

## THE DEVELOPMENT AND USER ACCEPTANCE SURVEY OF ASTAXANTHIN INFUSED TOPICAL MOISTURIZER



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### ABSTRACT

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Astaxanthin was a beneficial carotenoid in the nutraceutical, food, cosmetics, and feed industries. The new astaxanthin infused topical moisturizer was developed using astaxanthin, Manuka honey, and Neutrogena Hydro Boost gel moisturizer. The astaxanthin was extracted from *Haematococcus pluvialis* subjected to the in-house astaxanthin extraction procedure developed by the Microalgae Research Laboratory, UiTM Puncak Alam, Selangor. Fifteen topical moisturizer samples were developed via Box-Behnken Design. The moisturizer properties were significantly influenced by astaxanthin, Neutrogena Hydro Boost gel moisturizer, and Manuka honey content based on the User Acceptance Survey done on 250 respondents. This study observed response surface regression, analysis of variance, and response optimization for appearance, aroma/smell, and texture. The optimum levels of the three factors were astaxanthin is at 1 ml, Neutrogena Hydro Boost gel moisturizer is at 20 ml, and Manuka honey must be less than 10 g which is at 0.9192g, as 69% of respondents like the composition.

**Contribution/ Originality:** The current work contributes to a new formulation of topical moisturizers comprising astaxanthin, Manuka honey and Neutrogena Hydro Boost gel moisturizer. These three compounds were extensively reported in the previous literature regarding their benefits. Yet, a minimal study was done to optimize the combined compounds and the research participants' acceptance of the combined compounds.

### 1. BACKGROUND

Microalgae are microscopic organisms representing various species present in the aquatic and terrestrial ecosystem. Microalgae's unique ability as a source of many biochemical compounds allow them to be utilized for industrial and environmental applications [1]. Microalgae have a wide range of biotechnological uses due to their high nutritional values and are widely used in nutraceuticals, pharmaceuticals, and industrial applications [2]. Since decades ago, microalgae have been used to produce dietary supplements, cosmetics products, biofertilizers, and food additives. *Haematococcus pluvialis* is a unicellular alga that produces a high amount of astaxanthin, giving many benefits to human health due to its antioxidant effect against diseases such as diabetes, cancers, cardiovascular disease, inflammation, and ulcer [3]. Among the primary sources of astaxanthin, such as shrimp and *Phaffia rhodozyma* yeast, microalgae *Haematococcus pluvialis* is one of the most appealing sources as it can produce up to 2%–3% dry weight [4]. Astaxanthin has many potential uses in human health and nutrition [5]. Astaxanthin, with the

molecular formula of 3,3'-dihydroxy-4,4'-diketo- $\beta$ - $\beta$  carotene, are carotenoid compounds that exhibit high instability and poor water solubility, which can restrain their usage in pharmaceutical products [6]. Various stress situations, such as high temperature, high light, high salinity, and nutritional deficiencies of nitrogen and iron, will enhance the high level of astaxanthin [7]. Astaxanthin shows anticancer, antiaging, and antioxidant abilities in cosmetics industries.

According to Tominaga et al. [8] long-term preventive astaxanthin intake may prevent age-related skin degradation and preserve skin conditions associated with environmental exposure due to its anti-inflammatory properties. However, due to the insufficient data on the incorporation of astaxanthin in skincare applications, it could be noted that the cosmetics and skincare application of astaxanthin is still limited. Thus, this study was conducted to develop a new astaxanthin topical moisturizer by using the astaxanthin extracted from *H. pluvialis* using in-house Malaysian virgin coconut oil. Coconut oil is effective in retaining 90% of astaxanthin form when heated at 90°C for 8 hours [9]. This study aimed to optimize the ratio of astaxanthin (extracted in Malaysian virgin coconut oil (MVCO)), Manuka honey and Neutrogena gel moisturizer via Box-Behnken design and conducting a User Acceptance Test of the Astaxanthin topical moisturizer comprising of Appearance, Aroma/Smell and Texture to 250 research participants. Consequently, this study may be beneficial in developing astaxanthin-based skincare products in the future.

