The effect of vanillin and ginger powder addition on Chlorella-milk beverage

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Abstract. The study aimed to determine the effect of vanillin addition in the organoleptic acceptance of Chlorella-milk beverages. Besides, the protein and total phenolic content were measured in each formula. The sensory acceptance was determined with a 9-scale hedonic score. Just-about-right (JAR) test was done to determine the intensity of each formulation sensory attribute according to panellists’ preferences. The control sample was Chlorella milk which did not contain vanillin or ginger powder. Other samples were the Chlorella-milk beverages which added by 0.05% v/w vanillin powder (F1), 0.1% v/w vanillin powder (F2), 0.05% v/w ginger powder (F3), and 0.1% v/w ginger powder (F4). Sample F2, adding 0.1% v/w vanillin powder, had the best overall acceptance score of 7.10 ± 1.242. The control sample had to be improved by reducing the intensity of the Chlorella aroma. The addition of vanillin reduced respondents’ perception of the Chlorella aroma intensity. The total phenolic content was increased by adding vanillin and ginger powder. There was no significant difference in the protein content. In conclusion, adding vanillin powder to the Chlorella-milk beverages increased organoleptic acceptance. Adding vanillin and ginger powder did not affect protein content negatively but increased the total phenolic content of Chlorella-milk samples.

1 Introduction

Humans have used microalgae since the ancient Chinese civilization over 2000 years ago, but their application in commercial products only began in the last few decades [1]. From various variants of microalgae, Chlorella sp. is the most commonly cultured and used in different fields. Chlorella is usually used for high protein supplements for humans and livestock and various cosmetics and pharmacy products [2]. Around 60% dry weight of Chlorella sp. is the protein that consists of essential amino acids. Chlorella sp. also contains vitamins, minerals, and antioxidants, which provide health benefits [3].

It is well known that microalgae have a high nutritional value, but their application in the food industry is still minimal. The reason for the lack of microalgae application in the food industry is their sensory profile that is less liked for consumption: green herbs aroma, woody, fishy, grains-like, seaweed-like, green vegetable (spinach, cabbage), rancid, and fruity [4]. The unpreferable sensory profile of Chlorella needs to be improved to increase the acceptance of Chlorella products, for example, by using the flavor masking agent. Spices are usually selected for flavor masking agents because the undesirable profile of Chlorella is mostly related to the aroma. Besides, spices have a unique and strong aroma and taste while giving piquancy and color in some variants [4,5]. Spices were also chosen because they contain many bioactive compounds such as polyphenol, antioxidants, and essential oil, potentially enhancing the functional beverage's nutritional value [6].

Vanilla and ginger are the common spices used in food products. Vanilla is one of the most popular plants that are used as flavoring agents in various beverage products. Vanilla contains volatile compounds such as o-guaiacol, p-cresol, p-vinyl guaiacol, p-hydroxybenzaldehyde, vanillic acid, and vanillin, which contribute to the forming of the sweet vanilla aroma [7]. Ginger, while not having the same sweet aroma profile as vanilla, is also a potential candidate for a flavor masking agent. Ginger has a unique aroma and pungent flavor, which can be used for flavor masking, which is developed by the volatile compound in the rhizome, called oleoresin [8]. Besides, ginger is known for its healthy properties such as anti-inflammatory, antioxidant, hypertension reliever, and anti-carcinogenic [9].

In this research, we examine the effect of adding vanillin and ginger to the basic formulation of Chlorella-milk beverages. The addition of those spices is expected to mask the unpleasant flavor of Chlorella. Besides, we also measured the total phenolic compound and protein to test if the flavor masking may change the chemical composition.

2 Materials and methods
2.1 Chlorella-milk beverage formulation

There are two variables in the formulation which are two spice types and two concentration variants. The basic formulation that is used is 50 mL pasteurized milk, 1.25% w/v Chlorella biomass from “Earth Circle”, 5% w/v sugar, and drink water (used for diluting the dry ingredients before added to the milk). The other combinations are basic formulation added with 0.05% w/v vanillin powder, basic formulation added with 0.1% w/v vanillin powder, basic formulation added with 0.05% w/v ginger powder, and basic formulation added with 0.1% w/v ginger powder (Table 1).

