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Optimization of sauce formulation from sea grape (*Caulerpa racemosa*) protein hydrolysate using response surface methodology

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Abstract

Sea grape (*Caulerpa racemosa*) is an edible green alga which contains a high amount of protein with a seafood flavor, which can be released via protein hydrolysis. Our present work was designed to determine the optimal concentration of palm sugar and carboxymethylcellulose (CMC) in sea grape protein hydrolysate sauce. The process optimization was performed to evaluate multiple parameters such as viscosity, color properties, and hedonic score. Five different levels of palm sugar and CMC were designed using a central composite design to yield sauce with a maximum hedonic score. We developed different mathematical models in this work, where viscosity, color properties (L^*, a^*, b^*), and hedonic score were modeled using 2FI (two-factor interaction), quadratic, and linear models, respectively. The numerical optimization revealed that the predicted optimal solution was palm sugar and CMC concentration with generated viscosity, L^*, a^*, b^*, and hedonic score of 0.893 poise, 23.296, 7.300, 19.565, and 3.522, respectively, and a desirability value of 0.839. GC-MS data associated with referred information represented that sea grape sauce had four compounds with a typical seafood flavor, namely, nonanal, hexanal, (*E*)-2-octenal, and octanoic acid; some native palm sugar flavors; and Maillard reaction products (MRP). Our study suggests that sauce is a potential value-added product from sea grape protein hydrolysate.

Keywords Green algae sauce · Protein · Palm sugar · CMC · Central composite design

Introduction

Food utilization is one of the pillars of food security that motivate researchers to explore novel food sources towards meeting sustainable development goals (Pérez-Escamilla 2017). As an archipelagic country, Indonesia certainly has many foodstuff candidates in water, which are potentially beneficial for good health and well-being. Therefore, based on the National Research Master Plan of the Ministry of Research and Technology (2016), well-grounded studies on the comparative and competitive advantages of aquatic commodities in Indonesia or other archipelagic countries are crucial (Ministry of Research, Technology and Higher Eduction Indonesia 2016). Among the potential aquatic commodities in Indonesia, macroalgae or seaweeds are very valuable with more than 500 species which have not been completely explored (Wibowo 2019).

Caulerpa racemosa, colloquially known as "sea grape," is among hundreds of abundant algal species in Indonesia and tropical countries such as the Philippines, Vietnam, Singapore, Malaysia, Thailand, Taiwan, China, Fiji, and West Pacific Coast (Horstmann 1983; Titlyanov et al. 2016). Sea grape has a protein content of about 21.7% (w/w) with a very specific seafood-like flavor (Ma'ruf et al. 2013). This algal species is consumed by coastal people in a fresh condition for salad, due to its umami taste (de Gaillande et al. 2017), and in biscuits (Kumar et al. 2018). Our previous work investigated some seafood flavoring compounds in sea grape protein hydrolysate, such as aldehydes, ketones, and fatty acid derivatives, in which the previous study reported that the investigated compounds had a seafood flavor identity. Dang

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et al. (2015) stated that protein hydrolysis theoretically could yield free amino acids and some glutamic acid– or aspartic acid–rich oligopeptides, which are sources of umami taste.

Based on the characteristics of protein hydrolysate in our previous work, our present study focused on the utilization of sea grape protein hydrolysate to develop a sauce product. Based on a data collection survey on outer-ring fishing port development in the Republic of Indonesia in 2010, sauce is one of processed fishery products (Japan International Cooperation Agency 2010). In general, sauce has the combination taste of salty, umami, and sweet and is viscous and dark palm in color. These characteristics are determined by the ingredients of the sauce. Previous studies by Witono et al. (2015) and Tian et al. (2018) demonstrated that palm sugar and carboxymethylcellulose (CMC) determined the palatability of the sauce.

Palm sugar is a natural sweetener made from sap or nectar collected from flowers of several species of palms which is mainly composed of sucrose and is used in various traditional food and beverages in Indonesia, the Philippines, Thailand, Malaysia, and India (Saputro et al. 2019). In soy sauce production, palm sugar improves the flavor characteristics of the soy sauce due to the typical palm volatile compounds, while in bakery products such as cookies, palm sugar influences their textural properties (Apriyantono et al. 1996; Suwansri et al. 2009). CMC is a negatively charged cellulose derivative which is soluble in water at any temperature. CMC is commonly used in beverages to provide a rich mouthfeel. CMC plays a role in stabilizing protein in a solution by acidifying the protein itself to prevent precipitation. CMC is added to syrup and sauce formulations to increase the viscosity and the consistency of the product (Ergun et al. 2016).

Response surface methodology (RSM) is an efficient statistical technique used in various experiments in agricultural and biological sciences with a reduced number of trials. This technique evaluates the effects of independent variables and their mutual interactions on dependent variable/s (Roj et al. 2015). Our study was designed to determine the optimal amount of palm sugar and CMC by employing a central composite design (CCD).

