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THE USE OF ENCAPSULATED OLIVE OIL AND VINEGAR POWDER, IN THE FORMULATION OF REFRIGERATED BREADED FOODS, HAS A SYNERGISTIC EFFECT ON INCREASING THEIR SHELF LIFE

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Abstract: *Refrigerated breaded foods (preserved at 4ºC), such as croquettes, nuggets, and breaded meat and fish fillets, have a relatively short shelf life of only 8 days, which prevents products manufactured in Spain from being marketed in other European countries. For this, a shelf life of at least 15 days is required when stored refrigerated at 4 ºC. That is why different techniques have been investigated to increase its useful life. One of them has been the use of extra virgin olive oil (EVOO) encapsulated in alpha-cyclodextrin, mixing it with the breadcrumbs. In this way, a significant increase in useful life is achieved, up to 14 days. In this work, the possible synergistic effect of the simultaneous use of vinegar powder (in the formulation of the core of the croquettes) with encapsulated EVOO (mixed in breadcrumbs) has been studied. The shelf life has been evaluated by determining the evolution of the microbial count in this product (total mesophilic aerobes, enterobacteria, lactic acid bacteria, psychrophiles, and molds and yeasts). The new technology also achieved better preservation of its physicochemical properties (pH and color) through storage, being perceived as well as healthier and tastier than control ones. With this new technology, an increase in the shelf life of the croquettes has been achieved to exceed 21 days, packaged in trays with modified atmosphere (MAP) and stored at 4ºC.*

Keywords: Virgin olive oil, vinegar powder, α-cyclodextrin, inclusion complex, food safety.

1. INTRODUCTION

Today´s consumers look for easy ready-to-cook products that are not only healthy but also safe, in a context where new strategies to produce processed food as naturally as possible is desirable [1] [2]. Consumers also demand additive-free products processed with barely synthetic chemicals. On this basis, different preservation methods such as modified atmosphere packaging (MAP) have been proposed to extend shelf-life and ensure the microbiological stability of the product [1]. Besides, the incorporation of natural additives as new ingredients in the composition of foods has gained great popularity because of their bioactivity and its potential to replace damaging preservatives. However, the distinctive formulation of an ideal ready-to-cook breaded food product such as croquettes is of high interest owing to its impact on organoleptic characteristics [3]. Thus, natural preservatives that are nowadays proposed must extend product shelf-life without compromising sensory quality. Vinegar is a natural preservative that has a proven effect on lowering microbial spoilage of food, therefore being a possible alternative to chemical additives [4]. On the other hand, the consumption of fried breaded food like croquettes has been related to some health problems such as cardiovascular risk factors [5]. For all this, the purpose of this experimental investigation was to study the combined effect of standard vinegar powder formulated croquettes in addition to encapsulated extra virgin olive oil (EVOOαCD) mixed in breadcrumbs as an alternative to chemical preservatives in extending the shelf-life of breaded products, combined with MAP.

2. MATERIAL AND METHODS

2.1. Materials

The ingredients used for the preparation of the croquettes were purchased at a local supermarket (Cartagena, Spain), except for the xanthan gum (Doscadesa S.L; Murcia, Spain) and wheat breadcrumb (Frumen Desarrollos Alimentarios S.A.; Guadalajara, Spain). The encapsulated extra virgin olive oil within α-cyclodextrin (EVOOαCD) [6] and the standard vinegar powder (VP) were prepared and provided by Bioencapsulation and iPackaging S.L. (Murcia, Spain).

2.2. Preparation of croquettes and packaging conditions

For the croquettes´ core formulation, two types of dough were prepared according to the recipe described in the patent ES2440092B1 [7] by following the procedure detailed in [8], with some modifications. Vinegar croquettes (T1 and T2) incorporated VP inside the filling dough, while normal croquettes (C1 and C2) without VP inside the core were investigated as control samples (Table 1). After preparation, the dough was cooled down at room temperature and refrigerated at 4 ºC for 45 min prior to manually molding it into individual croquettes with cylindrical shapes in a laminar flow cabinet (ISO 5; equivalent to 100 FED STD 209E class). Croquettes were then coated in industrial albumen and breaded afterwards with normal wheat breadcrumbs (without EVOOαCD) or mixed wheat breadcrumbs (2:1 normal wheat breadcrumbs:EVOOαCD (*weight:weight)*). Thereafter, croquettes were kept at -20 ºC for 24 h before packaging in plastic trays under a modified atmosphere (70% N_2, 30% CO_2) (Efaman, Efabin; Murcia, Spain) inside a cleanroom (ISO 7; equivalent to 10.000 FED STD 209E class) at 8 ºC, and finally stored at 4 ºC for 3 weeks. Physicochemical (pH and color), microbiological and sensory analysis of three replicates were analyzed at each sampling time.

