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Sensory, physico-chemical and microbiological properties of cooked ham with *β-ciclodextrin* loaded with coriander and pimento essential oils

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Abstract

This paper presents the sensory, physico-chemical (protein, total fat, moisture and ash contents, pH, water activity, lipid oxidation) and microbiological (Total Viable Counts, Enterobacteriaceae) properties of cooked ham manufactured with different concentrations of β-cyclodextrin (0.01%, 0.02%, 0.03% w/w) loaded with coriander and pimento essential oils stored at 3°C ±0.5°C for 28 days. The results obtained in this study showed that the use of β-cyclodextrin (0.02% w/w) loaded with coriander and pimento essential oils is the best way to use it in the production of cooked ham, providing a gradual release of coriander and pimento essential oils and protection from the interaction with the other food ingredients.

Keywords: cooked ham, coriander and pimento essential oil, β-cyclodextrin, properties evaluation

1. Introduction

In recent years, food production and consumption requirements were changed a lot. Today's consumers are aware that food serves not only the simple role of satisfying the condition of hunger, but are also responsible for the pleasure of eating, and also for their health. In this context, food producers have increased concerns towards achieving innovative products that satisfy both the taste and health of consumers. Research in the past decade, in the field of innovative food, such as functional foods, have shown that the preparation of these foods has manifested in three trends: the use of traditional technologies and classical fortification of impact foods with different biocomponents (vitamins, fish oil, minerals, iodine, folic acid, probiotics, etc.); using special technology, which prevents bioactive components damage such as encapsulation, immobilization in matrices and films; using the concept of "nutrigenomics" in designing innovative food.

Due to the toxic and carcinogenic properties, many synthetic additives are used in limited quantity or even banned. For this reason, consumer demands for meat products made with natural food additives as an alternative to replacing synthetic food additives has increased [1]. These natural additives improve the quality of the meat without leaving residues in the product or environment [12]. The essential oils, obtained using different extraction methods [3] from vegetable sources such as flowers, buds, leaves, roots and seeds [4,5] are some of the best alternative considering their antimicrobial and antioxidant properties [6,7].

Due to the biological properties and characteristics of flavor, coriander (*Coriandrum Sativul L.*) and pimento (*Pimento Dioica*) essential oils has been

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widely used for centuries in food, especially in the meat industry [8-11]. After numerous studies, it was demonstrated that coriander and allspice essential oils presents antibacterial and antifungal properties [12, 13] but also antioxidant properties [14]. Due to genotoxicity and other side effects, the concentration of coriander and allspice essential oil in meat products is subject to laws and regulations. Flavor and Extract Manufacturer`s Association (FEMA) has approved the use of coriander essential oil in meat products in concentrations ranging between 43-68.5 ppm [15] as a flavor ingredient safe for consumer health, and the maximum level of eugenol, primary compound in the allspice essential oil, admitted in meat products is 2.5 ppm [16].

Depending on the concentration used, some of the essential oils may adversely alter the sensory properties of the product. Essential oils can be added to meat products both in the free state but also encapsulated in various edible polymers or biodegradable films thus providing a slow release both inside the products and on its surface [17] providing additional protection [18]. The encapsulation of essential oils is a good way for their use in the production of meat products, thereby providing protection against interaction with other food ingredients [4]. Thus, one of the most commonly used methods for the encapsulation of essential oils is encapsulation in β-cyclodextrin due to the high degree of encapsulation but also of release. Del Valle (2004) [17] reported that the use of cyclodextrins in meat products improves the texture, especially for pork jerky type products. Using β-cyclodextrin as encapsulating material ensures safekeeping of essential oils as well as a slow-release compared to other encapsulating materials. In Japan, cyclodextrins have been approved as ‘modified starch’ for food applications for more than two decades, serving to mask odours in fresh food and to stabilise fish oils. One or two European countries, for example Hungary, have approved β-cyclodextrin for use in certain applications because of its low toxicity [17].