Materials and methods

Materials

Sea grape (*Caulerpa racemosa*) was confirmed by the Research and Development Center of Oceanology, Jakarta, Indonesia, collected from the Brackish Water Aquaculture Center Jepara, Central Java, with a coordinate position of 6°35'11.9"S 110°38'39.8"E. Bromelain enzyme was supplied by PT. Bromelain Enzyme, Lampung. Glucose syrup 10% (w/v) was supplied by UD. Denly, Surabaya. Palm sugar solution 15% (w/v) was supplied by Nira Murni, Probolinggo. Filter paper no. 1 was supplied by Whatman, UK. Food-grade CMC (3% w/v) was supplied by PT. Bratachem, Surabaya.

Dried seaweed preparation

Sea grape was cleaned immediately after collection by using freshwater thoroughly to remove sand, debris, epiphytes, and other extraneous matter. The seaweed was then transported to the laboratory and put in a box covered with banana leaf inside. Samples were then air-dried at room temperature. The dried sea grape was then stored in plastic bags with silica gel to control the storage environment.

Preparation of sea grape protein hydrolysate

Sea grape protein hydrolysis was prepared based on Laohakunjit et al. (2014), summarized in Online resource 2. Two grams of dried sea grape was mixed with 100 mL of distilled water and then the pH was adjusted to 4.0 with drops of citric acid. The mixture was then pre-incubated at 50 °C for 10 min, and then bromelain enzyme with an amount of 50% (w/w) from the protein content in air-dried sea grape was poured into the mixture and further incubated for 3 h at the same temperature. The reaction was then terminated by heating to 95 °C for 15 min. After incubation, the sample was centrifuged at 867 × *g* for 15 min; the supernatant was decanted and filtered through filter paper. The sea grape protein hydrolysate was then collected in dark glass bottles and stored at − 4 °C until further use.

Production of sea grape protein hydrolysate sauce

The technical process in producing sea grape protein hydrolysate sauce was previously evaluated. The complete formulation followed the experimental design of Online resource 1; protein hydrolysate (mL), palm sugar (mL), and glucose syrup (mL) were mixed and cooked at 80 °C for 15 min, and then CMC gel (mL) was incorporated and cooked at 80 °C for a further 15 min. The sauce was then cooled to air temperature and then stored at 4 °C for further analyses. The production scenario is summarized in Online resource 2.

The viscosity of the sauce was analyzed by using a standard method of the Ostwald viscometer that used water as the comparing liquid. The viscosity was determined by the following equation:

$$\frac{\eta_0}{\eta} = \frac{\rho_0 \cdot t_0}{\rho \cdot t} \quad (1)$$

where η_0 = viscosity of the comparing liquid (poise), η = viscosity of sauce (poise), ρ_0 = pressure in the comparison liquid (dyne cm^{-2}), ρ = pressure in the sample fluid (dyne cm^{-2}), t_0 = comparing liquid flow time, and t = sauce flow time.

Sauce color was expressed as L^*, a^*, b^*, representing lightness, redness, and yellowness, respectively, analyzed by using a Color Reader CR-10 from Minolta Inc., Japan (Amin et al. 2018).

Hedonic test

Hedonic testing was carried out by 50 non-trained panelists aged 20–35 years with Indonesian nationality and conducted in a clean room with a controlled air temperature of 24 °C. Four hedonic attributes (appearance, taste, aroma, and color) were measured through a hedonic instrument based on a scaling guide of sensory evaluation of Peryam and Pilgrim (1957) in the Society of Sensory Professionals (2019). Each attribute was scaled 1–9 points, where 9: like extremely; 8: like very much; 7: like moderately; 6: like slightly; 5: neither like nor dislike; 4: dislike slightly; 3: dislike moderately; 2: dislike very much; and 1: dislike extremely. Panelists were required to mask their tongue with mineral water prior to testing each sample.

Identification of volatile compounds by using GC-MS

The investigation of the volatiles from the sauce was performed according to a method of Laohakunjit et al. (2014). The analysis was carried out on a set of headspace solid-phase micro-extraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) system (GC 7890A; MS 5975C, Agilent Technologies, USA). The algal sample was placed into a 22-mL vial and heated at 60 °C for 10 min in a GC-MS heating block for headspace analysis. Volatile compounds were absorbed onto an SPME fiber (50/30 μm DVB/Carboxen/PDMS StableFlex; Supelco, USA) for 20 min. After equilibrium, the SPME fiber was desorbed into the injector port at 250 °C for 20 min. The injector was operated in split-less mode. Helium was used as the carrier gas at a constant velocity of 1.0 mL min^{-1}. Volatile compounds were separated using a DB-WAX capillary column (30 m × 250 μm × 0.25 μm; J&W Scientific Inc., USA). The oven temperature program was as follows: initial temperature of 55 °C, increased to 180 °C at 5 °C min^{-1}, increased to 200 °C at 8 °C min^{-1}, and held at 200 °C for 10 min. Volatile compounds were detected using MSD (scan range of m/z 29–550) at 230 °C. MS results were then recorded using electron impact ionization at 70 eV. The total ion count (TIC) was recorded and used for data identification and quantification (area). The TIC was compared with the spectral component database known in the GC-MS library (NIST-14). The flavor information of each single compound detected by GC was referred to the flavor database of www.flavornet.org and some previous work.

