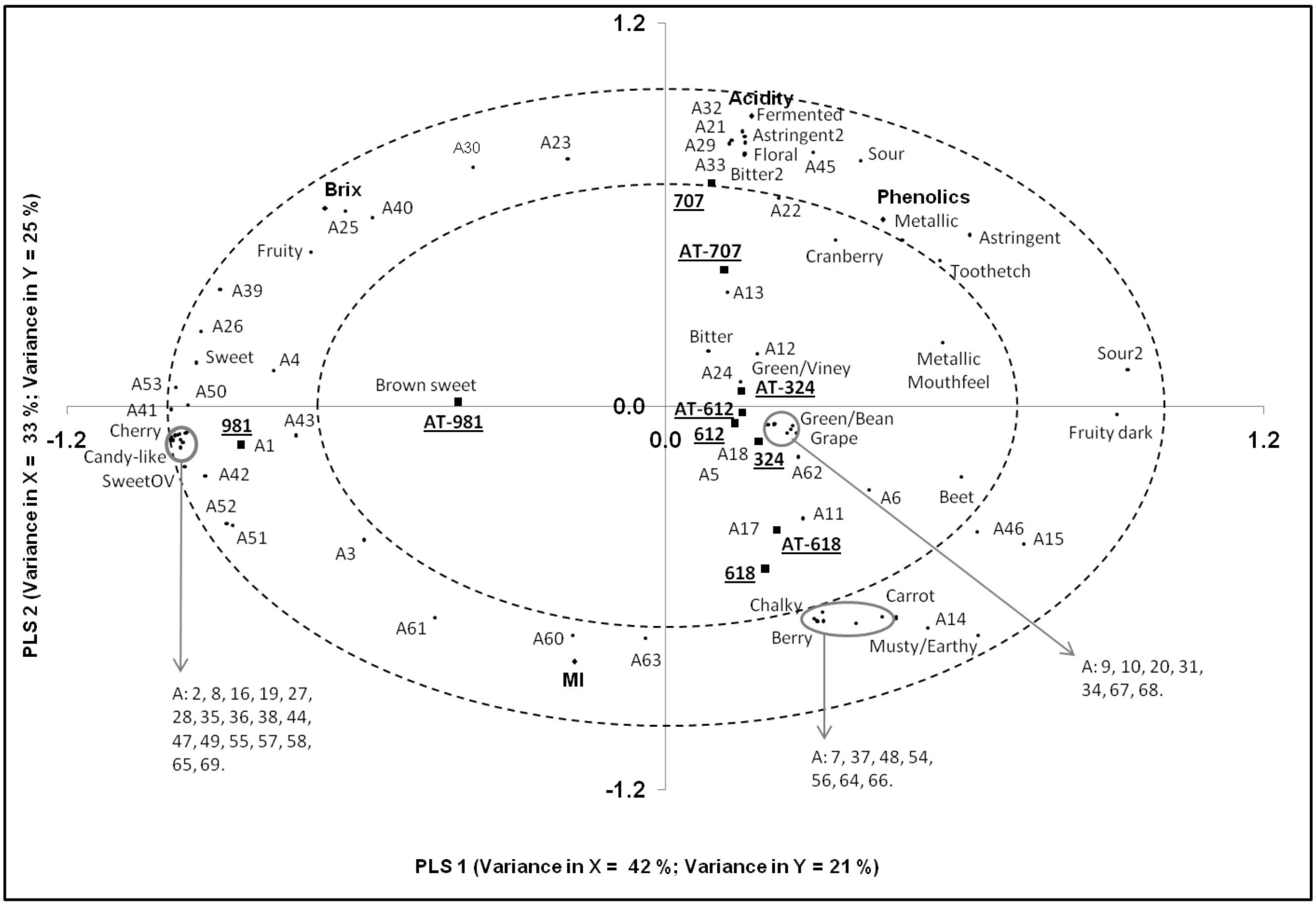

Figure 1. PLS regression map showing the relationship among instrumental and sensory data in all studied juices. *Legend*: Juice samples: indicated in bold and underlined font. Samples with an "AT" before the sample code represent samples "after" albedo treatment. ● Sensory attributes and instrumental volatile compounds. ♦ Physico-chemical data: Brix-TSS, Acidity-TA, MI-MI, Phenolics-TPC.