Table 1. Croquettes´core formulation without vinegar powder (VP) as control (C) or incorporating VP (T) and breaded with normal wheat or wheat mixed with extra virgin olive oil encapsulated within α-cyclodextrin (EVOOαCD).

Treatment	Core	Outer crust
C1	-	Wheat
C2	-	Wheat-EVOOαCD
T1	VP	Wheat
T2	VP	Wheat-EVOOαCD

2.3. Physicochemical analyses

The physicochemical quality of croquettes during storage was determined by pH and color analyses. A croquette solution was obtained after mixing with distilled water using a household blender, and a digital pH-meter (Crison GLP 21; Barcelona, Spain) was used for the pH determination of three replicates per sample. The outer crust color of the uncooked samples was measured using a colorimeter (Konica Minolta CR-400 Chroma Meter; Nueva Jersey, EE.UU.). The colorimetric results (L^*, a^* and b^*) were obtained by measuring three different portions of each replicate per sample.

2.4. Microbiological analysis

For microbiological quality determination through refrigerated storage, total mesophilic aerobes, psychrophiles, enterobacteria, lactic acid bacteria, molds and yeasts were analyzed. At each sampling day (1, 6, 10, 13, 17 and 20), three replicates per sample were evaluated. Briefly, 2 halves of croquettes (≈15 g) per replicate were mixed with 135 mL of sterilized buffered peptone water and a dilution series was subsequently done. Aliquots of 1 mL were pour-plated into Plate Count Agar (PCA), Violet Red Bile Dextrose Agar (VRBD) and De Man Rogosa and Sharpe Agar (MRS) for the microbial count of total mesophilic aerobes (30 ºC/48 h), psychrophiles (4 ºC/7 days), enterobacteria (37 ºC/48 h) and lactic acid bacteria (30 ºC/48 h), respectively, while aliquots of 0,1 mL were spread-plated into Rose Bengal Agar for yeasts and molds (25 ºC/7 days). Results were reported as log of colony forming units (CFU) per gram (log CFU/g).

2.5. Sensory analysis

Sensory evaluation of croquettes was conducted inside a room equipped with individual tasting booths at day 0 of storage. Before tasting, croquettes were baked in a household hot air oven (180 ºC for 10-15 min) (model 3HB4841X0, BSH; Zaragoza, Spain). Cooked samples were served in plastic plates coded with random letters, and panelists used still water to clean the palate between samples. The panel consisted of 7 members (60% women, 40% men, aged 20-50 years) who were given written instructions for a satisfactory tasting of the samples. Appearance, aroma, texture, flavor and overall general acceptance were assessed using 5-point hedonic scale of acceptability (5: excellent; 3: acceptable; 1: extremely bad).

2.6. Statistical analysis

The results were subjected to statistical processing using RStudio software. Statistical analysis was executed at a 95% confidence level ($p<0,05$), performing a one-way ANOVA analysis of variance and a Tukey HSD test. Data is shown as the mean value of three replicates along with its standard deviation.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analyses

The pH results of control and vinegar croquettes during 20 days of shelf-life evaluation are shown in Table 2. At the beginning of refrigerated storage time, pH values of croquettes ranged from 6.7 to 6.9, quite like those reported in refrigerated rainbow trout croquettes [9]. No significant differences were found in control croquettes (C1 and C2) between normal wheat and mixed wheat breadcrumbs. The addition of VP and EVOOαCD (T2) reduced the pH by 0.2 units (day 1), in comparison to control croquettes. pH values changed also during the rest of the shelf-life. A marked increase in the pH value of control croquettes breaded with normal wheat breadcrumbs (C1) was observed after 17 days of storage. It may be related to the progressive increase in microbial growth and the deterioration of samples. On the other hand, the combined effect of VP and EVOOαCD in the rest of the treatments limited pH variations and showed pH < 6.90, reaching a better preservation of the products [9] [10].