Experimental design for optimization using RSM

In our preliminary experiment, we have investigated the effect of palm sugar and CMC on the quality of sea grape protein hydrolysate sauce (unpublished data). The best combination was then used as the center point of the study, designed using a central composite design (CCD) (Online resource 3). The CCD was fixed by the statistical software Design-Expert (version 7.1.6, Stat-Ease Inc., USA) with five center points (Online resource 4). The model chosen for this study was according to the sequential lack-of-fit test, R square, and adjusted R square (Montgomery 2005; Roj et al. 2015). The desired goals for each factor and response were chosen through a numerical optimization technique of multiple responses such as maximizing, minimizing, target, within range, and none. Each factor was then weighted 3–5, as the degree of importance. Our main goal of this present study was a customer-accepted product; therefore, the hedonic test was the highest degree of importance (Online resource 5).

Statistical analysis

The design, regression analysis, analysis of variance (ANOVA) and the numerical optimization technique of multiple responses were conducted by using Design-Expert software (version 7.1.6, Stat-Ease Inc., USA). The confidence level of this study was 95% (0.95) or p value < 0.05.

Verification of the optimization model

The verification of the optimal solution was carried out in the laboratory with triplicate replication. The difference between predicted and verified yields was used to validate the model built where a difference of less than 5% was proposed a valid model. The difference was determined by the following equation (Wu et al. 2008):

$$\%\text{difference} = \frac{\text{Validation} - \text{Prediction}}{\text{Validation}} \times 100 \quad (2)$$

Results

Analysis of surface response methods in the production of sea grape protein hydrolysate sauce

The models were obtained for each different response (Online resource 6) based on sequential model sum of squares (SMSS), which involved multiple analyses such as assessment of lack of fit, and determination of p value, R square and adjusted R square. According to those analyses, we developed two-factor interaction for viscosity analysis, quadratic model for color analysis (L^*, a^*, and b^*), and linear model for hedonic test. The Adeq precision of each was greater than 4. The lack-of-fit assessment showed that most responses other than lightness had a p value higher than 0.05.

The effect of palm sugar and CMC concentration on viscosity

According to Online resource 6, the suggested mathematical model for viscosity was two-factor interaction (2FI) with R square of 0.795. This value represented that palm sugar (X_1) and CMC (X_2) affected the viscosity by 79.49%. The 2FI equation obtained from the experimental design for viscosity is shown below:

$$\eta = 0.71 + 0.42X_1 + 0.49X_2 + 0.39X_1X_2 \quad (3)$$

Based on Eq. (3), there was a positive correlation between the concentration of CMC and palm sugar on viscosity, where palm sugar had a lower coefficient than CMC. This model indicated that CMC had relatively more impact on viscosity than palm sugar. The p value of palm sugar and CMC was 0.0168 and 0.0084 ($p < 0.05$), respectively (Table 1). These results showed that CMC and palm sugar significantly affected the viscosity of the sea grape sauce. In line with the developed mathematical model, Fig. 1a shows that CMC had a steeper trend than palm sugar, which demonstrated that CMC had more effect on viscosity than palm sugar.

The effect of palm sugar and CMC concentration on lightness (L^*)

The mathematical model suggested in the analysis of L^* was a quadratic model with an R-square value of 0.985, which demonstrated that palm sugar and CMC affected lightness (L^*) by 98.52% (Online resource 6). The quadratic polynomial model equation obtained from the experimental design is shown below:

$$L^* = 23.43 + 2.74X_1 + 0.37X_2 - 0.040X_1X_2 - 1.81X_1^2 - 0.16X_2^2 \quad (4)$$

The p value of palm sugar and CMC in L^* response was 0.0001 and 0.0451 (Table 1), respectively, while Eq. (4) shows that the coefficient of palm sugar was 2.74 and that of CMC was 0.37. This model then informed that palm sugar and CMC had a significantly positive correlation with lightness, where palm sugar had relatively more impact on lightness than CMC. This information was supported by Fig. 1b, where variable palm sugar has a steeper trend than CMC. The steeper the trend, the more impact generated by the independent variable.

The effect of palm sugar and CMC concentration on redness (a^*)

The developed mathematical model of redness (a^*) response was a quadratic model, with an R square of 0.9887 (Online resource 6) and no significant lack of fit (Table 1). The quadratic model equation obtained from the experimental design is shown below:

$$a^* = 7.42 + 0.89X_1 - 0.11X_2 - 0.05X_1X_2 + 0.20X_1^2 + 0.054X_2^2 \quad (5)$$

The p values of palm sugar and CMC for redness analysis were both below 0.05. The coefficients of palm sugar and CMC in Eq. (5) were 0.89 and − 0.11, respectively. This data indicated that palm sugar could significantly increase the redness, while CMC reduced the redness of the sauce. This information was relevant to Fig. 1c, which shows that there was a significant increase of redness from 10 to 20% for palm sugar and a slight decrease of redness from 2 to 4% for CMC.

