Optimizing the preparation of seaweed-based edible cones with response surface methodology

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In the present study, we have prepared seaweed-based nutritious edible cones through per cent optimization of composite flour level using Box–Behnken design for response surface methodology. The three independent variables of the statistical design were pearl millet, wheat and seaweed powder from *Ulva lactuca* and *Gracilaria edulis*, and overall acceptability (OAA) and retention time (RT) were considered responses. A second-order quadratic polynomial equation was applied to the dataset of all responses to make predictions. *U. lactuca*-incorporated cones showed that the overall desirability function fits with the quadratic model at 99.77% significance level at a combination of 27.75% for pearl millet, 7.38% of wheat and 0.986% for *U. lactuca* with a predicted OAA of 8.2 and RT of 23.93 min, whereas in *G. edulis* cone, at 99.34% level of significance, the point prediction was at 27.94%, 7.95% and 1.006% for pearl millet, wheat and *G. edulis* with a predicted OAA of 8.331 and RT of 24.6 min.

Keywords: Composite flour, edible cones, *Gracilaria edulis*, response surface methodology, *Ulva lactuca*.

SEAWEEDS are known as 'superfoods' of *Gracilaria edulis* (GE: red algae) and *Ulva lactuca* (UL: green algae) selected for this study are found along the Indian coast. They are excellent sources to improve human nutrition, with considerable amounts of proven biologically active compounds like antioxidant, antimicrobial, antifungal, anti-inflammatory, antidiabetic, anticancer and antitumoral[1–5]. Seaweeds could be an excellent option for designing innovative foods, which are gaining growing interest among consumers and the food industry[6,7]. Numerous studies have explored the integration of seaweeds and/or their extracts into various food matrices, such as meat, cheese, pasta, etc., to improve nutritional and textural attributes, enhance antioxidant capacity and extend the shelf-life of products[8–10].

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Ice-cream cones play a pivotal role in the production and promotion of novelty-frozen desserts[11]. Sugar cones, often referred to as rolled ice cream cones, are made by rolling flat, waffle-like structures into conical shapes while they are still warm and malleable[12]. The primary ingredients for making ice-cream cones include wheat flour and sugar, with additional ingredients such as salt, water, emulsifier, preservatives, colourants, leavening agents and oils. Notably, refined wheat flour is a key ingredient in this process, but it contains low levels of essential amino acids and dietary fibre[13]. In response to this challenge, alternative cereal options derived from grains other than wheat have gained prominence. Among these, pearl millet (*Pennisetum glaucum* L.) is the fourth most important cereal in India, following rice, wheat and sorghum. It offers an economical source of staple food with comparatively high nutritional value. Kigozi *et al.*[14] have devised a production method for sorghum-based ice-cream cones and assessed their overall acceptability (OAA) and functionality, including retention time (RT).

A statistical method known as response surface methodology (RSM) is used to optimize the level of components and process conditions. It can assess the effect of various parameters and their inter-relations on the dependent variables and can be used to fit a quadratic polynomial model equation by selecting various design experiments. It also presents a good experimental design and result. This will reduce the number of trials, rendering it a straightforward and efficient choice compared to alternative statistical methods of optimization[15]. This design has been widely used to optimize the concentration of ingredients for developing bread sticks[16], vermicelli[17], edible composite films[18] and noodles[19]. Hence, the present study was conducted to optimize the extent of natural green ingredients for formulating seaweed-based, ready-to-eat, novel, tasty and healthy ice cream cones that do not contain preservatives, and are non-toxic with good OAA and RT. Moreover, the incorporation of *G. edulis* and *U. lactuca* will initiate a new area of blended seaweed foods that will aid in leveraging the health and functional gains of marine seaweeds.

