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# The Development and Consumer Acceptance Analysis of *Chlorella vulgaris* Infused Strawberry Honey Jam

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## ABSTRACT

*Chlorella vulgaris* is green microalgae belonging to the phylum Chlorophyta. This microalga is known for its high nutrient content, such as protein, chlorophyll, vitamins, and minerals. Through various research, *Chlorella* contributed to the enhancement of human health, including becoming an immune booster by increasing the activity of the immune system. In addition, it can act as a functional food that will provide nutritional support for humans. Due to that, *Chlorella* has been used for various applications in the industry like pharmaceuticals, cosmetics, and food products. However, the microalgae traits of having seafood taste and smell are prominent, resulting in unpalatable food in society. Therefore, this study aims to optimise the *C. vulgaris*, strawberry jam, and honey concoction in developing a new Chlorella-based confectionery food by examining appearance, texture, taste, and aroma parameters. Box-Behnken Design (BBD) is used to create an experimental design matrix of 15 samples with three factors: *Chlorella*, honey, and strawberry jam, at three distinct levels. An average score based on the 9-point Hedonic scale for each parameter was obtained from a survey participated by 62 participants and was then investigated using response surface methodology (RSM). The results from RSM analysis showed that weak interactions are reflected in combinations of *Chlorella* and honey, *Chlorella* and strawberry, and honey and strawberry. The desirability of jam from the optimisation plot indicated that the optimum levels of the three factors were 1.5 ml *C. vulgaris*, 3 g honey, and 16.5 g strawberry jam. This study provides an overview of the opportunity to develop new products and improvements to increase consumer acceptability by optimising physical parameters to develop nutritional food.

**Keywords:** *Parameter, Response Surface, Interaction, Optimisation*

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## INTRODUCTION

Microalgae are microscopic algae that reside in saline or freshwater environments. The group of microalgae contains diverse photosynthetic, prokaryotic, and eukaryotic microorganisms. Microalgae have simple cellular structures and generally possess a high growth rate [1]. Microalgae are a great source of nutritional value as they are well known for their chemical composition. Some of the valuable molecules from microalgae are polyunsaturated fatty acids, pigments and antioxidants [2, 3].

*Chlorella* is a eukaryotic green microalga capable of producing energy within several hours. It has a high photosynthesis potential and needs only a trivial amount of nutrients, carbon dioxide, oxygen, and sunlight [4]. The unicellular green microalgae, *Chlorella vulgaris*, belongs to the domain Eukaryota, kingdom Protista, division of Chlorophyta and genus *Chlorella* following its scientific classification. *Chlorella* derives from the Greek word 'chloros', meaning green, and 'ella' refers to microscopic size in Latin [5]. By having similar metabolic pathways and life cycles to the higher plant, these microalgae can proliferate quickly and have been used as an example of organisms for photosynthesis and carbon dioxide assimilation research.

Microalgae play a significant role as a source of natural products and bioactive compounds, replacing synthetic products. The genus *Chlorella* is sometimes used in the cosmetics and pharmaceutical industries and as a food substitute because it includes protein, carotenoids, polysaccharides, vitamins, and minerals. In particular, *C. vulgaris* is known for ascorbic acid, vitamins, tocopherol, minerals, polysaccharides [6], proteins, carotenoids, chlorophyll a and b, and pheophytin a [7]. The high productivity of *C. vulgaris* resulted in it being one of the most common microalgae biomass used for various biotechnological applications [6].

In *C. vulgaris*, high amounts of essential nutrients allow it to provide multiple nutrients required by the human body. *Chlorella* is regarded as a functional food in Japan because it consists of all the vital nutrients needed and can perform many therapeutic activities in the human body. It is possible as *Chlorella* communicates with the human body system by promoting the body’s ability to balance, heal, and be revitalised. *Chlorella* can supply essential nutritional components that are lacking in the modern diet. Besides, vitamins in *C. vulgaris* are bio-chelated; they are easier to absorb and assimilate due to their incorporation into amino acids [8, 9].

Moreover, *Chlorella* has been developed as a healthy food product in advanced countries like Germany, China, and Japan. *Chlorella* has been proposed as human food for its high protein contents and richness of vitamins, minerals and carotenoids [1]. Over the past few years, researchers have shown interest in their high-value chemical compounds in various microalgae applications in different fields of industry, such as cosmetics, pharmaceuticals and food [10].

