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Effects of Adding Green Grape Juice to *Cerasus humilis* Juice on Chemical Composition and Antioxidant Properties

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Abstract: The objective of this investigation was to enhance the sensory appeal of *Cerasus humilis* (*C. humilis*) juice and ascertain potential interactions among bioactive compounds within a functional juice matrix. Sensory attributes, color, antioxidant potential, polyphenol and vitamin C (VC) concentrations, and sugar levels were assessed. Diverse dosage combinations were examined to determine their impact on the quality of both *C. humilis* and Green grape juices. Among the formulations, CG5 exhibited elevated antioxidant activity and polyphenol content (455.02 mg TE/100 ml and 173.1 mg/100 ml, respectively), coupled with favorable consumer preference. Notable variations in compound content were observed across the seven analyzed juices. In the blended juices (CG1–CG5), interactions between distinct juices influenced VC and sugar levels, with other bioactives contributing to beneficial modifications, displaying a progressive rise. This study innovatively crafted delectable and convenient *C. humilis* juice, upholding its intrinsic high antioxidant activity, thereby presenting a novel avenue for marketable functional juice offerings.

Keywords: *C. humilis*; green grape; antioxidant properties; polyphenolic compounds; functional fruit juice

1. Introduction

Currently, a heightened pursuit of superior quality living has propelled consumers' expectations, particularly within the realm of food. A balanced diet and consistent physical activity are pivotal in sustaining consumer well-being [1]. Concurrently, health hinges on the assurance of high-quality, safe food, thereby accentuating the significance of food quality. Responding to consumer preferences, numerous food producers have ventured into crafting wholesome, secure food items, prioritizing nutritional value [2,3]. Natural products endowed with sensory allure and heightened health merits resonate favorably with consumers [2,3]. Additionally, the surge in demand for functional foods, renowned for bolstering immunity and averting ailments, is evident [4]. These functional foods wield potent health-preventive attributes, bridging the divide between pharmaceuticals and nutrition [5,6]. A diverse array of functional foods, including grains [7], fruits, and vegetables, cater to consumer nutritional requisites. Fruits, being a repository of vitamins, minerals, amino acids, and bioactive compounds, constitute an indispensable facet of human sustenance [8]. Fruit-derived antioxidant-rich compounds are renowned for curbing disease and oxidative harm [9–11], particularly exemplified by VC, flavonoids, and phenolic compounds. Thus, the allure of antioxidant-rich foods and their processed derivatives captivates both consumers and scientific researchers [12,13]. Distinct fruit compositions in juices confer variable antioxidant capacity and oxygen radical scavenging prowess. Fruit juices endowed with sweet-tangy symphony potentially exhibit heightened bioactivity relative to their singular counterparts [14,15].

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For functional fruits, medicinal edible varieties stand out, exemplified by Lycium and *C. humilis* [16,17]. Renowned for its substantial biomass, *C. humilis* emerges as a health-promoting fruit, earning monikers like "calcium fruit" and "natural calcium powder" due to its exceptional calcium content [18]. The aromatic pulp of *C. humilis* boasts remarkable nutritional value, abundant in diverse glycolic acids [19], vitamins, mineral elements [20], and potent antioxidant compounds [21]. *C. humilis* holds significant potential across the food, nutrition, health, and medical sectors, generating considerable interest and exploration [22], marking a recent focal point for research and utilization. Nonetheless, consumer acceptance of *C. humilis* remains hindered by its inherent sourness, bitterness, and limited storability. Presently, *C. humilis* has found favor in the form of processed products like dried fruit, jam, juice, and fruit wine, gaining popularity as a novel choice for calcium supplementation among the elderly and children. Regrettably, the direct consumption of *C. humilis* juice proves unpalatable, necessitating the addition of numerous additives during further processing. This trajectory contrasts with consumer demand for palatable and healthful functional foods. Notably, the realm of *C. humilis* juices, whether singular or mixed, remains unexplored, creating a void in *C. humilis* development. To bridge this gap, we introduced high-sugar Green grape juice as an augmentation to *C. humilis* juice, aiming to enhance both taste and nutritional value.

