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BREAD ROPE SPOILAGE DEVELOPMENT

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Abstract. Rope spoilage of bread is produced by bacteria belonging to *Bacillus* genus. Insufficient cleaning of the wheat before the milling operation contributes to obtaining flour with a high degree of contamination with these bacteria which produce thermoresistant endospores and further maintain their viability in the baking process. After baking, thermoresistant endospores can pass into the vegetative form and can multiply, causing spoilage of bread, which is manifested by the occurrence of an unpleasant odour, and the core of the bread becomes sticky. In this study, samples of bread from second-grade wheat flour were obtained. The kinetics of bacterial growth in the core of the thermostated bread at 37 °C for 84 hours was determined. It was found that immediately after baking the degree of contamination of the bread core was $1.28 \cdot 10^4$ cfu/g, and after 40 hours of thermostating the degree of contamination of the bread core increased by 2.85 logarithmic cycles, at this bread sample were observed the first signs of the rope spoilage (unpleasant odour). Thus, bread obtained from highly contaminated flour can contain a very large number of bacteria belonging to *Bacillus* genus, having no signs of rope spoilage, and thus the consumption of this bread may be dangerous to the consumer's health.

Keywords: *bread, bacteria grow, unpleasant odor, sticky core.*

Rezumat. Boala întinderii a pâinii este produsă de bacteriile aparținând genului *Bacillus*. Curățarea insuficientă a grâului înainte de operația de măcinare contribuie la obținerea făinii cu un grad ridicat de contaminare cu aceste bacterii, care produc endospori termorezistenți și astfel își păstrează ulterior viabilitatea în procesul de coacere. După coacere, endospori termorezistenți pot trece în formă vegetativă și se pot înmulți, cauzând astfel alterarea pâinii, care se manifestă prin apariția unui miros neplăcut, și miezul pâinii devine lipicios. În această cercetare au fost obținute probe de pâine din făină de grâu de calitatea a doua. S-a determinat cinetica creșterii bacteriilor în miezul pâinii termostatate la 37 °C timp de 84 de ore. S-a constatat că imediat după coacere gradul de contaminare a miezului pâinii a fost de $1.28 \cdot 10^4$ u.f.c./g, iar după 40 de ore de termostatare gradul de contaminare a miezului pâinii a crescut cu 2.85 cicluri logaritmice, la această probă de pâine s-au observat primele semne ale bolii întinderii (mirosul neplăcut). Astfel, pâinea obținută din făină foarte contaminată poate conține un număr foarte mare de bacterii din genul *Bacillus*, neavând semne ale bolii întinderii, și astfel consumul acestei pâini poate fi periculos pentru sănătatea consumatorului.

Cuvinte cheie: *pâine, creșterea bacteriilor, mirosul neplăcut, miezul lipicios.*

1. Introduction

There are many species of microorganisms that can cause the spoilage of bakery products. Rope spoilage is the most important and most common defect of bread caused by the activity of bacteria. The microbial agents that produce the alteration are part of the genus *Bacillus*, highlighting the following species: *Bacillus subtilis* [1-3], *Bacillus subtilis* subsp. *mesentericus* [4-7]. In some research, the important role of *Bacillus licheniformis* [8] and *Bacillus amyloliquefaciens* [9] bacteria in the production of this defect is highlighted. Some researchers consider that the initiators of rope spoilage in bakery products are also bacteria of the species *Bacillus cereus* [10], *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus polymyxa*, *Bacillus sonorensis*, *Bacillus megaterium*, *Paenibacillus polymyxa*, but their role is less important [2, 8, 11].