Samples 324 and 612 had a similar position in Figure 1. These samples had the lower content on total aromatic compounds and similar MI, but different TPC and different sensory profiles. Fifteen different sensory attributes were detected in samples 324 (Figure 2), and only ten in sample 612 (Figure 5). Although the TPC content of the original sample 324 was significantly higher than that of sample 612, the intensities of bitterness and astringency (parameters related with phenolics in literature, e.g., Vardin and Fenercioglu [31], were scored higher in sample 612. These attributes might be slightly masked in sample 324 due to the presence of other flavors that were not present in sample 612, e.g., cranberry or grape. Sourness and astringency have been reported as attributes that dislike consumers in pomegranate fresh juice [11]. In this way, Granato *et al.* [32] reported that pomegranate juices were characterized by high levels of astringency, and concluded that this may hinder their sensory acceptance; however, this problem could be overcome by including a health claim on the label, then the possible beneficial health effects probably drive their consumption. Other options to mask high astringency is to use a mixture of pomegranate cultivars, including sweet pomegranates, such as *Mollar de Elche*; the sweetness of this type of cultivars will help in masking excessive astringency. Sample AT-324 had higher sourness and astringency than original sample 324; these two attributes were not affected in sample AT-612, maybe because they were already high in the original/control juice. These results showed that consumer overall liking of sample 324, and the samples corresponding to cluster 2 [14] may be affected if macerated with albedo, but the effect of the treatment may not affect sample 612 (representative of cluster 4 as reported by Koppel and Chambers [14]). In addition, both AT-samples had new flavor notes that were not present in the original juices: green-viney and green-bean, respectively. These green flavors could to be related with the increase in hexanal (A12) and 2-hexenal (A13) concentrations (Table 2).

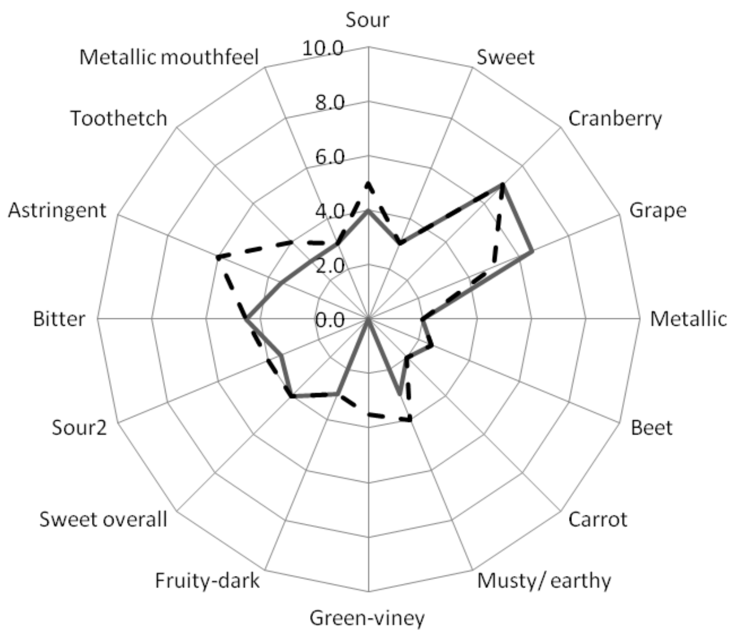

Figure 2. Spider plot showing the differences in the flavor characteristics of sample 324 "before" and "after" albedo treatment. *Legend*: Control juice indicated with a continuous line; AT-juice indicated with a discontinuous line. A numerical scale from 0 (representing "none") to 15 (representing "extremely strong") was used to obtain the data; the upper part of the scale is not shown in the graph. Differences can be considered significant ($p < 0.05$) when the difference between values were higher than 0.5 units.