The objectives of this study were to optimise the *C. vulgaris* powder, strawberry jam, and honey ratio via Box-Behnken design and conduct a Consumer Acceptance Analysis of the *Chlorella* Strawberry Honey Jam comprising of Appearance, Taste/Flavour, Aroma/Smell and Texture to 62 randomly selected participants. Additionally, this study may be beneficial as a reference for developing Chlorella-based confectionery products in the future.

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## EXPERIMENTAL

### *Cultivation and Production of Chlorella vulgaris Powder*

*C. vulgaris* was grown in a photobioreactor and supplied with the Chlorella Optimize Medium (COM-MRL) formulation developed by Microalgae Research Laboratory (MRL) UiTM and was subjected to optimal MRL *C. vulgaris* culture conditions. *C. vulgaris* powder was produced based on the MRL Optimal Chlorella Powder production method.

### *Preparation of Chlorella vulgaris Infused Strawberry Honey Jam*

Three factors were tested, consisting of *C. vulgaris* powder, strawberry jam, and honey ratio. A homogenous solution was created by mixing 5 g of *Chlorella* powder with 10 ml of water. *Chlorella* solution was supplied at 3 different levels i.e., 0.5 ml (-1), 1 ml (0) and 1.5 ml (+1). Strawberry jam was added to the ingredients at three levels consisting of 25 g (-1), 50 g (0) and 100 g (+1). Honey as an extra sweetener was added at 3 g (-1), 5 g (0) and 10 g (+1). Fifteen (15) new ingredients were formed with three centre points (all ingredients were supplied at the middle point or (0) level) and were developed using Box-Behnken Design (Design of Experiment (DoE) methodology) as shown in Table 1.

**Table 1:** Box-Behnken experimental design

| Factors | Box-Behnken Codes | Value | Unit |
|---|---|---|---|
| | -1 | 0.5 | |
| *Chlorella* | 0 | 1.0 | ml/L |
| | +1 | 1.5 | |
| | -1 | 3.0 | |
| Honey | 0 | 5.0 | g/kg |
| | +1 | 10.0 | |
| | -1 | 25.0 | |
| Strawberry Jam | 0 | 50.0 | g/kg |
| | +1 | 100.0 | |

### *Consumer Acceptance Analysis*

For the sensory quality evaluation of the newly formulated jam, 62 people were selected. The selection was made based on a random selection to avoid bias. The sensory attributes, namely appearance, taste/flavour, aroma/smell, and texture parameters, were graded based on the 9-point Hedonic scale. Each participant was

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given 15 jam samples in 30 ml plastic containers and a survey link (https://forms.gle/bo5paz3kS4iaiPaCA) via Google form to obtain feedback on the newly developed jam samples.

### *Statistical Analysis*

Box-Behnken Experimental Design was used to obtain average scores of 15 jam samples in each parameter consisting of appearance, taste/flavour, aroma/smell, and texture. In addition, Response Surface Analysis (Main effects, interaction effects and response surface plots) was used to analyse the results to determine the *C. vulgaris* ratio that can be accepted by the participants using software called Minitab. A response optimisation (predicted responses, desirability, and optimisation plot) was used to construct the optimal formulation of *C. vulgaris* infused strawberry honey jam. Analysis of Variance (ANOVA) on the response surface regressions was carried out and considered statistically significant when $P < 0.05$.

## RESULTS AND DISCUSSION

### *Box-Behnken Design of Response Surface Methodology (RSM)*

The experimental design was precisely designed according to the Box-Behnken Design (BBD). The three factors comprising *Chlorella*, honey and strawberry jam were assigned to Box-Behnken codes (-1, 0, +1) and given their values and units. Fifteen combinations of experiments for the comprehensive three factors and three levels were constructed. The responses calculated were average scores from 62 participants for the appearance, texture, taste and aroma parameters as presented in Table 2.

### *Response Surface Analysis of Appearance*

*C. vulgaris* is green due to its various chlorophyll pigments [11]. The presence of anthocyanin in a strawberry gives it a striking red colour [12]. Meanwhile, the colour of honey is related to temperature, manufacturing and storage process, and the primary factor is the floral source [13].