The effect of palm sugar and CMC concentration on yellowness (b^*)

The quadratic model with R square of 0.988 was developed to synthesize information on the effect of palm

Table 1 F and p values of independent variables resulting from analysis of variance (ANOVA)

	η (poise)		L^*		a^*		b^*		Hedonic score	
	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value
Model	5.43	0.0234	93.01	0.0001	122.31	0.0001	112.95	0.0001	7.61	0.0095
X_1—palm sugar	9.74	0.0168	332.20	0.0001	576.72	0.0001	375.31	0.0001	27.24	0.0012
X_2—CMC	13.18	0.0084	5.93	0.0451	8.84	0.0207	90.85	0.0001	1.76	0.2267
X_1X_2	4.19	0.0800	0.035	0.8560	0.91	0.3716	2.93	0.1304	1.18	0.3142
X_1^2	0.014	0.9083	126.63	0.0001	24.56	0.0016	93.36	0.0001	4.90	0.0624
X_2^2	0.015	0.9070	0.96	0.3594	1.87	0.2134	7.67	0.0277	3.99	0.0860
Lack of fit	0.30	0.8253	8.87	0.0306	2.33	0.2163	3.82	0.1140	0.59	0.6569

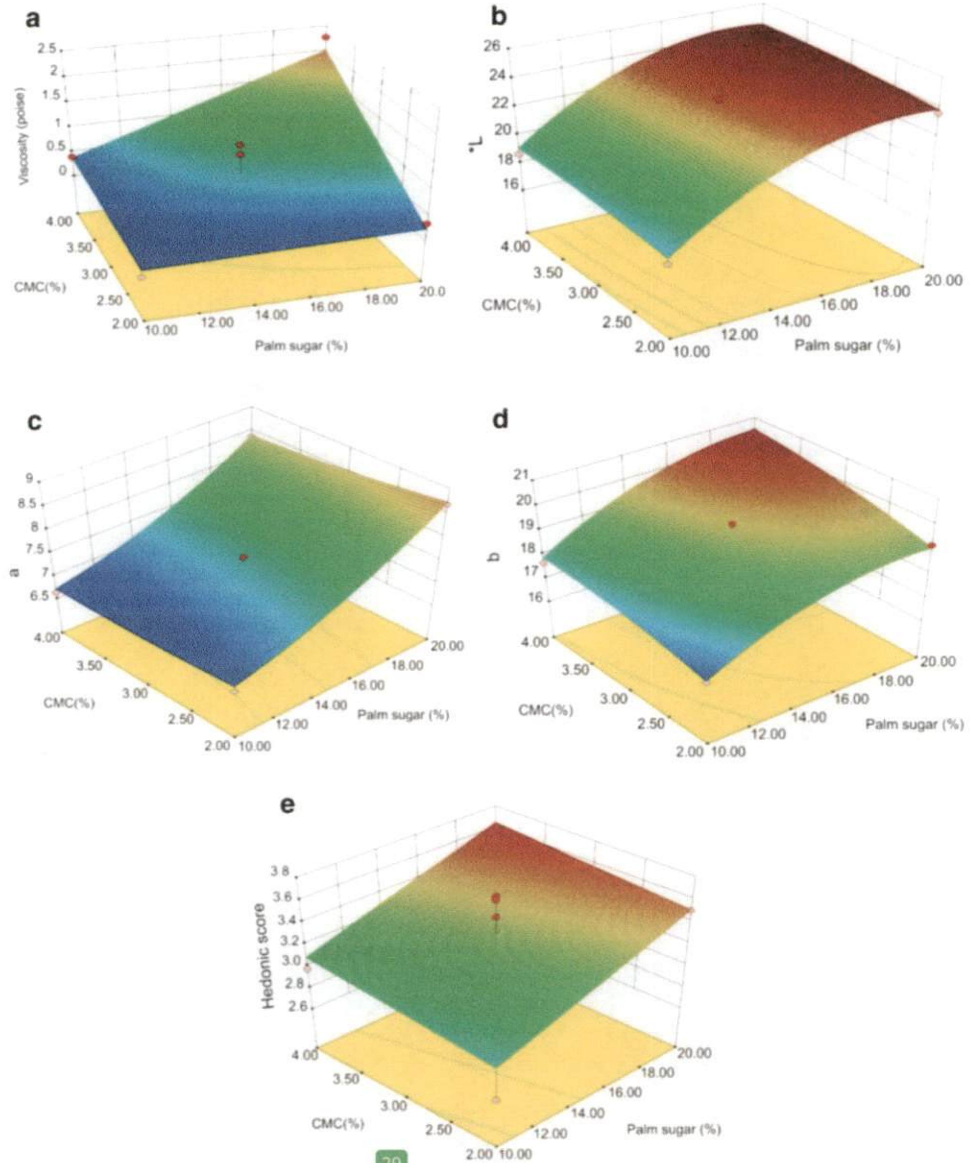

Fig 1 Surface plots of the optimization model. **a** Viscosity. **b** Lightness (L^*). **c** Redness (a^*). **d** Yellowness (b^*). **e** Hedonic score

sugar and CMC on yellowness (b). The equation of this response is shown below:

$$b^* = 19.45 + 1.81X_1 + 0.58X_2 + 0.15X_1X_2 - 0.68X_1^2 - 0.18X_2^2 \quad (6)$$

