IMPROVEMENT OF FOOD SAFETY AND SHELF LIFE OF REFRIGERATED CROQUETTES BY USING EVOO ENCAPSULATED IN CYCLODEXTRINS IN BREADCRUMBS

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Abstract: *Despite the desired sensorial characteristics proportionated by deep-fat frying, the fried breaded products are poorly perceived to consumers for health concerns. In the present study, the addition of 0 % and 33 % EVOO-α-CD in wheat and corn breadcrumbs in croquettes manufacturing was studied. The processing steps for 33% EVOO-α-CD croquettes consisted of pre-cooking under short-wavelength infrared oven and final cooking by hot air (home) oven, while control (0 % EVOO-α-CD) croquettes were no pre-cooked, and final cooking was by deep-fat frying. The physicochemical and sensory quality, and fat, fiber and acrylamide contents of croquettes were studied during refrigerated storage (at 4 ºC for 20 days). Higher fiber content and lower acrylamide and fat levels were found in samples fortified with 33 % of EVOO-α-CD. Microbial load of mesophilic microorganisms were decreased when incorporating EVOO-α-CD, as it reduced up to 5 log CFU/g. In conclusion, a fried-free infrared-baked croquette with EVOO-α-CD reduces fat content and acrylamide formation, and extends its refrigerated shelf life, obtaining a healthier product.*

Keywords: Extra Virgin Olive Oil, Low-fat content, High-fiber content, Inclusion complex, Acrylamide.

1. INTRODUCTION

Food market trends are focused on the development and/or improvement of products such as ready meals that are healthier and more nutritious, due to the growing awareness of healthier lifestyles, hence healthier eating. The high fat content of ready meals, as breaded meat products, range from one third to 50% of their weight. It is caused by the procedure of pre-cooking (the deep-fat frying operation) currently used in this food industry, which threatens seriously their demand [1], [2].

Croquettes are globally recognized as one of the most popular ready-to-cook fried foods, hence they are highly demanded. Their shape and taste (composition) vary in light of consumption trends of cultural preferences and their quality relays on its formulation and processing [1], [3].

The present study was carried out to evaluate the effect of incorporating extra virgin olive oil (EVOO) encapsulated in α-CD (alpha-cyclodextrins) in croquettes formula combined with a short-wavelength infrared baking-procedure. The purpose of this study is the recreation of a fried-free healthier croquette version by employing baking procedures and the addition of 33 % of EVOO-α-CD in the breadcrumbs used in manufacturing of croquettes. Sensory prefe-

rence, nutritional status and acrylamide levels were evaluated in order to achieve a healthier consuming option of a ready to cook meal for consumers.

2. MATERIAL AND METHODS

2.1. Materials

Croquette's ingredients were all purchased, except for the hydrocolloid, from a local supermarket in Cartagena (Murcia, Spain). Xanthan gum was supplied by Doscadesa S.L. (Molina de Segura, Murcia, Spain). Wheat breadcrumbs were purchased from a local supermarket in Cartagena, while corn breadcrumbs were supplied by the company Fripozo S.A. The EVOO-α-CD complex (in powder form) was prepared and supplied by the company Bioencapsulation and iPackaging S.L. (Fuente Álamo, Murcia).

2.2. Preparation of croquettes

Croquettes were prepared following the recipe of the patent ES2440092A1 [4]. Briefly, 100 g of croquettes (~12 g/croquette) required 80 g of EVOO, 160 g of unsalted butter, 60 g of fresh onion, 300 g of wheat flour, 1200 g of whole milk, 3 g of salt, and 5 g of xanthan gum. Once the dough was prepared, it was left to cool at room temperature for 10 min and then refrigerated (4 ºC) for 45 min. Obtained croquette dough was manually shaped in croquettes units, battered in 0.25 % xanthan gum, and breaded with either wheat (W) or corn breadcrumbs (C) with 0% or 33% of EVOO-α-CD powder. The EVOO-α-CD inclusion complex was prepared according to [5]. Croquettes with 0% of EVOO-α-CD had no pre-cooking, and final cooking was carried out by deep-fat frying (following market's guidelines). Croquettes with 33% of EVOO-α-CD in breadcrumbs were precooked under a short-wavelength infrared oven (at 360 ºC for 1.34 min) (in a roller grill model CT3000B; Roller Grill International, Bonneval, France) and final baking was effectuated with a home hot air oven (forced convection, at 150 ºC for 5-20 min) (oven model HBC36P753; Bosch, Germany) (this manufacturing method was named as new technology, NT). Then, croquettes were frozen at -20 ºC for 24 h and, then, thawed and stored at 4 ºC for 3 weeks' time, analyzing physicochemical characteristics, microbiology, sensory acceptation, fat content, fiber content, and acrylamide content, throughout storage.

