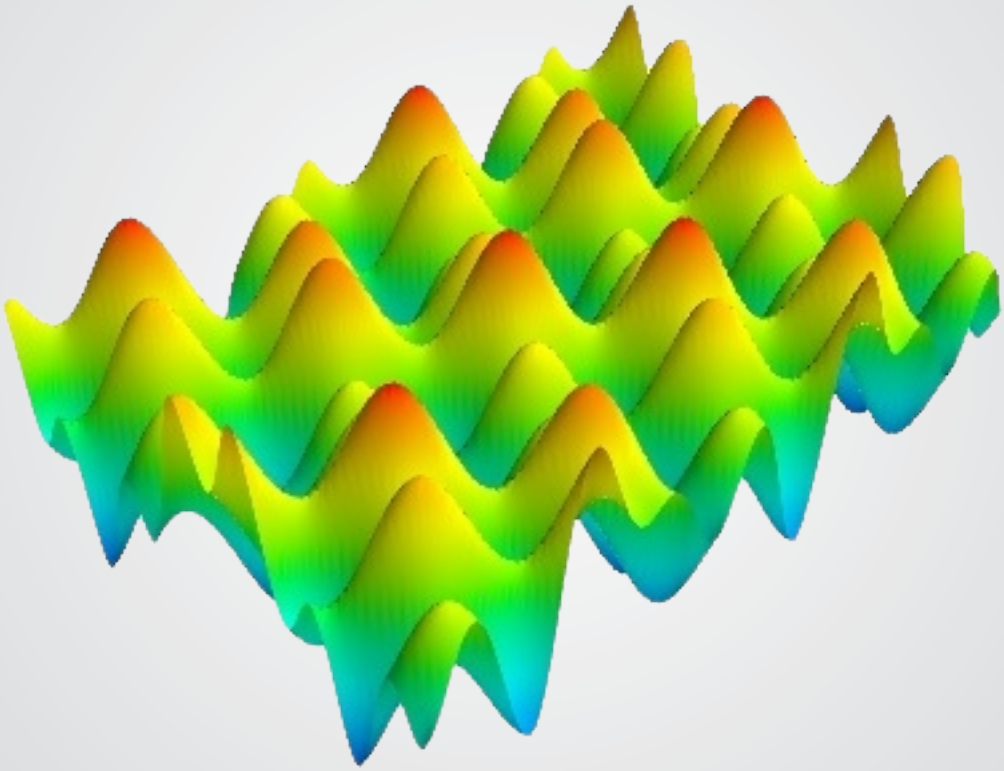
Journal of Food and Packaging

Science, Technique and Technologies



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ISSN 1314-7773

ISSN 1314-7773

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Journal of **FOOD** and **PACKAGING** Science, Technique and Technologies

Year VI, №13, 2017

National Academy of Packaging - Bulgaria

Web-site: http://mahvp.uft-plovdiv.bg/

Address: 26, Maritza blvd., 4002, Plovdiv, BULGARIA

Phone: +359 32 603 814
+359 888 14 2468
+359 899 170 453

E-mail: jfpstt@gmail.com
mahvpuht@gmail.com

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Study on the influence of packaging on the rustic sirloin quality

Buculei Amelia[1] , Țurcan Paulina[1]

[1]*Faculty of Food Engineering, University Stefan Cel Mare of Suceava*

Abstract. *The packaging market is geared towards various technologies that can reduce raw material consumption, increase labor productivity both during making the packaging itself and product packaging, the increasing conversation period and product shelf life, combine different types of materials in order to grow the quality of packaging and reduce the adverse impact on the environment.*

The extending of the product shelf life is directly influenced by the quality of the interaction that is made between the packaging method - packaging itself - product. In this context, the properties of the packaging take on a primary role in guaranteeing the protection and conservation functions. To carry out the research, different methods of packing the rustic sirloin have been studied throughout its shelf life. The samples were tested for physical-chemical organoleptic and standards in force. At the end of the storage time, muscle packed in a modified atmosphere has a higher value, observing his case and some stability in timp.pH's an indicator of freshness, it can be mentioned that both products have kept quality time.

Introduction

Over the last decade, extending the shelf life of the food is the most important additional function of the packaging method. Thus, a lot of new methods have been developed that are used worldwide for the purpose of packaging food, which is based on creating a modified atmosphere in which the product is introduced. (1).

In order to achieve the modified atmosphere in the packaging, a number of techniques have been determined to attain favorable results in this aspect. These include: vacuum packing, packing in a modified gas atmosphere, EMAP (Equilibrium Modified Atmosphere Packaging), active packing.

Packing fresh meat or meat products is aimed at slowing down microbiological activity, increasing enzyme activities that optimizes the mildew, reduces weight loss and keeps red color of meat on the entire supply chain, where needed. Dehydration, oxidation of lipids, discoloration and loss of flavor are basic processes to be taken into account when preserving meat and meat products (2).

The shelf life of the meat is of considerable importance in the retail market. The shelf life is defined as the time it takes for the product to run from its packaging to final use as long as the product properties remain acceptable to the consumer. Shelf life is determined by several properties including appearance, texture, flavor, color and nutritional value (3).

When considering the shelf life of a meat product, some people make a distinction between expendability and durability. The durability is the amount of time that passes from the date of meat packing until it can no longer be consumed or becomes unfit for human consumption due to the growth of harmful microorganisms.The expendability can be defined as the time browsed by the meat piece from its placement on shelf until color change. This red color change in bright red, cherry-red or other color, such as brown, is caused by a modification in myoglobin (4).

Materials and methods

To carry out the research, different methods of packing the rustic sirloin have been studied throughout its shelf life. The analysis carried out was: the determination of total protein substances in meat and meat products, done using the Kjeldahl method according to STAS 9065/4-81, nitrite determination, done using the Griess method according to STAS 9065/7-1974, humidity determination, pH determination, fat content determination, sensory analysis based on point scale.

In this case study, the sensory analysis of the "rustic sirloin" was done using the analytical method, with a 20-point appraisal system, which is highly used and allows the product to be

categorized according to the number of points received. On the basis of this scheme, in order to compare the quality of these products, the results of the analysis carried out on the first day of the study of the unpackaged product were selected as the reference, the others being the result of the 14th day of the vacuum-packed product and, respectively, in modified gas atmosphere.

The determination of these physico-chemical properties took place in four sessions at established intervals in order to observe their evolution throughout the study through the two packing methods.

Results and discussions

In order to see the interferences and differences between these products more clearly, we have developed a representative diagram for this purpose (Figure 4.6):

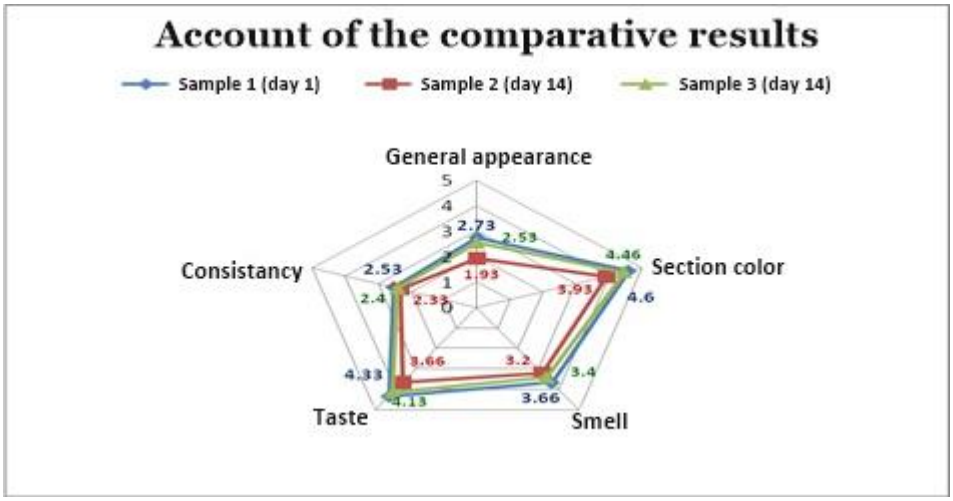

Fig 1. *Qualitative assessment of the two products packaged in vacuum and in modified gas atmosphere (* sample 1 - Unpacked sliced rustic sirloin, ** sample 2 - Vacuum packed sliced rustic sirloin, *** sample 3 - Sliced rustic sirloin packed in modified gas atmosphere)*

By taking the graphical representation of the products as a whole, it can be observed a more uniform distribution of the characteristics for the first product, the one packed in the modified gas atmosphere, compared to the same product on the first packing day. Due to this result, we can say that there is a better correlation between the characteristics of the first product, which gives, as a whole, a product of superior quality.

The water content, the main factor in determining the quality of meat products and in ensuring conservability, is schematically represented in the following figure:

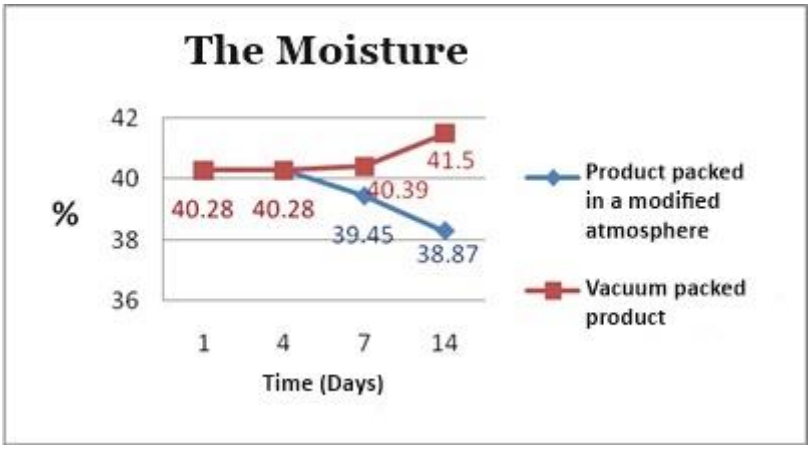

Fig.2. *Product moisture variation*

As can be seen in the figure above, the moisture remains the same until day 4, then rising by 1.22 % for the vacuum-packed product, however, in the case of the one packed in the modified gas atmosphere it decreases; it appears that the last product may have a longer shelf life than the first one, which may be more prone to the action of various alteration factors. Even if the products have different variations, they fit very well into the quality standard of the assortment.

The amount of protein that products hold over the 14 days is shown in the following graph:

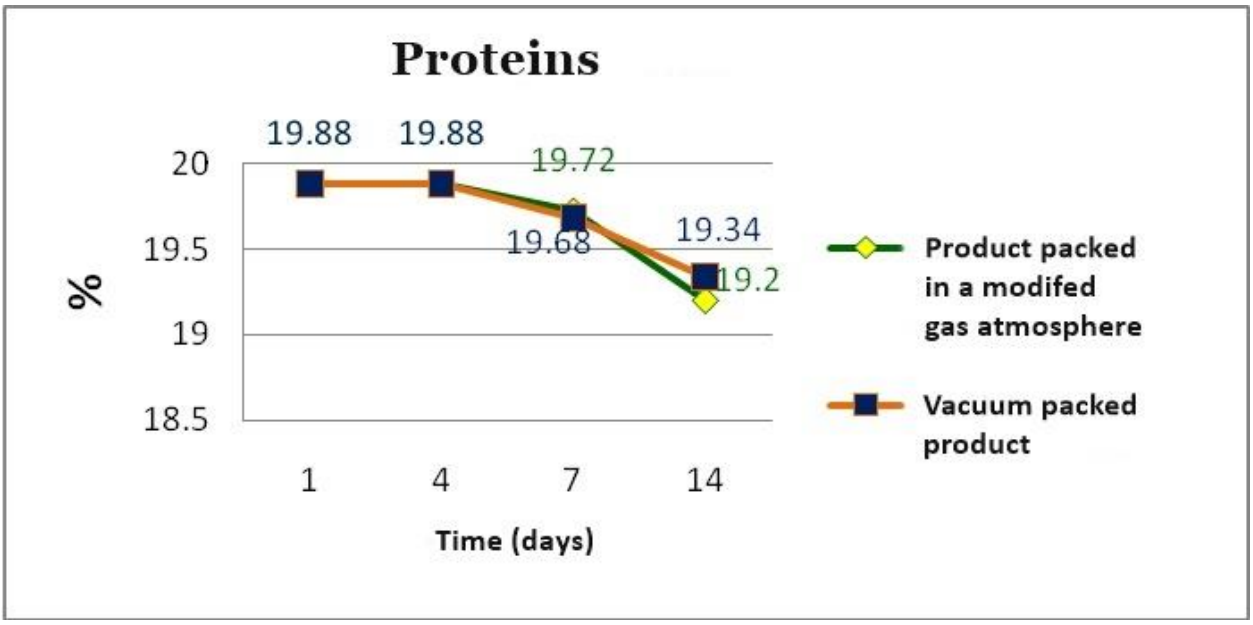

Fig. 3 *Evolution of the protein amount*

Total determined protein provides these products with a particular nutritional value and also classifies them at a good quality level (considering the admissibility conditions). With regard to these results arising from this analysis period, it is possible to observe the almost constant percentage of both products during the first 7 days, then decrease to about 19.2 in the case of modified atmosphere packaging , respectively to 19.34 for the other product. This decrease may be due to the unfavorable action of oxygen on proteins, as other authors (5) said that

the protein oxidation process of beef samples packed in a modified gas atmosphere high oxygen content is increasing with the passage of time. However, this correlation is dismissed by other researchers (6,7,8), which states that the nitrogen content measurement provides only a general indication on protein oxidation.