In the above equation, palm sugar and CMC had a positive correlation with yellowness. This showed that the more the palm sugar and CMC, the more intense the yellowness. Both factors had a significant effect on yellowness as the p values of palm sugar and CMC were 0.0001 and 0.01, respectively (Table 1), while the coefficient of palm sugar was 1.81 and that of CMC was 0.58. Further representation by a surface plot (Fig. 1d) showed that the trend of yellowness by palm sugar was steeper than CMC. This information suggested that palm sugar has more impact on yellowness than CMC in the present work.

The effect of palm sugar and CMC concentration on hedonic response

The hedonic response was the crucial factor in obtaining customer-accepted sea grape sauce. This test engaged non-trained panelists who contributed to our present study. The response was investigated by using a linear model with R square of 0.644 (Online resource 6). The polynomial model is as follows:

$$\text{Hedonic score} = 3.45 + 0.28X_1 + 0.071X_2 - 0.082X_1X_2 \quad (7)$$

In the above equation, palm sugar and CMC have a positive correlation with the hedonic score as each variable has a positive value. The p value of palm sugar was 0.0012, and that of CMC was 0.022 (Table 1). This information revealed that palm sugar significantly affected the hedonic response, while CMC did not significantly affect the hedonic response. The non-significant effect of CMC on the hedonic response could be in line with the coefficient value of CMC, which was relatively much lower than that of palm sugar. Figure 1e shows a steeper trend of the hedonic response by palm sugar.

Optimization and model verification

Based on the criteria set up for the numerical optimization (Table 2), the optimal solution contained concentrations of palm sugar and CMC of 14.43% and 3.49%, respectively, which yielded the desired characteristics of the final sauce (Table 2). The desirability of this prediction was 0.839 (near 1.000), which indicated that the prediction was accurate. The percentage of difference between prediction and validation values of all responses was less than 5%, which could show that the prediction performed in the present study was accurate.

Profile of volatile compounds in hydrolysate and sauce

Volatile compounds from sauce, produced by the optimized formulation, were identified by using GC-MS. The volatile compounds were classified into hydrocarbon compounds, aldehydes, ketones, alcohols, and fatty acids. As shown in Table 3, the protein hydrolysate had 58 detected compounds and the sauce had 34 detected compounds. Hydrocarbons were the most detected compounds in protein hydrolysate, while alcohols were the most detected compounds in sauce. In protein hydrolysate, all the hydrocarbon compounds were lost due to sauce production, while sauce production itself could generate newly detected hydrocarbon compounds such as isoamyl acetate, D-limonene, 5-ethyl-1,3-cyclohexadiene, octanal, (*Z*)-2-heptenal, naphthalene, 9-methyl-acridine, methoxy-phenyl-oxime, and felbamate.

The aldehyde class compounds in protein hydrolysate such as heptanal, (*E*,*E*)-2,4-decadienal, 2-heptadecenal, 2-(phenylmethylene)-octanal, and benzaldehyde were lost after sauce production; however, three compounds were retained, such as nonanal, hexanal, and (*E*)-2-octenal.

The number of alcohol class compounds increased after sauce production, where four alcohols of protein hydrolysate were lost. However, nine new compounds were detected in sauce, namely, eucalyptol, 1-hexanol, 2-ethyl-phenol, 1-octanol, 2,3-butanediol, [*R*-(*R**,*R**)]-2,3-butanediol, 3-methyl-2-octanol, dimethyl-silanediol, and 2,4-di-*tert*-butylphenol. Three ketone group compounds (*trans*-β-ionone, 3,3-dimethyl-2-hexanone, and 2,3-octanedione) were not detected in sauce, but new compounds including 6-methyl-2-heptanone, styrene, 1-hydroxy-2-propanone, and benzophenone were detected. The fatty acid octanoic acid was detected in both protein hydrolysate and sauce, while sauce production generated three newly fatty acids—acetic acid, nonanoic acid, and *n*-hexadecanoic acid.