In this study, we have systematically investigated the amalgamation of *C. humilis* juice and Green grape juice at varying ratios, aimed at achieving an optimally palatable mixed functional juice. The interplay of bioactive constituents between these diverse juices, along with their subsequent impact on sensory attributes and physicochemical characteristics of both *C. humilis* and Green grape juices, encompassing color, sugar content, VC levels, antioxidant activity, and polyphenol composition, was meticulously examined both pre and post-blending. By delving into the intricacies of juice blending and the resultant alterations in bioactive constituents, this research offers valuable insights into the development of ecologically sustainable and health-enhancing juice products.

2. Materials and Methods

2.1. Experimental materials

The fruits of *C. humilis* No. 6 and Green grape were used as research materials. fruits of *C. humilis* No. 6 were provided by *C. humilis* planting base in Suiling County, Suihua City, Heilongjiang Province, China. Green grape was purchased from Carrefour supermarket in Lesong Square, Harbin City, Heilongjiang Province, China.

2.2. Juice production

The process of production of juice from *C. humilis* and Green grape involved three main technological steps:

Processing of C. humilis juice. *C. humilis* was ground (50% water added) in a wall breaker (L12-Energy61, Jiu Yang Co., Ltd., Shenyang, China) and then 0.02% pectinase was added to the pulp in a water bath at 50°C for 3 h. In the action of a press (Ganggu Oolong, Taobao, Changsha, China) in order to obtain the juice.

Processing of Green grape juice. During the production of juice, Green grape is ground in a wall breaker and then pressed directly with a press to obtain juice.

Juices from *C. humilis* and Green grape were mixed at 34/66, 37/63, 40/60, 44/56 and 50/50 ratios immediately after obtaining. Due to the strong sour taste of *C. humilis* fruits, a higher amount of Green grape juice needs to be added to the *C. humilis* juice [23].

Then, the juice products were sterilized by heating at 100°C for 5 min and placed in glass jars, pasteurized (10 min), and cooled to 20°C [14]. Finally, seven different juices were obtained (Table 1). Each sample was prepared in three replicates.

Table 1. The resulting products.

No	Symbols	Product
1	C1	100% CJ
2	CG1	34% CJ + 66% GJ
3	CG2	37% CJ + 63% GJ
4	CG3	40% CJ + 60% GJ
5	CG4	44% CJ + 56% GJ
6	CG5	50% CJ + 50% GJ
7	G1	100% GJ

Note: *C. humilis* juice (CJ); Green grape juice (GJ).

2.3. Consumer evaluation

The sensory assessment criteria for the processed juice in this study adhered to the guidelines outlined in the national standard "GB/T16290-1996." Evaluation of the juice products was conducted using a 10-point scale methodology, with emphasis on taste, aroma, color, and consistency attributes. A panel of 20 randomly selected consumers participated in the sensory analysis. The coded samples, presented in uniform containers, were evaluated by the panelists under controlled conditions at a temperature of 20°C [24].

2.4. Chemical analyses

VC content was ascertained employing the method outlined by Vigneshwaran et al. [25], based on the reaction of 2,6-dichlorophenol-indophenol with VC within the samples, under acidic conditions. An ultraviolet-visible spectrophotometer (UV-1800 model, Shimadzu, Japan) was employed to measure the absorbance at 518 nm. The quantification, presented as mg/100 ml fresh weight (FW), was carried out following established procedures. Sugars were quantified using the HPLC-ELSD technique, following the protocol delineated by Oszmianski and Lachowicz [26]. Replicating each measurement thrice ensured accuracy. The outcomes were reported in mg/ml FW.

2.5. Colour parameters

The color attributes (L^*, a^*, b^*) of both C. humilis and Green grape juices were assessed through reflectance measurements, employing a color spectrophotometer (CM-5, Shimadzu, Japan). The methodology outlined by Wojdyło et al. [27] was followed for sample measurement. Measurements were conducted using a white ceramic reference plate ($L^* = 93.92$; $a^* = 1.03$; $b^* = 0.52$). Each reported data point represents the mean of three separate measurements.

