Development of Effervescent Tablets Containing Elderberry Vine Tea Compound and Evaluation of Their Antioxidant Activity

Wanhao Sun

College of Food Science & Nutritional Engineering, China Agriculture University, Beijing, China

Correspondence should be addressed to Wanhao Sun; 15558141912@163.com

Received 28 August 2023; Revised 20 November 2023; Accepted 22 November 2023; Published 7 December 2023

1. Introduction

The evolving dietary habits and sedentary lifestyles of contemporary populations have led to an upsurge in free radicals within the body, attributed to factors such as high-fat, high-sugar fast food, and limited physical activity [1]. The consequential accumulation of free radical-induced damage has been linked to a spectrum of organ-specific ailments, such as Alzheimer’s disease [2], atherosclerosis, diabetes, and radiation-induced afflictions [3]. In this context, antioxidants have emerged as potent defenders against oxidative stress, curbing the impact of free radicals on cellular and tissue integrity [4]. Natural sources of antioxidants include vitamin C, carotenoids, polyphenols, and flavonoids [5].

Elderberries, distinguished for their high flavonoid content, particularly anthocyanins, stand out as a prime repository of these bioactive compounds among various berry species [6]. Studies have shown that ripe elderberries contain 47% flavanol glycosides, 34% anthocyanins, 14% flavanols, and 3% hydroxycinnamic acids [7]. Scientific investigations have also affirmed the efficacy of elderberries in promoting mitochondrial and cellular revitalization, sustaining cellular equilibrium, and retarding the aging process [8]. This botanical asset, replete with antioxidant potency, emerges as an ideal candidate for innovative product development. Nonetheless, the utilization of elderberries remains confined to juices, fruit teas, and jams, which struggle with preservation challenges and susceptibility to significant anthocyanin losses during processing [9].

Vine tea, derived from the dried tender leaves of Ampe- lopsis grossedentata [10], exhibits significant antioxidant potential due to its rich content of 36 bioactive compounds, including gallic acid, butyric acid, ellagic acid, and myricetin, as demonstrated by the Folin-Ciocalteu assay [11]. However, vine tea products still remain in a preliminary processing stage due to geographical constraints and the inherent astringent taste [12]. Notably, conventional brewing methods only facilitate the infusion of a fraction of
bioactive components into the tea broth, leading to the underutilization of resources. To address these limitations, ultra-micropulverization technology emerges as a promising avenue. This technology can effectively enhance the content of water-soluble carbohydrates and the extraction of terpene synthase (TPS), thereby bolstering the scavenging activity on OH radicals [13]. Although widely adopted in black tea, green tea, Pu’er tea, and Oolong tea processing [14], its application to vine tea is largely unexplored.

Based on the aforementioned information, it is evident that elderberry and vine tea are rich in antioxidants. These properties position elderberry as a promising candidate for treating various inflammatory diseases including Huntington’s disease [15], atherosclerosis [16], and UVB-induced skin photoaging [17]. Similarly, vine tea, as a traditional Chinese herbal medicine, is frequently utilized to alleviate arthritis symptoms [18], liver fibrosis [19], and atherosclerosis [20]. In a strategic endeavor to unlock the untapped potential of vine tea and fully utilize the inherent qualities of elderberry, this study introduces a pioneering approach. It deploys ultra-micropulverization technology for the treatment of vine tea, seamlessly integrated with the use of effervescent tablets. That is because elderberry extract and ultra-micro vine tea powder are highly dehydrated and hygroscopic, while the anthocyanins present in the extracts are sensitive to environmental conditions [21]. As a result, it imposes high costs of storage and transportation. To overcome this challenge, the manufacturing of tablets is considered a distinctive technique [22]. Tableting stabilizes the physical and chemical properties of the product, extending its shelf life. Furthermore, tablet production offers advantages such as improved appearance and increased consumer acceptance [23].

