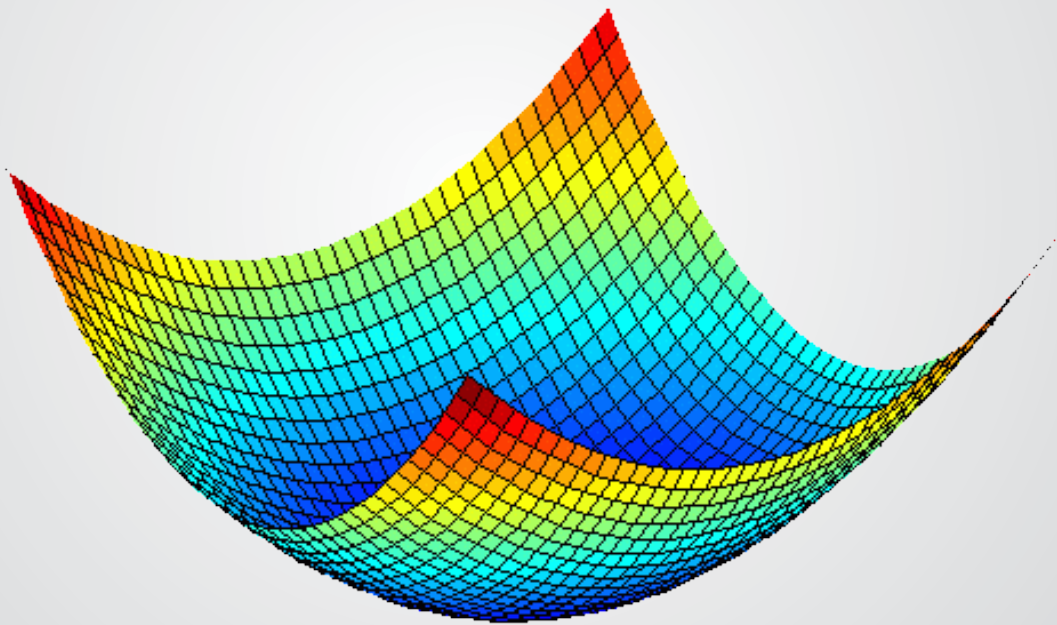




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## CONTENTS

1. SELECTION OF NITROGEN SOURCES IN ORDER TO INCREASE COLLAGENOLYTIC ACTIVITY OF BASIDIOMYCETE COPRINUS LAGOPIDES SUBMERGED CULTURE 5  
*Fedoryuk E.D., Shamtsyan M.M.*
2. COMPARATIVE QUALITATIVE ANALYSIS OF THE USE OF PAPER AND COMPUTER 9  
*V. Lasheva, A. Blazheva, H. Lasheva*
3. A STUDY ON THE STABILITY OF THE FOOD-PACKAGE SYSTEM 12  
*A. Buculei, A.-Mihaela Pşibilschi*
4. STUDYING THE POSSIBILITIES OF USING OF ESSENTIAL OILS IN DAIRY PRODUCTS. 3. BASIL (*Ocimum basilicum*) 17  
*I. Kostova, D. Dimitrov, M. Ivanova, R. Vlaseva, S. Damyanova, N. Ivanova, A. Stoyanova*
5. THERMODYNAMIC PARAMETERS DURING THE EXTRACTION OF ESSENTIAL OIL BEARING AND PHARMACEUTICAL PLANTS. 8. VIRGINIA FLUE-CURED TOBACCO – CONCENTRATED AROMATIC PRODUCTS 22  
*V. Popova, S. Tasheva, S. Damyanova, A. Stoyanova*
6. COMPUTER TECHNOLOGIES FOR ANALYSIS AND SYNTHESIS OF ELECTROMECHANICAL SYSTEMS IN FOOD INDUSTRY 27  
*L. F. Kostadinova-Georgieva*
7. STAND FOR TESTING THE INFLUENCE OF VARIOUS FACTORS ON TABLETING COFFEE 33  
*I. N. Shopov*
8. NON-TRADITIONAL YOGURT FROM COW'S MILK ENRICHED WITH PEA PROTEIN 38  
*I. Dobrev, R. Denkova, Z. Denkova, E. Filipov, V. Yanakieva*
9. SCREENING OF BACTERIAL LIPASE PRODUCERS 45  
*M.A. Pushkarev, T.B. Lisitskaya, V.P. Morozkina, V.A. Galynkin, A.V. Garabadzhiu, G.V. Kozlov*

10. STUDIES OF PHYSICAL AND TECHNICAL CHARACTERISTICS OF BIOWASTE AS COMPONENTS OF GASIFICATION	48
<i>A.A. Osmak, A.A. Seregin</i>	
11. EFFECT OF TEMPERATURE ON THE PROCESS OF PRESSING ORGANIC RAW MATERIALS	52
<i>D.V. Rindyuk, N.V. Ryabokon</i>	
12. MEASURES TO PREVENT EMERGENCY SITUATION	55
<i>N. V. Ryabokon, O. O. Seregin, O. P. Slobodjan, D. V. Rindyuk, O. O. Osmak</i>	
13. ROLE OF NUTRITION AND THE USE OF SPECIAL EXERCISE IN DIABETES	59
<i>G. Scherbakova</i>	
14. MODELLING OF PROCESS IN TWIN-SCREW DOUGH-MIXING MACHINES	64
<i>O. Kudinova, O. Kravchenko, I. Lytovchenko, Y. Telychkun, O. Gubenia, V. Telychkun, I. Dovgun</i>	
15. A COMPUTER PROGRAM FOR CALCULATING THE PLASTIC VISCOSITY OF CHOCOLATE MASS USING THE CASSON METHOD	69
<i>B. Milenkov, M. Hadzhikinova, D. Hadzhikinov, D. Hrusavov</i>	
16. INVESTIGATION OF LIPASE FROM ASPERGILLUS CARBONARIUS UPON THE QUALITY OF BREAD	73
<i>R. Chochkov, I. Sopotenska, V. Chonova, G. Karadzhov</i>	
17. STUDIES REGARDING THE INFLUENCE OF THE LAYER TYPE UPON THE BREAD QUALITY WITH DIFFERENT QUANTITIES OF FOOD ADDITIVES	77
<i>Buculei A., Poroch-Serițan M., Ailenei M., Constantinescu G.</i>	
18. INFLUENCE OF GUM ARABIC AND STARCH AS HYDROCOLLOIDS ON THE QUALITY OF EMULSION TYPE OIL-WATER IN FOOD	82
<i>O. Lugovska, V. Sidor</i>	

## SELECTION OF NITROGEN SOURCES IN ORDER TO INCREASE COLLAGENOLYTIC ACTIVITY OF BASIDIOMYCETE *COPRINUS* *LAGOPIDES* SUBMERGED CULTURE

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**Abstract.** The present paper describes the process of selecting of nitrogen sources and ratios of carbon (C) and nitrogen (N) sources in the composition of the nutrient medium in order to increase the collagenolytic activity of the submerged culture of basidiomycete *Coprinus lagopides*. The amounts of accumulated protein and biomass and pH of the culture liquid of the fungus as well as collagenase activity were determined. Submerged cultivation was carried out in nutrient medium with different proportions of C and N sources for 7 days. As N sources peptone, urea, ammonium nitrate and ammonium citrate were used, wherein the C source was glucose. The highest collagenolytic activity was detected on the 6th day of cultivation on glucose-peptone medium with a ratio of C and N source, equal to 5:1. High collagenase activity of producer was also observed on the 7th day of cultivation in the medium with urea at a ratio of C and N source, equal to 10:1. Thus, there is the opportunity to replace the conventionally used expensive source of N in the nutrient medium (peptone) by more cheap (urea), which significantly reduces the cost of the final product - a fungal collagenase.

**Keywords:** deep cultivation, basidiomycete *Coprinus lagopides*, collagenolytic activity, collagenase

### I. Introduction

One of the main problems of our times is to develop a waste-free technology by maximizing the involvement of protein secondary resources and wastes of the meat-processing industry. Ensuring sustainable lift in this direction can be achieved through the development of highly efficient enzyme preparations.

Proteolytic enzymes today amount about half of the production of enzyme preparations in the world market. Their performance is far superior to synthetic catalysts. They are highly specific with respect to their substrates and accelerate strictly defined chemical reaction without of formation of byproducts.

Practical use of enzyme preparations for the treatment of meat industry wastes show, that not all enzymes, which have high proteolytic activity, gave the required effect. This is due to the presence of proteins in such wastes, which are difficult to cleave by digestive enzymes. One of the main such hardly digestive proteins is collagen. One of the main ways to solve this problem is the use of collagenase - unique enzymes that hydrolyze specific peptide bonds in collagen, that are resistant to hydrolysis by other enzymes.

Collagenases, which are used nowadays, have some significant drawbacks. The most well-known producer of collagenase - the bacterium *Clostridium*

*hystoliticum* is the causative agent of gas gangrene, resulting in increased requirements of safety at all stages of production and sales [1]. When collagenase is obtained from king crab its hepatopancreas is used (organ, combining the functions of the liver and pancreas) [2]. Crab collagenase is safe for humans, but has limitations for industrial production, have seasonal character and a significant variability in the degree of purity and activity of the enzyme.

Due to these reasons, the most important issue seems to be finding of collagenase producers, which would be free from the above disadvantages. To our opinion higher fungi - basidiomycetes could be promising producers of collagenases. A complex of activities aimed at the discovery of new enzymes synthesized by higher fungi presents the great interest, collagenolytic enzyme is not the exception. The use of higher fungi as producers of collagenase gives the opportunity to culture the producer under controlled conditions and environments, which leads to a relative standardization of the product and the possibility of enzyme production on an industrial scale.

At the department of technology of microbiological synthesis of St. Petersburg State Institute of Technology (Technical University) (SPTI) basidiomycetes for high levels of collagenolytic activity were screened. This study shows that the highest collagenase activity had

*Coprinus lagopides*. Thus, this fungi is not well studied yet, the objectives of this study were:

1) to carry out submerged cultivations of the fungus *Coprinus lagopides* on media with different nitrogen sources;

2) to study of the dynamics of changes in pH, the amount of accumulated biomass and protein concentrations in the deep culture of the producer;

3) to study of the effect of different nitrogen sources and the ratio of carbon and nitrogen sources in the culture media on the enzymatic activity of the *Coprinus lagopides* submerged culture.

## II. Materials and methods

### 2.1. Strain of basidiomycete *Coprinus lagopides*

The strain of the basidiomycete *Coprinus lagopides* was obtained from the museum of basidiomycetes of the department of Technology of Microbiological Syntheses of SPTI. Maintenance of museum culture was carried out by periodic subcultures (every two months) on a solid nutrient medium - Saburo agar medium.

Composition of the medium Saburo, g/l:  
glucose – 40, peptone – 10, agar – 20-25.

### 2.2. Chemicals and reagents used

Anhydrous glucose, dry fermented peptone for bacteriological purposes, urea, ammonium nitrate and ammonium citrate, sodium chloride, potassium dihydrogen phosphate, potassium hydrogen phosphate, magnesium sulfate, calcium chloride, iron sulfate heptahydrate, zinc sulfate heptahydrate and fodder yeast extract were used for the preparation of nutrient medias.

Hydroxide, sodium citrate, sodium carbonate, copper sulfate pentahydrate, bovine serum albumin and Folin - Chokalteu reagent used in determining the protein in the culture liquid.

Disodium hydrogen phosphate dihydrate, sodium chloride, calcium chloride hexahydrate, isopropanol (2-Propanol), ninhydrin, L - leucine and collagen were used to determine collagenolytic activity.

### 2.3 Cultivation

Cultivation of the producer was performed in three stages:

1) growing the producer culture in test tubes on sloped agar medium;

2) obtaining of the inoculate in flasks with beads;

3) submerged culturing the fungus in media containing various sources of nitrogen (peptone, urea, sodium citrate and ammonium nitrate) and the ratios of the carbon (glucose) and nitrogen sources in the culture media (Table 1), the cultivation was conducted for 7 days.

Culture broth was sampled on the 3rd, 4th, 5th, 6th and 7th day. Determine pH and the amount of accumulated biomass. The resulting (native) solution was used for further study and determination of protein concentration and the collagenolytic activity.

**Table 1.** Composition of semi-synthetic nutrient media (in 1 liter of distilled water)

The components of the nutrient medium, g	Ratio of carbon and nitrogen source				
	1,2:1	1,5:1	3:1	5:1	10:1
Carbon source	10	10	10	10	10
Nitrogen source	8,33	6,67	3,33	2	1
NaCl	0,5	0,5	0,5	0,5	0,5
KH <sub>2</sub> PO <sub>4</sub>	0,6	0,6	0,6	0,6	0,6
K <sub>2</sub> HPO <sub>4</sub>	0,4	0,4	0,4	0,4	0,4
MgSO <sub>4</sub>	0,5	0,5	0,5	0,5	0,5
CaCl <sub>2</sub>	0,05	0,05	0,05	0,05	0,05
FeSO <sub>4</sub> ×7H <sub>2</sub> O	0,005	0,005	0,005	0,005	0,005
ZnSO <sub>4</sub> ×7H <sub>2</sub> O	0,001	0,001	0,001	0,001	0,001
Yeast extract	2	2	2	2	2

### 2.4. Determination of the amount of accumulated biomass

Amount of accumulated biomass was determined after its filtration and drying at 50°C. Native solution was subsequently used for the determination of pH, protein concentration and collagenolytic activity of macromycetes deep culture.

### 2.5. Determination of the protein concentration in the native solution

To determine the amount of protein formed during submerged cultivation required for the subsequent calculation of the specific activity of the enzymes, the method of Lowry was used [3].

### 2.6. Determination of the collagenolytic activity of native liquid

Collagenase activity was determined by ninhydrin method [4]. The method of the activity measuring is based on the ability of the enzyme to break down the collagen with the release and transfer to a solution the hydrolysis products. Concentration of released products is determined spectrophotometrically. The amount of enzyme (in micrograms), which being exposed to collagen hydrolysis products equivalent to 1 µg of L-leucine, under standard test conditions was adopted as a unit of collagenolytic activity (UCA). Collagenolytic activity in 1 ml of substance A<sub>κ</sub> was calculated by the formula (1):

$$A_{\kappa} = a \times V \times 2,5, \quad (1)$$

where  $a$  is the difference in concentration of the test  $C_i$  and control  $C_o$  solution ( $\mu\text{g} / \text{ml}$ ) and  $V$  is the volume of the test solution (ml).

Specific collagenase activity was calculated as the ratio of total collagenolytic activity to the protein concentration in the producer fluid culture.

### 2.7. Statistical processing of the experimental results

Statistical analyses of the results were carried out by determining the confidence intervals of the values obtained using the Student's coefficient in the Microsoft Office Excel 2007. Deviations from the mean values considered statistically significant if the confidence level was greater than or equal to 95%. Number of replicates in all dimensions is varied (from 4 or more). The experimental results were expressed as mean value  $\pm$  standard deviation.

## III. Results and discussion

According to the data obtained it can be concluded that the highest amount of culture biomass was produced on the 5th day of culturing, when urea was used as a nitrogen source and the least - when fungus was cultured on ammonium sources of nitrogen. Glucose-peptone medium occupies an intermediate position by the number of accumulated fungus biomass.

Culturing on the medium with urea was accomplished with strong alkalization while the use of ammonium nitrate as a nitrogen source leads to strong acidification of the medium. Submerge culture pH in the medium containing glucose: ammonium citrate was slightly acidic. Fungic cultivation on glucose-peptone culture medium pH accompanied by a transition from a weakly acidic to weakly alkaline.

The highest concentration of protein in the fungus culture broth is achieved on 4-5th days of cultivation on a glucose-peptone medium with a ratio of carbon and nitrogen sources, equal to 1,2:1, and the least - by culturing on ammonium nitrogen source. Medium with urea occupies an intermediate position by the number of accumulated protein.

The data obtained (Figures 1-4) suggest that the highest specific collagenolytic activity *Coprinus lagopides* exerts on the 6th day of cultivation on the medium where the nitrogen source is peptone and a ratio of carbon and nitrogen source, equal to 5:1. High specific collagenase activity was also observed on the 7th day of cultivation on the medium with urea at a ratio of carbon and nitrogen source, equal to 10: 1.

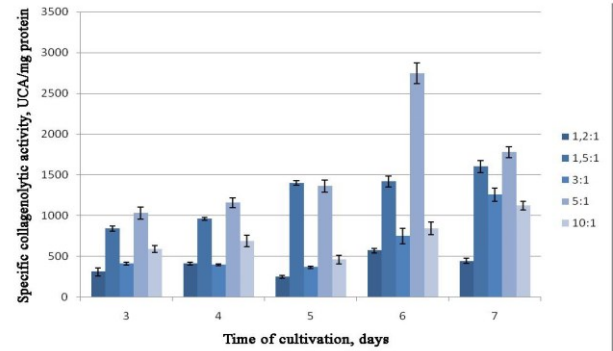


Figure 1. Specific collagenolytic activity level in case of growth on glucose-peptone culture medium

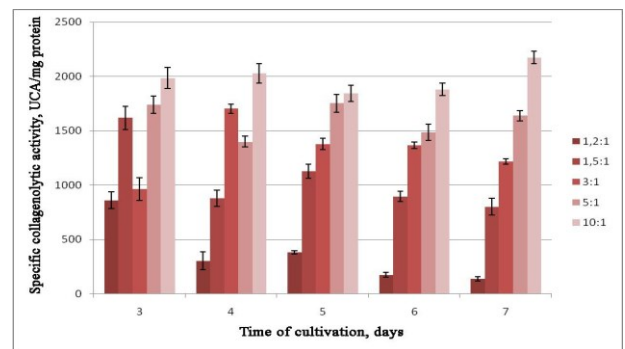


Figure 2. Specific collagenolytic activity level in case of growth on the medium with urea

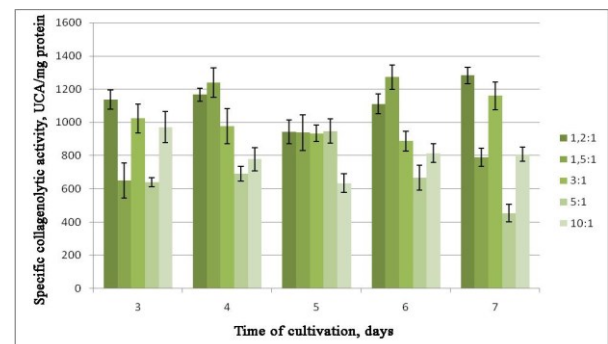


Figure 3. Specific collagenolytic activity level in case of using ammonium nitrate as the source of nitrogen

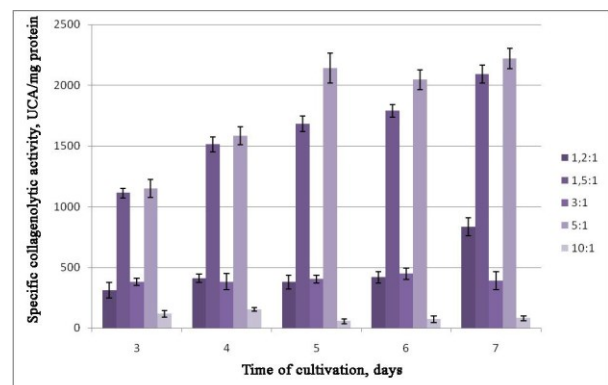


Figure 4. Specific collagenolytic activity level in case of growth on the medium with ammonium citrate

#### **IV. Conclusions**

The use of higher fungi as producers of collagenase provides an opportunity to realize production of this enzyme under controlled conditions and environments, which leads to a relative standardization of culture media and the possibility of enzyme production sustainable and on the industrial scale.

Highest fungus *Coprinus lagopides*, has a high collagenase activity and is a promising producer of highly active collagenase.

According to experiments, the most specific collagenolytic activity basidiomycete *Coprinus lagopides* submerged culture showed on the 6th day in a medium, wherein the nitrogen source is a peptone at a ratio of carbon source and nitrogen, equal to 5:1. High specific collagenase activity producer was also observed on the 7th day of cultivation in the medium with urea at a ratio of carbon and nitrogen source, equal to 10:1.

The results can provide the possibility of

replacing the conventionally used expensive source of nitrogen in the nutrient medium (peptone) for rather cheaper source (urea), which significantly reduces the cost of the resulting product - a fungal collagenase. Thus, fungal collagenolytic enzyme has not only technological but also economic advantages compared with currently existing collagenase preparations.

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## COMPARATIVE QUALITATIVE ANALYSIS OF THE USE OF PAPER AND COMPUTER

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**Abstract.** *The aim of the study was to undertake a survey of the Bulgarian undergraduate and graduate students about their preferences for the use of a computer or pen and paper when writing and reading in the learning process and leisure, what positions occupied at that and what use the computer in everyday life . The study was conducted in Sofia, Bulgaria in the period April-June 2014. It involved 38 undergraduate and graduate students from Sofia, Bulgaria, 25 of which were bachelors aged 19-25 years and 13 were Master's and Doctoral students aged 23 -26 years of different specialties: printing, computer science, psychology, pulp and paper, chemical engineering, marketing, industrial management, social psychology, accounting.*

*In accordance with the requirements to the students were given 4 questions in Bulgarian, the answers were also in Bulgarian.*

**Key Words:** paper, computer, writing, reading

**Question 1.** *Describe the differences you find when using a pen and using the computer. Furthermore describe what you like and dislike about both these modalities.*

### **Advantages**

The three main advantages of using computers in writing referred by students and PhD students are:

- Facilitates the work
- Allows correction and editing text
- Writing computer is faster

Furthermore, students prefer to use a computer when writing in the learning process and the work, because:

- It allows more readable and beautiful shape
- There is an opportunity for rapid reproduction of documents
- There are other options besides writing text, e.g. drawings, tables, formulas, equations, etc.
- The text can be easily sent to remote recipients
- There is an opportunity to change the font color, highlight and thicken
- Reduced paper costs
- The text written on a computer can be copy on paper.

### **Disadvantages:**

All participants have identified as the main disadvantage the fatigue and damage of the eyes when writing computer

-writing a formula or equation wastes more time than writing with a pen

- Authenticity of the text and the personality of the writer's handwriting and personal message are lost

-Make blatant spelling errors due to pressing a wrong button and using symbols inconsistent with spelling, then waste time for correcting

-Availability of electricity and internet is need, can be used only in a particular place

-The computer is heavy and uncomfortable to wear compared to the pen

**Conclusion:** The main part of students and doctoral students prefer to write courses and theses by computer, as the text is more beautiful, there is an opportunity for changing the text and text can be easily multiplied and sent to remote recipients. They do not use computer during lectures.

### **Writing with a pen on paper**

#### **Advantages**

Most of the students indicated as main advantage of writing with a pen that:

- Pen does not fatigue eyes
- People are more concentrated and studious when writing with a pen
- Pen is more convenient and available - only one sheet of paper is need
- The writing is memorized faster
- Can be used in any location, no need of electricity
- Cheaper, it is always at hand

#### **Disadvantages**

Most of the students indicated as main disadvantage of writing with a pen that

- It does not allow corrections and editing of text
- If there is an error, the text should be written again, if the document is official
- Writing by pen is slower and more laborious
- Paper is need
- The ink may cause smearing and stain the text

**Conclusion:** Only five of the surveyed students said that they prefer to use a pen instead of computer, others think that writing on a computer has many advantages and they use it extensively in there practice. Writing with a pen is more available and cheaper and allows in-depth consideration of the text. The opinion of one of the respondents can be given as an example of the preferences of the students –“I like the pen when I make plans, consider project and want to remember something. I like computer when I have to write an official document because it is tidier and more presentable.

**Question 2. Describe which differences you find in reading paper and reading on screen. Furthermore, describe what you like and dislike about both these modalities**

#### **Reading paper**

##### **Advantages**

All surveyed students said that they prefer to read from paper as:

- Reading is easier and does not damage the eyes
  - it is more pleasant
- The necessary passages can be highlighted
- reading is possible in time of travel and holidays
- reading is not dependent on the availability of electricity and internet
- People may take a comfortable position - lying on sofa

##### **Disadvantages**

Several responses indicated that improper posture while reading can hurt the spine

#### **Reading on screen**

##### **Advantages**

The main advantages in reading on screen are:  
-It's easier to find relevant information in large document

- It is faster to find required topic

Others mentioned advantages are:

- People can read without lighting
- Electronic books are lighter and with less volume than paper books
- EBooks can be read in time of traveling and holidays

##### **Disadvantages**

The main disadvantages in reading on screen are:

Tired eyes, headaches, need of more frequent breaks for a rest

Moreover, as other disadvantages are reported:

-More difficult to remember the text

-Ability to distraction

Radiation

-Several responses indicated that improper posture while reading can hurt the spine

**Conclusion:** The majority of the surveyed students prefer to read from paper mainly because of tradition (the characteristic appearance of the book), less eye fatigue, the comfort, the ability to highlight and easily memorizing. Some of them indicated that they prefer to print screen text on paper and then read it.

**Question3. Think now of the gesture and postures you assume in reading and writing using paper and on a screen. Reflect and describe them.**

There are many different opinions in responses of this question. Most of the respondents said that in their opinion they have poor posture while using the computer and reading and writing of paper, which has negative consequences for their health (hunching and stiffness). Many of them lay reading paper and sit during reading and writing on computer. There are students who read and write on a computer in bed.

**Question 4. Think now to you use of the computer/internet. This tool allows multimodal communication (images/videos, texts, sounds music and so on). How do you use it? Reflect on your personal experience and then describe it.**

There are different answers of this question; the students gave several applications on the computer in his personal life and training. The most important of these are:

- Communication
- Quick access to information about events, facts, weather, news, fashion
- Information on activities related to work or training
- Listening to music
- Relationship with social networks
- Writing essays, theses and more.
- Entertainment
- Movies
- Images
- Links with relatives who do not live in the country or in the city
- Banking



- Shopping
- Watch Videos
- Games
- Research

### **Conclusion**

The analysis of the information received indicates a general trend of increasing utilization of the computer in everyday life. There is a clear willingness of respondents to use a computer instead of paper. In conclusion it can be said that all respondents reported that extensively use computers in everyday life, learning and work, and cannot imagine life without them.

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## **A STUDY ON THE STABILITY OF THE FOOD-PACKAGE SYSTEM**

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**Abstract:** *This paper presents the way in which the shelf life of canned foods can be influenced by their interactions with the material (tin and iron) of the container. The most common form of these interactions is the corrosion either by etching or by pitting. There are several factors to be taken on account, which influence the installing of corrosion and the dissolution of tin or iron into the foodstuff. While the higher concentrations of tin affect both the shelf life of the products and the human health the high levels of iron affect only the shelf life but are beneficial to human health.*

*The first two of these are outside the scope of this paper, the remainder of which will concentrate on container interactions, both with the can contents and with the external environment. Regarding the interactions between the can and its contents, all foods interact with the internal surface of the can in which they are packed. In the unlacquered form, only tinplate has any corrosion resistance to the acids found in foods; all the other metals must be lacquered. Even tinplate must be lacquered where particularly aggressive products are packed, such as tomato purée, or where there is danger of pitting corrosion or surface staining (for example, in meat products)*

**Keywords:** components migration, food stimulants, inner protection lacquers, outer protection lacquers, inner welding protection lacquers, outer welding protection lacquers approval.

### **I. Introduction**

The food stuff products, an important part of the production of goods, must arrive at the consumer in packed state. This fact imposes the necessity of preserving the product during its shelf life ensuring at the same time the economic efficiency of the production and retail processes on the internal and external markets.

Thus, the quality of the packing materials and of the packages as well as the packing technologies must be at the level of those used in the developed countries and must respond to the requests imposed especially by the EU market.

The safety of the packing materials is based on the assurance that during the contact with the foods no unsafe chemical substances migrate from the material into the foods. The requests regarding the food safety are continuously increasing as well as the preoccupation for the European Committee legislation and the national legislation.

The stability of the food-package system when preserving the foods in cans is influenced by several factors:

- the fabrication materials for the metallic cans and caps;
- the protection materials for the metallic cans and caps;
- the characteristics of the packed foods;
- the preservation processes of the foods in these types of cans;

- the interaction between food- package by the determination of the components migration processes.

For most of the cases of foods packed in metallic cans some of their unique properties are destroyed to ensure their storage on a long term (for both solid foods and beverages) with the preservation of the safety of the quality and nutritional value of the products. These things are obtained by maintaining the integrity of the cans that prevent any type of contamination after the foods processing. The integrity of the metallic cans is based almost 100% on the properties of the different materials used for their construction especially on the inner protection layers the ending seals and the closures. Besides the obtainance on a long term of a shelf life for the package these materials must not contaminate themselves the foods at levels that can be damaging for the human health and besides must not cause any change in the appearance of the food.

### **II. Materials and methods**

The lab experiments surveyed the interaction between product and package during the time of storage (2 years). There were used two types of food products in metallic cans made of tin plate, three pieces drawn with 2 lacquering systems:

- white sulphur resistant lacquer (epoxy-phenolic, pigmented aluminium);
- yellow acido resistant lacquer (epoxy-phenolic).

The verification was lead on single lots obtained from the production of a vegetable can manufacturer (peas and tomato pasta). From each lot was taken a sample in accordance with the STAS 3730-92 requests. The cans were stored in the conditions mentioned in the product requirements. In order to study the interaction between the product and metallic package/ can, the canned foodstuff was periodically analyzed, during the storage time for the organoleptic, physico-chemical (heavy metals content) and microbiological characteristics.

The testing for the components migration followed the rules of the normative aspects for the

hygienic sanitary approval of the food packagings respectively: The Health Minister Order no. 975/1998, The HG norms no. 1197/2002 and the following additions HG no. 512/2004 and HG no. 559/2004, the methodologic indicator no. 173/1984 and the romanian standards aligned to the EU directives regarding the testing of the materials and articles in contact with the foods. There were chosen conditions similar to those applied in practice. The food simulants and the extraction reports were presented in the tables 2,3,4 and 5. The followed indicators are subsequently presented, in table 1, on resins groups according to their chemical nature:

**Table 1.** *The followed indicators on resins groups according to their chemical nature*

<b>Nr.c rt.</b>	<b>Resins groups</b>	<b>Name</b>	<b>Followed indicators</b>
<b>1</b>	<b>Protection epoxyphenolic lacquers</b>	HE 1526 gold (1 layer), HE 1526 gold (2 layers), PL 1333-16, Vitalure 334, Vitalure 344, Vitalure 345, DZ 036 SPT, Lacquer L 3311, Lacquer L 3312	The components global migration The organoleptic modifications of the samples and extraction liquids The ceasing of heavy metals in acetic extract: Pb and Cd Epychlorohydrin ceasing Phenolic derivates ceasing Bisphenol A ceasing UV fluorescence
<b>2</b>	<b>Protection modified epoxy lacquers</b>	PL 1014 gold, OV 1128-01	The components global migration The organoleptic modifications of the samples and extraction liquids The ceasing of heavy metals in acetic extract: Pb and Cd Epychlorohydrin ceasing Phenolic derivates ceasing Bisphenol A ceasing UV fluorescence
<b>3</b>	<b>Protection modified polyester lacquers</b>	Powder N 49291/W	The components global migration The organoleptic modifications of the samples and extraction liquids The ceasing of heavy metals in acetic extract: Pb and Cd The ceasing of acetaldehyde in acetic extract UV fluorescence
<b>4</b>	<b>Protection epoxy amine lacquer</b>	PPG 3190-822A	The components global migration The organoleptic modifications of the samples and extraction liquids The ceasing of heavy metals in acetic extract: Pb and Cd Epychlorohydrin ceasing Phenolic derivates ceasing Amines ceasing Formaldehyde ceasing UV fluorescence

### **III. Results and discussions**

Due to the complex interactions between the foods and metallic cans there can be led predictive tests upon the cans performance but they are quite limited. The cans testing on a long term in the preservation conditions of the foods is required for their entire period of shelf life that is between 1 and 5 years. The packagings manufacturer and the one

that packs the foods are responsible for all the aspects regarding the safety of the foods and the potential substance migration. It is essential that any type of restriction regarding the available materials for those that create the packagings not to refer at the high microbiological and chemical safety that the metallic cans offer. Generally the existing regulations referring to the matallic cans refer

separately to the metallic and nonmetallic components of the cans.