2.6. Total polyphenol

Total polyphenols were quantified following the Folin-Ciocalteu method as outlined by Lachowicz et al. [28]. Briefly, samples were homogenized with distilled water and the Folin-Ciocalteu phenol reagent. The addition of a 200 g/L sodium carbonate solution ensued, inducing reaction at ambient temperature under light protection for 1 hour. Subsequently, absorbance at 765 nm was recorded using a UV-Vis spectrophotometer (UV-1800 model, Shimadzu, Japan). Total polyphenols were expressed as milligrams of gallic acid equivalent (GAE) per 100 ml FW.

2.7. Antioxidant activity

Free radical scavenging activity (DPPH)was determined following the method described by Suja et al. [29], preparing a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in absolute ethanol. The radical scavenging activity (%) was determined using the following formula:

$$(A0-A1)/A0 \times 100$$

A0 = the absorbance of control; A1 = the absorbance of standard [30].

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted following the protocol outlined by Fu et al. [31]. Briefly, a total of 200 µl of the extracted solution was combined with 2.8 mL of FRAP solution, comprising 0.1 M acetic acid buffer (pH = 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1. The resulting sample mixture was vigorously shaken and incubated in the dark at room temperature for 10 minutes. For the blank, the extracted solution was substituted with 40% (v/v) methanol. Subsequently, the absorbance was measured at 593 nm using a spectrophotometer (UV-1800 model, Shimadzu, Japan). The FRAP results were quantified as milligrams of Trolox equivalents (TE) per 100 ml FW.

2.8. Statistical analysis

The data were analyzed using Origin 2021, SPSS, and Microsoft Excel 2020. The Student's t-test, one-way analysis of variance, and correlation analysis were applied to detect the differences and correlations between experimental groups. A p-value of 0.05 or lower was considered to indicate statistical significance.

3. Results and Discussion

3.1. Consumer evaluation of the tested products

The sensory evaluation results for *C. humilis* and Green grape juices based on comprehensive attributes including taste, aroma, color, and consistency are depicted in Figure 1. Overall, the outcomes indicate that all juices exhibit appealing color profiles (≥ 6.63). Notably, for Green grape juice, approximately 8.5 (G1), containing 60% (CG3), 56% (CG4), and 50% (CG5) Green grape juice, exhibited comparable elevated taste scores of 7.77, 8.13, and 8.67, respectively. Conversely, consumer evaluations rated *C. humilis* juice and the blend with 66% Green grape juice (C and CG1) with the lowest taste scores (3.5 and 5.4, respectively). The most elevated aroma ratings were observed for CG1 (7.76), CG4 (7.76), CG5 (8.37), and G1 (8.1), while C1 (6.43) and CG2 (5.7) received the lowest aroma ratings according to consumer assessments. In terms of consistency, C1 (8.66) achieved the highest scores, whereas CG4 (8.5) and CG5 (8.0) demonstrated superior status; conversely, CG1 (4.8) received the lowest status scores.

Overall, the Green grape juice (G1) exhibited superior product ratings, along with the blends incorporating 60%, 56%, and 50% Green grape juice (CG3, CG4, and CG5, respectively). These formulations demonstrated heightened consumer appeal, marked by enhanced sensory attributes. Conversely, *C. humilis* juice (C1) and the blend containing 66% added Green grape juice (CG1) were deemed categorically unacceptable, as higher proportions of *C. humilis* juice led to perceptible deterioration in overall taste profile. The inherent delicate and sweet flavor profile of Green grape juice is widely recognized. In stark contrast, the pronounced sourness, bitterness, and distinctive off-putting aroma of *C. humilis* fruit rendered it unpalatable to consumers. The judicious incorporation of Green grape juice yielded a noteworthy enhancement in the taste profile of *C. humilis* juice, rendering it more amenable to consumer preferences.