Bacteria that cause rope spoilage in bakery products are not part of the epiphytic microbiota of cereal plants and do not have the possibility to grow on their surface. The concentration of bacteria of the genus *Bacillus* on the surface of cereal plants is very low (approximate 5 cfu/g), but these bacteria are present in the soil, where their concentration can reach 10^5 cfu/g soil, and the main way in which they get to the grain mass is represented by the dust particles that attach to the grains during the harvesting and primary processing of the wheat. Additionally, during storage under improper conditions, when the cereals undergo the process of self-heating, the number of bacteria of the genus *Bacillus* increases considerably. Therefore, the concentration of these bacteria in the mass of cereals can exceed $3{\cdot}10^4$ cfu/g [11]. Subsequently, these bacteria, which become part of the cereal microbiota, pass into the flour in the process of milling the grains. Insufficient cleaning of the grain surface before the grinding operation contributes to the obtaining of flour with a high degree of microbial contamination [6, p. 231].

Flour is widely known as the main source of rope-producing bacteria in dough intended for manufacturing bakery products [12]. It is also considered that the flours with a higher degree of extraction (especially wholemeal flour), which contain more particles from the grain shell, are more contaminated with bacteria that produce the rope spoilage in bread, than those with a lower degree of extraction (white flour) [13]. Moreover, the environment of processing (air, the working surface of the equipment) or other raw materials such as yeast or additives may also be contaminated with *Bacillus* [14]. The application of hygiene procedures in the bread-making section is an important condition for preventing the ropiness in bread [7].

Bacteria of the species *Bacillus subtilis* are gram-positive, and bacillary in shape, the length and the width of cells are 2–5 µm and 0,4–0,6 µm. Have the ability to form oval-shaped spores smaller than the cell size, and centrally located [1, 15]. The colonies of bacteria, that cause rope spoilage in bakery products, have an irregular shape, sometimes circular, and have different sizes when cultured on Nutrient agar medium. The surface of the colonies is smooth, with a buttery sheen, slightly wrinkled. The color of the colonies is white, sometimes pink, translucent, or milky. The margin of the colonies is wavy or lobed [16]. The species of bacteria that cause the rope spoilage in bread are amylase producers, and thus, are characterized by amylolytic capacity [8,12]. At the same time, bacteria from the *Bacillus subtilis* species have the ability to produce enzymes, which catalyze protein hydrolysis. Thus, the degradation of the starch and proteins in the core of the bread under the action of these bacteria is considered the main cause of the occurrence of the signs of spoilage [6, p. 232].

Rope spoilage occurs more frequently in bakery products during the warm period of the year [6, p. 232; 4, p. 441]. Spores of bacteria, that cause rope spoilage of bread, are resistant to heat treatment, and because the temperature in the bread core achieves usually values of 95-100 °C for several minutes, these bacteria can survive [14]. Ropiness occurs particularly when warm (25-30 °C) and humid (water-activity, ⩾0.95) environmental conditions allow spore germination [1]. The first signs of this alteration, which can occur after 24-48 hours of bread storage, can be observed by changing the odour of the bread, which becomes uncharacteristic for the product. If the spoilage progresses, then the odour intensifies and becomes rotten; outbreaks of bacteria that appear as yellow-brown or pink-brown spots can be observed in the core; the porous structure of the core deteriorates, gaps appear, and the core becomes sticky and when broken it stretches into very thin mucilaginous wires, that is why this alteration is named „rope spoilage" or „ropiness". At the strong development of the spoilage, the core turns into a compact and dark mass, with a specific pungent and unpleasant smell, from which the name „potato disease" comes [6, p. 232; 5, p. 441; 17, 18]. In addition to the change of sensory properties of bread, the growth and development of rope-producing bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*) are accompanied by an accumulation of toxins, which can pose safety risks for consumers and can be implicated in producing of foodborne diseases [19].

The aims of the present study were to demonstrate the rate of bacterial growth in the bread core and to establish the correlation between bacterial growth and the occurrence of the signs of rope spoilage.

