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A NEWLY DEVELOPED KELULUT HONEY DRINK ENRICHED WITH SEAWEED BY USING DESIGN OF EXPERIMENT APPROACH

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ABSTRACT

A novel functional drink, *Kelulut* honey enriched with seaweed (designated as *K*hS), was successfully developed by using design of experiment (DOE) approach in this study. A two-level-factor Full Factorial Design (FFD), 2^2, was employed to screen the interactions among the two major composition (*Kelulut* honey and seaweed) as well as to analyse the effect of these ingredients towards the sensory and proximate properties of the newly developed product. The design of experiment (DOE) and data analysis were executed by using Design- Expert Software. Two main approaches of hedonic tests, i.e., the measurement of preference and the measurement of acceptance were applied during sensory evaluation. Results indicated that amount of seaweed and honey used during the preparation of *K*hS did affect the moisture content and pH of the end product significantly. Sensory evaluation revealed that the overall acceptability score of the newly developed *Kelulut* honey enriched with seaweed drink was prefered moderately by most of the panelists and thus it could be a favourable product to be commercialised in the market.

Keywords: Design of Experiement; Full Factorial Design; *Kelulut* honey; Proximate; Seaweed; Sensory

INTRODUCTION

In the recent years, modern consumers have become more health conscious and investigators seeking natural nutritrion alternatives for use in foods are increasing tremendously. Consumers believe more that food work directly to their health (Mollet & Rowland, 2002). Today, the role of foods are not only to satisfy hunger and to supply necessary nutrients for human but also to overcome nutrition related diseases and to improve physical and mental well being of the consumers (Menrad, 2003; Roberfroid, 2000). In this regard, functional food can be consumed for health promotion and disease prevention. A functional food is a typical food that has specific nutrients added to it, like vitamins or minerals, fiber, or probiotics or prebiotics. Example of functional product is functional drink (Siro et al. 2008).

In the present study, a functional drink was produced with stingless bee honey and red seaweed as the main ingredients. Stingless bee honey is a natural sweet nutritious food that contains abundant source of antioxidant and phenolic content (Sousa et al. 2016; Tuksitha et al. 2018). In Malaysia, stingless bee honey is also known as *Kelulut* honey. *Kelulut* honey is well known as natural dietary antioxidant and contains up to 10 times higher antioxidant than ordinary honey and contain lower fermentable sugar (Chua et al. 2013). An extensive study on honey by

Vol. 16. No.3 (1-10), ISSN: 1823-884x

Moniruzzaman et al. (2017) reported that the phenolic compounds present in honey possess several medicinal benefits such as antioxidant, antibacterial, and antimicrobial properties.

At the same time, global demand for seaweed foods or known as algae foods is expanding due to its functional and nutraceutical benefits (Wells et al. 2017). The widespread interest in seaweed foods due to their potential nutritional and bioactive compounds is evident in numerous recent reviews (Fleurence & Levine 2016; Tiwari & Troy, 2015). Seaweeds are photosynthetic organisms which constitute a total of 25 to 30 species, with a great diversity of forms, sizes and colours. As seaweeds are photosynthetic organisms, they produces free radicals and other oxidative reagents when they are exposed to high oxygen concentrations and light. Hence, the natural antioxidants in seaweeds are important bioactive compounds which play an important role against various diseases as well as against oxidative damage of cells (Karawita et al. 2007).

The present study attempted to screen the main effect and interactions between the two major ingredients, i.e., stingless bee honey and seaweed, on proximate profile and sensory of the final end product by using Design of Experiment (DOE) approach. While the researchers defining the physicochemical of stingless bees honey according to their botanical origins and species, the information on its food products still remained scarce in the literatures. In order to determine the impact of the combination of the ingredients towards the nutritional and proximate profile of the end product, design of experiment (DOE) is the best solution to reduce the number of experiment runs and costs. In addition, application of full factorial design could help to relate the factor and interaction beween the factors by giving a relationship coded equation.

MATERIALS AND METHOD

Honey Samples Collection

The processed *Kelulut* honey was provided by Rimbunan Hijau Bee Farms Sdn. Bhd, Sibu, Sarawak. The *Kelulut honey* provided is from *Heterotrigona itama* species with nectar source are mainly from *Acacia mangium* trees and flowers.