Table 1. Chlorella beverage formulations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milk (mL)</th>
<th>Chlorella (%)</th>
<th>Sugar (%)</th>
<th>Water (%)</th>
<th>Vanilli n Powder (%)</th>
<th>Ginger Powder (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>1.25</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F1</td>
<td>50</td>
<td>1.25</td>
<td>5</td>
<td>10</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>50</td>
<td>1.25</td>
<td>5</td>
<td>10</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>50</td>
<td>1.25</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>F4</td>
<td>50</td>
<td>1.25</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

2.2 Hedonic and JAR test

Hedonic and JAR test was done simultaneously with the same set of 5 samples. For this research, 30 untrained panellists have voluntarily participated in the sensory test, which was done by the in-home test. Each panellist received a set of five samples consisting of 1 of each formulation, and each sample will be marked with a random 3-digit code. There were five questions on the hedonic test with nine levels of the hedonic scale from extremely dislike (1) to extremely like (9). While the next 5 question for JAR test had 5 level bipolar scale option (1 = too weak, 2 = a little too weak, 3 = just about right, 4 = a little too strong, 5 = too strong).

2.3 Total phenolic compound

Total phenolic compound analysis was done using the Folin-Ciocalteu method according to Ertan et al. [10], with Gallic acid as the standard solution. The working solution was prepared by diluting the Folin-Ciocalteu reagent with distilled water in a ratio of 1:10. Sample or standard (1 mL) was mixed with 5 mL Folin-Ciocalteu working solution and incubated for 3 minutes in obscurity. The mixture was then mixed with 4 mL Na2CO3 (75 g/L), incubated in the dark for 2 hours at room temperature, and centrifuged at 4,200 rpm for 10 minutes. The supernatant was measured spectrophotometrically at 760 nm against distilled water. Results were expressed as Gallic Acid Equivalents (GAE) per liter samples. The analysis was done in three replications for each sample.

2.4 Total protein

Protein content analysis was done using Kjeldahl method with modification according to Mega [11]. Standardization of HCl was done with Na2B4O7 as the primary standard solution. Sample (1 mL) was pipetted to a Kjeldahl flask and added with 10 g K2SO4, 0.1 g CuSO4.5H2O, and 15 mL H2SO4. The Kjeldahl flask was shaken until evenly mixed and heated for around 45 minutes until the sample color became light green. Samples were allowed to cool at room temperature, followed by the addition of 100 mL of distilled water and 1 g of Zn to the sample. The sample was then transferred to a digestion tube and placed in a protein distiller. During the distillation process, 30 mL H3BO3; 70 mL NaOH and 50 mL H2O were added to the same digestion tube. An Erlenmeyer flask was placed to collect the distillate. The distillate was added with ten drops of Toshiro indicators and titrated with standardized HCl until the sample reached the equivalence point. The analysis was done in three replications for each sample. The titration volume was recorded to calculate the protein with the formula:

\[
\text{Protein content (\%) = \frac{\text{Volume of titration} \times \text{Normality of HCl} \times 14.007 \times 6.38 \times 100\%}{\text{Weight of sample}}} \]

2.5 Statistical analysis

The statistical differences between means of samples for organoleptic acceptance, total phenolic content, and protein content, were evaluated using one-way ANOVA on IBM SPSS. The analysis was then followed by a Tukey post-hoc test with a significant level (α) of 0.05. Pearson’s correlation analysis is used to determine the correlation of the acceptance level of each attribute: color, aroma, taste, texture, with the overall acceptance level.
2.6 Penalty analysis

JAR test results are the intensity of sensory attributes: green color, Chlorella aroma, sweetness, milk flavor, and smoothness. Penalty Analysis was conducted to determine which attribute that need to be improved in each formulation. Penalty analysis was done manually, which consisted of 3 main steps. The first step was calculating the respondent percentage for each category (too little, JAR, and too much). The second step was calculating the mean drop, which can be done by subtracting the acceptance level average of too little/too many groups from the acceptance level average of the JAR group. The last step was to make a plot with the x-axis representing respondent percentage and the y-axis representing a mean drop.