Discussion

Our present work has established different models predicting the effect of palm sugar and carboxymethyl cellulose (CMC) on the viscosity, lightness, redness, yellowness, and hedonic score of sea grape sauce. The present work performed multiple-parameter determination to assess the usability of the mathematical model in solving the problem of sea grape sauce production. Each parameter, such as R square, adjusted R square, lack-of-fit test, model p value, PRESS (predictive sum of squares), and Adeq precision, determined in each response fitted different models in our present work. The viscosity response, color properties (L^*, a^*, b^*), and hedonic score fitted 2FI, quadratic, and linear models, respectively. Myers et al. (2016) stated that the model used for optimization in response surface methodology is not limited to quadratic or

Table 2 Optimal solution of sauce production with desirability value of 0.839

	% palm sugar	% CMC	Viscosity (poise)	L^*	a^*	b^*	Hedonic score
Prediction	14.43	3.49	0.893	23.296	7.300	19.565	3.522
Validation			0.866	23.200	7.250	19.540	3.475
% difference			2.980	0.412	0.685	0.128	1.334

Table 3 Comparison of volatile compound profiles between sea grape protein hydrolysate and sauce

Compound name	Area		Flavor information
	Sea grape protein hydrolysate	Sauce	
Hydrocarbon			
(*E*)-5-Octadecene	545,271	-	Mild hydrocarbon
Cyclododecane	8,094,564	-	Paraffin
Pentadecane	36,746,798	-	Mild odor
2-Methyl-octadecane	9,939,301	-	Fuel-like
Naphthalene	3,489,521	-	Coal tar
4-Methyl-pentadecane	7,586,870	-	Mild odor
4-Methyl-tetradecane	2,030,225	-	Gasoline-like
3-Methyl-pentadecane	1,951,461	-	Mild odor
Heptadecane	2,377,317	-	Oily, fuel-like
2,4-Dimethyl-1-decene	7,895,366	-	Gasoline-like
2,6,10-Trimethyl-pentadecane	1,922,734	-	Mild odor
1-Pentadecene	379,884	-	Mild
1-Heptadecene	14,847,828	-	Alkenes
6-Propyl-tridecane	2,559,134	-	Gasoline-like
1,1′-Oxybis-hexadecane	624,923	-	Gasoline-like
8-Heptadecene	842,648	-	Fatty
4-Ethyl-2,6-dimethyl-pyridine	1,059,506	-	Meaty, roasted
4-Methyl-octadecane	6,547,385	-	Fuel-like
2,6,11-Trimethyl-dodecane	1,388,212	-	Coconut, fatty, waxy
Eicosane	500,421	-	Waxy
3-Methyl-heptadecane	6,702,007	-	Oily, fuel-like
(*E*)-2-Tetradecene	3,446,940	-	-
Caryophyllene oxide	1,653,683	-	Spicy, woody, terpenic
Tetratriacontyl pentafluoropropionate	257,903	-	Rancid
Lilial	711,488	-	Floral, muguet, watery, green, powdery, cumin
2-Ethylhexyl salicylate	915,979	-	Mild, orchid, sweet, balsam
Isopropyl myristate	10,808,599	-	Oily, fatty
9-Methylheptadecane	400,989	-	Odorless
2-Methyl-1-propyl-naphthalene	4,563,575	-	Sweet, floral, woody
1-Acetyl-4,6,8-trimethylazulene	4,257,626	-	Green, spicy, sweet
Versalide	401,571	-	Sweet, musk
Diisobutyl phthalate	781,319	-	Ester odor
β-Myrcene	54,813,414	-	Peppery, balsam
trans-β-Ocimene	44,610,997	-	Oily, sweet
cis-β-Ocimene	13,428,806	-	Oily, sweet
Neo-allo-ocimene	9,783,229	-	Sweet, floral, nut, skin, peppery, herbal, tropical
Allo-ocimene	7,083,843	-	Sweet, floral, nut, skin, peppery, herbal, tropical
β-Ocimene	7,070,023	-	Oily, sweet
Terpinolene	6,288,406	-	Oily
(+)-4-Carene	3,346,070	-	Sweet, pungent
Allo-neo-ocimene	2,671,790	-	Sweet, floral, nut, skin, peppery, herbal, tropical
1,3-Bis(1,1-dimethylethyl)-benzene	896,547.12	-	Cooked beef
Isoamyl acetate	-	1,825,881	Oily, banana odor
D-Limonene	-	14,406,843	Hydrocarbon
5-Ethyl-1,3-cyclohexadiene	-	3,314,085	Colorless liquid, fruity
Naphthalene	-	1,528,063	Sweet, floral, woody

Table 3 (continued)