Guo et al. [23] substantiated that the majority of *C. humilis* fruits exhibit high acidity, rendering them unsuitable for direct consumption and more appropriate for subsequent processing. In parallel, bog bilberry juice displays a markedly low pH (pH < 3.0) in contrast to other prevalent fruit juices, resulting in excessive sourness and precluding immediate consumption [32]. Remarkably, Green grape juice boasts elevated sugar levels and a nearly colorless appearance, rendering it a highly promising candidate for juice blending. The incorporation of 50% Green grape juice effectively mitigated the acidity of *C. humilis* juice without inducing color alteration. Collectively, consumer preferences skewed toward juices that harmoniously balanced acidity and sweetness, thus garnering superior ratings. Notably, taste assumes a pivotal role in the sensory evaluation of comestibles [33]. The sensory perception of pear and cranberrybush juice is notably influenced by the sugar-acid ratio, a quotient defined by the relationship between total soluble solids extract and total titratable acidity—a factor that significantly influences consumer predilections. This observation aligns with the findings

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of Lachowicz and Oszmianski [14], who emphasize that skillful blending of sweet and tart juices in appropriate proportions resonates as a judicious strategy to cater to consumer preferences.

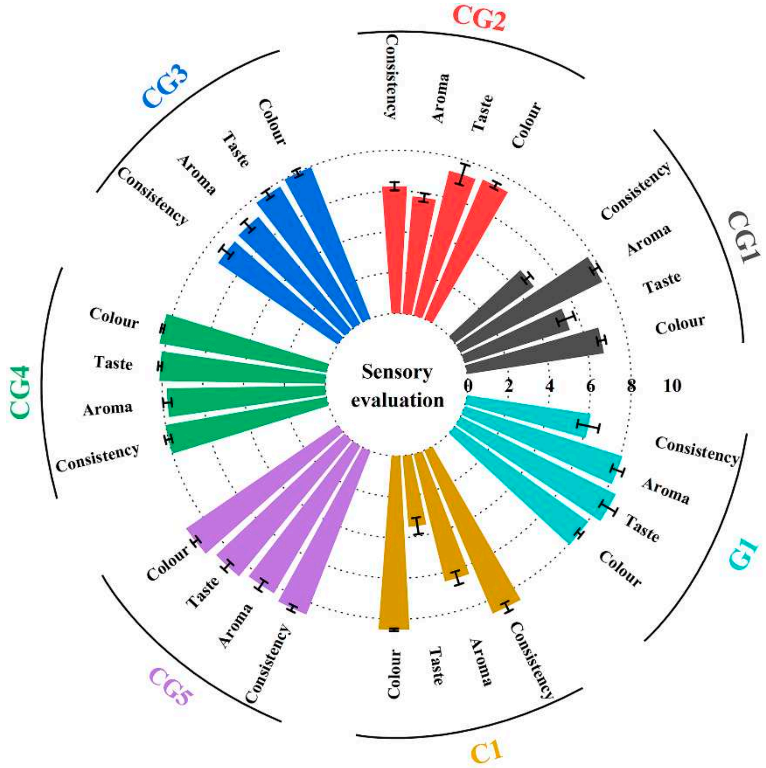

Figure 1. Consumer evaluation of different types of juices.

3.2. Chemical composition

With respect to VC content, C1 exhibited the highest concentration at 135.5 mg/100 ml, representing a substantial fivefold increase compared with G1 (Figure 2A). Generally, juices rich in *C. humilis* extract demonstrated heightened VC concentrations. *C. humilis* fruits have been recognized for their superior vitamin content relative to many other fruits [34], contributing to the pronounced VC elevation in C1 vis-à-vis G1. Nevertheless, the VC content did not exhibit a corresponding augmentation with increased *C. humilis* juice content, remaining relatively consistent across diverse juice types within a notably elevated concentration range. Evidently, the introduction of Green grape juice exerted an influence on VC levels within the composite juice, yet this interaction yielded favorable outcomes. Similar effects were observed in a blended juice composed of Brazilian Cerrado fruits (cagaita, mangaba, and marolo), leading to VC augmentation and flavor modulation [35]. Conversely, *C. humilis* juice manifested a sugar content of 112 mg/ml, significantly lower than Green grape juice (152 mg/ml) (Figure 2B). The addition of Green grape juice markedly enhanced the sugar content, albeit without a linear gradient. The altered sugar concentration in the blended juices indicates potential underlying reactions warranting further exploration. These findings collectively suggest that supplementing *C. humilis* juice with Green grape juice elevates both VC and sugar content, while concurrently influencing their respective increments. In the realm of food products, bitter and sour attributes tend to be aversive, whereas sweetness and freshness act as appetitive stimuli, commonly appealing to consumers' palates [36].