3 Results and discussion

3.1 The organoleptic acceptance

The result of the hedonic test of the acceptance level of all formulations is shown in Table 2. The result showed that the F2 sample had the highest acceptance score in the attributes of color, aroma, taste, and overall, with the average score of 7.03 ± 1.40, 7.13 ± 1.43, 6.96 ± 1.30, and 7.1 ± 1.24, respectively. Sample F1 had the most liked texture with an acceptance score of 7.26 ± 1.14. The result suggested the increment in the acceptance score in the Chlorella-milk beverages with the vanillin compared to the control sample. Samples obtained the highest acceptance score with the addition of 0.1% vanillin. The addition of higher ginger powder at 0.1% resulted in significantly decreasing acceptance of Chlorella-milk beverages.

Table 2. Acceptance level average for each formulation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.70 ± 1.51a</td>
<td>5.47 ± 1.72a</td>
<td>6.27 ± 1.96ab</td>
<td>6.57 ± 1.72ab</td>
<td>6.50 ± 1.72ab</td>
</tr>
<tr>
<td>F1</td>
<td>6.57 ± 1.59a</td>
<td>6.33 ± 1.52ab</td>
<td>6.77 ± 1.41b</td>
<td>7.27 ± 1.14b</td>
<td>7.03 ± 1.07b</td>
</tr>
<tr>
<td>F2</td>
<td>7.03 ± 1.40a</td>
<td>7.13 ± 1.43b</td>
<td>6.97 ± 1.30b</td>
<td>7.07 ± 1.36b</td>
<td>7.10 ± 1.24b</td>
</tr>
<tr>
<td>F3</td>
<td>6.80 ± 1.56a</td>
<td>5.77 ± 2.08a</td>
<td>6.03 ± 1.81ab</td>
<td>6.47 ± 1.55ab</td>
<td>6.40 ± 1.40ab</td>
</tr>
<tr>
<td>F4</td>
<td>6.27 ± 1.62b</td>
<td>5.37 ± 2.03a</td>
<td>5.47 ± 2.39a</td>
<td>5.73 ± 1.96a</td>
<td>5.60 ± 2.06a</td>
</tr>
</tbody>
</table>

The data were presented as mean ± standard deviation. Different superscript letter on mean values of samples indicates a significant difference between pairs of samples (p < 0.05) according to Tukey.

All attributes' acceptance levels positively correlated with the overall acceptance level, although some have a different magnitude of influence. For example, the taste attribute had the highest correlation coefficient with the overall acceptance with the r-value = 0.78. Therefore, the higher the taste acceptance level, the higher the overall acceptance and vice versa. Other than taste acceptance level, aroma and texture acceptance level also has a very strong correlation, while color has a weaker correlation even though it is still classified as a strong correlation.

3.2 Just-About-Right (JAR) and penalty analysis of Chlorella-milk beverages

JAR test is commonly used for developing new products, especially in the formulation, so the connection between the intensity of specific sensory attributes with the acceptance level can be observed, which will help improve consumer acceptability [12]. Xiong and Meullenet [13] stated that an optimal sensory attribute should have at least 70% of respondents answer just about right so that the majority can justify the opinion of the attribute in a perfect intensity. Mean drop represents the potential improvement that can be achieved if the attribute intensity is adjusted to JAR, where a negative value means that adjustment to JAR will decrease the acceptance level while a positive value means that adjustment will increase the acceptance level [12].

In this study, the JAR test was performed to determine the adequacy of the intensity of the sensory attributes: intensity of green color, Chlorella aroma, sweetness, milk flavor, and smoothness. The data obtained from the JAR test was the intensity categories which are too weak, a little too weak, just about right, a little too strong, and too strong. The distribution of the response can be seen in Figure 1. The result showed that sweetness intensity was optimal in control and sample F2. For the green color, the intensity was optimal in samples F2 and F3. While the sample F1 had an optimal level of smoothness.