2.3. Color determination

A portable colorimeter (Konica Minolta chromameter CR-400, Osaka, Japan) was used for croquette color assessment, both before and after final cooking (frying or baking). Measurements were taken on three different sides of each croquette, and the mean values of the parameters L^*, a^* and b^* were obtained. Color readings after final cooking for 0% EVOO-α-CD croquettes were taken after frying in sunflower oil at 180 ºC for 90 s, while for 33% EVOO-α-CD croquettes samples were taken at 5, 10 and 20 min after baking with air at 150 ºC.

2.4. Fat and fiber analysis

Croquette fat content was quantified according to the ISO 17059 method [6]. After weighing the croquette sample, it was placed in a 33 mm x 80 mm porous cellulose thimble (Whatman 10350240) covered with cotton. Fat was extracted at 69 ºC for 4 h in a solvent extractor, conventionally equipped, using 200 mL of n-hexane 96%. Solvent was later removed by a vacuum rotary evaporator at 40 ºC for 30 min at a rate of 40 rpm. Fat weight was calculated by subtracting the difference between the weight of the flask with the extracted fat and the weight of the empty flask. Quantification of total lipid content was determined dividing fat weight by the amount of sample. Fiber content was assessed following the enzymatic-gravimetric standard protocol.

2.5. Microbiological analysis

Samples of the four croquettes treatments (0 % and 33 % EVOO-α-CD croquettes breaded with corn or wheat breadcrumbs) were removed from storage at predefined times for microbiological evaluation (yeasts, molds, *Pseudomonas spp.,* enterobacteria, psychrophiles, mesophiles and lactic acid bacteria). Three replicates of 2 halves of croquettes (10 g) per treatment were homogeneously mixed for 1 minute in a stomacher (IUL Masticator 90002401, IUL S.A., Spain) with 90 mL sterilized buffered peptone water. 10-fold dilution series were performed for each microbial group. Mesophilic, psychrotrophic, Enterobacteria, *Pseudomonas spp*, and lactic acid bacteria were pour-plated, whilst molds and yeast were spread-plated. Plate Count Agar (PCA; Scharlau Chemie, Barcelona, Spain) was used for mesophilic, and psychrotrophic microorganism analysis (31 ºC/48 h and 4 ºC/7 days, respectively). Violet Red Bile Dextrose Agar (Scharlau Chemie, Barcelona, Spain) was employed for Enterobacteria microbial counts (37 ºC/48 h). Cetrimide Agar (Scharlau Chemie, Barcelona, Spain) was used for *Pseudomonas spp.* (37 ºC/48 h). De Man Rogosa and Sharpe Agar (Scharlau Chemie, Barcelona, Spain) was applied under microaerophilic conditions for lactic acid bacteria microbial count analysis (31 ºC/48 h), and Rose Bengal Agar (Scharlau Chemie, Barcelona, Spain) was used to enumerate molds and yeast (25 ºC for 5-7 days). Viable bacteria counted was reported as log colony forming units (CFU) per gram.

2.6. Acrylamide analysis

Acrylamide content was extracted using a commercial kit (DisQuETM 186006812/Oasis® MCX 3 cc; Waters) and analysed uwign HPLC-MS (Agilent 6240 ESI triple quadrupole) following the supplier guidelines. Estimation of acrylamide was carried out once croquettes were finally cooked: 0% EVOO-α-CD croquettes were deep-fried at 180 ºC/1.5 min while 33 % EVOO-α-CD croquettes were baked 150 ºC for 10 min. Analysis was done in triplicate.

2.7. Sensory analysis

Tasting of croquettes samples were conducted after its final cooking for consumption (by frying or baking, as previously described) throughout predefined shelf-life storage days periods (days 0, 4, 7, 11, 14 and 20). The evaluation was carried out in a taste room at different shifts per taster. The sensory evaluation was carried following sensory sheet proposed by Jiménez-Martín *et al.,* (2016) [7]. Appearance, color, flavor, texture and aroma were evaluated on a descriptive 5-point scale, where 5 and 1 represented the maximum and minimum score of the sensory attribute, respectively.