Preparation of Dry Red Seaweeds

The dry red seaweeds (*Kappaphycus sp.*) were rinsed and macerated. Following this, the mixture was dried at room temperature until constant weight. The sample was kept at 4^{o}C for subsequent analysis.

Design of Experiment (DOE)

A full factorial design (FFD) comprising of two levels and two factors was applied in the present study (Table 1). The two-level-two-factor (2^2)FFD was employed to evaluate the effects of the two main factors, amount of *Kelulut* honey (X_1) and amount of red seaweeds (X_2) towards six responses, namely, vitamin C (Y_1), pH (Y_2), crude protein (Y_3), ash (Y_4), fat (Y_5) and moisture content (Y_6).

Vol. 16. No.3 (1-10), ISSN: 1823-884x

Table 1: **Experimetal factors and levels**

Factor	Notation	Unit	Low (-1)	Centre (0)	High(+1)
Amount of *Kelulut* honey	X_1	g	10	30	50
Amount of red seaweed	X_2	g	30	45	60

The total of 15 runs of FFD generated by the Design-Expert Version 10.0 (Stat-East, Inc). Additional three center point runs were included to the design in order to reveal the main factors. The data were fitted to the order of 2FI (two factor interaction) models, as shown in Equation 1.

$$Y=\beta_0+\beta_1 X_1+\beta_2 X_2+\beta_{12} X_1 X_2+\varepsilon \qquad \text{(Equation 1)}$$

Where, Y is predicted response and ε is a random error.

Samples Preparation

The mass of seaweed and *Kelulut* honey used were set to two (2) levels for estimation of the main effects and interactions. The samples were prepared as per experimental design generated by the software (Table 2). The mass of water was fixed at 70% of the total volume of the beverage. Proximate analysis was conducted on the samples produced after the mixing procedure.

Table 2: **Experimental Design**

Runs	Experimental factors	
	X_1	X_2
1	10	30
2	30	45
3	50	30
4	10	30
5	10	30
6	10	60
7	50	30
8	50	60
9	50	60
10	50	60
11	10	60
12	30	30
13	10	60
14	30	45
15	30	45

* X_1 = amount of *Kelulut* honey; X_2 = amount of red seaweed

Proximate Analysis

The moisture content (%), vitamin C (g/L), pH, crude protein (%), fat (%) and ash (%) were analysed according to AOAC (2016)

Vol. 16. No.3 (1-10), ISSN: 1823-884x

Vitamin C analysis

The vitamin C was determined by redox titration (AOAC 2016). The titration was carried out by titrating 25 mL of samples and 3 mL of 1% starch indicator using the iodine solution.

pH Analysis

The pH of *K*hS samples was determined according to AOAC method 962.19 (AOAC, 2016). A waterproof H160 pH meter (Hach, USA) was used to measure the pH value.

Crude Protein Analysis

Crude protein was determined using Kjeldahl method AOAC (2016). The *K*hS sample (2 g) was digested with digester machine (FOSS Labtec Line, Sweden) equipped with scrubber at 420°C for 1 h 15 min. After cooling, digester tube undergone distillation process with distillation machine (FOSS Kjeltec 8100, Sweden). Then, titration step was done by using 0.1 N of hydrochloric acid (HCl) with receiver solution consists of methyl red and bromocresol green as indicator in 0.4% boric acid. The amount of crude protein was found by multiplying the percent nitrogen by a factor, usually 6.25.

Ash Analysis

Ash content was measured according to AOAC Official Method 920.181 (AOAC, 2016). The crucible was preheated at 100℃ before use. The *K*hS sample (5.0 g) was put into the preheated crucible. The crucible together with the food sample was burned at 550℃ in a furnace (Nabertherm, Germany) overnight until a light grey ash was obtained. The whole crucible was then cooled in a desiccator before the weight of the content was taken. The percentage of ash was calculated by using Equation 2.

$$Ash\ (\%) = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100 \qquad \text{(Equation 2)}$$

Fat Analysis

The fat content was determined using semi-automated Soxtec extractions systems (FOSS, Sweden). The *K*hS sample (2 g) was mixed with celite (the filter aid material). Then, the paste was weighed directly into the thimble which was preheated in an oven for two (2) hours. The sample was inserted in the Extraction Unit and petroleum ether was added in a closed system to extract the fats since lipid fraction of the food is soluble in non-polar organic solvents. A four steps extraction procedure consists of boiling, rinsing, recovery and drying were carried out. The fat content was measure in the unit percentage (%).

