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Research and Development Institute, NakhonPathomRajabhat University.
85 Malaiman Rd., Muang, NakhonPathom, 73000, Thailand
Tel. (+66) 34 109 300 ext. 3909, (+66) 34 261 053
Fax: (+66) 34 261 053
E-mail: unsdgs2018@webmail.npru.ac.th
Website: http://dept.npru.ac.th/unsdgs2018

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Physicochemical properties, total phenolic content and antioxidant activities of aloe vera beverages

Sumeth Piayura[1,*], Chompoonuch Khongla[2], Wichuda Klawech[2], Jirayu Musika[3] and Chutikarn Kapcum[4]

[1]Department of Food Science and Technology, Faculty of Agricultural Technology,
Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand
[2]Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology
Isan, Nakhon Ratchasima, 30000, Thailand
[3]Department of Food Science and Technology, Faculty of Liberal Arts and Sciences,
Sisaket Rajabhat University, Sisaket, 33000, Thailand
[4]Department of Food Technology, Faculty of Technology, Khon Kaen University,
Khon Kaen 40002, Thailand

Abstract

The aim of this study was to investigate the physical and chemical properties, total phenolic content, and antioxidant activities of different proportions of Aloe vera juice (AVJ) that is used to manufacture Aloe vera beverages (AVB). Four AVB samples, called AVB1, AVB2, AVB3, and AVB4, were prepared by using different proportions of AVJ, grape juice concentrate (GJC), and sugar syrup (SS). These were made is ratios of 90:5:5, 90:0:10, 40:5:5, and 40:0:10 (% v/v/v), respectively. The results showed that pH and total soluble solid of the end products ranged from 4.64 to 4.75 and 9.05 to 11.12 oBrix, respectively. The different ratios of AVJ addition led to minimal changes in color. The AVB1 sample showed the highest total phenolic content (1.09 mg GA Eq./100 mL), followed by AVB2 (9.85 ± 0.41 mg GA Eq./100 mL). AVB1 and AVB2 showed the highest antioxidant activities as measured by metal chelating and the ABTS radical scavenging assay. The results, therefore, conclude that the addition of AVJ at 90% proportion showed significantly better improvements to the total phenolic content and antioxidant potential in AVB than 40% proportion.

Keyword: Aloe vera, beverage, total phenolic content, antioxidant activity

I. INTRODUCTION

Aloe vera (*Aloe barbasensis* Miller) is a perennial plant of Liliaceae family with turgid green leaves joined at the stem in a rosette pattern [1]. The gel of the leaves is associated with many polysaccharides. In fact, more than 200 bioactive chemicals have been found in A. vera gel which provide potentially positive effects on human health beyond basic nutrition [2]. The antioxidant compounds in A. vera may increase the stability and nutritional value of food [3]. Health benefits of A. vera include increasing high-density lipoprotein (HDL), reducing low-density lipoprotein (LDL) and blood sugar in diabetics, fighting acquired immune deficiency syndrome (AIDS), and improving the immune system.

The industries of A. vera products, such as beverage, dairy products, and food supplement, are very important to the economy and have been increasing year by year [4]. Additionally, A. vera has been widely utilized as a resource for functional food, especially healthy drinks contained A. vera gel [1]. Nowadays, Aloe juices with certain blends are very popular, for example lemon juice, sherbet, and electrolytes in sport drink, soluble fiber in a diet drink, vitamin B, amino acid and acetaminophen in a hangover drink, and vegetable juices in healthy drink. However, mucilaginous gel obtained from a fresh A. vera leave has a bitter taste and result in unpleasant taste sensations. The addition of some fruit juices is the easy technique to reduce the bitterness of the vegetable drink. However, the study of appropriate beverage formulation of A. vera gel blended with some fruit juices is still lacking. Therefore, the aim of this work was to evaluate the effects of the different concentrations of A. vera juice (AVJ) on physical and chemical properties, total phenolic content, and antioxidant capacities in A. vera beverage (AVB).

