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OPTIMIZING THE FORMULATION OF SEAWEED BASED EDIBLE CONES WITH RESPONSE SURFACE METHODOLOGY

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Abstract

The present study formulated seaweed based nutritious edible cones through percent optimization of composite flours, and the formulation was achieved though Box Behnken Design of Response Surface Methodology. The three independent variables of statistical design were pearl millet, wheat and seaweed powder from *Ulva lactuca* and *Gracilaria edulis*, and Overall Acceptability and Retention Time were considered responses. A second order quadratic polynomial equation was applied to the dataset of all responses to make predictions. *Ulva lactuca* incorporated cones shown 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% *Ulva lactuca* with a predicted overall acceptability of 8.2 and retention time of 23.93 min, whereas in *Gracilaria 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 *Gracilaria edulis* with a predicted OAA of 8.331 and retention time of 24.6 min.

Key Words: Seaweed cones, BBD, OAA, RT, Composite flours

1. INTRODUCTION

Seaweeds are known as "superfoods", of which the selected *Gracilaria edulis* (red algae) and *Ulva lactuca* (green algae) for this study were found along the Indian coast, are excellent sources of valuable content for human nutrition with considerable amounts of proven biologically active compounds like antioxidant, antimicrobial, antifungal, anti-inflammatory, antidiabetic, anticancer, antitumoral [1,2,3,4,5]. The selection of seaweed could be an excellent option for designing innovative foods, which are gaining growing interest among the consumers and food industry [6,7]. Numerous research investigations have explored the integration of seaweed and/or its extracts into various food matrices, such as meat, cheese, pasta [8,9,10] ,with the aim of improving nutritional and textural attributes, enhancing antioxidant capacity, and extending product shelf-life.

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 created by rolling flat waffle-like structures unto conical shapes while they are still warm and malleable [12]. The primary ingredients for crafting ice cream cones include wheat flour and sugar, with additional components such as salt, water, emulsifier, preservatives, colorants, 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 fiber [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*) stands out as the fourth most important cereal in India, following rice, wheat, and sorghum. It offers an economical source of staple food with comparatively higher nutritional value. [14] have devised a production method for sorghum-based ice cream cones and assessed their overall acceptability and functionality, including retention time.

A statistical method known as Response surface methodology (RSM) is harnessed to optimize the level of components and process conditions, and it can assess the effect of various parameters and their inter-relations on the dependent variables during technological operations, and can be used to fit a quadratic polynomial model equation through selection of various design experiments. It can also present good experimental design and result expression. It will reduce the quantity of trials, rendering it a straightforward and efficient choice when compared to alternative statistical methods accessible for the purpose of optimization[15]. This design was widely used to optimize concentrations of ingredients level for developing bread sticks[16], vermicelli [17], edible composite film[18], and noodles[19]. Hence, this investigation was executed to optimize the extent of natural green ingredients for formulating the seaweed-based ready to eat novel, tasty, and healthy ice cream cones that don't contain preservatives, and are non-toxic with good overall acceptability and retention time. Moreover, the incorporation of *Gracilaria edulis* (GE) and *Ulva lactuca* (UL) will trigger a new division of blended seaweed foods that will aid in leveraging the health and functional gains of marine seaweeds.

2. MATERIALS AND METHODS

2.1. Materials

Seaweeds (*G. edulis* and *U. lactuca*) were gathered at Hare Island, Thoothukudi, Tamil Nadu, India, rinsed with fresh water thoroughly for removing epiphytes, mud, sea sand and other unintended particles. Then the samples were shade dried, pulverized, sieved and stored for further usage. Pearl millet bought from a local super market was cleaned with drinking water, drained and dehydrated at 65^{0}C in a hot air oven, pulverized, sieved and used for preparation. Other ingredients like wheat flour, salt, sugar, leavened were purchased from D Mart, Thoothukudi. All the ingredients were packed in air tight containers for further usage.