2. Materials and Methods

2.1 Study of the rate of colonial growth of bacteria isolated from the spoiled bread core

The rate of colonial growth of bacteria that cause rope spoilage in bread was determined by harvesting of bacteria cells with a loop from an outbreak in the core of spoiled bread, introduction into saline and obtaining the suspension of bacteria, placing a drop of this suspension in the center of Petri plate with the nutrient medium distributed preventively and solidified, thermostating of the sample at 37 °C with simultaneous video recording for 22 hours and visual observation of the size of the colony. Nutrient agar (Peptic digest of animal tissue 5.00 g/L; sodium chloride 5.00 g/L; beef extract 1.50 g/L; yeast extract 1.50 g/L; agar 15.00 g/L) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used to cultivate the bacterial colony. Thermostating of the sample was performed in the thermostat ST1 (Pol-Eko-Aparatura, Poland). Video recording was made with the camera Logitech C270 placed in the thermostat.

2.2 Preparation of the bread samples and thermostating

Bread samples were obtained by the standard baking method [20; 21, p 18]. The mass of the bread samples was determined by weighing on a balance BSN-60D1.3 (Alex S&E, Republic of Moldova). The temperature of the bread core was measured by injecting the digital thermometer SH-113V1 (IsoLab, Germany) immediately after baking.

To obtain the bread samples, second-grade wheat flour, from the batch of flour received at the Cahulpan bread-making company (Cahul, Republic of Moldova), was used. This type of flour is used to manufacture the assortment of bread with a lower price compared to bread obtained from highest-grade wheat flour. At the same time, the bread prepared from the second-grade wheat flour is characterized by a higher content of dietary fiber due to the higher content of bran particles. But the higher the bran content in the flour, the higher the degree of contamination of the flour with sporulated bacteria can be, that is why this type of flour was chosen in this research. The following properties of the flour were determined: acidity by titrating the aqueous suspension with sodium hydroxide in the presence of the indicator phenolphthalein [22]; humidity by drying to the constant mass [23]; wet gluten content by manually washing the piece of dough [24]; ash content by complete burning of the sample at 600 °C for 6 hours [25]; the total number of sporulated bacteria by preparing the wash by mixing 10 g of flour with 90 cm^3 of sterile saline and agitating with an orbital shaker for 10 minutes at the rotation 250 min^{-1} and pasteurizing it at 85 °C for 10 minutes, preparing dilutions, inoculating the samples and cultivating on Nutrient agar medium at 37 °C for 48 hours [26].

For the preparation of dough, its were also used: baking yeast (species Sacharomycess cerevisiae, „Livivski Drijdji", „Compania Enzim" company, Lviv, Ukraine), salt (for food use, „Arteomsoli" company, Soledar, Ukraine), potable water (from the aqueduct, Cahul). The quality of the raw materials used in this research is regulated in legislative acts of the Republic of Moldova [27, 28].

The recipe presented in table 1 was used to prepare the dough.

Table 1

The recipe for the preparation of the bread samples

Raw materials	Amount, %
Second-grade wheat flour	100
Baking yeast	3
Salt	1,5

The amount of water required for the preparation of the dough was calculated according to the total content of dry substances in the raw materials and the desired humidity of the dough equal to 45%.

After the baking process, the bread samples, have been cooled for 1 hour and packed in polyethylene bags, placed in the thermostat, and kept for 88 hours at the temperature of 37 °C.

The following utensils and equipment were used: laboratory mixer FIMAR 7/SN TR 1 V CE, thermostat BJPX - Diego, leavening cabinet UNOX XLT 133, laboratory oven UNOX XFT 133, laboratory glassware and utensils.

2.3. Determination of the number of bacteria in the bread core

The determination of the number of bacteria in the core of bread was performed immediately after baking and after each approximately 12 hours of thermostating of the samples. The bread sample has been cut; the core of the bread has been chopped using the crusher First FA-5481-1. An aliquot of 10 g of the chopped bread core have been transferred into a flask and mixed for 10 minutes in 90 cm^3 of sterile saline using the orbital shaker OS-10 at the rotation 250 min^{-1}. Decimal dilutions were prepared from the obtained suspension according to standard methods of microbiological analysis [26]. Inoculations have been made from the last two or three dilutions (two from each dilution). The bacteria have been cultured in the thermostat ST1 (Pol-Eko-Aparatura, Poland) at the temperature 37 °C.