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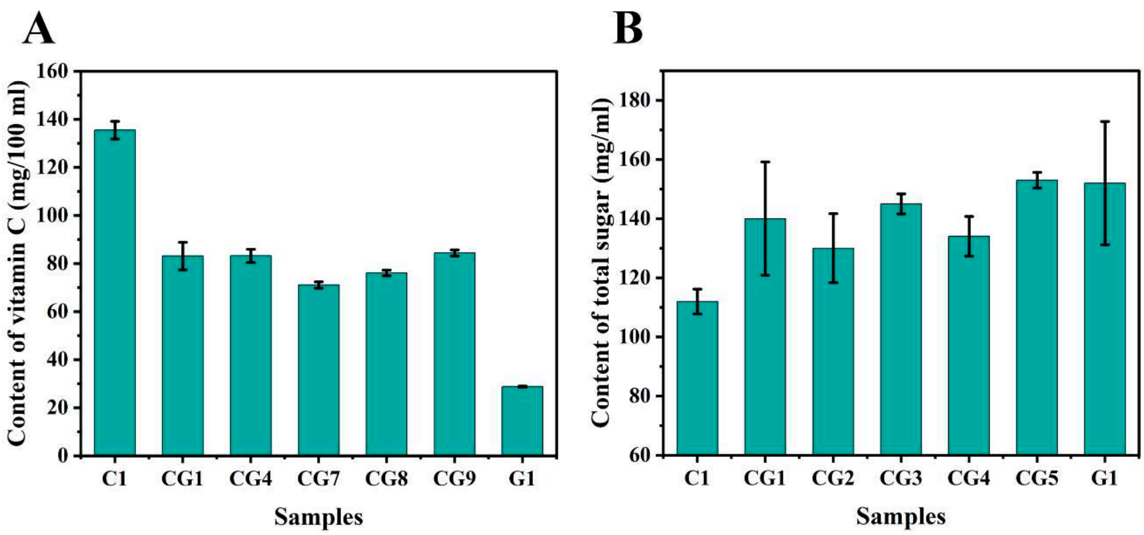

Figure 2. The content of total sugar in *C. humilis* and Green grape juice (A). The content of VC in *C. humilis* and Green grape juice (B).

3.3. Colour parameters of juices

Color stands as a pivotal determinant of juice quality [37], exerting a profound influence on consumers' initial perceptions and purchasing inclinations [27,38]. Table 2 outlines the color parameter values encompassing diverse types of mixed juice, including L*, a*, b*, ΔE, h°, and ΔC. Evidently, a substantial divergence in product color (C1, CG1–CG5, G1) is evident. Post-treatment, juice luminosity (L*) exhibited a progressive elevation, ascending from 40.06 in C1 to 99.82 in G1. Notably, incorporation of Green grape juice correlated with enhanced luminosity in *C. humilis* juice. The b* parameter spanned 46.49 in CG5 to 30.23 in CG1, signifying a yellowish shift attributed to Green grape juice. Meanwhile, parameter a* ranged from 66.64 in juice CG5 to 60.09 in juice CG1 upon immediate processing, denoting a heightened redness with reduced Green grape juice incorporation. Generally, a preference for reddish hues is discerned in juices.

Table 2. Colour parameters of analysed juices.

Samples	L*	a*	b*	ΔE	h°	ΔC
C1	40.06	66.94	66.74	-	-	-
CG1 (34)	62.50	60.09	30.23	43.40	26.70	37.15
CG2 (37)	61.56	61.65	33.09	40.28	28.24	34.06
CG3 (40)	59.38	63.91	36.12	36.33	29.47	30.77
CG4 (44)	56.69	65.11	40.63	31.01	31.97	26.17
CG5 (50)	53.69	66.64	46.49	24.40	34.89	20.25
G1	99.82	0.45	3.17	109.70	80.92	91.98

For a more accurate analysis of color variations among different juice types, a comparison of the parameter ΔE becomes imperative. This parameter quantifies the human eye's capacity to discern color disparities between two products, with varying levels of perceptibility. Specifically, for evaluators, a $\Delta E > 3$ denotes a visually perceptible color difference, while a $\Delta E < 3$ suggests challenges in distinguishing juice color differences [14,39]. The ΔE values, as presented in Table 2, distinctly elucidate disparities among the products (C1, CG1–CG5, G1), wherein $\Delta E > 3$. Consequently, the assessments offered by the evaluators, as depicted in Figure 1 through visual inspection of the color variations across diverse juices, exhibit a logical and well-founded correlation.