The combination of *Chlorella*, honey and strawberry in different ratios displayed different colours of jam samples, as indicated in Figure 1. Due to that, participants had contrasting opinions on their preferences in the colour of jam given in the consumer acceptance survey. Figure 2 represents the response surface plot on the average participants' scores for the appearance of the jam samples at three distinct levels of the combination among the factors. The factors in the jam sample comprise *Chlorella*, honey, and strawberry jam.

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**Table 2:** Box-Behnken experimental results

| Sample no. | Factors | | | Responses Average | | | |
|---|---|---|---|---|---|---|---|
| | ***Chlorella*** | **Honey** | **Strawberry Jam** | **Appearance** | **Texture** | **Taste** | **Aroma** |
| 1 | 1.5 ml | 10 g | 50 g | 6.44 | 6.74 | 6.53 | 7.03 |
| 2 | 1 ml | 3 g | 25 g | 5.90 | 6.05 | 6.10 | 6.13 |
| 3 | 0.5 ml | 5 g | 100 g | 7.48 | 7.19 | 6.98 | 7.10 |
| 4 | 1.5 ml | 5 g | 25 g | 5.02 | 5.05 | 5.26 | 5.31 |
| 5 | 1 ml | 10 g | 100 g | 6.84 | 6.60 | 6.85 | 6.66 |
| 6 | 0.5 ml | 10 g | 50 g | 6.98 | 6.66 | 6.71 | 7.08 |
| 7 | 1.5 ml | 5 g | 100 g | 6.53 | 6.53 | 6.55 | 6.81 |
| 8 | 0.5 ml | 5 g | 25 g | 6.65 | 6.61 | 6.60 | 6.69 |
| 9 | 1 ml | 5 g | 50 g | 6.60 | 6.66 | 7.13 | 6.97 |
| 10 | 1 ml | 5 g | 50 g | 6.58 | 6.60 | 6.68 | 6.58 |
| 11 | 1.5 ml | 3 g | 50 g | 5.87 | 6.15 | 6.27 | 6.23 |
| 12 | 0.5 ml | 3 g | 50 g | 7.44 | 7.02 | 7.08 | 7.23 |
| 13 | 1 ml | 5 g | 50 g | 6.48 | 6.35 | 6.58 | 6.68 |
| 14 | 1 ml | 3 g | 100 g | 7.15 | 6.89 | 7.16 | 6.97 |
| 15 | 1 ml | 10 g | 25 g | 5.16 | 5.53 | 5.26 | 5.63 |

The comparative response surface plot between *Chlorella* and honey in terms of appearance indicates that the participants favour the colour of the jam when *Chlorella* and honey are supplied least at -1. ANOVA on the response surface regressions for the interaction between *Chlorella* and honey was not significant as the value of $P > 0.05$. The appearance comparison of the response surface plot between *Chlorella* and strawberry jam yielded the highest point when *Chlorella* was at the lowest amount at -1 (0.5 ml), while the strawberry jam was highest at +1 (100 g).

However, the response surface regression for the *Chlorella* and strawberry jam interaction with $P > 0.05$ was insignificant. Besides, the preferred appearance among participants was when the strawberry jam was at the highest amount at +1 (100 g) and honey at the lowest, -1 (3 g) from the response surface plot between honey and strawberry jam. The interaction between strawberry jam and honey in response surface regression by ANOVA obtained a $P > 0.05$, suggesting insignificant.