Consumption of ham type meat products is relatively high because it is a high quality meat product, very popular, accounting for about 26% of meat products sold in Europe, especially in France, Spain and Italy [18]. There are different technologies for producing meat products depending on raw materials and processing conditions. The technological process for obtaining ham type meat product involves the use of the brine which is introduced by injection or immersion, followed by heat treatment. The final quality of the product depends both on the materials and on the processing technology used. The most important factors affecting the ham product quality are: the type of meat (raw material), the type and amount of spices, volume of injected brine, velocity and massaging time, heat treatment duration and temperature [19].

The aim of this research was to determine the sensory (taste and smell), physico-chemical (protein, total fat, moisture and ash contents, pH, water activity, lipid oxidation) and microbiological (Total Viable Counts, Enterobacteriaceae) properties of cooked pork ham manufactured with β-cyclodextrin (0.01%, 0.02%, 0.03% w/w) loaded with coriander and pimento essential oils stored at 3°C ±0.5°C for 28 days.

2. Materials and methods

Chilled pork thigh, was purchased from SC Fraher Distribution, having the following characteristics: lot no.105, temperature of 3.2°C when received and pH of 5.93. Coriander and allspice seeds from which the essential oils were extracted were bought from Supremia Grup SRL, Romania; additives (sodium nitrite and tripolyphosphate) from Haifa Chemicals Ltd., Israel; β-cyclodextrin from Merck. Co. KGaA, Germany and artificial membrane from SC. DARIMEX INTERNATIONAL, Romania. The other chemical reagents used are from the laboratories of the Faculty of Food Science and Engineering Galati.

2.1. Extraction of essential oils of coriander and allspice. Extraction of coriander and allspice essential oils from seeds was achieved by the hydrodistillation method proposed by Dong et al. (2011) [20] for 3 hours at 100°C using a Clevenger type equipment. The coriander and pimento EOs was kept in glass vials with plastic stoppers at a temperature of 4°C.

2.2. *Preparation of the encapsulated coriander and pimento EOs/β-CD complex.* For the preparation of the coriander and pimento EOs/β-CD complex it was used the co-precipitation method proposed by Dima et al. (2014) [21] using a ratio of coriander and pimento EOs: B-cyclodextrin of 15:85 (w/w). Coriander EO: pimento EO ratio was 3:1 (w/w).

2.3. Cooked ham manufacturing. In order to obtain the ham type meat product five technological recipes, presented in Table 1, were used. Pork meat (boneless thigh) was degreased to get a lean meat because a high fat content adversely affect quality of the ham. The meat was minced (chopped roughly) into pieces of 5-7 cm, with the grinding machine type K+G WETTER, Germany and tumblerized for 40 minutes on 10 rpm under vacuum 85 MPa with the tumbler type Eastman Outdoors 38229 Reveo MariVac, USA During tumblerizing, brine was added along with essential oils of coriander and allspice in free form and encapsulated in β-cyclodextrin according to the recipes shown in Table 1. The paste that was obtained was left to mature 24 hours, at 4°C; later, it was subsequently filled in artificial impermeable polyamide membrane of equal lengths, diameters and weights (L = 15cm, Ø = 50mm and m = 450g) with the filling machine Vemag ROBOT T-500. Ham sticks were scalded in thermal treatment cell Vemag type, Germany; a pasteurization program in stages was used to avoid excessive heating to the surface of the product [36], as follows: 65°C / 30 minutes; 70°C/30 minutes; 75°C for 55 minutes until it reached 71 ºC in the thermal center of the product. The ham sticks were then cooled in ice water (8-10ºC) in order to reduce as much as possible the cooling time since this final cooling stage from 40°C to 15°C is considered the most critical for rheological and sensorial properties. Ham sticks were stored in a WHIRLPOOL cooler at 3°C for 28 days. There were made 5 ham sticks in each sample, for sensory, physico-chemical and microbiological analysis.

2.4. Physico-chemical analyses

Physico-chemical analyzes were made every seven days the entire storage period of 28 days. Protein (ISO 937, 1978) [26], total fat (ISO, 1443, 1973) [27], moisture (ISO, 1442, 1997) [28] and ash contents (AOAC 2000) of cooked ham were determined according to methods recommended by the International Organization for Standardization. All analyzes were performed in duplicate.