Materials and methods

Materials

Seaweeds (*G. edulis* and *U. lactuca*) were collected from Hare Island, Thoothukudi, Tamil Nadu, India and rinsed thoroughly in fresh water to remove epiphytes, mud, sea sand and other unwanted particles. Then, the samples were shade-dried, pulverized, sieved and stored until further use. Pearl millet purchased from a local supermarket was cleaned using drinking water, drained and dehydrated at 65°C in a hot-air oven, pulverized, sieved and then used in the study. Other ingredients like wheat flour, salt, sugar, and leavened were purchased from a supermarket in Thoothukudi. All the ingredients were packed in airtight containers till further use.

Preparation of cones

The cones were prepared by blending composite flour of pearl millet (PMF), wheat (WF) and seaweed (SWP) with salt (0.3%), powdered sugar (7.5%), leavened (0.1%) and water (56%) using a Cephas 60 planetary mixer at low speed until a soft batter without clumps was formed. Next, the prepared batter was sheeted between preheated plates (150°C) of an electronic cone baker and baked for 4.5 min. Then, using a mould, the sheet was rolled into a cone shape and allowed to cool at room temperature before packing.

Optimizing processing parameters through RSM

For optimizing the percentage incorporation of PMF, WF and SWP for cone preparation, Box–Behnken design within the framework of RSM was adopted[20]. Three levels of PMF (*A*), WF (*B*) and SWP (*C*) were determined after reviewing preliminary studies performed at the Fish Processing Technology Department (Fisheries College and Research Institute, TNJFU, Thoothukudi), and 28% of PMF, 7.5% of WF and 1% of SWP were chosen as the centre points. Evaluation of OAA and RT of the cones served as the primary output parameters. Table 1 shows the codes and their respective independent variables. To develop the Box–Behnken quadratic design model, Design Expert 13.0 software was used, which allows comprehensive analysis with 17 experimental runs. Subsequently, the same software was used to generate response surface and contour plots.

Table 1. Independent variables and their levels

		Level		
Independent variable	Code	–1	0	+1
Pearl millet flour	*A*	26	28	30
Wheat flour	*B*	5	7.5	10
Seaweed powder	*C*	0.75	1	1.25

Determination of overall acceptability

A semi-trained assessment group ($n = 10$) was selected out of the Fish Processing Technology Department, Fisheries College and Research Institute (FCRI), Tamil Nadu Dr J. Jayalalithaa Fisheries University (TNJFU), Thoothukudi. The team members were chosen solely based on personal interest, scheduling and absence of food allergies. The evaluation team followed sensory parameters like appearance, colour, flavour, texture, taste and overall acceptability for rating the product based on a nine-point measurement, where 9 corresponds to ‘strongly like’, 8 to ‘very fond of’, 7 to ‘moderately fond of’, 6 to ‘slightly fond of’, 5 to ‘neutral’, 4 to ‘mildly dislike’, 3 to ‘moderately dislike’, 2 to ‘strongly dislike’, and 1 to ‘very strongly dislike’ (modified method of Peryam[21]).

Determination of retention time

For the assessment of cone retention capacity, or the time it takes for ice cream to seep through a specific cone, the prepared cones were placed in a test tube rack and filled with soft-serve ice cream. The time taken for ice cream to permeate through the exterior of a cone was measured in minutes and recorded as RT[22].

Data analysis

The analytical and quantitative data are presented as mean ± standard deviation (SD) of three replicates. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan’s post-hoc test. A significance level of $P < 0.05$ was used to determine statistical significance. Data analysis was conducted using SPSS software, version 20.

Results and discussion

Optimizing composite flour level for edible cones

To optimize the percentage ingredient levels for seaweed-based cones, 17 RSM combinations were devised both from *U. lactuca* and *G. edulis*. OAA and RT were the primary parameters considered for the preparation of cones[22]. Tables 2 and 3 represent the statistical analysis and interrelation between the independent (PMF, WF and SWP), and dependent (OAA, RT) factors. The model demonstrated significance in both instances, with a *P*-value less than 0.0001.