The third property analyzed was the total fat content expressed as a percentage, which is shown in the following scheme:

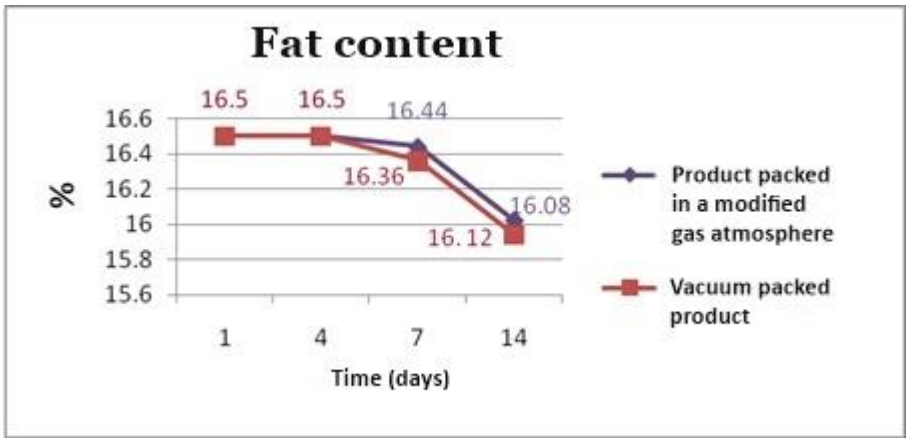

Fig. 4 *The fat content of the two products*

Regarding the lipid percentage content in time of the two products, it has a fairly low and very similar decrease in both products from 16.5% in the first 4 days, reaching 16.08% at the end of the period when packed in the modified atmosphere and 16.12% for vacuum treatment.

From the point of view of the quality standard, these products fulfill the quality requirements throughout the study.

In the case of sodium chloride, the percentage change is shown in the following figure:

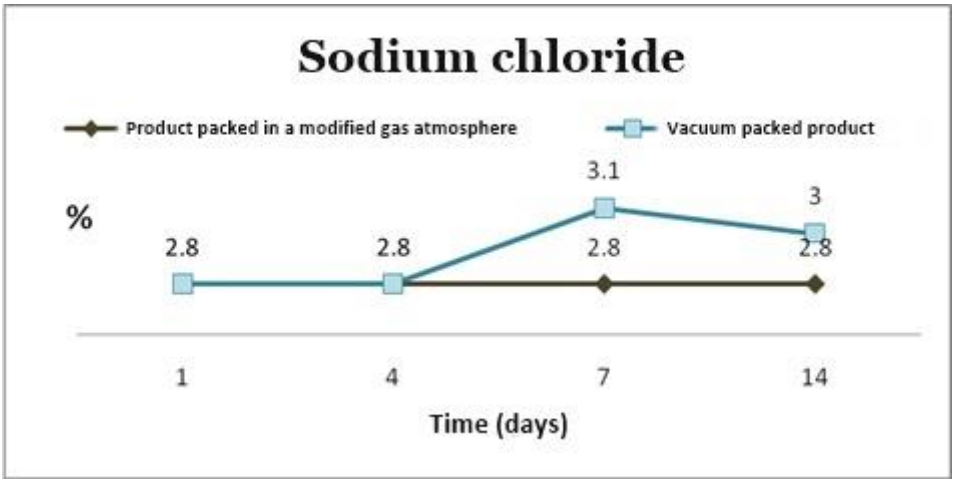

Fig. 5. *Comparative results of sodium chloride*

The results of the analyzes carried out for the determination of sodium chloride show a linear dispersion in the case of the product packed in a modified atmosphere, whilefor the other product the values increase from day 4 and show a slight decrease until the end of storage when the maximum permitted level for these products is achieved .

The sodium chloride is added in meat products to improve the taste and increase the preservation capacity, important points in determining the quality. From this perspective, we can say that the quality of the first product maintains its value constant in time.

The values resulting from the nitrate content are shown in Table 1:

Nitrate (mg/100g)	Day 1	Day 4	Day 7	Day 14
Product packed in a modified atmosphere	10.78	10.78	10.40	9.45
Vacuum packed product	10.78	10.74	10.28	9.12

Due to their destructive potential for human health, it is advisable to carry out repeated checks on their content in order to determine the quality of the product, as this parameter may suffer various changes during the storage of meat products. In this specific case, from the first day, the limit in the quality standard (7 mg / 100g) is exceeded by almost 4 milligrams more per 100 grams of product. However, together with other substances such as sodium chloride, they have a positive role in increasing their conservation capacity. Noteworthy is that their content decreases in time, from both products,the vacuum-packed product being marked by a remarkable decrease.

The last physico-chemical parameter that has been studied is the pH, represented in Figure 6.

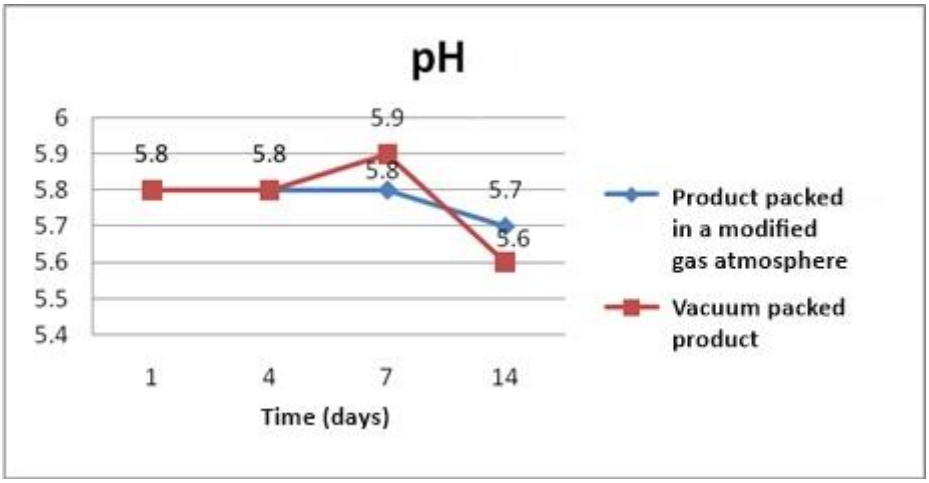

Fig 6. *PH variation during the study*

As outlined in the scheme, the pH values showed not too great differences during storage and between packing modes. The initial pH value was 5.8 and was thus maintained in the first period, then recorded an increase to day 7 in the case of vacuum packed product, then decrease in both products by the end of the period.

Conclusions

Despite of the most up-to-date analysis reports and published recommendations on possible

carcinogenic effects, smoked meat products have an increasing share in the total volume of production and, certainly, in sales. This market sector is of course favored by the Romanian national specificity, which contains a great variety of products of this kind, which are considered specialties, being consumed with great pleasure.

At the end of the retention time, the sirloin packed in the modified gas atmosphere has a higher value and some stability in time. Being a freshness indicator, the pH parameter results indicated both products have retained their quality over time. The pH variation is due to several factors such as the activity of bacteria, the degree of oxidation of meat-type proteins and others. Total determined protein provides these products with a particular nutritional value, and also classifies them at a good quality level (considering the admissibility conditions). Regarding these results arising from this analysis period, it is possible to observe the percentage being almost constant for both products during the first 7 days, then decrease to about 19.2 in the case of modified atmosphere packaging and a decrease to 19.34 for the other product.

In terms of the organoleptic analysis, since the closest values of characteristics to the first day are held by the product packed in a modified gas atmosphere, we can admit that this packaging method is ideal, because the products do not change their sensorial and organoleptic properties for a longer period comparing to the vacuum packed products.

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Investigation of juices with pulp obtained from stone fruit

Oksana LUGOVSKA[1]

[1]*Faculty expertise and health food products, National University of Food Technologies, Volodimirska 68, Kiev, Ukraine, e-mail:oksana.lugovska320@gmail.com*

Abstract: *This article is that juices occupy the leading place in the human diet. Along with the increase in the volume of juice production and the expansion of their range, the technology of their production is improved, with the purpose of preserving the biologically active substances of raw materials, improving the quality and nutritional value of the finished product. Current and necessary introduction of standards for methods of determining the indicators that allow establishing the naturalness of juices provide a regulatory framework for eliminating the sale of counterfeit products to consumers and create priority conditions for responsible manufacturers.*

This work is devoted to the examination of the chemical composition and nutritional value of juices with pulp obtained from stone fruits, to the conformity with the standard DSTU 4150: 2003 Juices, juice drinks, fruit nectars, vegetables and melons. General technical conditions.

Keywords: apricot, peach juice, minerals, vitamins, falsification

I. Introduction

Emulsions the juices contain vitamins and minerals, without which human life is impossible. They are not synthesized in the human body, but come into the body exclusively with food. Minerals play an extremely important role in the metabolic processes of the human body. They are necessary for the formation of the supporting tissues - bones, cartilage, teeth (calcium, phosphorus, magnesium, fluorine) involved in blood (iron, cobalt, copper, manganese, never), affect water metabolism, determining the osmotic pressure of blood plasma, they are the main parts of a number of hormones, vitamins, enzymes [1, 2].

Depending on the content in the body and its requirements, all the mineral elements are divided into macro- and microelements. Macro elements are elements, the daily need in human body is more than 100 mg. The macronutrients include sodium, potassium, calcium, magnesium, phosphorus, sulfur and chlorine. Body's need for micronutrients is milligrams or even micrograms per day (Iron, cobalt, iodine, fluorine, copper, manganese, zinc, selenium, etc.). The total content of all mineral substances is 3-5% of the mass of the human body. Their content in food is insignificant - 0.03-1.9%.

A deficiency in an organism of one or another microelement can arise for various reasons. Macronutrient and micronutrient deficiency can be traditional (linked to food raw materials and food) and alternative sources of food and biologically active substances.

Types of juices:

- restored;
- direct spin.

Recycled juice - the juice obtained by reduction of concentrated juice or concentrated puree or paste prepared drinking water in a ratio that ensures the preservation of the organoleptic, physical and chemical properties and nutritional value of juice from the same fruit, berries or vegetables, with the addition or without addition direct spinning juices, purees, concentrated natural aromatic substances obtained during the production of concentrated juice. Recovered juices

prepared as follows: concentrate is heated to a temperature of 100 degrees is maintained for several seconds, and then for a split second cooled to room temperature. Subsequently pour so much pure water as evaporated. Usually in such a drink is added vitamins, sugar.

It is also worth to say that the juices are lighted, unlit and juices with pulp. Juice with pulp is useful, because it has flavoring properties that are most suitable for fresh fruit and berries. But unliterated juice is saturated with vitamins more than illuminated [3].

Directly expressed juice - the juice obtained directly from fruits, berries or vegetables spin or centrifugation or wiping or other physical way to remove it.

Juices, depending on the type of raw material and the technology used, are made:

- illuminated;
- unclear;
- with pulp.

Requirements for the quality of raw materials. In juice production, different types of fruits and vegetables are used. The following groups of fruits are most significant: grain seed (apples, pears, etc.); Bone stone (apricots, peach, cherry, plum, etc.); Berries (black and red currants, gooseberries, raspberries, blueberries, etc.). Among vegetables, tomatoes should be distinguished, since tomato juice is the leader in the volume of production. Other vegetable juices, as a rule, contain several components: it may be carrots, pumpkin, beets and other vegetables. Requirements for raw materials intended for processing differ from requirements for fruits and vegetables for consumption in fresh form. So, for processing on the juice, fruit and berries with damaged skin (spots, burns) can be used, the size and shape of the fruit usually do not matter. However, it is unacceptable that the raw material is decayed - a small amount of rotten fruits or berries that have got into the processing can give an unpleasant smack of the whole batch of juice produced. In addition, such females may contain mycotoxin patulin [4].

Fruits and berries for making juices should be mature. Outstanding fruits have a faint color, high acidity, dense pulp. Juices from immature and underdeveloped fruits contain a smaller amount of aromatic substances, much lower than their quality and quantity when obtaining a concentrate of aromatic substances.

It is better to use autumn / winter autumn / winter varieties with juicy and sour - sweet flesh for the production of juices, as the fruits of summer maturity tend to produce less juice, less dry matter. Grapes with colored peel and unpolluted juice are not suitable for the production of natural juices. The mass fraction of sugars and acids determines the taste of juices. With high acidity and low sugar content juice turns out to be unhealthy. Some types of fruit and berry raw materials impose additional requirements. For example, grenades should have acidity within the range of 0.9-2.8%; It is better to use gooseberries with yellow color, as the red berry juice changes in color during processing and storage.

Reception, storage and intra-factory transportation of raw materials. Crystal sugar comes to enterprises, usually in bags by road. From cars, bags of sugar with the help of an electric car are transferred to the weight and after the determination of the mass stored in storage. The ladle bag is fed to the bunker with bags of sugar. Sugar is poured out of sacks into a bunker, from where it is fed to the preparation of sugar syrup and colander.

Essences, flavors, concentrates of beverages, concentrated juices, dyes and organic acids enter the plant, usually in a container of polymeric materials and, after proper recording, are kept in stock. To prepare working solutions, they raise the loader to collectors. After weighing these

products on their scales, using flexible hoses, the pump is pumped into collections for storage. Small batches are stored in transport containers. The juices and infusions are filtered on a lamellar filter, from which they are fed into pressure collections.

Preparation of water for technological purposes. As a rule, drinking water entering the production requires additional training and, above all, reduction of stiffness. At non-alcoholic drinks factories, biological water purification can be carried out by chlorination. Chlorination - a widespread method of biological water purification. The biological effect of chlorine consists in suppressing the metabolism and oxidation of the components of microorganism cells, which, as a result, they perish. This action is conditioned by the presence in chlorinated water of chlorinated acid and chlorine ion, directly interacting with the substances of the cell. Complete sterility of water during chlorination can not be achieved, since some microorganisms exhibit resistance to chlorine. The bactericidal effect of chlorine depends to a large extent on its initial dose and duration of contact with water. At a dose of chlorine 1 mg / l and the duration of contact for 1 hour, the number of bacteria decreases from 232000 in 1 cm3 of water to 180,000. Chlorine is readily soluble in water. At normal pressure and temperature of 10 ° C, its solubility is 9,75 g / l. Dissolving chlorine interacts with water and forms chlorine water, which is a strong oxidizer. The degree of hydrolysis of chlorine is determined by the pH of the medium. At pH 5, active chlorine is in water in molecular form, in the range of pH 5 ... 9.2 in water, chlorine oxalic acid predominates, and at pH> 9.2 - only ions C1.