## 2. DATA AND METHODS

### 2.1. Extraction of Astaxanthin in Malaysian Virgin Coconut oil from *H. Pluvialis*

The astaxanthin bearing *H. pluvialis* was obtained from Microalgae Research Laboratory (MRL), Faculty of Applied Sciences in UiTM Puncak Alam, Selangor. The *H. pluvialis* was subjected to MRL's in-house MVCO astaxanthin extraction. Astaxanthin paste in Malaysian virgin coconut oil was stored at 20°C until further use.

### 2.2. Preparation of Astaxanthin Topical Moisturizer in Neutrogena Gel Moisturizer

Three parameters were tested: astaxanthin in Malaysian virgin coconut oil, Neutrogena Hydro Boost gel moisturizer, and Manuka honey ratio. Neutrogena Hydro Boost gel moisturizer was supplied at 5 ml (-1), 10 ml (0), and 20 ml (+1). Astaxanthin in Malaysian virgin coconut oil was supplied and added to the Neutrogena Hydro Boost gel moisturizer at three different levels, which are at 0.25 ml (-1), 0.5 ml (0), and 1 ml (+1). Manuka honey was added to the moisturizer at 1 g (-1), 5 g (0), and 10 g (+1). The design of experiment method (DOE) used was Box-Behnken Design. The design of the experiment generated 15 formulations with one (1) centre point (all ingredients were supplied at the middle level (0 levels)). All 15 samples were distributed to the 250 respondents for the User Acceptance Survey. The Box-Behnken Design (Design of Experiment (DoE) methodology) is shown in Table 1.

### 2.3. User Acceptance Survey

Two hundred fifty respondents of various ages were selected to answer the User Acceptance Survey for the sensory quality assessment of the newly developed astaxanthin topical moisturiser. The attributes, namely 1) Appearance, 2) Aroma/Smell, and 3) Texture parameters, were graded based on the 9-point Hedonic scale. For many years, the nine-point hedonic scale has been widely used in rating scales to gather data on food or other samples' acceptance and serve as a reference point for results comparison. The stimuli (actual samples or food names) are shown singly and assessed on a scale ranging from "dislike extremely" to "like extremely" in the hedonic scale approach.

This study provided 15 moisturizer samples with different ingredients to the survey participants in 20 mL cups, including soap and tissues.

Table 1. Box-Behnken experimental design.

Factors	Box-Behnken Codes	Value	Unit
Astaxanthin	-1	2.50	ml/L
	0	5.00	
	+1	10.00	
Honey	-1	10.00	g/kg
	0	50.00	
	+1	100.00	
Neutrogena	-1	50.00	g/kg
	0	100.00	
	+1	200.00	

### 2.4. Statistical Analysis

Box-Behnken Experimental Design was used in this research. The Box-Behnken design is a response surface methodology (RSM) with only three levels. It is a unique three-level design because it does not have any points at the experiment region's vertices. The results may be significant if the points on the cube's corners represent level combinations prohibitively expensive to test or are impossible to test due to physical process constraints. The data was analyzed using surface plots to establish the astaxanthin ratio that the respondents will accept in the newly designed moisturizer formulation.

## 3. RESULTS AND DISCUSSIONS

### 3.1. Box-Behnken Design of RSM

According to the Box-Behnken Design (BBD), the experimental design was precisely designed. The three factors comprising astaxanthin, honey and Neutrogena Hydro Boost gel moisturizer were assigned to Box-Behnken codes (-1, 0, +1) and given their values and units. Fifteen (15) combinations of experiments for the comprehensive three factors and three levels were constructed. The responses calculated were average scores from 250 participants for the appearance, texture and aroma/smell parameters presented in Table 2.

Table 2. Box-Behnken experimental results.