The result, shown in Figure 1, showed that adding vanillin and ginger powder changed the intensity of the Chlorella aroma. In the control sample, 36.67% of respondents thought that the Chlorella aroma was too strong. In the sample F1, with the addition of 0.5% vanillin, 66% of respondents categorized the Chlorella aroma in JAR, and only 23.33% of respondents categorized the Chlorella aroma as too much. While, in
the F1 sample, 53.33% of respondents categorized the Chlorella aroma in JAR, and 30% of respondents categorized the Chlorella aroma as too low. The most remarkable showed in sample F4, in which 53.33% of respondents scored the intensity of Chlorella aroma too much. Ginger has a pungent aroma due to the gingerol. Therefore, the remarkable score in the Chlorella aroma in sample F4 may be because the respondent is misled between the pungent aroma and Chlorella aroma.

**Figure 1.** The percentage of Just-About-Right scale group responses in three levels (n = 30). A) Control; B) F1 - Chlorella milk beverages with 0.5% Vanillin; C) F2 - Chlorella milk beverages with 1% Vanillin; D) F3 - Chlorella milk beverages with 0.5% ginger and E) F4 - Chlorella milk beverages with 1% ginger.

The penalty analysis was conducted to understand the effect of each attribute’s intensity on the acceptability of the respondent. The evaluation of non-JAR that less than 20% of the respondent is considered insignificant. Therefore, the attribute with a percentage of respondents lower than 20% does not need to be improved even though the mean drop is high [14]. Respondent percentage and mean drop can be plotted so the identification of attributes that need to be improved can be made easily. The x-axis represents the respondent percentage, while the y-axis represents the mean drop.

Figure 2A shows the penalty analysis plot of control which suggests the necessary improvement of the basic formula of Chlorella beverage before adding a flavor masking agent. The penalty analysis plot of control suggested that the green color, Chlorella aroma, and milk flavor need to be improved. All three attributes were considered too much, which means the intensity of those attributes should be reduced to improve the acceptance level. Among the attributes that should be improved, Chlorella aroma has the highest mean drop and respondent percentage, which justifies that flavor masking is needed to reduce the Chlorella aroma. By improving them, the acceptance level will increase by 1.604.

In the F1 and F2 samples (Figure 2B and 2C), there was no attribute to be improved. Even though, there were 30% of respondents categorized the milk flavor as too much, the mean drop suggested that improvement will not affect the acceptance score. Besides, in sample F2, 30% of respondents thought that the Chlorella aroma was too low. However, this will not affect organoleptic acceptance. This result indicated that respondents justify that Chlorella gives an unpleasant aroma to the milk beverages aroma.
The result of sample F4 (Figure 2E) indicated that a higher percentage of ginger powder in the beverage formula reduces the intensity of sweetness. Besides, 53.33% of respondents categorized the Chlorella aroma as too much, which may be due to the ginger pungent aroma that misleads the consumer's perception. This result indicated that ginger could mask the Chlorella aroma. However, the higher percentage of ginger powder may result in a pungent aroma which is unpleasant to the respondent. Therefore, reducing the ginger percentage in the formulation will increase the acceptance level by 2.64 for sweetness and 1.69 for Chlorella aroma.

Figure 2. The penalty analysis plot for A) Control; B) F1 - Chlorella milk beverages with 0.5% Vanillin; C) F2 - Chlorella milk beverages with 1% Vanillin; D) F3 - Chlorella milk beverages with 0.5% ginger and E) F4 - Chlorella milk beverages with 1% ginger. TL = too low; TM = too much.

The use of vanillin and ginger powder aid on masking the unpleasant aroma that increase consumer acceptance on Chlorella-milk beverages. Vanillin powder could mask the Chlorella aroma due to the ethyl vanillin compounds contained in the powder. Ethyl vanillin is a flavouring agent that has the same aroma and flavor profile as vanilla. The chemical structure of ethyl vanillin is similar with vanillin, making ethyl vanillin often used as replacement of vanilla [15]. Ethyl vanillin has the ability to mask undesirable odor. Chlorella is known to have earthy aroma that could decrease organoleptic acceptance in food products [16]. Ginger is well known for its unique odor and flavour characteristics. The pungent flavor in ginger is caused by gingerols and shogaol [17]. A study by Vedashree et al [18] found that incorporation of dried ginger extract able to make food products such as ice cream had the desirable sensory attributes of ginger flavor. However, the amount of ginger incorporated to food products need to be considered as an increase of ginger added might decrease the aroma acceptance. The increase of ginger added to sweet food products that contain milk might reduce the milky and sweet aroma [18].

