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Properties of an Emulsion Gel with *Gracilaria fisheri* Agar, Whey Protein Isolate and Black Seed Oil

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ABSTRACT

Gracilaria fisheri agar has beneficial potential for human health; however, its application in food products needs to be incorporated into an emulsion gel due to its low gel strength. A response surface methodology with a central composite design was utilized to prepare emulsion gels containing 4–6% *Gracilaria fisheri* Agar (AGF), 4–8% Whey Protein Isolate (WPI) and 10–20% Black Seed Oil (BSO). The optimum levels of AGF, WPI, and BSO were determined to be 6%, 4% and 10% respectively to give the preferred gel strength of 966.24 g, water holding capacity of 96.12%, and DPPH antioxidant activity of 59.16%.

Keywords: antioxidant, emulsion gel, *Gracilaria fisheri* agar, optimization, properties

INTRODUCTION

Gracilaria fisheri is a red seaweed found mainly along the southern coast of Thailand and the east coast of peninsular Malaysia. Due to its relatively low gel strength, the utilization of *Gracilaria fisheri* Agar (AGF) in food products is in the form of composite material, specifically emulsion gel, rather than in its native form. The stability of the emulsion gel is attributed to its three-dimensional network structure, which prevents flocculation and coalescence of oil droplets (Li *et al.* 2020). The use of Black Seed Oil (BSO) as the oil phase in the emulsion gel may improve its oxidative stability, while emulsifiers such as Whey Protein Isolate (WPI) tend to form multiple viscoelastic films around the emulsion droplets, strengthening the network structure (Nor Hayati *et al.* 2020). However, the effect of AGF and its interaction with WPI and BSO on emulsion gel properties remains unexplored, which could provide a novel application of AGF in the food industry.

METHODS

The effects of three independent variables, namely *Gracilaria fisheri* Agar (AGF: 4–6%), whey protein isolate (WPI: 4–8%), black seed oil (BSO: 10– 20%), and their interactions on all dependent variables related to gel strength, water holding capacity, and DPPH antioxidant activity of emulsion gels, as well as optimization procedures on the AGF, WPI, and BSO amounts, were also studied using a response surface methodology. A total of twenty experimental runs were conducted, and the experimental data obtained were fitted to the generalized polynomial model. Numerical optimization was performed by the response optimizer using software to determine the exact optimum levels of individual and simultaneous multiple response optimizations. The Minitab software (Release 14) (Minitab Inc. USA) was used to create the experimental design, perform data analysis, and generate contour and surface plots, and the confidence level used was 95%. For validation, a one-sample t-test was employed to compare the experimental values (n=4) of gel strength, Water Holding Capacity (WHC), and DPPH antioxidant activity obtained from the optimal emulsion gel formulation with the predicted values (Fontes-Candia *et al.* 2021; Nor Hayati *et al.* 2020; Utama *et al.* 2018).

RESULTS AND DISCUSSION

The study revealed that the incorporation of AGF (4–6%) into emulsion gel containing WPI (4–8%) and BSO (10–20%) resulted in

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a gel strength of 291.61–927.04 g, WHC of 86.26–97.15% and DPPH antioxidant activity of 43.19– 59.56%. The data were adequately fitted to the quadratic model with high coefficients of determination (R^2=0.98–0.99). Importantly, no lack of fit was observed, indicating that the model accurately described the relationship between the variables (Table 1).

Moreover, the regression analysis revealed a significant ($p<0.05$) synergistic interaction between AGF and WPI, leading to an increase in the gel strength of the emulsion gel. In contrast, significant ($p<0.05$) antagonistic interactions between them led to a decrease in both WHC and DPPH antioxidant activity of the emulsion gel. This implies that when AGF and WPI were combined in certain proportions, the overall effectiveness of the gel in holding water and exerting antioxidant activity was reduced. Additionally, the linear effect of AGF was the most significant ($p<0.05$) in increasing both WHC and DPPH antioxidant activity, while the quadratic effect of AGF demonstrated the most significant ($p<0.05$) increasing effect on the gel strength of the emulsion gel. With this insight into the interaction effect, the levels of each AGF, WPI, and BSO were adjusted to achieve optimal properties of the emulsion gel. Consequently, the optimized emulsion gel was obtained at 6% AGF, 4% WPI, and 10% BSO, resulting in a preferred gel strength of 966.24 g, water holding capacity of 96.12%, and DPPH antioxidant activity of 59.16%. Importantly, all of these experimental values fell within the predicted values as indicated by the respective quadratic models (Table 2).

Table 1. Summary of ANOVA results for the fitted regression models[a]

Data Range	Gel strength (g) (Y_1)		Water holding capacity (%) (Y_2)		DPPH antioxidant activity (%) (Y_3)	
	Coefficient	p^*	Coefficient	p^*	Coefficient	p^*
Model	1928.02	0.001	55.1581	0.000	18.9175	0.024
Linear		0.000		0.000		0.000
Quadratic		0.000		0.001		0.000
Interaction		0.016		0.000		0.001
Lack of fit		0.959		0.740		0.067
R^2 value	0.981		0.995		0.991	
R^2 adjusted	0.970		0.991		0.984	

*[a]Coefficient value for each linear, quadratic and interaction term is not presented.

**Significant if $p<0.05$; DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2. The predicted, experimental and p-values of the optimized point on G. fisheri agar (6%), whey protein isolate (4%) and black seed oil (10%)

Responses	Predicted value (95% CI)	Experimental value (n=4)	p^*
Gel strength (g)	857.276−974.161	966.240	0.437
Water holding capacity (%)	95.6620−96.7702	96.1175	0.686
DPPH antioxidant activity (%)	58.8366−60.6880	59.1575	0.128

*Significant if $p<0.05$ determined by a one-sample t-test

CI: Confidence Interval

CONCLUSION

The study demonstrated that the addition of AGF, in combination with WPI and BSO, resulted in improved emulsion gel properties. The interaction between AGF and either WPI or BSO had a positive effect on the structural integrity and water retention of the emulsion gels, while also imparting antioxidant properties.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declares.

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