

Physicochemical Properties and Stability of Emulsions Containing Carotenoid-Rich Pumpkin Extract, Whey Protein Concentrate and Carboxymethylcellulose

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ABSTRACT

A response surface methodology with a central composite design was employed to investigate the effects of Carotenoid-Rich Pumpkin Extract (CE), Whey Protein Concentrate (WPC), and Carboxymethyl Cellulose (CMC) on the physicochemical properties and stability of emulsions. The WPC-CMC synergism resulted in the formation of a thick layer around the droplets, thus improving the emulsion system. The optimum levels of CE, WPC, and CMC were determined to be 6.00, 2.00, and 0.87%, respectively, to provide the desired emulsion with a pH of 4.18, a carotenoid degradation stability index of 0.88, a peroxide formation stability index of 0.77, and an apparent viscosity of 0.61 Pa.s.

Keywords: carotenoids, emulsions, physicochemical properties, stability, optimization

INTRODUCTION

Carotenoids are sensitive to environmental factors and are lipophilic, which limits their use in food applications. To overcome this drawback, carotenoids are encapsulated in the form of oil-in-water emulsions, which require precise manufacturing to ensure their effectiveness as delivery systems. This encapsulation approach also enhances their stability during manufacturing processes and storage (Pinna *et al.* 2022). One factor contributing to their stability is the presence of a polysaccharide-protein complex in the system, which imparts favorable interfacial properties and further aids in stabilization. In line with this, Kaade *et al.* (2022) demonstrated that the chemical stability of D-limonene-containing emulsions can be improved by employing a complex of whey protein and Carboxymethyl Cellulose (CMC). However, the effect of this complex on the physicochemical properties and stability of carotenoid-containing emulsions remains unknown. Therefore, this study investigated the effects of Whey Protein Concentrate (WPC) and CMC on the physicochemical properties and stability of the emulsions in the presence of Carotenoid-rich Extract (CE).

METHODS

Carotenoids in pumpkin were extracted by mixing the pumpkin pulp with a mixture of acetone and petroleum ether (1:1) and lyophilized to obtain CE. Oil-in-water emulsions were prepared using CE (2–6%), WPC (0.5–2.5%) and CMC (0.2–1%) with a 10% oil fraction based on a central composite design. The pH of the emulsions was determined using a pH meter. The total carotenoid content was determined at 450 nm using a UV-visible spectrophotometer (Yuan *et al.* 2008). The peroxide value was determined using the AOAC standard method. Apparent viscosity was measured using a programmable rotational viscometer. The experimental data obtained were fitted to a generalized polynomial model. Experimental design, data analysis and plotting of interaction, and response optimization were performed using Minitab software (Release 14) with 95% confidence interval. The best model fitted to each response was selected based on significant model fit ($p < 0.05$), non-significant lack of fit ($p > 0.05$), and high coefficient of determination values ($R^2 > 0.8$) (Nor Hayati *et al.* 2020).

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RESULTS AND DISCUSSION

The study revealed that the components of the emulsion (CE, WPC, and CMC) had a significant effect on the physicochemical properties and stability of the emulsions. Response surface models were successfully fitted to all variables with high coefficients of determination ($R^2=0.98-0.82$) (Table 1). CMC significantly ($p<0.05$) influenced most of the responses studied. The amount of CE influenced pH, stability against carotenoid degradation, and

peroxide formation. Increasing CE led to higher pH but decreased stability against peroxide formation, although it exhibited significant synergism with CMC.

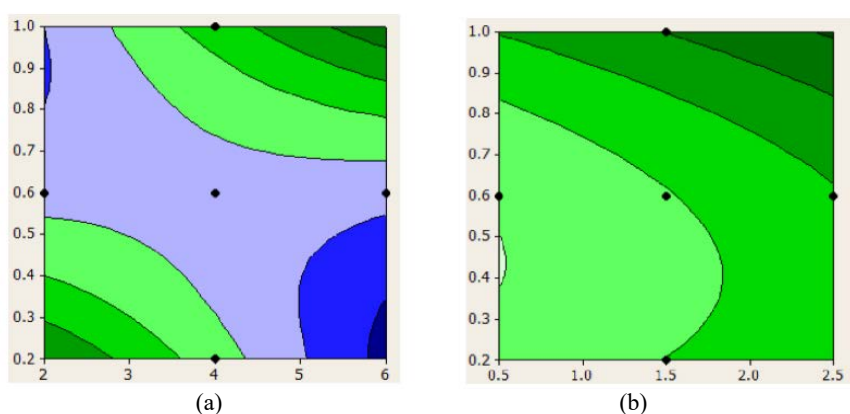
CE and WPC synergistically affected the stability against carotenoid degradation, while the strongest effect on physicochemical properties and stability came from the synergism between WPC and CMC due to the formation of a thick layer of protein-polysaccharide complexes that coated the droplets (Figure 1). The CMC-WPC synergism also increased the apparent viscosity

Table 1. The response surface models fitted to the experimental data

Responses*	Fitted model equations**
pH	$2.626 + 0.189X_1 + 0.220X_2 + 1.425X_3 - 0.005X_1^2 + 0.016X_2^2 - 0.525X_3^2 - 0.017X_1X_2 - 0.061X_1X_3 - 0.1031X_2X_3$ (1)
CD stability index	$0.770 - 0.164X_1 + 0.392X_2 + 0.229X_3 + 0.005X_1^2 - 0.081X_2^2 - 0.224X_3^2 + 0.038X_1X_2 + 0.127X_1X_3 - 0.384X_2X_3$ (2)
PV stability index	$1.680 - 0.196X_1 - 0.117X_2 - 2.076X_3 - 0.004X_1^2 + 0.001X_2^2 + 0.082X_3^2 + 0.021X_1X_2 + 0.311X_1X_3 - 0.016X_2X_3$ (3)
Apparent viscosity (Pa.s)	$0.376 - 0.096X_1 + 0.029X_2 - 1.068X_3 + 0.014X_1^2 + 0.014X_2^2 + 1.338X_3^2 + 0.015X_1X_2 - 0.038X_1X_3 + 0.075X_2X_3$ (4)

*CD: Carotenoid Degradation; PV: Peroxide Formation

** X_1 : Carotenoid-rich extract; X_2 : Whey protein concentrate; X_3 : Arboxymethy



CE: Carotenoid-Rich Extract; WPC: Whey Protein Concentrate; CMC: Carboxymethyl Cellulose

Figure 1. Interaction plots for (a) carotenoids degradation stability index and (b) peroxide formation stability index

of the emulsion. The optimal levels of CE, WPC, and CMC were found to be 6.00%, 2.00%, and 0.87%, respectively, resulting in a desirable emulsion with a pH of 4.18, a carotenoid degradation stability index of 0.88, a peroxide formation stability index of 0.77, and an apparent viscosity of 0.61 Pa.s. This optimization revealed that the emulsion could function as an optimum delivery system at this level of combination with such properties and oxidative stability, as CE was predicted to be the most stable to degradation. The experimental and predicted values were well within the predicted range and the adequacy of the final reduced models fitted by RSM was verified (Nor Hayati *et al.* 2020).

CONCLUSION

In this study, synergistic interactions were observed, particularly between WPC and CMC, resulting in significant effects on emulsion characteristics. Optimal levels of CE, WPC, and CMC were determined to achieve desirable emulsion physicochemical properties and stability. The adequacy of response surface models' adequacy was confirmed, supporting their reliability for emulsion formulation, as a potential delivery system for carotenoids.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declares.

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