**Table 2. Inner protection lacquers**

Nr. crt.	Name / Source	Chemical nature	Extraction medium	Extraction conditions/ Extraction ratio	Organoleptic changes		Global migration, ppm	Metals foil, ppm		Epichlorhydrin, ppm	Phenolic derivates, ppm	UV Fluorescence
					Sample	Extract		Pb	Cd			
1	<b>GOLD HE 1526-13</b> 1 layer ICI Packaging Coatings	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	7,75	-	-	absent	BPA: 1,6	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	9,75	0,012	0,000	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	4,75	-	-	-	-	-
2	<b>GOLD HE 1526-13</b> 2 layers ICI Packaging Coatings	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	9,0	-	-	absent	BPA: 2,4	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	11,5	0,019	0,000	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	5,5	-	-	-	-	-
3	<b>PL 1333-16</b> GRACE DAREX GmbH	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	9,5	-	-	absent	BPA: 2,2	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	16,5	0,015	<0,006	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	7,0	-	-	-	-	-
4	<b>VITALURE 344</b> <b>Code 16-4344 L</b> ICI Packaging Coatings	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	11,5	-	-	absent	BPA: 7	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	24,0	0,014	0,000	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	5,25	-	-	-	-	-
5	<b>VITALURE 334</b> <b>Code N 49234</b> ICI Packaging Coatings	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	19,0	-	-	absent	absent	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	26,75	0,048	<0,001	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	9,0	-	-	-	-	-
6	<b>GZ 036 SPT</b> POLONIA	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	10,5	-	-	absent	absent	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	18,25	0,007	0,001	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	6,5	-	-	-	-	-
7	<b>Sulforezistent lacquer L3312</b> POLICOLOR Romania	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	21,0	-	-	absent	BPA: 0,6	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	29,75	0,024	<0,006	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	6,5	-	-	-	-	-
8	<b>Acid-rezistant lacquer L 3311</b> POLICOLOR Romania	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	16,5	-	-	absent	BPA: 1	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	18,25	0,029	<0,006	-	-	absent
			i-octan	48h, t.c./ 1:1	unchanged	unchanged	-	-	-	-	-	-

**Table 3. Outer protection lacquers**

Nr. crt.	Name / Source	Chemical nature	Extraction medium	Extraction conditions/ Extraction ratio	Organoleptic changes		Global migration, ppm	Metals foil, ppm		Epichlorhydrin, ppm	Phenolic derivates, ppm	Formaldehyde, ppm	UV Fluorescence
					Sample	Extract		Pb	Cd				
1	<b>SE 1169-18</b> cover with <b>OV 1128-01</b> GRACE DAREX GmbH	Modified polyester Modified Epoxy-ester	distilled water	1h, 121°C/ 1:10	unchanged	unchanged	8,75	-	-	absent	absent	8,5	-
			acetic acid sol. 3 %	1h, 121°C/ 1:10	unchanged	unchanged	12,0	0,024	<0,006	-	-	-	absent
2	<b>PL 1014-69</b> gold GRACE DAREX GmbH	Modified Epoxy	distilled water	1h, 121°C/ 1:10	unchanged	unchanged	8,5	-	-	absent	BPA: 2,2	-	-
			acetic acid sol. 3 %	1h, 121°C/ 1:10	unchanged	unchanged	10,5	0,024	<0,006	-	-	-	absent
3	<b>VITALURE 345</b> <b>Cod 16-345L/</b> gold	Epoxy-phenolic	distilled water	1h, 121°C/ 1:10	unchanged	unchanged	10,0	-	-	absent	absent	-	-
			acetic acid sol. 3 %	1h, 121°C/ 1:10	unchanged	unchanged	17,5	0,026	<0,006	-	-	-	absent

	ICI Packaging Coatings												
4	<b>VITALURE 344 Cod 16-4344 L</b> gold ICI Packaging Coatings	Epoxy-phenolic	distilled water	1h, 121°C/ 1:1	unchanged	unchanged	11,5	-	-	absent	BPA: 7	-	-
			acetic acid sol. 3 %	1h, 121°C/ 1:1	unchanged	unchanged	24,0	0,014	0,000	-	-	-	absent
5	<b>Acid-resistant lacquer L 3311</b> POLICOLOR Romania	Epoxy-phenolic	distilled water	1h, 121°C/ 1:10	unchanged	unchanged	16,5	-	-	absent	BPA: 1	-	-
			acetic acid sol. 3 %	1h, 121°C/ 1:10	unchanged	unchanged	18,25	0,029	<0,006	-	-	-	absent

The most common form of this interaction is corrosion. In plain tinplate containers, this takes the form of etching or pitting corrosion, and staining of the surface may also occur. However, internal lacquers are available which reduce this effect by

providing a barrier between the food and the metal can wall. This also allows the use of other forms of metal container(e.g. tin-free steel or aluminium) which would otherwise be corroded very quickly.

**Table 4. Inner welding protection lacquers**

Nr. crt.	Name / Source	Chemical nature	Extraction medium	Extraction conditions/ Extraction ratio	Organoleptic changes		Global migration, ppm	Metals foil		Epichlorhydrin, ppm	Phenolic derivatives, ppm	Formaldehyde, ppm	Amines	UV Fluorescence,
					Sample	Extract		Pb	Cd					
1	<b>PPG 3190-822A</b>  HOBA Lacke und Farben GmbH	Epoxy-amine	distilled water	1h, 121°C/ 1:20	unchanged	unchanged	6,5	-	-	absent	absent	-	absent	-
			acetic acid sol. 3 %	1h, 121°C/ 1:20	unchanged	unchanged	8,5	0,017	<0,006	-	-	-	-	absent
			i-octan	1h, 121°C/ 1:20	unchanged	unchanged	8,0	-	-	-	-	-	-	-
2	<b>Powder N 49291/W</b>  ICI Packaging Coatings GmbH	Modified polyester	distilled water	1h, 121°C/ 1:20	unchanged	unchanged	7,5	-	-	-	-	-	-	-
			acetic acid sol. 3 %	1h, 121°C/ 1:20	unchanged	unchanged	10,5	0,005	<0,006	-	-	absent (acetaldehida)	-	absent
			i-octan	1h, 121°C/ 1:20	unchanged	unchanged	9,5	-	-	-	-	-	-	-

**Table 5. Outer welding protection lacquers approval**

Nr. crt.	Name / Source	Chemical nature	Extraction medium	Extraction conditions/ Extraction ratio	Organoleptic changes		Global migration, ppm	Metals foil, ppm		Epichlorhydrin, ppm	Phenolic derivatives, ppm	Formaldehyde, ppm	UV Fluorescence
					Sample	Extract		Pb	Cd				
1	<b>PPG 5020-801 A</b>  HOBA Lacke und Farben GmbH	acrylic	distilled water	1h, 121°C/ 1:20	unchanged	unchanged	8,0	-	-	-	-	absent	-
			acetic acid sol. 3 %	1h, 121°C/ 1:20	unchanged	unchanged	10,0	0,021	<0,006	-	-	-	absent

#### IV. Conclusion

On the basis of the tests results presented in tables 4-7 we can draw the followings:

- there were not registered organoleptic modifications regarding the appearance and smell of the samples or extraction liquids.

- the obtained values for the components global migration in all extraction environments were under the admitted value of 60 ppm;

- the ceasing of heavy metals were under the admitted values: the Pb much under 0,1 ppm, and the Cd under the detection value of the device of 0,006 ppm;

- the UV fluorescence indicates the absence of condensed polynuclear aromatic hydrocarbonates;

- there was not detected the presence of the following components: epychlorohydrin in the epoxy modified lacquers and epoxyphenolic lacquers, the acetaldehyde in the modified polyester lacquer, the amines in the modified epoxy amine lacquer.

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## STUDYING THE POSSIBILITIES OF USING OF ESSENTIAL OILS IN DAIRY PRODUCTS. 3. BASIL (*Ocimum basilicum*)

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**Abstract.** The possibility of using of the essential oil of basil (*Ocimum basilicum*.) in dairy products has been studied. The composition, antimicrobial properties and its effect on the microorganisms of starter cultures for dairy products has been studied. It was found that it exhibits high antimicrobial activity, but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. The essential oil of basil is a suitable natural addition to dairy products.

**Key words:** basil, essential oil, antimicrobial properties, microorganisms, lactic acid bacteria, minimum inhibitory concentration, minimum bactericidal concentration

### I. Introduction

Basil (*Ocimum basilicum* L.) is an annual herbaceous plant whose native land is Asia. Today it is grown in almost all countries with temperate, subtropical and tropical climate. Its over-ground part is used as a herb and spice. There are more than 100 varieties which differ in size, shape, color, aroma and composition. Basil contains essential oil, glycosides, tannins, organic acids, mineral salts. It is a source of vitamins K, A and C, mineral iron, calcium, manganese, magnesium, potassium. Basil has antiseptic, antispasmodic, analgetic, anti-inflammatory and slightly stimulating effect. It is used in infectious- inflammatory processes of urogenital and respiratory tract, tiredness and depression. Basil is a typical spice for many cuisines around the world. Fresh or dried, its over-ground part is used alone and in condiments in salads, soups, sauces, meat, poultry and fish dishes, in chesses, sausages, tinned food and pickles [3, 5, 6, 8].

Essential oil has a different composition depending on the type of basil and its origin. The oil produced in European countries, including our country is linalool type and contains linalool, geraniol, citronellol, bornylacetate, metilhavikol, nerol, eugenol, citric and others. The oil has strong antimicrobial activity and therefore is used in medicine and pharmacy. It is used in fragrance compositions for fine perfumery, soap, fragrance compositions of preparations for oral, deodorants, fragrance compositions for food- sauces, condiments, meat products, etc [3, 4, 7, 11]

The purpose of this work is to explore the possibilities for using of essential oil of basil in dairy products by examining the composition, antimicrobial properties, and its effect on

microorganisms by starter cultures for dairy products.

### II. Materials and methods

#### 2.1. Materials

2.1.1. Oil of basil has been used, provided by the company Vigalex Ltd, Sofia

2.1.2. Test microorganisms

To determine the antimicrobial activity of the oil of basil are used test cultures from NBIMCC - National Bank of Industrial Microorganisms and Cell Cultures, Sofia: Gram-positive: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633; Gram-negative: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017; Yeasts: *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231; Fungi: *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum*, *Fusarium moniliforme*.

2.1.3 Starter cultures

Two starter cultures have been used for white brined cheese: mikroMILK TBMC1, contains: *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactococcus lactis subsp. lactis*, *Lactobacillus lactis subsp. cremoris*, *Lactobacillus casei*;

„LB Bulgaricum” JSC LBB CM 310-40 with composition: *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactococcus lactis*.

#### 2.2. Methods

2.2.1. Determination of the composition of the essential oil of basil

The chemical composition of the oil was determined chromatographically. For GC analysis was used apparatus: GC 7890 A with MSD 5795 C; Temperature program 40°C for 3 min then 5°C/min to 300°C for 5 min, run time 60 min; Column: HP-5MS (30 m x 250 µm x 0.25 µm); Gas: helium with a flow rate of 1 cm<sup>3</sup>/min.

2.2.2. *Determination of the antimicrobial activity of oil of basil by agar diffusion method*

The experiments were conducted on Tryptic Soy agar (Biolife) – for bacteria and Sabourad- dextrose agar (Biolife) for the yeasts and fungi. The oil of basil was tested at concentrations of 100; 50; 10; 5; 1; 0,5; 0,1; 0,05 % in solvent 1% Tween 80.

The diameters of the zones of the growth inhibition were measured in mm with a digital caliper, such as up to 15 mm microbial culture is less sensitive; from 15 to 25 mm – sensitive and over 25 mm – highly sensitive.

The experiments were conducted in parallel with controls from the solvent, taking into account and correct its effect.

2.2.3. *Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

MIC and MBC were determined according to the methods described by Andrews [1], Barros et al. [2] and Smith-Palmer et al. [10].

2.2.4. *Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil of basil on the microorganisms in starter cultures*

The following concentrations of the oil were prepared: 1; 0,5; 0,1; 0,05 and 0,01% in solvent sterile 1% solution of Tween 80.

The growth of the bacterial cultures was assessed by comparing the number of the lactic acid bacterial in each suspension with that one of the control (bacterial suspension without oil). Inhibition of the growth of the lactic acid bacteria is read at that concentration of the essential oil in which is established 90% reduction in the number of lactic acid bacteria compared to the control samples.

2.2.5. *Studying of the influence of the oil of basil at concentrations suitable for the addition in food products on the microorganisms of the starter cultures*

The studying is conducted according to the procedures for determining to the total number of lactic acid bacteria in starter cultures [9].

All results are presented as average of three parallel experiments.

III. Results and discussion

Table 1 presents the chemical composition of the essential oil. The data shows that the oil contains 30 components (87,77% identified), the most of which are: β-linalool (49,73 %), 1,8-cineole (12,37 %) and α-bergamotene (7,26 %). Less than 3% are 26 compounds. The differences of the data in the literature are due to the type and origin of basil [3, 8, 11].

Table 1. Chemical composition of essential oil of basil (*Ocimum basilicum L.*)

Components	RI	%	Components	RI	%
α-Pinene	939	0,52	Neral	1238	0,16
Camphene	954	0,59	Geraniol	1253	0,15
β-Pinene	979	0,91	Linalyl acetate	1257	0,53
p-Cymene	1024	0,23	Geranial	1267	0,34
β-Phelandrene	1028	0,28	δ-Elemene	1332	0,26
1,8-Cineole (Eucalyptol)	1033	12,37	α-Elemene	1343	0,29
(Z)-β-ocimene	1038	0,54	Geranyl acetate	1381	0,65
(E)-β-ocimene	1050	1,84	β-Bourbonene	1384	0,42
cis-linalool oxide	1072	1,29	β-Elemene	1391	1,58
trans-linalool oxide	1082	1,36	α-Bergamotene	1434	7,26
β-Linalool	1098	49,73	α-Humulene	1454	0,24
Terpinen-4-ol	1177	0,19	(Z)-β-Farnesene	1457	0,33
α-Terpineol	1189	1,24	β-Selinene	1490	0,40
Estragole	1198	0,68	β-Bisabolene	1506	0,21
Nerol	1230	0,14	tau-Cadinol	1640	3,27

Figure 1 shows the distribution of the components of the oil in groups. Monoterpenes alcohols (51,16%) dominate, followed by monoterpenes and

sesquiterpenes hydrocarbons (respectively 17,19% and 10,99%).



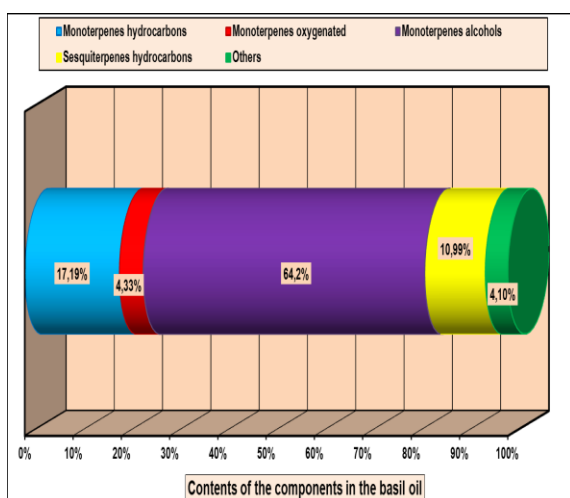


Figure 1. Distribution of the components of the oil

The results of the antimicrobial activity of the oil of basil are shown in Figure 2 and 3.

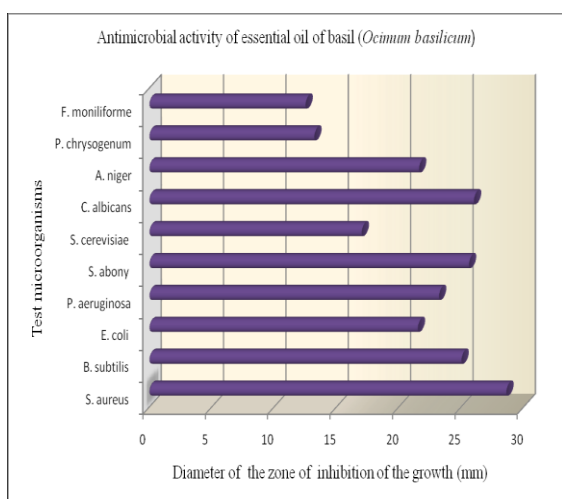


Figure 2. Antimicrobial activity of the essential oil of basil (*Ocimum basilicum*)

The obtained results show that the essential oil of basil (*Ocimum basilicum*) has clear pronounced activity to all tested microorganisms. There is high sensitivity to Gram-positive bacteria *Staphylococcus aureus* (28,1mm diameter of the zone) and *Bacillus subtilis* (25,1 mm diameter of the zone). In Gram-negative bacteria most is the impact of the oil on *Salmonella abony* (25,7mm diameter of the zone).

From the yeasts *Candida albicans* (26,1mm diameter of the zone) has high sensitivity to the

essential oil of basil while *Saccharomyces cerevisiae* is less sensitive.

The antifungal activity of the oil of basil is low with the exception of *Aspergillus niger*.

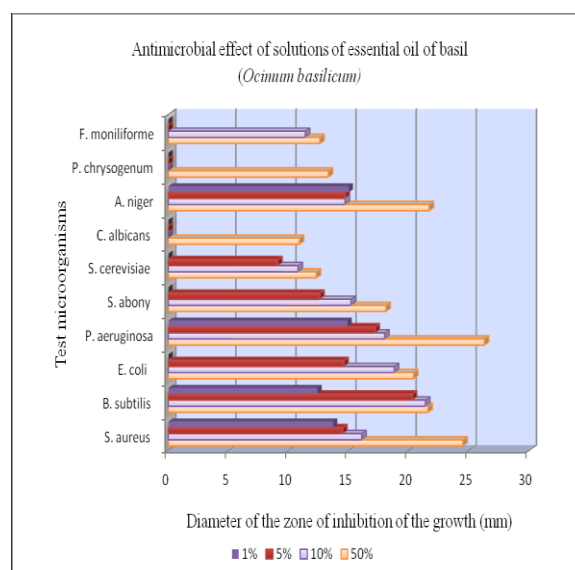


Figure 3. Antimicrobial effect of 50, 10, 5 and 1% solutions of the oil of basil

The essential oil of basil retains antimicrobial activity in 50% solution. It is highest against *Staphylococcus aureus* (24,5 mm zone of the inhibition of the growth), *Pseudomonas aeruginosa* (26,3 mm zone of the inhibition of the growth ) and *Aspergillus niger* (21,7 mm zone of the inhibition of the growth ). Gram-positive and Gram-negative bacteria are sensitive to the activity of 10 and 5% solutions of the basil oil. The growth of *Candida albicans* and *Penicillium chrysogenum* is not inhibited by these solvents. Resistant to 1% solution of the basil oil are studied Gram-positive, *Pseudomonas aeruginosa* from Gram-negative bacteria and from the fungi *Aspergillus niger*.

Since the essential oil of basil (*Ocimum basilicum*) has antibacterial activity in 1% solutions, to determine the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) experiments were conducted with solution of the oil in concentrations of 1; 0,5; 0,1; 0,05%.

Table 2. Effect of solutions of essential oil of basil on the growth of test microorganisms

Test microorganisms	Total number of viable colony forming units (cfu/ml)				
	Control	Basil – concentration of the oil			
		0,05%	0,1%	0,5%	1,0%
<i>Staphylococcus aureus</i> ATCC 6538	5,1x10 <sup>8</sup>	4,3x10 <sup>8</sup>	2,5x10 <sup>6</sup>	0	0
<i>Bacillus subtilis</i>	2,2x10 <sup>8</sup>	1,7x10 <sup>8</sup>	5,3x10 <sup>5</sup>	0	0

ATCC 6633					
<i>Escherichia coli</i> ATCC 8739	6,2x10 <sup>8</sup>	3,2x10 <sup>8</sup>	3,0x10 <sup>5</sup>	2,5x10 <sup>4</sup>	0
<i>Pseudomonas aeruginosa</i> ATCC 9027	3,0x10 <sup>8</sup>	2,4x10 <sup>8</sup>	5,0x10 <sup>4</sup>	0	0
<i>Salmonella abony</i> NCTC 6017	1,6x10 <sup>8</sup>	1,3x10 <sup>8</sup>	3,3x10 <sup>4</sup>	1,2x10 <sup>4</sup>	0

In Gram-positive bacteria and *Pseudomonas aeruginosa* minimum inhibitory concentration (MIC) of the essential oil of basil is 0,1%, and minimum bactericidal concentration (MBC) is 0,5%. In *Escherichia coli* and *Salmonella abony* MIC is 0,5%, and MBC – 1,0%. The antimicrobial activity of the studied by us basil oil confirms that according to the literature [7, 9, 11].

To be used in dairy products the essential oil should not inhibit the development of the lactic acid bacteria contained in the dairy starter cultures. In this connection, the influence on the lactic acid bacteria from the two starter cultures is analyzed. The obtained results are shown in Table 3.

**Table 3.** Effect of solutions of the essential oil of basil (*Ocimum basilicum*) on the growth of lactic acid bacteria from starter cultures for dairy products

Essential oil	Concentration (%)	Total number of viable colonies <i>Lactobacillus sp</i> (cfu/ml)		Total number of viable colonies <i>S. thermophilus L. lactis</i> (cfu/ml)	
		LBB CM 310-40	TBMC1	LBB CM 310-40	TBMC1
Control	0	3,1x10 <sup>7</sup>	2,8 x10 <sup>7</sup>	1,4x10 <sup>8</sup>	2,4x10 <sup>8</sup>
Basil	0,01	1,1x10 <sup>7</sup>	1,3x10 <sup>7</sup>	2,2x10 <sup>7</sup>	1,4x10 <sup>7</sup>
	0,05	5,6x10 <sup>4</sup>	4,1x10 <sup>5</sup>	6,4x10 <sup>4</sup>	3,7x10 <sup>4</sup>
	0,5	0	0	0	0
	1,0	0	0	0	0

The data shows that the growth of three kinds *Lactobacillus*, participating in the composition of the two starter cultures is not affected by the 0,01 % solution of the essential oil. 0,05 % solution of the oil substantially inhibits the growth of *Lactobacillus*. The number of viable colony forming units decreases strongly with both starter cultures. By increasing the concentration of the oil over 0,5% it does not observe growth of *Lactobacillus sp*. Therefore the MIC of the oil of basil against *Lactobacillus sp*. is 0,05 %, and MBC is 0,5 %.

The table also shows that the oil of basil affects analogically the growth of *Streptococcus thermophilus* and *Lactococcus lactis* – 0,01% weakly inhibits their development, 0,05% vastly inhibits

them and completely killing them over 0,5 %. Therefore MIC of the basil oil against *Streptococcus thermophilus* and *Lactococcus lactis* is 0,05 %, and MBC is 0,5 %.

The addition of essential oils in food products requires an appropriate amount so as not to deteriorate their quality. On the other hand, they must not have depressing effect on the specific microorganisms, involved in the preparation of the fermented milk product. In this connection, the influence of the basil oil is analyzed at concentrations 0,0008, 0,002 and 0,003%, which can be used in food products [3]. The obtained results are shown in Table 4.

**Table 4.** Effect of the essential oil of basil on lactic acid bacteria from starter cultures for dairy products.

Essential oil	Concentration (%)	Total number of viable colonies <i>Lactobacillus sp</i> (cfu/ml)		Total number of viable colonies <i>S. thermophilus L. lactis</i> (cfu/ml)	
		LBB CM 310-40	TBMC1	LBB CM 310-40	TBMC1

Control		$3,3 \times 10^7$	$2,8 \times 10^7$	$2,3 \times 10^8$	$2,5 \times 10^8$
Basil	0,0008	$2,9 \times 10^7$	$2,6 \times 10^7$	$3,1 \times 10^8$	$2,9 \times 10^8$
	0,002	$3,3 \times 10^7$	$2,0 \times 10^7$	$2,5 \times 10^8$	$2,7 \times 10^8$
	0,003	$2,8 \times 10^7$	$2,3 \times 10^7$	$2,9 \times 10^8$	$2,5 \times 10^8$

The growth of *Lactobacillus* in the two starter cultures used is not affected by the presence of the essential oil of basil in concentrations which can be used in food products. The number of colony forming units with the addition of essential oil is approximately equal to the number of the colonies in the control samples. *S. thermophilus* and *L. lactis* is also not sensitive to the used concentrations of the basil oil in the tested starters: LBB CM 310-40 and TBMC1.

The used amounts of the basil oil do not negatively affect the development of lactic acid bacteria in the studied starter cultures.

#### IV. Conclusion

The essential oil of basil exhibits high antimicrobial activity but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. Minimum inhibitory concentration of the basil oil against *Lactobacillus sp.*, *Lactococcus lactis* and *Streptococcus thermophilus* is 0,05 %, and minimum bactericidal concentration is 0,5%. The essential oil of basil is a suitable natural addition to dairy products.

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# THERMODYNAMIC PARAMETERS DURING THE EXTRACTION OF ESSENTIAL OIL BEARING AND PHARMACEUTICAL PLANTS. 8. VIRGINIA FLUE-CURED TOBACCO – CONCENTRATED AROMATIC PRODUCTS

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**Abstract.** The thermodynamic parameters characterizing the extraction of tobacco leaves (flue-cured Virginia, grown in Bulgaria) for obtaining the commercially-ready concentrated aromatic products concrete and resinoid have been determined – Gibbs free energy, activation energy, entropy and enthalpy of the process..

**Key Words:** Gibbs free energy, activation energy, entropy, enthalpy, tobacco, concrete, resinoid.

## I. Introduction

Tobacco (*Nicotiana tabacum* L.) has a long sometimes controversial history as an integral part of human life. It has been an object of thorough investigation for centuries now, turning it into one of the best studied crops in the world [10]. Parallel to its traditional role in the production of various smoking and smokeless products, tobacco has always attracted serious effort in terms of search for a broader scope of alternative, non-traditional applications – due to the versatility in its chemical composition, properties, socio-economic impact and health effects [1, 2, 3, 11]. As an essential oil-bearing plant known for centuries, tobacco has reasonably been considered a material for the production of aromatic products common to perfumery and cosmetics – by distillation and/or extraction with different volatile and non-volatile solvents, at a wide variety of applied techniques and conditions, stages of fractionation, purification and concentration of intermediate and final products. The range of the aromatic products obtained from tobacco typically includes: essential oil, concrete, resinoid, absolute, extracts with liquefies gasses, etc.

Concrete is an aromatic product obtained through extraction of fresh or dried plant materials with non-polar volatile solvents (hexane, petroleum ether, etc.), further concentrated by evaporation of the solvent [12].

Resinoid is an aromatic product obtained through extraction of dried plant raw materials with polar

solvents – ethanol, methanol, acetone, etc., followed again by removal of the solvent [12].

The objectives of this study were (i) to establish the basic thermodynamic parameters of the process of obtaining aromatic products (concrete and resinoid) from Virginia flue-cured tobacco, and (ii) to determine the influence of temperature on the efficiency of the extraction. The goal of the work was an in-depth characterization of the process of extraction of cured leaves from Virginia flue-cured tobacco, as the authors had already completed with respect to other types of tobacco grown in Bulgaria, such as Burley light air-cured [8].

## II. Materials and methods

### 2.1. Plant material

Commercially cured tobacco leaves of the type flue-cured Virginia, representing North Bulgaria growing region were used as initial plant raw material. The analytical samples included leaves from X, C and B stalk positions, represented in their natural proportions. Before extraction, tobacco leaves were oven dried at 40 °C, ground in a laboratory mill and sieved (mesh 0,11 cm).

### 2.2. Chemicals and reagents

Petroleum ether and ethanol (95 %) were provided by FILLAB (Plovdiv, Bulgaria).

### 2.3. Concentrated aromatic extraction products

All technological investigations were conducted in laboratory conditions.

Concrete was obtained by extraction with petroleum ether under the following conditions: static, batch mode; twofold extraction for 1 h and 0,5 h; temperature 20, 30 and 40 °C; hydromodule (raw material : solvent) – 1:10. The solvent was removed by evaporation on a rotary vacuum evaporator at water bath temperature 35 °C [5].

Resinoid was obtained by extraction with 95 % ethanol under the following conditions: static, batch mode; twofold extraction for 2,5 h and 2 h; temperature 20, 30, 40, 50, 60 and 70 °C; hydromodule (raw material : solvent) – 1:10. The solvent was removed by evaporation on a rotary vacuum evaporator at water bath temperature 70 °C [5].

#### 2.4. Coefficients of molecular diffusion

For the calculation of the coefficients of molecular diffusion of concrete and resinoid the following extraction conditions were applied: temperatures and raw material : solvent ratio – as specified above; duration = 1 h; at each 10 min interval the extract was removed by filtration and the remaining raw material was extracted with a new portion of fresh solvent. The values of the coefficients of molecular diffusion ( $D$ ,  $\text{cm}^2/\text{s}$ ) were calculated using the formula [12]:

$$D = \frac{\delta^2 (\lg E_1 - \lg E_2)}{\pi^2 (\tau_1 - \tau_2)}$$

where:  $\delta$  is  $\frac{1}{2}$  of lamina thickness (cm),  $E_1$ ,  $E_2$  are product yields (%), and  $\tau_1$ ,  $\tau_2$  determine time (s).

All results were presented as mean values from three repetitions.

#### 2.5. Thermodynamic parameters

On the basis of extraction products' yields, the equilibrium constant of the process was determined and the thermodynamic parameters calculated – Gibbs free energy, activation energy, entropy and enthalpy of the process.

Gibbs free energy ( $\Delta G$ , J/mol) was determined by the equation [4, 6, 7, 13, 14]:

$$\Delta G = -R.T. \ln K, J/mol,$$

where:  $R$  is the universal gas constant (J/K.mol),  $T$  is temperature (K), and  $K$  is the equilibrium constant of the process.

The activation energy of the process was determined by the equation [4, 6, 9, 13, 14]:

$$E_{act} = 2,3.R.T.tg\alpha.$$

The plotted relationship between the equilibrium constant and the reciprocal value of the absolute temperature gave the angle to the abscissa ( $\alpha$ ).

The entropy was defined by the equation [15]:

$$\Delta S = \frac{(E_{act} + \Delta G)}{T}.$$

The enthalpy was determined by the equation [4, 15]:

$$\Delta H = \Delta G + T\Delta S.$$

All calculations were done for the respective mean values from the experiments.

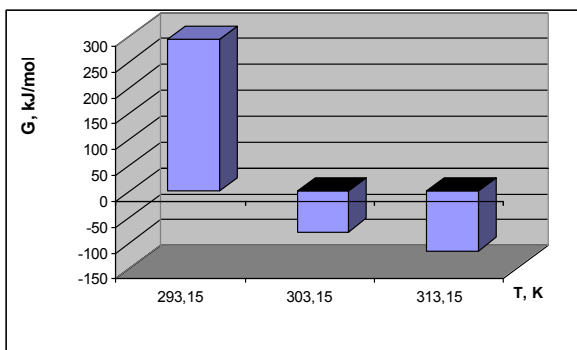
### III. Results and discussion

Extraction is the second most important method for obtaining aromatic substances from essential oil bearing plants, having the advantage to be carried out at lower temperatures than water/steam distillation. Principally, extraction of plant materials involves solid and liquid phase, and the structure of the solid matrix creates a significant resistance, making solid-liquid extraction much slower than liquid-liquid extraction. Typically the extraction of plant materials follows four stages: i) penetration of the solvent into the pores, ii) transfer of extractible substances to the phase barrier, iii) mass transfer from the barrier surface into the liquid phase, and iv) distribution of extracted substances within the liquid phase. The speed of the first two stages is much higher than that of the second two, making them decisive for the overall duration of the extraction process. The basic mechanism until the moment of deposition of substances onto the solid phase surface is diffusion. It is carried out in static state and is determined by Fick's 1<sup>st</sup> law of diffusion [12].

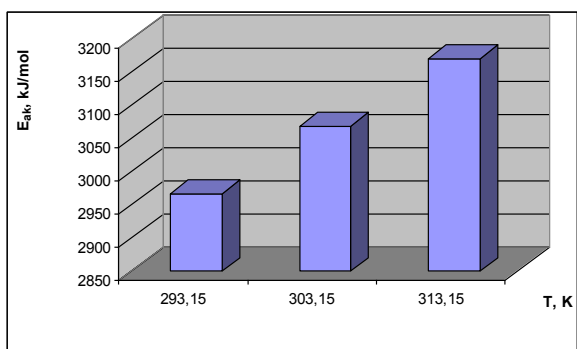
Generalized expression of the diffusion properties of the extracted material is the diffusion coefficient. It is characteristic for individual solid materials and depends on the structure and physical properties of the material and the solvent, as well as on the temperature and substance concentration. Diffusion coefficient is changed during the extraction, as plant tissues undergo physical and chemical transformations which alter their permeability. Diffusion coefficients are still being determined experimentally, due to the inhomogeneity and complexity of solid bodies [12]. Calculation of the diffusion coefficients was used to determine Gibbs free energy, the activation energy, entropy and enthalpy, for an in-depth clarification of ongoing mass transfer and thermal processes.

Figures 1–4 present the changes in Gibbs free energy, the activation energy, entropy and enthalpy of the extraction for obtaining the aromatic product concrete from Virginia flue-cured tobacco leaves.