2.4.1. pH measurements. pH measurements were performed in pH/Conductometer Consort C832 (Belgium), after homogenizing a 5g portion of the cooked ham in 45 ml of distilled water for 5 min. The measurements were performed in duplicate.

2.4.2. Water activity. The amount of water activity was determined through hygrometric method using the water activity analyzer GBX, consisting of an electronic data display (temperature, a_w), thermometer attached to the bottom of the device, a temperature sensor, a gold mirror and a fan. The reading of water activity (a_w) corresponds to the average of the last 40 instantaneous measurements made in the last 20 seconds.

2.4.3. Lipid oxdation. Determination of TBA-RS numbers. One of the most effective methods used to assess the degree of lipid peroxidation is the TBA method proposed by Ganhão, Estévez, and Morcuende (2011) [23]. This method is based on the reaction of the malonic aldehyde, which is formed from the degradation of lipid peroxides, with thiobarbituric acid. The condensation product is absorbed at a wavelength of 532 nm. Malonic aldehyde concentration was determined by the spectrophotometric method using UV VIZ JACSO 350 spectrophotometry device. The results are expressed in mg of malonic aldehyde / kg of product.

2.5. Microbiological analyses. Microbiological analyzes were performed in the microbiology laboratory of Food Science and Engineering Faculty, using AOAC methods described by Horwitz (2000) [24], every seven days for a period of 28 days. 10 g of each sample were transferred aseptically into sterile bags and 90 ml of sterile peptone water was added on each of them. The samples were stirred for 2 minutes with the homogenizer Bagmixer and decimal dilutions were performed for inoculation.

To determine the number of aerobic mesophilic bacteria, duplicate samples of each dilution were inoculated onto Plate Count Agar culture medium and thermostated for 48 hours at 37°C. Results were expressed in cfu / g of product.

To determine the number of Enterobacteriaceae, there were made inoculations in duplicate from decimal dilutions on 3M Enterobacteriaceae Count Plate. Petrifilms were thermostated for 24 hours at a temperature of 37°C and the results were expressed as the number of Enterobacteriaceae / g of product.

2.6. Sensory evaluation. Sensory analysis of ham type meat product was performed in the laboratory of Sensory Analysis of Food Science and Engineering Faculty on a group of 20 panelists (10 girls and 10 boys), students of the faculty who were trained during the course of sensory analysis. Sensory characteristics were measured on a scale of 0 to 12, 0 representing no flavor/taste and 12 representing the presence of strong flavor/taste. Assessments were noted in a sensory analysis bulletin proposed by Cristiane Marangoni and Neusa Fernandes de Moura (2011) [25].

2.7. Statistical analysis. The experiments were performed in duplicate and the results were presented as mean value, ± standard deviation. Comparison of the results was performed by ANOVA method using Turkey discrimination test at a confidence level of 95%. We used the program Sigma Plot 11.0, 2008.

3. Results and discussion

3.1. *Physico-chemical analyses. pH variation.* The physico-chemical properties of ham type meat product showed that the state of the coriander and allspice essential oils used (free or encapsulated) only influences pH and antioxidant activity expressed by TBA index. The test results are shown in Table 2 and are consistent with those obtained by other researchers [29-33].

The pH value explains a number of physicochemical and technological characteristics of meat and meat products, such as: freshness, color, tenderness, juiciness, water retention capacity and extractable protein content.

The study of pH variation in time (Figure 1) revealed its growth for all samples tested, as a result of increasing the activity of proteolytic enzymes and accumulation of the products with alkaline nature resulting from the degradation of proteins (ammonia, amino acids, amines, etc.).

P1 sample in which the mixture of essential oils was used in the free state has the pH values in the first 14 days, lower than the other samples, due to the acidic nature of the mixture of oils (pH 4.1). The pH of P1 sample ranges from 6.18 ± 0.017 and 6.42 ± 0.01, while for P3 sample with encapsulated essential oil, the pH ranges from 6.24 ± 0.011 to 6.45 ± 0.017. Although in the encapsulated oil samples the release of oils from microcapsules occurs over time, the pH of the samples increases because the alkaline components resulted from the biochemical processes are dominant.