Compound name	Area		Flavor information
	Sea grape protein hydrolysate	Sauce	
9-Methyl-acridine	-	6,517,349	Odorless
Methoxy-phenyl-oxime	-	48,254,444	Fresh fruity
Felbamate	-	879,508	Odorless
Aldehyde			
Nonanal	5,942,978	16,810,361	Geranium, plastic, marine, waxy, rose fresh, orange, fatty
Octanal	-	1,090,6047	Fruity, fatty, oily, creamy, sour
Hexanal	9,704,932	6,496,424	Fishy, grassy, leafy, green
(*Z*)-2-Heptenal	-	8,787,905	Green, fatty, oily, sweet
(*E*)-2-Octenal	1,264,738	4,112,965	Fatty, sweet, spicy, vegetable, fishy, oily, green
Heptanal	30,688,395	-	Burnt fat, citrus, rancid
(*E*,*E*)-2,4-decadienal	1,405,030	-	Fishy, beef, potato chips
2-Heptadecenal	3,263,594	-	Seaweed-like
2-(Phenylmethylene)-octanal	418,121	-	Grassy, leafy, green, fatty
Benzaldehyde	137,499,069	-	Sweet, oily, nutty, woody
Furfural	-	5,938,297	Bready
Benzeneacetaldehyde	-	3,570,022	Green, sweet, floral hyacinth
(*E*)-2-Decenal	-	9,094,016	Fatty
5-Hydroxymethylfurfural	-	4,448,580	Fatty, buttery
Alcoholic compound			
2-Phenoxy-ethanol	419,878	-	Mild, rose, balsam, cinnamyl
2,4-Di-*tert*-butylphenol	4,573,015	-	Fermented sausage
4-(1-Methylpropyl)-phenol	607,745	-	Phenol-like
Phenol	730,558	-	Phenol-like
Eucalyptol	-	3,084,553	Flavoring agents, spicy
1-Hexanol	-	1,305,829	Green, fruity, oily
2-Ethyl-phenol	-	2,460,741	Phenol
1-Octanol	-	4,825,763	Waxy, green, orange, aldehydic, rose, mushroom
2,3-Butanediol	-	40,061,120	Fruity, creamy, buttery
[*R*-(*R**,*R**)]-2,3-Butanediol	-	8,755,687	Fruity, creamy, buttery
3-Methyl-2-octanol	-	862,904	Waxy, green, orange, aldehydic, rose, mushroom
Dimethyl-silanediol	-	33,263,024	Odorless
2,4-Di-*tert*-butylphenol	-	3,822,425	Phenolic
3-Ethyl-2-pentanol	-	4,151,441	Fermented, bready, yeasty
Ketone			
trans-β-Ionone	1,260,274	-	Floral, woody, violet
3,3-Dimethyl-2-hexanone	1,112,026	-	Acetone like odor
2,3-Octanedione	1,101,425	-	Coffee, palm
6-Methyl-2-heptanone	-	4,871,330	Cheesy, fruity, green, spicy, fermented
Styrene	-	6,604,858	Spicy, coffee, nutty nuances
1-Hydroxy-2-propanone	-	1,310,935	Sweet, mint, fruity
Benzophenone	-	4,208,046	Balsamic
Fatty acid			
Octanoic acid	1,232,148	1,452,734	Fatty, rancid, vegetable
Acetic acid	-	27,961,994	Green, sour, vinegar
Nonanoic acid	-	5,035,295	Waxy
n-Hexadecanoic acid	-	4,020,739	Waxy, fatty

second-order polynomial, but the regression coefficients that have a statistically significant effect on response should be considered in the equation. Therefore, developing another model fitting to the experiment could be an appropriate technique.

The regression analysis and analysis of variance in Online resource 6 and Table 1 showed that most parameters required to fit the developed model meet the requirements, other than the lack of fit of lightness analysis. The models that met all the required parameters were reliable. The significant lack of fit could occur in lightness response, due to noise in the experiment (Behera et al. 2018). To anticipate the significant lack of fit of lightness response, we performed a model verification, where the experimental work was performed to compare and determine the level of difference between predicted and validated optimal solutions, determined in the numerical optimization step. The verification demonstrated that the difference level was less than 5%, where this value suggested that the models established were accurate and able to explain the effect of each independent variable on the dependent variable. However, a piece of previous work suggests 10% in the level of difference between predicted and validated values (Mabazza et al. 2020).

Our sauce production scenario in the present study utilized wet mixing, where all the ingredients mixed were in the form of solution in water. This method helped form sauce with an appropriate homogeneity. Further analyses indicated that the viscosity of sauce was significantly affected by palm sugar and CMC, where CMC had more impact than palm sugar. Ergun et al. (2016) state that CMC is a thickening agent, which is swollen in water with a stabilizing capacity. In our present study, CMC might acidify the protein in sauce, which prevented protein precipitation. This phenomenon caused increased viscosity and improved the quality and the consistency of the end sauce product. Palm sugar contains sucrose as the major saccharide, which is soluble in water. Palm sugar is produced from concentrated nectar, which before being molded into sugar, is very viscous (Saputro et al. 2019). Therefore, when it is diluted in water and then concentrated again through heating, the solution becomes viscous due to loss of water. The reduction of moisture content is followed by the increase of the final viscosity of the solution (Judoamidjojo et al. 1985). This information shows why palm sugar has been used for a long time as the main sweetener in sauce products. In general, the presence of CMC and palm sugar in the sauce formulation contributed to the number of dissolved particles in sauce. Witono et al. (2015) stated the friction between dissolved particles in a solution drives viscosity, where the greater the number of particles, the more viscous the solution. According to our findings, we suggest that palm sugar and CMC have a synergistic effect on the viscosity of the sauce.