Response surface designs are utilized to determine the best combination of inputs that yield the most favourable outcomes. This is achieved by minimizing the average prediction variance across the design spaces. Such designs are valuable tools for optimizing products and processes as they involve iteratively adjusting one or more inputs and analysing the impact of these adjustments on the output(s) [24]. Using this method, this study is aimed at the following:

1. Enhancing nutrient retention in vine tea through ultra-micropulverization
2. Alleviating the astringency of vine tea by harnessing the natural sweet and sour fruity flavour of elderberry
3. Developing an experimentally validated effervescent tablet formula that possesses strong antioxidant properties and is made of natural, healthy ingredients

Through the assessment of disintegration kinetics, foaming characteristics, tablet hardness, weight consistency, and free radical scavenging activities, this investigation comprehensively unravels the potential of this novel approach. This study not only elevates the nutritional profiles of elderberry and unlocks the untapped potential of vine tea products through meticulous processing and flavour enhancement but also lays the essential theoretical foundation to meet the evolving demands of modern society for antioxidant-rich functional foods.

2. Materials and Methods

2.1. Materials and Reagents. Vine tea: Zhangjiajie Baxingteng Tea Planting Farmers’ Professional Cooperative; elderberry extract: Xi’an Michel Biotechnology Co., Ltd.; mannitol, citric acid, sodium bicarbonate: Henan Gaobao Industrial Co., Ltd.; PEG-6000: Shandong Ruisheng Excipients Co., Ltd.; polyvinyl pyrrolidone: Zhejiang Huiquan Biotechnology Co., Ltd.; anhydrous ethanol: Jinan Qiwei Chemical Co., Ltd.; total flavonoid content assay kit, total phenol content assay kit, DPPH radical scavenging activity assay kit, hydroxyl radical scavenging activity assay kit, ABTS radical scavenging activity assay kit: Beijing Solarbio Science & Technology Co., Ltd. All the materials used are food grade.

2.2. Experimental Methods

2.2.1. Ultra-Micropulverization of Vine Tea. The vine tea was introduced into the oscillating pulveriser (MF-4A, Wenling Mingda Medicine Machinery Equipment Co., Ltd., China) and crushed for 5 minutes, and then sieved through a 100-mesh sieve to yield coarse-grinding powder. This preliminary powder was further ground using an ultra-micropulveriser (MFC-35B, Wenling Mingda Medicine Machinery Equipment Co., Ltd., China) in a single round of grinding for 10 minutes and then sieved through a 200-mesh sieve to obtain the ultra-micropowder [25].

2.2.2. Tablet Preparation. The acid-base separation semidry granule pressing method was employed to prepare the effervescent tablets [26, 27], as shown in Figure 1. Stevioside, citric acid, and elderberry extract powder were finely ground and sieved through a 120-mesh sieve and then blended with ultra-micro vine tea powder. After adding a 5% polyvinyl pyrrolidone (PVP) solution, the mixture was dried at 50°C. The resulting mixture was sieved again to produce acid granules. Alkali granules were prepared using a similar pathway. Both granules were then combined, and PEG-6000 lubricant was added. The mixture was pressed under 10 Mpa for 30 seconds using a tablet press (FW-5A, Tianjin Tianguang New Optical Instrument Technology Co., Ltd., China) to produce compound effervescent tablets with consistent composition.

2.2.3. Sensory Evaluation. After undergoing rigorous professional training, ten expert assessors, aged between 19 and 50 years, were selected to conduct sensory evaluations. The evaluation employed a double-masked methodology. The assessors evaluated preferences based on four dimensions. The detailed scoring is provided in Table 1.

To address varying levels of importance attributed to individual factors in the product evaluation, a weight distribution was established based on previous research [28]. Five experienced assessors independently assigned weights to a fuzzy subset $X = \{X_1, X_2, X_3, X_4\}$ of factors related to effervescent tablets, reflecting their perceived significance. Each $X_i$ value, ranging between 0 (not necessary) and 1 (very important), was rounded to one decimal place. Normalizing...
and averaging this data yielded the weight set $A = \{A_1, A_2, A_3, A_4\}$. The total sensory score was then computed using Equation (1).

$$\text{Sensory Score} = X_1 \times A_1 + X_2 \times A_2 + X_3 \times A_3 + X_4 \times A_4.$$  

\hspace{1cm} (1)

2.2.4. Single-Factor Experiments for Sensory Evaluation. This study employed single-factor experiments and focused on parameters intricately contributing to the overall perception of effervescent tablets. The levels of immobilization were determined in preliminary experiments: a disintegrant content of 50% at a 1.3 : 1 acid-to-base ratio, complemented by a 40% combination of elderberry extracts in a 4 : 1 ratio with ultra-micro vine tea powder, 2.5% stevioside, 4.5% mannitol, 2% PEG-6000, and 1% PVP.