II. MATERIALS AND METHODS

A. Preparation of Aloe vera juice

Fresh green and matured of A. vera leaves with uniform size were obtained from the Aloevera Herb International Co. Ltd., Bangkok. Grape juice concentrate (GJC), and sugar syrup (SS) were purchased from local store. The A. vera leaves were washed and kept in vertical position for about 1 h to facilitate the drainage of yellow liquid sap. The upper and lower rind portion was removed with the knife to separate the inner fillet portion, and further ground by blender and filtrated through the muslin cloth to obtained AVJ gel. Four different formulations of AVB containing different proportions of AVJ:GJC:SS (%v/v/v), namely AVB1 (90:5:5), AVB2 (90:0:10), AVB3 (40:5:5), and AVB4 (40:0:10) were prepared. The beverages were pasteurized at 85^{o}C for 15 min, and then stored in green glass bottles at refrigeration temperature (4 ± 1^{o}C) before analysis.

B. Physical and chemical analysis

The total soluble solids (TSS) was tested using a hand refractometer (HR-130, OPTIKA, Italy) and the pH value was

*corresponding author's e-mail: spiayura@gmail.com

measured by pH meter (pHMaster LAB, Dynamica Scientific Ltd., UK). Instrumental color measurement was carried out using colorimeter (Chroma Meters CR-400, Konica Minolta, UK) calibrated with black and white standards. The color parameters of lightness (L*), greenness/redness (-/+ a*), blueness/yellowness (-/+ b*), Chroma parameter (C) indicating color intensity, and hue angle (H^o) were directly recorded for each sample. H^o vary from 0^o (pure red color), 90^o (pure yellow color), 180^o (pure green color), and 270^o (pure blue color) [5].

C. Total phenolic content and antioxidant activities

The total phenolic content was estimated using a modified method of Follin-Ciocalteu method [6]. Briefly, 100 µL of AVB was dissolved in 2 µL of Na_2CO_3 followed by addition of 100 µL of Folin-Ciocalteu reagent .After allowing to stand for 30 min at room temperature, the mixture solution was measured the absorbance at 750 nm using UV–Vis spectrophotometer)Genesis 10 UV scanning, Thermo Fisher Scientific, USA .(The total phenolic content was expressed as mg gallic acid equivalent per 100 mL of AVB (mg GA Eq./100 mL sample).

ABTS radical scavenging assay (ABTS assay) was performed according to Wiriyaphan *et al*. [7]. $ABTS^{\bullet+}$ stock solution was prepared by mixing 7.4 mM of ABTS solution and 2.6 mM of potassium persulfate solution, in 10 mM phosphate buffer)pH 7.4(, and kept in the dark for 16 h .Fresh $ABTS^{\bullet+}$working solution was prepared by mixing $ABTS^{\bullet+}$ stock solution in 10 mM phosphate buffer)pH 7.4 (to attain the absorbance at 0.7 ± 0.02, at 734 nm. Twenty µL of AVB sample was mixed with 1980 µL of $ABTS^{\bullet+}$working solution, and then kept in the dark for 5 min before monitoring at 734 nm .Result was expressed as mg Trolox equivalents per 100 mL of AVB samples (mg Trolox Eq./100 mL sample).

Ferric reducing antioxidant power (FRAP assay) was carried out according to Wiriyaphan *et al*. [8] with slight modifications. Briefly , FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution. 200 µL of AVB was mixed with 1 mL of fresh FRAP reagent and then incubated for 1h at room temperature before measuring the absorbance at 593 nm .The result was expressed as mg Trolox equivalent per 100 mL of AVB sample (mg Trolox Eq./100 mL sample).

Metal chelating assay was measured according to Decker and Welch [9] with slight modifications reported by Wiriyaphan *et al*. [9]. Briefly, 100 µL of AVB was mixed with 50 µL of 2 mM $FeCl_2$ and 100 µL of 5mM 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine). The mixture was incubated at room temperature in the dark for 20 min .The color of ferrous iron–ferrozine complex was monitored at 562 nm .Result was expressed as mg EDTA equivalents per 100 mL of AVB sample (mg EDTA Eq./100 mL sample).