The color profile of the sauce was represented by the values of lightness, redness, and yellowness. According to the surface plots in Fig. 1b–d, all color properties presented a typical trend, where the more the palm sugar, the higher the lightness, redness, and yellowness of the sauce; however, CMC did not significantly affect the lightness and reduced the redness of the sauce. The trend also showed that palm sugar was the dominant influence on the color properties of the sea grape sauce. Indeed, palm sugar is dark brown in color, while CMC is white; thus, palm sugar would also play role in coloring the sauce. The color properties of sauce in our present study could be affected by two possible mechanisms, Maillard reaction and particle-particle interaction within the sauce solution. On the one hand, the Maillard reaction might occur during the sauce production as the ingredients contained sea grape protein and three saccharides, namely, palm sugar, glucose syrup, and CMC (Yang and Wei 2007; Witono et al. 2014). On the other hand, all ingredients mixed in the sauce formulation were water soluble, which made the solution become dense. The more the water-soluble components, the greater the number of particles in the sauce solution and a denser solution is obtained. Therefore, particle-particle interaction occurred, and then the color of the sauce became lighter. Saputro et al. (2017) stated that the more the sugar or carbohydrate in heated food ingredients, the more intense the Maillard reaction is, and the darker the color of the final product expected. Meanwhile, the food becomes lighter. Their study suggested that the color of the food was affected more by particle-particle interaction rather than the Maillard reaction.

Palm sugar had a positive correlation with the hedonic score, where the trend was like those of other responses such as lightness, viscosity, redness, and yellowness. In contrast, CMC had a negative correlation with the hedonic score, where the more the CMC, the lower the hedonic score obtained. Theoretically, too much CMC causes excess viscosity, which resulted in decreased customer preference. Therefore, the best hedonic score of formulated sea grape sauce in the present study had characteristics of viscosity, lightness, redness, and yellowness of 0.893 poise, 23.296, 7.300, and 19.565 respectively. This finding demonstrated the benefits of the use of response surface methodology with multiple responses, which could comprehensively solve the problem of production optimization of food products. According to the sauce formulation in this present work, we assumed that the taste was potentially formed by the combination between sea grape protein hydrolysate and palm sugar. A prior study by Tian et al. (2018) showed that sugar could intensify the savory taste of food.

GC-MS performed in this present study detected a typical seafood flavor in protein hydrolysate including octanal, benzaldehyde, nonanal and hexanal, which are native flavors in algae (Boonprab et al. 2006). These compounds are also found in fresh fish and shellfish (Alasalvar et al. 2005) and smoked fish (Guillen and Errecalde 2002; Guillen et al. 2006). Qi et al. (2017) demonstrated that hexanal, heptanal, and nonanal are compounds with a crab flavor identity. Some ketones such as pentadecane and octadecane are found in silver carp fish (Liu et al. 2009) and sesame paste (Shahidi et al. 1995). Octanoic acid, pyridine, and phenols are compounds that generate a shrimp flavor (Morita et al. 2003; Laohakunjit et al. 2014; Yu et al. 2010; Mouritsen et al. 2019). Four compounds with a typical seafood flavor in protein hydrolysate were detected in sauce (nonanal, hexanal, and (*E*)-2-octenal, and octanoic acid), while other compounds with a typical seafood flavor were not detected in the sauce. This information showed that the volatile compounds in protein hydrolysate, which were not detected in sauce, evaporated due to thermal processing in sauce production.

Most newly detected compounds in sauce had a bready/waxy/fatty flavor identity, and some compounds were native compounds in palm sugar such as nonanal, furfural, 5-hydroxymethylfurfural, *n*-hexadecanoic acid (Payet et al. 2005; Ho et al. 2007), and acetic acid (Saputro et al. 2019). Therefore, the integration area of nonanal was much higher than that of protein hydrolysate, due to the use of palm sugar in the sauce formulation. We detected volatile compounds in sauce, which typically formed through the Maillard reaction such as styrene (Goldmann et al. 2009), nonanal, octanal, furfural, benzene acetaldehyde, 5-hydroxymethylfurfural, 1-octanol, 3-methyl-2-octanol (Cui et al. 2017), 1-hexanol, 6-methyl-2-heptanone, and nonanoic acid (Karangwa et al. 2015). Using the combination of GC-MS data and referring to the previously published paper on flavor research, we suggest that the flavor of sauce was affected by seafood flavor from protein hydrolysate, native palm sugar flavor, and Maillard reaction product (MRP).

Conclusion

The present study employed a response surface methodology with central composite design to optimize the production of sea grape (*Caulerpa racemosa*) protein hydrolysate sauce, where multiple responses were mathematically modeled and validated. The analysis of variance demonstrated that palm sugar significantly affected all responses, while CMC affected all responses except the hedonic score. The optimal solution in the present work was conferred using palm sugar and CMC concentrations of 14.43% and 3.49%. The volatile component analysis revealed the presence of the typical seafood flavor in the sea grape sauce. To the best of our knowledge, the present work was the first study on the potential utilization of sea grape as the raw material of sauce with a seafood flavor. We suggest that our optimally formulated sea grape sauce could be worthwhile for the future commercialization of sea grape.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-020-02366-z.

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