Sample No.	Factors			Responses Average		
	Astaxanthin	Honey	Neutrogena	Appearance	Texture	Aroma
1	1.00 ml	10.00 g	10.00 g	6.67	6.00	5.80
2	0.50 ml	1.00 g	5.00 g	4.60	4.33	3.60
3	0.25 ml	5.00 g	20.00 g	3.73	5.80	4.47
4	1.00 ml	5.00 g	5.00 g	5.80	6.53	5.23
5	0.50 ml	10.00 g	20.00 g	4.93	6.53	5.93
6	0.25 ml	10.00 g	10.00 g	3.00	3.80	4.20
7	1.00 ml	5.00 g	20.00 g	8.00	10.27	11.13
8	0.25 ml	5.00 g	5.00 g	3.00	3.07	2.27
9	0.50 ml	5.00 g	10.00 g	4.47	5.20	4.27
10	0.50 ml	5.00 g	10.00 g	4.33	4.93	4.60
11	1.00 ml	1.00 g	10.00 g	6.53	5.60	5.80
12	0.25 ml	1.00 g	10.00 g	3.73	5.13	4.60
13	0.50 ml	5.00 g	10.00 g	4.13	4.80	5.07
14	0.50 ml	1.00 g	20.00 g	4.60	5.80	5.80
15	0.50 ml	10.00 g	5.00 g	4.20	4.13	4.13

### 3.2. Response Surface Analysis of Appearance

Response surface plot is the three-dimensional (3-D) response surface and contour plots. It visualised the relationship between response and experimental values for each variable parameter and the type of interaction between the test variables [10]. Astaxanthin caused the pink or red colour in seafood such as salmon, trout, lobster, and shrimp. The combination of astaxanthin, honey and Neutrogena Hydro Boost gel moisturizer in different ratios

displayed different colours of the astaxanthin moisturizer samples. The 250 participants had different preferences in the colour of astaxanthin moisturizer given based on the User Acceptance Survey. Figure 1 represents the response surface plot on the average participants' scores for the appearance of the astaxanthin moisturizer samples at three distinct levels of the combination among all the factors. The factors in the astaxanthin moisturizer sample comprise astaxanthin, honey, and Neutrogena Hydro Boost gel moisturizer.

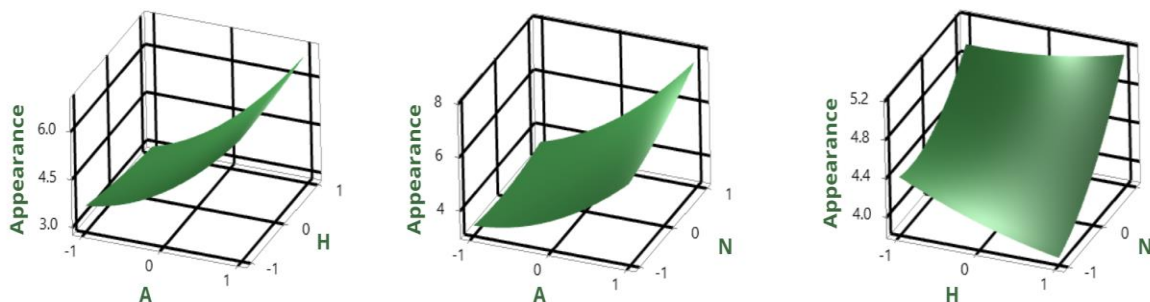


Figure 1. Response surface plots of appearance.

Note: The axes are denoted with A, N and H, which stands for Astaxanthin, Neutrogena and Honey.

The surface plot of astaxanthin and Manuka honey shows that the respondents like the appearance when the astaxanthin and Manuka honey are at level 1, astaxanthin at 1 ml and Manuka honey at 10 g. The surface plot for astaxanthin and Neutrogena Hydro Boost gel moisturizer also shows that the respondents favour them at level 1, with astaxanthin at 1 ml and Neutrogena Hydro Boost gel moisturizer at 20 ml. For the surface plot of Manuka honey and Neutrogena Hydro Boost gel moisturizer, the most favourable appearance is when both are at level 1, where Manuka honey is at 10 g, and Neutrogena gel moisturizer is at 20 ml. Analysis of variance for appearance is being tested at a 95% confidence limit. The interactions between all factors in response surface regression were conducted using ANOVA obtained  $P > 0.05$ .