Table 2. Analysis of variance (ANOVA) for the quadratic *Ulva lactuca* cone model

Source	Overall acceptability					Retention time				
	Sum of squares	d*f*	Mean squares	*F*-value	*P*-value	Sum of squares	d*f*	Mean squares	*F*-value	*P*-value
Model	5.80	9	0.6447	304.52	<0.0001	84.12	9	9.35	3780.91	<0.0001
A-PMF	0.0242	1	0.0242	11.43	0.0117	6.09	1	6.09	2463.47	<0.0001
B-WF	1.48	1	1.48	698.68	<0.0001	4.05	1	4.05	1637.05	<0.0001
C-SWF	0.0612	1	0.0612	28.93	0.0010	0.3828	1	0.3828	154.85	<0.0001
AB	0.1056	1	0.1056	49.89	0.0002	8.32	1	8.32	3366.81	<0.0001
AC	0.1260	1	0.1260	59.53	0.0001	0.3540	1	0.3540	143.21	<0.0001
BC	0.0272	1	0.0272	12.86	0.0089	0.1296	1	0.1296	52.42	0.0002
A^2	0.4132	1	0.4132	195.15	<0.0001	40.05	1	40.05	16199.13	<0.0001
B^2	1.20	1	1.20	565.52	< 0.0001	0.0186	1	0.0186	7.53	0.0287
C^2	1.99	1	1.99	942.06	<0.0001	20.97	1	20.97	8481.19	<0.0001
Residual	0.0148	7	0.0021			0.0173	7	0.0025		
Lack-of-fit	0.0073	3	0.0024	1.29	0.3912	0.0092	3	0.0031	1.52	0.3382
Pure error	0.0075	4	0.0019			0.0081	4	0.0020		
Correlation total	5.82	16				84.14	16			
R^2	0.9975					0.9998				
Adjusted R^2	0.9942					0.9995				
Predicted R^2	0.9779					0.9981				
Adequate precision	46.9398					175.8077				

Table 3. ANOVA for the quadratic *Gracilaria edulis* cone model

Source	Overall acceptability					Retention time				
	Sum of squares	d*f*	Mean square	*F*-value	*P*-value	Sum of squares	d*f*	Mean square	*F*-value	*P*-value
Model	10.29	9	1.14	1850.32	<0.0001	94.96	9	10.55	1327.65	<0.0001
A-PMF	0.0435	1	0.0435	70.42	<0.0001	4.99	1	4.99	628.25	<0.0001
B-WF	0.0545	1	0.0545	88.13	<0.0001	4.93	1	4.93	620.32	<0.0001
C-SWF	0.0136	1	0.0136	22.03	0.0022	0.1922	1	0.1922	24.18	0.0017
AB	0.2070	1	0.2070	335.07	<0.0001	8.04	1	8.04	1011.34	<0.0001
AC	0.2704	1	0.2704	437.64	<0.0001	0.0992	1	0.0992	12.49	0.0096
BC	1.20	1	1.20	1940.62	<0.0001	0.0552	1	0.0552	6.95	0.0336
A^2	5.67	1	5.67	9169.89	<0.0001	44.14	1	44.14	5554.08	<0.0001
B^2	1.61	1	1.61	2598.50	<0.0001	0.8254	1	0.8254	103.86	<0.0001
C^2	0.5457	1	0.5457	883.19	<0.0001	26.27	1	26.27	3305.39	<0.0001
Residual	0.0043	7	0.0006			0.0556	7	0.0079		
Lack-of-fit	0.0029	3	0.0010	2.79	0.1738	0.0265	3	0.0088	1.22	0.4118
Pure error	0.0014	4	0.0003			0.0291	4	0.0073		
Correlation total	10.29	16				95.01	16			
R^2	0.9996					0.9994				
Adjusted R^2	0.9990					0.9987				
Predicted R^2	0.9952					0.9951				
Adequate precision	105.6297					100.011				

