RESEARCH ON SOME MICROBIOLOGICAL QUALITY OF NATURAL SPICES USED IN THE FOOD

Gheorghe PUCHIANU[1], V.NECULA, Mihaela BABII, D.V. ENACHE

Abstract. *During the period 1.01.2012 - 30.04.2013 We examined a sample of 38 natural spices, taken from 17 commercial units. The diagnosis was made in the Laboratory Veterinary and Food Safety Brasov, using standardized methods: yeasts and molds, Enterobacteriaceae, Salmonella spp and Total Number of Germs . Each sample consisted of 5 units. The natural spices examined were contaminated grain of mustard yellow (parameters: Yeasts + Molds, Enterobacteriaceae and Total Aerobic Plate Count) and black pepper (the parameters: Yeasts + Molds and Total Aerobic Plate Count. Black pepper, cinnamon and bay leaves were irregular in a single parameter, and nutmeg, coriander and cumin were not contaminated with bacterial germs and molds. In terms of significance, the presence Enterobacteriaceae an indicator of faecal contamination, fungal contamination of a deterioration of the structural elements of defense, increased humidity and improper storage temperature, and an indicator of poor hygiene Total Aerobic Plate Count processing.*

Key words: natural spices, microbiological contamination, laboratory diagnosis

1. Introduction

Spices are produced without nutritional value (or low nutritional value), which is added to food in small quantities to give them special taste (taste, odor, flavor) higher, thus stimulating gastric secretions, appetite and digestion. They can lead to improved flavor, taste and increase shelf life of food is added.

Spices effect is caused by the presence of chemical composition seasoning properties: essential oils, aldehydes, esters, ketones, alcohols, hydrocarbons, terpene resins, etc.. This effect taste should not be used for hedging purposes or manufacturing deficiencies that occur as a result of alteration processes food. Unfortunately, some manufacturers sometimes used fraudulently spices to mask the smell and taste defects in materials prepared with questionable freshness.

Spices are classified according to the nature, origin and organoleptic characteristics of the following groups: natural spices themselves: flowers, fruit, leaves, rhizomes, seeds, bark of plants seasoning.

Use fresh or dried (and some frozen - ex. parsley, dill, lovage, etc.) or serve as raw material for extraction of substances seasoning, spices acidic seasoning products

[1] Prof.Dr.Transilvania University of Brasov - Faculty of Food and Tourism
E-mail: gpuchianu@yahoo.com

industry (table mustard, condiment sauces) oils ether and oleo-resins, spices salt (table salt).
The microorganisms that may be present in the spices are usually resistant to drought conditions, so that the seasoning can be found in most gram-positive bacteria or spores and fungal spores.
Initial micro spices derived from the plant and the soil were cultured at which the added microflora dust coming from their contaminated during the collection and handling of faecal contamination of rodents and insects, from the water used in processing, etc. Fungal contamination can come from first or added microflora during storage and transportation. The ground spices the total number of microorganisms is generally the same as that of the whole spices, if grinding was done under hygienic conditions.

2. Materials and Methods

During the period 1.01.2012 - 30.04.2013 we examined a number of 38 samples taken from 17 natural spices trading units.
Varieties studied were:
-yellow mustard seeds (Sinapis alba) - seeds mature, dry, spherical, with a diameter of 2.0-2.5 mm;
-black mustard seeds (Sinapis nigra) - mature seeds, dry, spherical, with a diameter of 1.0-1.5 mm brown red;
-black pepper (Piper nigrum) - unmatured whole grains, dry, wrinkled, pale gray-brown, 4-5 mm in diameter;
-ground black pepper - powder;
-cinnamon (Cinamomum ceylonicum) - shell, twisted dry;
-nutmeg (Myristica fragrans Houtte) - dried seeds, hulled, with a length of 15-30 mm, a thickness of 10-20 mm, the yellow-brown;
-coriander (Coriandrum sativum) - ripe fruits, dried spherical with a diameter of approx. 3 mm, yellowish-brown;
-cumin seeds (Carvum carvi) - ripe fruits, dried long 4-6 mm, color gray-brown;
-laurel (Laurus nobilis) - leaves 5-6 cm long and 2-3 cm wide, dry, shiny, green. (Table 1 and Photos 1- 9).
The diagnosis was made according to the standards of work, and ISO 21528-1 or 2 for *Enterobacteriaceae*, ISO 21527/1 or 2 for *yeasts and molds*, ISO 6579/2003 AC/2006 for detection of *Salmonella spp*. EN ISO 4833 for detection *Total Aerobic Plate Count*, samples made up of 5 parts, as follows:

Table 1. Varieties, number of samples and of establishments whwew they were taken of

No. Crt	Specification	No. establishments from which samples were taken	No. Samples /No. Units
1.	Yellow mustard seeds	2	6/30
2.	Black mustard seeds	1	3/15
3.	Black pepper grains	3	5/25
4.	Graund black pepper	3	4/20
5.	Cinnamon	2	7/35
6.	Nutmeg	2	3/15
7.	Coriander	1	2/10
8.	Caraway	1	2/10
9.	Dafin	2	6/30
	TOTAL	17	38/190

Photo 1. Yellow mustard seeds

Photo 2. Black mustard seeds

Photo 3. Pepper grains

Photo 4. Pepper ground

Photo 5. Cinnamon

Photo 6. Nutmeg

Photo 7. Coriander

Photo 8. Caraway

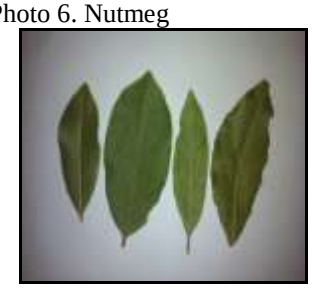

Photo 9. Dafin

Enterobacteriaceae detection. Use colony-count technique at 37^{o}C on solid medium violet red bile glucose agar (VRBG).
For the purpose of the laboratory has the following stages: making decimal dilutions, seed by pouring into each Petri dish 15 ml VRBG, previously cooled to 37^{o}C to 47 ^{o}C in a water bath, the incubation for 24 hours at 37 ^{o}C; examination for the presence of *Enterobacteriaceae* colonies considered, calculation of the number of CFU of *Enterobacteriaceae* per gram. Typical colonies are pink to red or purple (with or without halos precipitates) are selected at random five such colonies for passage to confirmatory biochemical tests; alleged *Enterobacteriaceae* colonies were restreaked on nonselective medium, oxidase reaction and confirmed using biochemical, oxidase test and fermentation test.
Colonies that are negative at oxidase test and positive at glucose test confirmed as *Enterobacteriaceae* (Photo 10).

Photo 10. Oxidase and fermentation tests

Determination of yeasts and molds. The procedure establishes general guidelines for counting viable yeasts and molds in products intended for human consumption colony count technique at a temperature of 25^{o}C. *Yeasts and molds* - are microorganisms that form colonies at 25^{o}C specific selective medium.
To achieve diagnosis following steps have: they took two sterile Petri dishes. With a sterile pipette to last in each case 1 ml of the initial dilution. Each box denotes the number of sample dilution and time. They took two sterile Petri dishes. With another sterile pipette, to each 1 ml of dilution trcut 10^{-1} (liquid product) or 1 ml of 10^{-2} dilution (other products) in each box. In some cases, the procedure was repeated with different dilutions. There are many dilutions were prepared so that in a Petri dish to obtain more than 10 and less than 150 colonies. It was poured approximately 15 ml of yeast extract medium cloranfenicol dextrose agar (MEDDCA) and / or agar DG 18, previously melted and kept at 45^{0}

± 1^0 C in a water bath, in each Petri dish. The time between the end of the initial suspension preparation (dilution 10^{-1} or if the product is liquid) and when it is poured into the boxes Petri did not exceed 15 min. The inoculum was mixed thoroughly with the environment, and the mixture was allowed to solidify, after which the Petri dishes were placed on a flat, cold. Witness box was prepared with 15 ml medium for checking sterility. Aerobic incubation was performed at 25^0C boxes for 4 or 5 days, after which we proceeded to calculate the number of yeasts and molds per gram or ml of sample from the number of colonies obtained in the dilution chosen boxes that enable a significant result .
After three, four and five days of incubation colonies were counted in each Petri dish is retained the boxes that contained less than 150 colonies.