Table 1. Technological recipes for obtaining ham type meat products

Sample code	Degreased pork thigh (Kg)	Functional aditives (brine)			Spices Esential oil/ inclusion complex			
					Free essential oils		Inclusion complex of β-cyclodextrin / essential oil (g/Kg)*	Cooled water (ml)
		Salt (g/Kg)	Sodium nitrate (mg/Kg)	Sodium tripolyphosphate (g/Kg)	Coriander (mg/Kg)	Allspice (mg/Kg)		
P_0	2.5	16	120	4	0	0	0	250
P_1	2.5	16	120	4	60	2.5	0	250
P_2	2.5	16	120	4	0	0	0.1	250
P_3	2.5	16	120	4	0	0	0.2	250
P_4	2.5	16	120	4	0	0	0.3	250

* maximum amount of β-cyclodextrin allowed is 5 mg / kg body weight per day [22].

Table 2. Physico-chemical analysis of ham type meat product during the entire storage period of 28 days at 3°C

Date (days)	Sample	Humidity %	Protein%	Lipids %	Ash %
26.02.014 *(1)*	P0.	74.98±1.251	18.535±1.031	2.538±0.021	2.965±0.001
	P1.	73.245±3.830	19.029±3.118	3.896±0.074	2.675±0.008
	P2.	73.59±2.018	19.212±2.215	3.210±0.08	2.689±0.021
	P3.	75.118±5.123	17.640±0.723	3.976±0.072	2.644±0.014
	P4.	73.967±1.611	19.027±1.335	4.203±0.112	2.586±0.023
05.03.014 *(7)*	P0.	74.572±2.164	18.241±0.628	3.585±0.043	2.845±0.035
	P1.	74.7005±1.153	18.423±2.441	3.586±0.008	2.695±0.121
	P2.	70.9168±2.601	19.040±2.127	5.550±0.065	2.732±0.017
	P3.	73.3321±4.131	20.534±3.217	5.682±0.516	2.736±0.023
	P4.	74.5373±3.182	18.510±1.321	3.015±0.017	2.910±0.017
12.03.014 *(14)*	P0.	75.2861±4.221	18.143±1.657	2.403±0.026	3.108±0.005
	P1.	72.5908±4.142	18.582±4.215	5.404±0.033	3.033±0.081
	P2.	72.4015±2.762	18.136±2.221	6.597±0.362	3.188±0.640
	P3.	72.1417±5.171	18.002±2.183	6.853±0.091	3.067±0.027
	P4.	72.7131±1.925	18.952±1.526	4.453±0.028	2.905±0.039
19.03.014 *(21)*	P0.	74.5675±3.424	29.049±3.021	3.193±0.004	3.072±0.019
	P1.	75.4472±6.326	18.313±0.623	3.049±0.023	2.819±0.071
	P2.	73.7742±1.622	18.416±1.363	4.512±0.061	2.634±0.011
	P3.	74.8239±3.125	18.247±1.621	3.514±0.235	2.678±0.052
	P4.	74.2574±2.426	17.719±0.831	3.259±0.010	2.957±0.071
26.03.014 *(28)*	P0.	74.4967±3.311	18.407±1.121	3.299±0.401	3.196±0.623
	P1.	73.8720±5.758	18.209±2.025	3.881±0.278	2.553±0.238
	P2.	75.9460±4.217	18.271±2.135	2.577±0.020	2.838±0.023
	P3.	72.6285±2.376	18.384±1.721	4.506±0.005	3.059±0.126
	P4.	71.5247±3.271	18.789±2.113	5.263±0.637	2.893±0.017

* The experiments were performed in duplicate and the results are given as mean values ± standard deviation at a confidence level of 95%