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3.4. Total phenolic compounds and Antioxidant activity

Figure 3 illustrates the total phenolic compound (TPC) concentrations in both C1 and G1 juices, as well as their blends. The TPC content in *C. humilis* juice measured 267.48 mg/100 ml, a remarkable 9.7-fold increase over that in Green grape juice. The introduction of G1 had a pronounced impact on the TPC levels within the resultant mixture. Consequently, the inclusion of G1 contributed to the preservation of elevated TPC content. Notably, the CG5 juice, which exhibited the most favorable taste profile, displayed a TPC content of 173.1 mg/100 ml. This value surpassed that of half the combined total of C1 and G1 by 25.56 mg/100 ml, suggesting that the addition of Green grape juice did not adversely impact the TPC content of the juice; in fact, it appeared to confer a beneficial effect. It is pertinent to mention that the TPC content in *C. humilis* itself was determined to be 393 mg/100 g, which is 1.5 times higher than that of *C. humilis* juice [31]. In contrast, Citrus × Clementina Hort juice displayed TPC concentrations ranging from 29.46 to 31.12 mg/100 ml [12], significantly lower than those observed in CG5. Nonetheless, the TPC content in Green grape juice remains unexplored, necessitating further investigation.

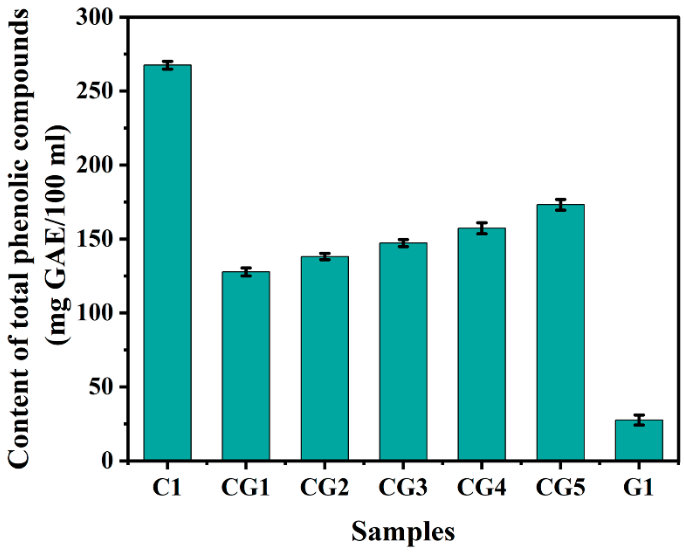

Figure 3. Effect of interactions on total phenolic compounds in juice after addition of Green grape juice.

The antioxidant activity (AA) of the examined juices was evaluated through the utilization of the DPPH and FRAP methodologies (Figure 4). The outcomes from both DPPH and FRAP assessments displayed a parallel trend across *C. humilis* and Green grape juice blends. In the realm of juice analysis, the most notable DPPH free radical scavenging rates were discerned in C1 and CG5: 86% and 57.7%, respectively (Figure 4A). Conversely, the lowest scavenging activity was observed in Green grape juice at 11%. Incorporation of Green grape juice (G1) led to an elevation in AA. The capacity to reduce ferric ions, as determined by the FRAP assay, exhibited a range from 35.96 mg TE /100 ml in G1 to 850.8 mg TE /100 ml in C1. In comparison with Green grape juice, AA values were augmented by approximately 9.47 (CG1), 10.39 (CG2), 11.52 (CG3), 12.28 (CG4), and 12.65 (CG5) (Figure 4B). Notably, all products demonstrated robust AA stability under the FRAP test, with values of 850.8 and 35.96 mg TE/100ml for C1 and G1, respectively, showcasing an ascending trajectory from CG1 to CG5 (340.68–455.02 mg TE/100 ml). The analysis, juxtaposing the sensory evaluations of the juices against the AA results, revealed an absence of correlation between potential AA and sensory appraisal outcomes. Intriguingly, the product with the highest AA (C1) was deemed less acceptable to consumers, yet garnered favor due to its nutritional value and quality. Of note, the most appealing product (CG5) also exhibited an elevated AA content. In conclusion, consumers can tailor their juice selection to their preferences, prioritizing either heightened functionality or superior taste.