2.2.5. Response Surface Design. Building upon the outcomes of the single-factor experiments, a four-variable, three-level Box-Behnken design was employed. The utilization of Design-Expert 8.0 facilitates multiple regression analyses and the subsequent optimization [29].

2.2.6. Determination of Physicochemical Properties

(1) Disintegration Time. Following the guidelines of the Chinese Pharmacopoeia 2020 [30], an effervescent tablet was placed in a 250 mL beaker with 200 mL of distilled water at a temperature of $20 \pm 5^\circ$C, and the time required for complete disintegration was recorded.

(2) Foaming Quantity. For accurate measurements, the effervescent tablets were first ground into powder and then tested for foaming quantity [31]. The weight of effervescent tablet powder before being placed into an aqueous beaker ($M_0$), the total mass before foaming ($M_1$), and the final weight of the residue after foaming ($M_2$) were determined using an electronic balance (FA2104N, Shanghai Jingqi Instrument Co., Ltd., China). The foaming quantity was computed using Equation (2), which represented the amount of CO$_2$ gas generated in milligrams per gram of effervescent tablet.

$$\text{Foaming Quantity (mg/g)} = \frac{M_1 - M_2}{M_0} \times 1000.$$  

\hspace{1cm} (2)
(3) Average Weight and Weight Variation. Twenty effervescent tablets were selected to compute the average weight. The actual weight of each tablet was compared to the average value [30].

(4) Hardness. The hardness was evaluated using a texture analyser (TA-XT Plus, SMS Corporation, UK) with a 2 mm cylindrical probe, a force sensing element ranging up to 100 N. The probe extraction distance was set at 2 mm, and the detection speed was 60 mm/min. The starting force was 0.4 N, and the maximum force value was measured [32].

(5) Moisture Content. The moisture content of the effervescent tablets was determined using the direct drying method [30]. A weighing bottle was placed in a vacuum-drying oven (DZF-6024, Shaoxing Shangli Instrument Co., Ltd., China) and heated until a consistent weight was attained. Subsequently, a precise quantity of the effervescent tablet (M4) was weighed and added to the same bottle. The bottle was then capped, and the total weight (M4) was recorded. The bottle and its contents were then placed back into the drying oven until a stable weight was reached (M5). The moisture content was calculated as Equation (3).

\[
\text{Moisture Content (g/100g)} = \frac{M_4 - M_5}{M_5} \times 100. \tag{3}
\]

(6) Colour. Following the recommendations of Commission Internationale d’Eclairage (CIE), the colour was represented by \(L^*\) (brightness/darkness), \(a^*\) (redness/greenness), and \(b^*\) (yellowness/blueness) values, utilizing a colour meter (ZE6000, Nippon Denshoku, Japan). Additionally, chroma (C), hue angle (h), and yellowness index (YI) were calculated through the following equations [33].

\[
C = \sqrt{a^{*2} + b^{*2}}, \\
h = \tan^{-1}\left(\frac{b^*}{a^*}\right), \tag{4}
\]

\[YI = 142.86 \times \frac{b^*}{L^*}.
\]

2.2.7. Determination of Antioxidant Content

(1) Total Flavonoid Content (TFC). The TFC was determined using a colorimetry method involving \( \text{NaNO}_2 \cdot \text{Al(NO}_3\text{)}_3 \cdot \text{NaOH} \) [34]. The sample was extracted in 70% alcohol for 10 minutes and neutralized with \( \text{Na}_2\text{CO}_3 \) (0.5 mL, 20%, w/v). Subsequently, the reaction mixture was diluted to 50 mL using distilled water, and its absorbance was measured at 517 nm. The TFC was quantified as gallic acid equivalents (GAE) per gram of dry extract.