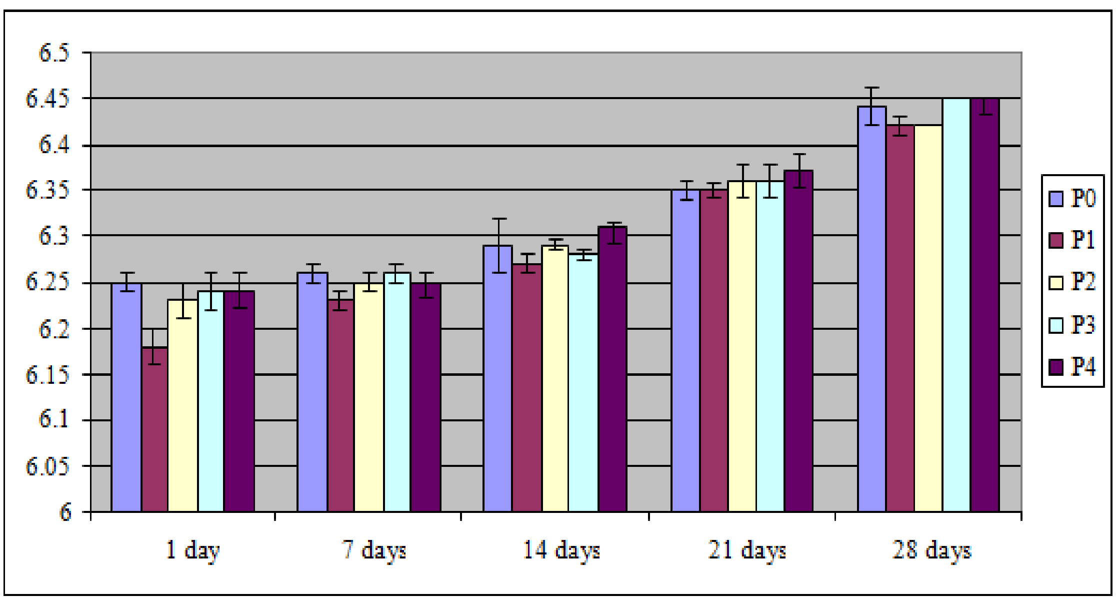

Figure 1. pH variation of the cooked ham during storage

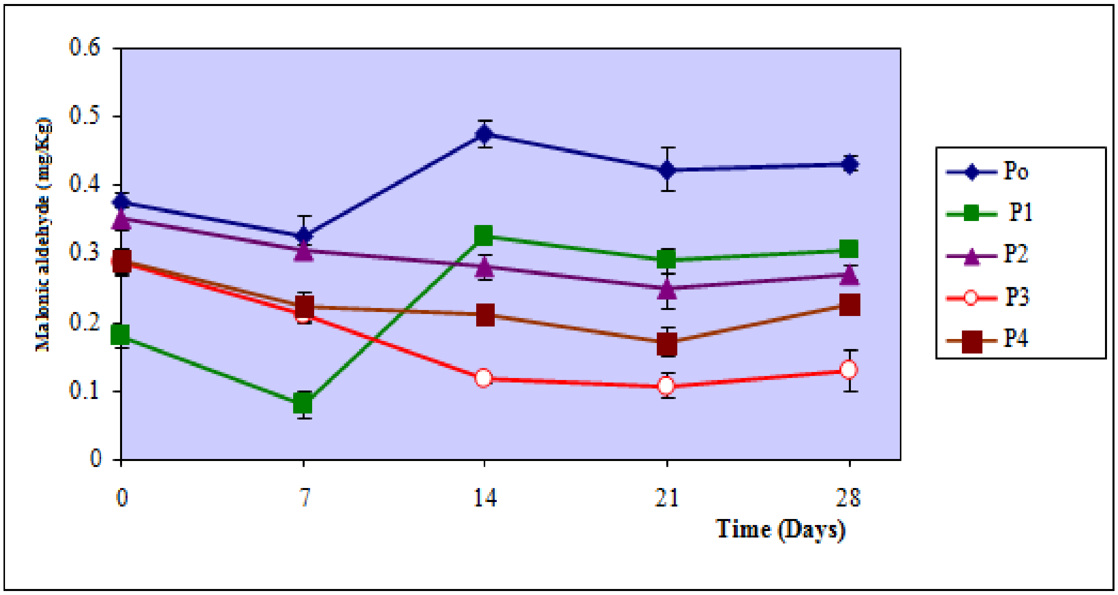

Figure 2. Changes in the rate of lipid oxidation of the ham type product mixed with coriander and allspice open and microincapsulated essential oils