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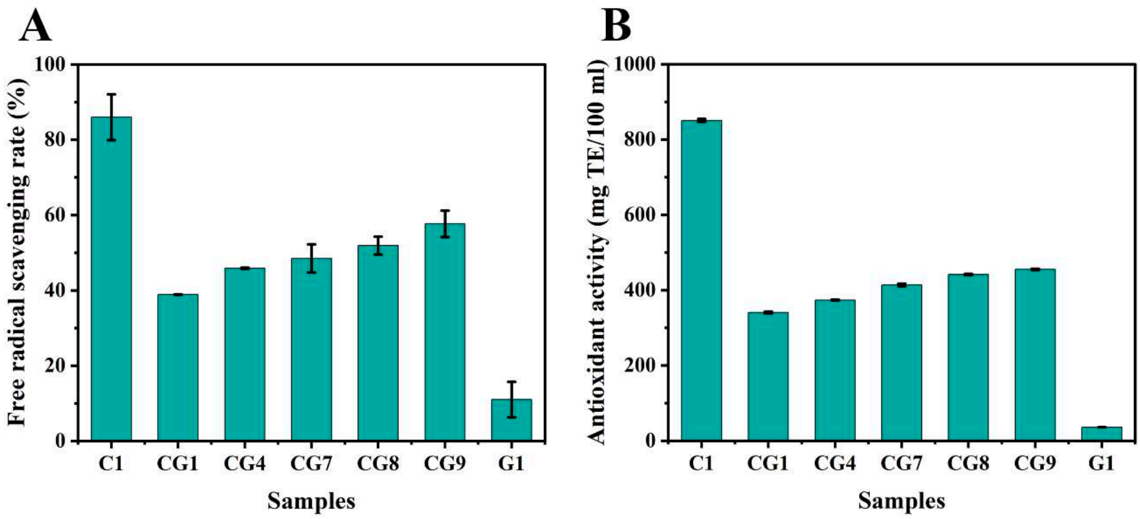

Figure 4. The analysis of antioxidant activity in *C. humilis* and Green grape juice. (A) DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging capacity. (B) FRAP, ferric reducing antioxidant power.

4. Conclusions

In this study, Green grape juice was harnessed to enhance the palatability of *C. humilis* juice and elucidate alterations in bioactive constituents within this functional amalgamation. The incorporation of natural Green grape juice yielded a marked enhancement in taste for *C. humilis* juice, concurrently minimizing reliance on artificial additives. Consumer evaluations corroborated superior scores for CG1–CG5 vis-à-vis C1, with CG5 notably securing the highest rating, attaining widespread consumer acceptance. The interplay between *C. humilis* juice and Green grape juice engendered discernible shifts in VC and sugar content across various blended formulations. Notably, this interaction yielded a favorable outcome, fostering an equilibrium of sour and sweet nuances that resonated more compellingly with consumers. Color attributes exhibited robust stability, manifesting a reddening trend commensurate with heightened *C. humilis* juice content. Remarkably, barring G1, the surveyed juices showcased commendable antioxidant activity. Particularly striking were C1 and CG5, exhibiting notable values of 850.8 mg TE/100 ml and 455.02 mg TE/100 ml, respectively. In summation, the infusion of Green grape juice emerged as a salutary practice, preserving the integrity of bioactive constituents within the juice matrix. This stratagem presents a commendable avenue to curtail the reliance on synthetic additives, facilitating the production of health-conscious, secure functional juices for discerning consumers. The pertinence of this approach extends to the food industry at large, offering applicability to diverse juice variants and promising augmented commercial value.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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