Moisture Content

The moisture content of *K*hS samples was measured using moisture analyzer (Sartorius, Germany).

Sensory Evaluation Test

The sensory evaluation involved a hedonic rating test which is used to get the subjects acceptance on the final product (product with highest amount of honey), such as, appearance, color, smell, mouth-feel texture, taste and their overall acceptability. Panelists were encouraged to add some

Vol. 16. No.3 (1-10), ISSN: 1823-884x

recommendations on how the *Kelulut* honey drink enriched with seaweed could be improved. Sample '216' was prepared with 140 g of macerated seaweed, 840 g of *Kelulut* honey and 2286.62 g of water were added to prepare 70 cups of drinks. Sample '216' represented the final product the highest level of total phenolic content. All the samples were kept under refrigeration until sensory tests were performed. The samples were served chilled during the test.

RESULTS AND DISCUSSION

Design of Experiment (DOE)

As shown in Table 3, the *K*hS samples were slightly acidic (pH ranging from 3.20 to 3.50) due to the presence of organic acids in *Kelulut* honey. Trace of vitamin C (0.03 g/L to 0.10 g/L) was found in the samples as well. Alvarez-Suarez et al. (2010) reported that *Kelulut* honey contains low content of vitamins. On the other hand, no fat was detected among all the samples and thus this newly developed beverage could be categorised as functional drink with low level of cholesterol detected. Besides, ash content and crude protein were in the range from 0.07 to 0.12% and 0.00 to 0.17%, respectively.

Table 3: **Results on Proximate Analysis**

Run	Amount of seaweed, X_1 (g)	Amount of honey, X_2 (g)	Vitamin C, Y_1 (g/L)	pH, Y_2	Crude protein, Y_3 (%)	Ash, Y_4 (%)	Fat, Y_5 (%)	Moisture content Y_6 (%)
1	30	45	0.08	3.34	0.09	0.09	0.00	84.31
2	30	45	0.08	3.35	0.00	0.08	0.00	83.65
3	10	60	0.05	3.23	0.00	0.07	0.00	78.77
4	10	30	0.10	3.28	0.09	0.09	0.00	79.13
5	30	45	0.08	3.34	0.09	0.09	0.00	83.64
6	50	60	0.10	3.39	0.09	0.09	0.00	85.05
7	10	60	0.05	3.22	0.17	0.08	0.00	75.38
8	10	30	0.05	3.27	0.17	0.07	0.00	80.14
9	50	30	0.05	3.49	0.09	0.11	0.00	88.78
10	50	60	0.05	3.37	0.09	0.11	0.00	86.12
11	50	30	0.05	3.50	0.17	0.12	0.00	89.2
12	50	60	0.05	3.37	0.00	0.09	0.00	85.64
13	10	60	0.10	3.21	0.09	0.10	0.00	78.28
14	10	30	0.03	3.28	0.17	0.11	0.00	79.52
15	50	30	0.05	3.50	0.00	0.10	0.00	90.36

Statistical Analysis

Table 4 shows the summary of ANOVA for the corresponding model. In regard of significant model, the factors, X_1 and X_2 had significant interaction towards the Y_2 and Y_6 of the samples. The combination of both factors could affect the pH value of the samples.