(2) Total Polyphenol Content (TPC). The TPC was determined using the Folin-Ciocalteu spectrophotometry method with gallic acid as the external standard [35]. The sample was extracted in 70% alcohol for 10 minutes and neutralized with \( \text{Na}_2\text{CO}_3 \) (0.5 mL, 20%, w/v). Subsequently, the reaction mixture was diluted to 50 mL using distilled water, and its absorbance was measured at 760 nm. The TPC was quantified as gallic acid equivalents (GAE) per gram of dry extract.

2.2.8. Determination of Antioxidant Capacity

(1) DPPH Radical Scavenging Activity. Incorporating adaptations from the study conducted by Wu et al. [36], after being dissolved in 70% ethanol, the samples underwent 20 minutes of ultrasonication, and different sample concentrations were prepared: 0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.7 mg/mL, 1.0 mg/mL, 3.0 mg/mL, 5.0 mg/mL, 7.0 mg/mL, and 9.0 mg/mL. The experimental setup utilized a 96-well plate, with each initially receiving 100 \( \mu \text{L} \) of 0.1 mM DPPH chromogenic reagent, followed by the addition of 100 \( \mu \text{L} \) of the specific sample solution. Subsequently, the absorbance was measured at 517 nm with a microplate reader (ThermoFC, Shanghai Yilang Electromechanical Equipment Co., Ltd., China) after 30 min incubation at room temperature. DPPH radical scavenging activity was assessed using the following equation:

\[
\text{DPPH(% inhibition)} = \left(1 - \frac{A_1}{A_0}\right) \times 100\%, \tag{5}
\]

where \( A_1 \) and \( A_0 \) represent the absorbance of the sample and blank, respectively.

(2) Hydroxyl Radical Scavenging Activity. Following the methodological guidelines stipulated by the kit, the samples are dissolved in 70% ethanol and underwent 20 minutes of ultrasonication. Then, the same concentration ladder as the DPPH radical scavenging experiments was prepared. Subsequently, the reaction solution was transferred to a 96-well plate, incubated for 20 min at room temperature, and absorbance at 550 nm was measured using a microplate reader equipped with a 1 cm optical aperture. Hydroxyl radical scavenging activity was assessed using the following equation:

\[
\text{Hydroxyl (% inhibition)} = \left(1 - \frac{A_1}{A_0}\right) \times 100\%, \tag{6}
\]

where \( A_1, A_0 \), and \( A_0 \) represent the absorbance of the control, sample, and blank, respectively.

(3) ABTS Radical Scavenging Activity. Incorporating adaptations from the study conducted by Thaipong et al. [37], the ABTS working solution was prepared as follows: 5 \( \mu \text{L} \) of a 7.4 mM ABTS radical solution was mixed with 88 \( \mu \text{L} \) of a 2.6 mM potassium persulfate solution. The mixture was allowed to stand at room temperature in the dark for 12 to 16 hours. Prior to measurement, the working solution was diluted with 80% ethanol to achieve an absorbance range of 0.7 ± 0.05 at 734 nm. Then, 20 \( \mu \text{L} \) of sample solutions with the same concentration ladder as the DPPH radical scavenging experiments were combined with 200 \( \mu \text{L} \) of the diluted...
ABTS solution. After incubating for 6 minutes, the absorbance at 734 nm was measured spectrophotometrically. The ABTS radical scavenging activity was assessed using the following equation:

$$\text{ABTS(%inhibition)} = \left(1 - \frac{A_f}{A_0}\right) \times 100\%,$$

where $A_f$ and $A_0$ represent the absorbance of the sample and blank, respectively.

2.2.9. Statistical Analysis. Data are presented as the mean ± SD. The differences among the groups were compared by one-way analysis of variance (ANOVA). Correlations were calculated by the Pearson linear regression analysis. All statistics were analysed by Design-Expert (V8.0.6.1), SPSS (27.0), and GraphPad Prism (9.5.1). A $P$ value < 0.05 indicates statistical significance. Unless otherwise noted, all data were generated from three independent experiments.

3. Results and Discussion

3.1. Weight Distribution for Sensory Evaluation. To comprehensively understand the preferences of the assessors and their collective impact on the overall sensory experience, the weight distribution of key sensory factors in the effervescent tablet assessment is presented in Table 2.