D. Statistical analysis

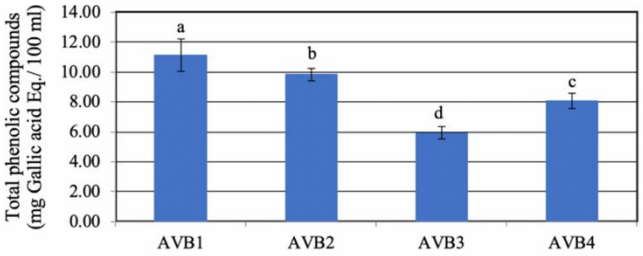
The experiments were performed with arrangements in a completely randomized design)CRD (and the mean value ± standard deviations were presented .Data analyses were performed by ANOVA)analysis of variance (and computed by the SPSS statistics for Windows, Version 17.0) SPSS Inc., Chicago, U.S.A .(.Duncan's new multiple range test)DMRT (was used to determine significant differences among results and statistical significance was accepted at the 95 % probability)$p \leq 0.05$(level.

III. RESULTS AND DISCUSSION

A. Physicochemical characteristic of Aloe vera beverage

The results obtained with respect to physical and chemical characteristics are presented in Table 1. Four blends of AVB with different proportions of AVJ, GJC, and SS caused little change of pH varied from 4.64 to 4.75. The TSS of AVB varied between 9.35 to 11.10 °Brix which was insignificantly affected by AVJ concentrations ($p > 0.05$).

For color characteristics were observed that L* of 90% AVJ addition samples (AVB1 and AVB2, by 53.42 and 56.50, respectively) showed higher than 40% AJV addition (AVB3 and AVB4, by 59.51 and 59.80, respectively), whereas b* was observed with the higher value in AVB1 and AVB2, indicated that AVB with 90% AVJ addition exhibited more dark-yellow. While a* showed negative value in all samples, indicating a slight green color. Comparable result was previously reported by Di Scala *et al*. [10], the initial colorimetric parameter L*, a*, and b* of fresh A. vera gel were 52.00, -3.75, and 18.72, respectively. With regard to C and H^o value, there were no significant differences in C and H° value of all samples. The samples presented the C and H^o values ranging from 10.73 to 13.12 and 92.28 to 95.52, respectively, indicated that they had bright yellow color. In general, high H^o value together with low C value is often indicative of a dull color [11].



Bars with differences letters indicate means with significant different ($p \leq 0.05$).

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

Fig. 1. Total phenolic content of each A. vera beverages (AVB).

B. Total phenolic content and antioxidant activities

Phenolics are considered as the main component of the plant, which can suppress free radicals [12]. The total phenolic content are shown in Fig. 1. It was found that AVB1 had the highest total phenolic content with the value 11.16 ± 1.09 mg GA Eq./100 mL, followed by AVB2 and AVB4, respectively, (9.85 ± 0.41 and 8.07 ± 0.52 mg GA Eq./100 mL, respectively). Comparable results were previously reported by Hulle *et al.* [13], who reported that the phenolic content for untreated and thermal treated of A. vera beverage mixed with

TABLE I. Physicochemical characteristic of each Aloe vera beverages (AVB).

Parameters	Values **			
	AVB1	AVB2	AVB3	AVB4
Biochemical attributes				
pH	4.68 ± 0.05 ^{a,b}	4.64 ± 0.06 ^{a}	4.75 ± 0.02 ^{b}	4.67 ± 0.08 ^{a}
TSS (^{o}Brix)^{ns}	11.10 ± 0.85	10.12 ± 0.34	9.05 ± 3.05	10.35 ± 1.12
Color				
L*	53.42 ± 3.68 ^{b}	56.50 ± 1.80 ^{a,b}	59.51 ± 3.22 ^{a}	59.80 ± 4.70 ^{a}
a*	-1.10 ± 0.67 ^{b}	-0.60 ± 0.28 ^{a,b}	-0.72 ± 0.36 ^{a,b}	-0.48 ± 0.39 ^{a}
b*	11.92 ± 2.39	14.45 ± 1.82 ^{a}	10.69 ± 3.03 ^{b}	11.64 ± 0.92 ^{b}
Chroma (C)^{ns}	^{a,b}	13.12 ± 0.90	10.73 ± 3.00	11.65 ± 0.90
Hue (H^{o})^{ns}	11.99 ± 2.34	92.28 ± 1.71	94.54 ± 3.24	92.50 ± 2.05
	95.52 ± 4.00			

**: Values are expressed as mean ± S.D. (n = 6).

$^{a-b}$: Superscript letters with different letters in the same column indicate significant difference ($p \leq 0.05$).

ns: non-significant.