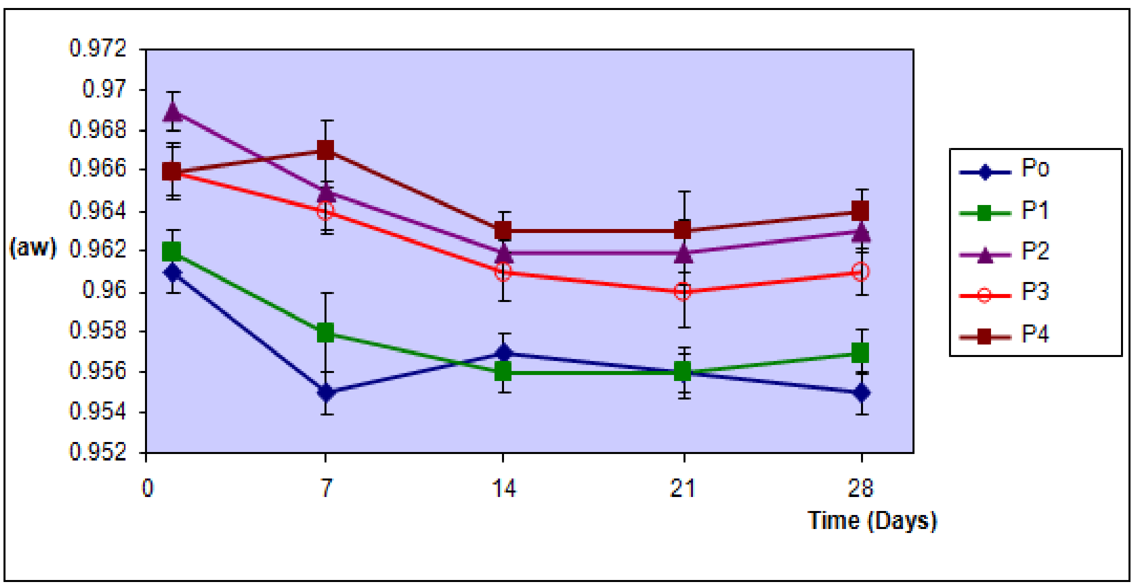

Figure 3. Water activity variation of ham type meat products, during storage

Table 3. Microbiological analyses results of ham type meat products

Sample	Number of storage days (3°C)	Total number of mesophilic aerobic bacteria ufc/ g product	Number of enterobacteriaceae/ g product
P_0	1	<10	<10
	7	<10	<10
	14	<10	<10
	21	<10	<10
	28	$2{,}9 \times 10^3$	$1{,}25 \times 10^2$
P_1	1	<10	<10
	7	<10	<10
	14	<10	<10
	21	<10	<10
	28	6,9 x 10	1 x 10
P_2	1	<10	<10
	7	<10	<10
	14	<10	<10
	21	<10	<10
	28	$2{,}4 \times 10^3$	9,7 x 10
P_3	1	<10	<10
	7	<10	<10
	14	<10	<10
	21	<10	<10
	28	$3{,}24 \times 10^2$	7,4 x 10
P_4	1	<10	<10
	7	<10	<10
	14	<10	<10
	21	<10	<10
	28	$9{,}2 \times 10^2$	9,1 x 10

pH, along with other factors, such as: water activity, ionic strength, content of myofibrillar protein, the presence of additives (tripolyphosphates), the salt content, the degree of comminution of the meat and massaging conditions influence the moisture content of the meat products.

There was no significant change in water content in the analyzed products because we used an polyamide impermeable artificial membrane (table 1).