Photo 10. *Penicilium urtice* and *Fusarium nivale*, medium colonies DDCA (original)

Photo 11. *Aspergillus citrinum, Penicilium urticae* medium colonies DDCA (original)

Photo 12. *Rodothorulla rubra, Rodothorulla mucilaginosa* medium colonies DDCA (original)

Number of yeasts and molds per gram or ml was calculated using the following formula:

$$N = \frac{\Sigma C}{(n_1 + 0{,}1\, n_2) \times d}$$

where: ΣC – amount of colonies counted in all boxes, n_1 – number of boxes retained colonies first dilution, n_2 - number of boxes held at the second dilution, d – the dilution of which the first counts were made (for example 10^{-2}).
The result is expressed as a number between 1.0 and 9.9 multiplied the 10x where x is the power of 10.
If there is no initial colony suspension in appropriate containers as starting material was solid, the number of yeasts and molds per gram of product has been reported to be less than 10. If the sample boxes there is no colony was originally the product liquid, the number of yeasts and molds per milliliter product reported as being less than one.
Detection of bacteria in the genus *Salmonella.* To prepare the suspension of basic fraction analyzed by 25 g was added to 9 volumes (225 ml) of BPW (APT) previously brought to ambient temperature. This mixture was treated in a

stomacher for one minute, avoiding foaming and removing the air from the bag as possible.

Subsequently, detection of *Salmonella spp* requiring completion of four successive phases:

1.Preenrichment in selective liquid media: BPW was inoculated at room temperature with the sample for analysis, then incubate it at 37 º C ± 1 º C for 18 h ± 2 h;

2.Enrichment in selective liquid medium: Rappaport-Vassiliadis medium with soya (RVS bullion) and Muller-Kauffmann broth Tetrathionate / novobiocin (bullion MKTTn) culture inoculated with peptone water obtained after inoculation. RVS broth is incubated at 41.5 ° C ± 1 ° C for 24 hours ± 3 hours and MKTTn broth at 37 ° C for 24 hours ± 3 hours.

Photo 13.
Rappaport –Vasilliadis medium
before and after incubation

Photo 14.
Müller –Kauffman medium
before and after the incubation

3.Isolation and identification: the cultures obtained after inoculation RVs and MKTTn. Inoculate two solid selective media: xylose-lysine-deoxycholate agar (XLD agar) any other solid selective medium complementary to XLD agar and especially suitable for lactose-positive Salmonella isolation (eg we used the Rambach). XLD and Rambach agar medium incubated at 37 º C ± 1 º C and examined after 24 h ± 3 h

4.Confirmation of the identity - after allegedly Salmonella colonies grew, they've isolated and confirmed the identity by biochemical and serological tests.

Determination of Total Aerobic Plate Count (APC). Establishes the presence or absence of aerobic microorganisms in food products examined by colony counting method at a temperature of 30°C.

The principle of the method consists in sowing towards a solid poured into two Petri dishes with a quantity of sample for analysis and sowing decimal dilutions of the sample to be analyzed or mother suspension (initial). Working dilutions were

prepared so much that in a Petri dish to obtain more than 300 colonies. Sowing is done by embedding the solid culture medium or a sample of a known amount of work represented by a range of gram or decimal dilutions in Petri dishes incorporation after solidification environment.
Working methodology involves the following steps:
-Sample preparation for analysis and initial dilution.
With a sterile pipette put 1 ml of the original solution in a test tube containing 9 ml of sterile dilution. Are then dilutions, the concentration difference is 10x (decimal dilutions)
-Seeding and Incubation:
1.Take two Petri dishes in which sterile pipette 1 ml sample analyzed. Take two other sterile Petri dishes. With a new sterile pipette is inserted in each case 1 ml of the first decimal dilution of the sample to be analyzed (10^{-1}). Repeat this procedure with the following dilutions using some new sterile pipette for each decimal dilution.
2.Pour into each Petri dish that was filed about 15 ml inoculum agar or nutrient agar at 45 ±1oC white. Mix thoroughly inoculated culture medium and allowed to solidify putting Petri dish on a cold surface and horizontal.
3.Incubate the Petri dishes with the lid down. Incubation was at 30 ±1oC for 72±2 hours.
-Interpretation - after the period of incubation is done in counting colonies in each Petri dish containing no more than 300 colonies. (Photo no. 15 and 16)
-Calculating - to hold cans containing 15 to 300 colonies in the two successive dilutions.
-Expression of results.

Photo 15.
Total aerobic plate count (10^{-1})

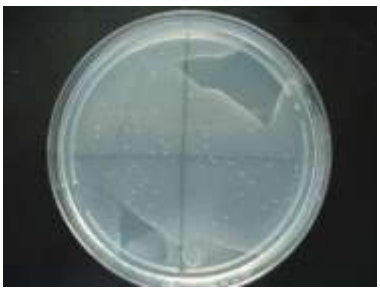

Photo 16.
Total aerobic plate count (10^{-3})

For counting boxes are used which contain less than 300 colony and at least 15 colonies. The number of organisms per ml or g of the product, is calculated as a weighted average of the following formula:

$$N = \frac{\Sigma C}{(n_1 + 0{,}1\, n_2) \times d}$$

where: ΣC – amount of colonies counted in all boxes, n_1 – number of boxes retained colonies first dilution, n_2 - number of boxes held at the second dilution, d – the dilution of which the first counts were made (for example 10^{-2}).