Evidently, taste ($X_3$) and aroma ($X_4$) emerge as central considerations, both bearing a weight of 0.3. This emphasis resonates with earlier findings by Liao et al. [38] and Chen [39], where the forced-decision method corroborates the significance of these attributes. Appearance ($X_1$) and liquor colour ($X_2$) carry equal weights of 0.2 each. Their acceptability is more intricately linked to the operational parameters. Therefore, the total sensory score can be calculated as equation (8), which better encapsulates the evaluative priorities.

$$\text{Sensory Score} = \text{Taste} \times 30\% + \text{Aroma} \times 30\% + \text{Appearance} \times 20\% + \text{Liquor Colour} \times 20\%.$$

3.2. Investigation of Single-Factor Experiments. The results of single-factor experiments are shown in Figure 2. Figure 2(a) reveals a distinct sensory score trend concerning the elderberry extract-to-ultra-micro vine tea powder ratio. The score exhibits a biphasic pattern, characterized by an initial increase followed by a decline, reaching its pinnacle at a ratio of 4:1. This optimal ratio engenders a sensorial experience dominated by a pronounced fruity aroma, complemented by the unique flavour of vine tea. The visual aspect also contributes to the sensory appeal, as the colour showcases transparency and a pleasing peach hue. At lower ratios (below 3:1), the presence of insoluble material becomes noticeable. This can be attributed to the gradual increase of insoluble substances in vine tea, such as cellulose and lignin, which leads to the formation of a natural precipitate [40]. Additionally, lower ratios accentuate the bitter and astringent taste of vine tea, as supported by the study conducted by Wang et al., which attributes this taste to the terpenoid content in vine tea, particularly limonoids [41, 42]. At higher ratios (above 5:1), the solution exudes a robust fruity flavour, along with an appealing rosy red hue, but the tea flavour becomes less pronounced. Therefore, the response surface design selects the ratios of 3:1, 4:1, and 5:1 as the parameter range.

Compared to other nonnutritive sweeteners such as aspartame and acesulfame potassium, steviosides stand out as noncaloric sucrose substitutes and noncariogenic sweeteners, with fewer adverse effects on the neurological system [43]. The influence of total stevioside content on sensory scores, as depicted in Figure 2(b), follows a nuanced trajectory: a gradual ascent succeeded by a notable decline, reaching its pinnacle at 3%. As the quantity augments, the sweetness of the solution gradually intensifies, attaining a balance of moderate sweetness and tanginess. However, surpassing the 3.5% threshold leads to the emergence of a melange of bitterness and metallic attributes. This observation aligns with the findings of Espinoza et al. [44], and the potential cytotoxicity also sets a limit to its extensive addition [45]. Considering both the gustatory experience and safety concerns, this work focuses on a parameter range of 2.5%-3.5% for the addition of stevioside, which aligns with the range established by Skapska et al. in the context of functional fruit-herbal beverages [46].

In comparison to sodium dodecyl sulfate and magnesium stearate, PEG-6000 exhibits the advantage of being free from metal ions, thus effectively circumventing the catalysis of anthocyanin oxidation [47]. The impact of PEG-6000 addition on the sensory score of effervescent tablets, illustrated in Figure 2(c), unfolds in a two-phased manner, with an initial ascent followed by a subsequent descent, culminating at its zenith at 3%. Within this context, a PEG-6000 addition of less than 2% yields a rough tablet surface prone to detachment. Conversely, a concentration surpassing 5% poses the risk of punch sticking [48]. Consequently, a judicious parameter range of 2%-4% has been delineated.

The quantity of disintegrant holds sway over both taste and disintegration time at a predefined ratio. Figure 2(d) underscores this ascending-then-descending pattern, with the zenith achieved at 50%. Given the fixed acid-to-base ratio of 1.3:1 in disintegrants, the solution’s acidity proportionally escalates with an augmented disintegrant content. Notably, a disintegrant content less than 45% yields a bland disintegrated solution, while an excess of 55% elicits an irksome acidity. This typically necessitates the introduction of sugar to attain a balanced sour-sweet profile [49]. The amount of disintegrant also profoundly influences the disintegration time: a higher quantity of disintegrant correlates with progressively shorter disintegration times. Ultimately, this trend culminates in a disintegration time of less than 4 minutes with a disintegrant content exceeding 45%, which remains well within the acceptable range for commercially available edible effervescent tablets [50]. A comprehensive synthesis of sensory evaluation and disintegration time considerations yields a parameter range of 45%-55% for optimal response surface design.
Table 2: Weight distribution for sensory evaluation.