2.2. 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%) by using a Cephas 60 planetary mixer at low speed until the formation of soft batter without clumps. Then, the prepared batter was sheeted between the preheated plates (150^{0}C) of electronic cone baker and baked for 4.5 min duration, then by using the mold, the sheet was rolled in to the shape of a cone and allowed to cool at atmospheric temperature before packing.

2.3. 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 Response Surface Methodology (RSM) was put into practice [20]. Three levels of PMF (A), WF (B) and SWP (C) were determined after reviewing the preliminary studies performed at the Fish Processing Technology department, and 28% of PMF, 7.5% of WF and 1% of SWP were chosen as the center points. Evaluation of cones overall acceptability (OAA) and retention time (RT) served as the primary output parameters. Table 1 displays the coded values alongside their respective independent variable values. To create the Box-Behnken quadratic design model, Design Expert 13.0 software was utilized, which allowed to conduct a comprehensive analysis with 17 experimental runs. Subsequently, the same software was used to generate response surface and contour plots.

2.4. Determination of overall acceptability

A semi-trained assessment group of (n=10) was selected out of the Fish Processing Technology department, FCRI, TNJFU, Thoothukudi and the evaluation team members were chosen solely in accordance with personal interests, scheduling, and absence of food allergies. The evaluation team followed sensory parameters like appearance, colour, flavour, texture, taste and over all acceptability for rating the product based on a 9-points 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 "strongly dislike" (modified method of [21]).

2.5. Determination of retention time

The assessment of cone retention capacity, or time it took for ice cream to seep through a specific cone, involved placing the prepared ice cream cones on a test tube rack and filling them with soft-serve ice cream. The time duration it took for the ice cream to permeate through the cone's exterior was measured in minutes and recorded as the cone's retention time [22].

2.6. Data analysis

The experiment's analytical and quantitative data were presented as the 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 employed to determine statistical significance. The data analysis was conducted using SPSS software, Version 20.

3. RESULTS AND DISCUSSION

3.1. Optimizing the composite flour level for edible cones

For optimizing the percentage ingredients level for seaweed-based cone, seventeen (17) RSM combinations were formulated both from *U. lactuca* and *G. edulis*. OAA and RT as the primary parameters to be considered a criterion for the formulation of cones [22]. The quadratic model statistical analysis and inter-relation in between the input factors i.e., PMF, WF and SWP with respect to OAA and RT for dependent values were furnished in Table 2 and 3 for *U. lactuca* and

G. edulis cones. The model demonstrated significance in both instances, with p-values less than 0.0001.

3.2. Optimization of *U. lactuca* (UL) based ingredients 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 the WF has a higher influence than the ULSWP and PMF on overall acceptability, where as in the case of retention time, 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 employed. A 'p' value of less than 0.05 indicates the model's adequacy [23,24]. The lack of fit test yielded a 'p' value of 0.0001 for both OAA, and RT. In this investigation, 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 the model's significance [25,26]. The adjusted coefficients of determination for OAA and RT were 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 production of UL–based edible cones, with OAA and RT as desired parameters. The optimal explanatory model equations for OAA and RT are as follows

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

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

Where A = PMF, B=WF, C=ULP

Two-dimensional contour plots and three-dimensional response surface plots were utilized to elucidate the influence of various combinations of factors on OAA and RT, as depicted in Fig 1 & 2. These graphical representations provided insights into the interplay between the factors tested. The presence of significant interactions between 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 (Fig 1 A and 1B) and 3D response plots (Fig 1D and 1E) illustrated substantial interactions between PMF, WF and ULSWP. Conversely, Fig 1C and 1F displayed circular contour plots, indicating that the interaction between WF and ULSWP was not statistically significant. For RT, the elliptical 2D contour plots (Fig 2 A and 2C) and 3D response plots (Fig 2D and 2F) pointed to noteworthy interactions between PMF & WF, and WF & ULSWP, respectively. In contrast, the interaction between PMF and ULSWP was not deemed significant as evidenced by the circular contour plot in Fig 2B. The observed convex shapes of the three-dimensional response surface plots (Figure 1D-F & Figure 2D-F) under optimum conditions[23] indicated the presence of well-defined optimal parameter combinations within the respective models. These results underscore the robustness of the experimental findings.