Vol. 16. No.3 (1-10), ISSN: 1823-884x

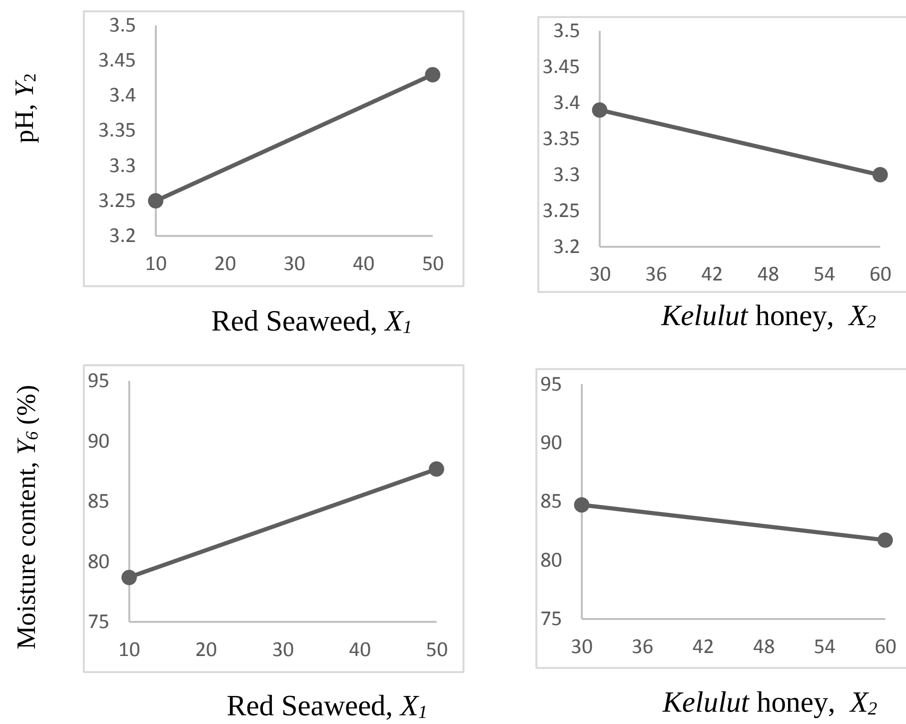
Table 4: Summary of ANOVA p-values for Corresponding Model

	p-values					
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Model	0.8209	< 0.0001*	0.4840	0.1030	ND	< 0.0001*
X_1	0.7787	< 0.0001*	0.2897	0.0370*	ND	< 0.0001*
X_2	0.4068	< 0.0001*	0.2897	0.1659	ND	0.0003*
X_1X_2	0.7787	< 0.0001*	0.7172	0.8232	ND	0.1556
Curvature	0.3874	0.8775	0.4241	0.2866	ND	0.2122

ND = Not detected ; * Significant interaction ($p < 0.05$)

Figure 1 reveals the interaction plot of the significant model. The results indicated that when the mass of seaweed (X_1) increased, the pH (Y_2) and moisture content (Y_6) of the end product increased significantly. On the other hand, when the mass of *Kelulut* honey (X_2) increased, the pH and moisture content of the product reduced. In this case, X_1 portrayed a positive effect on pH and moisture content while X_2 was vice versa.

Figure 1: **Interaction Plot between Significant Experimental Factors and Responses**



The coded mathematical relationships of pH, Y_2 is presented in Equation 3 while moisture content, Y_6 is presented in Equation 4.

$$\text{pH, } Y_2 = 3.34 + 0.094\, X_1 - 0.094\, X_2 - 0.016\, X_1X_2 \qquad \text{(Equation 3)}$$

$$\text{Moisture content, } Y_6 = 83.20 + 4.49\, X_1 - 1.49\, X_2 \qquad \text{(Equation 4)}$$

Vol. 16. No.3 (1-10), ISSN: 1823-884x

The model equation is used to identify the significant of factors and their interaction effects. The high moisture content of seaweed would increase the moisture content of end product which agreed with the outcome of Xiren & Aminah (2017) who reported that the moisture content of *Kappaphycus alvarezii* from Langkawi and Sabah, Malaysia was around 80%.

Following the statistical analysis of results through ANOVA, the confirmation tests on the Equation 3 and Equation 4 were performed. By using Design Expert Version 10.0 Software (Stat-Ease, Inc), the proposed solution with the best desirability value of 0.973 (almost equal to 1.000) was chosen where 10 g of seaweed and 60 g of *Kelulut* honey were used. The amount of *Kelulut* honey added was set to maximum level for the purpose to maximize the total phenolic content of the final product produced (Wong et al. 2018). The two significant responses as mentioned above, i.e., pH and moisture content of end product, were analyzed and compared with the predicted values (Table 5). The percentage of difference of Y_2 and Y_6 were all below 10%. The pH value of sample during confirmation testing was found slightly higher which might be due to the fact that different batches of *Kelulut* honey was used. As mentioned by Alvarez-Suarez et al. (2010), the composition of *Kelulut* honey is dependent on seasonal, geographical and environmental factors.