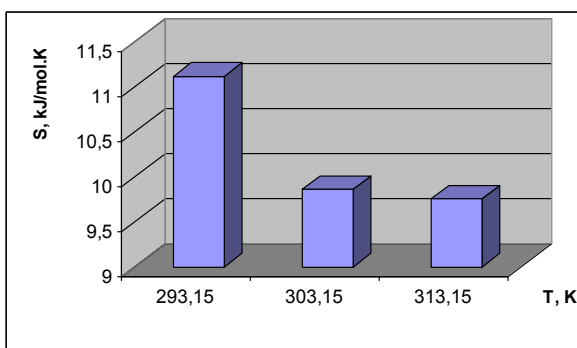




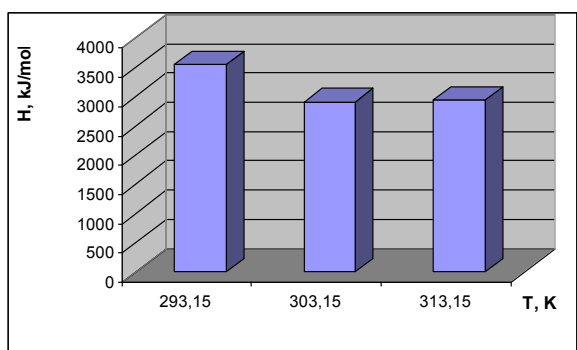
**Figure 1.** Extraction of concrete – change of Gibbs free energy in dependence on temperature



**Figure 2.** Extraction of concrete – change of activation energy in dependence on temperature



**Figure 3.** Extraction of concrete – change of entropy in dependence on temperature

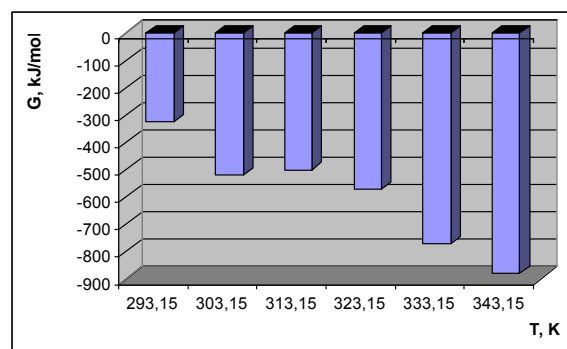


**Figure 4.** Extraction of concrete – change of enthalpy in dependence on temperature

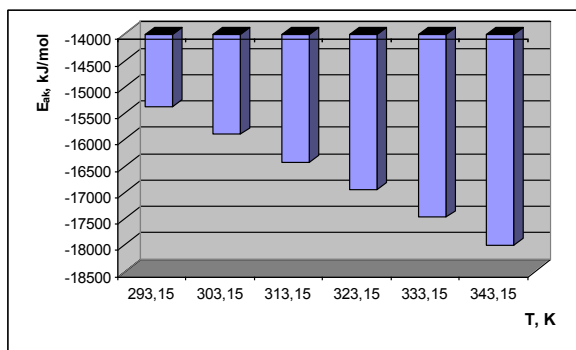
The change of Gibbs free energy is indicative of the maximum amount of work obtainable from a thermodynamic system in a reversible process, still preserving a state of equilibrium with respect to temperature and pressure. Its change predicts the direction of irreversible isobaric-isothermal processes, as well as the settlement of thermodynamic equilibrium [4]. The values presented on Fig. 1 clearly show that Gibbs free energy attains its maximum at temperature 20 °C (293,15 K). At the same temperature, the activation energy is down to its lowest value, meaning that the system requires minimum energy to complete the process. The calculated values for entropy and enthalpy, on their turn, are highest at 20 °C and decline with the rise of temperature.

Comparing these results to previously calculated values of the four thermodynamic parameters of the extraction of concrete from another type of Bulgarian tobacco – Burley air-cured [8], it turns obvious that the minimum in the activation energy is observed once again with temperature 20 °C, but there are different trends in the influence of temperature on Gibbs energy, entropy and enthalpy. These variations could be attributed to the well-known differences in the chemical composition of the initial tobacco material (the two tobaccos represent light and dark types) and to the various interactions occurring in the matrix during the extraction of concrete with the specific solvent.

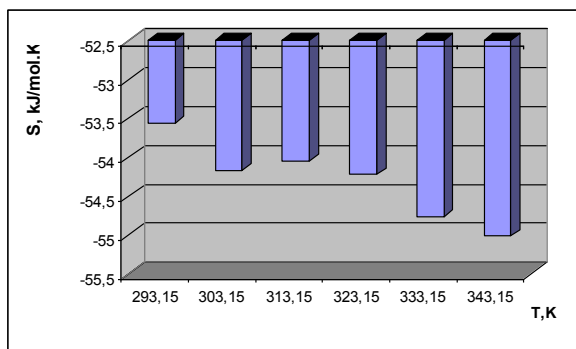
The same four thermodynamic parameters – Gibbs free energy, the activation energy, entropy and enthalpy of the process were investigated, but in the case of extracting Virginia flue-cured tobacco leaves for obtaining the aromatic product resinoid. The changes in the calculated values as a function of temperature are presented on figures 5 – 8.



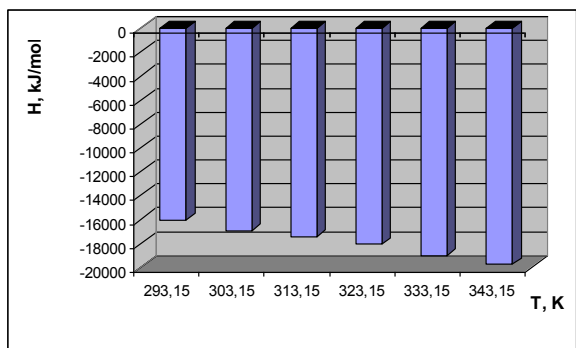
**Figure 5.** Extraction of resinoid – change of Gibbs free energy in dependence on temperature



**Figure 6.** Extraction of resinoid – change of activation energy in dependence on temperature



**Figure 7.** Extraction of resinoid – change of entropy in dependence on temperature



**Figure 8.** Extraction of resinoid – change of enthalpy in dependence on temperature

Data show that the activation energy of resinoid extraction from Virginia flue-cured tobacco is minimal at 20 °C (293,15 K), with a stable trend of increase at higher temperatures (30 – 70 °C). Calculations concerning the rest of the thermodynamic parameters – Gibbs energy, entropy and enthalpy, show the opposite trend – the respective maximums are at temperature 20 °C (293,15 K). These findings would mean that for a higher efficiency of the process of Virginia tobacco leaves' extraction with the specific solvent it would be most favourable to establish the system working at that temperature. The negative sign of the

calculated values of Gibbs energy, activation energy, entropy and enthalpy suggest that chemical interactions between the solvent and the raw material occur during the process.

If a comparison is drawn between current results and these obtained for the extraction of resinoid from Burley air-cured tobacco [8], it turns out that there is an almost complete correspondence in the pattern of all four studied thermodynamic parameters. Therefore, it could be concluded that the extraction of resinoid from tobacco leaves is characterized by process parameters that do not change with the type of tobacco – either flue-cured Virginia or air-cured Burley.

#### IV. Conclusions

This study has allowed for the first time to be determined the thermodynamic parameters – Gibbs free energy, activation energy, entropy and enthalpy, of the extraction process for obtaining aromatic products from Virginia flue-cured tobacco, contributing to a more in-depth understanding of the extraction process.

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## COMPUTER TECHNOLOGIES FOR ANALYSIS AND SYNTHESIS OF ELECTROMECHANICAL SYSTEMS IN FOOD INDUSTRY

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**Abstract:** *The problems concerning the approaches for analysis and synthesis of wide spread electromechanical transport systems in food industry are discussed in the paper. New methods with application of computer technologies in these processes are explored. Some important advantages of using Matlab and Simulink as program mediums for analysis and synthesis of electric drive systems are presented. A Simulink model of a control system for AC electric drive is presented. The responses to typical control and disturbing inputs are examined and the results are graphically presented. The possibilities for optimal tuning of the system are suggested.*

**Key Words:** transport electromechanical systems, computer modeling, Simulink models of electric drives

### I. Introduction

Transport systems in the food industry meet the following basic functions: transportation; arrangement; coordination – spatial and productivity; calibration; technological impact (during cooling and drying). Viewed as an object of control, transport systems can be reduced to: electro-mechanical part that contains the operating mechanism connected by the transmission to the electric motor; power semiconductor converter converting electrical energy between the power source and the electric motor; information (control) part that serves to control the power semiconductor converter and provides the desired properties of the electric drive. The requirements to the control of the electromechanical transport systems in the food industry can be reduced to the following: soft start and soft stop for reducing the wear on bearing elements in the kinematic chains; variable speed control over a wide range to ensure optimal coordination and technological impacts on productivity and ensuring optimal energy regimes during load changes.

The analysis and synthesis of the described electromechanical systems are complex tasks. The solution of this problem consists of series of decisions and a set of interrelated tasks, ranging from the preparation of the terms of reference and ends with the surrender of the working project documentation. These problems in the past 10 years are decided on an entirely new basis. This is related to the appearance of powerful computing technology, based on application software packages. Implementation of computer models for these systems is a creative task. A major problem of the study is the adequate use of application programs to solve specific tasks. Therefore, in addition to the mandatory analyzing the physical processes in these

electromechanical (sometimes called mechatronic) systems it is necessary to know the possibilities and the specificities of application packages for solving specific tasks. There are packages such as OrCAD PSpice and OrCAD Capture combine that deliver a complete circuit simulation and verification solution [1], but for modeling the mechanical part of the systems one should use another different package. The MathWorks, Inc. supplies the software MATLAB® and Simulink® with its large libraries and especially SimPowerSystems, and Simscape, providing tools for modeling and simulation of multi-domain physical systems, such as those with mechanical and electrical components [2]. The experience in the analysis of static and dynamic characteristics of DC and AC motors in the programming environment Matlab [3] gave rise to the next step – to use Simulink for structure modeling of the entire electromechanical system. Simulink provides the researcher a variety of options ranging from structural (mathematical) representation of the system and ending with the generation of code for programming the microprocessor in accordance with the structural scheme of the model. The main problem when modeling multi-domain physical systems is widely varying range of the measures of different types of inertia. For example, the difference between the mechanical and electromagnetic inertia can be more than ten orders of magnitude and respectively such is the difference between the duration of transients.

The objectives of this study is to suggest an algorithm for decomposition of a complex electromechanical system and on its base to define the steps of modeling the elements, analyzing the responses of the system to the control input and to the disturbances in Simulink.

## **II. Materials and methods**

The object of study is the electromechanical system with general structure of three parts: electromechanical part; power semiconductor converter (PSC) and control block (CB). The electric machine (EM) is the main element in the electromechanical part. It works as a converter of electrical to mechanical energy at motor mode, as a converter of the mechanical to electrical energy at generator mode and as a converter of electrical and mechanical energy to thermal energy at breaking mode of operation. The transmission (T) and the working machine (WM) are the mechanical elements of the electromechanical part. The PSC connects the source of electrical energy to the electric machine and adjusts the parameters (voltage and frequency) of the machine. The sensors (S) receive information about the current behavior of the elements and transmit it to the CB. The CB processes the information from the feedbacks and the reference input and forms a control signal which is applied to the input of PSC for the proper control of the EM parameters.

The algorithm of the design of such electromechanical system may be formulated as follows: selection of EM and T according to the type of the WM and the work that must be performed; selection of PSC according to the type of EM and the requirements for operating modes of the WM; mathematical description of the mechanical part of the system as an object of control; mathematical description of the PSC as an element of the control system; mathematical synthesis of controller based on the obtained object for control and formulated requirements to its behavior in statics and dynamics; creating structure models based on the mathematical descriptions of the elements; testing the behavior of the model with control and disturbing inputs; setting the parameters or changing the structure of the controller for providing the desired system behavior; a physical implementation of the system.

In the conducted study we have used the simplest mathematical model for the mechanical part – one mass model or solid attached mass of inertia to the motor shaft. The electric machine is a three-phase squirrel cage motor with rated power 22 kW and voltage 380 V. A frequency control of the induction motor has been chosen for motor speed regulation in a wide range under the rated speed. For enabling a fast transient response the Field Oriented Control or Vector control is suggested. Field Oriented Control (FOC) of a 3-phase AC motor involves imitating the DC motors' operation. All controlled variables are transformed to DC instead of AC via mathematical transformation. The goal is to control torque and flux

independently. There are two mathematical transforms in the FOC algorithm – Clark Transform, Park Transforms and their inverse. The Clarke transform uses three-phase currents  $I_A$ ,  $I_B$  and  $I_C$  to calculate currents in the two-phase orthogonal stator axis:  $I_\alpha$  and  $I_\beta$ . These two currents in the fixed coordinate stator phase are transformed to the  $I_{sd}$  and  $I_{sq}$  currents components in the d, q frame with the Park transform. The currents  $I_{sd}$ ,  $I_{sq}$  and the instantaneous flux angle  $\theta$ , calculated by the motor flux model, are used to calculate the electric torque of an AC induction motor [4, 5].

For realizing the models we have used the program medium of MatLab 7.12.0 (R2011a). The MatLab package name comes from the combination of Matrix Laboratory, mainly focused on processing of datasets (matrices and vectors). MatLab provides the richest library of functions the only problem is quickly to find those needed to solve a specific task. The entire library of functions is divided into sections. More general functions are part of the core of MatLab. The specific to a given area functions are included in a sections, named toolboxes. The full package Simulink contains about 30 sections. Simulink provides a graphical user interface (GUI) for building models as block diagrams. Simulink also includes a comprehensive block library of sinks, sources, linear and nonlinear components, and connectors and one can also create custom blocks. The interactive graphical environment simplifies the modeling process, eliminating the need to formulate differential and difference equations in a language or program. Simulink software is integrated with the MATLAB environment. It requires MATLAB to run, depending on it to define and evaluate model and block parameters. Simulink can also utilize many MATLAB features. For example, Simulink can use the MATLAB environment to: define model inputs; store model outputs for analysis and visualization; perform functions within a model, through integrated calls to MATLAB operators and functions. The Simulink software includes 16 standard block libraries. Some of them are Continuous blocks; Discrete blocks; Math operations; Signal routing; Sinks; Sources; User-Defined Functions etc. The Discrete library contains blocks that represent discrete time functions, such as Discrete transfer function and Discrete-time integrator. The Discrete-Time Integrator block in place of the Integrator block creates a discrete system. With the Discrete-Time Integrator block is possible to define initial conditions on the block dialog box or as input to the block to define an input gain (K) value, to define upper and lower limits on the integral. The block can integrate using the Forward or Backward Euler, and

Trapezoidal methods. The library User-defined function provides a MATLAB Function block, for including MATLAB code in models that generate embeddable C code.

Most important in the study of electromechanical systems is SimPowerSystem package. SimPowerSystems software belongs to the Physical Modeling product family and operates in the Simulink environment. The models are built using simple click and drag procedures. The SimPowerSystems has an object oriented libraries, where the Electric drive library is included. In the Extra Library a Discrete Control block is organized which contains discrete filters of first and second order and discrete controllers. There is a separate library "Machines".

The mathematical description of a system is given with the equations: (1) to (3) for the rotor and (4) to (6) for the stator of the AC machine; (7) the torque, obtained by the AC motor as an electromechanical converter and (8) a balance of moments (torques) of the machine shaft [4].

$$u_A = R_A i_A + \frac{d\psi_A}{dt} \quad (1)$$

$$u_B = R_B i_B + \frac{d\psi_B}{dt} \quad (2)$$

$$u_C = R_C i_C + \frac{d\psi_C}{dt} \quad (3)$$

$$u_a = R_a i_a + \frac{d\psi_a}{dt} \quad (4)$$

$$u_b = R_b i_b + \frac{d\psi_b}{dt} \quad (5)$$

$$u_c = R_c i_c + \frac{d\psi_c}{dt} \quad (6)$$

$$\vec{M} = k(\vec{\psi} \times \vec{i}) \quad (7)$$

$$J \frac{d\vec{\omega}_m}{dt} = \vec{M} - \vec{M}_n \quad (8)$$

The equations contain the instantaneous voltages  $u$ , currents  $i$  and magnetic fluxes  $\psi$  of the stator and the rotor, and the active resistance  $R$  of the phase windings. Typically, the windings are connected symmetrically so  $R_A = R_B = R_C = R_S$  is the active resistance of the stator winding,  $R_a = R_b = R_c = R_R$  - active resistance of the rotor winding.

In the equation (8):  $J$ ,  $\text{kgm}^2$  is the moment of inertia of the shaft of the machine, taking into account both the inertia of the machine, and converted to the shaft inertia of the operating mechanism and transmission;  $\omega$ ,  $\text{rad/s}$  is an angular

velocity of the shaft of the machine;  $\vec{M}_H$  (Nm) – torque of the operating mechanism, aligned with the shaft, as a whole, it may be a function of the speed and angle of rotation.

Because the models used to be detailed, we could create subsystems to represent the model. A standard Simulink method is used to do this. All the elements are enclosed in a bounding box with the mouse and then "Create Subsystem" from the „Edit window" is being selected.

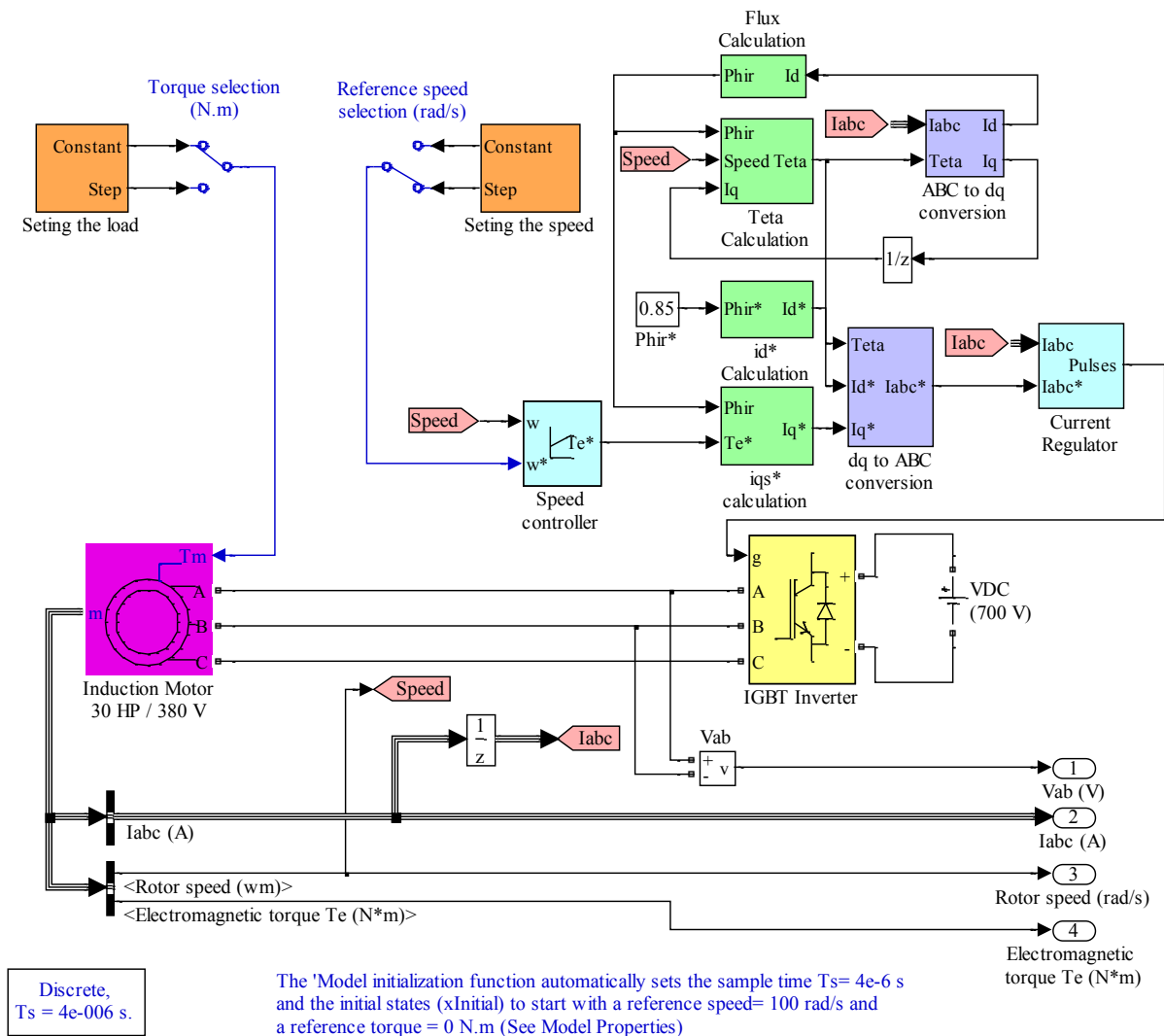
### III. Results and discussion

The created model of the described electromechanical system is presented in Figure 1.

A three-phase motor rated 30 HP (22kW), 380 V, 2940 rpm is built with standard Simulink® block from the library SimPowerSystems>Extra Library>Machines. The base frequency of the sinusoidal reference wave is 50 Hz. The machine's rotor is short-circuited. The load torque  $T_m$ , applied to the machine's shaft is manually set by choosing one of the outputs of the block "Setting the load". The initial motor speed is set to zero.

The induction motor is fed by a PWM inverter, built with a block from the library SimPowerSystems > PowerElektronics > Universal Bridge. The block implements a bridge of IGBT-Diodes. Series RC snubber circuits are connected in parallel with any of the switches. The inputs of the Inverter are connected to a DC voltage source of 700 V. The outputs of the Inverter are applied to the stator windings of the Asynchronous Machine block. The firing pulses to the inverter are generated by the Vector control system, built from blocks of the SimPowerSystems library. The speed control loop uses a proportional-integral controller to produce the torque reference  $T_e^*$ . The motor flux and its position the angle  $\theta$  are calculated in the blocks "Flux calculation" and „ $\theta$  calculation". The phase currents  $I_{abc}$  are converted to quadrature-axis currents  $i_q$  and  $i_d$ . The reference  $i_q^*$  which controls the motor torque is calculated in the block "i<sub>qs</sub>\* calculation". The motor flux is controlled by the direct-axis current reference  $i_d^*$ , which is calculated in the block „i<sub>d</sub>\* calculation". The block "dq to ABC" is used to convert  $i_d^*$  and  $i_q^*$  into current references  $i_a^*$ ,  $i_b^*$ , and  $i_c^*$  for the current regulator which produces the control pulses for the Inverter.

Six sink and source blocks are used in the model to send signals to any destination from blocks that have the specified tag, these are feedback signals of the motor speed and currents.



**Figure 1.** Simulink model of a frequency controlled AC motor for driving transport systems in food industry

The two setting blocks provide both constant and step reference speed and torque selection. Two blocks Bus Selector are used to accept a bus as an input. The first selects the stator current and the second – the rotor speed and electromagnetic torque  $T_e$ . A Voltage Measurement block provides signal for saving the data.

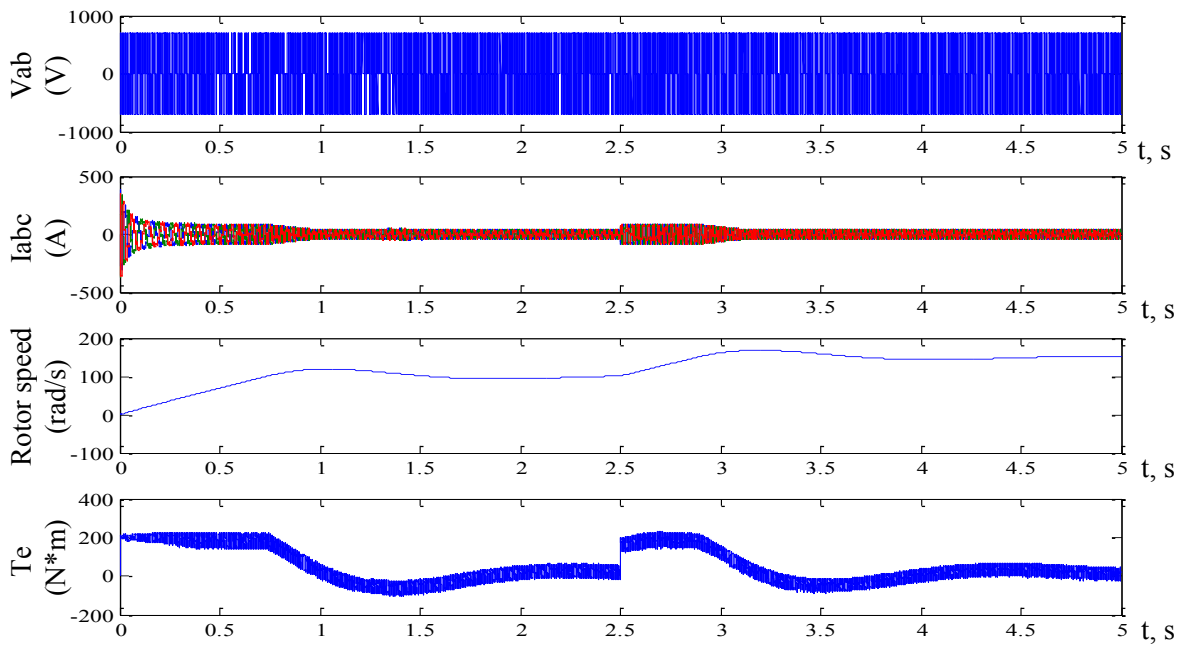
Four Sink Block Parameters provide an output ports for the voltage  $V_{ab}$ , currents  $I_{abc}$ , rotor speed and Electromagnetic torque.

Before starting the simulation in the Simulation/Configuration Parameters menu the simulation start time is set to (0), stop time – to (5s), Solver options type (fixed step) and solver (discrete).

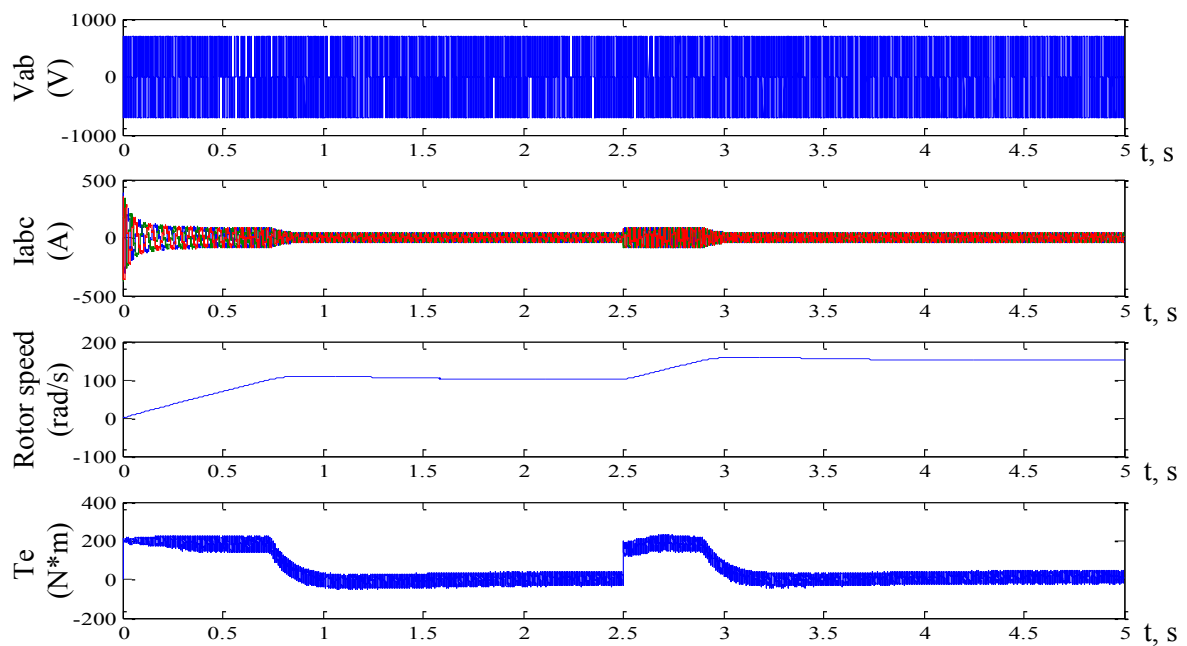
In Figure 2 the responses of the system to a change in the reference speed at no load at the shaft of the motor, applied at moment  $t=0$  is constant 100 rad/s

and the load at the shaft is zero (idle start). At  $t=2,5$  the reference speed changes from 100 rad/s to 150 rad/s.

From the diagrams it is observed that in the motor currents and in the electromagnetic torque waveform the noise is introduced from PWM inverter. The motor's inertia prevents this noise from appearing in the motor's speed waveform. The steady-state speed is reached after 2-2,5s. There are oscillations in the transient function of motor speed. As such oscillations are not desired a new tuning of the speed controller has been done. The results of the new simulation are presented in Figure 3. The transition process for the speed of motor can be adopted for technically optimal. The electromechanical system reaches its steady-state at time 2s with up regulation under 20%. The system has no steady error.



**Figure 2.** Transient processes with oscillation of the inverter output voltage ( $V_{ab}$ ) motor current ( $I_{abc}$ ), motor speed ( $\omega_m$ ) and electromagnetic torque ( $T_e$ ) to a change in the reference speed at no load



**Figure 3.** Optimal transient processes of the inverter output voltage ( $V_{ab}$ ) motor current ( $I_{abc}$ ), motor speed ( $\omega_m$ ) and electromagnetic torque ( $T_e$ ) to a change in the reference speed at no load

In Figure 4 the responses of the system to a change in the load torque (the disturbance) at the shaft of the motor are shown. At starting process the limit of the electromagnetic torque developed by the motor is 200Nm. At steady state condition (no load mode) the average value of  $T_e$  is zero. At time 2,5 s the load increases with a step of 80Nm and as a response the instantaneous value of the motor speed

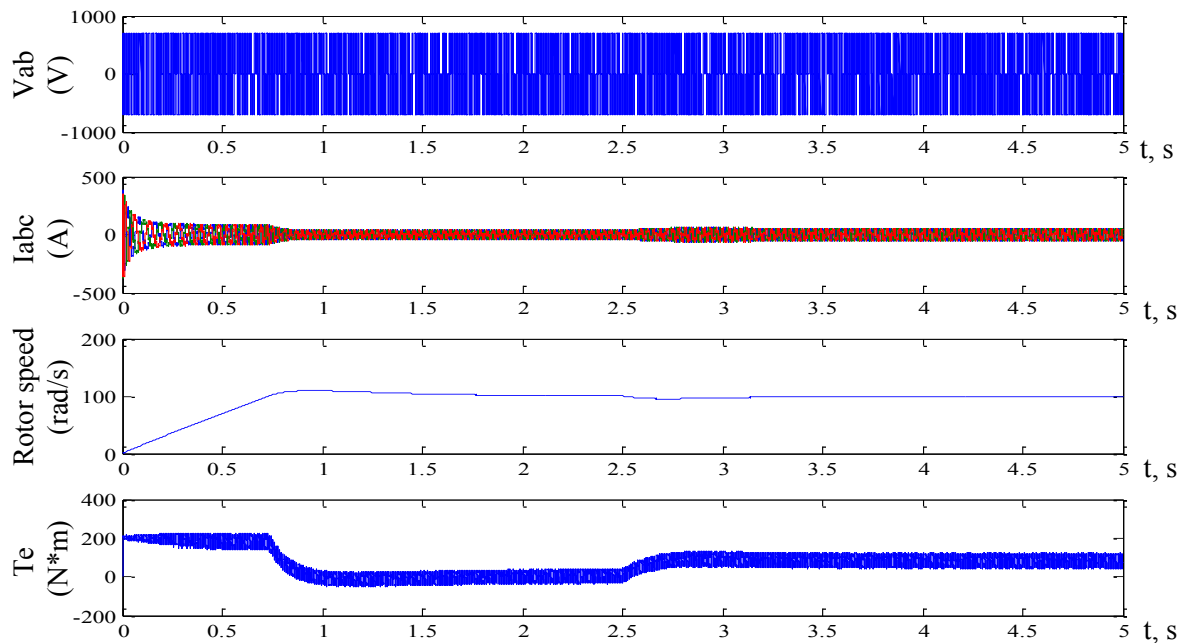
decreases not more than 15%. The transient process for the motor speed lasts about 2s and the system reaches its steady-state at the set speed of 100 rad/s and electromagnetic torque of 80 Nm.

For better observation of the PWM inverter's output it is better to use the zoom on the voltage  $V_{ab}$  waveform.



All the data of the observed parameters have been saved during the simulation and can be used as initial

states for future simulations.



**Figure 4.** Optimal transient processes of the inverter output voltage ( $V_{ab}$ ) motor current ( $I_{abc}$ ), motor speed ( $\omega_m$ ) and electromagnetic torque ( $T_e$ ) to a change in load torque

#### IV. Conclusions

The program medium of MatLab and Simulink is very suitable for the analysis of the electromechanical systems. This analysis includes interactions between mechanical, electrical, and control part of a system. This is possible because all the parts of the simulation interact with the extensive Simulink modeling library.

The results shown in the paper after testing the system to control inputs and disturbances show, that it is possible to find out after several steps the optimal tuning of the controllers for the desired and most convenient behavior of the system.

The system can be easy tested at any initial conditions and input signals. The responses of the system can be assessed at no load and at rated load mode of operation.

The simulation is necessary for obtaining the values of any of the parameters of the system which sets the system at a limit of stability. As at simulation are used some linear models of real elements it is important to define the reserve in stability to ensure it for the real physical system.

The first step to implementation the system in real world is to provide a test of a virtual object in Simulink combined with real controllers ( $\mu P$  or DSP).

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## STAND FOR TESTING THE INFLUENCE OF VARIOUS FACTORS ON TABLETING COFFEE

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**Abstract:** Recently, tableting is very popular in coffee production through so called technology ESE. ESE standard tablets are produced by technology that is the know-how of the manufacturers. This hampers the creation of methodologies for the design of machinery for tableting and packaging of coffee, and ultimately leads to monopolization of the market for tableting machines. To eliminate this gap in the knowledge about the process of tableting coffee it is necessary to identify the factors influencing it and to design a stand on which to imitate the target process.

**Keywords:** stand, coffee, "coffee pods", tableting

### I. Introduction

In the food industry there is an increasing application of tableting using different materials: sugar, concentrates, powders, tea, coffee, spices, food additives, etc. Tableting is a mechanical process of getting products or semi-finished products in the form of small, with a particular shape, tablets of bulk or powder materials [1].

It is mainly used to improve the technological properties of food materials, to prevent sticking, to allow the use of the material in small and equal portions, to improve hygiene, to increase the duration of storage, to give compactness of the device, and to ease the transportation. Recently tableting is very popular with the coffee production through so called technology ESE (fig.1).