### 3.3. Response Surface Analysis of Texture

The texture of a product is essential because different skin types absorb thicker creams and thinner liquids differently. The effects and experience of using a product may differ. Based on the survey, the highest total score was when astaxanthin was at level 1, Manuka honey at level 0, and Neutrogena Hydro Boost gel moisturizer at level 1. The lowest total score was when the astaxanthin was at level -1, honey at level 0, and Neutrogena Hydro Boost gel moisturizer at level -1. The surface plot of astaxanthin and Manuka honey demonstrates that the optimal value of astaxanthin and Manuka honey is level 1. The astaxanthin quantity is 1 ml, and the Manuka honey quantity is 10 g. The surface plots for astaxanthin and Neutrogena Hydro Boost gel moisturizer indicate that respondents preferred 1 ml for astaxanthin and 20 ml for Neutrogena Hydro Boost gel moisturizer at level 1. The surface plot of Manuka honey and Neutrogena Hydro Boost gel moisturizer demonstrates that the most favourable ingredients occur when Manuka honey is at level 1 and Neutrogena Hydro Boost gel moisturizer is at level 1 as well. The Manuka honey is 10 g, and the Neutrogena Hydro Boost gel moisturizer is 20 ml. The response surface plots of texture are shown in Figure 2.

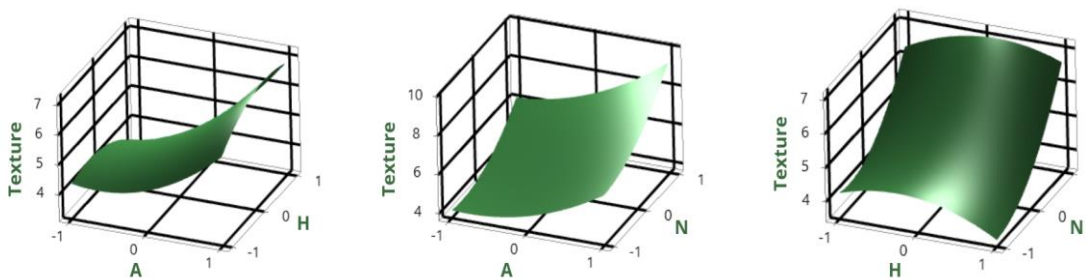


Figure 2. Response surface plots of texture.

Note: The axes are denoted with A, N and H, which stands for astaxanthin, Neutrogena and honey.

The p-value for texture from response surface regression indicates that astaxanthin and Neutrogena Hydro Boost gel moisturizer are significant. The ANOVA for texture yields a p-value of 0.853 for Manuka honey linear regression, indicating insignificant response surface regression because it is greater than 0.05. Astaxanthin and Neutrogena Hydro Boost gel moisturizer have p-values of 0.018 and 0.029, respectively. Both astaxanthin and Neutrogena Hydro Boost gel moisturizer had a P-value less than 0.05, which is significant. For square regression, the p-values for astaxanthin with astaxanthin, Manuka honey with Manuka honey, and Neutrogena Hydro Boost gel moisturizer with Neutrogena Hydro Boost gel moisturizer are all non-significant, namely 0.237, 0.334, and 0.285. The p-values for astaxanthin combined with Manuka honey, astaxanthin combined with Neutrogena Hydro Boost gel moisturizer, and Manuka honey combined with Neutrogena Hydro Boost gel moisturizer is 0.462, 0.665, and 0.513, respectively, and are not significant. The R-square value is 84.37%, and the data is plotted commonly. The hold values, 0, indicate the starting point of each ingredient.

### 3.4. Response Surface Analysis of Aroma/Smell

One reason for preferring one moisturizer over another is the aroma/smell. The highest total score for aroma/smell was 167 when the astaxanthin is at level 1, Manuka honey at level 0 and Neutrogena Hydro Boost gel moisturizer at level 1. In contrast, the lowest total score was 34 when the astaxanthin was at level -1, Manuka honey at level 0 and Neutrogena Hydro Boost gel moisturizer at level -1. The response surface plots of aroma are illustrated in Figure 3.