3.2. Lipid oxidation. When preparing ham type meat products, raw material passes through various technological processes, such as aging, massaging and boiling which favors lipid oxidation as a result of their interaction with oxygen or with different pro oxidants [34]. The low rate of lipid oxidation observed in ham is due to the antioxidant capacity of the additives used, such as nitrite and tripolyphosphate. Sodium nitrite is known as a good antioxidant as a result of complexation of the iron (prooxidant) from the hemoglobin pigment (heme).

In ham type meat products analysis, the control sample (P0) used was a ham type product which had only sodium nitrite as an antioxidant. For product P1, the same amount of sodium nitrite was used and additionally an amount of a mixture of allspice and coriander open essential oils was added. For products

P2, P3, P4 we used the same amount of sodium nitrite and we added different amounts (table 1) of β-cyclodextrin complex / essential oil. Changes in the rate of lipids oxidation of the five analyzed ham type products during storage for 28 days is shown in fig 2.

From Figure 2 it can be observed that on the first day of storage, the control sample P0 shows a high degree of lipid oxidation, expressed as a high value for the content of malonic aldehyde (0.3759 ± 0.021 mg/kg) compared with the sample P1 (0.1782 ± 0.028) containing a mixture of essential oils in the free state. The other samples P2, P3, P4, where the essential oils are encapsulated in β-cyclodextrin, malonic aldehyde content values on the first day of analysis, is lower than that of the control sample, due to the presence of the essential oils located on the outside of the β-CD molecules cavity [21]. After seven days, the degree of oxidation of the lipids decreases for all the samples, but with different rates. Thus, the lowest oxidation rate was observed in the P1 sample, due to the antioxidant activity of non-encapsulated essential oils, whose molecules in direct contact with the lipid molecules inhibited the oxidation process. The rate of lipid oxidation in the samples P_2, P_3, P_4, is greater than that of the P1 sample but lower than the one of the control sample ($P < 0.05$).

The fact that for each sample (P_2, P_3, P_4), the decrease of malonic aldehyde content after seven days of storage is not significant ($P > 0.05$), explains the slow release of the essential oil of β-CD complex [21].

In the fourteenth day of storage, there was an increase in lipid oxidation process for samples P0 and P1, which means that the antioxidant activity of coriander and allspice essential oils decreased. However, for samples P_2, P_3, P_4, from the seventh day, there was a decrease in the content of malonic aldehyde, which explains a low rate of lipid oxidation, and also an increase in antioxidant activity of essential oils obtained from β -CD. Of the samples P_2, P_3, P_4, the most pronounced decrease in aldehyde content P3 test malonic has, on day 14 (0.1067 ± 0.0178 mg / kg) and then remains fairly constant until day 28 to a storage, the control sample corresponds to a malonic aldehyde content of 0.4305 ± 0.021 mg/kg.

Among the samples P_2, P_3, $P_{4,}$ sample P3 has the most pronounced decrease in malonic aldehyde content, on day 14 (0.1067 ± 0.0178 mg/kg) and then it remains fairly constant until day 28 of storage, when the malonic aldehyde content of the control sample reaches 0.4305 ± 0.021 mg / kg.

The results obtained, which are consistent with [31], show that the best antioxidant activity was met in the product P_3, containing 0.5 g β-CD complex / essential oil, followed by product P_4 which contains 0.75 g β-CD complex/essential oil and P_2 product, with 0.25 g β-CD complex/essential oil. The increase in lipid oxidation of the product P4, compared to the product P3 may be explained by the decrease of β-CD solubility, which determines an uneven distribution of β-CD complex/essential oil in the weight of the product.

3.3. Water activity. Water plays an important role in ensuring the quality and safety of food. The presence of water in foods influence their texture and favors the growth of microorganisms which cause food spoilage. Water also provides conditions for the deployment of chemical and enzymatic reactions that are responsible for color, taste, smell and physical, chemical and nutritional stability of food. The results of the water activity variation in the ham-type meat products, during the storage period are presented in FIG. 3 and are consistent with those found by [30, 35]. On the first day of storage, water activity values of the prepared products are between 0.961 ± 0.021 for the control sample, which contains only sodium nitrite and 0.969 ± 0,0,013, for P2 sample which contains a mixture of coriander and allspice essential oils encapsulated in β-CD.

