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IMPACT OF MICROBIOLOGICAL QUALITY OF THE RAW MATERIAL AND THE TECHNOLOGICAL PROCESS IN THE MICROFLORA OF FINAL PRODUCT (CONFECTIONERY PRODUCTS)

Amilda Ballata[1*], Laura Shabani[1], Klodiola Dhamo[2]

[1]Department of Industrial Chemistry, Faculty of Natural Sciences, University of Tirana, Boulevard Zogu I nn., 1000 Tirana, Albania
[2]Department of Medical Education, Aldent University, Dibra street 235,1000 Tirana, Albania

[*]e-mail: amilda.ballata@fshn.edu.al

Abstract

The purpose of the study is to evaluate the microbiological quality of raw materials and the impact of the technological process used in the final quality of the some confectionery sweet products.

For this purpose several microbiological parameters were analyzed as: total aerobic mesophilic bacteria, yeasts, molds and coliform bacteria. These parameters were determinate in both, raw materials (cocoa, flour, eggs powder milk) and in the respective final product (Dolce with chocolate-coated and dolce non chocolate-coated). Colony forming unit counting method was used for quantitative evaluation, using decimal scale dilution and plate count agar cultivation method. Standard and selective media were used: Plate Count Agar (PCA) for total aerobic mesophilic bacteria, Potato Dextrose Agar (PDA) for yeasts and molds, and Mc Concey for coliform bacteria. By comparing the microbiological indicators in the raw materials and the final product, were evaluated the impact of the physical-chemical parameters (pH, Aw) of the raw materials and the effects of the technological process on the resistance of the microorganisms. Products are assessed according to the limit values of the number of microorganisms defined in the Codex Alimentary of one EU country.

In our study all samples were contaminated by total count bacteria. From raw material samples the highest of total count bacteria load was in flour samples 10^3 cfu/g while wasn't in egg powder samples. Also the samples of the final products resulted a total bacteria load of 10^2cfu/g. Yeast was resulted in cocoa samples and also in the final product samples (chocolate-coated sweet).

The study found that the technological process also quantity and mostly the type of microorganisms present in the raw material affects the microbiological quality of the final product.

***Key words**: Sweet with chocolate coated, Miicrobial quality, Raw materials, Cocoa, flour, Eggs powder, Bacteria.*

1. Introduction

In confectionary industry various critical parameters regarding quality were used: control of raw material, environmental factor, processing techniques, etc. Quality control procedures therefore need to be tailored to fit the requirements of each individual company, and indeed each manufacturing operation within the company. On arrival at the factory, the first task is to ensure that the raw material is in a satisfactory condition so they should be accompanied by an analysis sheet.

The primary requirements for adequate control of the manufacturing process are clearly defined recipes, manufacturing procedures and specifications. In applying this, the process is analyzed in detail, all operations which are critical to product quality are identified, and appropriate controls introduced, e.g., weights, mixing times, temperatures, vacuum levels, steam pressures, etc., The majority of dough preparation for confectionary product is baked to a temperature sufficient to kill any harmful bacteria present. However, surface contamination can always occur in the subsequent handling of products, after they have cooled down can be subject to microbiological contamination.

Good hygiene practices are therefore of paramount importance [1]. The number of incidents of microbiological contamination of foodstuff is increasing rapidly, and foods which were previously thought to be safe are increasingly found to be not so, and today's consumer is far more aware of the problem [2]. Confectionery products are rich in nutrients and high moisture content, and as a result they are an environment suitable for the development of microorganisms. Mould and bacteria are greater risk to the confectionery industry and fewer types of yeast. *Rhizopus* spp., *Penicillium* spp. and *Aspergillus* spp. are often found in these products. Bacteria that cause more problems are species of the genus *Bacillus* and *Staphylococcus aureus*, *Enterobacter* spp, *Salmonella* spp. [3].