Optimization of U. lactuca-based ingredient levels

Among the three independent variables, the *P*-value of WF, *B* ($P < 0.0001$) was lower than that of *U. lactuca* powder, *C* ($P < 0.0010$) and PMF, *A* ($P < 0.0117$), indicating that WF has a greater influence than ULSWP (*Ulva lactuca* seaweed powder) and PMF on OAA, whereas in the case of RT, all the three ingredients showed a high and equal influence ($P < 0.0001$). To assess the adequacy of the selected model in describing the observed data, the lack-of-fit test was used. A *P*-value less than 0.05 indicates the adequacy of the model[23,24]. The lack-of-fit test yielded a *P*-value of 0.0001 for both OAA and RT. In this study, a notably high coefficient of determination (R^2) was observed for OAA (0.9975) and RT (0.9998), indicating a strong model-data fit. Additionally, the adjusted coefficient of determination was considered as a measure of significance of the model[25,26]. The adjusted coefficient of determination for OAA and RT was calculated as 0.9942 and 0.9995 respectively. Furthermore, the predicted R^2 values closely matched the adjusted R^2 values for both response variables. Consequently, the model was deemed appropriate for optimizing the

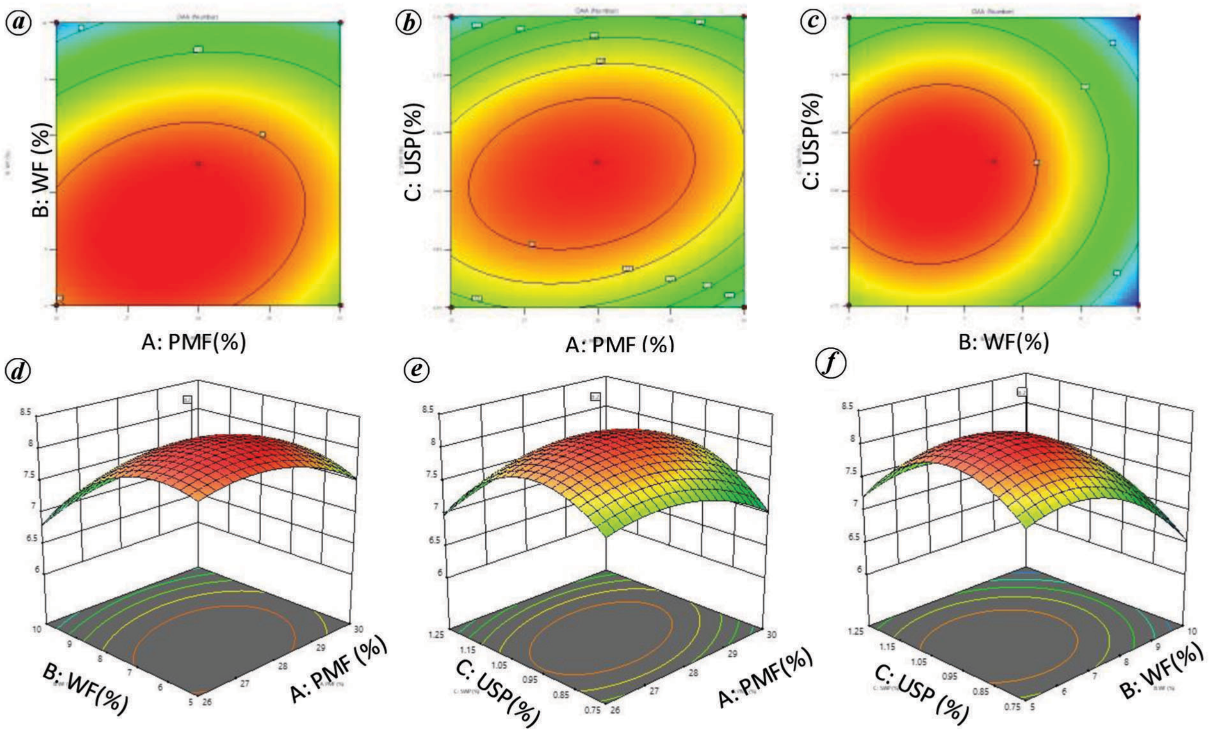

Figure 1. Contour and response surface analysis plots depicting the impact of flour of pearl millet (PMF), wheat (WF) and *Ulva lactuca* seaweed (ULSWP) on overall acceptance.