Samples 981 and AT-981 had the higher concentration in total volatile compounds and also the higher TSS. As discussed previously, most benzene derivatives (A41–A50) were found in these two samples, especially high was the content of benzaldehyde (cherry, bitter almond). In addition, a considerable amount of esters (A22–A38) and terpenes (A51–A68) were found in these samples, making their aroma profile the most complicated (Figure 3). Despite this high amount of volatiles, only ten flavor attributes were detected in these samples (representative of cluster 5 as reported by Koppel and Chambers [14]), characterized by candy-like and cherry attributes, and a high overall sweetness.

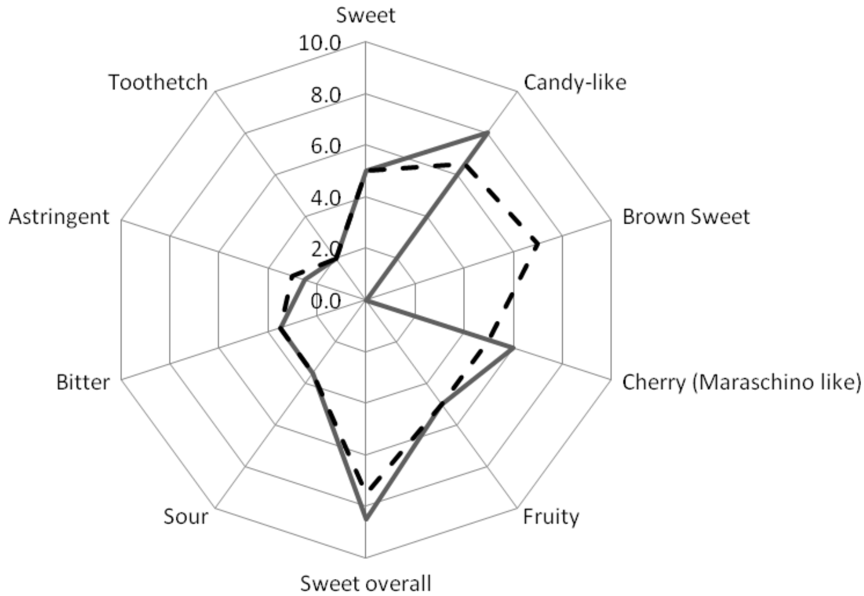

Figure 3. Spider plot showing the differences in the flavor characteristics of sample 981 "before" and "after" albedo treatment. *Legend*: Control juice indicated with a continuous line; AT-juice indicated with a discontinuous line. A numerical scale from 0 (representing "none") to 15 (representing "extremely strong") was used to obtain the data; the upper part of the scale is not shown in the graph. Differences can be considered significant ($p < 0.05$) when the difference between values were higher than 0.5 units.

Samples 618 and AT-618, the ones with the higher MI, were characterized by dark-fruity, musty/earthy and carrot flavors, and also for a chalky mouthfeel (representative of cluster 1 as reported by Koppel and Chambers [14]). As can be seen in Figure 4, bitterness and astringency of these samples were considerably affected by the treatment (maceration with pomegranate albedo). Although some new volatile compounds were detected in the sample AT-618 (e.g., 3-hexenal, 2-hexenal, acetic acid butyl ester, 4-methyl benzaldehyde, α-terpineol), no new flavor notes were found by the panel.

Samples 707 and AT-707 were representative of cluster 3 (fermented flavor and a toothetch mouthfeel; Koppel and Chambers [14]). These samples had also high sourness, bitterness and astringency, persistent attributes which appeared a second time during the testing with a high intensity as well. Figure 6 shows the changes in the sensory profile from sample 707 to sample AT-707. As can be seen, mainly bitterness was affected in this sample, so it is assumable that consumer overall liking may be affected as well. This original sample was characterized for its high TPC, and also for its high bitter and astringent character. These results seemed to confirm the relationship between these sensory attributes and the phenolic compounds (Figure 1), but only when the concentrations are high, and depending on the original flavor profile of the product (as demonstrated when comparing samples 612 and 324).