Eggs used as raw material or added to milk for the production of creams may be sources of *Salmonella* spp. Microbiological quality of raw milk can affect the product's microflora as psychotrophic bacteria, Gram negative, coliforms bacteria, lactic acid bacteria, *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp. and yeasts, and moulds. Flour microflora is composed of a variety of yeasts and moulds microorganisms *Penicillium* spp., *Rizhopus* spp., *Aspergillus* spp., thermophilic and thermoduric bacteria and pathogenic bacteria like: *Bacillus cereus*, *Clostridium botulinum* etc. [4].

The study of the microorganisms nature (including their source, growth and characteristics), is the key to the microbial control of confectionery products. Therefore, respecting the steps of the technological process, specific to each set of sweets and working parameters (time, temperature and relative air humidity) can provide product safety [5].

2. Materials and Methods

The purpose of the study is to evaluate the microbiological composition of raw materials and the impact of the technological process on the microflora of the finished product filled with cream (cream filled product with chocolate coating or not).

Objectives of the strudy were:

- Determination of microbiological parameters: total mesophilic bacteria load, yeast, mould, coliform bacteria
- Analysis of the microbiological parameters at the specific technological steps of each product chain, and
- Evaluation of hygiene practices, technological processes, environment and personnel.

Studied samples were taken from: raw materials of cocoa, flour, eggs, cream fillers, and product samples at specific stages of the technological process up to the packaged product. Samples represent products manufactured in country. Two types of product have been studied: final cream filled bakery product with or without chocolate coatings.

For the microbiological control of each final product, parallel samples of raw materials and product samples are analysed at different stages of the technological process production (thermal treatment, UV sterilization, keeping in refrigerator) including packaged products (cream filled bakery product with chocolate coating or not). This was done in order to study the effect of each technological process on present microflora (how the microflora changes from raw materials to the final product). Microbiological analyses were carried out on a different production date. The microbiological parameters analysed are: total aerobic mesophilic bacteria, yeasts, moulds and coliforms bacteria/other enterobacteria. Decimal dilution method was used, followed by quantitative determination of microorganisms by plate count agar in standard media (PCA for total aerobic mesophilic bacteria, PDA for yeast and Czapek for moulds) and selective media (Mc Conkey for coliforms bacteria and other enterobacteria) [6]. Then, the colony counting technique was used. The results were organized in the respective tables for each sample.

For the calculation of microbiological loads following formula was used:

$$N = \sum C / V\,(n_1 + 0.1\,n_2) \times H$$

Legend: $\sum C$ - number of colonies counted of all Petri dishes, V - cultivation volume, n_1 - number plate with first dilution, n_2 - number plate with second dilution, H - dilution factor corresponding to the first dilution.

From the colonies developed at Petri dishes were prepared various microscopic slides and fast simple biochemical tests (catalyze, oxidize test). Morphological data from microscopic examination and cultural observation from the phenotypic description of the colonies developed on the respective media help to determine the genera of the microorganisms present in the analyzed samples.

For evaluation of hygienic practices of technological process, environment and personnel were used different methods: for paper packaging was used plate count agar method in PCA, and Mc Conkey media, for personnel fecal coliforms sanitary indicator - fermentation tubes method, and for the air microflora was used sedimentation methods in agar media.

Microbiological results were evaluated based of Guidelines for the microbiological examination of ready- to- eat foods [7].

Table 1. Guidelines levels for determining the microbiological quality of ready-to-eat foods

Parameters	Microbiological Quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous
Standard plate Count				
Level 1	$< 10^4$	$< 10^5$	$\geq 10^5$	
Level 2	$< 10^6$	$<10^7$	$\geq 10^7$	
Level 3	N/A	N/A	N/A	
Indicators				
Enterobacteriaceae	$<10^2$	10^2 - 10^4	$\geq 10^4$	
Escherichia coli	<3	3-100	≥ 100	**
Pathogens				
Staphylococcus coagulase+	$<10^2$	10^2 - 10^3	10^3- 10^4	≥ 10^4 SET+ve
Bacillus cereus and other pathogenic *Bacillus* spp.	$<10^2$	10^2- 10^3	10^3- 10^4	$\geq 10^4$
Salmonella spp.	Not detected in 25 g			Detected
Yeast	$< 10^1$			
Mould	$<10^1$	$<5*10^2$		

3. Results and Discussion

Results from microbiological analysis of raw materials are presented in Table 2.