production of UL–based edible cones, with OAA and RT as the desired parameters. The optimal explanatory model equations for OAA and RT are as follows

$$\text{OAA} = +8.17 - 0.0550A - 0.4300B - 0.0875C + 0.1625AB + 0.1775AC + 0.0825BC - 0.3133A^2 - 0.5333B^2 - 0.6882C^2,$$

$$\text{RT} = +23.91 - 0.8725A + 0.7113B - 0.2187C - 1.44AB - 0.2975AC - 0.1800BC - 3.08A^2 - 0.0665B^2 - 2.23C^2,$$

where *A* is PMF, *B* the WF and *C* is ULP.

Two-dimensional contour plots and three-dimensional response surface plots were used to elucidate the influence of various factors on OAA and RT (Figures 1 and 2). These graphical representations provide insights into the interplay between the factors tested. The presence of significant interactions between the factors was assessed through the shape of the contour plots. Specifically, circular contour plots indicated negligible interactions, whereas elliptical contour plots indicated significant interactions, in accordance with established methodology[24]. In the context of OAA, the elliptical 2D contour plots (Figure 1 *a* and *b*) and 3D response plots (Figure 1 *d* and *e*) illustrate substantial interactions among PMF, WF and ULSWP. Conversely, the circular contour plots reveal that the interaction between WF and ULSWP is not statistically significant (Figure 1 *c* and *f*). For RT, the elliptical 2D contour plots (Figure 2 *a* and *c*) and 3D response plots (Figure 2 *d* and *f*) reveal interactions between PMF and WF, and WF and ULSWP respectively. In contrast, the interaction between PMF and ULSWP is not deemed significant, as revealed by the circular contour plot (Figure 2 *b*). The observed convex shapes of the 3D response surface plots (Figures 1 *d–f* and 2 *d–f*) under optimum conditions indicate the presence of well-defined optimal parameter combinations within the respective models[23]. These results underscore the robustness of the experimental findings.

Optimization of G. edulis-based ingredient levels

Among the three input factors, the *P*-value of PMF, A and WF, *B* ($P < 0.0001$) was lower than that of GESWP (*Gracilaria edulis* seaweed powder), *C* ($P < 0.0022$), indicating that PMF and WF have a greater influence than GESWP on OAA. In the case of RT, the *P*-value of GESWP, *C* ($P < 0.0017$) is also higher than those of PMF and WF ($P < 0.0001$). The *P*-value for the lack-of-fit test for OAA and RT was 0.0001, indicating a significant level of fit. Further, a high coefficient of determination was observed for both OAA (0.9996) and RT (0.9994), implying strong model-data agreement. Adjusted coefficients of determination were also estimated, resulting in values of 0.9990