3.Results and Discussions

Microorganisms that can contaminate spices are classified impaired and pathogenic microorganisms.

Microorganisms of impaired - can cause food spoilage and subsequent spices plus. Impaired bacterial spices when it occurs, is usually caused by bacteria of the genus Bacillus (*B. subtilis, B.licheniformis, B.megaterium, B.pumilis, B.brevis, B.cereus* etc.) and *Clostridium.* Altered fungal spices is commonly produced by molds (*Aspergillus glaucus, Aspergillus niger, Penicillium spp*), some of which are able to synthesize mycotoxins dangerous to consumer health.

Pathogenic microorganisms - spices can sometimes contain antimicrobial agents that can be caused by food poisoning, especially sporogenous bacteria, anaerobes such as *Clostridium perfringens, Bacillus cereus* and *Bacillus subtilis* rare. Nesporogene gram negative bacteria also can be found in spices, usually as a result of recent contamination, eg: *Escherichia coli* and *Salmonella spp Staphylococcus aureus* is rare in spices, although bacteria resistant to dryness. *Coliform bacteria* are often found in spices, but their presence indicates, generally, recent contamination, due to their sensitivity of dryness.

Towards detecting *yeasts and molds* were examined 38 samples. Each sample was composed of five units, which means that the total number of tests performed was 190 (Table 2).

It is found that the total number of 33 samples examined were consistent fits within the values of admissibility (maximum 2 units of each sample showed values between 10^5 and 10^6 or lower values of 10^5) and a number of 5 samples representing a rate of 13.2% were non-compliant (less than 2 units of each sample showed values between 10^5 and 10^6 or greater than 10^6). Most non-compliant samples were found in black pepper (50% of total samples examined). Considerable percentage of non-compliance were recorded in other kinds of natural spices namely 16.7% from yellow mustard seeds and 14.3% the cinnamon.

This can be explained if the pepper that is a less stable microbiologically than grains, being devoid of structural elements of defense, which is why mold cleaner come in direct contact with the nutrients necessary for their development. The fungal contamination of the samples examined were determined product moisture and storage temperature likely factors that favored the emergence of alterative changes.

Table 2. Samples examined for the detection Yeasts and Molds

No. Crt	Specification	No. Samples /No. Units	Results obtained					
			Compliance			Non compliance		
			No.	%	Values cfu/g	No.	%	Values cfu/g
1.	Yellow mustard seeds	6/30	5/25	83,3	$1,2x10^3$ $1,3x10^4$	1/5	16,7	$1,11x10^6$ $1,43x10^6$
2.	Black mustard seeds	3/15	3/15	100	$1,2x10^2$ $4,3x10^4$	-	-	
3.	Black pepper grains	5/25	5/25	100	$1,25x10^3$ $2,43x10^4$	-	-	
4.	Graund black pepper	4/20	2/10	50,0	$9,4x10^4$ $9,8x10^4$	2/10	50,0	$1,85x10^6$ $2,18x10^6$
5.	Cinnamon	7/35	6/30	85,7	$4,7x10^4$ $6,5x10^4$	1/5	14,3	$1,03x10^6$ $1,09x10^6$
6.	Nutmeg	3/15	3/15	100	$8,3x10^4$ $1x10^6$	-	-	
7.	Coriander	2/10	2/10	100	$5,5x10^4$ $5,7x10^4$	-	-	
8.	Caraway	2/10	2/10	100	$1,8x10^3$ $2,9x10^4$	-	-	
9.	Dafin	6/30	5/25	83,3	$3x10^5$ $2,6x10^4$	1/5	16,7	$1,17x10^5$ $1,33x10^5$
	TOTAL	38/190	33/ 165	86,8		5/25	13,2	1,17x105 2,18x106

Towards detection of bacteria of the family *Enterobacteriaceae* were examined 38 samples. Each sample consisted of 5 units. (Table 3).