In order to improve the biological state, water is chlorinated after filtration. Due to the oxidative action of chlorine, the degree of water color decreases, smells and odors disappear. Chlorination also helps to remove iron and manganese from the water. Organic compounds of iron under the influence of chlorine are destroyed, divalent iron passes into trivalent and as a result of hydrolysis and precipitates in the form of hydroxide iron. Manganese oxidizes and falls into sediment. In the production of soft drinks, chlorination of water should be accompanied by dechlorination, since residual chlorine gives it an unpleasant taste and smell. in addition

II. Materials and methods

Analytical research methods conducted an examination of the chemical composition and nutritional value of juices with pulp obtained from stone fruits, to the compliance with the standard DSTU 4150: 2003 juices, juice drinks, fruit and berry nectars, vegetables and melons. General specifications [7,11,14,15].

Dry matter and mass fraction were measured by physicochemical drying method in a drying cabinet; Mono- and disaccharides - by a sugar meter (pic. 1)

Pic. 2 Sugar meter

Sugar meter could have measured Brix:

★ Material & Structure & Range: Copper construction, main prism + daylight plate + calibration screw + rubber grip + focus

adjustment + eyepiece. Adjust prism when can't see scale clearly, get accurate scale. Soft rubber eye piece for comfortable viewing. Measures brix is 0-32%, minimum scale is 0.2

★ Features: High quality and accurate testing result. Heavy-duty and light weight, portable and compact, small volume, convenient to carry. ATC (Automatic Temperature Compensation, 10℃~30℃) for accurate readings, without having to re-calibrate after changes in ambient working temperature

★ Utilities: Designed for testing the amount of sugars in fruits, vegetables and grasses and quality control in production, very helpful in agriculture, food manufacturing and field operation. For measuring brix, a refractometer is easier and more accurate than a hydrometer

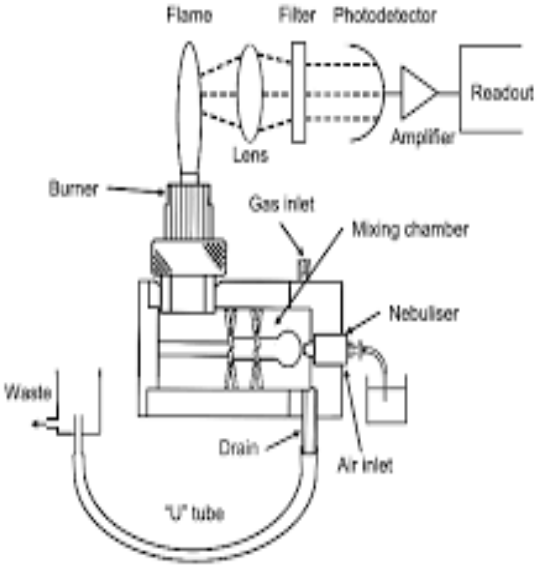
★ High quality, sturdy design, compact in size and light in weight, packed with a plastic case, convenient to keep up and carry around. Zero-line adjustable visual sharpness setting at the eyepiece. Uses ambient light only which means battery or power source is not required

★ Full kit: Storage case, include refractometer, mini screwdriver (to calibrate to "0") for adjusting, pipette, cleaning cloth, and English manual.

Na, K, Ca and vitamin C content were measured by the method of flame photometry with a flame photometer PFP-7 (pic. 2) designed to determine the concentration of ions (Na, K,). The PFP7 is a low temperature, single channel flame photometer that is designed for the routine determination of sodium, potassium, calcium, barium and lithium concentrations. The flame failure safety system makes these products ideal for use in industrial and educational environments. The use of fine and coarse sensitivity controls allows for accurate measurements each and every time.

PFP7 Industrial Flame Photometer

• Designed for industrial analysis
• Supplied with Na, K, Li, Ba and Ca filters
• Low temperature, single channel
• Flame failure safety system
• Operates with propane, butane, natural gas or LPG



Pic. 2 Flame photometer PFP-7

The object of research is apricot and peach juice with pulp with the addition of emulsions. Organoleptic indicators of juices with pulp (apricot and peach):
appearance and consistency-homogeneity of the liquid with a uniformly distributed flesh; taste and aroma, characteristic of this type of juice; color - a homogeneous, characteristic of this kind of fruit from which the juice is made.

We will analyze the organoleptic, physico-chemical parameters of juices and the content of micro and micronutrients, vitamins for compliance with the standards.
Analyzing the quality indices of the juices studied, investigate whether there is a violation of the parameters of the technological process of making juices and their falsification.

Table 1 - Physico-chemical parameters of juices according to the standard

Fruit juices with pulp	Mass fraction,%		pH, not more than
	Soluble dry matter, not less than	Titrated acids per citric acid, not more than	
Apricot	11,2	1,10	4,0
Peachy	12,0	0,65	4,0

Table 2 Nutritional value of 100 g of juices according to the standard

Name of fruit juice with pulp	Proteins, g	Carbohydrates, g	Mineral substances, mg						Vitamins, mg				
			Na	K	Ca	Mg	P	Fe	β-каротин	B_1	B_2	PP	C
Apricot	0,9	11,0	1	153	14	4	13	0,3	1,3	0,01	0,02	0,15	12,0
Peachy	0,5	10,0	16	190	11	9	19	0,3	0,3	0,02	0,04	0,40	2,0

III. Results and discussion

As a result of the use of research methods, the chemical composition of juices, measured dry matter, pulp, mineral matter and vitamins were investigated.

The results of the research are presented in Table 3.

Table 3 Chemical composition and nutritional value of juices with pulp obtained from stone fruit

Indicator	Apricot juice	Peach juice
Chemical composition, g /100 g		
Dry matter, %	11,5	13,3
Mass fraction of pulp, %	35	35
Mono and disaccharides	13,70	11,12
Мінеральні речовини, мг/ Mineral substances, mg 100 g		
Na	*1,55*	*1,45*
K	160	180
Ca	12,5	14,0
Vitamins, β-carotene, mg /100 g	4,70	6,0
Vitamin C	***4,70***	***6,0***

From the studied quality indicators of apricot juice with pulp, does not meet the requirements of the standard content of vitamin C - decreased from 12 mg / 100 g to 4.7 mg / 100 g., peach juice does not meet the requirements of the standard content of Na – reduced from 16 mg / 100 g. To 1.55 mg / 100 g. When carrying out sensory analysis for organoleptic parameters, all the juices for color, aroma, consistency meet the standard. Physicochemical indicators also corresponded to normative documents. In the study of physico-chemical parameters in peach juice, the higher content of dry matter (12) but less acidity (0.65) compared with apricot juice (11.2 and 1.1), respectively.

IV. Conclusions

The analysis of organoleptic, physico-chemical parameters of juices and the content of micro and microelements, vitamins for compliance with the standards has been carried out.

Analyzing the quality indices of the juices studied, there is a violation of the

parameters of the technological process of making juices.

This is evidenced by:

• reduced vitamin C content in apricot juice. Therefore, in order to replenish the supply of vitamin C in the body, it is necessary to turn to plant products during their flowering. It is also known that vitamin C is lost during heat treatment.

• Reduced Na content in peach juice. Falsification of juices was discovered, using more complex imitation of natural product, using different types of forgings - apple puree added to peach juice, etc.

It is clear that the content of vitamin C and trace elements in various fruits depends on the soil they are grown from, the climate from the time of ripening, the amount of precipitation that falls, and, finally, on the time and method of storage. Even in different fruits of the same species, there is always a certain difference, and this is explained by different numbers of different laboratories. The technology of juicing is particularly influential, namely the temperature processing modes.

Consequently, the declared indicators of investigated juices from the trading network do not meet the requirements of the state standard [8, 9,10,12,13,].

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Identification of a strain of the genus *Lactobacillus,* isolated from spontaneously fermented yogurt

Rositsa DENKOVA[1], Desislava TENEVA[2], Bogdan GORANOV[3], Zapryana DENKOVA[4], Zoltan URSHEV[5], Teodora KOPARANOVA[4], Kristina IVANOVA[2], Georgi KOSTOV[6]

[1]Department of Biochemistry and Molecular Biology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria, e-mail: rositsa_denkova@mail.bg

[2] Food Research and Development Institute, Plovdiv, Bulgaria

[3]LBLact, Plovdiv, Bulgaria

[4]Department of Microbiology, University of Food Technologies, Plovdiv, Bulgaria

[5]LB Bulgaricum, Sofia, Bulgaria

[6]Department of Wine and Brewing, University of Food Technologies, Plovdiv, Bulgaria

Abstract. *Probiotic strains are included in the composition of probiotics and functional foods because their intake enhances human health. The search for newly isolated strains of lactobacilli and the examination of their probiotic potential is one of the main trends in contemporary nutritional science. Each newly isolated strain has to be identified first before the subsequent examination of its probiotic properties. A newly isolated strain of the genus Lactobacillus, Lactobacillus Pr2, was identified using biochemical and molecular-genetic methods (ARDRA-analysis and sequencing of the gene for the 16S rRNA) as a representative of the species Lactobacillus delbrueckii ssp. bulgaricus. Lactobacillus delbrueckii ssp. bulgaricus strains are successfully included in the composition of starters for a variety of probiotic and functional foods. Thus, a number of studies would be performed to determine the probiotic potential of Lactobacillus delbrueckii ssp. bulgaricus Pr2 and its possible application in the composition of starters for functional foods.*

Key Words: probiotic, *Lactobacillus*, API, sequencing, ARDRA

I. Introduction

Probiotic strains are included in the composition of probiotics and functional foods because their intake enhances human health. The search for newly isolated strains of lactobacilli and the examination of their probiotic potential is one of the main trends in contemporary nutritional science. Each newly isolated strain has to be identified first before the subsequent examination of its probiotic properties.

According to modern polyphase taxonomy, the combination of phenotypic and molecular-genetic methods for the typing and subtyping of bacteria, and in particular of lactobacilli, is applied.

Lactobacilli are similar in their phenotypic and physiological characteristics. Because of this, their differentiation is a difficult process. This requires the use of additional methods for their reliable identification and characterization. With the advances in molecular biology, a set of molecular DNA-based techniques replace phenotypic identification methods. Unlike phenotypic methods, molecular-genetic methods are much more logical, faster, more reliable and more reproducible, and can differentiate between species and types within the species that are otherwise indistinguishable when applying phenotypic identification methods.

The aim of the present study was to identify a strain of the genus *Lactobacillus* isolated from spontaneously fermented yogurt.

II. Materials and methods

1. Microorganisms

The studies were conducted with *Lactobacillus* Pr2, isolated from spontaneously fermented yogurt.

1.2. Reference cultures: *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 20081, *Lactobacillus delbrueckii* ssp. *lactis* DSM 20072, *Lactobacillus helveticus* DSM 20075, *Lactobacillus casei* ssp. *rhamnosus* LMG 6400.

2. Nutrient media

2.1. LAPTg10–broth. Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6-6.8 and Tween 80 - 1cm^3/dm^3 was added. Sterilization - 20 minutes at 121 °C.

2.2. LAPTg10–agar. Composition (g/dm^3): LAPTg10 – broth, agar - 20. Sterilization - 20 minutes at 121 °C.

3. Determination of the biochemical profile

The API 50 CHL system (BioMerieux SA, France) was used to identify the studied *Lactobacillus* strain based on the consumption of 49 carbohydrate sources. Fresh 24-hour culture of the strain was centrifuged for 15 min at 5000 x g. The resulting biomass sludge was washed twice with PBS buffer and resuspended in API 50 CHL medium, a component of the kit used. The API strips were placed in the incubation box, the microtubules were inoculated with the prepared cell suspension and sealed with sterile liquid paraffin. The results were recorded at the 24th hour and at the 48th hour of incubation at optimal temperature for the growth of the studied strain (37°C). The recording of the results was performed based on a color change in comparison with the control (microtubule 0). The changing of the color from blue to green to bright yellow were reported as positive results. The results obtained were processed with apiweb^R identification software and the studied strain was identified with the corresponding level of confidence.

4. Genetic methods

4.1. Isolation of total DNA

The isolation of the genomic DNA was performed with E.Z.N.A. DNA isolation kit according to the manufacturer's instructions.

4.2. 16S rDNA amplification and 16S ARDRA

All PCR reactions were performed using a PCR kit PCR VWR in a volume of 25 µl in Progene cycler (Techne, UK) according to the manufacturer's instructions, using 50 ng total DNA of the studied strain and 10 pmol primers in each reaction. DNA from the strain was amplified using universal primers for 16S rDNA-27f (5'AGAGTTTGATCMTGGCTCAG3') [1] and 1492r (5'ACCTTGTTACGACTT3') [1]. The amplification program included: denaturation - 95 °C for 3 min, 35 cycles - 93 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, final elongation - 72 °C for 7 min.

The resulting PCR product was separated into three aliquots and each aliquot was treated for 24 hours with a restriction enzyme (*Eco* RI, *Hae* III or *Alu* I) (Boehringer Mannhem GmbH, Germany) with a concentration of 10 units/µl.

The resulting restriction products were visualized on a 2% agarose gel stained with an ethidium bromide solution (0.5 µg/cm^3) using UVP Documentation System (U.K.).

4.3. Purification of the product from the PCR reaction (16S rDNA) from a TAE-agarose gel

The purification of the 16S rDNA was performed with a DNA purification kit (GFX Microspin TM) according to the manufacturer's instructions.

4.4. Sequencing of the 16S rRNA gene

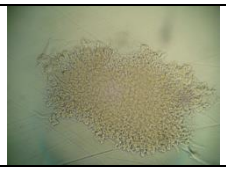
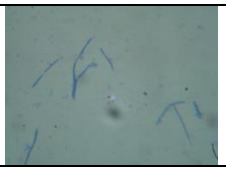
The partial sequencing of the 16S rRNA gene with the two universal primers (27f and 1492r) was performed by the Sanger method by "Macrogen Europe Laboratory", The Netherlands. Using the *CLC Sequence Viewer* software, the entire sequence of the 16S rRNA gene was obtained, and the resulting whole sequence was compared with the on-line database sequences via the BLASTn algorithm. Thus, the studied strain was identified to the species level with the corresponding confidence level.