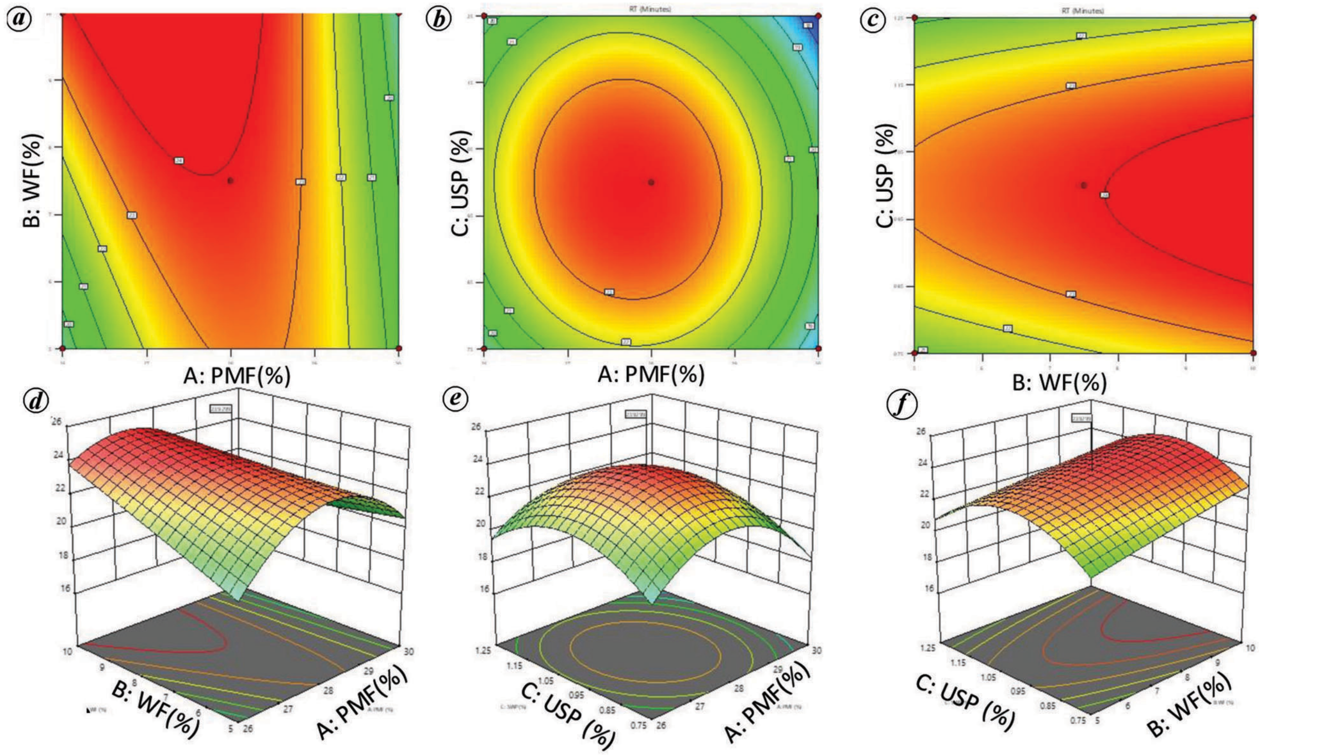

Figure 2. Contour and response surface analysis plots depicting the impact of PMF, WF and ULSWP on retention time.