3.3. Optimization of *G. edulis* based ingredients

Between the three input factors, the value of ‘p’ for PMF, A & WF, B ($p<0.0001$) was lower than that of GESWP, C ($p<0.0022$), indicating that the PMF and WF have a higher influence than the GESWP on overall acceptability, where as in the case of retention time also p value of GESWP, C ($p<0.0017$) were higher than the p value of PMF and WF ($p<0.0001$). The p value for the lack of fit test, concerning OAA and RT was found to be 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 concordance. Adjusted coefficients of determination were also calculated, resulting in values of 0.9990 for OAA and 0.9987 for RT, signifying the model's robustness in explain the variations in these response variables. Additionally, the predicted R^2 values exhibited favorable agreement with the adjusted R^2 values for both the responses. Consequently, the model's suitability was affirmed. The most optimal explanatory model equations for OAA and RT were as follows:

$$OAA = +8.34+0.0738A+0.0825B-0.0412C+0.2275AB-0.2600AC+0.5475BC-1.16A^2-0.6175B^2-0.3600C^2$$

$$RT = +24.45-0.7900A+0.7850B-0.1550C-1.42AB-0.1575AC+0.1175BC-3.24A^2-0.4427B^2-2.50C^2$$

where A = PMF, B=WF, C=GESWP

2-dimensional contour plots and 3-dimensional response surface plots, illustrating different combinations tested, are provide in Fig 3 and 4 for the evaluation of OAA and RT concerning GESWP cones. Regarding OAA of GESWP cones, the elliptical 2D contour plots (Fig 3B and 3C) and 3D response surface plots (Fig 3E and 3F) clearly depicted the presence of significant interactions between PMF & GESWP and WF & GESWP, respectively. Conversely, the interaction between PMF and WF was not found to be significant (Fig 3A & D). For RT, the circular contour plots (Fig 4 B & 4E) indicated that the interaction between PMF and GESWP was not deemed significant. This was further validated by the absence of an elliptical contour plot. The convex shapes observed in the three-dimensional response surface plots (Figure 3D-F & Figure 4D-F) are indicative of well-defined optimal conditions within the respective models. These findings affirm the robustness and suitability of the experimental data.

4. Conclusions

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

5. Acknowledgment

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6. Conflict of Interest

The authors confirm that there are no conflicts of interest to disclose.

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Table 1 Independent variable and its levels

Independent variables	**Codes**	**Levels**		
		-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

Table 2: Analysis of Variance (ANOVA) for the quadratic *Ulva lactuca* cone (ULC) model

	OVERALL ACCEPTABILITY					**RETENTION TIME**				
Source	**Sum of squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of squares**	**df**	**Mean Square**	**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		
Cor Total	5.82	16				84.14	16			
R 2	0.9975					0.9998				
Adj R 2	0.9942					0.9995				
Pred R 2	0.9779					0.9981				
adeq precision	46.9398					175.8077				

Table 3: **Analysis of Variance (ANOVA) for the quadratic *Gracilaria edulis* cone (GEC) model**

	OVERALL ACCEPTABILITY					**RETENTION TIME**				
Source	**Sum of squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of squares**	**df**	**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		
Cor Total	10.29	16				95.01	16			
R^2	0.9996					0.9994				
Adj R^2	0.9990					0.9987				
Pred R^2	0.9952					0.9951				
Adeq precision	105.6297					100.011				

Figure 1 Contour and response surface analysis plots depicting the impact of PMF, WF and ULSWP on overall acceptance

