Inhibition and reduction of general microflora with natural antimicrobial agents (oregano), in poultry products, Albania

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Abstract. Food safety today is one of the top priorities for consumer health. The study aims to realize products of pure poultry with high antimicrobial values. The most important point of studies of this type is the determination of lower antimicrobial concentrations that inhibit the action of harmful microorganisms. The methodology of this study is based on the microbiological analysis of chickens in the poultry plants of Patos (Fier) and Boboshtica (Korça) untreated and after treatment with oregano (natural antimicrobial agent). From the laboratory analysis it was found that there is a significant difference from part to part in the untreated samples. The surface offers less load compared to the inside and neck area associated with packaging techniques and hygienic conditions during processing. In untreated Patos chickens the bacterial load dominates, the fungal one is almost negligible, in untreated Boboshtica chickens the bacterial and fungal load dominates, where both signs are significantly reduced after treatment.

Key words: safety, natural antimicrobial agent, oregano, neck, abdominal part, poultry.

1. Introduction

The chicken production industry is closely related not only to the technological side, such as optimal parameters, technological conditions of slaughter, the degree of mechanization, organization of wards, but also to the quality side of the raw material and final products. Chicken meat and their by-products belong to the large group of food products consumed by the Albanian population (Jay, 2000). Their consumption has increased and this is related to:

1. Albanian biodiversity and the existing tradition for the consumption of this product;
2. At the relatively normal price at which poultry products are offered to the consumer;
3. In the nutritional values they provide, evidencing a relative reduction of the fatty part which would cause possibly irreparable problem of the cardiovascular system.

The fatty part identified or chemically assessed in poultry industry products depends on the variety selected and the food that specific breeds use. Albanian companies market poultry products in competition with their sister products at home and abroad. It is this competition that requires a clear and complete system of control and certification. Quality, safety and control are obliged to be performed referring to the law "On Service and Veterinary Inspectorate". The prospects of the country in terms of the use of this type of food require rigorous hygienic-sanitary and veterinary measures, optimal parameters of storage of products in refrigeration systems, implementation of existing laws, accurate certification of local products, implementation of new norms and rules, in accordance with those of the European Union to prevent and manage the potential risk of these products. (Tribune expert, 2004)

The methodology of experimental work offers a range of experiments of chemical and microbiological nature of control and quality as well as modern methods to reduce microbial contamination of these products. The main directions of the organization of scientific research are listed as follows:

1. In the factors that affect the increase of microbial load in a poultry product, to be offered to the consumer safely;

2. in determining the ways to intervene in the process of colonization of *Salmonella* spp. inside the chicken digestive tract and to minimize the spread of colonized microorganisms to other edible parts using antimicrobial agents. The most important point of studies of this type is the determination of lower antimicrobial concentrations that inhibit the action of harmful microorganisms (USDA, 2005b).

The main purpose of scientific research is to reduce the degree of resistance of pathogenic bacteria and their distribution. Referring to the global trends to provide BIO type products (natural products without chemical interference) are balanced scientific research studies that tend to preserve and provide products that have an advantage in the poultry industry, using natural antimicrobials, mainly oils essential (Prifti, 2007). The action of substances of pharmaceutical nature, antibiotics, is effective especially in those genera and species that are sensitive and very sensitive to the action of the above substances (Kallço, 2005). But, since currently the consumption of chicken meat has significantly increased, if it is treated with antibiotics the load of antibiotics in the human body continuously increases by increasing the degree of immunity and making the organism resistant when it is in specific pathology needs the

use of antibiotics. This is the main reason that specific studies in the US and in European Community countries use essential oils of plant origin that do not generally spoil the naturalness of the finished products. As essential oils: Clove oil; oregano oil; thyme oil (Nestle, 2001).

2. Material and Methods

Two mass consumable samples were used, Korca Chickens "Boboshtica" and Patos Chickens. They are champions who come from two different areas of the country which together with the product offer changes that are reflected in the variety, hygiene, safety and packaging. Samples were analyzed first untreated and then treated with antimicrobial agents. (USDA, 2005a)

The samples taken for analysis were not treated during the control in full because theoretical data accept visible changes in the microbial load passing from the surface areas to those of the interior. It is accepted from the literature that the inner part generally offers, due to the tissue structuring of the protein composition, a greater microbial load compared to the surface area, which is influenced by the environmental conditions and the existence or not in abundant amounts of adipose tissue. It should be noted that the neck area has a load several times greater than other areas (Varnam & Sutherland, 1995). Likewise, areas of the interior within or away from major organs present different burdens. If we return to the parts that are packaged, the contacts that the surface has with the surrounding environment must be evaluated, the substrate that the surface offers to the microorganisms in the white meat (Yang et al., 2001). In order to create a complete picture, the analysis of certain parts was done: surface, interior (part of the thighs, white meat and the area near the organs, the part of the neck) (Harley & Prescott, 2002). The microbiological control was carried out taking into account the control methods in the Technical Microbiology practicum (Frashëri, 1982a). MPA terrain were used for bacteria, YM-Broth for yeast. This terrain was chosen because it is known for efficient results for bacterial and yeast load, the Çapek terrain, which due to the naturalness of the preparation without using antibiotics against bacteria, offered after 24 hours and 48 hours the bacterial load. The dilution method was applied, respectively: 1:10 (first dilution); 1:100 (second dilution); 1:1000 (third dilution). It was worked with two parallels for each sample and the results were checked after 24-hours, 48-hours and 7-days for molds (Frashëri, 1982b). Each specified fraction of the selected products was analyzed in the same way to provide an opportunity to compare results.