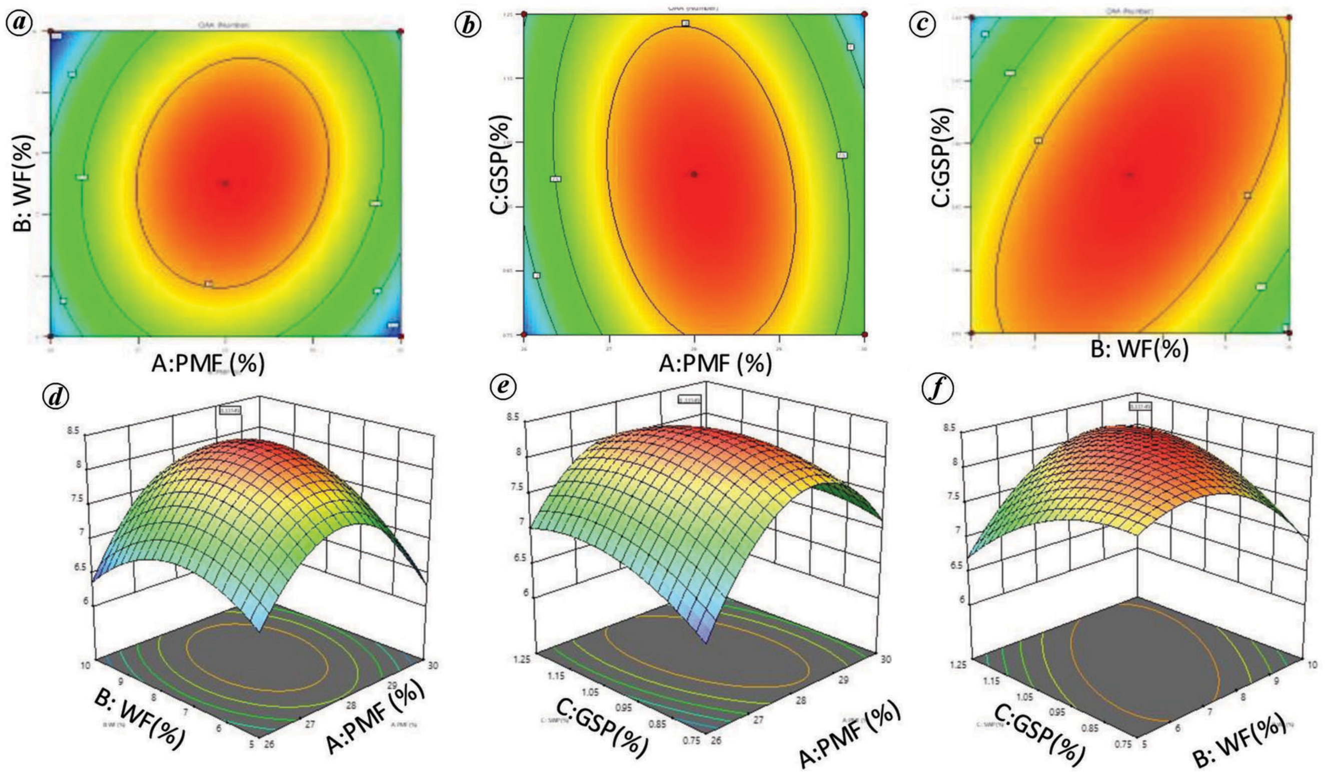

Figure 3. Contour plots and response surface analysis plots depicting the impact of PMF, WF and *G. edulis* seaweed powder (GESWP) on overall acceptability.