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

TABLE II. Antioxidant activity of A. vera beverages (AVB).

Sample	ABTS radical scavenging activity (mg Trolox Eq./100 mL)	FRAP value (mg Trolox Eq./100 mL) ^{ns}	Metal chelating activity (mg EDTA Eq./100 mL)
AVB1	3.08 ± 0.71^{a}	1.44 ± 0.18	5.61 ± 0.49^{a}
AVB2	3.34 ± 0.68^{a}	1.60 ± 0.42	4.75 ± 0.69^{a}
AVB3	1.41 ± 0.23^{b}	1.33 ± 0.27	2.27 ± 0.91^{b}
AVB4	3.23 ± 0.75^{a}	1.56 ± 0.05	2.26 ± 0.78^{b}

Values are expressed as mean ± S.D. (n = 6).

$^{a-b}$: Superscript letters with different letters in same row indicate significant difference ($p \leq 0.05$)

ns: non-significant.

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

litchi fruit was 33.6 and 42 mg GA Eq./100 mL, respectively. Whereas, total phenolic content of pure A. vera gel contained 37.70 mg GA E/q.100 mL, [10]. However, total phenolic content in AVB samples were lower than the previous studies, this might be due to the different of concentrations, thermal treatments and kinds of fruit juice blending as well as growth conditions of plant.

The antioxidant activities of plant extracts have been broadly used as the important parameter in order to evaluate their bioavailability as medicinal foodstuffs [12]. In this study, antioxidant activities were determined in accordance with three methods, including ABTS assay, FRAP assay, and metal chelating assay. The scavenging activity for the ABTS radical varied from 1.41 to 3.34 mg Trolox Eq./100 mL (Table 2). The samples of AVB1 and AVB2 showed the highest for ABTS assay (3.08 ± 0.71 and 3.34 ± 0.68 mg Trolox Eq./100 mL, respectively), which showed the similar trend to metal chelating assay (5.61 ± 0.49 and 4.75 ± 0.69 mg EDTA Eq./100 mL, respectively). Although, antioxidant activity evaluated by FRAP assay was not significantly ($p > 0.05$) different between each sample. These results indicated that high concentration of AVJ addition impacted on greater antioxidant activities. The sample with high concentration of AVJ had greater amount of phenolic compound that highly correlated with antioxidant activity. The ability of phenolic compound involves antioxidant activity with different mechanism, including scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations [14]. Hęś *et al.* [15] confirmed the metal chelating ability of aloe extract. Moreover, Nejatzadeh-Barandoz [16] noticed the ability of A. vera gel extract on ferric ion reducing powder. In generally,

phenolic compounds can play an important role on reducing power activity which is based on chelation of Fe^{2+} ions in a quantitative manner by the reagent ferrozine, resulting in the formation of a complex with Fe^{2+} ions. Therefore, the chelating ability influences other scavenging activities of free radicals which protect the organisms against oxidative damage [17, 18]. Nevertheless, the concentrations of phenolic compounds are not the only factor influencing antioxidant capacity, but their structural arrangements (number and position of hydroxyl groups, double bonds, and aromatic rings) also play the key role [19].

V. CONCLUSION

Based on the results, the concentration of AVJ leads to minimal changes in physical and chemical properties, including pH, TSS, and color of the AVB. The AVB samples with 90% proportion of AVJ addition promoted a significant increase in antioxidant activities by metal chelating agent assay and ABTS assay, and total phenolic content, but did not affect to FRAP assay value. Consequently, it can be concluded that the AVB samples with 90% AVJ addition was the suitable condition of healthy beverage. However, further clinical trials regarding these claims are necessary before accurate conclusions regarding these health benefits can be made

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