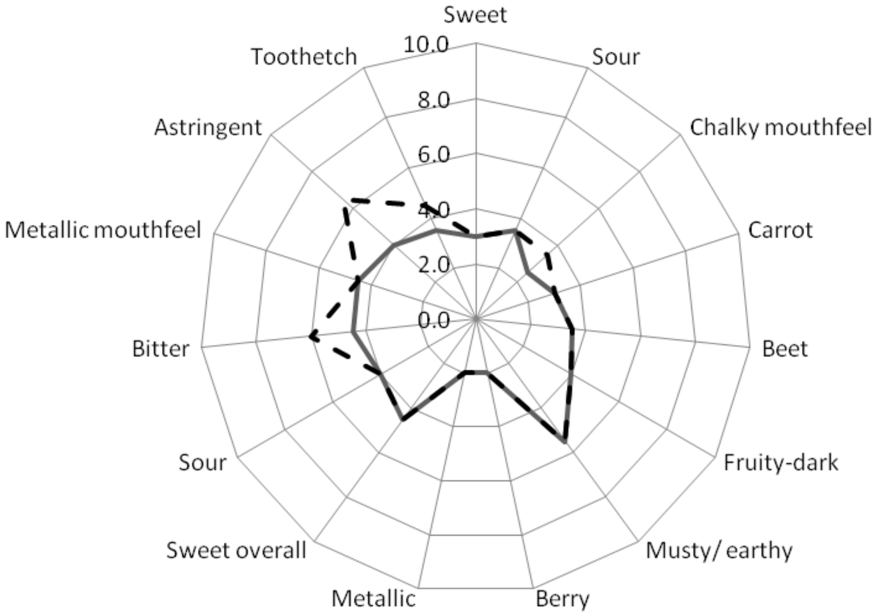

Figure 4. Spider plot showing the differences in the flavor characteristics of sample 618 "before" and "after" albedo treatment. *Legend*: Control juice indicated with a continuous line; AT-juice indicated with a discontinuous line. A numerical scale from 0 (representing "none") to 15 (representing "extremely strong") was used to obtain the data; the upper part of the scale is not shown in the graph. Differences can be considered significant ($p < 0.05$) when the difference between values were higher than 0.5 units.