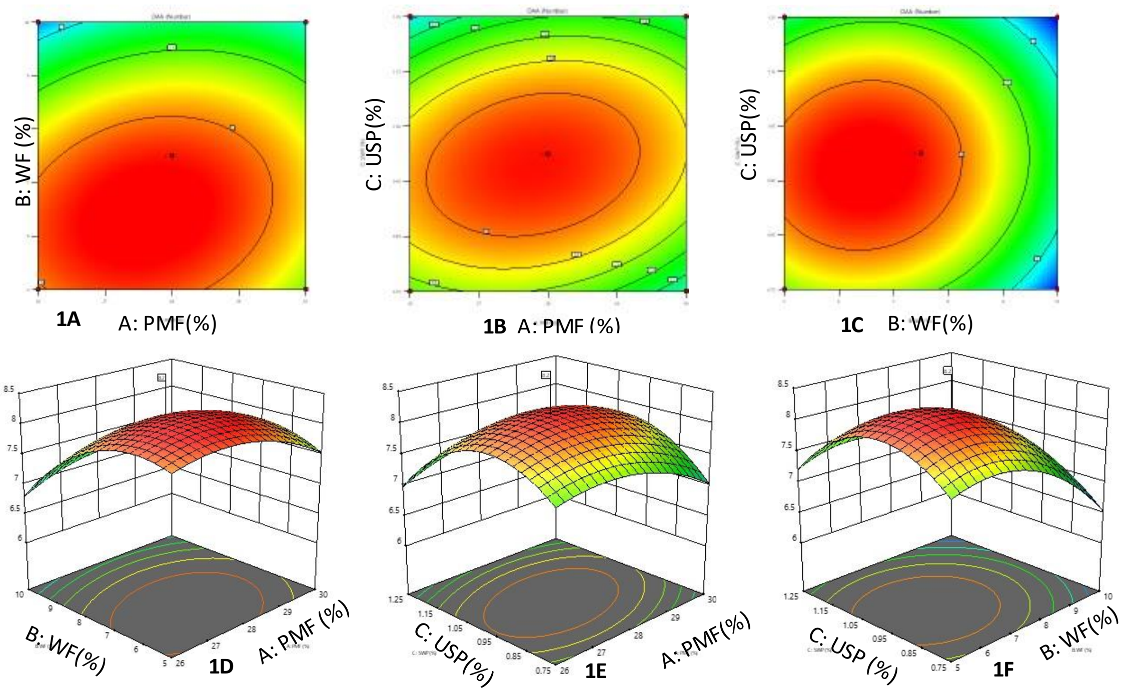

Figure 2 Contour and response surface analysis plots visualizing the impact of PMF, WF and ULSWP on Retention time