Table 2. pH of normal croquettes (C) or VP (T) and breaded with normal (C1, T1) or mixed wheat breadcrumbs (C2, T2) during cold storage at 4 ºC (mean(n=3)±SD).

Treatment	Time (days)					
	1	6	10	13	17	20
C1	6.9 ± 0.1^{Aa}	6.7 ± 0.1^{Aa}	6.7 ± 0.2^{Aa}	6.7 ± 0.2^{Aa}	7.3 ± 0.1^{Ab}	7.3 ± 0.2^{Ab}
C2	6.8 ± 0.1^{Aa}	6.8 ± 0.1^{Aa}	6.8 ± 0.1^{Aa}	6.9 ± 0.1^{Aa}	6.9 ± 0.1^{Ba}	7.3 ± 0.1^{ABb}
T1	6.8 ± 0.1^{ABac}	6.7 ± 0.1^{Aa}	6.9 ± 0.1^{Acd}	7.0 ± 0.1^{Ad}	6.9 ± 0.1^{Bcd}	7.4 ± 0.1^{ABb}
T2	6.7 ± 0.1^{Ba}	6.9 ± 0.2^{Aa}	6.8 ± 0.1^{Aa}	6.9 ± 0.1^{Aa}	6.9 ± 0.2^{Ba}	7.4 ± 0.1^{Bb}

Different uppercase letters within each column indicate significant ($p<0.05$) differences between treatments. Different lowercase letters within each row indicate significant ($p<0.05$) differences between storage time per treatment.

Regarding color, differences ($p<0.05$) were found in the L^* values of samples during shelf-life (Table 3). All treatments were in the bright part of the scale (+L^*), but results showed that EVOOαCD incorporation in the outer crust better preserved the luminosity of normal and vinegar croquettes (C2 and T2) throughout storage. On the contrary, the surface of samples breaded with normal wheat breadcrumbs (C1 and T1) was markedly darkened at the end of the storage period. This darkening effect was also observed for the a^* value. Samples with no EVOOαCD on the outer crust showed a rusty and reddish color (+a^*). Changes in L^* and a^* values during shelf-life were also reported by [11]. The breaded procedure also significantly impacted the b^* value. Samples presented a noticeable yellow color (+b^*), due to the yellowness of wheat breadcrumbs. At the starting day of the storage period, encapsulated EVOOαCD croquettes had a greater yellow character that was not statistically significant. Croquettes with no encapsulated EVOOαCD maintained a similar yellowish color during the 20 days of cold storage, no changes ($p>0.05$) were reported. The formulation with VP and normal wheat breadcrumbs showed a marked yellow color on the first day of shelf-life. The addition of VP in combination with EVOOαCD (T2) had the lowest yellow character, which was different to the rest of the treatments. These changes in color values may have been related to the changes in the formulation of croquettes here evaluated [9].

Table 3. Color (L^*, a^*, b^*) of normal croquettes (C) or VP (T) and breaded with normal (C1, T1) or mixed wheat breadcrumbs (C2, T2) during cold storage at 4 ºC (mean(n=3)±SD).