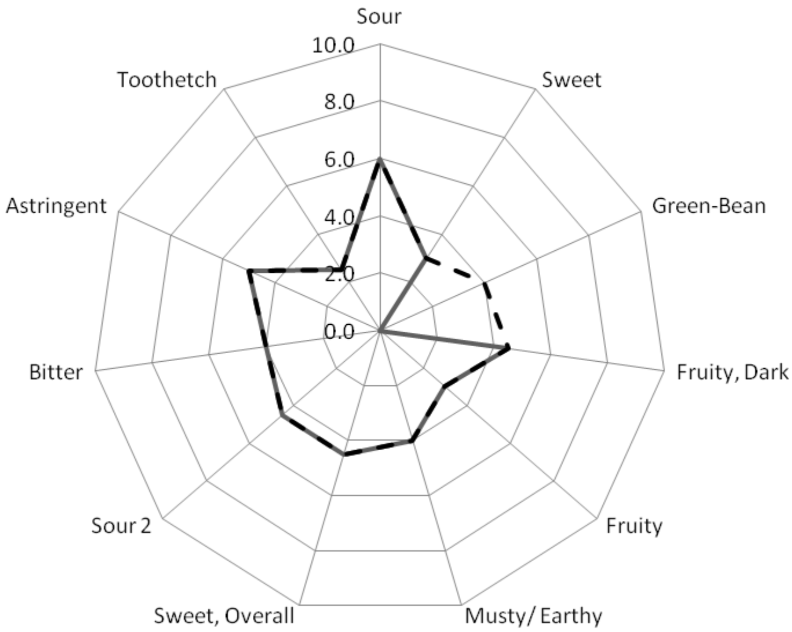

Figure 5. Spider plot showing the differences in the flavor characteristics of sample 612 "before" and "after" albedo treatment. *Legend*: Control juice indicated with a continuous line; AT-juice indicated with a discontinuous line. A numerical scale from 0 (representing "none") to 15 (representing "extremely strong") was used to obtain the data; the upper part of the scale is not shown in the graph. Differences can be considered significant ($p < 0.05$) when the difference between values were higher than 0.5 units.

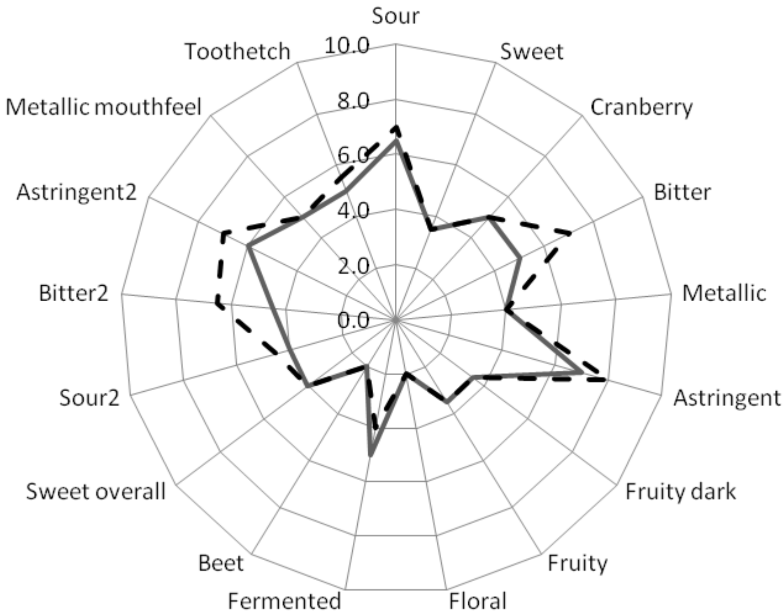

Figure 6. Spider plot showing the differences in the flavor characteristics of sample 707 "before" and "after" albedo treatment. *Legend*: Original juice indicated with a continuous line; AT-juice indicated with a discontinuous line. A numerical scale from 0 (representing "none") to 15 (representing "extremely strong") was used to obtain the data; the upper part of the scale is not shown in the graph. Differences can be considered significant ($p < 0.05$) when the difference between values were higher than 0.5 units.

4. Conclusions

Maceration of pomegranate juices with 10% pomegranate albedo for 24 h had no significant effects on TSS, TA, and MI in any of the five pomegranate juices. Maceration with albedo significantly increased TPC in all samples, with increases being 1.3-2.7-fold. Some volatile compounds appeared after the maceration with albedo, for example hexanal, 2-hexenal, and 3-hexenal, which brought green flavor notes to some of the samples (AT-324, and AT-612). Astringency and bitterness of some juices were higher after the maceration, but not in all samples. Samples representing clusters 4 and 5 as described by Koppel and Chambers [14] (musty/earthy and candy-like and sweet overall flavors, respectively) had new flavor notes after albedo addition (green-bean and brown sweet), but sourness, bitterness or astringency were not affected. Maceration with 10% pomegranate albedo seemed to be a good strategy to elaborate healthy and competitive juices, at least to all this companies which products belong to clusters 4 and 5, and have low values of TPC. Consumer studies should be conducted to confirm the impact that the increases in bitterness and/or astringency may have in the acceptance of products from clusters 1, 2 and 3.

Author Contributions

L.V-A. and E.C. planned and designed the experiments. L.V-A. performed the experiments. L.V-A. and A.A.C-B. analyzed the data. L.V-A. wrote the manuscript. L.V-A., E.C., and A.A.C-B. edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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