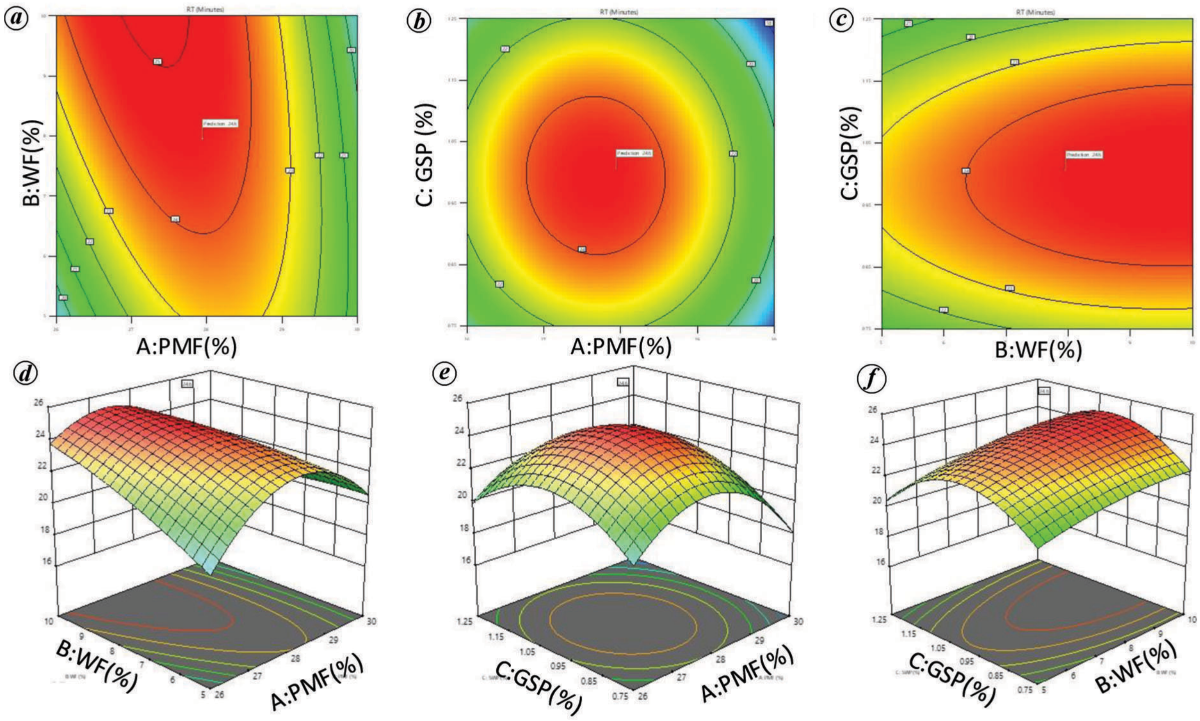

Figure 4. Contour plots and response surface analysis plots depicting the impact of PMF, WF and GESWP on retention time.

for OAA and 0.9987 for RT, signifying the robustness of the model in explaining the variations in these response variables. Additionally, the predicted R^2 values exhibited favourable agreement with the adjusted R^2 values for both responses. Consequently, the suitability of the model was affirmed. The most optimal explanatory model equations for OAA and RT are as follows

$$\begin{aligned}\text{OAA} = &+ 8.34 + 0.0738A + 0.0825B - 0.0412C \\ &+ 0.2275AB - 0.2600AC + 0.5475BC \\ &- 1.16A^2 - 0.6175B^2 - 0.3600C^2,\end{aligned}$$

$$\begin{aligned}\text{RT} = &+ 24.45 - 0.7900A + 0.7850B \\ &- 0.1550C - 1.42AB - 0.1575AC \\ &+ 0.1175BC - 3.24A^2 - 0.4427B^2 - 2.50C^2,\end{aligned}$$

where A is PMF, B the WF and C is GESWP.

The 2D contour plots and 3D response surface plots, illustrating the different combinations tested, are shown in Figures 3 and 4 for the evaluation of OAA and RT respectively, for GESWP cones. With regard to OAA of GESWP cones, the elliptical 2D contour plots (Figure 3 *b* and *c*) and 3D response surface plots (Figure 3 *e* and *f*) clearly depict the presence of significant interactions between PMF and GESWP, and WF and GESWP respectively. Conversely, the interaction between PMF and WF is insignificant (Figure 3 *a* and *d*). For RT, the circular contour plots (Figure 4 *b* and *e*) reveal that the interaction between PMF and GESWP is insignificant. This was further validated by the absence of an elliptical contour plot. The convex shapes observed in the 3D response surface plots (Figures 3 *d–f* and 4 *d–f*) are indicative of well-defined optimal conditions within the respective models. These findings affirm the robustness and suitability of the experimental data.

Conclusion

The model validation through three independent trials resulted in a mean value of OAA and RT of 8.19 and 24.04 min respectively, for *U. lactuca* cone and 8.33 and 24.6 min respectively, for *G. edulis* cone at a 99% confidence level. The cones without seaweed incorporation (control) and seaweed cones showed no statistical difference, indicating that the seaweed-based cones can be sensorily acceptable, and the RT of seaweed cones had increased by an average of 5.15 min than the control cones.

Conflict of interest: The authors declare that there are no conflict of interest.

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