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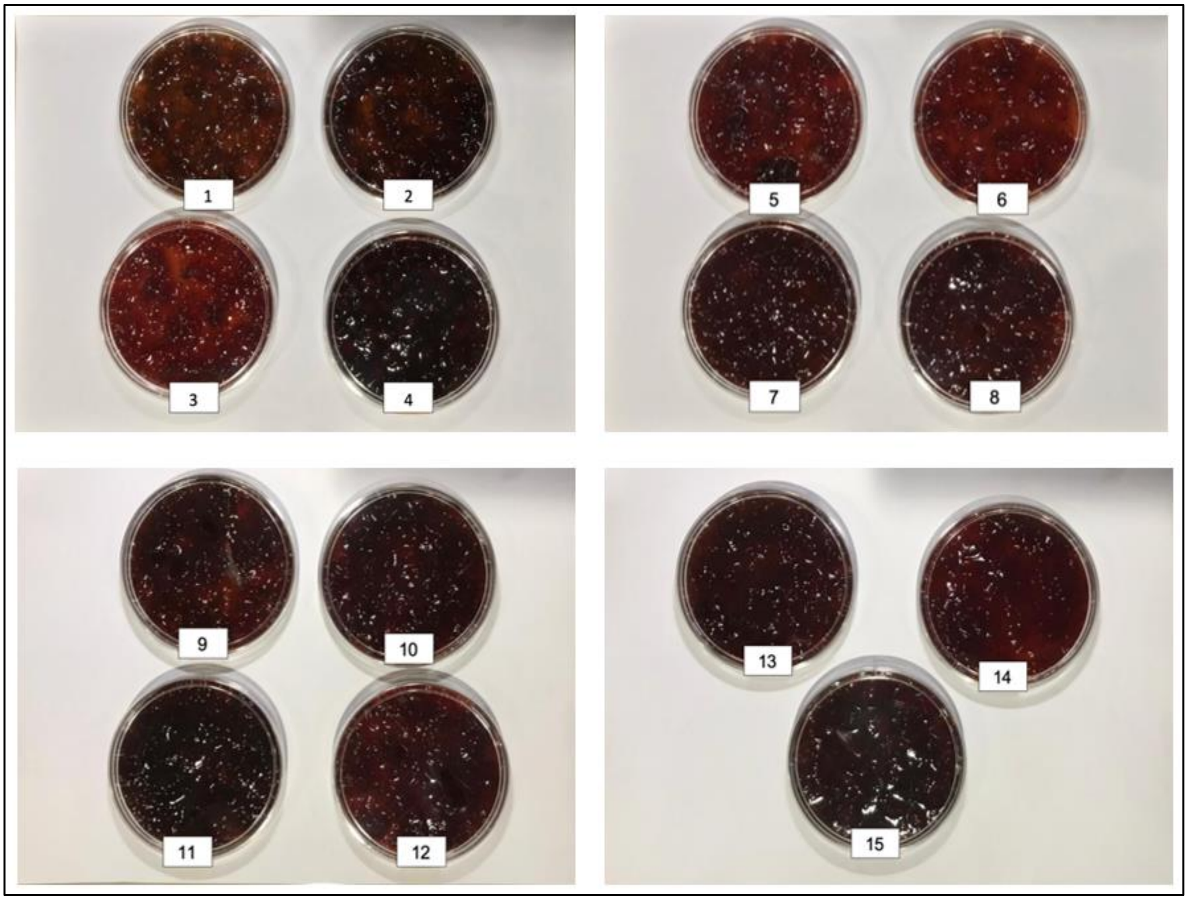


**Figure 1:** The colour and appearance morphologies of the 15 formulations of the *Chlorella*-Honey Infused Strawberry jam based on the Box-Behnken design. The number indicates the experimental formulation highlighted in Table 2

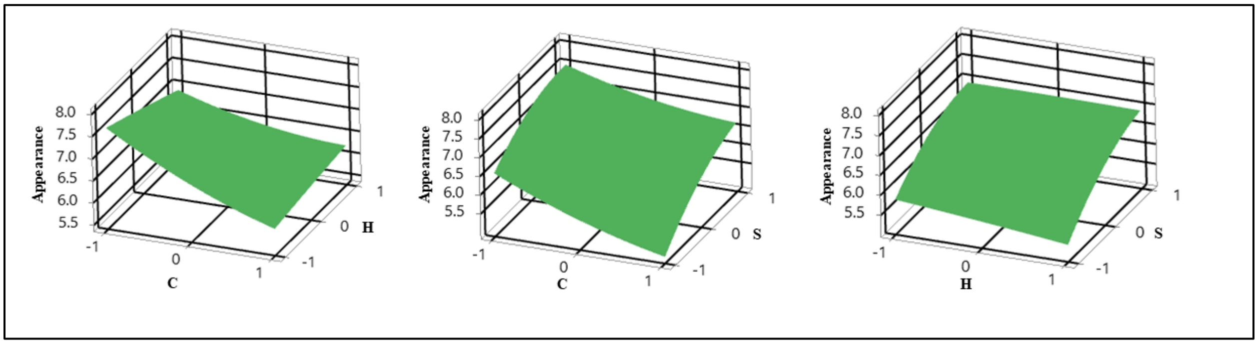


**Figure 2:** Response Surface Plots of Appearance. The axes denoted with C, H, and S are *Chlorella*, Honey, and Strawberry Jam, respectively