According to Guidelines for the microbiological examination of ready- to- eat food referring to the microbiological parameter of total bacteria:

- Flour sample is considered of 'less satisfying' category and with the higher load of total bacteria 10^4 cfu/g.
- Cocoa, egg powder, and cream samples are considered of 'satisfying' category.
- Referring to the coliforms bacteria parameter: all the samples are considered to be of 'less satisfying' category (Figure 1).

All samples have resulted in bacteria of the genus: *Bacillus* spp. (*B. cereus*), *Pseudomonas* spp., *Flavobacterium* spp. Dominating moulds belonged to the genus: *Peniciullium*, *Mucor*, *Apergillus*, while yeasts to the pigmented pink yeast (*Rhodotorula*). The presence of coliforms bacteria shows lack of hygiene practices in the processing industries of these materials (personnel, environment, machinery, etc.).

Microorganisms found in cream-filled bakery product without chocolate coatings are presented in Table 3.

The sample of baked dough after the bakery is not filled with the cream, due to the high temperature effect (268 ^{0}C/6.4 min), results only with a few spores of mould. The baking process eliminated the microflora carried by the raw material. At the time of the chocolate decor, the sample showed a total bacterial load of 10^3 cfu/g due to the characteristics of the product itself (pH - 7.0, product moisture content 19.55%) and the environment (relative humidity = 42%, T = 19.7 ^{0}C).

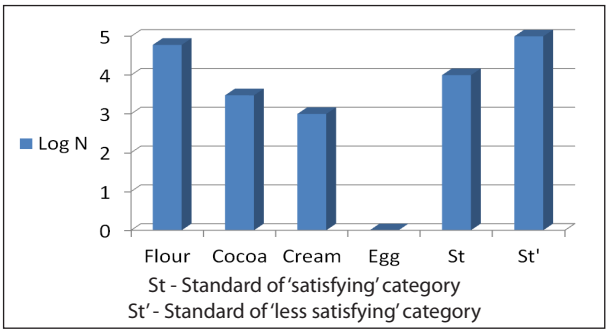

Figure 1. Logarithm N of total bacterial load of raw materials

Table 2. Microbiological results of raw materials

Parameters	CFU per gram			
	PCA (total bacteria)	PDA (yeast)	Czapek (mould)	Mc.Conkey (coliforms)
Flour	6×10^4	20	5	9×10^1
Cocoa	3×10^3	3	2	7×10^1
Egg powder	-	-	-	-
Cream	1×10^3			2×10^3

Figure 1. Development of different microbial cultures colonies in PCA Petri dishes from the cultivation of flour sample

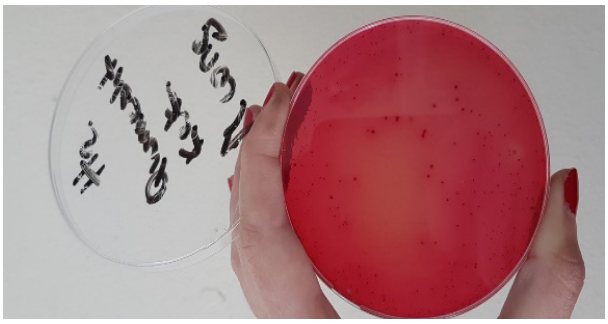

Figure 2. Colifom bacteria colony in Mc Conkey Petri dishes cultivated from cream sample

Figure 3. The development of pink pigmented yeast colonies in PDA Petri dishes present in flour sample

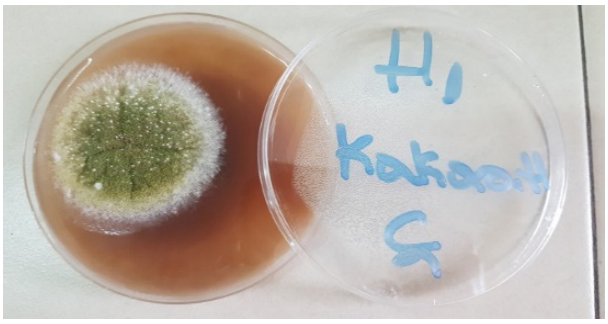

Figure 4. The presence of mould *Penicillium* spp. in Czapek present in cocoa sample