Treatment		Time (days)					
		1	6	10	13	17	20
L^*	C1	75.7 ± 1.2^{ABa}	73.4 ± 0.9^{Aab}	74.4 ± 1.5^{Aab}	73.6 ± 2.8^{Aab}	70.5 ± 0.6^{Ab}	70.2 ± 2.7^{ACb}
	C2	75.2 ± 1.6^{ABab}	76.7 ± 0.2^{Ba}	75.0 ± 1.4^{Aab}	75.1 ± 1.6^{Aab}	74.7 ± 0.4^{Bab}	73.5 ± 0.7^{ABb}
	T1	77.5 ± 0.5^{Aa}	74.7 ± 0.9^{ACb}	70.2 ± 0.6^{Bcd}	71.0 ± 1.6^{Ad}	68.9 ± 0.2^{Ccd}	68.3 ± 1.1^{Cc}
	T2	74.6 ± 0.4^{Babc}	76.6 ± 0.6^{BCa}	75.9 ± 0.7^{Aa}	73.6 ± 0.5^{Abc}	72.8 ± 0.8^{Dc}	75.2 ± 1.5^{Bab}
a^*	C1	6.9 ± 0.5^{Aab}	5.2 ± 0.9^{Aab}	5.6 ± 0.4^{Aa}	5.7 ± 0.7^{Aa}	7.6 ± 0.2^{Ab}	7.3 ± 1.0^{Aab}
	C2	5.7 ± 1.2^{Aa}	4.7 ± 0.4^{Ba}	4.7 ± 0.4^{Ba}	4.6 ± 0.3^{Ba}	4.2 ± 0.3^{Ba}	4.4 ± 0.2^{Ba}
	T1	5.9 ± 0.7^{Aa}	6.6 ± 0.9^{Aa}	6.7 ± 0.2^{Ca}	5.9 ± 0.5^{Aa}	6.0 ± 0.2^{Ca}	6.7 ± 0.5^{Aa}
	T2	7.3 ± 0.5^{Aa}	5.2 ± 0.3^{ABbc}	5.2 ± 0.2^{ABbc}	5.6 ± 0.2^{ABb}	4.4 ± 0.4^{Bc}	4.6 ± 0.4^{Bbc}
b^*	C1	44.2 ± 0.6^{Aba}	43.8 ± 0.9^{ABa}	43.8 ± 0.9^{Aa}	43.3 ± 0.3^{Aa}	44.3 ± 0.2^{Aa}	43.3 ± 0.4^{Aa}
	C2	46.2 ± 2.3^{Aa}	40.9 ± 0.6^{ACb}	39.6 ± 0.3^{Bbc}	40.0 ± 1.2^{Bbc}	39.0 ± 0.8^{Bbc}	37.6 ± 0.5^{Bc}
	T1	41.8 ± 0.9^{Ba}	44.4 ± 2.1^{Ba}	43.9 ± 0.7^{Aa}	42.0 ± 1.8^{ABa}	41.1 ± 0.4^{Ca}	41.7 ± 0.5^{Ca}
	T2	46.0 ± 0.5^{Aa}	39.2 ± 0.2^{Cbd}	39.5 ± 0.3^{Bb}	39.3 ± 0.7^{Bbd}	36.7 ± 0.7^{Dcd}	37.9 ± 0.7^{Bd}

Different uppercase letters within each column indicate significant ($p<0.05$) differences between treatments. Different lowercase letters within each row indicate significant ($p<0.05$) differences between storage time per treatment.

3.2. Microbiological analysis

The microbial counts evolution of total mesophilic aerobes and enterobacteria of the investigated croquettes is

shown in Table 4. The incorporation of encapsulated EVOOαCD in the breading together with the VP in the dough showed a synergistic antimicrobial effect. During day 1 of storage, microbiological counts of total mesophilic aerobes were <1 log CFU/g for all treatments. Throughout storage time, the total mesophilic aerobes of C1 treatment increased gradually, exceeding 8 log CFU/g after 20 days of storage (Figure 1a). Differences ($p<0.05$) were found in microbial counts of total mesophilic aerobes between C1 and croquettes with mixed wheat breadcrumbs: 5.0 log CFU/g for C2 and 3.3 log CFU/g for T2. These results are in agreement with the reducing microbial growth effect exhibited by EVOOαCD when mixed in corn or wheat breadcrumbs reported by [8]. Samples that incorporated VP inside the core formulation (T1 and T2) showed ≈ 2 log CFU/g up to day 17 of refrigerated storage, while C1 croquettes surpassed 7 log CFU/g. Thus, VP had a strong antimicrobial potential when added to the filling dough of refrigerated breaded food. At day 20 of storage time, enterobacteria cell counts showed a humongous growth for C1 treatment (6.2 log CFU/g), whereas viable cell counts were outside the detection limit (< 1 log CFU/g) in samples formulated with VP and breaded with EVOOαCD (Figure 1b). Lactic acid bacteria counts were below the detection limit throughout the refrigerated shelf life of all treatments (results not shown). On day 17, psychrophile counts were ≈ 3 log CFU/g in C1 samples, but reached > 7 log CFU/g after 20 days. Psychrophile counts remained < 1 log CFU/g in the rest of the treatments (data not shown). No viable cell growth was shown for molds and yeasts during the first 15 days of storage. However, on day 20 were found ≈ 4 log CFU/g in normal formulation croquettes with normal wheat breadcrumbs (data not shown). The combined use of EVOOαCD and VP in this novel formulation reduced microbial cell counts at approximately 6 log CFU/g and extended the shelf-life more than 20 days (the 7 log CFU/g load was not exceeded at the final time of storage).