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The interactions between all factors in response surface regression were conducted using ANOVA obtained $P > 0.05$. It was defined as insignificant due to an insufficient number of respondents in the user acceptance test. Nevertheless, from the data analyses, fortification of the green biomass colour of *Chlorella vulgaris* and the light-yellow colour of honey in a jam was preferred at the lowest amount. On the other hand, the amount of strawberry jam was selected as the highest amount as more participants preferred the strawberry jam's bright red colour. The data in this research is correlated to previous research that observed strawberry jam colour positively influences the overall liking of the strawberry jam post specific storage duration [14].

### *Response Surface Analysis of Texture*

Strawberry jam is generally a mixture of strawberry fruit, sugar and pectin, thus producing a thick and soft texture [15]. While honey appeared in sticky liquid form [16], it contained several sugar components [17]. With 15 different sets of combinations of the *Chlorella*, strawberry jam and honey, the texture of jam samples ranges from a viscous jam to a watery jam. The texture of *Chlorella*, honey, and strawberry jam at three levels is evaluated among the participants via survey and then analysed using a response surface plot analysis as shown in Figure 3.

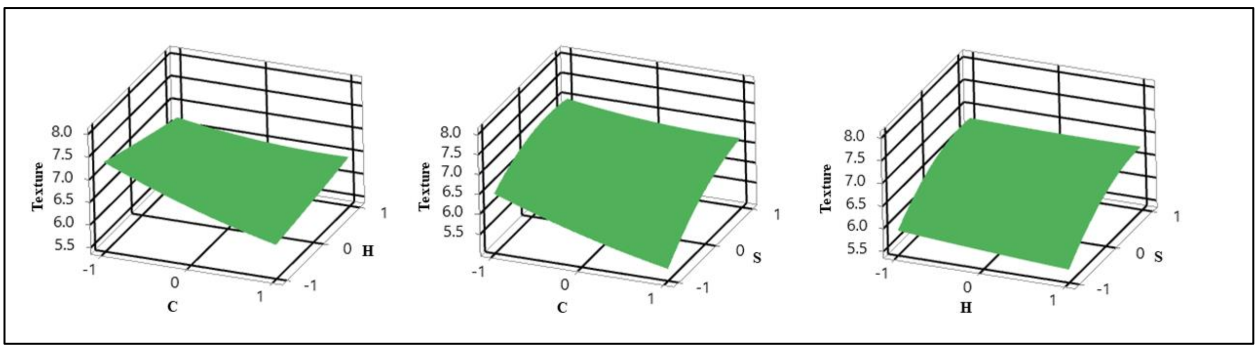


**Figure 3:** Response Surface Plots of Texture. The axes denoted with C, H, and S are *Chlorella, Honey* and *Strawberry Jam*, respectively

When both *Chlorella* and honey were administered at the lowest value of -1, the comparative response surface plot relating to the texture of the jam samples obtained the highest score among participants. The interaction between both factors using Analysis of Variance (ANOVA) shows a $P > 0.05$, indicating insignificant. The texture's comparative response surface plot between *Chlorella* and strawberry jam yielded the highest score among participants when *Chlorella* was supplied at the lowest value at -1 (0.5 ml) and strawberry jam at the highest value at +1 (100 g). However, with a $P > 0.05$, the response surface regression for *Chlorella* and strawberry jam interaction was insignificant. According to the response surface plot between honey and strawberry jam, participants preferred the texture of jam when honey was provided at the lowest level at -1 (3 g) while the strawberry jam was at the highest level at +1 (100 g). In response to

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surface regression, the interaction between honey and strawberry jam had a value of *P* > 0.05, implying that it was not significant.