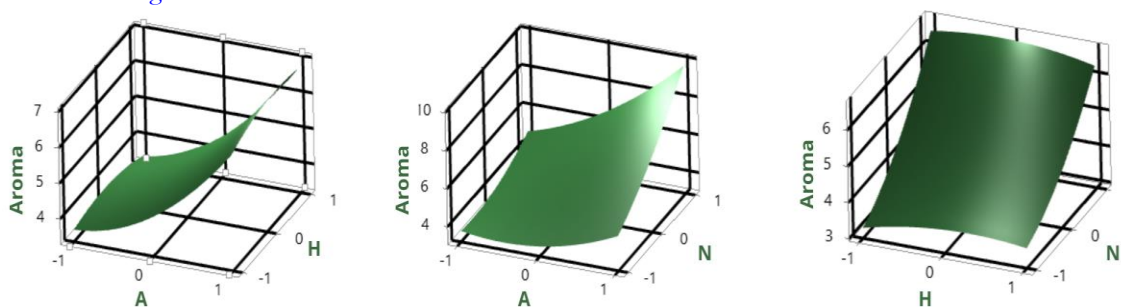


Figure 3. Response surface plots of aroma.

Note: The axes are denoted with A, N and H, which stands for Astaxanthin, Neutrogena and honey.

The surface plot of astaxanthin and Manuka honey shows favourable when the astaxanthin and Manuka honey are at level 1. The amount of astaxanthin is 1 ml, and Manuka honey at 10 g. The surface plot for astaxanthin and Neutrogena Hydro Boost gel moisturizer shows that the respondents like them at level 1, astaxanthin at 1 ml, and Neutrogena Hydro Boost gel moisturizer at 20 ml. The surface plot of honey and Neutrogena Hydro Boost gel moisturizer shows the most favourable appearance is when Manuka honey at level -1 and Neutrogena Hydro Boost gel moisturizer at level 1. The honey is at 1 g and Neutrogena Hydro Boost gel moisturizer at 20 ml. The p-value of response surface regression for aroma shows that astaxanthin and Neutrogena Hydro Boost gel moisturizer are significant. The analysis of variance for aroma resulting from the p-value for linear regression of Manuka honey is 0.945, and it is not significant because it is larger than 0.05. The p-value of astaxanthin and Neutrogena Hydro Boost gel moisturizer is 0.019 and 0.022, respectively. The P-value for astaxanthin and Neutrogena Hydro Boost gel moisturizer is significant as the value is less than 0.05. For square regression, the p-value of astaxanthin with astaxanthin, Manuka honey with Manuka honey, and Neutrogena gel moisturizer with Neutrogena Hydro Boost gel moisturizer are not significant is 0.356, 0.747, and 0.532, respectively. For interaction regression, the p-value of astaxanthin with Manuka honey, astaxanthin with Neutrogena Hydro Boost gel moisturizer, and Manuka honey with Neutrogena Hydro Boost gel moisturizer are 0.884, 0.217, and 0.884, respectively, and is not significant. The R-sq is 83.86% which means the data is in a normal plot. The hold values of 0 indicate the starting point of each ingredient.

3.5. Response Optimization

Response optimization determined the best formulation of the ingredients of astaxanthin topical moisturizer. The starting point of astaxanthin, Manuka honey, and Neutrogena Hydro Boost gel moisturizer are at level -1 as it is the lowest point. The local solution shows that the best formulation of the astaxanthin topical moisturizer is when astaxanthin and Neutrogena Hydro Boost gel moisturizer are at level 1 and Manuka honey at below level 1 (0.919192). The weight of Manuka honey must be lesser than 10 g. The predicted response optimization plots are shown in Figure 4.