<table>
<thead>
<tr>
<th>Professional assessors</th>
<th>Degree of affiliation</th>
<th>Normalization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Average</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Figure 2: Optimal parameter ranges via single-factor experiments. Data are represented mean ± SD ($n = 10$). Bars labelled with distinct letters (A, B, C, D, and E) indicate statistical significance at $P < 0.05$. (a) Elderberry extract-to-ultra-micro vine tea powder ratio: influence on sensory scores. (b) Total stevioside content: impact on sensory scores. (c) Total PEG-6000 content: effect on sensory scores. (d) Total disintegrant content: relationship with sensory scores.
3.3. Response Surface Optimization and Analysis

3.3.1. Response Surface Design and Analysis of Variance. Guided by the results gained from the single-factor experiments, this study conducted the response surface design according to 2.2.5. The sensory scores \((Y)\) serve as the response values, and the factors and levels are shown in Table 3. Detailed results of these multifaceted experiments are presented in Tables 4 and 5.

Through regression fitting and variance analysis, a quadratic multinomial regression equation that describes the relationship between sensory scores \((Y)\) and the four independent variables is derived in the following equation:

\[
Y = 8.39 - 0.23A + 0.46B - 0.13C - 0.037D + 0.21AB - 0.02AC - 0.15AD + 0.022BC + 0.005BD + 0.06CD - 0.013A^2 - 0.25B^2 - 0.21C^2 - 0.65D^2.
\]

As shown in Table 5, the regression equation model boasts a \(F\) value of 144.98, accompanied by a low significance level \((P < 0.0001)\). This outcome substantiates the model's profound significance and its congruence with the established equation. In contrast, the lack of fit test reveals an \(F\) value of 0.55, coupled with a \(P\) value of 0.7861, surpassing the conventional significance threshold of 0.05. Thus, this particular term stands as inconsequential, signifying a marginal model error. The metrics of \(R^2 = 0.9941\) and \(Adj R^2 = 0.9873\) illustrate that this model elucidates 98.73% of the variance observed in sensory scores. The coefficient of variation (CV) is a mere 0.7%, undercutting the accepted 5% threshold, a testament to the model's robust adaptability.

The orchestration of influence among the quartet of factors shaping the overarching scores of the effervescent tablets emerges with utmost clarity from the discerning lens of the \(F\) and \(P\) values in the ANOVA. This hierarchical arrangement unfolds as follows: reigning supreme is the total stevioside content \((B)\), positioned above the ratio of elderberry extract-to-ultra-micro vine tea powder \((A)\), succeeded by the total PEG-6000 content \((C)\), and culminating with the total disintegrant content \((D)\). Systematically, the facets that wield a resounding impact, adorned with a statistical significance of \((P < 0.01)\), reveal themselves as the primary actors, namely, terms \(A, B,\) and \(C,\) alongside the secondary personas \(B^2, C^2, D^2,\) and the intriguing interactions of \(AB\) and \(AD\). Marching to a slightly muted tune but nevertheless embracing significance \((P < 0.05)\), the primary role is attributed to term \(D\). However, adopting a cloak of insignificance \((P > 0.05)\), the secondary term \(A^2,\) and the cross terms \(AC, BC, BD,\) and \(CD\) fade into the backdrop. A refined optimization model (Equation (10)) emerges.

\[
Y = 8.39 - 0.23A + 0.46B - 0.13C - 0.037D + 0.21AB - 0.15AD - 0.25B^2 - 0.21C^2 - 0.65D^2.
\]

3.3.2. Interactive Analysis. In order to gain insights into the combined effects of different factors on the sensory score, this study performed an interactive analysis. The response surface and contour maps are presented in Figure 3, offering a visual representation of their effects on the sensory score.