The response surface regression for the interaction between all factors using ANOVA had a *P* > 0.05. It was deemed insignificant due to the lack of participants' participating in the survey. Based on the analysis obtained, people preferred *Chlorella* to be added at the lowest amount. Moreover, adding honey to the jam at the lowest amount was preferable—meanwhile, more participants adored the soft and thick texture of the strawberry jam at the highest amount.

### *Response Surface Analysis of Taste*

*C. vulgaris* has seafood and a slightly bitter taste because it is commonly grown in water habitats [18]. Thereby, a combination of sweet and sour strawberry [19] and the sweet flavour of honey [16] in a jam can reduce the unpleasant taste of *Chlorella*. The taste of each jam sample varies depending on the ratio of the combination of *Chlorella*, honey and strawberry jam. A response surface plot is used to analyse participants' average scores regarding the taste of the combination between *Chlorella*, honey and strawberry jam, as presented in Figure 4.

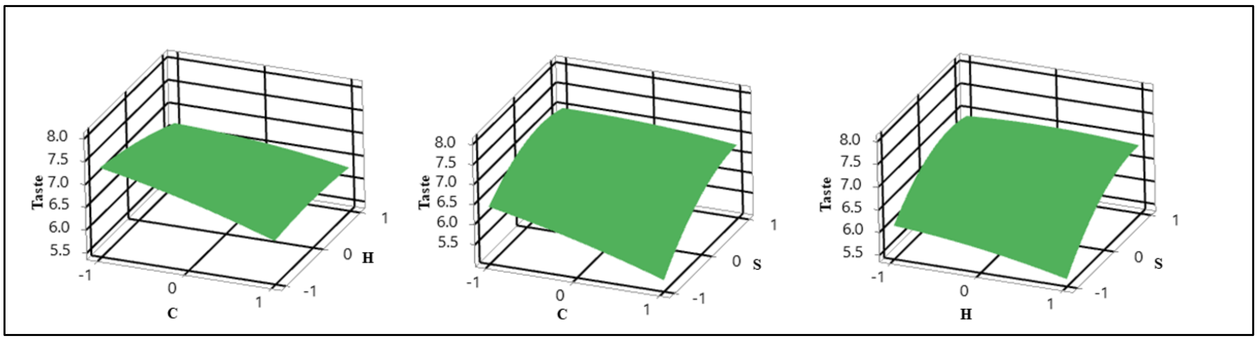


**Figure 4:** Response Surface Plots of Taste. The axes denoted with C, H, and S are *Chlorella*, Honey, and Strawberry Jam, respectively

The comparative response surface plot on the taste of the jam samples received the most significant score if both *Chlorella* and honey were supplied at the lowest value (-1). The interaction between the two factors using Analysis of Variance (ANOVA) has a *P* > 0.05, signifying that it was not a significant result. As *Chlorella* was provided at the lowest value at -1 (0.5 ml) and strawberry jam at the highest value at +1 (100 g), the taste's comparative response surface plot produced the highest score among participants. However, the response surface regression for the *Chlorella* and strawberry jam interaction was insignificant with a *P* > 0.05. Based on the response surface plot between honey and strawberry jam, the participants preferred the taste of the jam when honey was supplied at the lowest level at -1 (3 g), and strawberry jam was provided at the highest level at +1 (100 g). The interaction between honey and strawberry jam obtained a value of *P* > 0.05, indicating that it was not significant.

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In the ANOVA analysis, the interaction of all factors gives a value of $P > 0.05$. The result was considered insignificant as there was a lack of participants testing the jam. Nevertheless, the participants favoured the addition of *Chlorella* and honey at the lowest level due to its strong taste that might affect the original taste of jam. On the other hand, the strawberry jam was required at the highest value among the participants as they preferred the sweet-sour taste of strawberries.

### *Response Surface Analysis of Aroma*

*Chlorella* has a strong smell with a green appearance, similar to seafood, affecting consumers' acceptability [20]. Meanwhile, the volatile organic compounds (VOC) in a strawberry produce a unique sweet aroma of the strawberry fruit that is pleasant among people [12]. Honey's aroma is sweet, fresh and fruity [13].