Sample 1, which contains a mixture of free essential oils, has less water activity in the first 21 days of storage, because free oil has the ability to reduce the water activity as a result of the decrease in molar fraction of water in the mixture and also the decrease of vapor pressure at food-air equilibrium. Similar explanations are expressed by Walstra (2003) [36]. The decrease in water activity for the products P2, P3, P4 is evidence of slow release of coriander and allspice essential oils encapsulated in β-CD.

The values of water activity is not correlated with the water content determined gravimetrically due to the presence of ionizable substances in products content, which influences the water activity [36].

3.4. Microbiological analyses. Microbiological analyses made for these products revealed the occurrence of aerobic mesophilic bacteria and also of enterobacteria only after 28 days of refrigerated storage (Table 3).

From Table 3 it can be observed that the number of microorganisms contained in the product with free essential oils, is less than in the control sample, which does not contain essential oils. For the products with encapsulated oils, the number of mesophilic bacteria appeared after 28 days of storage is less than in the product with coriander and allspice encapsulated essential oils due to its slow release.

3.5. Sensory evaluation.Although three ham type meat products (P2, P3, P4) with coriander and allspice microencapsulated essential oil were obtained, only P3 was chosen for research for two reasons: the amount of powder (β-cyclodextrin with coriander and allspice essential oil) added in brine and the degree of solubilization of the powder (β-cyclodextrin with coriander and allspice essential oil) in brine.

Given these considerations, after the first day of storage, it was observed that the flavor and taste of coriander/allspice perceived by the panelists during the tasting of P1 product was very strong (12 of the panelists said that the consumption of the product is almost unacceptable, in sensorial terms). Instead, the smell of coriander/allspice perceived by the panelists for P3 product was lower than the taste of coriander/allspice of the same product, due to the masking of the odor by encapsulating the essential oils in β-cyclodextrin. This aspect highlights the slow and gradual release of essential oil of coriander and allspice encapsulated in β-cyclodextrin during mastication due to changes in pH. During chewing, the acidity of the oral cavity increases to pH values between 5.5-6 at which level the release of coriander/allspice essential oil from β-cyclodextrin is slow and constant at a rate of 50% in the first 14 days of storage.

Although the amount of powder (β-cyclodextrin with allspice and coriander essential oil) added in P4 product of 0.75g (0.03%) was higher than the one added to P3 product of 0.5g (0.02%), P4 product had a lower score for odor and taste perception of coriander/allspice compared to P3 product, due to the unsolubilization of the amount of powder in brine while stirring. Although P0 product does not contain coriander/ allspice essential oil, it obtained a very low score for perception of smell of coriander/allspice, possible due to the strong odor during the tasting of P1 product.

During the 28 days of storage were not observed major differences in the perception of smell and taste of coriander/allspice for P1 and P3 products, these features remaining constant as follows: for P3 product (odor of coriander $<$ coriander taste, allspice smell $<$ taste of allspice) due to the use of coriander/allspice essential oils in encapsulated system in β-cyclodextrin; for P1 product (coriander smell $\geq$ coriander taste; allspice smell $\geq$ allspice taste) due to the use of coriander/allspice free essential oil and due to the impermeability of the membrane.

4. Conclusions

Coriander and allspice essential oils have strong and pungent taste and smell, which could reject consumers; for this reason, the use of such oils in order to increase the shelf-life, without the product being rejected by the consumer, on sensory basis, encapsulation in β-CD may be a method for reducing these drawbacks. The optimal dose of β-CD complexed with a mixture of coriander and allspice essential oils was the appropriate one of 0.2 g / kg of meat (0.02%), according to International Standards on the use of cyclodextrins in food, extending the shelf life of the ham type meat product with a low content of salt, nitrite and tripolyphosphate.

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Compliance with Ethics Requirements. Authors declare that they respect the journal’s ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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