**Fig.1** A tablet, an operating position of coffee and the sign of the technology ESE

ESE (which stands for 'easy serving espresso') is a technology for simplification in the preparation of good espresso with a few simplistic actions through the use of 'servicing' (individually prepackaged) doses containing 7 grams of ground coffee, pressed and hermetically sealed between two layers of filter paper. E.S.E. tablets are prepackaged disposable doses of coffee that was roasted, measured, pressed and hermetically sealed between two layers of filter paper. The quantity of coffee, the fineness of grinding and the pressure are set so as to obtain the

best espresso. The technical characteristics of the ESE dose are consistent with the camera contained in the ESE espresso machine. ESE standard is more easily recognizable to consumers and has the following advantages [2]:

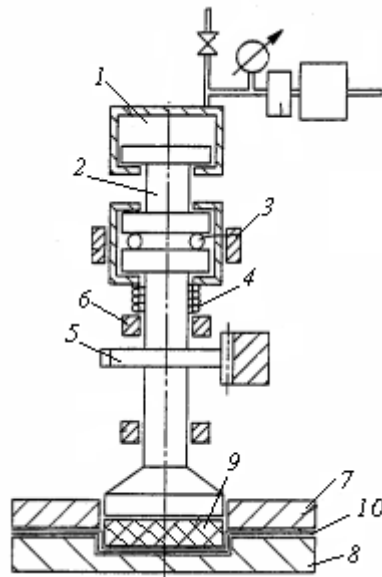
- easy to be used
- saves time
- easy to be cleaned
- Eco-friendly
- easy compatibility between the machines and the tablets

- freedom of choosing different brands of machines and tablets on the market
- constant quality espresso.

ESE standard tablets are produced by technology that is the know-how of the manufacturers. Reports of factors influencing tableting process and the limits within which their values change are not published. This hampers the creation of methodologies for the design of machinery for tableting and packaging of coffee, and ultimately leads to monopolization of the market for tableting machines. To eliminate this gap

in the knowledge about the process of the coffee tableting it is necessary to identify the factors influencing it and to create a stand on which to imitate the target process.

## **II. Formulation of basic functions, the structure and the technical requirements for the construction of the stand for testing the tableting coffee process.**



**Fig. 2** Working position of the machine for coffee tableting according to the patent for an invention of Illy

In the majority of the machines for coffee tableting are used embodiments of the structure, which is published in the patent of the ESE system creator - Illy [3].

The figure (Figure 2) shows the operating position of a coffee tableting machine of the company (creator of ESE system) - Illy. It consists of a pneumatic cylinder 1, a piston - punch 2, bearing unit 3, a spring for automatic raising of the piston 4, unit for rotating the punch at the end of compression 5, guide 6, guides of the strip of filter paper 7 and 8. 9 and 10 denote respectively the coffee tablet and the filter cloth. Due to the need of closing the tablet with filter paper the compression process is one-sided, as to expel the air a further rotating motion is used.

The operation of the machine in the operating position is as follows: Upon applying pressure in the

pneumatic cylinder 1, the piston 2 together with the punch is moved downwards by the action of the rack and the gear wheel 5 is rotated. The friction is reduced by the bearing unit 3. Within the matrix 8 is formed the tablet 9, which in the next position is covered with a filter paper adhered to the lower paper layer 7.

Factors influencing the tableting process can generally be divided into factors associated with the characteristics of the material entering the machine and factors related to the specifics of the tableting process - figure 3. In the work [4] the factors influencing coffee tableting process are detailed and hypotheses about changes in quality of coffee beverage caused by each of them are constructed.



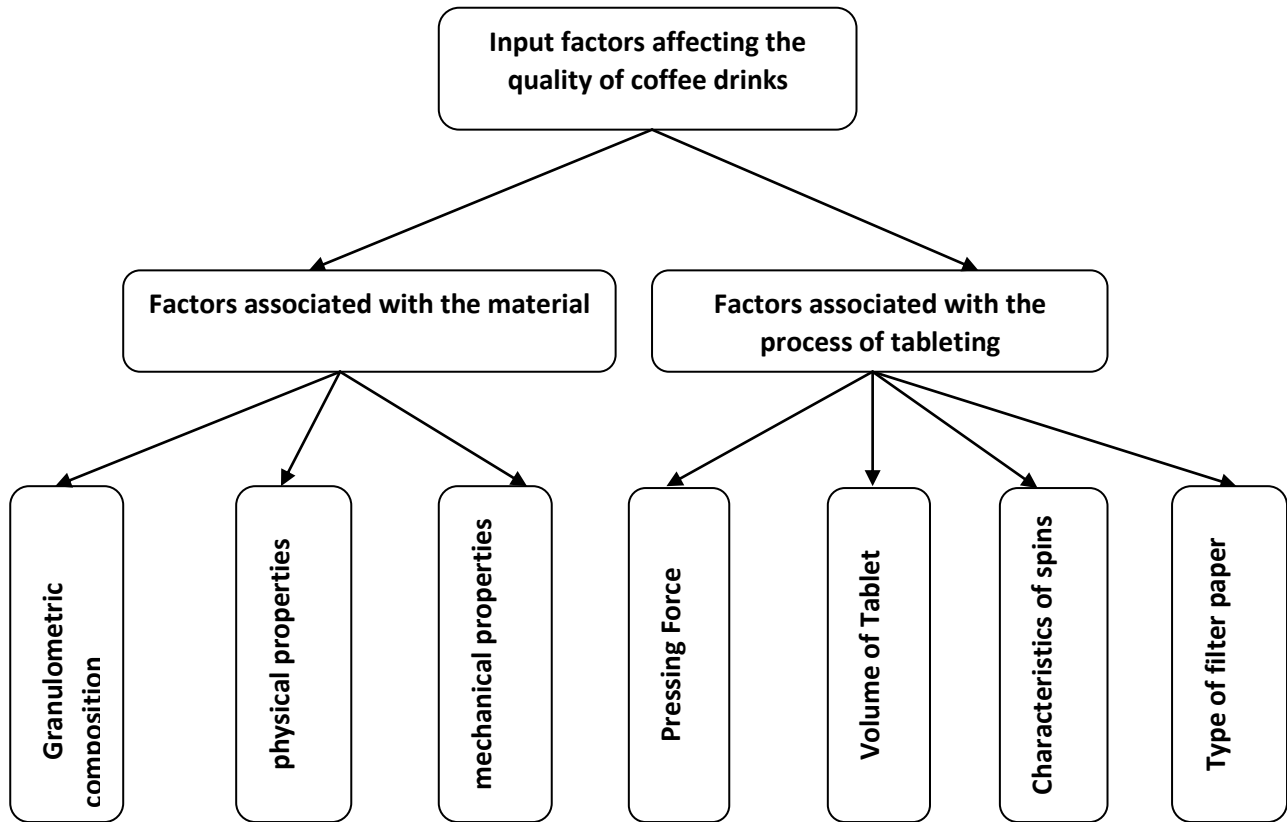


Fig. 3. Classification of the factors affecting the quality of the coffee beverage in the process of coffee tableting

Analysis of the structure of the operating position of the coffee tableting machine and the factors influencing the process, and hence the quality of the coffee beverage gives the opportunity to determine the structure and the functions which the stand must do (fig.4):

- Ability to change the volume of the tablet.
- Ability to change the effort of pressing, and thus the pressure on the powder / coffee /.
- Ability to measure the effort of pressing.
- Ability to change the angle of rotation of the punch.
- Ability for handling the filter paper in the required by the ESE standard form.
- Ability to stick the filter paper.

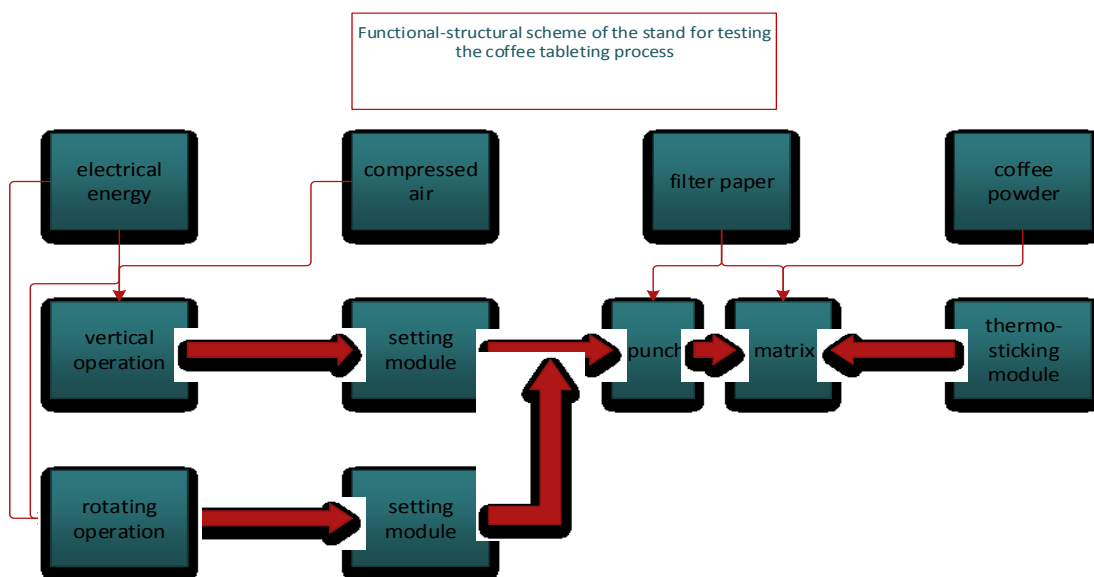


Fig. 4. Functional-structural scheme of the stand for testing the coffee tableting process

### III. Structure of the stand for testing the coffee tableting process

In the Department of machines for food industry at Technical faculty of University of Food Technologies in Plovdiv, a 3D model construction of the stand for testing the coffee tableting process is created.

The stand (fig.5) consists of a stand 1, a unit changing the effort of pressing 2, an adjustable stop to stop at various points to change the volume of the tablet, a unit for travelling 3 containing slider fitted with punch 4, matrix 5, a unit for rotation with adjustable stop and a scale to measure 6 and a base plate 7.

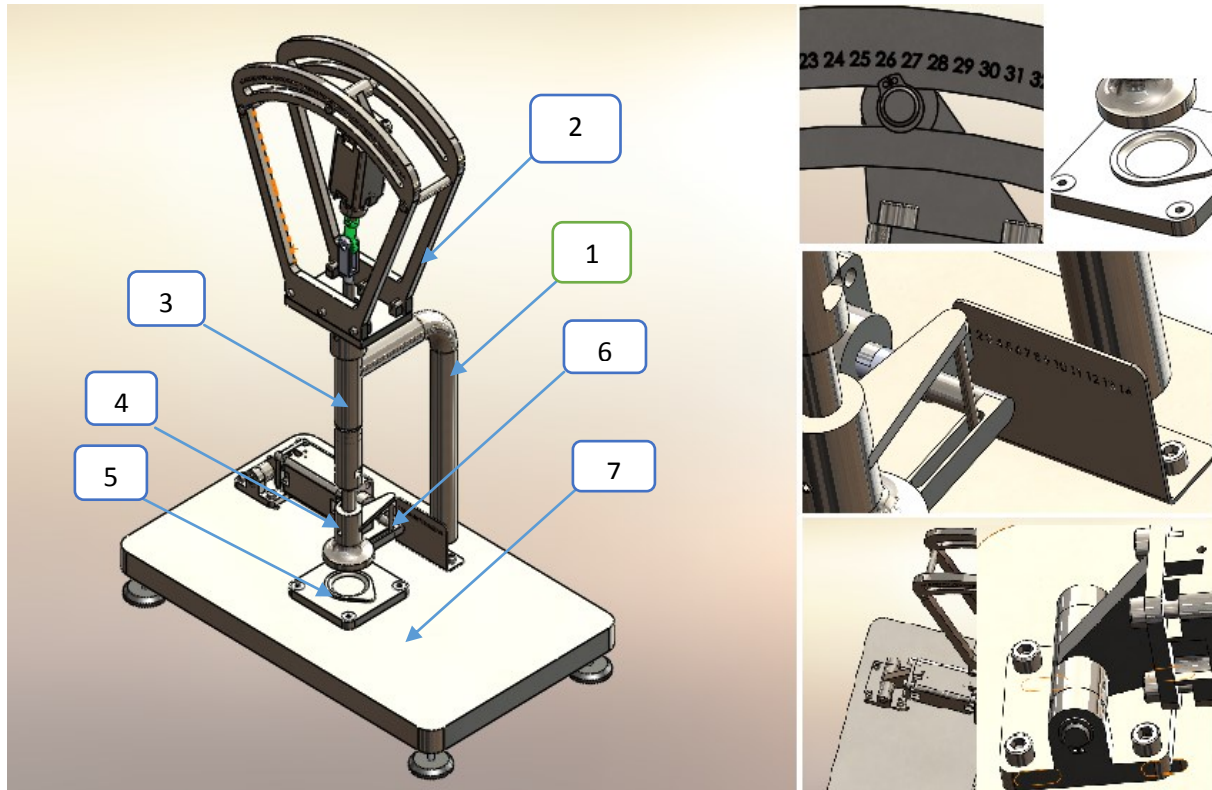


Fig. 5. Stand for coffee tableting process /common look and close up the main units /

The operation of the stand is as follows (fig.5):

In coffee grinder / outside the stand / with a possibility for adjusting the particle size of the milled particles is prepared coffee, which will be subjected to compression. Before the start of individual preparation of tablets, the top and bottom filter paper for future tablets are molded on the stand. On the bottom of the mold (5) is placed molded sheet of filter paper. It is packed with 7 grams of coffee by dosing spoon. The pneumatic cylinder of the unit for changing the force of the compression (2) is tilted in the required angle, so that the projection of the force developed by the pneumatic cylinder in the vertical axis corresponds to the necessary for the experiment. The rotation angle of the punch (6) is set by moving the adjustable support. The air supply to the vertical cylinder is provided, while at the end of travel the plunger rotating is carried out (4). The plunger rises (4) and on the compressed tablet is placed manually molded filter paper, and then the plunger (4) is lowered again on the tablet. In the closed position of

the plunger (4) and the matrix (5) the thermo-sticking unit comprises a paper / not shown /, and the two parts of the filter paper - an upper one and a bottom one are sealed. This tablet is ready for extraction.

### IV. Conclusions

1.The problem of the lack of knowledge about the factors influencing coffee tableting and how they affect the quality of the coffee beverage, which in turn makes it impossible to create a formalized methodology for the design of a coffee tableting machine.

2.The functions needed to carry out the future stand for testing the tableting process are defined.

3.A functional structure diagram of a stand for testing the coffee tableting process is created.

4.A 3D model construction of a stand for testing the coffee tableting process is created.

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## NON-TRADITIONAL YOGURT FROM COW'S MILK ENRICHED WITH PEA PROTEIN

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**Abstract:** Proteins of plant origin present a good alternative for partial substitution of animal milk in the production of different dairy foods and beverages. Selected strains of lactobacilli *Lactobacillus acidophilus* 2; *Lactobacillus delbrueckii ssp. bulgaricus* BB; *Lactobacillus delbrueckii ssp. bulgaricus* GB; *Lactobacillus casei ssp. casei* 1Ch; *Lactobacillus helveticus* I were examined for their ability to grow in cow's milk enriched with pea protein. The best mixing ratio of pea milk and 10% skimmed cow's milk to produce quality products with each strain was determined. Yogurts with high concentration of viable cells (over  $10^9$  cfu/cm<sup>3</sup>) and moderate titratable acidity, which can be administered as functional foods, providing the body with the necessary amount of useful microflora to conduct its inherent preventive and prophylactic function, were obtained.

**Keywords:** *Lactobacillus*, pea protein, pea milk, functional food, yogurt

### I. Introduction

To restore and maintain the balance of the microflora in the gastrointestinal tract the introduction of beneficial microorganisms (lactobacilli and bifidobacteria) by concentrates of viable cells called probiotics or functional foods is done. To perform their inherent preventive and prophylactic role the viable cell concentration of lactobacilli and bifidobacteria should exceed  $10^8$  cfu/g/cm<sup>3</sup>.

Generally, probiotic bacteria are introduced in the organism in the composition of yoghurts and other fermented yogurt beverages [7, 9, 10]. Today, the administration of probiotic preparations in the composition of non-milk-based foods and beverages or in the form of tablets, capsules, lyophilized preparations is gaining more and more popularity [2, 11].

Soy milk is an excellent substrate for the development of lactobacilli and bifidobacteria with probiotic properties. There has been success in replacing part of cow's milk with soy milk in the production of a number of products available on the market [3]. By culturing symbiotic or single lactobacilli or streptococci strains Denkova and Murgov, 2001 [5] obtained soy fermented foods such as soy yogurt, soy acidophilic milk (with a probiotic strain of *Lactobacillus acidophilus*) and soy bifid yogurt (with a probiotic strain of *Bifidobacterium bifidum*), as well as soy yogurt beverages (acidophilic-bifid beverage). The concentration of active cells exceeded  $10^9$  cfu/cm<sup>3</sup> and the shelf life of the soy fermented foods was over 20 days in a

refrigerator ( $4\pm 2^\circ\text{C}$ ). The authors found that the flavoring substances such as lactic, acetic, tartaric and malic acid, acetaldehyde, diacetyl generated by the metabolism of lactobacilli and bifidobacteria removed soy off flavor and soy fermented foods were accepted by the consumer.

Inspired by the success in the partial replacement of cow's milk with soy milk, researchers worldwide continue exploring the possibilities of other vegetable proteins to be used as substitutes for fresh cow's milk. Currently, another vegetable attracted researchers' attention - peas *Pisum sativum* [1, 12].

Dry field peas and other grain legumes, commonly referred to as pulses are the edible seeds of the pods of legume plants and are common worldwide. Peas are extensively used as sources of starch, fiber and protein due their economic viability with regard to fractionation, extensive growth worldwide and the simplicity of the dehulling (removal of the protective, high-fiber outer seed coat) procedures used in their processing. Pea beans also contain vitamins (A, E, H, PP, B vitamins, vitamin C (in a raw state)) and  $\beta$ -carotene, minerals (iron, zinc, copper, manganese, aluminum, boron, molybdenum, fluorine, vanadium, nickel, titanium, silicon, lead, selenium, zirconium, cobalt, chromium, potassium, phosphorus, sulfur, chlorine, calcium, magnesium, sodium). All substances in the pea beans have a beneficial impact on the body health. Peas are useful in a number of adverse health conditions like anemia and obesity. They also improve the functioning of the liver, kidneys and the cardiovascular system. Green pea beans exhibit antiseptic properties [8].

From an economic perspective pea protein costs are almost half as much as milk protein and 25% less than soy protein. In addition, the price of pea protein is relatively stable and resistant to fluctuations [8].

Several processes for the extraction of the gliadin and glutenin (core proteins of peas) can be applied. One option is water-vapor extraction of pea beans to obtain pea milk, rich in biologically active substances. But the most commonly used method is the grinding of the pea beans and concentrating the pea protein by drying to obtain an isolate which is subsequently resuspended to receive "pea milk". The pea protein isolate has a number of beneficial functional qualities such as good solubility in water, stability at high temperatures, good foaming capacity and high oil in water emulsifying power [6] and good stability in terms of shearing and retorting [8, 13].

The pea milk is mixed with skimmed cow's milk at various ratios and is utilized by the microorganism as a novel substrate.

The aim of the present paper is to examine the possibilities of supplementation of skimmed cow's milk with pea milk for the obtaining of fermented probiotic foods.

## II. Materials and Methods

### Microorganisms

In the present research were used probiotic strains of lactic acid bacteria from the genus *Lactobacillus*: *Lactobacillus acidophilus* 2; *Lactobacillus delbrueckii* ssp. *bulgaricus* BB; *Lactobacillus delbrueckii* ssp. *bulgaricus* GB; *Lactobacillus casei* ssp. *casei* 1Ch; *Lactobacillus helveticus* I.

### Media

Sterile skimmed cow's milk with titratable acidity of 16-18°T.

LAPTg10-broth medium. Composition (g/dm<sup>3</sup>): peptone (Fluka) - 15,0; tryptone (Difco laboratories) - 10,0; yeast extract (Scharlau) - 10,0; glucose - 10,0; Tween 80 (MERCK) - 1,0; pH 6.6 to 6.8.

LAPTg10-agar medium. Composition (g/dm<sup>3</sup>): LAPTg10-broth medium; agar - agar - 15,0.

Medium for detection of molds and yeasts in milk and dairy products at 25°C according to ISO 6611/2004;

Baird Parker agar base (*Staphylococcus aureus*);

Medium for detection and isolation of *Salmonella* sp. by horizontal process according to BS EN ISO 6579/2003;

Chromocult TBX-agar (*E. coli*);

PCA agar (medium for the determination of TBA (total bacterial abundance) according to BS ISO 6610:2002;

Pea milk.

### Preparation of pea milk

Raw peas (*Pisum sativum* L.) were ground to flour in the laboratory mill. Samples (35 g) of ground pea flour were extracted with 140 ml of 50 mM Tris-HCl (pH 8.8) for 1 h at 4 °C with constant agitation and subsequently centrifuged (20 000 g, 20 min). The extraction was repeated twice. The supernatant, containing albumins and globulins, was dialysed at 4 °C for 48 h against distilled water and later on lyophilised [14, 15]. Pea isolate, containing 81.34% pea protein, was obtained and was used to prepare 10% pea isolate milk.

### Determination of the titratable acidity

Ten cm<sup>3</sup> of each sample were mixed with 20 cm<sup>3</sup> of distilled water. The titratable acidity was determined by titration of each sample with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink colour persisting over 1 min. One Torner degree (°T) corresponds to 1 cm<sup>3</sup> 0.1 N NaOH, needed for the neutralisation of an equivalent amount of organic acid, contained in 100 cm<sup>3</sup> of the cultural medium [4].

### Determination of the concentration of viable cells

Appropriate serial dilutions in saline solution of the obtained yogurts were prepared and the spread plate method was applied. 0.1 cm<sup>3</sup> of the last three dilutions was used to inoculate LAPTg10-agar (for the enumeration of lactobacilli and streptococci) or the respective elective solid medium (for the enumeration of the specific microorganisms). The inoculated Petri dishes were incubated for 3 days at optimum temperature for the growth of the respective microorganisms until the appearance of countable single colonies. The count of the colonies was then used to estimate the number of bacteria in the original sample.

Organoleptic assessment was performed in accordance with BDS 15612-83

### Statistical analysis

Data from triplicate experiments were processed using MS Office Excel 2010 software, at level of significance P<0.05.

## III. Results and discussion



The ability of probiotic lactobacilli strains to grow in skimmed cow's milk enriched with 4% pea isolate was examined.

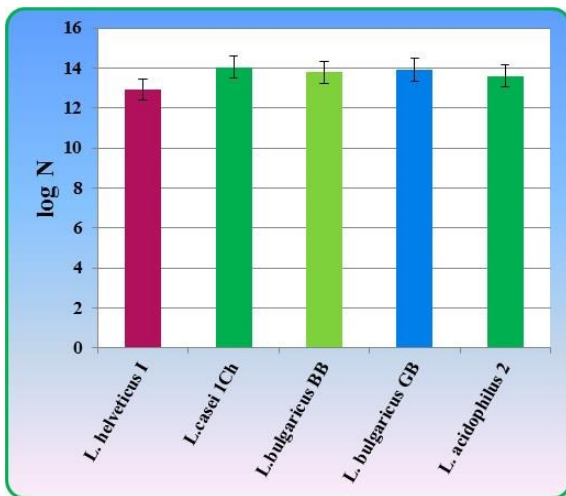
First, the microbial status of the cow's milk enriched with 4% pea isolate was determined (Table 1).

**Table 1.** Microbial status of skimmed cow's milk enriched with pea isolate

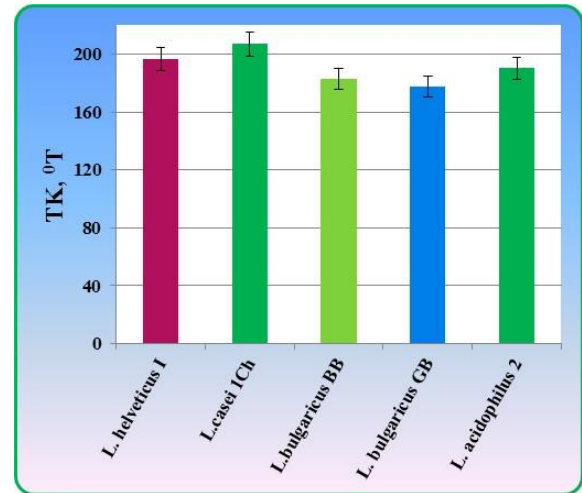
TBA, cfu/cm <sup>3</sup>	Specific microorganisms, cfu/cm <sup>3</sup>			Molds and yeasts, cfu/cm <sup>3</sup>
	<i>E.coli</i> (TBX-agar)	<i>St.aureus</i>	<i>Salmonella</i> sp.	
<1	<10	Not found	Not found	<10

The different lactobacilli strains were cultured under optimum conditions for 3-17 hours at 30-37°C. The results of the experimental studies are shown on Fig. 1a and Fig. 1b.

The experimental data show that the probiotic *Lactobacillus* strains can grow in skimmed cow's milk enriched with pea isolate. The titratable acidity of the medium was in the range between 120 and 200°T (Fig. 1b) and the concentration of active cells reached 10<sup>13</sup> - 10<sup>14</sup>cfu/cm<sup>3</sup> (Fig. 1a).



**Fig. 1a.** Concentration of viable cells of non-traditional yogurts, obtained with cow's milk, enriched with 4% pea isolate.



**Fig. 1b.** Titratable acidity of non-traditional yogurts, obtained with cow's milk, enriched with 4% pea isolate.

The prepared yogurts with *Lactobacillus acidophilus* 2; *Lactobacillus delbrueckii* ssp. *bulgaricus* BB; *Lactobacillus casei* ssp. *casei* 1Ch; *Lactobacillus delbrueckii* ssp. *bulgaricus* GB; *Lactobacillus helveticus* I possess not only the beneficial properties of the pea protein but also deliver high concentrations of viable cells of probiotic bacteria (1.10<sup>13</sup> - 1.6.10<sup>14</sup> cfu/cm<sup>3</sup>), which increases their biological value.

With the selected strains of probiotic lactic acid bacteria were prepared yogurts with skimmed cow's milk enriched with pea milk. The pea isolate concentration ranged from 0% to 10% in order to determine the optimum concentration of pea isolate for the preparation of good quality yogurts with each of the selected strains. The process was carried out at optimum temperatures for each strain for 4-17 hours.

Experimental data suggested that the best *Lactobacillus acidophilus* 2 yogurt was prepared with 4% pea isolate (Table. 2, Fig. 2a, Fig. 2b). Unfortunately, due to the high titratable acidity of the obtained product the probiotic strain *Lactobacillus acidophilus* 2 was not included in the further studies. Similar studies were conducted with the other probiotic cultures. The yogurts with best organoleptic properties were obtained with *Lactobacillus delbrueckii* ssp. *bulgaricus* BB in skimmed cow's milk enriched with 4% pea isolate and *Lactobacillus delbrueckii* ssp. *bulgaricus* GB in skimmed cow's milk enriched with 2% and 4% pea isolate (Table 3 and Table 4). The resulting yogurt contained significant amount of beneficial microflora (more than 10<sup>12</sup> cfu/cm<sup>3</sup>) (Fig. 3a and Fig. 4a) and titratable acidity of the medium reached between 90 and 130°T (Fig. 3b and Fig. 4b).

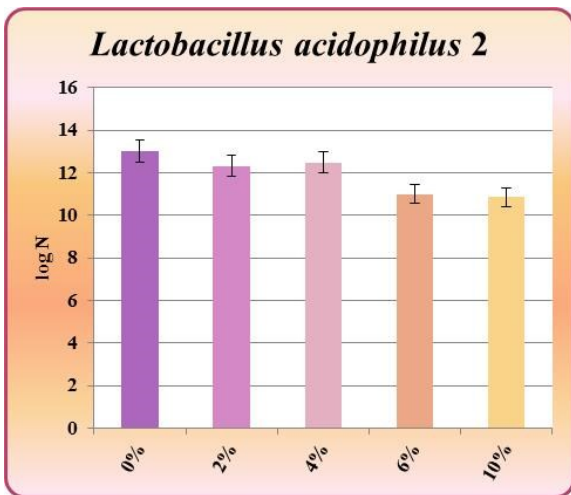


**Table 2.** Organoleptic evaluation and microscopic pattern of the pea yogurts obtained with *Lactobacillus acidophilus 2*

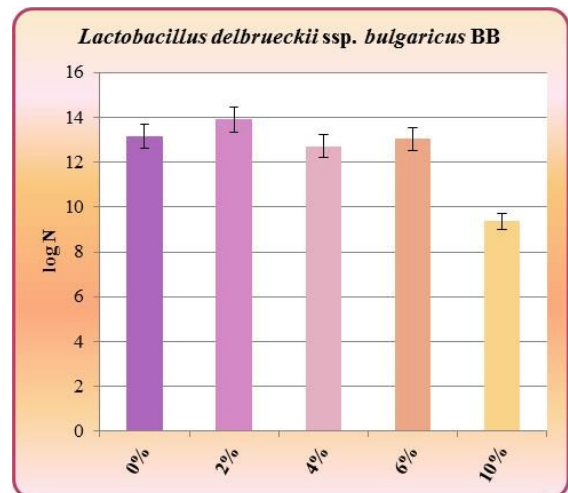
Pea isolate, %	Organoleptic evaluation	Microscopic pattern
0 %	Typical acidophilic yogurt flavor with clear lemon off flavor	Rich microscopic pattern Short thickened rods
2 %	Typical acidophilic yogurt flavor, no lemon off flavor	Rich microscopic pattern Short thickened rods
4 %	Thick in texture, pea off flavor	Rich microscopic pattern Short thickened rods
6 %	Thick in texture, pea off flavor	Rich microscopic pattern Short thickened rods; appearance of chains
10 %	Thick in texture, pea off flavor	Short rods, slow cell division, long chains

**Table 3.** Organoleptic evaluation and microscopic pattern of the pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus BB*

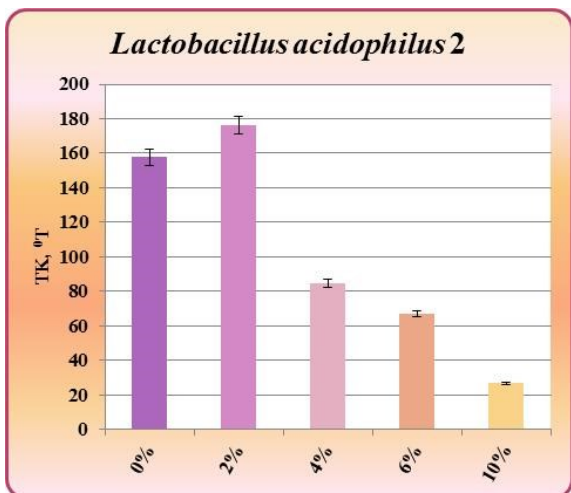
Pea isolate, %	Organoleptic evaluation	Microscopic pattern
0 %	Pleasant yogurt flavor	Rich microscopic pattern Short thickened rods
2 %	Less acidic; pleasant yogurt flavor	Rich microscopic pattern Short thickened rods
4 %	Lighter taste; less acidic; thicker in texture; pleasant yogurt flavor, no pea off flavor	Rich microscopic pattern Short thickened rods; appearance of chains
6 %	Thick in texture, pea off flavor	Smaller amount of single rods, predominant chains
10 %	Thick in texture, pea off flavor	Smaller amount of single rods, predominant chains



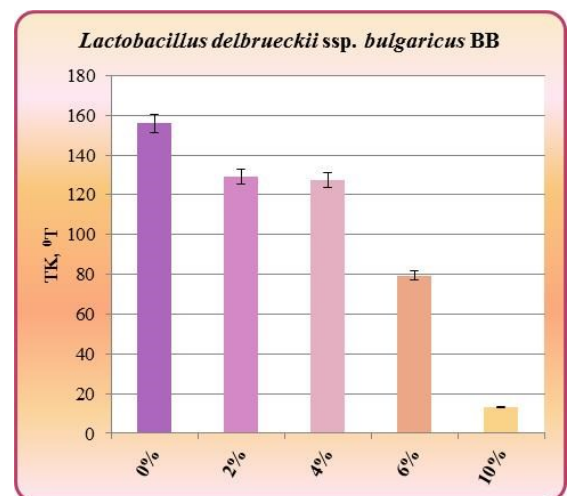
**Fig. 2a.** Concentration of viable cells of pea yogurts obtained with *Lactobacillus acidophilus 2*.