Both strawberry and honey have a favourable sweet aroma and can conceal the smell of *Chlorella* when it is added. A total of 15 samples were produced with the combination of *Chlorella*, honey and strawberry at three different levels ranging from lowest, moderate and highest to test the aroma of jam samples that were acceptable to the participants. The aroma of the combination of *Chlorella*, honey and strawberry jam was evaluated using a response surface plot as presented in Figure 5.

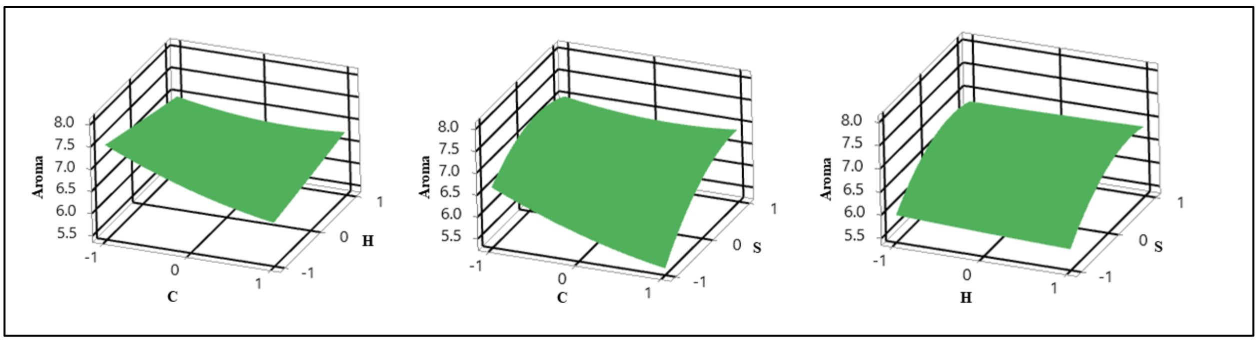


**Figure 5:** Response Surface Plots of Aroma. The axes denoted with C, H, and S are *Chlorella*, Honey, and Strawberry Jam, respectively

According to the response surface plot between *Chlorella* and honey in terms of aroma, participants liked the colour of the jam when *Chlorella* and honey were provided at the lowest level (-1). The interaction between *Chlorella* and honey was not significant in response surface regression by ANOVA, with $P > 0.05$. Regarding the aroma for response surface plot between *Chlorella* and strawberry jam, *Chlorella*'s highest point was obtained at its lowest level at -1 (0.5 ml). The strawberry jam was at its highest level at +1 (100 g). However, with a $P > 0.05$, the interaction between *Chlorella* and strawberry jam was insignificant. Participants preferred the aroma when the strawberry jam was at the highest level at +1 (100 g) and honey at the lowest level at -1 (3 g) on the response surface plot between strawberry jam and honey. In response-surface regression by ANOVA, the interaction between honey and strawberry jam had a $P > 0.05$, indicating insignificant.

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All interactions of factors in response surface regression were analysed using ANOVA, which yielded a $P > 0.05$. It was deemed insignificant because there was an inadequate number of participants in the survey. However, based on the aroma data analysis, the seafood smell of *Chlorella* was preferred at the lowest level among the participants. The participants also chose honey as the lowest amount to be added to the jam. The strawberry jam yielded the highest level as the fruity smell of strawberry is preferable among participants.

### *Response Optimisation*

Response optimisation analysis was conducted to observe the optimal level of the parameter appearance, taste/flavour, aroma/smell, and texture based on the requirement to maximise consumer responses. Moreover, the analysis of response optimisation calculated the individual desirability for each parameter. These values were combined to give the value of composite desirability, which is the predicted acceptance among the participants. As shown in Figure 6, the predicted responses with desirability were identified for the four parameters on the optimum level.

The optimisation plot maximises the number of scores for each parameter when all the factors are at optimum levels with a high predicted response [21, 22]. The optimisation plot represented the effects of each parameter (columns) on the three factors in the jam formulation (rows). Based on the graph, when the ratio of *Chlorella,* honey and strawberry jam was optimal, the expected maximum response for appearance is 479.6 with desirability of 68%. The texture obtained an estimated maximum response of 456.7 and desirability of 59%. The value of taste estimated maximum responses is 454.3, and the desirability is 55%.