3.3 Effect of vanillin and ginger to total phenolic and protein content

The results of the total phenolic analysis are shown in Table 3. The result suggested that the total phenolic content significantly increased in samples with vanilla and ginger addition. In samples F1, F2, F3, and F4, an
increase in phenolic content is possibly due to phenolic compounds such as flavonoids, organic acids, and amino acids contained in vanilla and ginger. *Chlorella* is known to have phenolic compounds such as organic acids and flavonoids. *Chlorella pyrenoidosa* contain flavonoids such as caffeine, protocatechuic acid, catechin, epicatechin, epigallocatechin-gallate, caffeyl-glucose, and dihydroquercetin-7,4'-dimethyl-ether [19]. Vanillin powder contains ethyl vanillin that has a phenolic hydroxyl group on its structure and is known to be the major phenolic constituent in vanilla products [20, 21]. Ginger is rich in phenolic content such as pyrogallol p-hydroxybenzoic acid, furelic acid, p-coumaric acid, gallic acid, and caffeic acid. Ginger also contains vanillin, which is also a phenolic compound [22].

### Table 3. Total phenolic content of *Chlorella* beverage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolic Content (mg GAE/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>266.16±2.63a</td>
</tr>
<tr>
<td>F1</td>
<td>304.50±1.34b</td>
</tr>
<tr>
<td>F2</td>
<td>324.84±5.64d</td>
</tr>
<tr>
<td>F3</td>
<td>313.17±0.37m</td>
</tr>
<tr>
<td>F4</td>
<td>316.60±4.87d</td>
</tr>
</tbody>
</table>

The data were presented as mean ± standard deviation. Different superscript letter on mean values of samples indicates a significant difference between pairs of samples (p < 0.05) according to Tukey.

The results of protein content analysis are shown in Table 4, suggesting that adding vanilla and ginger did not significantly affect the protein content of *Chlorella* beverages. In this research, all beverages contained the same amount of *Chlorella*. In addition, the result indicated that vanillin and ginger did not adversely affect the protein content in products. *Chlorella* has been known to have high protein content, about 55 – 60%, that is considered safe to be consumed by humans. The protein of *Chlorella* has become a potential source of high-quality protein due to the presence of essential and non-essential amino acids in *Chlorella* itself. Around 35% of amino acids in *Chlorella* consist of lysine, leucine, isoleucine, tyrosine, tryptophan, and valine [23], [24], [25], [26].

### Table 4. Protein content of *Chlorella* beverage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.00±0.14a</td>
</tr>
<tr>
<td>F1</td>
<td>2.07±0.19b</td>
</tr>
<tr>
<td>F2</td>
<td>2.17±0.22a</td>
</tr>
<tr>
<td>F3</td>
<td>2.10±0.04a</td>
</tr>
<tr>
<td>F4</td>
<td>2.19±0.04a</td>
</tr>
</tbody>
</table>

The data were presented as mean ± standard deviation. Different superscript letter on mean values of samples indicates a significant difference between pairs of samples (p < 0.05) according to Tukey.

### 4 Conclusion

Basic formulation or control sample of *Chlorella*-milk beverage needs to be improved according to the penalty analysis plot for control which showed that *Chlorella* aroma is too strong. According to hedonic test result, sample F2 which is the basic formulation with addition of 0.1% v/w vanillin has the highest organoleptic acceptance. Addition of vanillin and ginger powder increase total phenolic content of samples due to the presence of phenolic compounds in vanillin and ginger and did not give negative effect on protein content of *Chlorella*-milk beverages.

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### References