2.8. Statistical analysis

Results were statistically analyzed using Rstudio software, performing a one-way ANOVA statistical analysis followed by the Tukey HSD test at a 95 % confidence level. Data is expressed as the mean value with its standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Color

Visual appearance and color are critical factors in a consumers purchasing decision [8]. Wheat-breadcrumbs control samples did not show color differences ($p > 0.05$) (data not shown) compared to NT during storage (see Figure 1a), whereas corn-breadcrumbed NT croquettes presented lower yellow notes (b^*) and higher lightness (L^*). However, despite this slight difference in shade, the panelists evaluated both treatments positively.

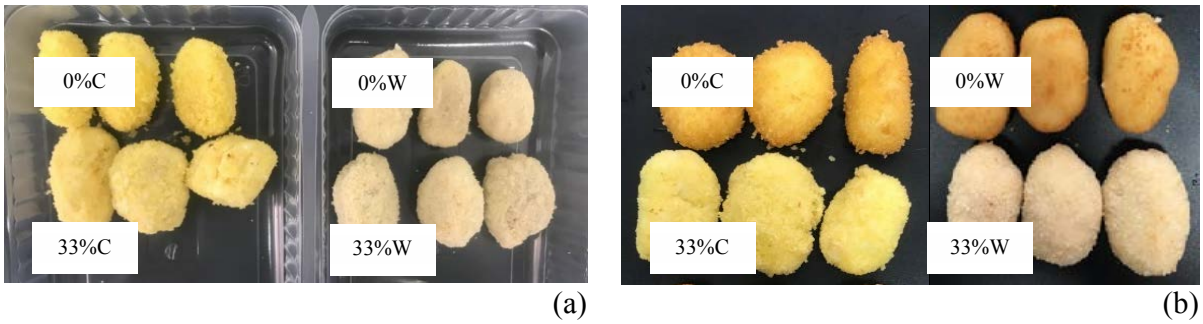

Figure 1. Croquettes breaded with 0% or 33% of EVOO-α-CD in corn (C) or wheat (W) breadcrumbs and precooked according to the amount of EVOO-α-CD incorporated: 0% no pre-cooking, 33% short-wavelength infrared precooking. (a) At storage before final cooking for consumption; and (b) Cooked for consumption (with 0% deep- fat frying at 180 ºC for 90 s; and 33% by baking in convection oven at 150 ºC for 10 min.

Consumers have control over selecting the final cooking method for ready to cook meals. However, 33% croquettes samples have been manufactured to be baked by the consumer in a hot-air oven. This way, fried- free croquettes can be introduced in the market as a healthier version of this traditional product all the while maintaining its organoleptic characteristics, including its oily nuances and crunchiness. 33% EVOO-α-CD croquettes samples (for both corn and wheat breadcrumbs) after baking at low temperature for a period of 5 to 20 min did not reach the expected visual appearance of a fried breaded product ($p < 0.05$) (data not shown). As observed in Figure 1b, the crust-color darkened but the golden-yellow color characteristic of a fried product was not obtained. Nevertheless, these samples were not rated negatively visually. On the other hand, panelists rated the 33%-C samples as more appetizing than 33%-W samples, as they had a darker color, more appealing to the eye. The color parameter in final cooking could be improved by raising the final cooking temperatures and thus favoring the Maillard reactions (180-200 ºC) and/or with the addition of colorants such as paprika, turmeric or beta-carotene.

3.2. Fiber and oil content

Fiber content increased significantly for 33 % EVOO-α-CD croquettes samples when compared to 0 % EVOO- α-CD croquettes samples by 14.3 and 46.2 % for corn and wheat breadcrumbs, respectively. This significant increase in fiber, primarily due to α-cyclodextrin, as it is a soluble fiber, provides extra nutritional value, increasing the benefits of refrigerated breaded products for marketing and consumption. The difference in fiber content between the two types of breadcrumbs can be attributed to their particle size. Thus, higher similarity in granulometry terms between breadcrumbs and the EVOO-α-CD powder will result in higher adhesion of the EVOO-α-CD to the croquette.