At the same time, the aroma has predicted maximum responses of 464.5 with 59% desirability. Based on the response optimisation analysis, the optimum levels with the maximum response obtained were *Chlorella* at -1, honey at -1 and strawberry jam at 0.333. The optimisation plot was used to create a new composition of the jam formulation to appeal to the participants. Thus, with a consumer acceptance of 60.2%, the participants will favour the new formulation of *C. vulgaris* infused strawberry honey jam when the three factors of the jam are supplied at 0.5 ml *C. vulgaris*, 3 g honey, and 16.5 g strawberry jam.

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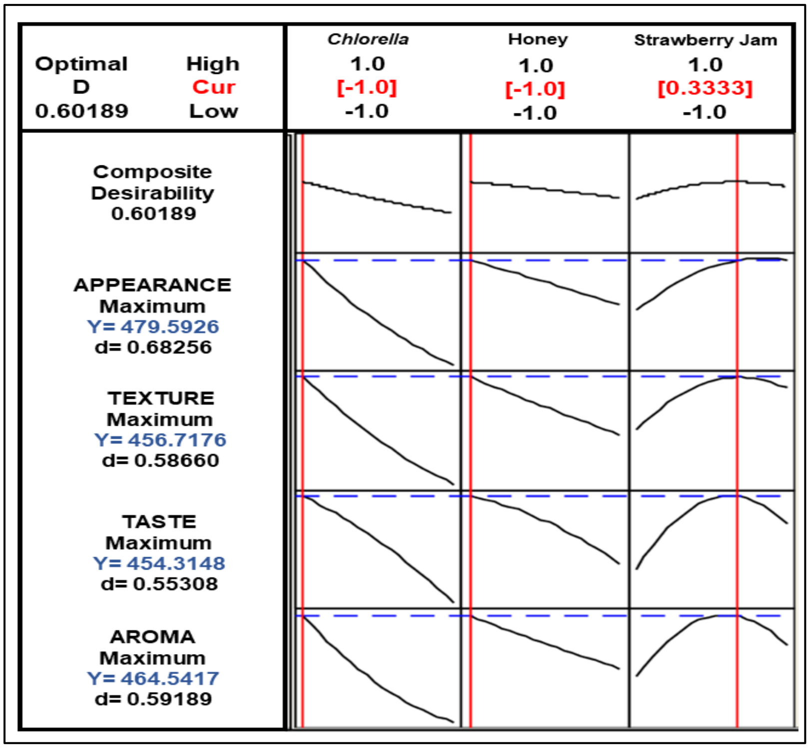


**Figure 6:** Response Optimisation Plot analysis of the optimal formulation of *Chlorella*, honey and strawberry jam against the appearance, texture, taste and aroma of the newly formulated Chlorella – Honey Strawberry jam

## CONCLUSION

This study evaluated the properties, and overall acceptance of *C. vulgaris* infused strawberry honey jam related to appearance, taste/flavour, aroma/smell, and texture. As the assigned values of *Chlorella*, strawberry and honey formulations were not significant, a response optimisation was conducted to determine the optimal formulation. From the response optimisation analysis, the optimum values for the jam formulation are 0.5 ml *Chlorella*, 3 g honey and 16.5 g strawberry jam. The participants preferred the addition of *C. vulgaris* in a jam at the lowest amount. Due to the insufficient number of participants, the analysis indicates that the interactions between the factors are weak. More participants will be required for future studies to obtain accurate optimal formulation before the new *Chlorella*-Honey Strawberry jam can be commercialised in the near future.

Therefore, increasing the number of participants in the acceptance survey of the jam will produce a significant finding. Significant data can be obtained by creating a new formulation of *C. vulgaris* infused strawberry honey jam to segregate the levels of each factor. Moreover, further research needs to be conducted for this product development. In addition to its nutrients, *C. vulgaris* may give health advantages

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to humans. Therefore, by optimising the consumers' acceptability of *C. vulgaris*, it can be concluded that this study provides insight into developing commercial *Chlorella*-based food products in the future.

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## AUTHOR'S CONTRIBUTION

Nur Aisyah carried out the research, analysed the data, wrote and revised the article. Khairul Adzfa designed the research, analysed the data, supervised research progress, reviewed the article, and approved the submission. Muhamad Helmi, Sitti Rahma and Mohd Shafiq reviewed the article.

## CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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