Table 5: Confirmation Test

Responses	Predicted values	Experimental values	Percentage of difference (%)
Y_2	3.22	3.48	8.07
Y_6 (%)	77.21	77.56	0.45

Sensory Evaluation Test

After the average score for each attribute was calculated, the profile for the Sample '216' was generated. Sample '216' is the final product with the highest honey content and total phenolic content. According to the radar chart (Figure 2), it has a good taste, however the sweetness needs improvement. Some panelists stated that Sample '216' has a strong aftertaste which was not very agreeable. A solution should be investigated to reduce the aftertaste. The results from the questionnaire indicated that the texture, appearance and color of Sample '216' are acceptable. There are suggestions that the natural chewiness of algae should be maintained and the maceration duration of seaweed should be shortened in order to have bigger size of seaweed particle in the drink. On the other hand, the aroma acceptance of Sample '216' is not high because the panelists considered the unique smell of stingless bee honey as pungent. Last but not the least, the sensory analysis shows that the overall acceptability of the *Kelulut* honey drink enriched with seaweed (*K*hS) was 6.57, i.e., "like moderately" by most of the panelists.

Vol. 16. No.3 (1-10), ISSN: 1823-884x

Figure 2: **Radar Chart Representing The Acceptance Score of Sample '216'.**

CONCLUSION

In conclusion, the Design of Experiment (DOE) is an useful tools to be applied during development of new food products. The results of present study suggested that the amount of seaweed and honey added during the preparation stage had significantly affected the pH and moisture content of the final product. The overall acceptability of the newly developed *Kelulut* honey functional drink also indicated the marketability potential of the product. To the best of our knowledge, this is the first report on the development of new beverage from *Kelulut* honey enriched with seaweed.

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REFERENCES

Alvarez-Suarez, J. M., Giampieri, F., Gonzalez-Paramas, A. M., Damiani, E., Astolfi, P., Martinez-Sanchez, G., …& Battino, M. (2012). Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food and Chemical Toxicology*, 50, 1508-1516.

AOAC (2016). Official methods of analysis of AOAC International (20th ed.). Association of Analytical Communities, Gaithersburg, MD, USA.

Chua, L. S., Rahaman, N. L. A., Adnan, N. A., Tan, E. & Tjih, T. (2013). Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of Analytical Methods in Chemistry*, 2013, ID 313798, 1-8.

Codex, A. (2001). Revised codex standard for honey. *Codex Alimentarius.*

Fleurence, J. & Levine, I. (Eds) (2016). Seaweed in health and disease prevention. Academic Press.

Vol. 16. No.3 (1-10), ISSN: 1823-884x

Karawita, R., Senevirathne, M., Athukorala, Y., Affan, A., Lee, Y.J., Kim, S. K., Lee, J. B. & Jeon, Y. J. (2007). Protective effect of enzymatic extracts from microalgae against DNA damage induced by H 2 O 2. *Marine Biotechnology*, 9(4), 479-490.

Menrad, K. (2003). Market and marketing of functional food in Europe. *Journal of Food Engineering*, 56, 181–188.

Mollet, B. & Rowland, I. (2002). Functional foods: At the frontier between food and pharma. *Current Opinion in Biotechnology*, 13, 483–485.

Roberfroid, M. B. (2000). GR Gibson & CM Williams (Eds.), Functional foods. Concept to product. Woodhead Publishing Limited, Cambridge, England, 9–27.

Siro, I., Kapolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance-A review. *Appetite*, 51(3), 456-467.

Sousa, J. M., de Souza, E. L., Marques, G., Meireles, B., de Magalhães Cordeiro, Â. T., Gullón, B. & Magnani, M. (2016). Polyphenolic profile and antioxidant and antibacterial activities of monofloral honeys produced by Meliponini in the Brazilian semiarid region. *Food Research International*, 84, 61-68.

Tiwari, B. K. & Troy, D. J. (2015). Seaweed sustainability–food and nonfood applications. *In Seaweed Sustainability*, 1-6.

Tuksitha, L., Chen, Y. L. S., Chen, Y. L., Wong, K. Y., & Peng, C. C. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia Pacific Entomology*, 21(2), 563-570.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E. & Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, 29(2), 949-982.

Wong, P., Hii, S. L., Lim, S. L. & Suzy, R. A. G. (2018). Development of new stingless bee honey-algal drink by using statistical approach. *Proceedings of the Agricultural and Food Mechanization (NCAFM)* (pp. 130-132). Penerbit MARDI.

Xiren, G. K. & Aminah, A. (2017). Proximate composition and total amino acid composition of *Kappaphycus alvarezii* found in the waters of Langkawi and Sabah, Malaysia. *International Food Research Journal*, 24(3), 1255-1260.

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