Table 3. Microbiological results of cream-filled bakery product without chocolate coatings

Samples	CFU per gram			
	PCA (total bacteria)	PDA (yeast)	Czapek (mould)	Mc Conkey (coliforms)
Baked dough without cream	-	-	2 *Penicillium* spp.	-
With cream and the chocolate décor	1×10^3 *Bacillus* spp. *Flavobacterium* spp.	-	1 *Penicillium* spp.	1.2×10^2
Held in refrigerator (7 °C/5min.) before UV radiation	2×10^2 *Flavobacterium* spp. *Pseudomonas* spp. *Bacillus* spp.	-	1 *Penicillium* spp.	-
After UV radiation	8×10^1 *Bacillus* spp.	-	-	-
Packaged product	6×10^1 *Bacillus* spp.	-	-	9×10^1

Keeping products under refrigeration conditions (7 °C/5 min.) has contributed in the reducing of the microbial load 10^2 cfu/g. Then was used UV radiation treatment which decreases the microbial load in the order of 8×10^1 cfu/g. The packaged product, shows a reduced number 6×10^1 cfu/g (microflora is represented by thermo-resistant sporogenic bacteria, mainly to *B. cereus*) and there is a presence of coliforms bacteria of the order of 10^1 cfu/g.

According to the guidelines for the microbiological examination of ready-to-eat food, the final product results of 'satisfactory' category, referring to the total bacteria load and 'less satisfying' category for coliforms bacteria < 10 - < 4×10^2 cfu/g (Figure 2).

Results from cream filled bakery product with chocolate coatings microbilogical analysis are presented in Table 4.

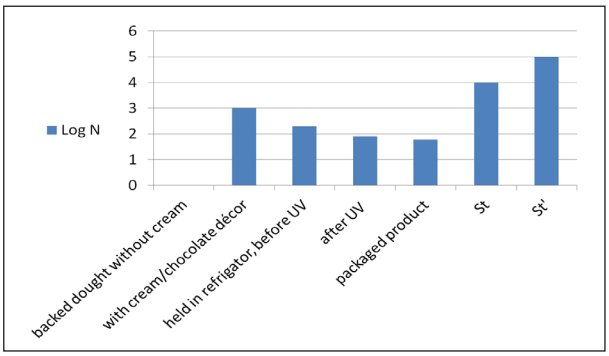

Figure 2. Logarithm N of total bacterial load of cream filled bakery product without chocolate coating

Table 4. Microbiological results of cream filled bakery product with chocolate coatings

Samples	CFU per gram			
	PCA (total bacteria)	PDA (yeast)	Czapek (mould)	Mc Conkey (coliforms)
Baked dough without cream	-	-	2	-
Baked dough with cream	10^2 *Bacillus* spp. *Flavobacterium* spp.	-	-	-
With cream and chocolate coated	3 x 10^2 *Bacillus* spp. *Flavobacterium* spp.	-	1 *Penicillium spp.*	1.5 x 10^2
Held in refrigerator at 7° C before UV radiation	1 x 10^2 *Bacillus* spp. *Flavobacterium spp.*	20	-	8 x 10^1
After UV radiation	8 x 10^1 *Bacillus* spp. *Flavobacterium* spp.	-	-	-
Packaged product	1 x 10^2 *Bacillus* spp.	-	1 *Penicillium spp.*	5 x 10^1

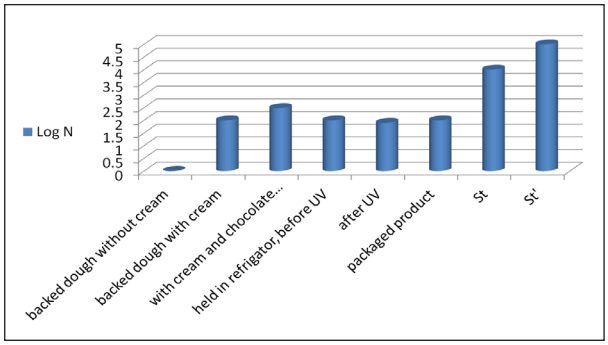

Figure 3. Logarithm N of total bacterial load of cream filled bakery product with chocolate coating