2.4. The test for the occurrence of the rope spoilage

After about every 12 hours of thermostating, one of the bread samples has been analyzed organoleptically for unpleasant odours and other signs of rope spoilage, such as sticky and changed color core [21, p 20]. For each analysis, the bread sample has been unpacked, it has been broken in two halves, and immediately the core has been smelled. After this, the appearance and colour uniformity of the core has been examined. The stickiness of the core has been examined by the touch.

2.5. Calculation methods

The Excel programme from the Office 2010 suite was used to process experimental data. The necessary calculations were performed using this application, the data obtained from the microbiological analysis of the bread samples were graphically represented and the function was chosen for the mathematical description of the kinetics of bacterial growth in the core of bread.

3. Results

3.1. Colonial growth rate of bacteria isolated from spoiled bread core

Figure 1 represents images showing an increase in the size of the bacteria colony every two hours of thermostating.

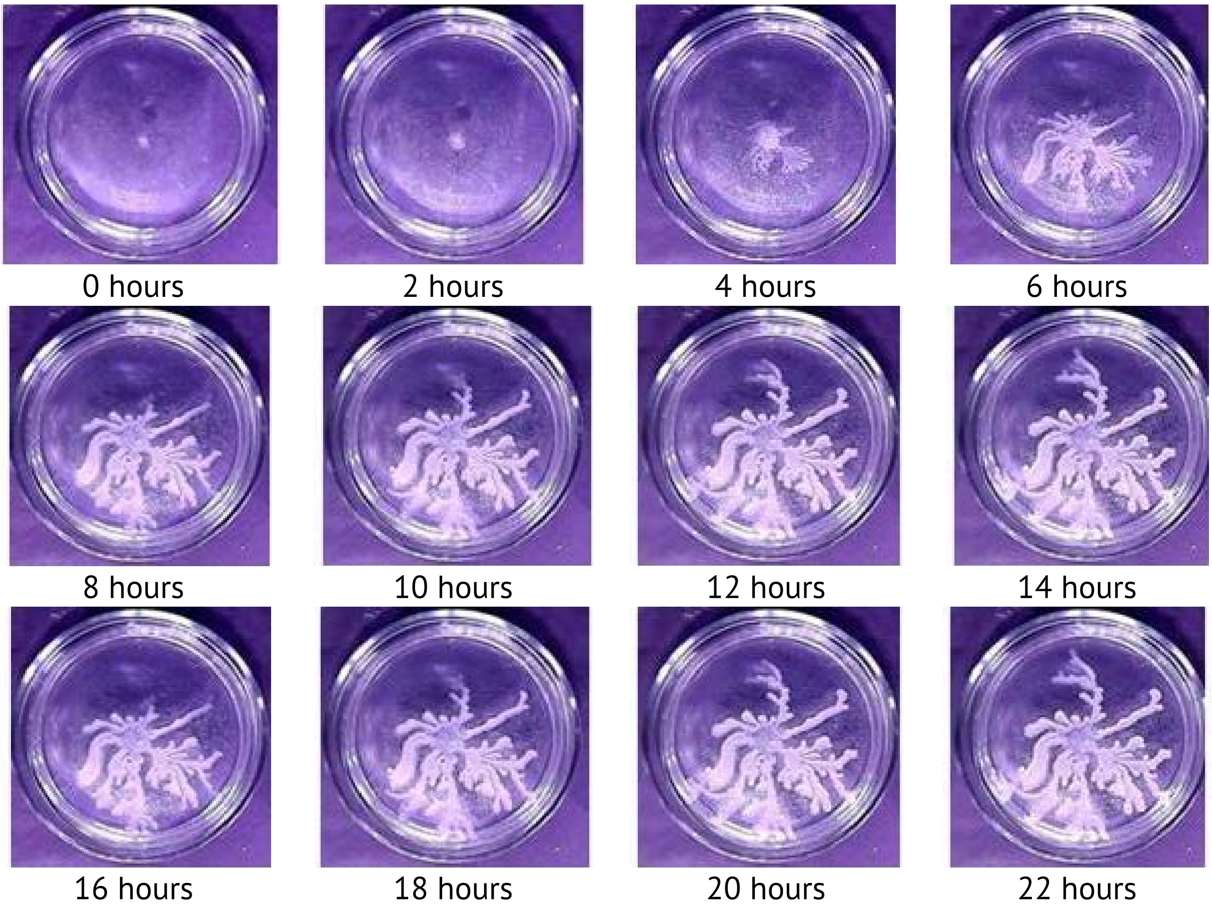

Figure 1. Colonial growth of bacteria isolated from spoiled bread core.

It was found that after 6 hours of thermostating the size of the colony reached about 50 mm in diameter, and after 22 hours it occupied the entire surface of the Petri plate, which demonstrates the high growth rate of these bacteria and proves that the number of bacteria in the core of the bread can quickly reach a level sufficient to produce rope spoilage in a very short time (sometimes even after a few hours) [6, p. 232; 12].

3.2. The bread samples and the properties of the flour