**Fig. 3a.** Concentration of viable cells of pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus BB*.



**Fig. 2b.** Titratable acidity of pea yogurts obtained with *Lactobacillus acidophilus 2*.

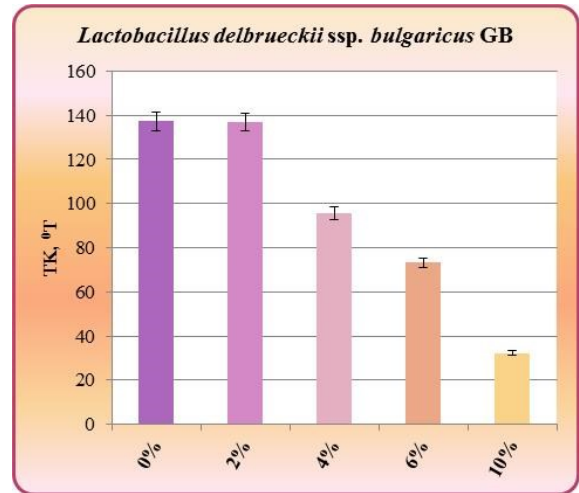


**Fig. 3b.** Titratable acidity of pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus* BB.

Yogurts enriched with pea isolate with good organoleptic evaluation were obtained with the probiotic strains *Lactobacillus helveticus* I and *Lactobacillus casei ssp. casei* 1Ch as well (Table 5 and Table 6). The change in the concentration of viable cells and the titratable acidity of both yogurts are shown on Fig. 5a, Fig. 5b, Fig. 6a and Fig. 6b.

**Table 4.** Organoleptic evaluation and microscopic pattern of the pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus* GB

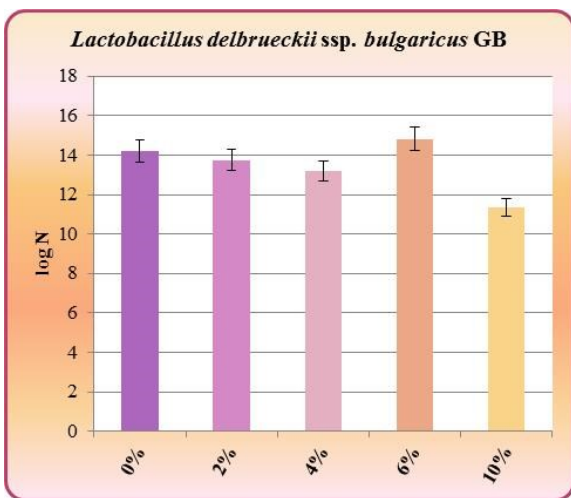
Pea isolate, %	Organoleptic evaluation	Microscopic pattern
0 %	Pleasant yogurt flavor	Rich microscopic pattern Short thickened rods
2 %	Pleasant yogurt flavor	Rich microscopic pattern Short thickened rods
4 %	Pleasant mild yogurt flavor	Rich microscopic pattern Short thickened rods; appearance of chains
6 %	Thick, pea off flavour	Rich microscopic pattern Short thickened rods
10 %	Thick, pea off flavour	Small amount of cells



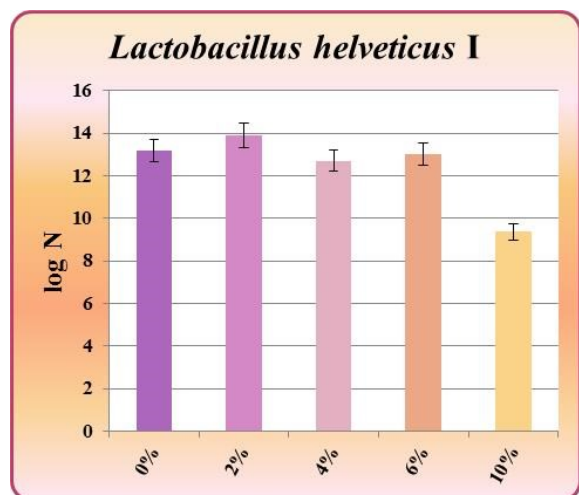
**Fig. 4b.** Titratable acidity of pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus* GB.

**Table 5.** Organoleptic evaluation and microscopic pattern of the pea yogurts obtained with *Lactobacillus helveticus* I

Pea isolate, %	Organoleptic evaluation	Microscopic pattern
0 %	Yogurt flavor; fruity off flavor	Rich microscopic pattern Short thickened rods
2 %	Yogurt flavor; pea off flavor	Rich microscopic pattern Short thickened rods
4 %	Pea off flavor	Rich microscopic pattern Short thickened rods
6 %	Pea off flavor	Rich microscopic pattern Short thickened rods
10 %	Pea off flavor	Small amount of cells



**Fig. 4a.** Concentration of viable cells of pea yogurts obtained with *Lactobacillus delbrueckii ssp. bulgaricus* GB.



**Fig. 5a.** Concentration of viable cells of pea yogurts obtained with *Lactobacillus helveticus* I.

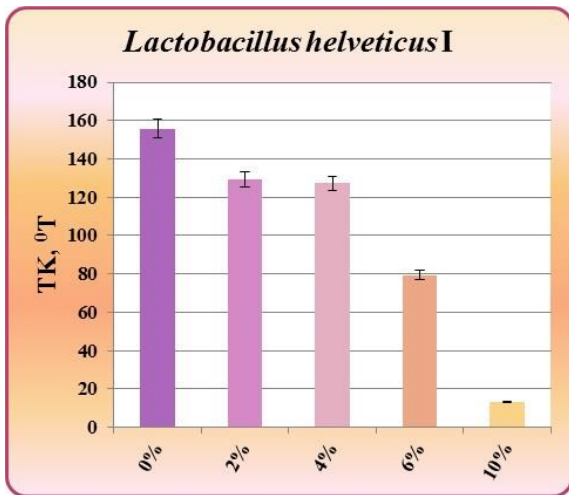


Fig. 5. Titratable acidity of pea yogurts obtained with *Lactobacillus helveticus I*.

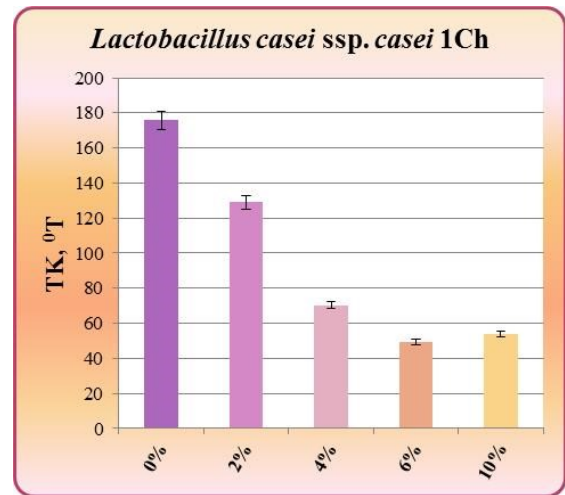


Fig. 6b. Titratable acidity of pea yogurts obtained with *Lactobacillus casei ssp. casei 1Ch*.

Table 6. Organoleptic evaluation and microscopic pattern of the pea yogurts obtained with *Lactobacillus casei ssp. casei 1Ch*

Pea isolate, %	Organoleptic evaluation	Microscopic pattern
0 %	Pleasant yogurt flavor, cheesy off flavor	Fine short rods
2 %	Pleasant yogurt flavor, thick in texture, no pea off flavor	Fine short rods
4 %	Thick in texture, pea off flavor	Fine short rods, appearance of chains
6 %	Thick in texture, pea off flavor	Larger amount of chains
10 %	Thick in texture, pea off flavor	A huge amount of chains, slow cell division

The increase in the amount of pea isolate led to the appearance of pronounced off flavor in all yogurts. In the experiments with *Lactobacillus helveticus I* even the addition of small amounts of pea isolate gave the yogurts pronounced pea off flavor. Therefore, *Lactobacillus helveticus I* was not included in the further research.

With the selected concentrations of the pea isolate for each strain yogurts were prepared and stored at refrigerated conditions ( $4\pm 2^\circ\text{C}$ ) for 15 days.

Upon storage of the yoghurts in a refrigerator for 15 days the number of living cells of the probiotic cultures decreased with 1-2log N and the titratable acidity increased to 120 - 140°T (Table 7, Fig. 7).

Table 7. Change in the titratable acidity of the yogurts enriched with pea isolate during storage at refrigeration temperature ( $4\pm 2^\circ\text{C}$ ) for 15 days

Probiotic strain	Pea isolate, %	Day 1	Day 5	Day 10	Day 15
<i>L. d. ssp. bulgaricus</i> BB	4 %	95,00	101.15	108.3	136.8
<i>L. casei ssp. casei</i> 1Ch	2 %	105,45	123.10	132.1	132.05
<i>L. d. ssp. bulgaricus</i> GB	2 %	108,30	112.10	118.1	120.2
<i>L. d. ssp. bulgaricus</i> GB	4 %	74,10	94.50	119.7	122.55

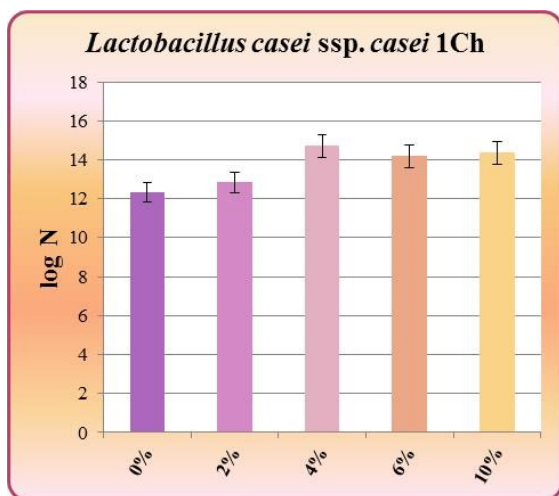


Fig. 6a. Concentration of viable cells of pea yogurts obtained with *Lactobacillus casei ssp. casei 1Ch*.

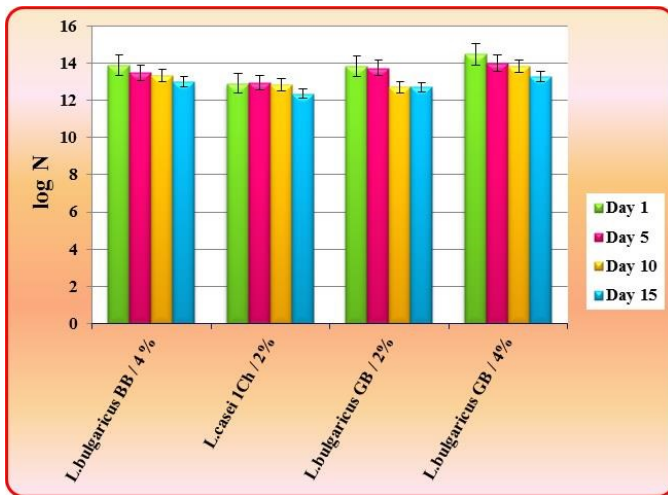


Fig. 7. Change in the concentration of viable cells of the yogurts enriched with pea isolate during storage at refrigeration temperature ( $4\pm 2^{\circ}\text{C}$ ) for 15 days.

From the obtained yogurts enriched with pea isolate only those with the probiotic strain *L. bulgaricus* GB allowed 15 days of refrigerated storage, maintaining moderate titratable acidity, while the other yogurts enriched with pea protein were suitable for consumption for only 10 days of storage in a refrigerator (Table 7). These yogurts maintain high concentration of active cells in the process of storage (Table 7).

#### IV. Conclusion

Probiotic *Lactobacillus* strains able to grow in skimmed cow's milk enriched with pea isolate were selected: *Lactobacillus acidophilus* 2; *Lactobacillus delbrueckii* ssp. *bulgaricus* BB; *Lactobacillus delbrueckii* ssp. *bulgaricus* GB; *Lactobacillus casei* ssp. *casei* 1Ch; *Lactobacillus helveticus* I. Screening to determine the best percentage of addition of pea isolate to the 10% skimmed cow's milk to produce quality yogurts using each of the selected strains was carried out. Non-traditional pea protein yogurts with high concentration of viable cells (over  $10^9$  cfu/cm<sup>3</sup>) and moderate titratable acidity which can be stored in a refrigerator for at least 10 days were obtained. They can be used as fermented probiotic foods, providing the body the necessary amount of useful microflora to conduct its inherent preventive and prophylactic role.

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## SCREENING OF BACTERIAL LIPASE PRODUCERS

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**Abstract.** Bacterial cultures from the collection of the Department of Technology of Microbiological Synthesis of the St.Petersburg State Technological Institute were screened for their lipase producing ability. Among the tested cultures were 30 strains of bacteria. It was found that 19 bacterial cultures are able to produce extracellular lipase. The values of the activity and specific activity were found during submerged cultivation of the lipase producers. Dynamics of lipase accumulation by two bacterial cultures *Bacillus thuringiensis* and *Rhodococcus equi* was monitored.

**Key Words:** screening, extracellular, lipase, bacteria, specific activity, submerged cultivation, dynamics of lipase accumulation

### I. Introduction

Sources of lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are various organisms. They are produced by plants, animal and microorganisms. Lipases of microbial origin, mainly bacterial and fungal, represent the most widely used class of enzymes in biotechnological applications due to their stability, selectivity, and broad substrate specificity [1]. The extracellular bacterial lipases are of considerable commercial importance, as their bulk production is much easier. However, the high cost of lipases limits their application in industry and makes it relevant to search for new lipase producers among different groups of microorganisms.

Lipases find promising applications in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical industry, the dairy industry, the agrochemical industry, paper manufacture, nutrition, cosmetics, and pharmaceutical processing [2].

The aim of the current study was screening of lipase producers among the 30 cultures of bacteria from the collection of the Department of Technology of Microbiological Synthesis of the Saint-Petersburg State Institute of Technology.

### II. Materials and methods

The object of the current study were strains of bacteria cultures: *Agrobacterium sp.*, *Arthrobacter citreus*, *Arthrobacter globiformis*, *Azotobacter chroococcum*, *Bacillus cereus*, *Bac. mesentericus*, *Bac. megaterium*, *Bac. mycoides*, *Bac. polymyxa*, *Bac. subtilis*, *Bac. subtilis* 79 (329), *Bac. thuringiensis*, *Flavobacterium sp.*, *Flavobacterium sawor*, *Micrococcus corallines*, *Micrococcus*

*polychromus*, *Mycobacterium album*, *Mycobacterium brevicola*, *Rhodococcus equi*, *Mycobacterium luteum*, *Mycobacterium phlei*, *Nocardia rubra*, *Sarcina erythromyxa*, *Sarcina flava*, *Sarcina lutea*, *Mycobacterium sp.*, *Staphylococcus citreus*, *Pseudomonas sp.*, *Pseudomonas aurantiaca* 336/547, *Pseudomonas fluorescens*. Pure cultures were taken from the collection of microorganisms of the Department of Technology of Microbiological Synthesis of the Saint-Petersburg State Institute of Technology.

Stock cultures were cultivated on nutrient agar slants at a temperature of 28-30 °C for 2-4 days.

Screening of lipase producers was carried out in two stages. In the first step the presence/absence of the lipolytic activity was determined in agar medium. At the second step lipolytic activity was measured by quantitative method in liquid medium.

**Agar medium for screening of the bacterial strains for lipase production** has the following composition in (g/l): peptone – 10,0; NaCl – 5,0; K<sub>2</sub>HPO<sub>4</sub> – 1,0; CaCl<sub>2</sub> – 0,1; agar – 20,0. The medium was sterilized by autoclaving at an excess pressure of 50,7 kPa for 30 minutes. Separately prepared 20% aqueous solution of Tween 80 was sterilized at an excess pressure of 50,7 kPa for 30 minutes in an autoclave, and added to sterile medium. The concentration of Tween 80 in the culture medium was 5% (w/v). The culture medium was poured into sterile petri dishes. The agar plates were line inoculated with the bacterial strains and the plates were incubated at 30 °C for 1-5 days. Lipolysis was indicated by the appearance of zone of precipitated insoluble calcium salts of fatty acids around the line of inoculation. Cultures that formed such zones were selected for further studies.

**Submerged cultivation** of lipase producers was carried out in Erlenmeyer flasks with 100 mL culture medium on a rotary shaker (rotational speed - 230 min<sup>-1</sup>) at 28-32 °C. Inoculum for submerged culture was grown in two stages. In the first stage bacterial stains were cultivated on nutrient agar slants at a temperature of 28-30 °C for 2-4 days.

In the second stage bacterial cultures were introduced with inoculation loop into the liquid medium from agar slant. Liquid medium has the following composition in (g/l): olive oil – 5,0; Tween 80 – 5,0; peptone – 3,0; KH<sub>2</sub>PO<sub>4</sub> – 1,0; yeast extract – 1,0; NH<sub>4</sub>NO<sub>3</sub> – 1,0; MgSO<sub>4</sub>×7H<sub>2</sub>O – 0,5. Inoculum was cultivated for one day on a rotary shaker. Amount of inoculum was 5% of the volume of the sterile basal medium. Cultivation was performed for 1 to 7 days.

Cells from the culture liquid were separated by centrifugation at 4000 g. Protein concentration and lipase activity were determined in the supernatant.

**Lipase assay** was performed using titrimetric method [3]. Tween-80 was used as the lipase substrate. One ml of culture supernatant was added to 5 ml of a 40% solution of Tween-80 and 4 ml 0,5 M phosphate buffer (pH 7.0).

The enzymatic reaction was performed at 37 °C, shaking at 250 rpm for 24 hours. The reaction was stopped by addition of with 30 ml of 95% ethanol. The amount of fatty acids released from the Tween 80 was determined by titration with 0,05 M NaOH. One unit (U) of lipase activity was defined as the amount of enzyme capable of releasing one μmol of oleic acid per minute.

### III. Results and Discussion

In the first step screening was carried out in agar medium. The ability to produce extracellular lipase by bacterial cultures was determined by the formation of zone of calcium salts of fatty acids around the line of inoculation. Observation was carried out on the first, second and fifth days of cultivation. Growth of colonies was found among all bacterial cultures on the first day of cultivation. The screening of bacterial cultures for lipase production on solid agar medium is shown in Table 1.

Significant number of bacterial cultures produce extracellular lipase as shown in Table 1. On the first day of the cultivation zone of hydrolyses appeared in only 4 cultures - *Rhodococcus equi*, *Mycobacterium phlei*, *Sarcina erythromyxa*, *Mycobacterium sp.* On the second day of cultivation zone of hydrolyses was observed in 11 other cultures - *Staphylococcus citreus*, *Sarcina lutea*, *Sarcina flava*, *Mycobacterium*

*luteum*, *Bac. subtilis* 79 (329), *Bac. thuringiensis*, *Flavobacterium sp.*, *Bac. polymyxa*, *Bac. mesentericus*, *Azotobacter chroococcum*, *Arthrobacter citreus*. Totally 19 bacterial cultures showed lipolytic activity on the fifth day of cultivation.

Bacteria strains selected during the first stage of screening cultured in a liquid medium. Lipolytic activity and protein concentration was determined in the cultural liquid. Results obtained at the 3rd day of the cultivation, are given in Table 2.

**Table 1.** Screening of the bacterial strains for lipase production on solid agar medium

№	Culture	Lipolytic activity		
		Day		
		1	2	5
1	<i>Agrobacterium sp.</i>	-	-	-
2	<i>Arthrobacter citreus</i>	-	+	+
3	<i>Arthrobacter globiformis</i>	-	-	+
4	<i>Azotobacter chroococcum</i>	-	+	+
5	<i>Bac. megaterium</i>	-	-	-
6	<i>Bac. mesentericus</i>	-	+	+
7	<i>Bac. mycoides</i>	-	-	-
8	<i>Bac. polymyxa</i>	-	+	+
9	<i>Bac. subtilis</i>	-	-	-
10	<i>Bac. subtilis</i> 79 (329)	-	+	+
11	<i>Bac. thuringiensis</i>	-	+	+
12	<i>Bacillus cereus</i>	-	-	-
13	<i>Flavobacterium sawor</i>	-	-	+
14	<i>Flavobacterium sp.</i>	-	+	+
15	<i>Micrococcus corallines</i>	-	-	-
16	<i>Micrococcus polychromus</i>	-	-	-
17	<i>Mycobacterium album</i>	-	-	-
18	<i>Mycobacterium brevicola</i>	-	-	+
19	<i>Mycobacterium luteum</i>	-	+	+
20	<i>Mycobacterium phlei</i>	+	+	+
21	<i>Mycobacterium sp.</i>	+	+	+
22	<i>Nocardia rubra</i>	-	-	+
23	<i>Pseudomonas aurantiaca</i> 336/547	-	-	-
24	<i>Pseudomonas fluorescens</i>	-	-	-
25	<i>Pseudomonas sp.</i>	-	-	-
26	<i>Rhodococcus equi</i>	+	+	+
27	<i>Sarcina erythromyxa</i>	+	+	+
28	<i>Sarcina flava</i>	-	+	+
29	<i>Sarcina lutea</i>	-	+	+
30	<i>Staphylococcus citreus</i>	-	+	+

\* - wide zone

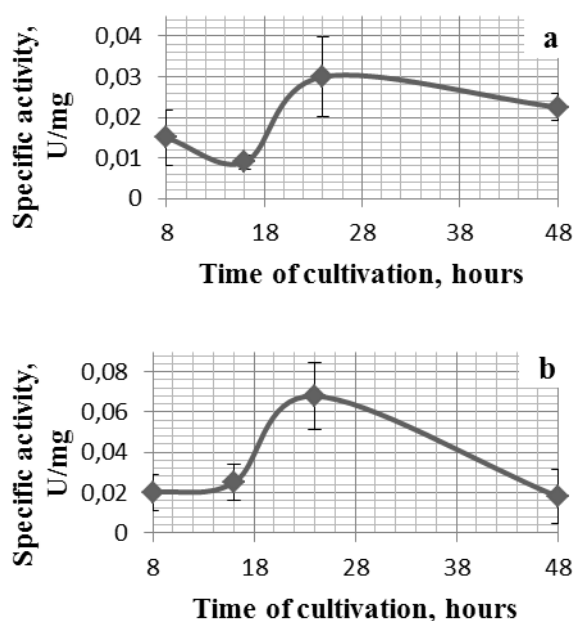
According to the data, shown in table 2, nine bacterial cultures can be picked out: the highest specific activity among bacterial culture showed *Mycobacterium sp.*, as well as the cultures of *Mycobacterium phlei*, *Arthrobacter citreus*, *Rhodococcus equi*, *Bac. mesentericus*, *Bac. polymyxa*, *Bac. thuringiensis*, *Flavobacterium bravor*, *Arthrobacter globiformis*.



Dynamic of lipase accumulation by two bacterial cultures *Bacillus thuringiensis* and *Rhodococcus equi* is shown in Figure 1.

**Table 2.** Screening of the bacterial strains for lipase production in liquid medium

№	Culture	Volumetric activity, U/ml	Protein concentration, mg/ml	Specific activity, U/mg
1	<i>Arthrobacter citreus</i>	0,241±0,012	0,359±0,019	0,670±0,070
2	<i>Arthrobacter globiformis</i>	0,155±0,008	0,303±0,016	0,510±0,050
3	<i>Azotobacter chroococcum</i>	0,109±0,007	0,449±0,023	0,240±0,027
4	<i>Bac. mesentericus</i>	0,330±0,016	0,540±0,028	0,610±0,060
5	<i>Bac. polymyxa</i>	0,299±0,015	0,517±0,025	0,580±0,060
6	<i>Bac. subtilis</i> 79 (329)	0,043±0,002	0,359±0,018	0,121±0,012
7	<i>Bac. thuringiensis</i>	0,229±0,014	0,404±0,020	0,570±0,060
8	<i>Flavobacterium sawor</i>	0,283±0,014	0,494±0,025	0,570±0,050
9	<i>Flavobacterium sp.</i>	0,118±0,006	0,730±0,030	0,161±0,012
10	<i>Mycobacterium brevicola</i>	0,082±0,005	0,620±0,030	0,132±0,014
11	<i>Mycobacterium luteum</i>	0,052±0,002	0,509±0,026	0,102±0,013
12	<i>Mycobacterium phlei</i>	0,297±0,017	0,382±0,015	0,780±0,080
13	<i>Mycobacterium sp.</i>	0,321±0,016	0,359±0,019	0,890±0,090
14	<i>Nocardia rubra</i>	0,064±0,003	0,239±0,012	0,269±0,027
15	<i>Rhodococcus equi</i>	0,278±0,014	0,449±0,023	0,620±0,060
16	<i>Sarcina erythromyxa</i>	0,116±0,006	0,860±0,040	0,136±0,014
17	<i>Sarcina flava</i>	0,064±0,003	0,438±0,023	0,147±0,015
18	<i>Sarcina lutea</i>	0,127±0,008	0,359±0,017	0,350±0,040
19	<i>Staphylococcus citreus</i>	0,030±0,002	1,305±0,068	0,023±0,002



**Figure 1.** Dynamic of lipase accumulation by *Bacillus thuringiensis* (a) u *Rhodococcus equi* (b)

For both cultures peak of activity occurs at the end of the first day of cultivating. It indicates a significant rate of biosynthesis of lipases by bacteria. Further optimization of the medium composition and cultivation conditions will increase lipase activity.

#### IV. Conclusions

As a result of screening of 30 bacterial cultures were found 19 strains of lipase producers. It might be well to point out that current study does not answer the question of what lipase producer has the greatest potential. Firstly, liquid culture media used for screening, probably is not ideal for all the producers. It is necessary to optimize composition of the medium (different carbon and nitrogen sources, various lipase inducers, and other factors, for example, metal ions Ca, Mg, Fe, etc) for maximization of the enzyme production. Secondly, the potential application of a particular lipase is defined not only by its high activity, but also by its substrate specificity (regiospecificity, FA-specificity, enantiospecificity), as well as its thermal stability and resistance to organic solvents.

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## **STUDIES OF PHYSICAL AND TECHNICAL CHARACTERISTICS OF BIOWASTE AS COMPONENTS OF GASIFICATION**

**A.A. Osmak, A.A. Seregin**

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*Proved promising use of plant biomass as a fuel due to the widespread introduction of bioenergy technologies. Justified the use of biomass for heat and electricity. The basic reasons for the use of organic materials as a source of renewable energy. The examples of industrial use of energy from biomass in the EU.*

*Investigated the variation of the calorific value of the product gas from the humidity of the incoming raw materials. By experiment investigated the suitability of various types of plant biomass (sunflower husks, hulls of buckwheat and oats) to further thermochemical conversion for alternative fuel.*

*The results of the technical analysis (moisture, ash, calorific value) of a number of analytical samples of agricultural waste.*

*Studied the elemental composition of certain types of plant material, which suggests that these agricultural residues are highly reactive fuel with a high yield of volatiles.*

**Keywords:** biomass, sunflower husks, buckwheat husks, oat hulls, wood, peat.

### **I. Introduction**

In recent years, in the world there is great interest in the use of biomass for heat and electricity, its involvement in the energy balance of the country. This is indicated by numerous studies in the European Union and the United States, aimed at identifying the best ways of using biomass energy [1, 3, 6].

Ukraine has significant biological resources, including the processing of biowaste in the form of food raw materials that can be used as alternative or additional fuels. At present, the consumption of biomass is limited mainly to wood and waste wood processing industry: about 1 million. Tons of fuel per year is used for heating private houses, as well as enterprises of timber and woodworking industries of the country [2, 4, 5].

Studies show that by diversifying sources of energy fuels country can expect the replacement of about 10 million tons of fuel per year, which is certainly a positive factor in ensuring the country's energy independence. Undoubted is the fact that the involvement of biological resources in the fuel balance of the country will benefit the environment as a result of the combustion of biomass, both directly and in different versions with coal-biomass mixtures, environmental performance processes of thermal processing of raw materials in which there is much biomass better than conventional [2, 6].

Biomass energy has significant advantages compared to fossil fuels and a number of other renewable energy sources, ensuring energy supply, improving living standards, increasing welfare. Energy systems based on biomass are a potential mechanism contributing to sustainable development

and environmental protection. Among the main reasons for this attention is worth noting:

- widespread availability, even in remote areas: biomass fuel is available wherever there are trees and crops, as well as processed food products and fibers;

- resource is used if necessary: biomass is to be stored fuel energy source, which at any moment could be used to power, unlike other renewable energy sources, which are characterized by irregular and / or seasonality;

- versatility: Biomass is a potential source of primary energy - liquid, gas, heat and electricity;

- no effect on the climate: Provided environmentally sound preparation and combustion, biomass energy does not cause climate change and greenhouse gas emissions.

Therefore plant biomass due to such their basic characteristics as renewability of this type of fuel, ecological cleanness in comparison with other types of fuel, no impact on the balance of free carbon in the atmosphere, leading to development of the "greenhouse effect", is considered one of the most "noble" fuels and is considered in many countries as a promising source of energy in the near future.

### **II. Materials and methods**

In the laboratory, it is examined the suitability of various types of plant biomass to further thermochemical conversion for alternative fuel.

Taking into account the technical characteristics of the plant biomass for the further work was selected sunflower husks, buckwheat and oat hulls, wood waste (chips), and peat.

Ash content was determined according to GOST 1.1.022-90, in accordance with GOST 27314-91

humidity, volatile substances according to GOST 6382-91, carbon and hydrogen in accordance with GOST 24081-88, GOST 8606-93 sulfur, and oxygen from the difference of 100% - programming components. The heat of combustion was determined according to GOST 147-95.

### III. Results and discussion

According to the authors, to begin the process of large-scale introduction of bioenergy technologies is necessary with the introduction of modern boilers, gas generators for the thermochemical conversion of renewable raw materials of organic origin. All other technologies for energy production from biomass (biogas, liquid fuels, energy crops) are no less important priority, but at this stage of technology development in our country are not able to quickly replace the traditional types of fuels for the production of thermal energy with the lowest investment costs and the shortest period payback.

On the basis of scientific research team of the Department of Theoretical Mechanics and resource-saving technologies of the National University of Food Technologies (NUFT) prepared a set of design documentation for which was made a prototype gas-generating energy complex Huck-3 (Figure 1).

Enterprise-based UV "Saharenergoservis" AK "Suter", p. Ustimovka, Vasilkovskaja district., Kyiv region. together with employees NUFT conducted preliminary and acceptance tests of the prototype Huck-3.



Figure 1. Prototype gas-generating energy complex Huck-3

In the study it was found that the gas generator is working steadily on raw humidity 20 ... 30%. Stuck of fuel in the mine was not observed. The produced Synthesis gas had a composition typical for generating gases produced in the so-called "reverse" process. The Calorific value of the product gas was also responsible standard size and ranged from 3.6 ... 4.94 MJ / m<sup>3</sup>. The Calorific value of the gas depended on the initial moisture content of the fuel (see Fig. 2).

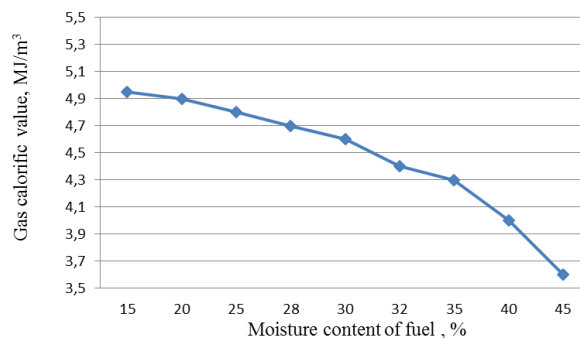


Figure 2: A plot of the calorific value of the product gas from the initial moisture content of fuel

The composition of plant biomass and, consequently, its physical and technical characteristics depend on the origin [2]. Significant impact on the gasification of biomass have characteristics such as moisture and ash content [7].

Moisture may be condensed and adsorbed, the amount of the latter depends on the ambient humidity. Wood moisture content may be 50% [2]. Agricultural waste, such as straw, contains about 10 ... 12% of water [2]. Moisture reduces the efficiency and effectiveness of the use of plant biomass as a fuel and also increases the cost of transportation.

Mineral content of the plant biomass varies within a wide range. Wood contains about 0.5% ash consisting essentially of carbonates, carboxylic acid salts and small amounts of silicon and sunflower husk it is to 30% [2]. The water-insoluble inorganic compounds reduce the enthalpy of the biomass.

For The generalization of the physical and technical characteristics of different types of biomass, we used the results of studies of a number of agricultural waste (husks of sunflower seeds, buckwheat husks, oat hulls), and the data of foreign authors [2, 7].

Results of analyzes based on the mass of workers:

Name	Indicator
Humidity,%	45,5
Ash,%	1,35

Carbon,%	30,5
Hydrogen,%	1,8
Sulphur%	0,05
Nitrogen,%	0,3
Oxygen%	10,1
Net calorific value, MJ / kg	8770

The elemental composition of the fuel ash is shown below:

Compound	Amount%
SiO <sub>2</sub>	25,74
TiO <sub>2</sub>	0,75
Al <sub>2</sub> O <sub>3</sub>	4,9
Fe <sub>2</sub> O <sub>3</sub>	3,38
CaO	43,81
MgO	6,43
SO <sub>3</sub>	0,15

Composition of the fuel and ash according to foreign researchers on the dry weight does not differ from that shown above. Moisture can vary from 45% to 60%.

Waste wood processing plants, as a rule, are dry sawdust, wood shavings and lumpy waste. An exception is the fine dust from sanding chipboard containing abrasives and resin. Humidity of the sample taken from the point of collection of waste in the open air was about 25%, and the operating heat of combustion of about 17.35 MJ / kg.

Specific weight of the sample taken is only 150 kg/m<sup>3</sup>. About half of the waste is "soft" with a particle size of about 0.2 ... 1 mm; others - swarf and chips with a maximum size of 50 mm.

The table shows the results of the technical analysis of a number of analytical samples of agricultural waste. In actual agricultural production humidity can be somewhat higher (2 ... 4%), and the heat of combustion, respectively, lower. The difference in the ash content is determined by the presence of extraneous inorganic inclusions.