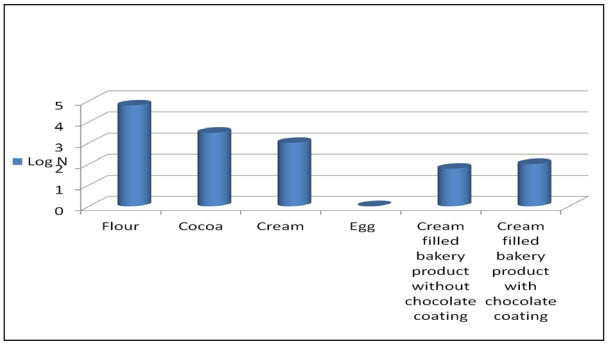

Figure 4. Logarithm N of total bacterial load of raw material and final products

The sample of baked dough when the bakery is not filled with the cream, due to the high temperature effect (268 ° C/6.4 min), results only with a few spores of mould. The baking process eliminated the microflora carried by the raw material. The sample of creamy dough resulted in a total bacterial load of 10^2 cfu/g (due to the high bacterial load of raw material that is used). The chocolate coating process is reflected by a slight increase in total bacteria (from cocoa and air contact).

Keeping in the refrigerator 7 °C reduces the bacterial load, and resulted with some pink pigmented colonies of yeast (*Rhodotorula*). UV radiation treatment reduced the bacterial load of 10^1 cfu/g.

The packaged product shows an increase quantitative bacterial content and results with the presence of coliform bacteria due the packaging.

Referring to the guidelines for the microbiological examination of ready-to-eat food, the final product results of a 'satisfying' category referring to the total bacterial load and 'less satisfying' category to coliforms bacteria (Figures 3 and 4).

Referring to the results obtained for the assessment of hygiene practice of technological process, environment and personnel we observed that paper packaging in PCA, Mc Conkey media did not resulted in microbial loading. Fecal coliforms sanitary indicator for personnel showed that from 21 examined samples 29% resulted positive. The air microflora made by sedimentation methods in agar media resulted in a good quality but with the presence of some moulds.

Air microflora in the production department is dominated by *Bacillus* spp., *Flavobacterium* spp., *Pseudomonas* spp., and by moulds *Aspergillus* spp., *Penicillium* spp. and *Mucor* spp.

4. Conclusions

According to the Guidelines for the Microbiological Examination of Ready-to-eat Food:

- All raw material samples have resulted in a 'less satisfying' category < 10^1 - < 4 x 10^2 cfu/g referred to the coliforms bacteria which is reflected in the final product samples.

- The presence of coliforms bacteria shows lack of hygiene and sanitation practices in the raw materials and product processing industry (personnel, machinery, processing lines, pipettes, creams, environmental cleaning techniques etc.)

- By the phenotypic description of colonies and rapid biochemical evidence we determined presence of: bacteria - *Bacillus* spp., *Flavobacterium* spp., *Pseudomonas* spp., moulds - *Penicillium* spp., *Aspergillus* spp., *Mucor* spp., and pink pigmented yeast *Rhodotorula* spp.

- The quality of the final product is influenced by the microflora of raw materials, mainly flour whose high microflora load is reflected in the dough, which falls after baking. So the process of baking is enough to prevent the high bacterial load in the flour.

- The use of raw materials, cream filling, and chocolate coating shows an additional microflora of the product under treatment.

- Keeping in the refrigerator 7 ^{0}C/5min. is a stabilization process of microflora and the UV radiation treatment improves the microbiological quality by reducing the microbial load carried by the raw materials.

- Packaging is a sterile process (carried out under aseptic conditions). The presence of coliforms bacteria in the final product is related to the hygienic quality of personnel that manipulate the product.

- These products are categorized within 'satisfying' category, but hygiene requirements remain to be improved.

5. References

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