III. Results and discussion

1. Phenotypic characteristics of Lactobacillus Pr2

The characterization of newly isolated strains begins with an assessment of culture purity, macroscopic and microscopic morphological control. The micro- and macromorphological characteristics of the lactobacilli isolate are presented in Table 1.

Table 1. *Colonial and cellular characteristics of Lactobacillus Pr2.*

Strain	Colonial characteristics		Cellular morphology	
	Description of the colonies	Visualization	Description of the cells	Visualization
Pr2	Star-shaped colonies with irregular ends, protruding, whitish, 2-3 mm in diameter		Long thick rods with rounded ends, single, in pairs or in chains	

When cultured on LAPTg10-agar medium, the isolated strain grew in the form of protruding, whitish colonies, 2-3 mm in size, with irregular shape and jagged edges. The cells were single and in chains and were long rods with rounded ends.

After characterization according to the basic morphological criteria (Table 1), the studied strain was subjected to determination of its biochemical profile with the API 50 CHL rapid identification system (Biomerieux, France). According to its ability to utilize 49 carbon sources (Table 2) and the subsequent software processing with apiweb^{R}, it was established that the strain *Lactobacillus* Pr2 belonged to the species *Lactobacillus delbrueckii* ssp. *bulgaricus* with a confidence level of 87.1%.

Table 2. *Ability of Lactobacillus Pr2 to utilize the 49 carbon sources included in the API 50 CHL identification system.*

#	Carbohydrate	Pr 2
1	**Glycerol**	-
2	**Erythriol**	-
3	**D-arabinose**	-
4	**L-arabinose**	-
5	**Ribose**	-
6	**D-xylose**	-
7	**L-xylose**	-
8	**Adonitol**	-
9	**β-metil-D-xyloside**	-
10	**Galactose**	-
11	**D-glucose**	+ (90 – 100 %)
12	**D-fructose**	+ (90 – 100 %)
13	**D-mannose**	+ (90 – 100 %)
14	**L-sorbose**	-
15	**Rhamnose**	-
16	**Dulcitol**	-
17	**Inositol**	-
18	**Manitol**	-
19	**Sorbitol**	-
20	**α-methyl-D-mannoside**	-
21	**α-methyl-D-glucoside**	-
22	**N-acetyl-glucosamine**	+ (90 – 100 %)
23	**Amigdalin**	-
24	**Arbutin**	-
25	**Esculin**	-
26	**Salicin**	-
27	**Cellobiose**	-
28	**Maltose**	-
29	**Lactose**	+ (90 – 100 %)
30	**Melibiose**	-
31	**Saccharose**	-
32	**Trehalose**	+ (90 – 100 %)
33	**Inulin**	-
34	**Melezitose**	-
35	**D-raffinose**	-
36	**Amidon**	-
37	**Glycogen**	-
38	**Xylitol**	-
39	**β-gentiobiose**	-
40	**D-turanose**	-
41	**D-lyxose**	-
42	**D-tagarose**	-
43	**D-fuccose**	-
44	**L-fuccose**	-
45	**D-arabitol**	-
46	**L-arabitol**	-
47	**Gluconate**	-
48	**2-keto-gluconate**	-
49	**5-keto-gluconate**	-

API 50 CHL assays provide a rapid and reproducible identification of some types of lactobacilli, but sometimes with an insufficiently high percentage of discrimination. Using only the classical phenotypic physiological and biochemical characteristics does not always allow a meaningful distinction to be made between the different types of lactobacilli, especially considering that phenotypic variability observed in lactobacilli [2]. Therefore, according to the modern concepts of taxonomic determination of lactobacilli, molecular-genetic methods have to be applied as well and, especially in cases of relatively low discrimination rates provided by classical methods.

2. Molecular-genetic characterization of Lactobacillus Pr2

Lactobacillus Pr2 was subjected to ARDRA analysis with the enzymes *Hae* III (Fig. 1), *Alu* I (Fig. 2) and *Eco* RI (Fig. 3) to confirm the results for its species identification obtained by the conventional identification method.

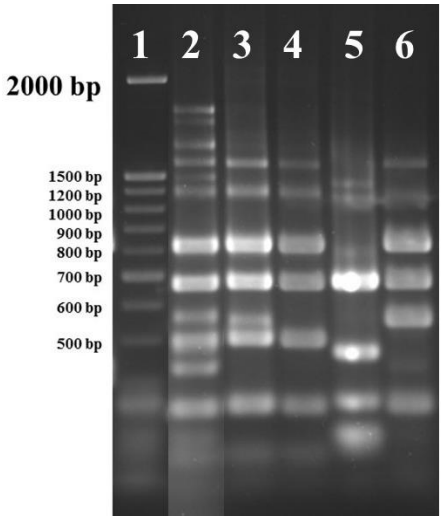


Figure 1. *Restriction profile with Hae III.*

1. 100 bp Plus DNA Ladder
2. *Lactobacillus* Pr2
3. *L.d.* ssp. *bulgaricus* DSM 20081
4. *L.d.* ssp. *lactis* DSM 20072
5. *L. helveticus* DSM 20075
6. *L. casei* ssp. *rhamnosus* LMG 6400

The restriction profile of *Lactobacillus* Pr2 with *Hae* III was similar to that of *Lactobacillus*

delbrueckii ssp. *bulgaricus* DSM 20081 and *Lactobacillus delbrueckii* ssp. *lactis* DSM 20072 (Fig. 1), which required ARDRA analysis to be performed using other enzymes - *Alu* I and *Eco* RI.

The restriction profile of *Lactobacillus* Pr2 with the restriction endonuclease *Alu* I was again similar to that of *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 20081 and *Lactobacillus delbrueckii* ssp. *lactis* DSM 20072 (Fig. 2). The profile of *Lactobacillus* Pr2 with *Eco* RI (Fig. 3) confirmed that *Lactobacillus* Pr2 was a *Lactobacillus delbrueckii* ssp. *bulgaricus* strain.

As a result of the research, the species identification of *Lactobacillus* Pr2 strain was confirmed. For its complete identification, another molecular-genetic method for genotyping - sequencing of the 16S rRNA gene - was applied. The results of the 16S rDNA sequence analysis referred *Lactobacillus* Pr2 strain to the species *Lactobacillus delbrueckii* ssp. *bulgaricus* with a percentage of similarity between the sequence of the 16S rDNA of *Lactobacillus* Pr2 and the partial sequence of the 16S rDNA of *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 - 99% (Fig. 4).

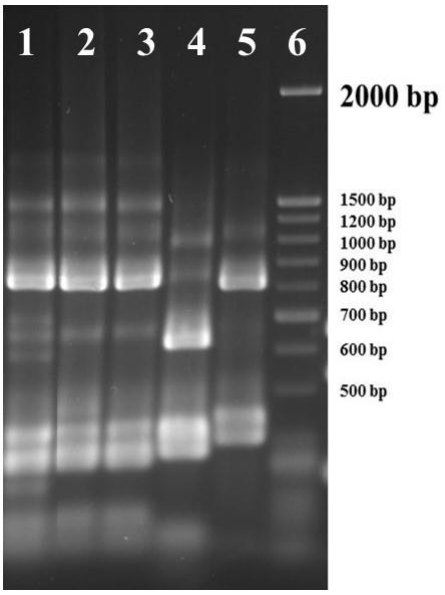


Figure 2. *Restriction profile with Alu I.*

1. *Lactobacillus* Pr2
2. *L.d.* ssp. *bulgaricus* DSM 20081
3. *L.d.* ssp. *lactis* DSM 20072
4. *L. helveticus* DSM 20075
5. *Lactobacillus casei* ssp. *rhamnosus* LMG 6400
6. 100 bp Plus DNA Ladder

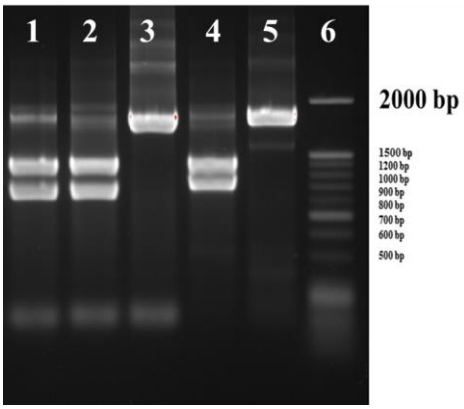


Figure 3. *Restriction profile with Eco RI.*

1. *Lactobacillus* Pr2
2. *L.d.* ssp. *bulgaricus* DSM 20081
3. *L.d.* ssp. *lactis* DSM 20072
4. *L. helveticus* DSM 20075
5. *L. casei* ssp. *rhamnosus* LMG 6400
6. 100 bp Plus DNA Ladder

IV. Conclusions

The newly isolated strain *Lactobacillus* Pr2 was identified by the application of cultural, microbiological, physiological, biochemical (API 50 CHL) and molecular-genetic (ARDRA and sequencing of the gene for the 16S rRNA) methods as representative of the species *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Lactobacillus delbrueckii* ssp. *bulgaricus* strains are successfully included in the composition of starters for a variety of probiotic and functional foods. Thus, a number of studies would be performed to determine the probiotic potential of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 and its possible application in the composition of starters for functional foods.

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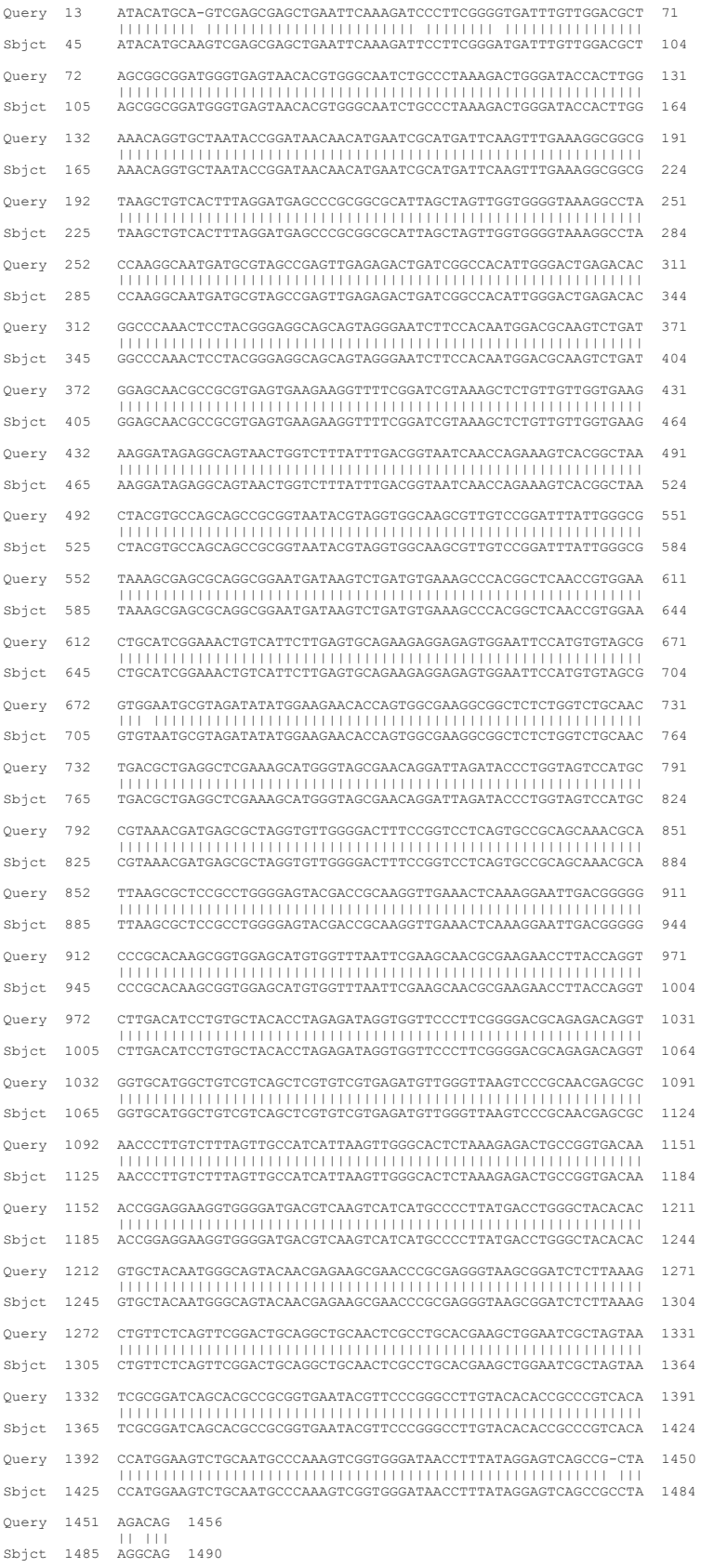

Figure 4. *Comparison of the Lactobacillus Pr2 16S rDNA sequence and the partial 16S rDNA sequence of Lactobacillus delbrueckii ssp. bulgaricus ATCC 11842.*

Examination of some probiotic properties of *Lactobacillus delbrueckii ssp. bulgaricus* Pr2, isolated from spontaneously fermented yogurt

Desislava TENEVA[1], Bogdan GORANOV[2], Rositsa DENKOVA[3], Zapryana DENKOVA[4], Teodora KOPARANOVA[4], Kristina IVANOVA[2], Georgi KOSTOV[5]

[1] *Food Research and Development Institute, Plovdiv, Bulgaria*

[2]*LBLact, Plovdiv, Bulgaria*

[3]*Department of Biochemistry and Molecular Biology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria, e-mail: rositsa_denkova@mail.bg*

[4]*Department of Microbiology, University of Food Technologies, Plovdiv, Bulgaria*

[5]*Department of Wine and Brewing, University of Food Technologies, Plovdiv, Bulgaria*

Abstract. *The intake of probiotic preparations and probiotic foods helps restore the balance of the gastrointestinal microflora and has beneficial effects on the overall health status of the organism. Some probiotic properties of a newly isolated and identified strain Lactobacillus delbrueckii ssp. bulgaricus Pr2 were examined: profile of antibiotic resistance, survival in the model conditions of the gastro-intestinal tract, antimicrobial activity against pathogens and the possibility of conducting industrial processes – culturing and freeze-drying. The studied strain survived the model conditions of the gastrointestinal tract, maintaining enough viable cell concentrations, demonstrated high antimicrobial activity against pathogens and allowed the conduction of culturing and freeze-drying with reaching and maintaining of high concentration of viable cells. After further examinations of the probiotic properties of Lactobacillus delbrueckii ssp. bulgaricus Pr2, the strain can be included in the composition of probiotic preparations and starters for probiotic foods whose consumption would improve contemporary man's health.*

Key Words: probiotic, *Lactobacillus*, API, stomach, antibiotic resistance, freeze-drying

I. Introduction

The major regulators of the balance of the gastrointestinal microflora are lactobacilli: *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*. Thus, their daily intake is of utmost importance [7]. They are widely applied in the composition of probiotics and as starter cultures for fermented food products such as yoghurt, white brine cheese, yellow cheese, kefir, etc..