Eight bread samples of prismatic shape were obtained, each with a mass of 450 ± 4 g. The temperature in the core of the bread immediately after baking reached values of 96 ± 0.5 °C.

Table 1 shows values for the main properties of the flour used in the baking tests. Values of the physicochemical indices of flour (acidity, humidity, wet gluten content and ash content) are within the limits provided in the regulations for wheat flour in the Republic of Moldova [29].

At the same time, it was found that the total number of sporulated bacteria is more than 10^3 cfu/g, which means that this sample of flour is characterized as being heavily contaminated [30, p. 78].

Table 2

Properties of the flour used in baking tests

Properties and units of measure	Value	Permissible rates
Acidity, degrees of acidity	2.7 ± 0.1	max. 3.8
Humidity, %	12.8 ± 0.2	max. 15
Wet gluten content, %	33.8 ± 0.2	min. 20
Ash content, %	0.79 ± 0.1	max. 1.25
Total number of sporulated bacteria, cfu/g	$1.5 \cdot 10^3$	-

3.3. The kinetics of the growth of bacteria in the core of bread and the occurrence of signs of the rope spoilage

Figure 2 shows the kinetics of bacterial growth in the core of bread. It was found that the number of bacteria in about 90 hours of thermostating increases by 6.7 logarithmic cycles. The degree of core contamination at the end of thermostating was $6.98 \cdot 10^{10}$ cfu/g. Kinetics of increasing the number of bacteria in the core of bread is characterized by an exponential function, the value of the square of Pearson's correlation coefficient being greater than 0.9.

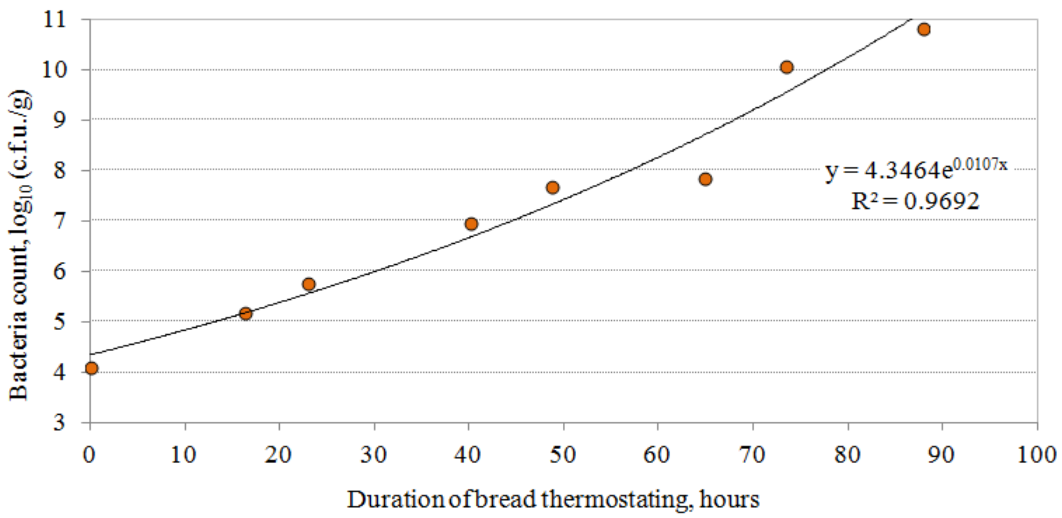

Figure 2. Kinetics of bacterial growth in the core of spoiled bread.