As illustrated in Figure 3(a), the 3D surface plot representing the interaction between the elderberry extract-to-ultra-micro vine tea powder ratio and total stevioside content exhibits a relatively steep slope, accompanied by a large ovality in the contour map. These characteristics indicate a significant impact of this interaction on the sensory score \((P < 0.05)\). According to the results of the single-factor experiment, as the elderberry extract-to-ultra-micro vine tea powder ratio decreased, the main constraint on sensory scores was the bitterness of the vine tea, but the refreshing sweetness of the stevioside can mask this drawback well. As mentioned in previous studies, dry stevia leaves have been used to sweeten traditional bitter drinks such as mate tea [51]. On the other hand, the berry sweetness of the elderberry itself and the herbal sweetness of the stevioside are somewhat incompatible when the above ratios are too high, even leading to slightly astringent aftertaste. Thus, within certain limits, the interaction of these two factors \((AB)\) should not be omitted.

Similarly, in Figure 3(b), as the elderberry extract-to-ultra-micro vine tea powder ratio and total disintegrant content increase, the sensory score initially rises and then declines. The evident curve radius of the response surface and the large ovality of the contour map further emphasize the considerable influence of this interaction \((AD)\) on the sensory score \((P < 0.05)\). It is supposed that at the lower the elderberry extract-to-ultra-micro vine tea powder ratio, citric acid can act as a bitter taste modifier similar to stevioside. This hypothesis is confirmed by Sotoyama et al., who found that citric acid can suppress the bitter taste of olopatadine hydrochloride orally disintegrating tablets [52]. Similarly, in higher ratio, a sour-sweet solution presents a balance of both sour and sweet flavours. Many drinks on the market such as fruit vinegars are made using this principle of neutralization [53].

Conversely, the interactions between the other four groups of factors \((AC, BC, BD,\) and \(CD)\) show relatively weaker effects on the sensory score, as evident from Figures 3(c)–3(f). These findings are consistent with the ANOVA results, reinforcing the notion that these interactions may have less pronounced impacts on the overall sensory evaluation. Of these, the amount of total PEG-6000 does not have a significant interaction with any of the other variables, which attributes to the fact that at this range of additions, it has little effect on mouthfeel and mainly affects the morphology of the tablets [54].

3.3.3. Validation of Optimum Conditions. Based on the interactive analysis, this study proceeds to validate the optimal conditions, which encompass an elderberry extract-to-ultra-micro vine tea powder ratio of 3.8: 1, coupled with precise content of stevioside, PEG-6000, and disintegrant at 3.41%, 2.73%, and 49.87%, respectively. This configuration
is projected to yield a sensory score of 8.62. However, considering practicality, a refined adjustment was made; the elderberry extract-to-ultra-micro vine tea powder ratio remains at 3.8:1, while the stevioside, PEG-6000, and disintegrant contents are adjusted to 3.4%, 2.7%, and 50%, respectively. In line with these parameters, a trio of parallel tests is executed, culminating in a sensory score of 8.57 ± 0.11. Impressively, the relative error value stands at a mere -0.58% in comparison to the prognosticated value. This notable convergence underscores the potential of these optimized conditions. In the forthcoming phase, comprehensive assessments encompassing both physical and chemical attributes will illuminate the potency and promise of this refined formulation.

3.4. Quality Inspection of Effervescent Tablets. Following the procedure outlined in Sections 2.2.6 and 2.2.7, a comprehensive evaluation of the physicochemical properties of the effervescent tablets is conducted. Notably, the mean disintegration time is determined to be 184.50 ± 4.65 s, accompanied by a foaming quantity of 30.64 ± 2.10 mg/g, and a weight variation of less than ±5%. These three essential
parameters demonstrate compliance with the specifications delineated in the 2020 edition of the Chinese Pharmacopoeia [30], indicating that the product has an appropriate disintegration rate, effervescent effect, and consistent tablet quality. The texture analyser data reveal that the tablets exhibit a hardness of 54.59 ± 3.41 N. This property is crucial as it ensures the tablets can withstand the various stages of production, packaging, and transportation without being damaged [55].