Microorganisms included in the composition of probiotics must meet the following requirements [8]:

1. to be part of the natural microflora in humans and animals.

2. to have the ability to adhere to epithelial cells or cell lines.

3. to survive in the stomach and intestines, i.e. to survive under acidic pH conditions in the stomach and to be resistant to the action of bile juice in the intestines.

4. to be able to proliferate in the gastrointestinal tract - by predominantly utilizing the substrate to suppress and expel pathogenic and toxigenic microorganisms from the biological niche.

5. to allow industrial processes (culturing freeze-drying, etc.) and maintain high concentration of viable cells during the conduction of the processes and storage.

6. to possess antimicrobial activity against conditionally pathogenic, carcinogenic and pathogenic microorganisms, which is associated with the inactivation of their enzyme systems, the overcoming of their adhesion, suppression of their growth and ejection from the biological niche, resulting in the normalization of the gastrointestinal microflora.

7. to produce antimicrobial substances.

8. to modulate the immune response.

9. to be safe for clinical and nutritional applications.

In recent years, particular attention has been paid to probiotic bacteria of human origin as components of probiotics and probiotic foods as well as preparations for targeted therapy of certain types of diseases. Isolation of new lactobacilli strains from different sources and examination of their probiotic

properties allows the selection of strains with probiotic potential for incorporation as components in probiotic fermented foods.

The aim of the present study was to examine some probiotic properties of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 isolated from spontaneously fermented yogurt.

II. Materials and methods

1. Microorganisms

1.1. The studies were conducted with *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2, isolated from spontaneously fermented yogurt, part of the culture collection at the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria.

1.2. Test microorganisms.

Test pathogenic microorganisms: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella* sp. (clinical isolate).

2. Nutrient media

2.1. MRS–broth. Composition (g/dm^3): casein peptone - 10; yeast extract - 4; meat extract - 8; glucose - 20; K_2HPO_4 - 2; sodium acetate - 5; diammonium citrate - 2; $MgSO_4$ - 0.2; $MnSO_4$ - 0.04; Tween 80 - 1 cm^3; pH = 6.5. Sterilization - 15 minutes at 118 °C.

2.2. MRS–agar. Composition (g/dm^3): MRS-broth, agar - 20. Sterilization - 15 minutes at 118 °C.

2.3. Saline solution. Composition (g/dm^3): NaCl - 5. Sterilization - 121 °C for 20 minutes.

2.4. LAPTg10–broth. Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6-6.8 and Tween 80 - 1cm^3/dm^3 was added. Sterilization - 20 minutes at 121 °C.

2.5. LAPTg10–agar. Composition (g/dm^3): LAPTg10 – broth, agar - 20. Sterilization - 20 minutes at 121 °C.

3. Determination of the antibiotic resistance profile

The laboratory testing of the sensitivity of the studied strain to antibiotics was performed by the Bauer, Kirby et al. (1966) [1] disc-diffusion method (DDM). Fresh 24-hour culture of the strain was used to inoculate MRS-agar plates. Standard antibiotic-impregnated paper disks were placed in the inoculated Petri dishes. The plates were incubated for 48 hours at optimum temperature for the growth of the studied strain (37°C). The diameters (in mm) of the sterile zones formed around each of the antibiotic disks were recorded. The following indications were used: R - resistant (d_{zone}<8 mm), SR - intermediately sensitive (d_{zone}= 8 - 16 mm), S - sensitive (d_{zone}>16 mm).

4. Determination of the survival at low pH in the presence of pepsin and at low and neutral pH in the presence of pancreatin [2]

Fresh 24-hour culture of the studied strain was centrifuged for 15 min at 5000 x g. The resulting biomass sludge was washed twice with PBS buffer and resuspended to the original volume in PBS buffer. 0.2 cm^3 of the cell suspension were incubated with 5 cm^3 of a buffer solution with pH = 2 containing 0.5% NaCl and pepsin (3.2 g/dm^3) (Sigma, 2.500-3.500 U/mg protein), buffer solution with pH = 4.5 and pancreatin and a buffer solution with pH = 7 and pancreatin at a temperature optimal for the growth of the studied strain (37°C). The study was conducted for 24 hours, taking samples at the 0th, the 2nd, the 4th and the 24th hour to determine the total number of viable cells (cfu/cm^3).

5. Determination of the survival at different concentrations of bile salts (by the method modified by Denkova Z., 2005 [4])

Fresh 24-hour culture of the studied strain was centrifuged for 15 min at 5000 x g. The resulting biomass was washed twice with PBS buffer and resuspended to the original volume in PBS buffer. 0.2 cm^3 of the cell suspension were incubated with 5 cm^3 of MRS broth medium with different concentrations of bile salts (0%, 0.15%, 0.3%, 0.6%, and 1%). The mixtures were incubated for 24 hours at optimum temperature for the growth of the studied strain (37°C) and aliquots to determine the total number of viable cells (cfu/cm^3) were taken at the 0th, the 2nd, the 4th, the 6th, the 8th , and the 24th hour.

6. Determination of the antimicrobial activity against pathogenic microorganisms – well-diffusion method

Culture liquid (CL), biomass (B) and neutralized acellular supernatant (pH=6.5) (NASN) obtained from a 24-hour culture of the studied strain were used to determine the antimicrobial activity. The antimicrobial activity was tested against the following pathogenic microorganisms: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella* sp. (clinical isolate). Each of the test microorganisms (concentration of 10^7cfu/cm^3) was inoculated into Petri dishes with MRS-agar medium and wells with a diameter of 7 mm were prepared after the hardening of the medium (final concentration of 10^5cfu/g). 0.1 cm^3 of CL, B, and NASN were pipetted into the wells and allowed to diffuse into the

agar for 1 h at 4 °C. The Petri dishes were incubated at 37 °C for 24 hours, after which the inhibition zones in mm were recorded.

7. Determination of the number of viable cells

Appropriate tenfold dilutions of each sample in saline solution were prepared. 0.1 cm^3 of the last three dilutions were spread plated on MRS-agar medium or LAPTg10-agar medium. The inoculated Petri dishes were incubated at 37°C for 48-72 hours until the appearance of countable single colonies. The number of colonies was used to estimate the number of viable lactobacilli cells (cfu/cm^3).

8. Freeze-drying

Primary treatment involved dilution of the culture of the studied strain *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2, equilibration, dosing, immobilization of the cell suspension by retention in the corresponding polymer matrix that also acted as cryoprotective media.

The immobilization matrix used was hydrocolloid and contained 4% solution of high-ester apple pectin and 1.2% sodium alginate solution in a ratio of 1 : 1, respectively. A mechanical method for immobilizing the microbial cells was applied, i.e. cell binding to the carrier was accomplished by mixing the cell suspension with the carrier (the hydrocolloid matrix) in the following sequence of the process steps:

1. Obtaining of the cell suspension by culturing the strain of MRS-broth medium for 24 hours at 37 °C.

2. Mixing the obtained suspension with the polymer solution at a temperature of 40-45 °C and homogenization for 1 hour and 30 minutes in a reactor with a stirring rate of 500 rpm.

The prepared mixture was subjected to freeze-drying. After lyophilization, the freeze-dried preparation was ground in an "Erveka" granulator. The lyophilized concentrate was packed in three-layer aluminum foil packs hermetically sealed under vacuum.

9. Microbiological studies of the freeze-dried concentrate - BDS 1670-82 and Ordinance No 5 of MH - SG 39/84, BDS EN ISO 4833: 2004.

Indicators:

- lactic acid bacteria, CFU/g;
- total number of mesophilic aerobic and facultative anaerobic bacteria - CFU/g;
- *Escherichia coli* in 0.1 g of the product (ISO 16649-2: 2001);
- pathogenic microorganisms, including *Salmonella* sp. (BDS EN ISO 6579: 2003) in 25.0 g of the product;
- coagulase-positive staphylococci in 1.0 g of the product (BDS EN ISO 6888-1: 2005 + A1: 2005);
- sulphite-reducing clostridia in 0.1 g of the product (ISO 15213: 2003);
- microscopic mold spores, CFU/g (BDS ISO 6611: 2006);
- yeast, CFU / g (BDS ISO 6611: 2006);

10. Processing of results

All experiments were performed in triplicates. Data was processed using Microsoft Office Excel 2013 software, using statistical functions to determine the standard deviation and the maximum estimation error at a level of significance $\alpha < 0.05$.

III. Results and discussion

1. Antibiotic resistance of Lactobacillus delbrueckii ssp. bulgaricus Pr2

Knowledge of the antibiotic resistance of probiotic lactobacilli is essential and one of the conditions for the selection of potentially probiotic strains. It is an essential criterion in the selection of probiotic cultures due to the possibility of combined antibiotic and probiotic therapy in order to restore the normal microflora of the gastrointestinal tract and/or the urogenital tract [2]. In addition, normal bacteria, including lactobacilli, can serve as a source of antibiotic resistance genes, transferring them to various pathogenic microorganisms [5].

20 antibiotics with different mechanisms of action most commonly used in medical practice were selected and the sensitivity of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 was investigated. The results obtained from the disc-diffusion method are summarized in Table 1.

Lactobacillus delbrueckii ssp. *bulgaricus* Pr2 was resistant to penicillin, cefamandole, tetracycline and ciprofloxacin.

The presence of acquired antibiotic resistance factors is considered highly undesirable in Europe [3]. Precise analysis of antibiotic resistance spectrum data in the selection of probiotic strains due to the possibility of transferring genetic elements inducing resistance is needed [5]. Rules and criteria for the assessment of transferable resistance genes in starter cultures and probiotics for use in animals and humans have been developed.

Table 1. *Antibiotic sensitivity of Lactobacillus delbrueckii ssp. bulgaricus Pr2.*

#	Mechanism of action	Antibiotic		Concentration	Pr2
1	Inhibitor of cell wall synthesis	Penicillin	P	10 µg/disc	R
2		Azlocillin	Az	75 µg/disc	S
3		Piperacillin	P	100 µg/disc	S
4		Ampicillin	A	10 µg/disc	S
5		Oxacillin	O	1 µg/disc	S
6		Amoxicillin	Ax	25 µg/disc	S
7		Vancomycin	V	30 µg/disc	S
8		Cefamandole	Cm	30 µg/disc	R
9	Inhibitor of protein synthesis	Tetracycline	T	30 µg/disc	R
10		Doxycycline	D	30 µg/disc	S
11		Gentamicin	G	10 µg/disc	S
12		Kanamycin	K	30 µg/disc	S
13		Tobramycin	Tb	10 µg/disc	S
14		Amikacin	Am	30 µg/disc	S
15		Lincomycin	L	15 µg/disc	S
16		Chloramphenicol	C	30 µg/disc	S
17		Erythromycin	E	15 µg/disc	S
18	Inhibitor of DNA synthesis or cell division	Rifampin	R	5 µg/disc	S
19		Nalidixic acid	Nx	30 µg/disc	S
20		Ciprofloxacin	Cp	5 µg/disc	R

d <8 mm - R, at 8 <d <16 mm - SR, d> 16 mm - S.

Screening for potentially transmissible resistance to chloramphenicol, clindamycin, erythromycin, gentamicin, rifampin, tetracycline in lactobacilli is recommended [3]. *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 should be tested for potentially transmissible tetracycline resistance (Table 1).

2. In vitro determination of the ability of Lactobacillus delbrueckii ssp. bulgaricus Pr2 to survive in conditions simulating the different parts of the gastrointestinal tract

Probiotic strains should have the ability to survive in the conditions of the gastrointestinal tract in order to be used in the composition of probiotic preparations. Therefore, in a series of experiments, the resistance of the cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 in artificially created conditions of the gastrointestinal tract - pH = 2 + pepsin, pH = 4.5 + pancreatin and pH = 7 + pancreatin were examined (Fig. 1). In a parallel experiment the tolerance of the strain to different concentrations of bile salts (0%; 0.15%, 0.3%, 0.6%, and 1%) was examined as well (Fig. 2).

The cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 were most sensitive to pH=2 + pepsin. At pH = 2 + pepsin, the viable cell concentration started reducing from the very start of the process and at the 24th hour the concentration of active cells was 2x10^{1}cfu/cm^{3}. At pH = 4.5 + pancreatin at the 24th hour the number of active cells reached 3x10^{3}cfu/cm^{3}. At pH = 7 + pancreatin - at the 24th hour it was 6x10^{3}cfu/cm^{3}.

Under acidic conditions (pH = 2 + pepsin) the number of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 was reduced by 5 logN for 24 hours. At pH = 4.5 + pancreatin by the 24th hour the reduction in the viable cell count was 3 logN, and at pH = 7 + pancreatin the reduction was 2.8 logN (Fig. 1).