The same grounds and the same method were applied to the samples treated with antimicrobial agents, the interpretation of the results of which will be given in detail in this experimental part.

3. Results and Discussions

The results of the experiment for the untreated samples are presented in full in Tables 1 and 2. In Table 1, the microbiological evaluation of the chickens of Boboshtica (Korçe) is presented in detail. The microbiological control to verify the safety of the product was analyzed on the skin, the inside, the part of the thighs, the inside in the area of the digestive system. (Forsythe & Hayes, 1998). This anatomical division was made based on the data of the literature on the control of the surface and interior and on the changes offered by the area surrounding the digestive apparatus, the latter being related to the significant substrate for the growth of microorganisms.

Table 1. Microbiological control of chickens from Boboshtica, Korçë (sample not treated with oregano).

No.	The part taken for analysis	MPA terrain					YM-Broth terrain				ÇAPEK terrain					
		Incubation time					Incubation time				Incubation time					
		24 hours cfu/g			48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		7 days cfu/g	
		Dilution	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	surface (skin)	1	16	No counting	No counting	No counting	-	No counting	No counting	No counting	No counting	No counting	No counting	No counting	60	No counting
		2	14	14	No counting	No counting	-	25	No counting	No counting	-	-	No counting	No counting	5	17
		3	4	1	4	14	-	-	5	11	-	-	-	-	17	29
2	Inner (white meat)	1	No counting	No counting	No counting	No counting	-	-	53	54	No counting	No counting	No counting	No counting	-	45
		2	5	5	51	10	-	1	No counting	18	-	1	-	-	-	-
		3	-	-	-	2	19	8	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting
3	inside (close to organs)	1	No counting	22	No counting	12	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	1	1
		2	No counting	5	No counting	No counting	310	No counting	No counting	No counting	-	-	-	-	-	-
		3	No counting	No counting	No counting	No counting	1	2	No counting	No counting	No counting	No counting	No counting	No counting	1	1

Table 2. Microbiological control of Patos chickens (sample not treated with oregano).

No.	The part taken for analysis	MPA terrain					YM-Broth terrain				ÇAPEK terrain					
		Incubation time					Incubation time				Incubation time					
		24 hours cfu/g			48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		7 days cfu/g	
		Dilution	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	surface (skin)	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	60	No counting
		2	960	750	No counting	No counting	448	504	No counting	No counting	-	-	No counting	No counting	5	17
		3	110	720	860	824	50	45	178	220	-	-	-	-	17	29
2	Inner (white meat)	1	No counting	No counting	No counting	No counting	37	320	No counting	No counting	70	60	108	No counting	-	-
		2	124	207	728	628	180	192	No counting	No counting	23	20	96	48	-	-
		3	4	9	28	44	-	-	88	104	-	-	28	45	-	-
3	The neck	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	3	-
		2	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	-	-
		3	800	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	-	-

Table 3. Microbiological control of chickens from Boboshtica, Korcë (sample treated with oregano).

No.	The part taken for analysis	MPA terrain					YM-Broth terrain				ÇAPEK Terrain					
		Incubation time					Incubation time				Incubation time					
		24 hours cfu/g			48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		7 days cfu/g	
		Dilution	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	surface (skin)	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	528	480	No counting	No counting	2	-
		2	65	58	67	63	92	120	112	158	72	68	96	112	-	1
		3	11	20	17	38	28	36	33	48	-	6	15	28	1	-
2	Inner (white meat)	1	No counting	No counting	No counting	No counting	232	248	No counting	No counting	640	648	648	720	2	5
		2	158	160	178	168	160	138	166	156	42	48	49	62	-	1
		3	25	29	28	35	18	28	25	36	1	-	6	2	-	-
3	inside (close to organs)	1	No counting	No counting	No counting	No counting	180	220	197	228	172	164	176	178	-	1
		2	19	33	40	37	40	41	48	44	38	24	40	36	-	2
		3	13	-	21	8	3	4	24	15	-	-	-	7	-	1

Table 4. Microbiological control of Patos chickens (sample treated with oregano).