The colour properties provide an objective assessment of the product’s appearance. The $L^*$, $a^*$, and $b^*$ values indicate a relatively brighter appearance with a red shift on the red-green axis and a reduced yellow shift on the blue-yellow axis. C, $h$, and YI values suggest a softly saturated colour resembling natural berries, akin to jambolan effervescent tablets developed by Minh [56]. Notably, no signs of artificial colour additions are observed.

Regarding ingredient content, the moisture content is determined to be 3.52 ± 0.13 g/100 g. This value falls below the national standard limit of 5% [30]. The lower moisture content can prolong the shelf life and help mitigate the risk of microbial contamination. Moreover, these effervescent tablets exhibit a remarkable TFC of 9 g/tablet and TPC of 9.84 ± 4.95 mg GAE/g. Due to the abundance of flavonoids and polyphenols, along with their C6-C3-C6 basic structural units and numerous free phenol hydroxyl groups, the effervescent tablets are expected to exhibit a remarkable activity to scavenge free radicals by providing hydrogen protons [57]. The quantification of this activity will be discussed in the following section.

3.5. Detection of Antioxidant Activity. To assess the antioxidant capacity comprehensively, this study performs three established techniques: DPPH, hydroxyl, and ABTS radical scavenging activity experiments [58]. By comparing the antioxidant potential of solutions across diverse concentrations and ascertaining median effective concentration ($EC_{50}$) values, an insight into the product’s efficacy in combating oxidative stress can be gained. The radical scavenging rate curve is shown in Figure 4.

According to the $EC_{50}$ values, the inhibition rates of DPPH, hydroxyl, and ABTS radical are calculated as 0.4667 mg/mL ($R^2 = 0.9819$), 0.9772 mg/mL ($R^2 = 0.9932$), and 0.5014 mg/mL ($R^2 = 0.9856$), respectively. In comparison with other antioxidant solid beverages available in the market, this effervescent tablet demonstrates significantly lower $EC_{50}$ values than the Aronia melanocarpa effervescent tablet [59] and fermented Ginkgo biloba seeds solid beverage [60], while slightly exceeding the $EC_{50}$ of the Moringa oleifera leaf extract effervescent tablet [61]. It is noteworthy that the effervescent tablet’s raw material is not a direct anthocyanin extract from elderberries, but rather a concentrated powder designed to preserve taste. Despite this difference, the effervescent tablet still exhibits a well-balanced and satisfactory antioxidant activity, as indicated by its competitive $EC_{50}$ values.
Figure 3: Continued.
Figure 3: Response surface and contour maps for different interactive analyses. The curvature of the response surface plot and the elliptical shape of the contour plot reflect the magnitude of the impact of factor interactions on the sensory score. (a) Interaction between elderberry extract-to-ultra-micro vine tea powder ratio and total stevioside content. (b) Interaction between elderberry extract-to-ultra-micro vine tea powder ratio and total PEG-6000 content. (c) Interaction between elderberry extract-to-ultra-micro vine tea powder ratio and total disintegrant content. (d) Interaction between total stevioside content and total PEG-6000 content. (e) Interaction between total stevioside content and total disintegrant content. (f) Interaction between total PEG-6000 content and total disintegrant content.
4. Conclusion

In conclusion, this study has successfully developed an effervescent tablet with high levels of antioxidants. The optimized formulation comprises 50% total disintegrant with a balanced acid-to-base ratio of 1.3:1, a blend of elderberry extracts and ultra-micro vine tea powder constituting 40% at a ratio of 3.8:1, 3.4% stevioside, 2.7% PEG-6000, 2.9% mannitol, and 1% PVP. The resulting tablets exhibit an attractive external appearance, clear solution, delightful sweetness, captivating aroma, and commendable physicochemical attributes. Notably, the DPPH, hydroxyl, and ABTS radical scavenging activities are quantified with EC$_{50}$ values of 0.4667 mg/mL, 0.9772 mg/mL, and 0.5014 mg/mL, respectively, highlighting their robust antioxidant potential. This effervescent tablet not only offers a novel approach to harness the benefits of elderberry and vine tea but also presents an innovative concept for antioxidant-enriched functional foods. Future investigations could explore the aromatic characteristics of vine tea to further enhance the nutritional value of the product.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The author gratefully acknowledges the technical and financial assistance from the College of Food Science & Nutritional Engineering, China Agricultural University.

References