**Table.** *Physical and technical characteristics of agricultural waste*

indicator	Type of waste		
	sunflower husk	oat hulls	buckwheat husk
Moisture, W,%	8,4	9,87	6,5
Ash, A,%	2,7	4,78	7,95
Heat of combustion, Q, MJ / kg	16,89	14,4	15,82

In the literature [2], the following data on the net calorific value of a number of similar materials: rice husk – 13,3 MJ/kg, sunflower husks – 15,4 MJ/kg, straw – 15,7 MJ/kg.

Thus, the heat of combustion of agricultural residues changed within a narrow range of 13.3 to 17,0 MJ / kg, and it is quite high. Humidity of

natural waste is at a level of 10% and an ash content is not exceeding 8%.

Waste has similar cell composition with a carbon content of about 50% oxygen - 42%. The low sulfur content and a moderate nitrogen content indicates that the emissions of oxides of sulfur and nitrogen in any combustion technology is unlikely to exceed 600



mg/m<sup>3</sup>. It should also be noted that the said agricultural residues are highly reactive fuel with large (about 80%) of a volatile substance.

In contrast to the organic part the mineral composition varies within very wide limits. Especially it refers to silica (40 ... 87%), iron (0.2 ... 7.7%), calcium (0.6 ... 30.6%) and potassium (6.2 ... 20%). All elements except alkaline material can impact render on the pollution of the heating surfaces.

The melting temperature of ash (after ashing in a muffle) is about 1300 °C (1200 °C to 1400 °C).

Peat is a product of decomposition of plant residues and did not manage to decompose elements of plants, the degree of decomposition increases with increasing depth of peat. It is distinguished horse, lowland and mixed types of peat.

In its natural state turf is heavily watered, so it requires pre-drying. In the air-dry peat moisture is 15 ... 25% [2].

It should also be noted that the peat contains a large enough volume (to 36%) of mineral substances. It is explained by silt and the remains of mineral salts from plants. The composition of the mineral part of peat has a significant impact on the process of gasification.

The elemental composition of the peat is on average: 3,4 ... 9,4% hydrogen, 54 ... 60% carbon, 0,5 ... 3,0% nitrogen, 28,5 ... 39,5% oxygen, 0,1 ... 1,5% sulfur. The calorific value of the fuel mass of peat varies over a fairly wide range, and it is 19,7 ... 25,1 MJ / kg [2].

Pyrogenic peat decomposition process is similar to the process of decomposition of wood. Young peat composition distillate approaches to wood, and the old to the brown coals. When heating the peat to a temperature of 100 °C passes the drying process. Next, the process proceeds to the step of pyrolysis, where first is released CO<sub>2</sub> and H<sub>2</sub>O. The most intensive decomposition into gaseous constituents starts from 150 °C.

It should be noted that the timber in its various spatial axes has different coefficients of expansion. For example, pine wood along the fiber expands to 20 times greater than across the fiber. Therefore, when heated to pyrolysis temperature in the wood structure stresses arise leading to formation of macro-and microscopic cracks. Due to these cracks, fast equalization of temperature occurs and drying process is enhanced by increasing of the reaction surface of the piece of wood. In this stand volatile gases and vapors which are also comprised of hydrocarbon compounds, carbon monoxide, water vapor, resin and benzene. Remains solid residual carbon.

Share of the resulting volatile components is crucial to the regime of burning solid fuel. In the literature on this subject can be found very different data. The values vary in a wide range from 65 to 87% [2, 7]. The reason for their spread can be different methods of investigation: differences pyrolysis temperature, residence time of the particles, and others.

#### **IV. Conclusions**

Analysis and study of the properties of plant biomass as a fuel for gas plants is done, which included the systematization of data received by domestic and foreign authors, and the results of their own research to determine the physical and technical characteristics of the raw materials, the elemental composition of raw materials and ash. The results obtained are the characteristics of organic materials formed the basis for the development of processes of thermochemical conversion of plant biomass.

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# EFFECT OF TEMPERATURE ON THE PROCESS OF PRESSING ORGANIC RAW MATERIALS

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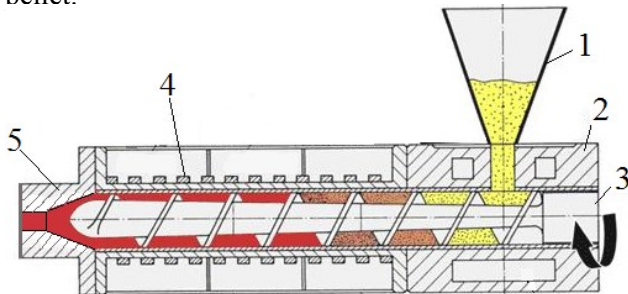
**Abstract:** The process of extrusion pelletizing particulate materials, such as sunflower and buckwheat husk, oak and pine chips, wheat straw and bran has been considered. It was investigated the effect of temperature of raw materials, compacting pressure and the diameter of the press-matrix on quality of fuel pellets.

**Keywords:** temperature, particulate materials, biofuels, pelleting.

## I. Introduction

Production of solid biofuels is an urgent requirement for fuel and energy complex of any developed country in the world, and therefore there is a requirement for research that would allow discovering the peculiarities of the process with granulation of fuel pellets.

In article [1] the problem of determining the optimal technical and technological parameters of the process of granulation extrusion was treated and was got the disperse of materials obtained depending influence of the main parameters of unit pressed pellet (Fig. 1) on the quality of the final product – the pellet.



**Figure 1.** Scheme of node compression screw extruder-granulator (1 – bunker, 2 - cylinder, 3 – screw, 4 – heater, 5 - forming head).

Regardless of the hardware design at process of granulation significant influence on the formation of granules of a material which has been granulated: raising of the temperature increases the density of granules, and also reduces energy consumption in the process of pressing [2].

The basic constituents of raw materials for solid biofuels are cellulose, hemicellulose and lignin. Lignin as an amorphous polymer is a kind of binding between cellulose fibrils, providing strength and rigidity of the cell wall (if cellulose with properties corresponding fixture, the lignin, which has a high compressive strength - concrete).

At low temperature processes (up to 160 ° C) reaction hydrolytic decomposition of carbohydrate

content and partial impolymerization of lignin to form low molecular weight fragments are predominant. Rising of the temperature of the process increases the degree of degradation of wood carbohydrates, and yet with lignin impolymerization reactions begins to compete re-polymerization his reaction.

Therefore, attached to change of the temperature of the process to 150-170 ° C of lignin in wood decreases with increasing temperature and process of lignin increases markedly, reaching 33-36% [3-7].

The aim of this work is to study the effect of temperature on the material quality (density) of the final product (pellets) considering structural and technological parameters of the equipment for its compaction during pressing extrusion.

## II. Materials and methods

To solve this problem a multifactorial experiment is proposed and to develop mathematical and statistical model of dependence of density fuel pellets produced from sunflower and buckwheat hulls, oak and pine shavings, wheat straw and bran from raw temperature, pressure and pressing channel diameter press-matrix.

$$\rho = f(t, P, d),$$

where

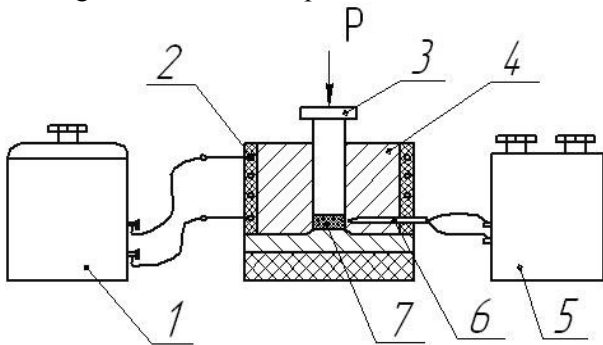
$\rho$  - density of pellets, kg/m<sup>3</sup>;  
 $t$  - temperature materials, ° C;  
 $P$  - pressure compression, MPa;  
 $d$  - diameter of the press matrix.

Factorial experiment of second order, which is used to describe non-linear objects, in our case, is represented by polynomial [8]:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_1x_3 + b_6x_2x_3 + b_7x_1x_2x_3 + b_8x_1^2 + b_9x_2^2 + b_{10}x_3^2$$



In Figure 2. a scheme of pilot plant for studying the influence of temperature on the process of pressing the materials is represented.



**Figure 2.** A scheme of pilot plant  
 1 - autotransformer 2 - electric heating coil, 3 - punch, 4 - press matrix, 5 – potentiometer, 6 - thermocouple, 7 – sample

Pressing is performing by using of a hydraulic press to press the matrix 4 with varying diameter punch 8–40 mm at a speed of pressing 0,005 m/s. Temperature press-matrix regulate by changing the voltage filed in the electric spiral 2 of isolated press-matrix 4. The temperature of press-matrix is measured by thermocouple 6, potentiometer connected to the direct current 5. Pressure was measured by a manometer.

The experiment was conducted for the following types of materials: sunflower and buckwheat husk, oak and pine chips, wheat straw and bran.

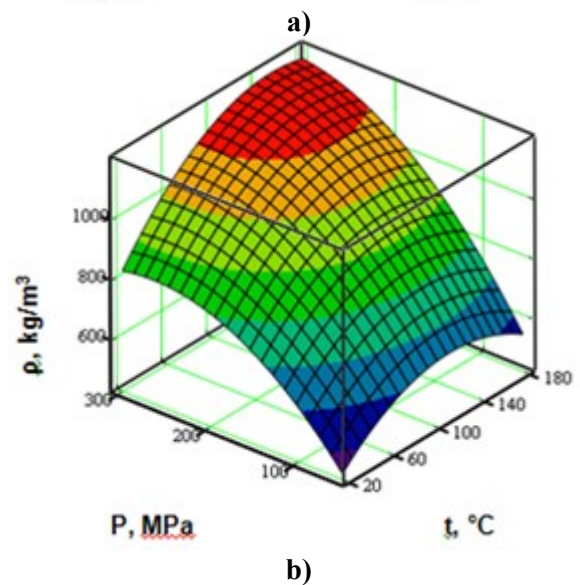
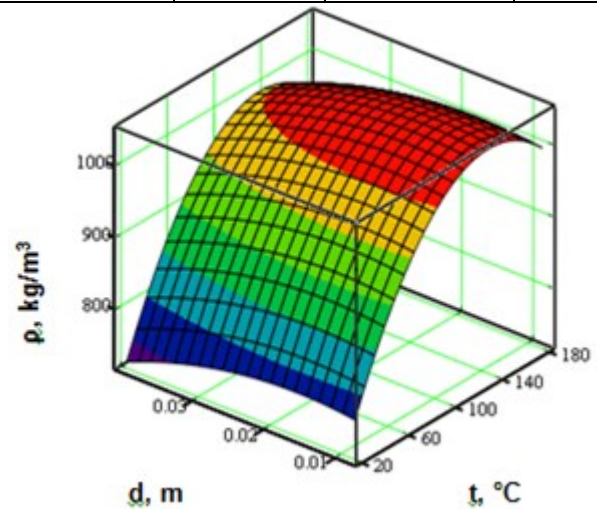
### III. Results and discussion

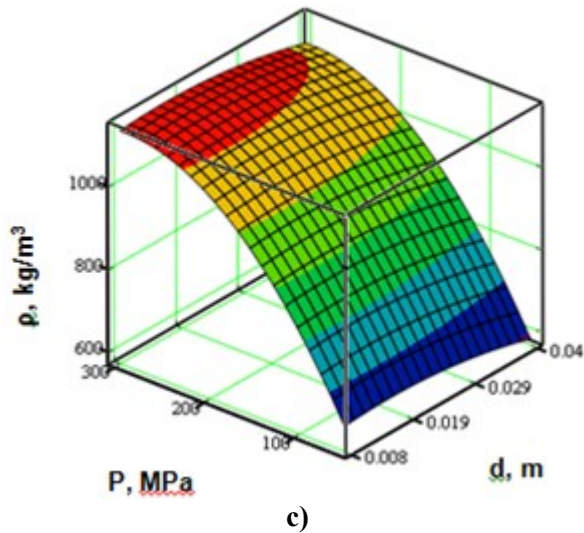
The analysis of the represented dependences showed that with increasing of temperature of the raw materials from 20 to 160 ° C increasing the density of obtained granules is observed. This is due to the intensification of the process of dissolution of lignin with increasing temperature.

It should be noted that a further increasing in temperature of material over 160 ° C is not appropriate because it has a little effect on increasing the density of granules, and thus leads to unnecessary energy costs. Also it is experimentally confirmed that the increase in compacting pressure and decreasing of diameter of press-matrix provides density increase granules.

**Table 1.** Intervals of variation

Intervals of variation	$x_1$ (t, °C)	$x_2$ (P, MPa)	$x_3$ (d, m)
Zero level	100	175	0,024
Step of variation	80	125	0,016
The lower level	20	50	0,008
The upper level	180	300	0,04





**Figure 3.** The dependence of the density of granules (husk of sunflower) from: a) diameter of d press matrix and temperature t of raw materials; b) compacting pressure P and temperature t of raw materials; c) compacting pressure P and diameter d press matrix

**Table 2.** The results of mathematical and statistical analysis of the experiments

Type of raw	Regression equation
Husk of sunflower	$\rho = 155,67 + 7,7184t + 2,356P + 8115,31d + 0,0017t \cdot P + 10,9375t \cdot d + 5,375P \cdot d - 0,02887t^2 - 0,00462P^2 - 241445,31d^2$
Buckwheat of husk	$\rho = 113,358 + 4,787t + 4,571P + 4229,391d + 0,0042t \cdot P - 13,535t \cdot d - 7,734P \cdot d + 0,0773t \cdot P \cdot d - 0,0206t^2 - 0,00852P^2 - 90132,813d^2$
Shavings of pine	$\rho = 293,741 + 5,379t + 3,175P + 8148,875d + 0,00289t \cdot P - 0,02107t^2 - 0,00605P^2 - 209035,156d^2$
Chips of oak	$\rho = 201,88 + 6,654t + 2,6715P + 6159,45d + 0,00114t \cdot P + 8,301t \cdot d + 5,437P \cdot d - 0,024t^2 - 0,00404P^2 - 217667,969d^2$

<b>Straw wheat</b>	$\rho = 35,176 + 6,598t + 3,792P + 7828,813d + 0,00265t \cdot P + 9,328t \cdot d - 0,02585t^2 - 0,0069P^2 - 201035,156d^2$
<b>Wheat bran</b>	$\rho = 330,27 + 2,446t + 3,385P + 2612,5d + 0,00333t \cdot P - 0,0118t^2 - 0,0064P^2 - 90472,6d^2$

#### IV. Conclusions

The obtained results should be used as a guideline at the organization of the technological process of granulation of dispersed materials by extrusion and at construction of related equipment.

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## MEASURES TO PREVENT EMERGENCY SITUATION

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**Abstract:** *Requirements to design and placement of dairy enterprises are specified. The basic stages of development, preconditions and signs of emergency situations in such enterprises are provided. On the example of dairy plant schedule of non-accidental stop of plant producing condensed milk cans it was developed.*

**Keywords:** emergency situation, dairy plants, civil protection, removal, localization, schedule of accident-free stopping.

### I. Introduction.

Processing enterprise is specified in the law of Ukraine "About Milk and milk products" as enterprise which buys milk and raw milk, which has manufacturing facilities and conditions for the production of dairy products with guaranteed quality and safety in accordance with the regulating documents.

The design, location, construction, technical re-equipment and reconstruction of milk processing enterprise (plant, shop) is to be carried out in accordance with acting safety requirements to design of the dairy industry; in accordance with public sanitary rules of planning and building of the human settlements, in accordance with building regulations and standards of technological design. Following of the above mentioned standards at all stages of the process, from the design of enterprise to the production of finished dairy products, is significant compulsory component of a comprehensive system of measures aimed at achieving of stable supply of customers with products of high-quality.

In accordance with its functional use the territory of milk processing plant is divided into before plant zone, industrial zone, utility and storage territories. In before plant zone administrative buildings, sanitary facilities, checkpoint, parking employees, are located. The industrial buildings, mechanical repair plants are located in the production zone; the boilers, pump stations, storage of ammonia, oil products, fuel, construction and reserve building materials, packaging are placed in utility and storage zone. It should be mentioned that around borehole sand spare tanks for drinking water controlled zone is located, and protective zone is located round the treatment plant.

Design of the main production shops of the plant includes taking into account their location in accordance with the process flow and at the same

time avoiding counter flows of raw materials and finished products [1].

Operating of dairy enterprises in full measure is enabled with plenty of technological equipment, proper working conditions workplace, safety of vehicles, machinery and other capital goods.

Dairy plants are among the biggest in the food industry in terms of volume of production as well as in terms of the number of workers. Sizes of production facilities, automation of processes, large equipment (vacuum evaporators, dryers of different type, containers for temporary storage of raw materials and finished products), and large scale are the factors showing the necessity for the protection to prevent extreme emergencies. Such protection on dairy enterprises is provided by system of civil protection.

Civil protection in Ukraine is a system of organizational, technical, sanitary, preventive and other measures that undertaken by the central and local executive bodies, local authorities and subordinated forces and means, enterprises, institutions and organizations regardless of ownership and by voluntary units[2]. They provide the implementation of measures for prevention and liquidation of emergency situations that threaten the lives and health of people and cause material damages in peace time and in times of crisis.

In foreign countries, civil protection, as a system of strategic viability of states, is designed to perform tasks that aimed at protecting of the population and economy from the situations of industrial and natural character.

Today almost all industrialized countries abroad are working to prevent natural and man-made disasters. This work is based on monitoring and forecasting. Each country develops and creates own variants of national civil protection structures. Despite individuality all these systems are primarily guided by principles taking into account human law of the Geneva Convention of 1949.

## II. Materials and methods.

Due to the scale of food enterprises, the number of working staff involved in production of consumer goods and due to quantity of technical equipment civil protection is of great importance in the food industry.

Considering the feasibility of the system described above, we should mention that dairy industry is no exception from general trends. Significant part of food industry enterprises can be treated as potentially dangerous objects.

Technical level of enterprises, concentration of production in big industrial cities, weak points of production location, organization of potentially dangerous activities in the zones of possible natural disasters should be treated as main factors affecting scale of the consequences of emergencies. Accidents often have catastrophic consequences accompanied by explosions, fires, radioactive and chemicals contamination of the environment [4].

The emergency situations are caused by violation of processes, equipment operation, temporary layoffs as a result of automatic safety locks and other local violations in the work of departments, sections and individual objects falling towers and power lines breaking.

Each industrial enterprise, including dairy plant, develops action plan of accident-free quick stop of production in case of an emergency (disaster). This plan should ensure decrease of risk of secondary damaging factors to the minimum. The feasibility of the plan and the willingness of staff to its execution are identified during civil protection (CP) testing. Set of required documents for testing developed beforehand. Stages of development of the accident, conditions and characteristics of its origin, means of liquidation and localization on the example of ammonia refrigeration unit are shown in the table 1 below.

**Table 1.** Stages of emergency development, conditions, signs of its occurrence, location and means of eliminating

Scenario name and stage of accident	Means of emergency protection	The sequence of actions
Exceed of parameters above critical values	To warn others about the danger, to use personal protection equipment, to do the emergency stop of	Use personal protection equipment. Disconnect compressor using the emergency shutdown.

	compressors, to switch on crash ventilation.	
Depreciation or material fatigue of equipment	Evacuate people from the danger zone, isolate the zone, prevent entering of unauthorized individuals, work only in protection clothing	Inform about emergency via system of alerts communication. Assess the situation. Disable bad block.
Mistakes of personnel servicing and repairing of equipment	Provide first aid to victims, direct victims from the zone of lesions for examination. In the case of intense gas leak, give it to evaporate, to apply water spray for its deposition. Isolate zone of emergency situation, notify management.	Block ways of getting ammonia in sewers, basements, tunnels. Neutralize gas using water hand barrels IBS-50, Surround danger zone and evacuate people. Provide assistance to victims. Send people to medical examination

## III. Results and discussion.

The civil protection involves development of schedule of accident-free stopping of certain production zone or plant as a whole in order to prevent accidents on dairy enterprises system of.

For plotting of the schedule, information about the content of preventive operations, their agents and duration is required (Table 2).

**Table 2.** Content warning operation of accident-free stop the plant for the production of condensed canned milk with sugar and their duration

Content of the operation	Executor	Start of operation, min	End of operation, min
Getting a signal	Responsible duty	1	2
Notification of plants	Manager, Service of alerts	2	5
Stop the raw	Heads of	3	6



feeding	departments, operators of industrial buildings		
Sequential shut down of batchers	Mechanical-engineer	4	7
Stop and disabling of equipment on all production lines	Mechanical-engineer	4	20
Evacuation of the personnel	Responsible duty	5	11
Disconnection of the voltage from power panels, switching off sources of emergency lighting	Duty electrician, chief of the plant	10	17
Covering the raw	Responsible staff	7	15
Blocking water and heating system	Duty mechanic	7	9
Turning off the power of plants on the main distributing board	Chief Energy	14	20
Disabling the well, blocking water	Mechanical-engineer	7	10
Reporting to the chief engineer about shutdown of the production department, or milk processing enterprises in general	Chief Engineer	18	22

Data from Table 2 shows that during emergency stop of enterprise the most intervals are spent for such operations as evacuation, stop and turn off equipment.

Schedule of trouble-free stop of the milk processing enterprises is shown on the example of prevention of emergency situations during the production of condensed canned milk with sugar.

Total technological scheme the production of sweetened condensed milk is shown on Fig. 1.

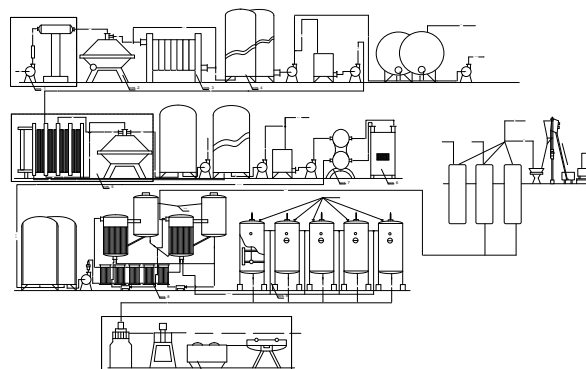


Figure 1. Technological scheme the production of condensed milk with sugar

Analyzing the data on Figure 1, we can say that the production of condensed milk cans requires the using of plenty of technological equipment and facilities.

Production of milk canned is performed in the following sequence: receiving milk (position 1), cleaning (position 2), cooling (position 3) and temporary reservation (position 4), normalization (position 5), homogenization (position 6), pasteurization (position 7), condensation (position 8), cooling (position 9), packing (position 10)[3]. In general, the production process provided such equipment and facilities as pumps for liquid and condensed products, cleaners, separators, tanks for interim reservation raw, pasteurizer-holder, tubular heat exchangers, homogenizers, vacuum evaporators, crystallizers, packing machines and so on.

Some of technical equipment (vacuum evaporators, crystallizers) used in the production of condensed milk cans has large size and requires significant cost of resources, regular and thorough examination of service ability and capacity.

Schedule of trouble-free stop of the plant producing canned milk is shown on Fig. 2.

In the dairy plants according to the order of the Ministry of Ukraine of Emergencies and Affairs of Population Protection from the Consequences of Catastrophe in Chernobyl № 288 of 15.05.2006 "Regulations setting, operation and maintenance of systems for early detection of emergency situations and notification of people at the moment of their occurrence" system of automatic early detection of emergencies, objective warning system, and on-call dispatcher service were established.

The plant which can threaten people's lives and can also cause damage is equipped by automatic systems for early detection of emergencies. This system includes technical sensors, sirens, etc. They

control the dangerous parameters of equipment and environment.

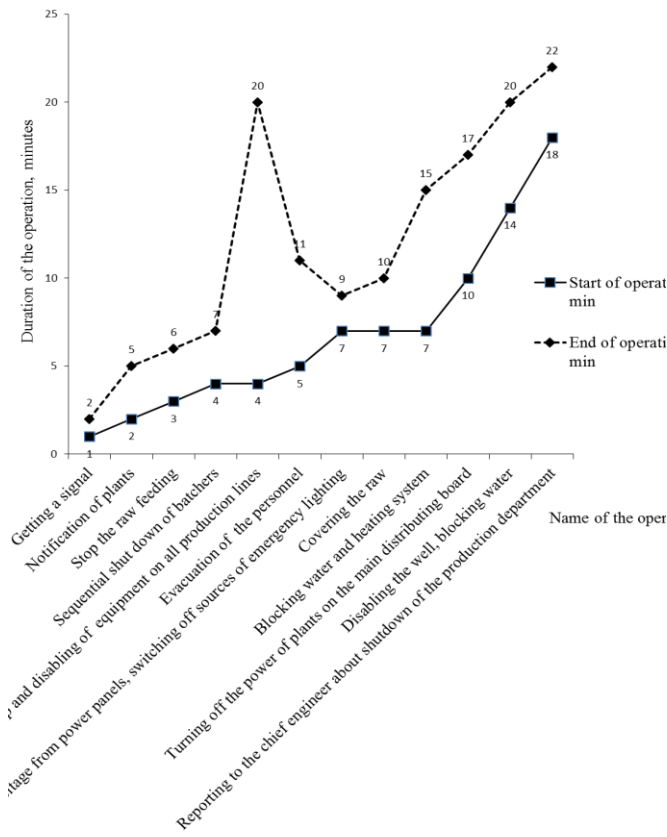


Figure2. Schedule of trouble-free stop of plant producing condensed milk cans

Object warning system provides alert of management and staff working in dairy plants on duty employees and responsible for shift in the rescue service of civil protection units, which are involved in joint action to eliminate and locate the accident. System includes an electric siren to send a warning signal "All attention", street speaker, illuminated signs (information board) subscriber radio receiving of the network public broad casting, centralized calling system, direct dial telephone between on duty manager and on duty employee of MIA.

The responsibility for timely notification of people who are on the territory of enterprise and attracting of the necessary capabilities of civil protection services to control and eliminate an emergency (accident) relies on manager.

The results of above mentioned notification and arrival capabilities CP on duty service manager reports to the authorized supervisor of liquidation (localization) of emergency.

On receiving of notification about the accident manager should immediately via using the object warning system, perform following steps: enable a remote sirens start to transmit sound electric siren which means "all attention!", transmit the appeal text to people who are at territory of the enterprise, via the hotline with another MIA must report about the occurrence of the situation at the plant to attract the necessary capabilities of CP services (Deadline for notification is 3 minutes. The time scheduled for collection capabilities of civil protection depends on the time of their gathering and the distance to the accident), to inform the company's management about emergency situation, to inform safety Inspector about the situation at the enterprise.

#### IV. Conclusions

The proposed engineering measures reduce the risk of accidents, fires, explosions, reduce material loss of plants, protect employees from entering into possible defer at zone of. A set of preventive measures ensures the security of all employees and, thereby, contribute to ensuring the necessary conditions of work of milk processing plant.

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## **ROLE OF NUTRITION AND THE USE OF SPECIAL EXERCISE IN DIABETES**

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**Abstract.** *Any type of diabetes plays an important role proper nutrition. Among the causes of diabetes are: lack of exercise, aimed at consumption, chronic stress, excessive mental work, eating meals with the dominance of fats and carbohydrates, chemical poisoning, sexual excesses and hereditary factors. In this article we show effect of exercises on the disease and some product that can influence on our condition. These specific exercises can improve the condition of patients.*

**Key words:** blood, glucose, eating, food, diabetes.

### **I. Introduction**

Diabetes mellitus is associated with metabolic disorders. It is based on the lack of assimilation of glucose. Second type of diabetes often occurs against a background of obesity.

Any type of diabetes plays an important role proper nutrition. In mild form of second type of diabetes diet is the main treatment. In moderate and severe form of the disease we need to combine diet with taking hypoglycaemic drugs or insulin.

### **II. Materials and methods**

Analysis of modern literature on the effect of diet and exercise on diabetes was conducted. The experience of the author to promote exercise and a special diet was accounted.

### **III. Results and discussion**

Basic dietary guidelines for patients with diabetes.

1. Compliance a diet: it is necessary to comply with a uniform distribution of carbohydrates throughout the day, and to correlate diet pill with features and / or insulin.

2. Did not starve. Meals take 4-5 times per day.

3. Food is considered in terms of how fast after absorption from the gastrointestinal tract can lead to increased blood sugar levels. From this perspective, all products can be divided into those that can increase blood sugar quickly, slowly or cannot at all increase. Both types of diabetes in need of a meal so that admission blood sugar was as a gradual rather than abrupt.

From the food should be excluded foods that contain carbohydrates that are rapidly absorbed: sugar, candy, honey, cakes, pies, jams, chocolate, ice cream, soft drinks, wine, and cream of rice cereal.

By products containing "slow" carbohydrates and are recommended for diabetes include: black rye bread, bread with wholemeal flour, bread with oats, oats, buckwheat and millet porridge.

4. If the patient can not give up sweets doctor can prescribed sweeteners: sorbitol, xylitol, fructose, saccharin, synthetic substances (aspartame, cyclamate). It should be remembered that aspartame can not be used for cooking and hot use more than 6 tablets per day. Saccharin is not recommended for use in the presence of liver disease and kidney disease, and the dose should not exceed 3 tablets per day.

5. Remember that fruits of one species, but of different sorts (eg equal weight sour and sweet apple) raise sugar. Fructose, contained in fruit, very quickly absorbed and apply according to the rapid sugar. Undesirable to use grapes (containing pure glucose), fruits with lots of fructose (fig, persimmon), dried fruits (raisins, prunes, dried apricots). Permitted are use of: apples, citrus fruits, watermelon, peaches, plums, pomegranates, cherries, strawberries, currants.

6. Vegetables should be limited with potatoes, corn and legumes (beans, peas) since they contain starch. Unlimited quantities can be eaten all kinds of cabbage, carrots, radishes, tomatoes, cucumbers, zucchini, eggplant, onions, lettuce, fresh herbs (parsley and dill).

7. Ration patient with diabetes should be enriched in fiber, which is found in sufficient quantities listed above "permitted" fruits and vegetables, as well as cereals and wholemeal bread. The best content in the diet foods: cereal - 40%; of vegetables - 51% of fruits and berries - 9%.

8. Use of alcoholic beverages to a patient with diabetes should be prohibited or greatly restricted. So how alcohol contributes to liver damage, pancreas, helps the sharp decrease of blood glucose.

Drinking alcohol while taking hypoglycaemic drugs can cause a number of complications, the most severe of which is the development of coma. Allowed are table wines with sugar content not exceeding 3-5% but not more than 150-200 grams at a time. For alcoholic beverages possible consumption of a single dose of vodka or cognac less than 75-100 g

9. Permitted drinks are unsweetened tea, coffee, milk, natural fruit and berry juice with sour-sweet varieties without added sugar, tomato juice, mineral water, extract of rose hips. Fruit juices with sugar are not recommended.

10. In the diet should be enough protein. Recommended low-fat varieties of meat (chicken, rabbit, beef) lean fish in boiled form (carp, pike, perch, cod). You must refrain from eating cooked sausages and frankfurters, as starch is used in their manufacture. Vegetable protein are found in soy, legumes, mushrooms.

11. It is Necessary to restrict the use of animal fats, replace them with vegetable as animal fat contains cholesterol and saturated fatty acids that promote the formation of atherosclerotic plaques in the blood vessels. These plaques are the cause of heart attack, stroke or gangrene due to blockage of blood vessels. Eat fats mainly in the form of vegetable oils (olive, sunflower, corn), since they contain unsaturated fatty acids, which help to reduce cholesterol levels.

12. When choosing food should be given preference to those that contain a sufficient amount of vitamins for deficiency, which occurs in diabetes: B vitamins (B1, B2, B6, B12), A, E, C in the diet is an important micronutrient availability zinc and chromium, which increase the effectiveness of insulin. It should be remembered about the need for correction of magnesium deficiency in diabetes, as it is involved in all types of exchange in the normal operation of the heart, regulates vascular tone, improves the properties of the blood. By eating foods containing magnesium (nuts, buckwheat porridge, etc.) it is absorbed only 40%. In addition, eating foods that contain lots of sugar or caffeine (tea, coffee, cola), any "refined cuisine" (including fast food), increased emotional and physical stress leads to latent magnesium deficiency. Reset all deficiency of these vitamins and minerals can not only using the appropriate foods, but also willing vitamins.

13. Diabetics are overweight body necessarily have 1-2 times a week to spend fasting days (apple, kefir, oatmeal, vegetable, etc.).

Patients with diabetes of any type must remember that they have to eat sugary foods exclusively when developing hypoglycemia (a condition related to the

sharp decrease in blood sugar and accompanied by severe weakness, sweating, tremor, loss of consciousness).

Modern pathogenetic treatment of diabetes is aimed at the highest possible compensation of metabolic abnormalities that occur in the body as a result of absolute or relative insulin deficiency. Currently, there are three ways to achieve compensation of diabetes: diet therapy, treatment drugs, dosed exercise.

None of them is not without drawbacks, and each requires a large collaborative effort on the part of the doctor and the patient. Along with the improvement of methods and patterns of diet and insulin, the creation and introduction of new drugs, a lot of attention recently attracted unconventional therapies.

The beneficial effects of physical activity on the course of diabetes are well known. It is connected with the fact that physical activity provides multilateral action and causes an increase in functional activity of various human organs and systems. When muscular work is enhanced blood flow to skeletal muscles, increases oxygenation of blood and oxygen supply is facilitated organs and tissues. Amplified metabolism, increases glucose uptake by cells. With sufficient compensation while working as a result of muscle contraction is increased revenues insulin from subcutaneous depot. There is also evidence that the working muscles release into the blood substances that have action similar to insulin. Exercise increases tissue sensitivity to insulin receptors.