It is found that the total number of 36 samples examined were consistent fits within the values of admissibility laid down in Regulation 2073/2005 (maximum 2 units of each sample had between 100 -1000 cfu / g or lower values 100), and a total of two samples representing a rate of 5.3% was inconsistent (less than 2 units of each sample are shown between 100 -1000 cfu / g or greater than 1000 cfu / g). Percentage most non-compliant samples were found in black pepper grains (20% of total samples examined). Considerable percentage of non-compliance were recorded in other kinds of natural spices namely 16.7% from yellow mustard seeds.

Enterobacteriaceae contamination can be explained by different sources of contamination secondary intervention most likely set of non hygienic conditions during harvest and handling operations, packing, knowing that the presence *Enterobacteriaceae* are indicators of faecal contamination of the substrates on which they are found.

Table 3. Samples examined for detection spices Enterobacteriaceaelor

No. Crt	Specification	No. Samples /No. Units	Results obtained					
			Compliance			Non compliance		
			No.	%	Values cfu/g	No.	%	Values cfu/g
1.	Yellow mustard seeds	6/30	5	83,3	$1,2x10^2$ $3.2x10^2$	1	16, 7	$4,6x10^3$ $4,8x10^3$
2.	Black mustard seeds	3/15	3	100	$1,2x10^2$ $1,65x10^2$	-	-	
3.	Black pepper grains	5/25	4	80,0	$3,3x10^2$ $5,1x10^2$	1	20, 0	$1,37x10^3$ $2,5x10^3$
4.	Graund black pepper	4/20	4	100	$7,2x10^2$ $8,3x10^2$	-	-	-
5.	Cinnamon	7/35	7	100	$2,2x10^2$ $2,7x10^2$	-	-	-
6.	Nutmeg	3/15	3	100	$3,1x10^2$ $3.5x10^2$	-	-	-
7.	Coriander	2/10	2	100	$1,8x10^2$ $2,6x10^2$	-	-	-
8.	Caraway	2/10	2	100	$8,8x10^2$ $1x10^3$	-	-	-
9.	Dafin	6/30	6	100	$6,3x10^2$ $7,1x10^2$	-	-	-
	TOTAL	38/190	36	94,7		2	5,3	$1,37x10^3$ $4,8x10^3$

Table 4. Samples examined for the detection of *Salmonella spp*.

No. Crt	Specificare	No. Samples /No. Units	Results obtained					
			Compliance			Non compliance		
			No.	%	Values cfu/g	No.	%	Values cfu/g
1.	Yellow mustard seeds	-	-	-	-	-	-	-
2.	Black mustard seeds	-	-	-	-	-	-	-
3.	Black pepper grains	-	-	-	-	-	-	-
4.	Graund black pepper	1/5	1/5	100	Abs.	-	-	-
5.	Cinnamon	-	-	-	-	-	-	-
6.	Nutmeg	-	-	-	-	-	-	-
7.	Coriander	-	-	-	-	-	-	-
8.	Caraway	1/5	1/5	100	Abs.	-	-	-
9.	Dafin	1/5	1/5	100	Abs.	-	-	-
	TOTAL	3/15	3/15	100	Abs.	-	-	-

Even if the national strategy does not provide detection of bacteria of the genus Salmonella germs on request benefi we examined three samples. Each sample consisted of 5 units. (Table 4).
It is found that all samples were compliant exams consecutively germs like *Salmonella* detection, which demonstrates that the harvest conditions were adequate and complied with human handlers hygiene, the presence of *Salmonella spp* (which belong to the family *Enterobacteriaceae*) representing indicators of faecal contamination.
The national strategy even if the program does not provide the total aerobic plate detection, we request benefi examined 10 samples, each sample being composed of 5 units. (Table 5).

Table 5. Samples examined for the detection of Total Number of Germs

No. Crt	Specificare	No. Samples /No. Units	Results obtained					
			Compliance			Non compliance		
			No.	%	Values cfu/g	No.	%	Values cfu/g
1.	Yellow mustard seeds	2/10	1	50	$3,2x10^4$ $6,2x10^4$	1	50	$1,23x10^5$ $1,42\ x10^5$
2.	Black mustard seeds	1/5	-	-	-	1	100	$1,42\ x10^5$ $2,25\ x10^5$
3.	Black pepper grains	2/10	2	100	$3,2x10^4$ $6,2x10^4$	-	-	
4.	Graund black pepper	2/10	1	50	-	1	50	$3,5\ x10^5$ $4,2\ x10^5$
5.	Cinnamon	4/20	4	100	$9,1x10^4$ $1x10^6$	-	-	-
6.	Nutmeg	1/5	1	100	$7,3x10^4$ $8,21x10^4$	-	-	-
7.	Coriander	-	-	-	-	-	-	-
8.	Caraway	1/5	1	100	$6,52x10^3$ $8,8x10^3$	-	-	-
9.	Dafin	-	-	-	-	-	-	-
	TOTAL	13/65	10	76,9		3	23	23,1