Table 4. Total bacterial count (log CFU/g) for total mesophilic aerobes (TMA) and Enterobacteriaceae (ENT) of normal croquettes (C) or VP (T) and breaded with normal (C1, T1) or mixed wheat breadcrumbs (C2, T2) during cold storage at 4 ºC (mean(n=3)±SD).

Treatment		**Time (days)**					
		1	**6**	**10**	**13**	**17**	**20**
TMA	C1	0.9 ± 0.1^{Aa}	1.0 ± 0.1^{Aa}	3.8 ± 0.3^{Ab}	3.7 ± 0.7^{Ab}	7.8 ± 1.0^{Ac}	8.0 ± 0.8^{Ac}
	C2	0.9 ± 0.1^{Aa}	1.2 ± 0.4^{Aa}	2.4 ± 1.3^{ABab}	1.3 ± 0.3^{Ba}	3.3 ± 0.3^{Bb}	5.0 ± 0.3^{ABc}
	T1	0.9 ± 0.1^{Aa}	1.2 ± 0.2^{Aa}	1.7 ± 0.6^{Ba}	1.0 ± 0.1^{Ba}	1.9 ± 0.7^{Ba}	1.7 ± 0.9^{Ca}
	T2	0.9 ± 0.1^{Aa}	1.1 ± 0.2^{Aab}	1.0 ± 0.1^{Ba}	1.0 ± 0.1^{Ba}	3.2 ± 0.4^{Bab}	3.3 ± 2.0^{BCb}
ENT	C1	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	1.3 ± 0.7^{Aa}	1.4 ± 0.8^{Aa}	1.0 ± 0.1^{Aa}	6.2 ± 1.0^{Ab}
	C2	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	1.4 ± 0.7^{Aa}	1.5 ± 0.9^{Aa}	1.0 ± 0.1^{Aa}	1.1 ± 0.3^{Ba}
	T1	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	1.5 ± 0.5^{Aa}	1.7 ± 0.6^{Aa}	1.0 ± 0.1^{Aa}	1.0 ± 0.1^{Ba}
	T2	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Aa}	0.9 ± 0.1^{Ba}

Different uppercase letters within each column indicate significant ($p<0.05$) differences between treatments. Different lowercase letters within each row indicate significant ($p<0.05$) differences between storage time per treatment.

3.3. Sensory analysis

Sensory evaluation of croquettes was done after hot air baking in a household air oven (180 ºC for 10-15 min), as previously described (data not shown). The breading process with normal wheat breadcrumbs or mixed wheat breadcrumbs with encapsulated EVOOαCD reported differences in the appearance of croquettes, although it did not affect their acceptance. Control and vinegar croquettes with encapsulated EVOOαCD showed a pleasant crunchy texture. No marked differences were found in creaminess when VP was incorporated, regardless of its breading procedure, whilst panelists highly appreciated vinegar croquettes above control ones. Normal and mixed wheat breadcrumbs croquettes showed no differences, although the addition of EVOOαCD was positively perceived as mild and less stuffy, in comparison to normal wheat croquettes. The incorporation of VP inside the filling dough alongside the encapsulated EVOOαCD resulted in a smooth flavor and a softer outer crust that contributed to its general acceptance.

4. CONCLUSIONS

The results obtained in this experimental investigation showed that the use of VP in combination with encapsulated EVOOαCD had a significant antimicrobial effect during refrigerated storage of uncooked

croquettes. Vinegar croquettes among the other treatments were most likely to reduce microbial counts (6 log CFU/g) through 20 days. The use of VP contributed to panelists preference over control croquettes prepared with normal formulation. Besides, the mixing EVOOαCD in the outer crust of croquettes was perceived as smoother flavor, while normal wheat croquettes were less appreciated by consumers. The synergistic effect of VP and EVOOαCD in combination with MAP packaging in clean conditions was effective in controlling microbial contamination at the refrigerated conditions studied. The results from this study contribute to the potential use of natural additives instead of conventional formulations to prevent microbial deterioration and to enhance the sensorial quality of breaded products.

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