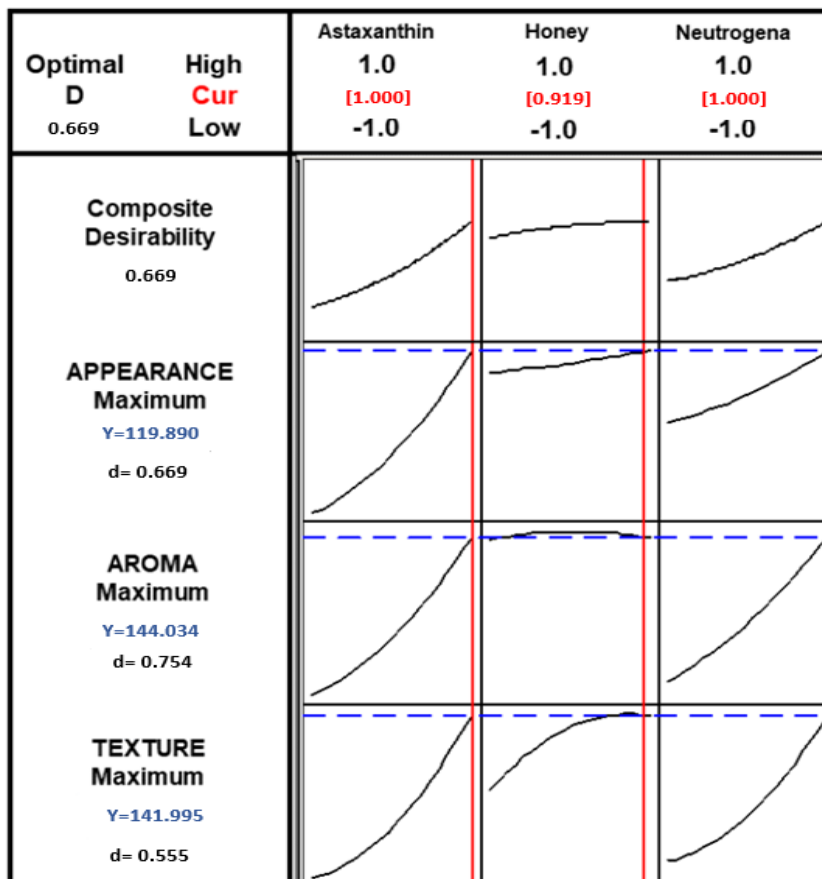


Figure 4. Response optimization plot.

The individual desirability of appearance, aroma/smell, and texture for local solution are 0.554746, 0.753682, and 0.716377, respectively, while the composite desirability is 69%. The 69% indicates that only 69% of the respondents like the astaxanthin topical moisturizer when the Neutrogena Hydro Boost gel moisturizer is at 20 ml, astaxanthin at 1 ml, and Manuka honey at 0.9192 g. This research's low percentage of composite desirability will be a baseline for future research regarding incorporating astaxanthin in a topical moisturizer. The global solution's desirability of appearance, aroma/smell, and texture is 0.554746, 0.753682, and 0.716377, respectively. The composite desirability for the global solution is 0.669075. Individual and composite desirability measures how well a combination of variables meets the responses' objectives. Individual desirability (d) assesses how settings optimize a single response; composite desirability (D) assesses how settings collectively optimize a collection of responses. Desirability has a value between 0 and 1. The value 1.0 represents the ideal situation, while 0 indicates that one or more responses are out of bounds. The desirability value indicates that there are not enough data obtained. More respondents are needed to participate in the testing of astaxanthin topical moisturizer. As shown in Figure 4, the predicted responses with desirability were identified for the four parameters on the optimum level.



#### 4. CONCLUSION

In this study, the best formulation was found when astaxanthin and Neutrogena Hydro Boost gel moisturizer were at level 1 and Manuka honey at 0.919192, while astaxanthin was at 1.0. Neutrogena Hydro Boost gel moisturizer is at 20 ml, and Manuka honey must be less than 10 g which is at 0.9192 g. The result obtained showed that the composite desirability is 0.6911, 69%. The desirability of less than 80% indicated insufficient data, mainly due to the COVID-19 pandemic. An improvement should be made by increasing the number of respondents, and their demographic should be expanded. Respondents should be of various ages, races, ethnicity, and gender. It can increase the accuracy of the data obtained. Next, the number of samples and the ratio of the three ingredients, astaxanthin, Manuka honey, and Neutrogena Hydro Boost gel moisturizer, should be increased. For example, the volume of the ingredients should be supplied in larger volumes, such as from 20 ml to 200 ml of Neutrogena Hydro Boost gel moisturizer.

#### Abbreviations

MVCO: Malaysian virgin coconut oil.

MRL: Microalgae Research Laboratory.

DOE: Design of the experiment method.

RSM: Response surface methodology.

BBD: Box-Behnken Design.

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