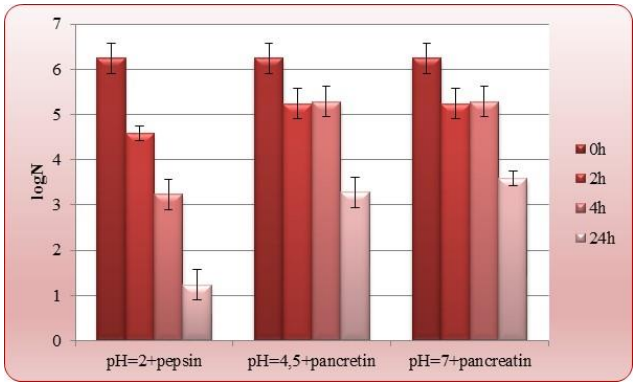

Figure 1. *Survival of Lactobacillus delbrueckii ssp. bulgaricus Pr2 under simulated conditions of the gastrointestinal tract - acidic pH (pH = 2) + pepsin, pH = 4.5 + pancreatin and pH = 7 + pancreatin.*

The cell is an open dynamic system and creates conditions for the entry of bile salts into the cytoplasm. The cytoplasmic membrane is made up of phospholipids containing fatty acids which, upon interaction with bile salts, form complex compounds (most of them with a crystalline lattice) which, under certain conditions, release the incorporated compound. This in turn disturbs the permeability of the cytoplasmic membrane and the transport of nutrients from the outside to the inside of the cell. Young growing cells are especially sensitive to the presence of bile salts. Cells in the stationary growth

phase survive this stress and form colonies on the surface of the agar medium. Lower concentrations of bile salts in the medium allowed surviving cells to grow, while higher concentrations (0.6%, 1%) retained their growth. The reduction in the concentration of viable *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 cells in the presence of different concentrations of bile salts began decreasing from the very beginning of the experiment, the degree of reduction being directly proportional to the concentration of bile salts in the medium (Fig. 2).

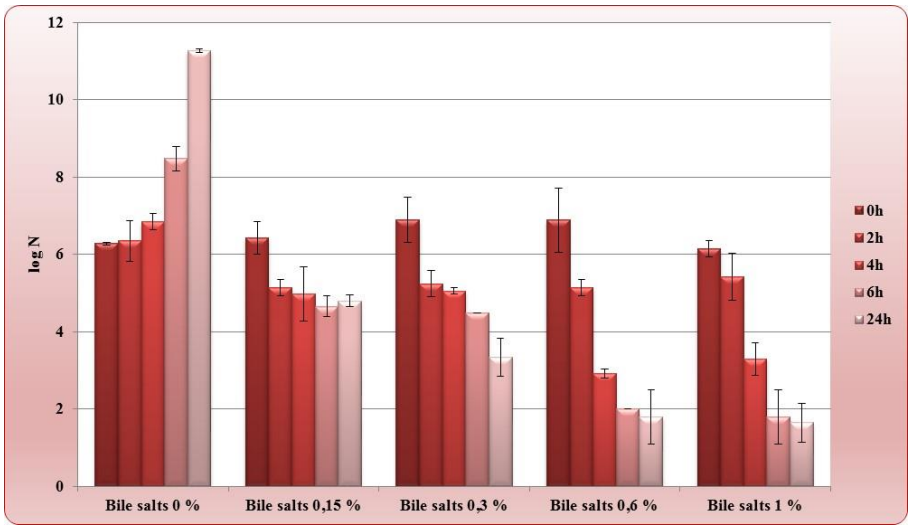

Figure 2. *Survival of Lactobacillus delbrueckii ssp. bulgaricus Pr.2 at various concentrations of bile salts in the medium.*

The reduction was 2 logN at 0.15% bile salts, 4 logN at 0.3% bile salts, 5 logN at 0.6% bile salts and 1% bile salts (Fig. 2). *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 survived at the different concentrations of bile salts while retaining significant amount of viable cells.

Lactobacillus delbrueckii ssp. *bulgaricus* Pr2 demonstrated the ability to survive in model conditions of the gastrointestinal tract, which is why it is considered to be a potentially probiotic culture. According to the *in vitro* assessment criteria for probiotic strains and the studies conducted, it can be concluded that the strain combines beneficial properties that identify it as promising strain for inclusion in the composition of probiotics and probiotic foods.

3. Antimicrobial activity of Lactobacillus delbrueckii ssp. bulgaricus Pr2

Several criteria are used in the selection of probiotic strains, one of the most important being their ability to enhance the host's natural protection against enteropathogens by producing antimicrobial substances or by competitively inhibiting and excluding these pathogens [6].

In this connection, the antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 by the well-diffusion method was studied (Table 2).

Table 2. *Antimicrobial activity of Lactobacillus delbrueckii ssp. bulgaricus Pr2 against pathogenic microorganisms. The values are in mm.* d_{well} *= 6mm.*

d_{zone}, [mm] / Strain (sample)		*Staphylococcus aureus* ATCC 25923	*Escherichia coli* ATCC 8739	*Listeria monocytogenes*	*Salmonella* sp.
	Culture liquid (CL)	11,00±1,00	10,00±0,00	14,50±0,50	8,00±0,00
	Biomass (B)	8,00±0,00	-	10,00±0,50	8,00±0,00
	Neutralized acellular supernatant (NASN)	-	-	9,00±0,00	-

Lactobacillus delbrueckii ssp. *bulgaricus* Pr2 demonstrated antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Listeria monocytogenes*, *Salmonella* sp. (clinical isolate), with the culture liquid having greater antimicrobial activity than the neutralized acellular supernatant.

The NASN did not inhibit the growth of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739 and *Salmonella* sp. (clinical isolate).

In the study of the antimicrobial activity of the studied strain against *Listeria monocytogenes*, the growth of the pathogen was suppressed by the culture liquid, the biomass and the neutralized acellular supernatant of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2.

The results obtained support the understanding of the mechanisms of the antagonistic action of probiotic strains. Probiotics are capable of influencing intestinal pathogens by producing antibacterial agents including lactic acid, acetic acid and antibiotic-like substances (bacteriocins) by competing for food components and/or attachment sites, by influencing the enzyme activity or by increasing the activity of macrophages and the levels of specific antibodies.

The conducted studies of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2, capable of inhibiting the growth of all the pathogens studied, is of interest for its therapeutic application as a probiotic culture. This determines the need for further complex characterization of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 as a probiotic strain.

4. Freeze-dried concentrate

Lactobacillus delbrueckii ssp. *bulgaricus* Pr2 was cultured under static conditions at 37°C for 24 hours. The obtained biomass was immobilized and freeze-dried in the presence of high-ester apple pectin and sodium alginate and the microbiological status after sublimation of the freeze-dried concentrate (Table 3) as well as the survival of the strain in the lyophilization process (Fig. 3) was determined.

Standard studies of the microbiological parameters of the lyophilized preparation demonstrated the absence of insemination with pathogenic microflora (Table 3).

The freeze-dried concentrate did not contain pathogenic microflora and met the standard microbial purity requirements. The absence of insemination with pathogenic microorganisms proved that the whole technological process was carried out in accordance with the sanitary norms and requirements.

Table 3. *Microbiological status of the freeze-dried preparation of Lactobacillus delbrueckii ssp. bulgaricus Pr2.*

Type of pathogenic microorganisms tested	Norm according to BDS	Analysis
1. Total number of mesophilic aerobic and facultative anaerobic bacteria	No more than 800	220 – 360
2.*Escherichia coli* in 0.l g of the product	Not to be found	Not found
3. Sulfite-reducing clostridia in 0.1 g of the product	Not to be found	Not found
4. *Salmonella* sp. in 25.0 g of the product	Not to be found	Not found
5. Coagulase-positive staphylococci in 1.0 g of the product	Not to be found	Not found
6. Microscopic mold spores, CFU/g	No more than 100	Not found
7. Yeast, CFU/g	No more than 100	Not found

The hydrocolloids used for the mechanical immobilization and as cryoprotectors during freeze-drying were highly effective and the survival rate of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 was high (Fig. 3).

Figure 3. *Survival of Lactobacillus delbrueckii ssp. bulgaricus Pr2 in the freeze-drying process.*

The results obtained show the effectiveness of the combined hydrocolloid matrix as an immobilization vehicle and cryoprotective medium in the freeze-drying process. The high survival rate of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 in the

lyophilized preparation was due to the optimal programming of the whole technological process - freezing conditions, suitable cryoprotection environment, freeze-drying regime conditions, properly defined cycle time, which ensured low residual moisture content of the final product. In addition, the freeze-drying technology itself prevented the growth of pathogenic microflora. The higher the degree of dehydration is, the less the survival of the pathogenic microflora is.

IV. Conclusions

Lactobacillus delbrueckii ssp. *bulgaricus* Pr2 demonstrated a number of probiotic roperties. *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 had high survival rate under model conditions of the gastrointestinal tract (pH = 2 + pepsin, pH = 4.5 + pancreatin, pH = 7 + pancreatin, high concentrations of bile salts), high antimicrobial activity against pathogenic microorganisms – *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella* sp. The freeze-dried concentrate of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 had high content of viable cells after lyophilization. After further examination of other probiotic properties of *Lactobacillus delbrueckii* ssp. *bulgaricus* Pr2 the strain can be included in the composition of probiotic preparations and starters for functional foods.

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Peculiarities of using numerical methods in design of technological equipment

PETROVA Yuliya [1], GOROKCHOVA Elena[2], AFENCHENKO Dmitrii[1]

[1]- Donetsk National University of Economics and Trade named after Mykhayilo Tugan-Baranovsky, Donetsk, e-mail: yunic@ukr.net
[2]- Specialised school of physics and mathematics №17, Donetsk, e-mail: dsfmh-17@mail.ru

Abstract. *The limits of use of design formulas and mathematical models of engineering calculation of structures based on durability, based on a resistance of materials and elasticity theory, which should be considered when using computer technology calculation and design elements and nodes constructions of various equipment, including equipment for the food industry are discussed*.

Key Words: numerical methods, modeling, stress-strain state, twist, bend.

I. Introduction

Currently, in the design of technological equipment in almost all branches has significantly increased interest in numerical methods, which allow directly to provide solutions of common equations for determining displacements, stresses, deformations and forces that arise in design or its constituent parts as a result of the use of mechanical forces, including taking into account the dynamic loads.

Universal software systems finite element analysis are quite popular with professionals in the field of automated engineering calculations and finite element solution of linear and nonlinear, stationary and non-stationary space problems in mechanics of deformable solid body mechanics and structures (including non-stationary geometrically and physically nonlinear problems of contact interaction of structural elements), tasks, fluid and gas mechanics, heat transfer, Electrodynamics, acoustics, as well as the mechanics of related fields.

Rival industrial companies are looking for ways to produce high quality products at minimal cost. Finite element analysis using software systems can help significantly reduce the costs of design and manufacturing, add confidence to the developer in the correctness of the decisions he made.

In some cases, test samples are undesirable or impossible. Computer software products are invaluable in situations like this, including such areas as the application of biomechanical prosthetics damaged joints in humans and the creation within the eye lenses. Other significant applications of computer finite element analysis programs relate to a wide range of applications: from heavy machinery products to integrated circuits.

At the stage of preprocessor preparation, the initial data necessary for the solution is specified. The user selects coordinate systems and types of finite elements, indicates elastic constants and physical and mechanical properties of the material, builds a solid model and finite element grid, performs the necessary actions with nodes and grid elements, specifies communication equations and constraints, and justifies the design scheme of the studied object.

In this scheme, some assumptions are made about the insignificant effect of individual structural elements, some kinematic constraints, some internal forces, the stress-strain state is justified, and the possibility of using known physical and mathematical laws is justified. At the same time, the accuracy of calculations inevitably decreases and the range of tasks that can be solved is reduced.

Taking into account the above and other assumptions, a mathematical model of the object under study is constructed. This model can represent the algebraic or differential dependencies of the interaction between individual elements of the design scheme, it may be a system of differential equations in ordinary or partial derivatives.

After solving the equations of the mathematical model, one can see and analyze the basic behaviors of the elements of the object under study; find the equation of motion and the relative location of individual nodes of the structure, velocities and accelerations, the forces of interaction between individual elements of the structure, internal force factors, stresses and displacements.

Simplification in the solution of these problems and assumptions in the deviation of the calculated force factors and deformations from actual values

depend on the accuracy of the design scheme and the mathematical model.

That is why, the use of computer calculations using programs with closed mathematical models and ready-made computational formulas can lead to errors that are invisible to the inexperienced calculator or an obvious absurd result of the calculation.

The reliability of the calculation when using the same program largely depends on the level of general engineering experience of the calculator, in particular his knowledge and skills in resisting materials, machine parts, etc.

However, in these disciplines, the boundaries of the application of calculation formulas are practically not discussed.

The purpose of the paper is to determine the limits of applicability of the material resistance formulas for the calculation of elastic systems subject to deformation of torsion and bending for their rational use in mathematical modeling and computer technologies for calculating elastic systems.

II. Materials and methods

The classical methods of the theory of the stress-strain state of the material resistance, the theory of elasticity, differential dependencies, the biharmonic equations of Sofia-Germain, the fourth-order characteristic equations were used in the work.

III. Results and discussion

Torsion of round rods. Torsion at the arrival of external forces in planes perpendicular to the geometric axis of the rod. A torsion-testing rod is called a shaft.

In the transverse (and longitudinal) resolution of the shaft, only tangential stresses arise [1].

$$\tau_\rho = \frac{M_x(x)}{J_p}\rho. \qquad (1)$$

where: $M_x(x)$ - the torque of the internal forces, is taken from the diagram of the torque moments of the external load;

$J_p = \frac{\pi d^4}{32}$ - polar moment of inertia of the cross-sectional area of a shaft of diameter d;

p – is the distance from the center of the cross section of the shaft to the considered site in the cross section.