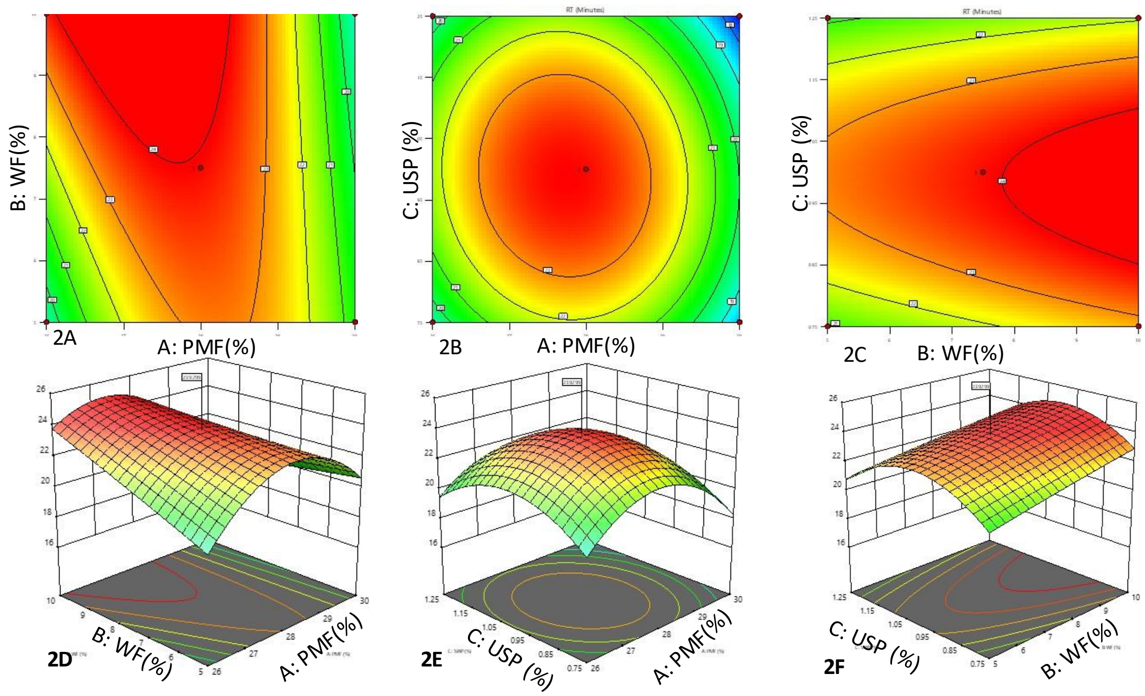

Figure 3 Contour plots and response surface analysis plots depicting the impact of PMF, WF and GESWP on overall acceptability

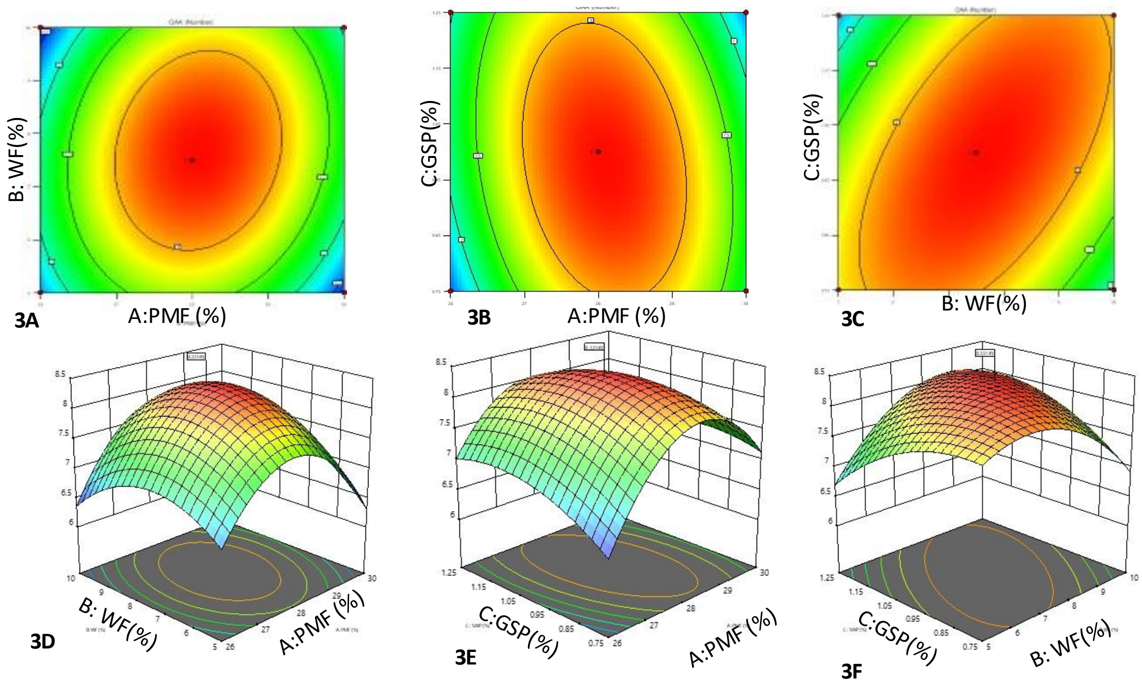

Figure 4 Contour plots and response surface analysis plots visualizing the impact of PMF, WF and GESWP on Retention time