It appears that out of the 10 examined samples of 7 were consistent fits within the values of eligibility, and a number of 3 samples representing 23% were irregular. Percentage most non-compliant samples were found in samples of yellow mustard seeds (100% of the samples examined). Considerable percentage of non-compliance were recorded in other kinds of natural spices namely by 50% from yellow mustard seeds and ground black pepper.
Percentages obtained are however irrelevant in terms of the number of samples examined, but they can still offer an insight aerobic microbial contamination of various kinds of natural spices.

The interpretation we made in relation to the provisions of Order 43/2012 of the President ANSVSA and Order 27/2011 (Table 6).

Table 6. Interpretation in relation to ANSVSA Provisions

Analysis to be performed	Sampling plan		Limitations	
			m	M
Enterobacteriaceae	5	2	100 cfu/g sau ml	1.000 cfu/g sau ml
Yeasts and molds	5	2	105 cfu/g	106 cfu/g
NTG	5	2		
Salmonella spp.	5	2	Abs	Abs

Conclusions

(1)In laboratory tests of 38 samples of natural seasonings, a total of five samples representing a rate of 13.2% were inconsistent in terms of fungal contamination, two samples representing a rate of 5.3% were infected with *Enterobacteriaceae* , the samples examined in the direction of detection of bacteria in the genus *Salmonella* were in line, and a total of 3 samples representing a rate of 23% were inconsistent in terms of *Total Number of Germs.*

(2)The natural spices examined were contaminated grain of mustard yellow (parameters: *Yeasts and Molds*, *Enterobacteriaceae* and *Total Number of Germs*) and black pepper (the parameters: *Yeasts and Molds* and *Total Number of Germs*). Black pepper, cinnamon and bay leaves were irregular in a single parameter, and nutmeg, coriander and cumin were not contaminated with bacterial germs and molds.

(3)*Enterobacteriaceae* presence of an indicator of faecal contamination, fungal contamination of a deterioration of the structural elements of defense, increased humidity and improper storage temperature, and an indicator of poor hygiene *Total Number of Germs* processing.

(4)Indicates the presence of bacteria usually recent contamination, due to their sensitivity of dryness. Some of the molds that contaminate spices may be able to synthesize mycotoxins dangerous to consumer health.

(5) Can contaminate food spices are incorporated microbiologic can produce their alteration.

References

[1] Bârzoi D., Meica S., Neguţ M.,1999, Food poisoning. Diaconu Coresi Publishing, Bucharest.

[2] Bârzoi D., Apostu S., 2002, Microbiology of food. Risoprint Publishing, Cluj Napoca.

[3] Necula V., Babii Mihaela, 2010, Food, nutrition and their impact on consumers. Transilvania University of Brasov Publishing.

[4] Necula V., Babii Mihaela, 2012, Sensory analysis of foods and food products. Transilvania University of Brasov Publishing.

[5] Puchianu G., 2012, Microbiological criteria for food safety and process hygiene. Transilvania University of Brasov Publishing.

[6] Răpuntean G, Răpuntean S., 1999, Special Veterinary Bacteriology. Publisher Tipo Agronomy, Cluj - Napoca.

[7] Rotaru O, Mihaiu M., Dan S., 2006, Control of animal health products. Publisher Risoprint Cluj - Napoca.

[8] Rotaru O, Mihaiu M., 2002, Pathology of the food. Todesco Publishing Cluj – Napoca

[9] Rusu L, Neagu M., 2011, Food Safety - Food Quality. Spiru Haret Publishing Science.

[10] Savu C., 2008, Hygiene and control of animal products. Signs Publishing.

[11] Ştirbu C., Turcu D., (1999) - Getting bacteriology. Publisher Brumaire, Timişoara.

[12] Order no. 17 Methodological Norms for the application of Timetable for surveillance, prevention and control of animal diseases, of those transmissible from animals to humans, animal welfare and environmental protection for 2011 and the surveillance and control program in the field of food safety 2011.

[13] Working methodology for detecting bacteria in the genus Salmonella - standard SR EN ISO 6579/2003 AC/2006 "Microbiology of food and animal feeding stuffs - Horizontal method for Salmonella spp research Commission Regulation no. 2073/2005 / EC.