Fat percentage at consumption moment arranged from highest to lowest was as follows: 0%-C > 33%-C > 0%- W > 33%-W. Samples breaded with corn breadcrumbs were 5.1% higher in fat content than samples breaded with breadcrumbs. Fat content was reduced by 80.1 and 50.7 % for 33 % EVOO-αCD croquettes samples, breaded with corn and wheat breadcrumbs, respectively. As for the absorbed oil content, the most significant reduction was that of the 33%-C-croquettes samples with respect to 0%-C- croquettes, followed by 33%-W- croquettes with respect to 0%-W-croquettes. For 0%-croquettes wheat breadcrumbs were able to absorb 23.3 % less oil in frying than corn breadcrumbs, while for 33%-croquettes the oil absorbed by wheat breadcrumbs was higher than that of corn breadcrumbs. This phenomenon may be because wheat breadcrumbs have a smaller particle size than corn breadcrumbs and are more similar in granulometry terms to that of the EVOO-αCD powder, favoring the adhesion of the mixture to the croquette in the breading

stage. Chen *et al.,* (2009) [8] evaluated, among other factors, the oil content absorbed in the crust (made with wheat and corn flour) of fish sticks during pre-frying (180 ºC for 30 s). These authors found a fat absorption of 3.1-8 %. These results are similar to those of our study, although flours are not as absorbent as breadcrumbs.

Table 1. Fat content and amount of oil absorbed in croquettes breaded with 0 % or 33 % EVOO-α-CD and with different precooking techniques (0 % no pre-cooking, and 33 % short-wavelength infrared precooking) and final consumption cooking techniques (0 % deep-frying 180 ºC/90s, 33 % baking in a convection oven at 150 ºC/10 min). (Mean values (n=3) ± standard deviation).

Treatment	% Pre-cooked fat (Sale point)	% Final cooking fat (Consumption point)	% Absorbed oil
0%-C	3.33 ± 1.27^{aA}	12.68 ± 0.24^{aB}	9.35 ± 1.29aC
0%-W	0.68 ± 0.40^{bA}	7.46 ± 0.26^{bB}	6.78 ± 0.48aC
33%-C	3.01 ± 0.45^{aA}	2.53 ± 0.51^{cA}	0 ± 0.68aC
33%-W	0.56 ± 0.37^{bA}	3.68 ± 0.23^{cB}	3.12 ± 0.44aB

[a-c] Different lowercase letters (superscripts) within each column indicate significant differences ($p < 0.05$).
[A-C] Different capital letters (superscripts) within each row indicate significant differences ($p < 0.05$). C = corn breadcrumbs, W = wheat breadcrumbs.

3.3. Microbiological analyses

The microbiological evaluation of the refrigerated croquettes during the storage period of 20 days at 4 ºC is shown in Figure 2. Throughout all storage, significant differences ($p < 0.05$) in bacterial counts of total aerobic mesophilic (Figure 2a), *Enterobacteriaceae* (Figure 2b), psychrophiles (Figure 2c) and yeasts (Figure 2d) was observed between 0%-croquettes and 33 % croquettes, for both types of breadcrumbs. Differences had an exponential tendency as refrigerated storage progressed; thus, most noticeable results were observed at the end of the storage period studied. Croquettes fortified with 33 % of EVOO-α-CD powder in breadcrumbs doubled shelf life in refrigerated storage for croquettes as it reduced viable counts up to 5 log CFU/g and showed no apparent growth of yeasts/molds on the surface of croquettes after 20 days of storage.

3.4. Acrylamide analyses

Acrylamide content for both cooking procedures was low. Nevertheless, croquette samples breaded with 33% EVOO-α-CD (pre-cooking under short-wavelength infrared oven) and final baked with hot air at 150 ºC for 10 min resulted in a lower acrylamide content (5 µg/kg) than croquette samples breaded with 0% EVOO-α- CD (without pre-cooking) and final deep-fat frying in sunflower oil at 180 ºC/ 1.5 min (20 µg/kg). Results are in agreement with Mesias *et al.,* 2020 [9] who studied acrylamide levels of various products provided by different food services. Results indicated mean values of acrylamide levels of 40 and 36 µg/kg for chicken nuggets and croquettes, respectively; whilst home-style mean values were significantly lower (22 µg/kg).