However, in patients with diabetes the body's response to physical activity, is that it metabolic effects may vary depending on the initial state compensation metabolism. Thus, at moderate hyperglycemia (blood glucose does not exceed 16.7 mmol / l) exercise lead to lower blood glucose.

We have over the years proven methods of yoga therapy of diabetes. Elements of one of the most productive ways to manage them, yoga became gradually root in European medicine, including clinical Diabetology.

Comparison of procedures from outside yoga techniques like physical therapy, which is one of the three "pillars" of compensation of diabetes, is in favor of yoga as significantly more effective, more productive and safer approach does not require, through a number of features, rigorous unit dosage.

With numerous set of asan we tested and used most accessible performed in sparing mode, a lightweight version that effectively affect the state of the endocrine, cardiovascular and immune systems, normalizing the digestive tract, reducing the need for the amount of food taken due to more complete

utilization eaten and accumulated as fat. The normalization of the patient's weight is like automatically without volitional attitudes and other usual violent action.

In pranayama procedure we introduced control pelvic down as one of the principal methods of healing. We introduce a procedure for breathing muscles that straighten the spine, with almost passive respiratory muscles: Ioha -wise, Beyond head on his knee, a pair of camel - rabbit, dog - snake.

With poses that stimulate the activity of the pancreas, we used a triple Cobra Locusts -bow, characterized in Indian texts like that "eradicates diabetes" and asana, normalizing activity timuyea, thyroid, pituitary ( Stand on the shoulders + fish ) and compensating immunodeficient ( Sphinx ).

Diabetes - a disease in which the blood contains large amounts of sugar. This occurs due to a lack of insulin action. Insulin - a hormone produced by the pancreas that regulates metabolism: fats, proteins and carbohydrates (sugars ). The main symptoms of diabetes are thirst, frequent urination, sometimes dehydration.

Among the causes of diabetes - lack of exercise, aimed at consumption, chronic stress, excessive mental work, eating meals with the dominance of fats and carbohydrates, chemical poisoning, sexual excesses and hereditary factors.

Based on the causes of diabetes should build the practice of yoga.

The second type of diabetes millitus - is a heterogeneous disorder that is characterized by a genetic predisposition and the relationship of insulin resistance with decreasing functionality Betta -cells of the pancreas.

It is often associated with high blood pressure, hypertriglyceridemia, and low levels of highly lipoproteins, which is an additional risk factor for atherosclerosis and cardiovascular disease.

For many people with diabetes it is very difficult to monitor blood glucose regularly, because such a plan conditioned requires significant changes in lifestyle and behavior.

Because of the chronic nature of diabetes patients often resort to preventive treatment to maintain itself, resulting in the order of health, mitigating complications using complementary therapy.

Yoga - This ancient, traditional Indian psychophysical and spiritual teachings, which we studied for several decades, in order to clarify the role of yoga in a positive impact on some heavy chronic diseases, including hypertension, asthma, obesity, neuromuscular diseases and mental disease.

There are several hypotheses to determine the biological mechanism that links the positive yoga impact with diabetes.

One hypothesis suggests that yoga is especially good for relieving stress and to achieve relaxation. Another - that the invasive nature of the impact yoga can achieve significant results quickly and without side effects.

The researchers conducted this experiment: 25 patients (including 10 women and 15 men) in the average age of 42, were selected by random sampling in September 2006 at a hospital Haridwar. For data collection methods were used prior and follow-up. Yoga classes were held for 63 days for all subjects. Glucose levels before and after eating were measured using a chemical analyzer RA- 50, in which previous and subsequent diastolic and systolic blood pressure measured by a mercury sphygmomanometer. Hypotheses were tested using t- test. We used the following yoga exercise: Lanhhu Shankhaprakshalana ( LSHP ) held once a week, Kundzhal kriya - twice a week, Uddiyana Bandha - 20 day approaches, Ahnisara Kriya - approaches 20 per day, but occupation ended ten minutes of Shavasana. Classes Kundzhal kriey not conducted on the day LSHP. All of the study participants recommended by doctors not to take medication at the time of the experiment.

Yogic cleaning practice has a direct effect on the allocation of pancreatic cells by restoring its alternation of tension and relaxation of the stomach ( Ahnisara kriya and Uddiyana Bandha ).

Shankaprakshalana improves blood circulation and purifies it by improving the supply of oxygen and nutrients to the epithelium of the gastrointestinal tract. It improves metabolism, tidies normalizes digestion and excretion, as well as assists the muscles to tighten up the skin and internal organs to position correctly.

Shankhaprakshalana cleanses and tones the digestive tract. This technique of yoga just cleans the small intestine. Typically, sparse food from the stomach to the intestine passes through the ring and into the large intestine in 3-4 hours. Practice Lahhku Shankaprakshalany activates and accelerates the process, which takes 30 minutes to an hour, running gear cleaning utilities.

Here are a few exercises for the treatment of diabetes

Mayyurasana. Kneeling, well put them to the side, connect the arms and put his hands back on the mat. Stretch his arms and slowly lower the body to put together the elbow. Legs, put together, pull out and making breath, tear them off the floor so that the body and legs are parallel to the floor. Lie in an

attitude as possible, concentrating attention on the stomach.

In addition to the prevention and treatment of diabetes exercise normalizes the liver and spleen, tones the kidneys, stomach and gland.

Dzhanurasana. Lie on your belly, toes and heels together, feet rest on toes, arms extended at your sides and back of her hand lying on the mat, chin rests on a mat. Push down the side, bend your knees and clasp hands ankles or feet in recovery. Make a calm breath through your nose and hold your breath after breath. Lift your head and upper body as high as possible and tear hips off the floor. Stay in this position as long as the breath after inhalation. In this position, you can swing forward and backward.

Chakrasana (outside wheel). Lie on your back with your hands put the floor behind and feet - about the buttocks. Raise your body off the floor as high as possible and bend. Hold the pose anywhere from 30 seconds to 3 minutes. The ideal is out when his hands and feet close together.

This exercise is very good not only in diabetes but also for liver, thyroid, eyes, reduces body fat in the abdomen, they remove the curvature of the spine.

Holosana.

Sit on the floor, legs straight, toes over, palms rest on the floor and directed forward. Raise your hands up and lean forward, holding the soles, and put his head on straight legs. Hold the pose to 1-2 minutes, directing attention to the waist. Not the recovery of pancreatic function, has a rejuvenating effect on the spine, tones the internal organs, increases sexual potency and helps with heartburn, bloating and increased flatulence.

Several recent studies have shown that yoga can effectively resist stress.

As a result, the practice decreases the formation of glucagon, launched relaxation processes are highlighted endorphins and possibly improves the effectiveness of insulin.

Relaxation that comes as a result of correct practice of yoga promotes abundant blood supply of muscle tissue, resulting in a possible improvement excretion of insulin receptors in the muscles, resulting in glucose utilization and lower blood sugar. Yoga exercises and conventional PT exercise improves hlikimia control, by increasing the amount of insulin receptor binding in patients with diabetes millitus second type.

Several studies have investigated the impact of yoga on the regulation of glucose. They noted a significant decrease in blood glucose concentration before and after meals, as well as reducing cortisol in serum. Cortisol is the main hormone of the adrenal glands. It affects the metabolism of carbohydrates,

proteins and fatty acids on maturation of white blood cells in hematopoietic tissue, fluid and electrolyte balance in the nervous system and the regulation of blood pressure.

Determination of cortisol can be used to characterize the state of the hypothalamic -pituitary axis. Studies have shown that the amount of cortisol in serum is reduced due to the practice of yoga. Yoga classes are not only able to pay development of diabetes, but also improve the body's resistance to psycho- physiological level. In conclusion, it should be noted that further detailed studies in order to clarify the effect of yoga exercises on the second type of diabetes.

Regarding this study clearly can conclude that yoga had a pronounced positive effect on a group of subjects with Type II diabetes with a mean age of 42 years.

Power yoga is manifested at many levels and in accordance with the right approach can be used to improve the functioning of the body, treatment, gaining peace and quiet inside, as well as to clarify understanding, recognize its nature and realization of self.

More from the arsenal of hatha yoga is definitely a useful exercise in diabetes will Nauli, in which the deep massage internal organs, including the pancreas.

#### **IV. Conclusions**

Our data demonstrate the effectiveness of treatments special exercise therapy as a subsidiary method of compensation of disturbed metabolism in diabetes. This method can be widely use in public health practice, in particular, can be successfully applied in terms of spa treatments in the schools of diabetes in the halls of physiotherapy.

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## **MODELLING OF PROCESS IN TWIN-SCREW DOUGH-MIXING MACHINES**

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**Abstract.** *Simulation of the kneading yeast dough kneading machine in continuous operation: To intensify the machining test during mixing, we suggested to use screw the working part of various modifications, which provide continuous transportation and intensive mechanical impact on the dough during kneading.*

*To study the process of kneading proposed design model using a software package Flow Vision. Investigated different types of kneading part: digitalete, spiral, ribbon.*

*Analysis of the results of modeling allowed us to offer the design a combined work part that provides a three-step process of knead the dough kneading machines in continuous action.*

**Keywords:** dough, knead, screw, mixing.

### **I. Introduction**

A wide application of the advanced dough-making technologies in bread production involves the use of intensive mechanical processing of the semi-finished products. In this regard, double-speed dough-mixing machines of the periodic operation with intensive dough kneading have become a frequent practice in bread making industry resulting a transition from a continuous stream of dough kneading to periodic one because there are no continuous dough-mixing machines of intensive action. The creation of the dough-mixing machines of continuous action is currently important.

The intensification of the mixing process can be performed by increasing the frequency of the body rotation or changing its structure that leads to changes in the structural and mechanical dough properties and provides the reduction of the duration of the dough fermentation.

During fermentation of the dough semi-finished products the change of their structural and mechanical properties occurs together with the microbiological processes that determines the state of the surface, the specific volume and porosity structure of the finished products.

### **II. Materials and methods**

A calculation model of dough kneading using the software package Flow Vision of the Russian firm "Tesis" is suggested to study a dough kneading process. This package is designed to model hydrodynamic processes in technical and natural conditions and to visualize these processes using the methods of computer graphics. It is based on the

analysis of the stress-strain state of the studied material by the finite-element method.

Having analyzed all basic mathematical models that are presented in Flow Vision the model of "incompressible liquid" is used. This model describes the motion of the viscous liquid, gas at small Mach numbers, small and large Reynolds numbers. There may be small changes in density; it means the presence in the dough a gas phase only as the air entrapped during mixing.

A mathematical model of the calculating process of dough kneading is based on the use of a well-known Navier-Stokes equation, energy, transfer convectional, and diffusive shift and viscosity changes of the matter during mixing.

To study different types of the mixing bodies is suggested on the basis of the theoretical studies (Fig. 1) that are placed in pairs in the mixing trough capacity, with the same pitch, the opposite direction of the helices and counter rotation. A combined working body consists of one helix of the solid screw that provides a directed movement of the components into a mixing zone of the tape working bodies; the solid screw delivers the mixed components into a zone of intensive mechanical processing by the screw with the varied pitch.

The components for dough kneading are transported into the receiving pipe and unloading of the kneaded dough is performed through the lattice with openings where the change of the cross-section enables to alter the time and intensity of mechanical processing.

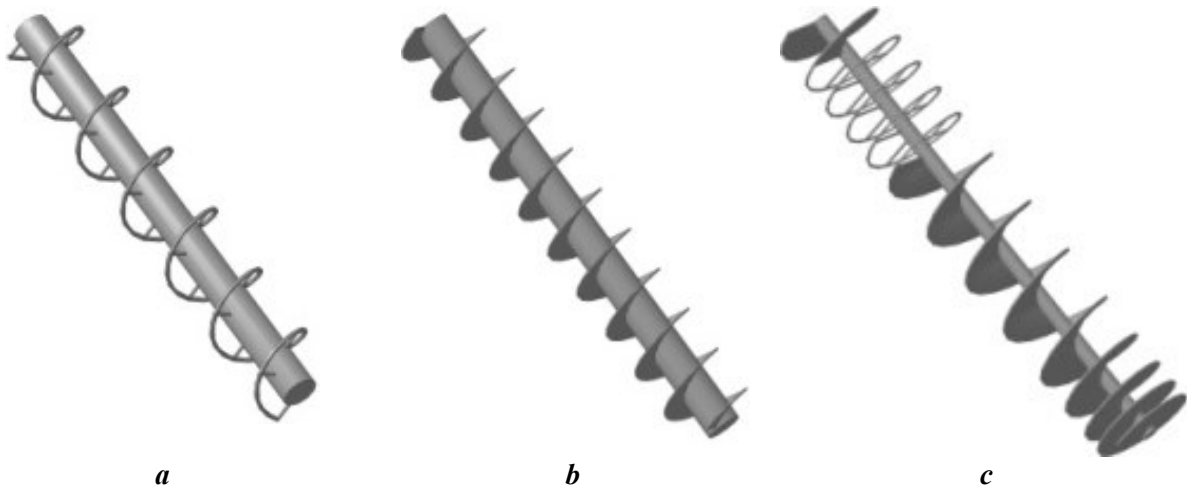
### **III. Results and discussion**

Intensive mechanical dough processing while kneading has a positive impact on the quality of the

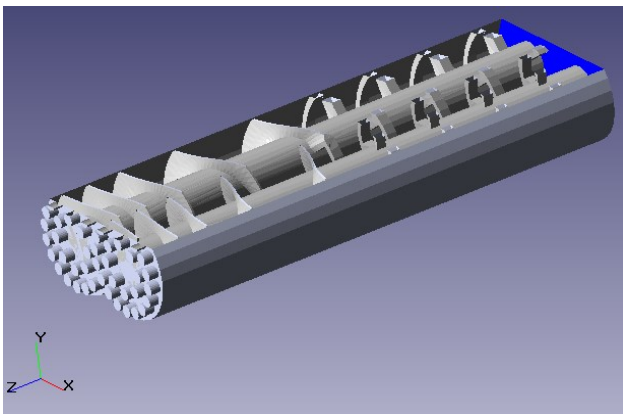
finished products with the use of the advanced dough-making methods. The application of the screw working bodies of various modifications is suggested as the working bodies that provide continuous transportation and intense impact.

Mixing the components occurs in machines with the screw working bodies due to the friction of the mixture on the walls of the screw and trough while moving and sliding. To provide the work of the screw it is necessary that the adhesive force of the mixture with the screw is less than its friction force on the walls of the trough.

Depending on the type and composition of the mixture various types of the screws are used: solid, tape. The mixing effect is higher in the tape working bodies than in the mixers with the solid screws. The redistribution of the particles in the tape mixers is due to the opposite motion of the mixture under the action of the tapes. The highest effect of mixing is reached with the screws that have a perforated surface of the helix, but at the same time they have a lower effect of transportation. Solid screws provide transportation and intensive mechanical dough processing [1].



**Fig.1.** Working Bodies of Various Configurations: Pin (a), Helical (b), Tape (c), Compound (d)



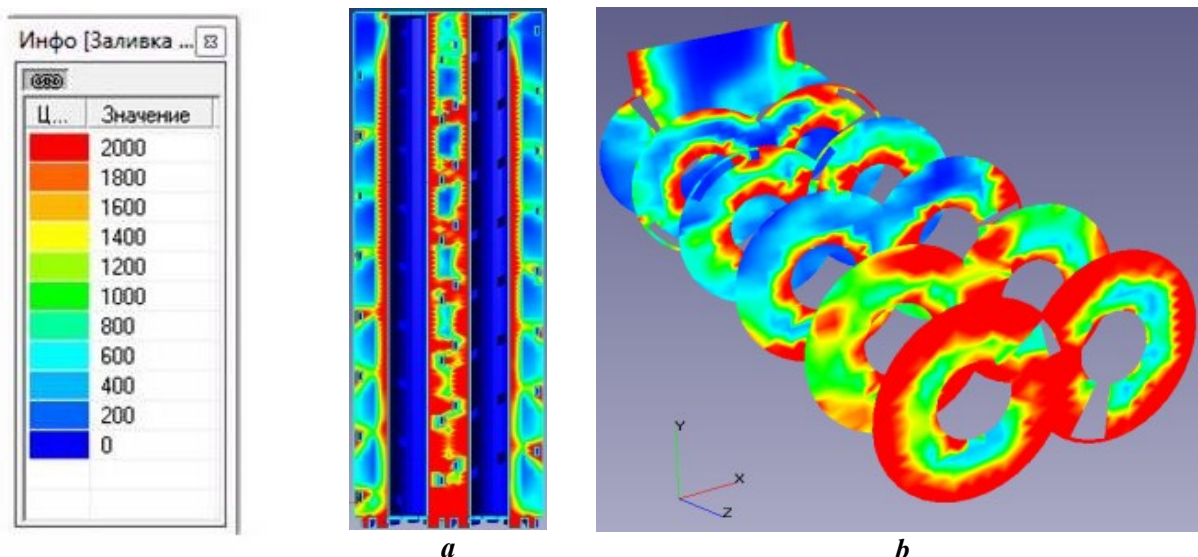
**Fig.2** The Calculated Model of the Compound Working Body

Geometric models are built using the software “Compass”, for the compound working body the model is shown in Fig. 2.

The results of the computational experiment are presented in the horizontal axial section and vertical sections for all investigated working bodies. The linking method enables to estimate the degree of influence of each working body in terms of kinetic energy dissipation.

The spiral working body (Fig. 3) is characterized by an intense equal impact throughout the length of the working body in the area of the flights engagement, and in the end an increase is caused by the resistance of the lattice.

The main energy consumption for internal friction occurs around the shafts of the screws caused by the highest gradient of the dough deformation speed.



**Fig.3** The Calculated Result of the Spiral Working Body: *a* – the Horizontal Axial Section; *b* – the Vertical Sections

During the study of the tape working body (Fig. 4) it was revealed that the most intense impact occurs in the area of the flights engagement and on the shafts surface. Such working body is characterized by the transporting function rather than mixing. Installed lattice at the output enhances the impact.

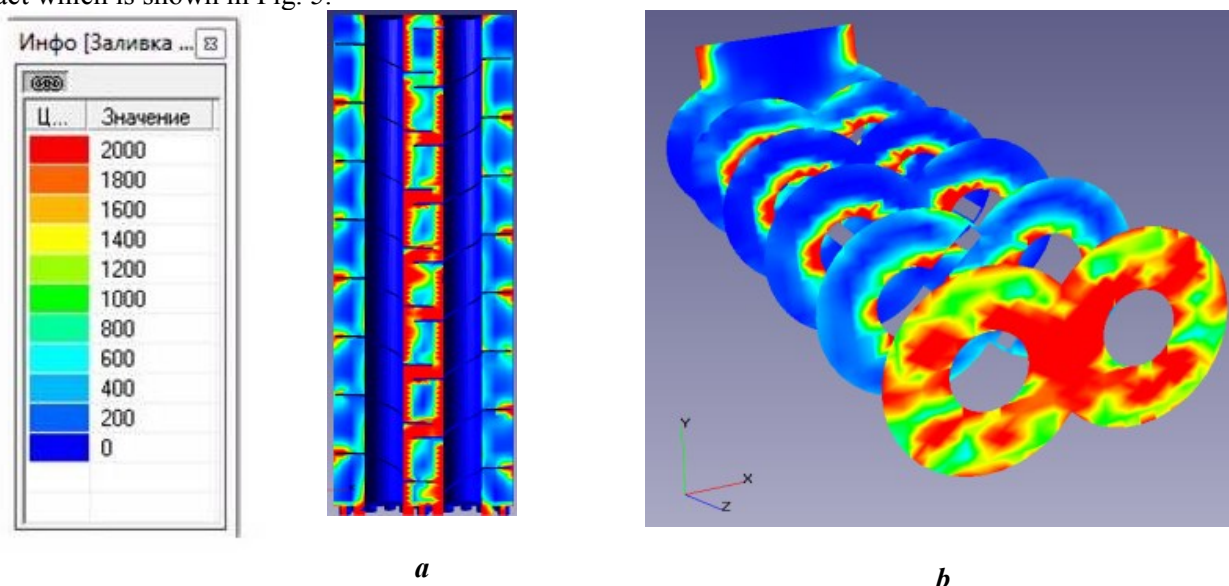
Due to the large total linear velocity of the working bodies at the fixed walls of the container the biggest curling of the product is marked in this area.

The pin working body possesses the gentlest impact which is shown in Fig. 5.

The axial velocity component of the product in this case is negligible, so the main energy consumption occurs in the area around the shafts during the mode of almost laminar moving.

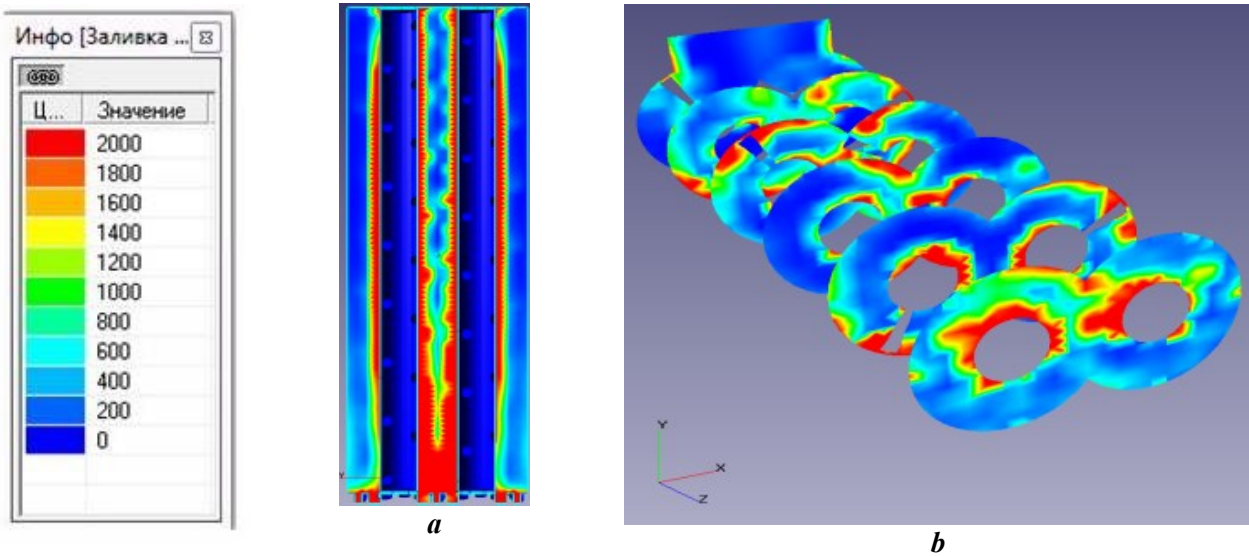
The increase of the impact in front of the lattice in engagement area occurs probably due to the low transporting capacity of the pin working body.

The construction of the compound working body and its calculations are suggested on the basis of the theoretical and experimental studies (Fig. 5).

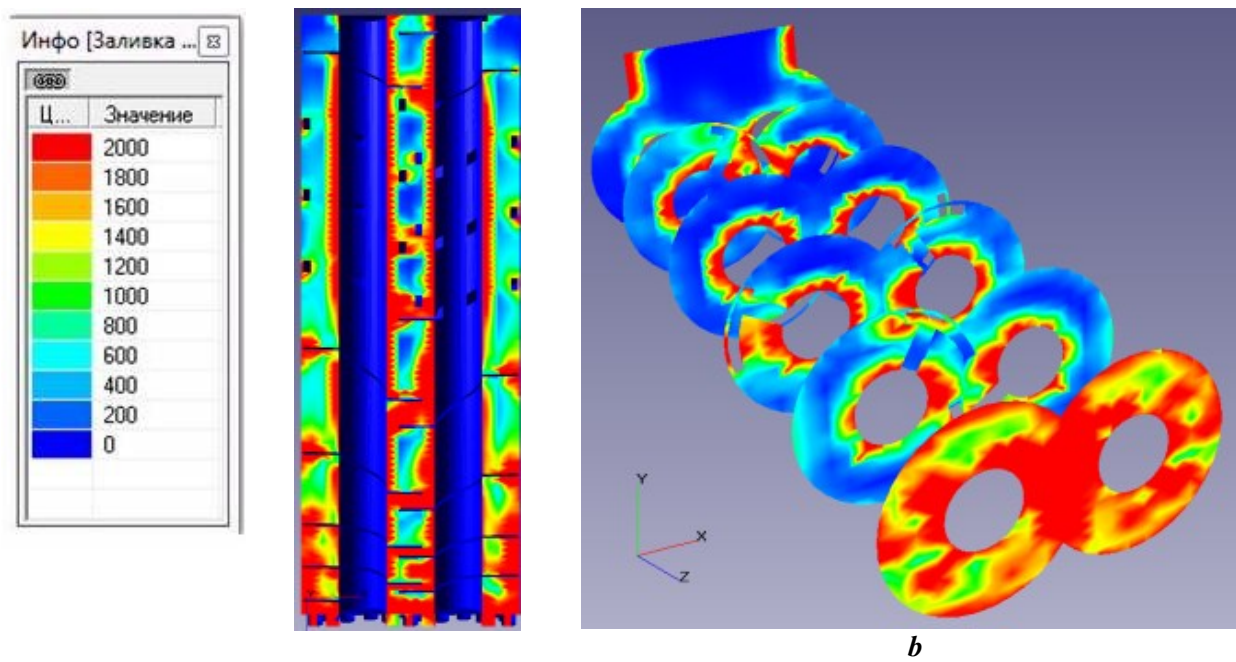


**Fig.4.** The Calculated Result of the Tape Working Body: *a* – the Horizontal Axial Section; *b* – the Vertical Sections





**Fig.5.** The Calculated Result of the Pin Working Body: *a* – the Horizontal Axial Section; *b* – the Vertical Sections



**Fig.6.** The Calculated Result of the Compound Working Body: *a* – the Horizontal Axial Section; *b* – the Vertical Sections

One solid tape flight to supply the raw materials into the mixing zone of the components by the spiral working body is set to enhance the transporting function of the compound working body, then the set solid tape flights that provide dough transportation into the third zone, while the second stage of kneading is carried out. The third stage – plasticizing – is carried out by the solid tape working body with a reducing pitch. Pitch reducing between the flights

contributes to intensive mechanical impact of the dough mass throughout the volume of the camera, as in the area of the flights engagement, and in the near-wall layer.

The dissipation of kinetic energy into heat happens more frequently in the places of the impact on dough of the working body; the greatest impact has a screw working body with a reduced pitch.

Modelling of the dough kneading process for different constructions of the working bodies of

continuous action is suggested using the software package Flow Vision. Data analysis enables to suggest a construction of the compound working body that provides a three-stage process of dough kneading in the dough-mixing machines of continuous action.

#### **IV. Conclusions**

Modelling of the dough kneading process for different constructions of the working bodies of continuous action is suggested using the software package Flow Vision. Data analysis enables to suggest a construction of the compound working body that provides a three-stage process of dough kneading in the dough-mixing machines of continuous action.

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## A COMPUTER PROGRAM FOR CALCULATING THE PLASTIC VISCOSITY OF CHOCOLATE MASS USING THE CASSON METHOD

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**Abstract.** We develop and implement an algorithm to determine the plastic viscosity of melted chocolate mass using the Casson's method. We collect data by measuring of the shear rate at 40° C using a Rheotest 2 viscosimeter. We test the software by running it with the collected data as input. Our algorithm validates input data by discarding samples that do not comply with the technological requirements of speed gradient.

**Keywords:** computer program, viscosity, chocolate.

### I. Introduction

With the development of the computer technologies, especially in the 1990s, the trend of continuous expanding of application of the computer techniques in food industry was increased. While many pre-built software packages exist, some problems require custom software solutions to be built. We present one such program for computing a plastic viscosity of melted chocolate mass using Casson's method. Viscosity is an indicator that to a large degree determines the properties of chocolate mass during the manufacturing of chocolate products. Rheologically, the melted chocolate mass is a non-Newtonian fluid and has an effective (structural) viscosity [4].

Using effective viscosity as a measure of the rheologic properties of chocolate mass creates some practical issues, as its value depends on the applied mechanical impact (shear rate). The ratio between shear stress ( $\tau$ ) and shear rate ( $D$ ) is non-linear [2]. The rheological properties of chocolate mass can be described correctly by Casson's equation, which postulates a linear relation between  $\sqrt{\tau}$  and  $\sqrt{D}$  [2, 7]. The benefit of characterising plastic viscosity of chocolate masses using Casson's plastic viscosity is that the measure can be expressed as a single value.

The international organisation of cocoa and chocolate (OICC) recommends that the viscosity of chocolate masses have to be determined using Casson's method [5]. A particular methodology has also been established [6]. According to this method, Casson's plastic viscosity ( $\eta_{CA}$ ) is calculated as follows:

$$\eta_{CA} = \left[ \frac{(1+a)\sqrt{\tau} - b}{(1+a)\sqrt{D}} \right]^2, \text{ Pa.s}, \quad (1)$$

where  $a$  is the ratio between the radii of the coaxial cylinders of the rheoviscosimeter;  $D$  is the shear rate that ranges between 5 and 60  $s^{-1}$ ;  $\tau$  - shear stress,

$Pa$ . The value of the parameter  $b$  is determined by graphically creating a rheographe with coordinates  $(1+a)\sqrt{D}$  -  $(1+a)\sqrt{\tau}$  using the experimental data and approximating it linearly. The origin of the line is extrapolated to the coordinate axis of shear strain. The resultant section defines the value of  $b$ .

The Casson shear stress (limit of fluidity)  $\tau_{CA}$  is calculated using equation (2)

$$\tau_{CA} = \left( \frac{b}{2} \right)^2. \quad (2)$$

The disadvantage of the method [6] is the requirement to apply a graphical method to determine the value of the parameter  $b$ . The aim of this work is therefore to develop a computer program to calculate the Casson's viscosity ( $\eta_{CA}$ ) of chocolate mass using formula (1) that automates the computation of  $b$  and  $\tau_{CA}$ .

### II. Materials and methods

In developing the software for computing Casson plastic viscosity using the OICC methodology we use the C++ programming language. It is an algorithmic language that makes it easy to encode the logical constraints of the method and also has a wide range of mathematical functions, describing the mathematical part [1]. To test the software we analyse three types of chocolate mass [3]. All analyses were carried out using a Rheotest 2 viscosimeter (Germany).

From the obtained data and the implemented constants of the viscosimeter corresponding of requirements of analysis, the programme calculates the values of the triples  $x_n = (1+a)\sqrt{D}$  and  $y_n = (1+a)\sqrt{\tau}$ , and after it computes the equation of a straight line in a place ( $y=Ax+B$ ). The corresponding coefficients are calculated using least squares. The program computes the Casson's viscos-

ities based on the value of a squared, and shear strain based on equation (2).

### III. Results and discussion

Figure 1 shows a block diagram of the program for measure of Casson's plastic viscosity.