In an arbitrary section of the shaft, normal and tangential stresses arise $\sigma_\alpha = \tau \sin 2\alpha$ and $\tau_\alpha = \tau \cos 2\alpha$, and at an angle of 45 degrees, the principal stresses $\sigma_1 = -\sigma_2 = \tau$,appear, Fig. 1.

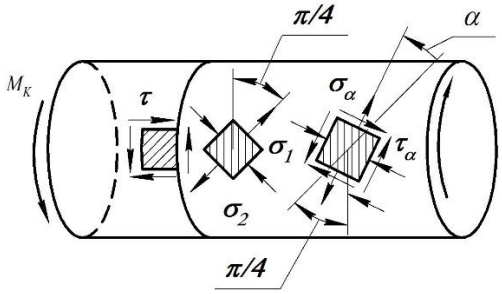

Figure 1. *Stresses in the torsion of the shaft*

Thus, the shaft is in a plane stressed state, and it should be checked for strength by the appropriate strength theory based on the principal stresses.

According to the III theory of strength, the design stress. $\sigma^{III}_{расч} = \sigma_1 - \sigma_2 = 2\tau \le [\sigma]$.It follows that the allowable shear stress is equal to half the permissible normal stress.

According to the IV theory, the strength condition has the form,

$$\sigma^{IV}_{расч} = \sqrt{\sigma_1^2 - \sigma_2^2 - \sigma_1 - \sigma_2} = \sqrt{3\tau} \le [\sigma],$$

whence the tangential stresses are 0.57 times less than normal stresses.

The deformations of the shaft, that is, the angles of twisting, are calculated according to Hooke's law

$M_{кр}(x) = GI_p \frac{d\varphi}{dx}$, whence we obtain

$$\varphi(x) = \int_l \frac{M_x(x)}{GJ_p} dx. \qquad (2)$$

Only with concentrated external loads, the torques of internal forces M_x=*const* and the angles of twisting can be calculated from formula

$$\varphi(x) = \frac{M_x}{GI_p} x. \qquad (3)$$

Limitations of the calculation formulas (1) - (3) in the textbooks are not given. However, the long shaft under the action of torque loses stability that is, curved along the helix. Such a state is limiting, and the critical moment is calculated by the formula [2]

$$M_{кр} = \frac{2\pi}{l} EI \ , \qquad (4)$$

where EI - is the bending stiffness.

If, in addition to the torque, a compressive force P is applied to the shaft, its critical value is equal to the strength of Euler

$$P_{кр} = \frac{\pi^2 EI}{(\mu l)^2}. \qquad (5)$$

However, at the same time the coefficient of reduction of the length of the round rod is calculated by the formula

$$\mu = \frac{2\pi EI}{\sqrt{(2\pi EI)^2 - (M_k l)^2}}. \qquad (6)$$

Therefore, the length of the shaft is limited by the value

$$l \le \frac{\pi}{\mu}\sqrt{\frac{EI}{P}}. \qquad (7)$$

From the side of the minimum value of shaft length, there is no restriction, since the shaft experiences a pure shear. Consequently, only in the case of pinching, the shaft length is limited by the Saint Venant condition, that is, its length must be greater than the diameter.

Bending. Bending arises from the action of forces and pairs of forces located in a plane passing through the main central axis of the rod.

When bending, the beam undergoes a plane stress state - normal stresses appear in the cross section, calculated according to the Navier formula

$$\sigma = -\frac{M_z}{J_z} y, \qquad (8)$$

where: M_z - the bending moment of internal normal forces with respect to the neutral z -axis, obtained as the sum of the moments of all external loads taken from one side of the cross-section considered (taken from the bending moment diagram);

J_z - the moment of inertia of the cross-sectional area of z the beam with respect to the neutral axis of the beam z, which passes through the center of gravity of the cross-section of the beam and is perpendicular to the main plane in which the load acts;

y - is the distance from the neutral axis to the layer where the voltage is calculated.

The tangential stress in the transverse (and longitudinal) sections of the beam is determined by the formula of Zhuravskii

$$\tau = \frac{Q(x)S_z(y)}{bJ_z}, \qquad (9)$$

where: - $Q(x)$ - is the transverse force;

$S(y)$ - is the static moment of the section plane;

b - beam width.

In the literature [3] it is proved that the Navier formula is applicable if the length of the beam exceeds the height four or five times, that is, short rods can not be calculated from beam formulas. In short rods, the material resistance hypothesis is not applicable, the cross sections are deplaned and the longitudinal normal stresses do not vary linearly, and vertical normal stresses can not be neglected. Such a short beam is called a beam-wall, and its calculation for strength and rigidity is based on the biharmonic equation of Sofia-Germain

$$\frac{\partial^4 \varphi}{\partial x^4} + 2\frac{\partial^4 \varphi}{\partial x^2 \partial y^2} + \frac{\partial^4 \varphi}{\partial y^4} = 0, \qquad (10)$$

using stress functions

$$\varphi = F(y)\cos\alpha x = F(x)\cos\frac{n\pi}{l}x \qquad (11)$$

subject to its subordination to the boundary conditions.

Such a function, when substituted in (10), leads to a differential equation with concerning $F(y)$

$$\alpha^4 F(y) - 2\alpha^2 F''(y) + F^{IY}(y) = 0. \qquad (12)$$

The solution of this equation in the form $F(y) = e^{ky}$, leads to a characteristic equation $k^4 - 2\alpha^2 k^2 + \alpha^4 = 0$, whose roots. $k = \pm\alpha$. Consequently, the solution of equation (11) takes the form

$F(y) = C_1 sh\alpha y + C_2 ch\alpha y + C_3 y sh\alpha y + C_4 y ch\alpha y.$

This is how the stress function is determined

$$\varphi = \left(C_1 sh\alpha y + C_2 ch\alpha y + C_3 y sh\alpha y + C_4 y ch\alpha y\right)\cos\alpha x$$

By this function, from differential dependencies

$$\sigma_x = -\mu q + \frac{\partial^2 y}{\partial y^2}, \sigma_y = -q + \frac{\partial^2 \varphi}{\partial x^2}, \tau_{xy} = -\frac{\partial^2 \varphi}{\partial x \partial y},$$

there are calculated normal and tangential stresses:

$$\sigma_x = \sum_{n=1}^{\infty} [\alpha\left(C_1 sh\alpha y + C_2 ch\alpha y + C_3 y sh\alpha y + C_4 y ch\alpha y\right) + 2\left(C_3 sh\alpha y + C_4 ch\alpha y\right)]\alpha \cos\alpha x, \quad (13)$$

$$\sigma_y = \sum_{n=1}^{\infty} \left(C_1 sh\alpha y + C_2 ch\alpha y + C_3 y sh\alpha y + C_4 y ch\alpha y\right)\alpha^2 \cos\alpha x \quad (14)$$

$$\tau = \sum_{n=1}^{\infty} [\alpha\left(C_1 ch\alpha y + C_2 sh\alpha y + C_3 y ch\alpha y + C_4 y sh\alpha y\right) + C_3 sh\alpha y + C_4 ch\alpha y]\alpha \sin\alpha x. \quad (15)$$

For a multi-span beam-wall with a height h equal to the span l from a uniformly distributed load q, stress diagrams are obtained at the upper edge of a beam-wall of unit width, the distribution of which is not comparable with the beam ones, Fig. 2.

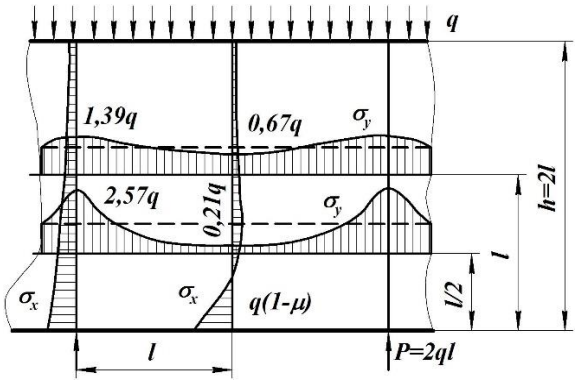

Figure 2. *Stress distribution in a multi-span wall-beam*

Regardless of the ratio *l/h*, the greatest normal stress occurs in the longitudinal direction at the lower edge of the beam-wall in the middle of the span and in this case is equal to

$$\sigma_x = -q\left(2\sum_{n=1}^{\infty}\cos\alpha x + \mu\right) = q(1-\mu) = 0{,}75q,$$

($\mu = 0{,}25$).

According to the beam formula (8) for h = l, in the middle of the span of a rigidly clamped beam tension $\sigma = \frac{M}{W} = \frac{ql^2}{24}\frac{6}{h^2} = 0{,}25q$, which is three times less than in the beam-wall.

This calculation allows us to determine the lower limit of the use of beam formulas. For the upper boundary, one should consider the calculation of flexible threads.

Flexible threads. Flexible threads are considered horizontal rods of great length, which experience only tensile stresses. However, small sections of flexible threads in the place of its fixation on the support and in the places of application of concentrated forces undergo bending.

In the tutorial [2], the calculation of flexible threads with allowance for bending is given. On the basis of the calculated formulas for longitudinal-transverse bending of a rod with a uniformly distributed load q and a tensile force N, the calculated bending moment formula

$$M(x)=\frac{q}{k^2}\left(1-\mathrm{ch}kx-\frac{1-\mathrm{ch}kl}{\mathrm{shkl}}\mathrm{sh}kx\right),\quad k=\sqrt{\frac{N}{EJ}}\,. \tag{16}$$

From this, it is clear that in short rods, that is, for small values of the argument of hyperbolic functions, can be considered $\mathrm{ch}kx=1+k^2x^2/2$, $\mathrm{sh}kx=kx$. Then from (16) we obtain the bending moment formula of a two-bearing beam

$$M(x)=\frac{ql}{2}x-\frac{qx^2}{2}.$$

For large rod lengths, when the argument $kx>4$ и $\mathrm{sh}kx\approx\mathrm{ch}kx$, from formula (16) we obtain the value of the bending moment

$$M(x)=\frac{q}{k^2}\left(1-\mathrm{ch}kx+\mathrm{sh}kx\right)=\frac{q}{k^2}\left(1-\mathrm{e}^{-kx}\right)\approx\frac{qEJ}{N}\,. \tag{17}$$

In flexible filaments, the bending stress can be neglected in comparison with the tensile stresses of the longitudinal force N. However, if the normal stress of the beam bending moment is greater than the stress of the longitudinal-transverse bend (17), then beam formulas should not be used. From these considerations, we obtain a criterion for the applicability of beam formulas

$$\frac{ql^2}{8}\geq\frac{qEJ}{N}, \tag{18}$$

but taking into account the longitudinal force $N=\frac{8}{3}f^2\frac{EJ}{l^2}$ and arrows sagging threads $f=\frac{5}{384}\frac{ql^4}{EJ}$ at a load satisfying the strength condition $q=8W\sigma/l^2$ we find that for

$$\frac{l}{h}\geq 2{,}88\sqrt{\frac{Ei}{\sigma h}},\quad i=\sqrt{\frac{J}{F}}, \tag{19}$$

the stretched rod should be considered a flexible thread.

IV. Conclusions

The calculation formulas for the resistance of materials in torsion are used when the shaft length is less than the critical value (7).

In the case of bending, the calculation formulas for the resistance of materials can be used if the length of the beam is more than four times the height and is less than the critical lengths and less than the critical length (19). It should be noted that these recommendations should be taken into account in any engineering calculations, including when using modern computer complexes for structural calculations for strength, rigidity and stability.

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Designing and creation of display and packaging for souvenir

D. A. Todorova, I. Z. Markova

University of Chemical Technology and Metallurgy, 8 Kl. Ohridski, 1756 Sofia, Bulgaria
e-mail: todorova.dimitrina@uctn.edu

Abstract. *This project aims to study the basic processes of design and manufacture of corrugated display and consumer packaging from folding cardboard, to the stage of their approved real sample. The focus group, for which the project has been advanced to, are young children. The structural and graphical design should be interesting and attractive to the attention of children and should enhance their ability to recognize and remember the types of the animals.*
For the structural design of the display and the package is being used, the well-known in the packaging industry software - Eng View Packaging and Display Designer. It is a CAD/CAM software, which enabled creation of every stage of the design and production process, starting from conceptual design to its three-dimensional representation, to the final arrangement of graphic design and management of machines for cutting the finished product.
The process from the idea to inception of the packaging is a workflow that should be conformable to the materials, technology, market and consumer needs and even more. The contemporary design of the packaging and their appropriate commercial realization involves the usage of a large number of software products that repeatedly shortened steps in the design but requires a specific knowledge of the exact materials of which the packaging should be produced and specific technologies that enable the successful implementation of the original idea.

Key words: structural design, display design, packaging design, packaging

I. Introduction

The total global production of packaging amounts to around 680-700 billion dollars, with the trend to reach 800 billion dollars [1,2]. According to the statistics for the 2014 the approximate percentage of the used types of packaging materials are as shown on Figure 1 [2,3].

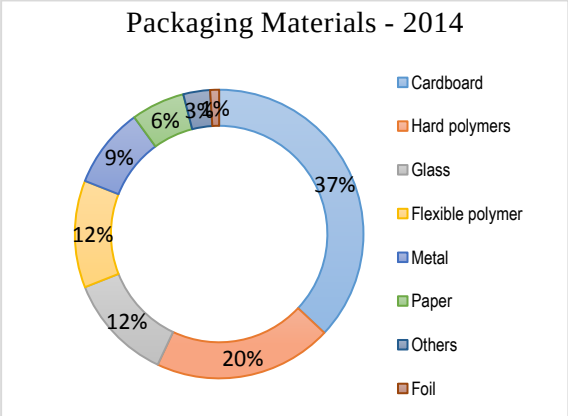

Fig. 1. *Packaging materials for 2014*

The packaging originated in antiquity and the original purpose was for protection and storage to transported items. Nowadays, the packaging performs more and more diverse functions. It has become a powerful tool of marketing and advertising and helps rapid and successful implementation of products on the market. Quality package however, should not only be attractive and modern, but also meet a series of requirements [4]. Packaging production requires the use of materials with specific properties. The correct choice of material for the production of paper and cardboard packaging is one of the main stages of development and design of packaging for a particular product.