No.	The part taken for analysis	MPA terrain					YM-Broth terrain				ÇAPEK terrain					
		Incubation time					Incubation time				Incubation time					
		24 hours cfu/g			48 hours cfu/g		24 hours cfu/g		48 hours cfu/g		24 hours cfu/g		48 hours cfu/gr		7 days cfu/g	
		Dilution	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	surface (skin)	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	10	4
		2	No counting	No counting	No counting	No counting	178	180	No counting	No counting	No counting	No counting	No counting	No counting	1	7
		3	348	298	360	308	124	136	248	296	120	148	158	151	1	3
2	Inner (white meat)	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	2	-
		2	898	882	960	1080	880	892	886	968	760	720	Pa nr	Pa nr	8	3
		3	436	328	448	346	380	404	428	408	132	148	260	216	3	1
3	The neck	1	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	No counting	27
		2	920	608	No counting	No counting	760	720	898	780	688	670	No counting	No counting	-	-
		3	588	288	No counting	No counting	312	448	348	451	124	184	208	218	-	-

There are obvious changes when passing from the first dilution to the third, when passing from one part taken for analysis to another. Particles present visible bacterial loads clearly and abundantly in the internal area close to the organs. There is a relatively low bacterial load in the thigh area and a normal bacterial load in the first dilution of the surface. With the passage of the incubation time from 24 hours to 48 hours, the microbial load of the surface changes significantly, it grows rapidly and the pigmented yellow and red bacteria dominate. In some cases, capsular formations are observed, especially in the YM-Broth terrain (in the bacterial load that this terrain provides). Perhaps this is related to the special role of the carbon sources present in the terrain. The YM-Broth medium provides a very limited yeast load as expected from the literature and also a bacterial load that stands out in the first 24 hours where there is mainly growth of colorless, non-pigmented bacteria. In the inner area near the organs, in contrast to the other parts, yeasts with a visible development of pseudomycelium result, which is not observed either on the surface or inside. This is expected due to the large load that is warned inside the organs. While the YM-Broth medium offers us yeast symbiosis conditions in a limited number but not pseudomycelial yeasts. Among the clearly developed fungal colonies, we mention *Mucor* spp. on the surface that may not be related to the surface but to the surrounding environment, the genus *Penicillium* spp. and *Aspergillus* spp. The number of *Mucor* colonies in the first dilution is very large, especially on the surface. The thigh area has a visible pleural burden which is completely replaced in the intra-organ part by bacteria and yeast with pseudomycelia. Table 2 provides the results of Patos chickens which are widespread in the market of the capital and in other parts of the country. The parts taken for analysis are: Surface; interior; the neck (Frashëri, 1982b).

The internal part has not been fully analyzed, but only the white meat has been selected, which has not been microbiologically checked in Boboshtica chickens. From the beginning, a very large microbiological load is noticeable compared to the chickens of Boboshtica. Bacterial colonies are evident in the MPA field as colorless transparent or pigmented colonies in a relatively large number already in the first 24 hours and with an innumerable level on the surface and neck. Meanwhile, the neck area offers even in the third dilution 800 colonies per plate, a very high figure. There are many colonies that have formed a visible capsule and symbiosis is observed within the bacteria themselves. White meat offers a limited load compared to the surface and neck. Whereas YM-Broth medium provides only bacterial colonies but not yeast.

Pseudomycelial yeasts are not observed. After 7 days in the Çapek field, a limited number of fungi are observed on the surface and there are none in the interior, while the genera *Aspergillus* spp. and *Cladospora* spp. are observed on the neck.

4. Conclusions and recommendations

Many conclusions have been reached from the experimental data of the untreated samples, which have been detailed and interpreted in the results section. In summary, they are presented as follows:

1. The microbial load of the chickens of Patos (Fier) is significantly greater compared to those of the Korça area. This may also be related to the presence of a higher amount of adipose tissue (triglycerides) in the production of Pathos chickens.

2. There is a noticeable difference from particle to particle. The surface offers less load compared to the interior and neck area related to packaging techniques and hygienic conditions during processing. The inside part offers visible changes in the thigh area, in the white meat and near the organs (abdominal part).

3. The area near the organs has a very large load, especially the one centered around the digestive system. The biggest load is the bacterial load. Large colonies and often developed microbial forms that live together and use the ground where they develop (symbiotic and metabiotic forms) are evident. Among the yeasts identified in the interior, those with pseudomycelium dominate, while the number of other yeasts is almost reduced.

4. The neck area is evidently very busy. This is also consistent with the theoretical material. It is theoretically accepted that slaughtering techniques can increase the microbiological load provided.

5. The very big difference that the two selected samples offer, leaves a path open for a detailed control of the new untreated samples to create a complete picture of the poultry products of the Albanian market.

6. Looking at the differences offered by the individual parts, it is also recommended to check the products sold in pieces, but also those destined for the following thermal treatment, when it comes to mass consumption (parts used in Fast Food).

7. The term safety takes on its own meaning from the products that are put on the market and is not left simply to forms of thermal treatment, but to the use of efficient methods on untreated samples with microbial load reducing agents including natural antimicrobials.

8. In untreated Pathos chickens the bacterial load dominates, the fungal one is almost negligible

9. In untreated Boboshtica chickens, bacterial and fungal load dominates, both of which are significantly reduced after treatment.

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