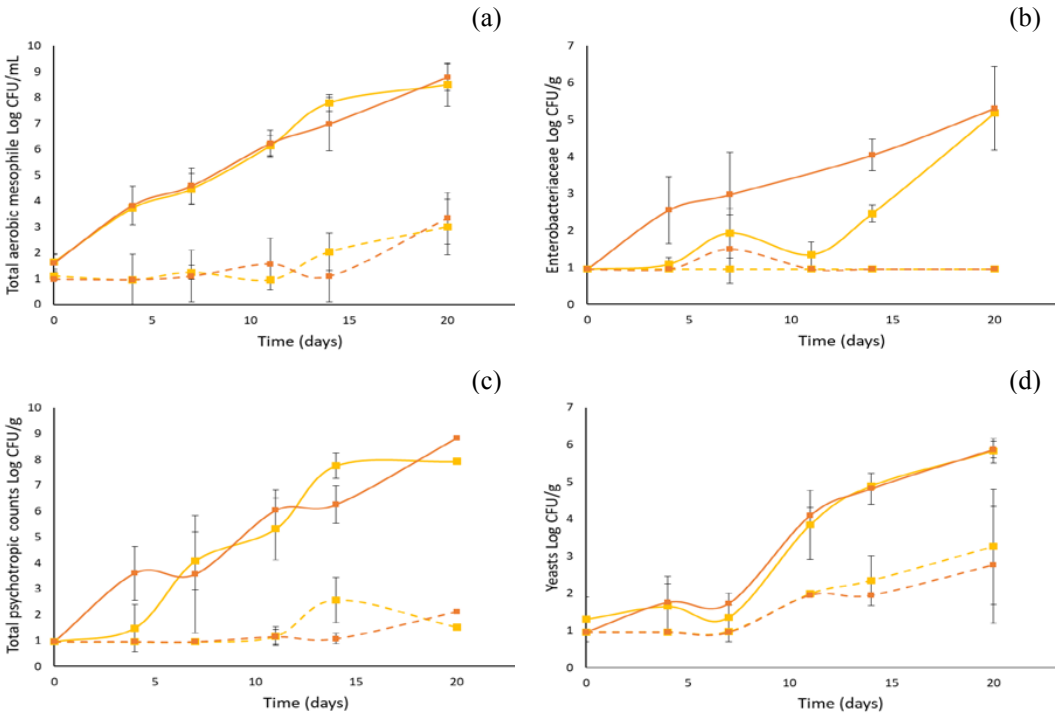

Figure 2. Bacterial counts over 20 days of refrigerated storage at 4 °C for (■) wheat breadcrumbs, and (■) corn breadcrumbs croquettes fortified with 0% (solid lines) or 33% EVOO-α-CD (dashed lines). Samples with 0% EVOO-α-CD have no pre-cooking and samples with 33% EVOO-α-CD have been short-wavelength infrared baked. Total bacterial count (log cfu/g) is shown for (a) total aerobic mesophiles, (b) *Enterobacteriaceae*, (c) psychrophiles, and (d) yeasts. Values are expressed as mean ±SD, where error bars represent standard deviations of the mean value.

3.5. Sensory analysis

Sensory analyses were performed on croquettes after the final cooking step for consumption, being deep-fat frying in a hot bath of sunflower oil at 180 ºC for 1.5 min for 0 % EVOO-α-CD croquettes samples; and baking in a domestic hot air oven at 150 ºC for 10 min for 33 % EVOO-α-CD croquettes samples. Significant differences ($p > 0.05$) were obtained in appearance between the kinds of breadcrumbs, where panelists preferred corn-breadcrumbs croquettes based on its coloration. On the other hand, during consumption no significant differences were found between croquettes fortified with 0 % or 33% EVOO-α-CD, revealing consumer's acceptation and no *apriori* organoleptic property changes. After chewing, panelists evaluated 0 % EVOO-α-CD croquettes as "heavier and oiler" while 33 % EVOO-α-CD croquettes as "lighter".

4. CONCLUSIONS

It was possible to develop an infrared precooked croquettes enriched with 33 % EVOO-α-CD in the breadcrumbs (w/w), to be commercialized as refrigerated product, which overall acceptation (with the exception of color) was better than control croquettes (with 0 % EVOO-α-CD). The outcomes of the current study indicated lower fat content and acrylamide levels, as well as higher fiber content and an extended refrigerated shelf-life when introducing 33 % EVOO-α-CD within the breadcrumbs.

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