Good planning includes many intermediate stages and functional tasks to be performed by the specialists from the operation departments in enterprises. It requires excellent estimates of profitability, unique design of the package, optimal pre-press, printing and finishing processes and also logistic processes before the product is released for production and subsequent distribution commercially [5].

The following Figure 2 shows the main stages to the typical design process.

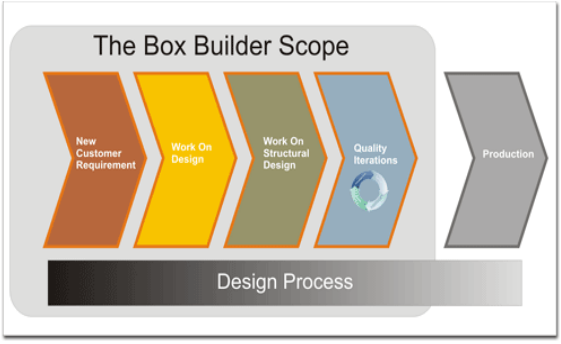

Fig. 2. *Main stages to the design process*

The different stages of the design process are [5]:

• New requirements or client needs: The client informs the designer of its packaging needs.

• The idea and the box type are considered: The best type of box to meet the client´s needs is considered.

• The structural aspects of the proposed box are worked on: Once the type of box to be designed is clear, the development of the box using a CAD tool begins.

• Modifications are made: Following the development of the first outline of the box, a prototype is made and the client´s comments and requested changes are used in order to redesign the box.

• Production stage begins: Once the client has accepted the design, the production stage begins.

Figure 3 shows the four most common phases in the process of transformation the model of the packaging to real designe.

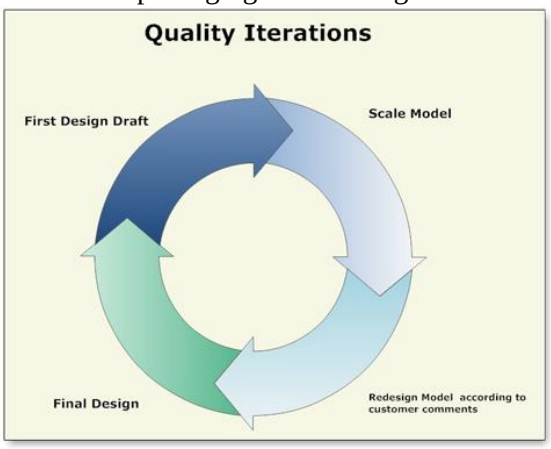

Fig. 3. *Phases in the modeling of the box design*

- Draft design: This is the first design drawn up in accordance with the client´s specifications.
- Prototype: Following the draft design, a prototype is made which is sent to the client.
- Redesign: Once the client has received the prototype, it can comment on it or request modifications. The model is then redesigned and sent to the client as before.
- Final design: Following modification, the client accepts the box design.

This project aims to study the basic processes of design and manufacture of display from corrugated cardboard and consumer packaging from folding cardboard for zoo souvenirs till the stage of their approved real sample.

The focus group, for which the project was advanced to, were young children and especially toddlers visiting Zoo. Thus the structural and graphic design should be attractive and very interesting to the attention of children and should enhance their ability to recognize and remember the types of the animals.

II. Materials and methods

For the structural design of the display from corrugated cardboard and the package from flexible cardboard was used, the well-known in the packaging industry software - Eng View Packaging & Display Designer Suite [6]. It is a CAD/CAM software, which enabled creation of every stage of the design and production process, starting from conceptual design to its three-dimensional representation, to the final arrangement of graphic design and management of machines for cutting the finished product.

The technological plan for the construction of the actual sample of the display and the consumer packaging of souvenir includes the following steps:

a) Creating the structural design of the display and consumer packaging.
b) Creating the graphical design of the display and the packaging
c) Print
d) Masking
e) Cutting the samples on a plotter.
f) Preliminary estimation for the total value of the actual samples

The materials that were chosen are:

Corrugated cardboard for the display supplied by Dunapack Rodina AD, Bulgaria is five-layer EC flute Board grade: R36 EC Weight, g/m^2-624, Thickness, mm - 5.4, Edge crush test ECT kN/m - 6,6, Bursting strength, kPa - 1125.

Folding box board for the consumer packaging is Gloss top side double coated with white back, GC1 Zenith – 250g/m^2 with Thickness, µm - 350, Brightness, % - 91,5, Smoothness, µm – 1,4 and Gloss 75º, % - 50.

The cardboard for the sleeve of the consumer packaging is Matt top side double coated with white back, GC1 Zenith – 200g/m^2 with Thickness, µm - 280, Brightness, % - 91,5, Smoothness and µm – 1,4

III. Results and discussions

3.1. Creating the structural design of the display and consumer packaging.

This phase involves a detailed consideration of the alternative solutions defined in the planning phase and results in the determination

of the most suitable proportions, dimensions and details of the structural elements and connections for constructing each alternative structural arrangement being considered.

Any creation of structural design of packaging begins with the decision for the product material, in our case - the corrugated board for display and folding box board for consumer packaging. Undoubtedly, this process often is bidirectional and may need to be adjusted after the creation of graphical design and it should be tailored to the final consumer conditions and the original idea.

The EngView Library has more than 1 500 parametric packaging designs for folding carton and corrugated board, as well as complex resizable structures for rigid board. It consists of ECMA and FEFCO standards as well as of many other designs developed as part of the software.

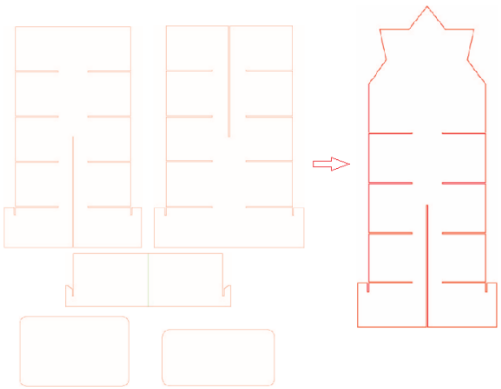

Fig. 4. *2D structural design of the corrugated display*

Figure 4 and Figure 5 presents the main elements of the corrugated display and their 2D and 3D structural design, before and after the adjustment of the structure corresponding to the specific graphic design corresponding to our purpose.

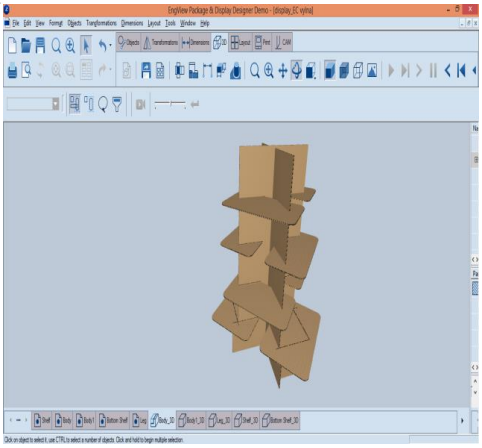

Fig. 5. *3D structural design of the corrugated display*

The structural design of the consumer packaging from the folding box board also have to be comply with several important aspects concerning the end product such as what is placed inside; what is its weight; what is its volume, where it will be placed into the package and also questions concerning the logistic of the package.

The structural design for both types of graphic design (wildlife and marine animals) for the consumer package is the same and is shown on Figure 6. It is from the Eng View Library under the code: EVF 11001 Reverse Regular Tuck Lock - width: 40mm, depth: 60mm and Height: 40mm.

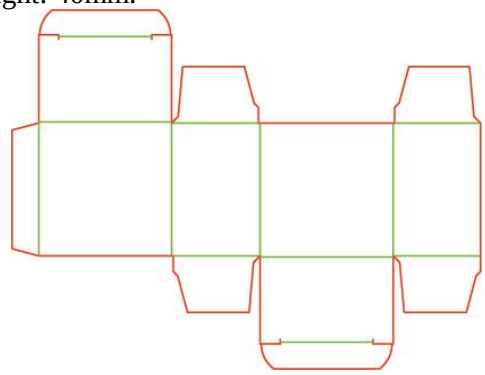

Fig. 6. *Structural design of consumer package*

The consumer package was construct with a "sleeve" in which the package was filled in. Its purpose was to give the package more attractive appearance as through non-standard construction will display and for which animal is intended to be. In Figure 7 is presented the structural design of the three types of foldable cardboard sleeve.

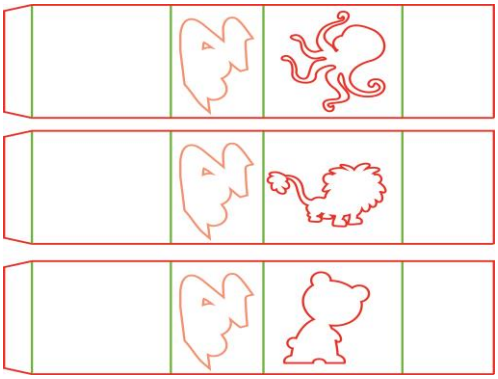

Fig. 7. *Structural design of the three types of foldable cardboard sleeve.*

3.2. Creating the graphical design of the display and the packaging

Largely the graphic design of the package determines the effectiveness of advertising of the product. The packaging should correspond the wishes of the consumers, thus the product

packaging becomes a means of communication between company, manufacturer and client.

This stage of the manufacturing process requires the designer to comply with many features:

Influence of the used colors for the graphic design of the product. The color is a fundamental property that causes certain visual sensations in accordance with the spectral composition and the intensity of the reflected radiation. It is a decisive factor in the aesthetic design of the packaging, conveys information about the status and quality of packaged goods. The colors contribute to raising awareness and providing psychological impact as well as the idea of the commodity. The importance of color is leading, as it is noticeable in the first environment. They are several main groups of colors:

Basic colors: red, yellow, blue;
Secondary colors: green, purple, orange, brown, pink;
Neutral colors: black, white, gray.

Different colors of objects around us affects differently the psychological state of the people. Colors should be in harmony with the advertising product, to refer to its nature and to balance the typical corporate style colors. Associations that are made in some basic colors are:

Yellow - light, heat, sun, summer and others.
Light blue - water, air, sky, wisdom, cold and others.
Green - nature, plants, ecology, peace and others.
Red - fire, ban, threat
White - purity, light, brightness and others.
Black - darkness, grief, sadness and others.

Shape - it can cause different reactions in the consumer and have a key influence in deciding when buying. The shape can be strictly geometrical, modified or free flowing but it bears information about the sizes of packaged goods.

The choice of font of text - should be legible font color should contrast with the background to make the information clear and understandable for the user.

The design of the package - is fully in compliance with what was intended to be presented. For the purposes of this work was used graphic images of animals that were easily recognizable for the focus group (toddlers) to which the products were intended. The 2D graphical design of the elements consist of the corrugated display are shown in Figure 8.

Fig. 8. *2D graphical design of the corrugated display*

The graphic design was consistent with the structural design of the package. It is divided, so that when you put the two main parts, each side of the display to be visible for each animal type (wildlife and marine animals).

3.3. Creating the graphical design of the consumer packaging

The graphic design of the packaging of the folding box board was also divided into two categories (wildlife and marine animals) and the 2D and 3D design is shown on Figure 9 and Figure 10. The logo was also presented in the graphical design and was placed at the same place where it should be placed in the sleeve. The used colors were chosen so that after placing the sleeve of the package, toddlers could be able to see only the contours lines of the shape. This effect could improve the ability of recognizing the design presented in the display.

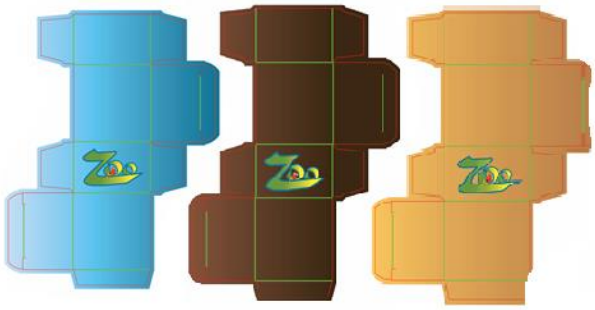

Fig. 9. *2D graphical design of the consumer package*

The main design trends are characterized by a clean, colorful look, white space, and subtle gradients. The design of fonts is more easy and dramatic. Whimsical, hand drawn illustrations aren't just for kids anymore. Retro styles are

inspired by the 80s and 90s, with bold colors, pixel art, playful geometric designs and patterns.

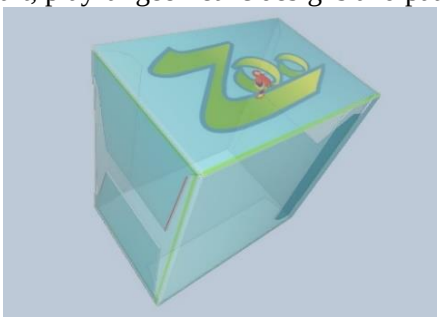

Fig. 10. *3D graphical design of the consumer package*

Photos and illustrations come to life with 2D animation and cinema graphs. Another trend is the deconstruction and distorting layouts in a seemingly random way. The films and TV influence also the graphic design [7,8].

According to this trend the final product, which is shown on Figure 11 could be of interest for the City Zoo.

Fig. 11. *The final product*

IV. Conclusion

The process from the idea to inception of the packaging is a workflow that should be conformable to the materials, technology, market and consumer needs and even more. The contemporary design of the packaging and their appropriate commercial realization involves the usage of a large number of software products that repeatedly shortened steps in the design but requires a specific knowledge of the exact materials of which the packaging should be produced and specific technologies that enable the successful implementation of the original idea.

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ISSN 1314-7773

Journal of **FOOD** and **PACKAGING** Science, Technique and Technologies

National Academy of Packaging-Bulgaria