It was also observed that the initial degree of contamination of the bread core was $1.28 \cdot 10^4$ cfu/g. And because bacteria of the genus *Bacillus* are considered pathogenic, bread with a high degree of microbial contamination can represent a risk to consumer health. These bacteria can be involved in the production of diseases with mixed etiology (intoxication and infection), and can produce emetic syndrome (nausea, vomiting) observed after 1-5 hours after consumption of the product [4, p. 441; 6, p. 28; 19].

At the same time, it was found that the first signs of bread rope spoilage occurred after 40 hours of thermostating – the organoleptic analysis of the bread revealed the characteristic odour of the alteration (Table 2).

Table 2

Correlation between the degree of bread core contamination and the occurrence of signs of rope spoilage

Duration of bread samples thermostating, hours	0	16	23	40	49	65	73	88
The occurrence of signs of the rope spoilage	-	-	-	±	+	+	+	+
The total number of bacteria in the core of the bread, cfu/g	$1.3 \cdot 10^4$	$1.5 \cdot 10^5$	$5.9 \cdot 10^5$	$9.0 \cdot 10^6$	$5.0 \cdot 10^7$	$6.9 \cdot 10^7$	$1.2 \cdot 10^{10}$	$6.9 \cdot 10^{10}$

Note: – no signs of rope spoilage; ± poor signs of rope spoilage (unpleasant odour); + pronounced signs of alteration (unpleasant odour and sticky core).

Analyzing the correlation between the degree of contamination of the bread core and the occurrence of the signs of rope spoilage, it was found that the unpleasant odour, characteristic to this alteration, appears at a fairly high degree of core contamination (10^6 cfu/g), which means that a person, who does not feel the odour of rope spoilage, can eat bread heavily contaminated with bacteria of the genus *Bacillus* and be at risk of food poisoning.

At a longer thermostating of the bread samples, there was an intensification of the unpleasant odour, the local change of the colour of the core and the occurrence of outbreaks of bacterial growth (zones of yellow-brown sticky core) (Figure 3).

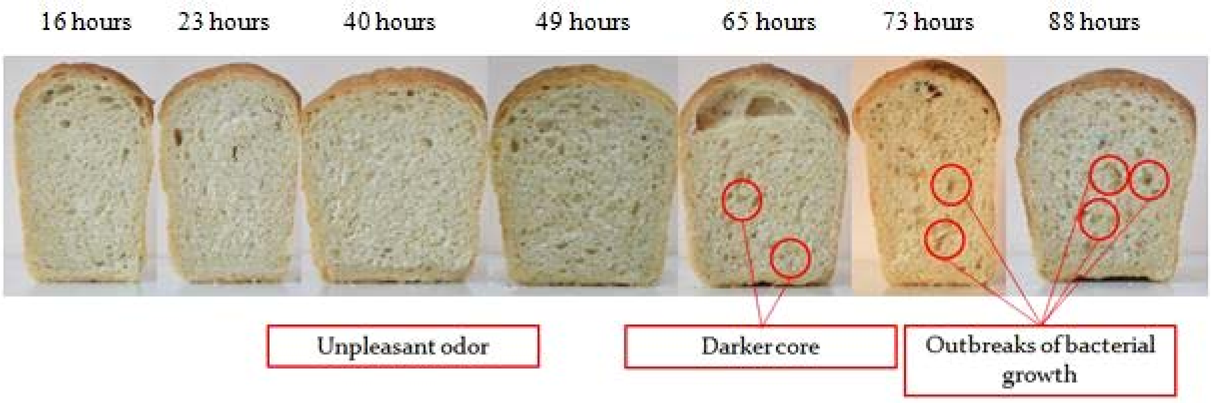

Figure 3. Signs of the occurrence of the rope spoilage in bread samples.

