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RESPONSE SURFACE MODELLING OF DRIED CHARD AND BLACK CURRANT ON CIE-a*, NOMb, AND MDA IN A REFRIGERATED COMMINUTED MEAT MODEL

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I. INTRODUCTION

In dry-cured sausages, synthetic nitrate and nitrite ensure a desirable colour via the formation of nitrosylmyoglobin, control lipid oxidation, and serve as powerful antimicrobial agents against spoilage and pathogenic organisms. However, the established association between nitrite/nitrate and the formation of nitroso-carcinogenic compounds has led to their reduction or elimination in meat products and the search for alternatives to their utilization [1]. The use of plant and fruit extracts as substitutes for nitrifiers has gained ground for processed meat applications. The incorporation, at the optimal levels, of these replacers should provide reformulated dry uncured sausages with colour and oxidative stability characteristics like those formulated with nitrifiers [2].

The study aims to evaluate, using experimental design, the effect of the inclusion of dried chard (DC) (*Beta vulgaris* L.) and aqueous black currant extract (BCE) (*Ribes nigrum* L.) on CIE a*-value, malondialdehyde (MDA), and nitrosylmyoglobin (NOMb) contents in a comminuted meat model under refrigerated storage (7 days at 4ºC).

II. MATERIALS AND METHODS

Chard was size reduced, dried at 60ºC for 72 h, and ground using knife mill. Dehydrated black currant was extracted with water (1:9, w/v) with the addition of Pectinex Ultra XXL and extracted in a rock and roller mixer for 60 min at room temperature. Before being incorporated into meat models, the extract was diluted with deionized water (1:1, v/v).

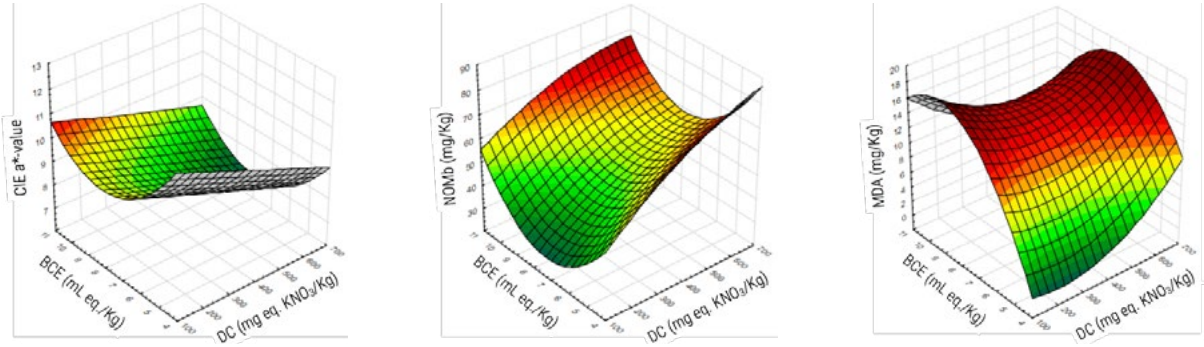
A three-level full factorial design was applied to modelized the effect of DC and BCE on CIE a*-value, NOMb and MDA contents. The incorporation of DC (mg KNO_3 equivalent/kg) and BCE (mL/kg) was studied at three levels (150, 300 and 600 for DC and 5, 7.5 and 10 for BCE).

The comminuted meat model was formulated with Iberian pig meat, salt (2%), sodium ascorbate (0.04%), DC and BCE (as required in the experimental design) and water (to equalize the volume of water added with BCE). Five samples/experimental groups were disposed in RODAC plates, sealed, and stored at 4ºC for 7 days in the dark. The meat model was designed to simulate the conditions of the initial stage temperature used for drying Iberian dry-cured sausages.

Nitrate quantification in chard was assayed by ion-pair reversed-phase liquid chromatography [3]. The HPLC analysis was performed in a Kinetex® 2.6 µm C18 (100 x 3.0 mm) column using 5 mM tetrabutylammonium hydrogen sulphate + 5 mM ammonium bicarbonate (pH 6.5 with NaOH) as mobile phase at a flow rate of 0.5 mL/min and detection UV/Vis at 218 nm. The AMSA meat colour measurement guidelines [4] were followed for the determination of the CIE a*-value. The NOMb content determination was conducted spectrophotometrically according to Lavado *et al.* [5]. The MDA concentration was assessed according to Lavado *et al.* [5]. The experimental data were processed using XLSTAT v. 2009 [6] and Statgraphics Centurion XVI [7].

III. RESULTS AND DISCUSSION

The experiments performed under different levels of DC and BCE provided information about the formation of NOMb, redness (CIE a*-value) and lipid oxidation in comminuted meat under refrigerated storage. Regression analysis revealed a good fit of the model for NOMb and MDA with determination coefficients (R^2) for the models with values of 0.971 and 0.950 for NOMb and MDA, respectively. The determination coefficient for the model of CIE a*-value was 0.845. The ANOVA results for each response variable indicated that DC and BCE on the model can explain the experimental variation for the NOMB (P > F = 0.0159) and MDA (P > F 0.0362); but not for CIE a*-value (P > F = 0.1796).



CIE a* = 8.25 – 0.88 * [DC] – 0.39 * [BCE] + 0.01 * [DC]^2 + 1.02 * [BCE]^2 + 0.09 [DC] * [BCE]; (R^2 = 0.845)

[NOMb] = 47.36 + 11.95 * [DC] – 0.26 * [BCE] – 2.84 * [DC]^2 + 11.86 * [BCE]^2 – 2.15 [DC] * [BCE]; (R^2 = 0.972)

[MDA] = 13.87 + 0.17 * [DC] + 3.02 * [BCE] + 1.99 * [DC]^2 – 4.26 * [BCE]^2 – 1.97 * [DC] * [BCE]; (R^2 = 0.950)

Figure 1. 3D Surface plot of CIE a*-value (left), NOMb (center) and MDA (right), against dehydrated chard (mg/kg) and black currant extract (mL/kg). (X-axis: Dehydrated chard (mg KNO_3 eq./Kg; Y-axis: Black currant extract (mL/Kg); Z-axis: CIE a*, [NOMb], [MDA]).

The optimal combination of DC and BEC was determined by maximizing the desirability of the responses. Ideally, the maximal desirability should be at the maximum value of CIE a* and the higher NOMb concentration and the lowest MDA contents. The obtained desirability was 0.776. The optimum level of addition was DC: 356.41 mg KNO_3 eq./Kg and BCE: 5.0 mL/Kg.

IV. CONCLUSION

The use of response surface methodology allowed the simultaneous effect of dehydrated chard and blackcurrant extract on CIE a*, NOMb and MDA formation to be studied. Further research should be undertaken to assay the obtained optimal levels of DC and BCE when added to uncured dry sausages.

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