These changes were observed at the values of degree of microbial contamination of the bread core of 10^7-10^{10} cfu/g.

4. Discussion

In this research, it was observed that the characteristic odour of rope spoilage of bread occurs at a very high degree of bacterial contamination of the core, and since the rope-producing bacteria possess the property of releasing toxins into the product in which they grow, there is a risk that after consuming of such bread some people will suffer from poisoning. Thus, to avoid the risk of ropiness in bread and minimize the risk of intoxication, the microbiological control of the quality of the received flour must be applied at the bakery factories and to avoid the use of flour heavily contaminated with bacteria of the genus *Bacillus* in the bread making process, this flour can be directed for the obtaining of other products such as dry pretzels or breadcrumbs (which during the manufacture are treated with high temperatures and are characterized by a low moisture content, which makes it impossible for these bacteria to survive). Dilution of the heavily contaminated flour batches with uncontaminated flour batches can also be applied [7].

At the same time, the classical methods of preventing the rope spoilage of bread can be applied, such as the use of sourdough [31-39], calcium propionate [40], and other bakery improvers [41], the effectiveness of which was demonstrated in many scientific papers.

Unconventional methods can also be applied, such as the treatment of the surface of wheat grains with continuous ultraviolet light, or light pulses, with the aim to reduce the contamination degree of the grains before milling [16].

Electromagnetic radiation with wavelengths range 200–280 nm (UV-C light) is called bactericidal or germicidal light because by treatment with radiation in this range, bacteria and viruses are effectively inactivated [42]. The inactivation of microbial cells, by treatment with ultraviolet light, is based on the damage of nucleic acids [43], which is the cause of the loss of ability of microorganisms to reproduce. Nucleic acids absorb ultraviolet light in the wavelength range between 200 and 310 nm. Absorbed ultraviolet light causes the breaking of bonds and the formation of pyrimidine, thymine, or cytosine dimers in the same strand of DNA or RNA. These dimers change the helical structure of the DNA, which disturbs the cell replication process; therefore, the microorganisms become inactive [42; 44].

The treatment with light pulses is an effective method used to destroy both vegetative forms and spores of microorganisms [45-47]. This technology uses short pulses of intense white light with wavelengths from 100 nm in the UV range to about 1100 nm in the near-infrared region. The inactivation of microorganisms by light pulses treatment is attributed to the effect of the high energy of a pulse and the UV component, which represents about 25% of the light pulse spectrum [45]. The biological mechanism of microorganisms' inactivation includes restructuring nucleic acids, denaturation of proteins, and damage to other cellular components [42].

5. Conclusions

Bacteria of the genus *Bacillus* can be characterized by a high rate of reproduction, which can be the cause of the rapid occurrence of signs of rope spoilage in bread when the flour is with a high degree of contamination. Bread obtained from flour with a high degree of contamination with sporulated bacteria can represent a risk to consumers because signs of the rope spoilage in bread (unpleasant odour, sticky core) appear when the degree of core contamination is over 10^6 cfu/g, thus at the degree of contamination below 10^6 cfu/g the consumer is not warned that the bread is contaminated. Bacteria of the species *Bacillus subtilis* are considered conditionally pathogenic and can cause gastroenteritis, therefore, in order to prevent the risk of food poisoning it is necessary to prevent contamination of flour with sporulating bacteria, or to apply the methods of inactivation of them during the bread making process.

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Conflicts of Interest: This research represents the continuation of the doctoral studies and contains in the introductory part some information taken from the doctoral thesis entitled "Improvement of the bread quality as a result of wheat decontamination by non-conventional methods" and carried out under the direction of scientific coordinator, Mrs. Turtoi Maria, Prof. Dr. Eng.

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