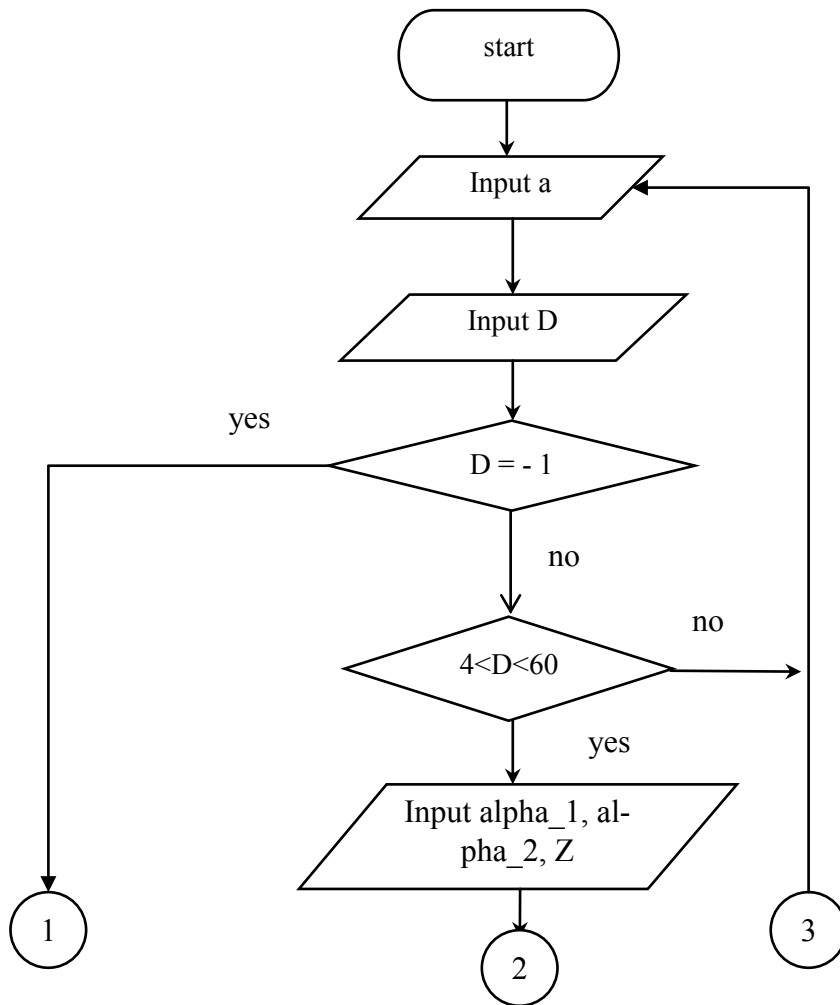


Figure 1. Block diagram for computing Casson's plastic viscosity

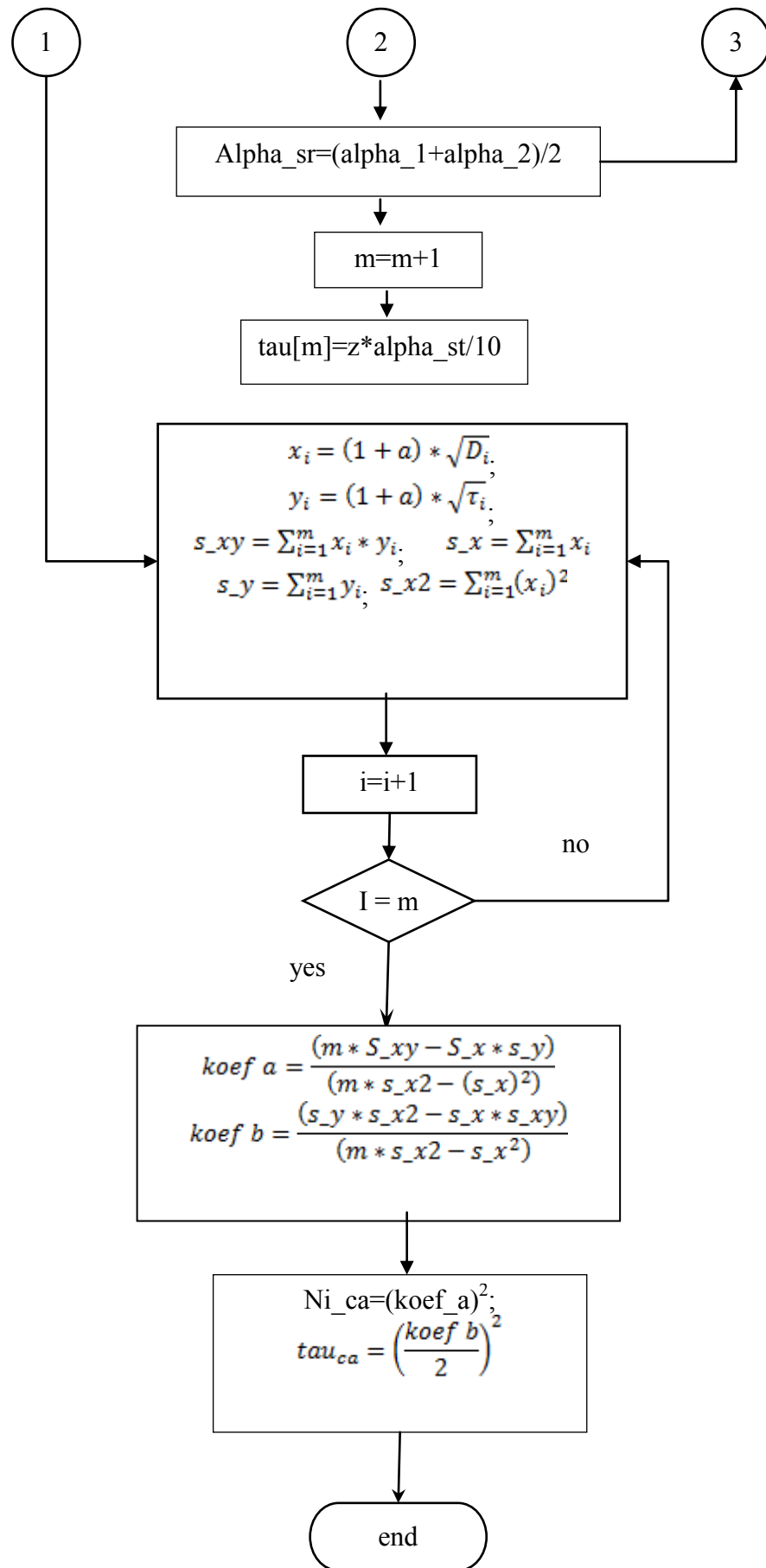


Figure 1.1. Block diagram for computing Casson's plastic viscosity

The application of program for calculating of the Casson's viscosity was implemented by the following algorithm:

Enter the ratio of the radii of the coaxial cylinder of the rheoviscosimeter ( $a$ ).

In an infinite cycle enter the values of the shear rate ( $D$ ) are introduces only these corresponding of  $5 < D < 60 \text{ s}^{-1}$  (in accordance with [7]) and storing them into a one-dimensional array.

Enter the values shown on the viscosimeter screen ( $\alpha$ ) for the respective shear rates towards increasing or decreasing values.

Enter a correction constant ( $z$ ) for the viscosimeter. This is read from the machine's user manual.

Compute the average values of  $\alpha$  of all readings while increasing and decreasing the values of  $D$  between  $5$  and  $60 \text{ s}^{-1}$ . Compute the shear stress ( $\tau$ ) for each value of  $\alpha_{cp}$ . Use  $-1$  as the value of the shear rate ( $D$ ) to exit the cycle.

In a separate loop for each value of  $D$  we compute and store in the separate arrays the following values:

$$x_i = (1 + a) * \sqrt{D_i},$$

$$y_i = (1 + a) * \sqrt{\tau_i}.$$

Compute  $\sum x_i * y_i$ ,  $\sum x_i$ ,  $\sum y_i$  and  $\sum (x_i)^2$ . These are needed to compute the coefficients in the equation  $y = Ax + B$  using the least squares method.

Casson's plastic viscosity ( $\eta_{ca}$ ) is computed by squaring the  $A$  coefficient. The shear strain is determined using formula (2).

#### IV. Conclusion

We develop a computer program that automates the application of graphical methods for determination of Casson's plastic viscosity and limit of fluidity of melted chocolate mass using the OICC methodology.

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## INVESTIGATION OF LIPASE FROM *ASPERGILLUS CARBONARIUS* UPON THE QUALITY OF BREAD

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**Abstract.** The influence of the lipase from *Aspergillus carbonarius* (0.06, 0.33 and 0.60 %) upon the quality of wheat bread was studied. The effect of lipase was observed on the baking characteristics – volume, loaf volume, weight, and sensory properties (volume, aroma, crust and crumb color, mastication, porosity, atypical flavor, and aftertaste). It has been found that the addition of lipase from *Aspergillus carbonarius* has a positive effect on bread characteristics - the volume and loaf volume increase. Sensory profile of bread with an addition of lipase is greatly improved - the volume, aroma and porosity were increased for all test samples.

**Key Words:** lipase, wheat bread, quality, sensory analysis

### I. Introduction

The use of enzymes in breadmaking is a long drawn practice for improving the dough properties and bread quality. The use of lipase as compared with other enzymes in the baking industry is very recent; it is interpreted as a relatively new and interesting ways for improving the quality of flour, dough and bread [4, 6, 7].

The lipase is from the group of hydrolases acting on ester bonds. Lipases hydrolyze carboxylic ester bonds in water-insoluble triacylglycerol esters of long chain fatty acids to diacylglycerol and a fatty acid [13, 14].

The use of exogenous lipase from different types in breadmaking has a positive effect on the volume of bread, and the development of the crumb [4, 6, 8, 9, 11, 12].

All hydrolytic enzymes have been shown to be effective in reducing the initial density and the increase of the specific volume of the bread. The lipase addition increases the bread volume significantly compared to the control sample (about 20-30 %). It increases the resistance of the walls and reduces the density of the air sacs, while improving retention of gas in dough. The higher quantity of lipase reduce the volume of bread, which is likely due to the longer action of the enzyme in the dough, resulting in the formation of drying of the dough and the formation of a dense and hard dough [5, 6, 7, 9]. In literature it's not found studies about the use of lipase enzyme from *Aspergillus carbonarius* on the quality of bread.

The general objective of the present study is to investigate the influence of lipase from *Aspergillus carbonarius* on wheat bread quality.

### II. Materials and methods

#### 2.1. Raw materials:

- Wheat flour with 10,6 % moisture, acidity – 2.2 °H;
- Water, according to BSS 2823-83 [2];
- Yeast, according to BSS 483 – 80.
- Salt, according to (№ 23 23/ 2001) [3].
- Enzyme – lipase from *Aspergillus carbonarius* with 1080 U/ g activity (isolated and prepared by UFT).

#### 2.2. Analytical methods:

**Baking method** – by methodology prepared in UFT - Plovdiv. The dough temperature is 29 - 30 °C, resting time – 20 min, bread formation, final fermentation (35 °C) for 40 – 45 min, baking temperature 220 - 230 °C [1];

- **Weight (g), volume (cc)** – according BSS 3412 – 79;

- **Index (H/D)** – the ratio of height to diameter of bread [1];

- **Loaf volume, cc/ g** – the ratio of volume to masse of bread [1];

- **Sensory analysis** – the evaluation is making according to ISO 8586-2:2008 with the next indicators: volume, odor, crust color, crumb color, mastication, porosity, atypical flavor, residual flavor (table 1). Before the analysis the degustators were prepared by the test procedure. Each degustator applied to the tasting map (scale for the intensity of



indicators) the values characterizing the strength of perceptions of each property. The arithmetic results are graphically shaped on a coordinate system. Each property receives a value, which forms the sensory evaluation of bread [9]. 24 students selected and trained according to ISO 8586-1 (third year students of Faculty of Food Technology of UFT), participated in the evaluation of bread samples. The students received encoded samples and questionnaires as well as instructions for the evaluation of the samples [10].

**Table 1.** Tasting card for index intensity of bread

№	INDEX	INDEX INTENSITY								
		1	2	3	4	5	6	7	8	9
1	Volume									
2	Aroma									
3	Crust color									
4	Crumb color									
5	Mastication									
6	Porosity									
7	Atypical flavor									
8	After taste									

The information contained on the sensory performs was indicated as:

**Legend:**

1 – extremely dislike;	6 – slightly like;
2 – very much dislike;	7 – moderately like;
3 – moderately dislike;	8 – very much like;
4 – slightly dislike;	9 – extremely like.
<b>5 – neither like nor dislike;</b>	

Based on study and analysis of the literature [8] led to the choice of the following quantities enzyme shown in table 2.

**Table 2.** Enzyme dosing

ENZYME	QUANTITY, %		
	A	B	C
<b>Lipase</b>	0,06	0,33	0,60

**III. Results and discussion**

After baking test, by methodology prepared in UFT - Plovdiv it is established the weight and volume of bread on the floor (table 3).

From the results of weight and volume of bread it is established that the addition of lipase in minimum and average quantity (sample A and B) has a positive effect.

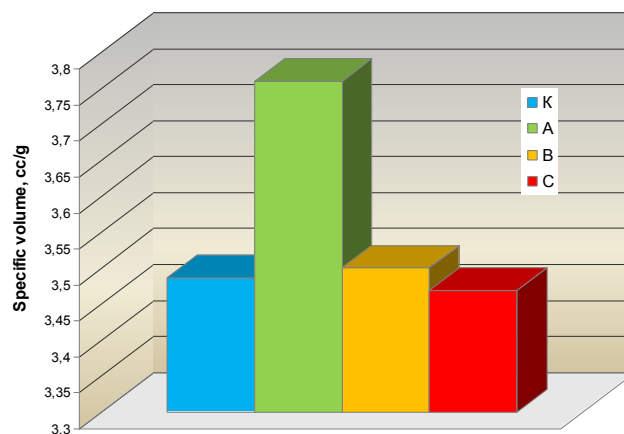
**Table 3.** Weight and volume of bread on the floor with lipase

Sample	Weight, g	Volume, cc
<b>K</b>	203.7	710
<b>A</b>	205.9	775
<b>B</b>	204.8	750
<b>C</b>	201.8	700

The weight of control sample is 2.2 g lower. The higher result is to sample A. The volume of bread is significantly increased. This is resulting from better rheological and gas retention properties of dough. It is obtained the best results with addition of minimum quantity of enzyme. The difference between the control sample and the sample A is 8.4 %. With maximum added quantity of lipase (sample C) a weight and volume of bread are smaller in comparison with the control sample. This indicates that the greatest quantity of enzyme reduces a quality of bread.

The loaf volume of bread is the ratio between the volume and a weight. The loaf volume helps to make more complete characteristic of bread.

The results of studies of the samples for loaf volume are presented in figure 1.



**Figure 1.** Loaf volume of bread

It is found that the highest loaf volume is obtained with an addition of minimum quantity of lipase (sample A) respectively 3,76 cc/g. The difference between the control sample and sample A in percentage is 7,4.

With addition of 0,33 % (sample B) of lipase there is an improvement also. The decrease in the loaf volume of bread with maximum quantity of lipase (control C) from control sample K is 0,5 %.

The index H/ D is determinate by the ratio of height to diameter of bread after baking process.

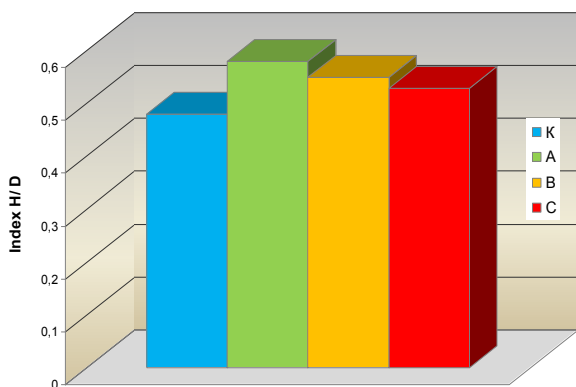


Figure 2. Index H/ D of bread

The results of studies of the samples index H/ D are presented in figure 2. It is found that the index H/ D with higher values is in addition of 0,06 % enzyme (0,58). The difference between the control sample and sample A is 0,10 units (17,2 %). The increase for the other two quantities fits in the difference between the control and test sample A with minimum addition of lipase. After baking test the results for weight and volume of tin bread are presented in table 4.

Table 4. Weight and volume of tin bread with lipase

Sample	Weight, g	Volume, cc
K	390	1090
A	398	1180
B	400	1160
C	385	1130

The results for tin bread are similar to the results for bread on the floor. The highest weight is obtained with addition of the small quantities of enzyme – sample A and sample B. With addition of the highest quantity of lipase (sample C), weight is decreasing below that the control sample.

The volume of all sample of tin bread with lipase is higher than of the control sample. The highest volume with an addition of 0,06 % lipase is 1180 cc. The difference between the volume of the control sample and those of tin bread with 0,06 % enzyme expressed as a percentage is 7,6. From these results it can be concluded that there is a correlation between the action of lipase and volume of dough. The bread on the floor which is made from a smaller weight of dough is optimal in terms of weight and shape of the enzyme from *Aspergillus carbonarius*.

It is established the positive effect of the enzyme at determination of the basic parameters of bread. It was obtained the same results. The results of addition of minimum and the average quantity of enzyme (0,06 and 0.33 %) are the best. On the other

hand the higher quantity of enzyme decreased all the values of quality index.

The results from sensory evaluation of bread samples containing different quantities of lipase compared to the control sample are shown in figure 3. It is found that all samples are smooth, with fine crust, no major cracks and bumps, with a characteristic color, which ranges from yellowish-beige to light brown.

After cutting is showed that the control sample has a greater tendency to curl, as its crumbs were more glued and enlarged. All experimental samples had better development and a more uniform crumb structure as compared to the control sample. The bread crumb color (sample K) is more yellow than the test samples. The taste of all breads is pronounced. At the time of removing the bread from the oven all test samples have an alien grassy flavor that eventually disappears and unaffected taste profile of bread. It is possible that this flavor can be separated due to the action of lipase low molecular weight fatty acids or of an ester-forming.

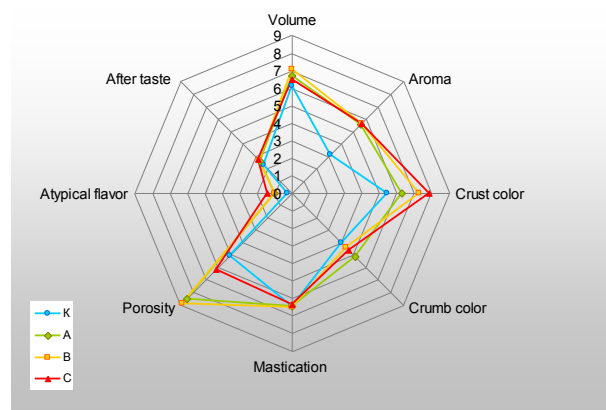


Figure 3. Sensory analysis of bread – comparison between the control samples and tests samples

It is found that volume of all samples with enzyme is increased in comparison with control sample. The highest volume is established in addition of 0,06 % enzyme. High quality of the samples A and B is established in the porosity. For all test samples there is a flavor increasing. By increasing the quantity of lipase intensity of bread crust is improved. Bread test samples have an atypical flavor but the values are in the category of „slightly perceptible”. Mastication and atypical flavor are without any changes in all samples. High quality of the samples at all levels of addition of lipase is established in the porosity. The difference between control sample and test sample B is 50 %. The crumb is perceived as more developed.

#### IV. Conclusions

The results from experiments indicated that addition of lipase from *Aspergillus carbonarius* have a positive effect upon the bread characteristics – volume and loaf volume increased.

The results from sensory analysis using hedonic rating showed that volume and the aroma of all samples with enzyme are increased. Sensory profile of bread with an addition of lipase is greatly improved - the volume, aroma and porosity were increased for all test samples.

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## STUDIES REGARDING THE INFLUENCE OF THE LAYER TYPE UPON THE BREAD QUALITY WITH DIFFERENT QUANTITIES OF FOOD ADDITIVES

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**Abstract:** In the paper we assessed the bread quality at different quantities of food additives by comparison of the physico-chemical and organoleptic properties as well as the reaction in different storage circumstances and different packagings compared with those of the control sample. The shelf-life from both the sensorial and microbiological point of view as well as the bread's quality is directly influenced by the used ingredients and the type of package.

The food packagings must be proper and ensure the protection and nutritional stability of the food stuff during the transportation, distribution and shelf life. Due to the close contact that exists between the food and its pack there is a possibility of transfer in the food of different physical, chemical and biological contaminants.

**Keywords:** packaging, bread, additive, food safety

### I. Introduction

Bread, being a daily consumed food essentially but perishable, it is important to be of a good nutritional quality and safe for the consumer. In the past years, the concept of the technological process of food production was completed in the sense that there have been taken into account a series of technological operations after baking: the cooling, the slicing in pieces, the packing of food as a whole or in sliced pieces as well as the bread's refrigeration to ensure a longer shelf life for it (Weber, 2000; Wan de Mareel et al., 2002).

At the end of the baking process when the bread is taken off the oven the cover temperature according to the baking type can arrive at 120 - 160°C, and the core at 95 - 98°C. When it arrives in the storage where the temperature is of 18<sup>o</sup> - 25<sup>o</sup>C and the relative humidity of air of 60 - 80%, the bread's rapid cooling process starts from the outer layers to the core (Kotsianis et al., 2002).

A risen problem for the bread's storage period would be the molding that is provoked by a large number of molds the most frequent being those of *Aspergillus* type (*A. Flavus*, *A. Fumigus*, *A. Niger*, *A. Nidulans*), *Mucor* type (*M. Mucedo*, *M. Pusillus*, *M. Spinus*), *Penicillium* type (*P. Expansum*, *P. Glaucus*). The molded bread modifies its coloration due to the molds colonies that have a specific colour, the taste and smell become unpleasant so that it becomes improper for consumption. The nourishing quality is also lost due to the mycotoxins secreted by some molds: *Aspergillus flavus*, *Aspergillus*

*ochraceus*, *Penicillium expansum*, *Penicillium viridicatum* (Banu, 1999).

The molds present in the flour and in the other primary and auxiliary materials are destroyed while baking thus the molding isn't due to the raw materials are contaminated with molds. The molds contamination of bread can occur in the following stages: 1) bread transportation between sections, 2) cooling and storage, 3) while slicing and packing (optional operations) (Banu et al., 1999, Popa & Belc, 2003).

The purpose of the paper is to assess the quality of bread with different quantities of additives by comparing the physico-chemical and organoleptic properties as well as the behaviour at storage in different types of packages compared with those of the control sample.

### II. Materials and methods

There were prepared four samples of bread of 500g with the following production recipe: 500 g wheat flour; 400 ml water; 25 g yeast; 15 g sugar; 10 g salt; in it there were introduced different quantities of the additive *Eka valore* (sample 1 - 0 g additive, sample 2 - 0,9 g additive, sample 3 - 1,5 g additive (the standard quantity frequently used for the bread flour enhancement), sample 4 - 2,5 g additive). The additive *Eka valore* (*Marathon Distribution Group, Pakmaya*) has in its composition dispersion stabilizer, soy flour, emulsifier, ascorbic acid, enzymes, cystein and calcium carbonate. The bread was obtained with the aid of special bread preparing automatic devices (Alaska, Romania).

By the organoleptic examination there were evaluated: the bread outer appearance, the symmetry of the form, the volume, the cover's colour and structure, the colour, the flexibility and porosity of the core, the smell, taste and foreign bodies presence (Pop, 2005). The organoleptic evaluation from the consumer's point of view was done on a group of 25 students from the Faculty of Food Engineering (Stefan cel Mare University of Suceava, Romania).

For to assess each bread sample there were analyzed the following physico-chemical properties: porosity (by weighting method), elasticity (by pressing a piece of core of a given height and form for a given amount of time and by measuring the height when it recovers after eliminating the pressure force), humidity, acidity (by the titrimetrical method) (Bordei, 2007). The three-dimensional structure of the core was studied with Optica SZM-2, fitted with a digital camera (magnification: eye 10x/22mm, objective 0.7x - 4.5x).

For to study the bread behaving stored in different types of packages most commonly used in

the household during 3 days time the four bread samples were sliced and packed as it follows: 1) without package, 2) in cotton towel, 3) in paper towel and 4) in polyethylene bags. The developed molds on the bread samples were studied at microscope (Motic, Nitech, Romania) on wet samples.

### III. Results and discussions

The first impact on the consumer with the food product is about the outer appearance of it. For the four bread samples immediately after baking the symmetry and regularity of the bread was controlled taking on account the following aspects: the bread volume, the general appearance of the cover surface, the colour and resistance. The bread samples with the additive quantities mentioned above are differ by the different traits of the outer appearance in comparison with sample 1. The results of the assessment for the four samples are presented in table 1.

**Table 1.** *The traits of the outer appearance of the fresh bread samples*

Sample	Sample 1 (bread with 0 g additive)	Sample 2 (bread with 0,9 g additive)	Sample 3 (bread with 1,5 g additive)	Sample 4 (bread with 2,5 g additive)
Analyzed trait				
Symmetry and regularity of the form	Incorrect	Incorrect	Symmetrical, normal	Incorrect
Bread volume	Well risen	Well risen	Well risen	Flattened
General appearance of the cover surface	Slightly cracked	Shinny, slightly cracked	Shinny	Rough cracked surface
Cover colour	Uneven	Uneven	Normal, even	Whitish, uneven
Cover resistance	Crispy	Slightly rough	Crispy	Brittle

The state and appearance of the core were verified by examination of the bread section. The results of the examination of the four bread samples are presented in table 2. The third sample has the best elasticity of the core. The wheat flour is unique in the way in which it forms the gluten structure in mixture with water to form elastic dough that can retain gases (Lorimer et al., 1991).

By adding soy flour the nutritional value of the wheat flour is enhanced for soy increases the proteic quality of the wheat flour by its lysine content, an amino acid that is not found in wheat. As well, by its riboflavin, pyridoxine, panthotenic acid, folic acid, niacin, inositol, calcium, phosphorus and iron content soy contributes to the nutritional equilibration of bread.

**Table 2.** *The appearance and state of the core of the fresh bread samples*

Sample	Sample 1 (bread with 0 g additive)	Sample 2 (bread with 0.9 g additive)	Sample 3 (bread with 1.5 g additive)	Sample 4 (bread with 2.5 g additive)
Analyzed trait				
Visual appearance of the slice of bread	Pleasant	Pleasant	Pleasant	Pleasant
General appearance of the core	Uneven	Even	Even	Uneven
The core's colour	Yellowish with flour traits	Yellowish	Yellowish	Yellowish, uneven, with flour traits
The porosity of the structure	Irregular vacuoles, wet core when testing	Big pores of oval form, unevenly distributed	Small pores of oval form; dry core when testing	Irregular vacuoles, wet core when testing
Elasticity	Elastic	Elastic	Very elastic	Non elastic



The resistance of the core at finger pressing	Comes back slowly at initial form	Comes back slowly at initial form	Comes back immediately at initial form	Doesn't come back at initial form
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The core texture is an important qualitative structural trait being influenced by the dimension and porres uniformity as well as by the thickness of

their layers. The three-dimensional structure of the core of bread in sample 1 is presented in figure 1.



**Fig. 1.** The three-dimensional structure of the bread core of sample 1 (magnification: 20x)

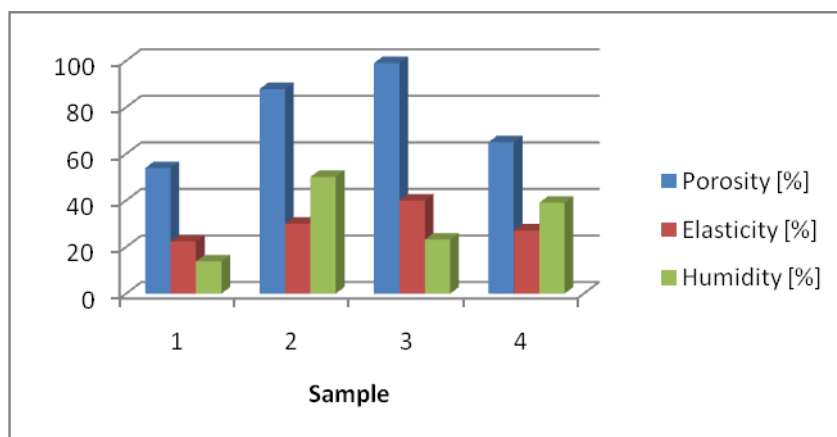
The bread samples were tested from the flavour, taste and microbial altering point of view. In this first step of characterizing the samples at the initial stage, the breads being fresh didn't have any signs of microbial altering or presence of foreign bodies.

By assessing the data obtained in the enquiry (the grades being between 1 and 6, the maximum being 6) resulted the fact that the students appreciated as favourite with a score of 300 points Sample no. 3

(bread with 1,5 g additive), followed by Sample no. 2 (bread with 0,9 g additive) with a score of 288 points. Sample no. 4 (bread with 2,5 g additive) obtained 280 points. The differences in the grades obtained are not significantly high - fact that shows that the bread being fresh doesn't present significant modifications in comparison with sample 1- the control sample (table 3).

**Table 3.** The traits regarding the smell and taste of the fresh bread samples

Sample	Sample 1 (bread with 0 g additive)	Sample 2 (bread with 0,9 g additive)	Sample 3 (bread with 1,5 g additive)	Sample 4 (bread with 2,5 g additive)
Analyzed trait				
Presence of foreign bodies	There were no foreign bodies observed			
Taste	Specific	Normal	Pleasant, sweet	Normal
Flavour	Specific	Pleasant	Pleasant	Pleasant

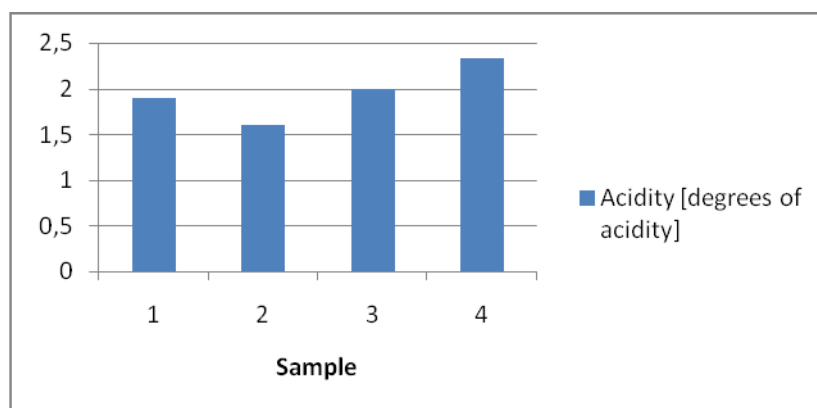


**Fig. 2.** Porosity, elasticity and humidity of the bread samples with different additive quantities: 1 - 0 g additive, 2 - 0,9 g additive, 3 - 1,5 g additive, 4 - 2,5 g additive

The physic-chemical traits are presented in figure 2 by the quantification of porosity, elasticity and humidity. The porosity was the highest, of 99%, registered for sample 3 with a content of 1,5 g additive, and for sample 4 with a content of 2,5 g additive the porosity was of 64,99%. The best elasticity is also in the case of sample 3 while for sample 2 we saw a medium elasticity and for sample 4 a low elasticity. Sample 3 presented low humidity

content in comparison with samples 2 and 4. According to the data in figure 2 it was seen that for the bread with additive the porosity, elasticity and humidity contents were enhanced in comparison with the control sample no. 1.

The bread with the highest additive content had a high acidity value in our case for the samples 4 and 2 (figure 3).



**Fig. 3.** Acidity of the bread samples with different additive quantities: 1 - 0 g additive, 2 - 0,9 g additive, 3 - 1,5 g additive, 4 - 2,5 g additive

From the studies led on the fresh bread we saw that the bread with 1,5 g of additive presented the best physic-chemical traits being appreciated by the consumers the most.

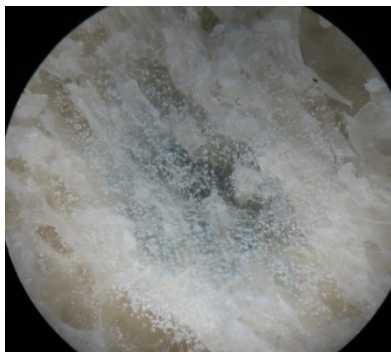
The samples no. 2 and no. 3 with contents of 0,9 g additive and respectively 1,5 g additive didn't present any altering after the first storage day in all four types of packages. The appearance of the samples is pleasant, the cores of the samples are soft, not losing the sensorial qualities and not presenting any traces of mold. Sample no. 4 with a content of 2,5 g of additive presents small variations in appearance and texture for the slice of bread packed in polyethylene package the core becoming gluey so that the product is rejected by the consumer. The other slices kept with a package and packed in cotton towel or paper towel presented a slightly obsolescence fact that can be accepted by some consumers. On the surface of all four bread slices in sample 3 there were no mold traits observed. In figure 4 it is presented the stereomicroscopic image of the mold that appeared on the bread slices from sample 1 bread kept for 2 days in polyethylene bag.

After the second day of storage sample no 2 of bread slices preserved in cotton towel, paper towel and polyethylene bag present slightly visible mold traits the core being almost gluey while the unpacked bread is very dry. The slice of bread sample no 3 kept in a polyethylene bag didn't suffer any

alterations from the core's elasticity point of view, it seemed fresh while the other slices of bread kept unpacked or packed in cotton towel or paper towel presented drying traits. The mold didn't develop on the slice of bread packed in cotton towel while on the surfaces of the other bread slices the mold traits appeared. The slice from the bread sample no 4 kept in polyethylene bag presents a gluey sticky core with visible traits of mold and the slices of bread of this sample kept unpacked or packed in paper or cotton towel dried out altering their colour and presenting visible traits of mold thus becoming improper for the consumption.

The bread slice from sample no 3 kept for 3 days in cotton towel presented only slightly visible traces of mold while after the third day of storage the mold was present on the surfaces of all the other bread slices from all the four samples.

After the microscopic examination the mold taken from the bread samples subjected to the study (that are rich sources of polysaccharides (amylase)) we saw that the mold was of *Aspergillus* type (figure 5). *Aspergillus* is a fungus formed by several species of molds that can be found in different climates all over the world. The *Aspergillus* species are aerobic and can be found in almost all environments that are rich in oxygen where they grow mostly under the form of molds.



**Fig. 4.** The mold present on the sample 1 bread slice stored for 2 days in polyethylene bag (magnification: 20x)



**Fig. 5.** The mold of *Aspergillus* type taken from the bread samples surfaces

#### IV. Conclusions

The adding of different quantities of additive in bread modifies significantly the physic-chemical properties as well as the organoleptic ones but in a smaller amount.

In our study it was observed the stability from the microbiologic point of view of a bread grade with different quantities of additive during a period of three days time kept in the most common ways of packing for the household: (1) without a package, 2) in cotton towel, 3) in paper towel and 4) in polyethylene bags.

After the results obtained it was observed the fact that the slice of bread from sample no 3 (with 1.5 g of additive) kept for three days in cotton towel presented the best stability from the microbiologic point of view. The packing of the bread slices in polyethylene bags, regardless the additive quantity suffers the most and rapid alterations from the physic-chemical properties point of view of the microbiologic stability and organoleptic qualities favoured also by the high relative humidity of the air from the waterproof pack to the water vapors.

Following the microscopic examination of the mold taken from the bread samples subdued to the study we saw the presence of a mold of *Aspergillus* type. By the molds development there are weight losses in bread, altering of the appearance due to the colonies characteristically coloured and taste altering as a results of the molds creation of some

compounds that give the characteristic mold trait, the bread becoming improper for consumption.

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## **Influence of gum arabic and starch as hydrocolloids on the quality of emulsion type oil-water in food**

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**Abstract.** *The paper systematically describes the basic theoretical information about the improvements in the production of emulsions. We consider the theory of a stable emulsion system, namely the particular use of raw materials and their properties analyzed the conditions necessary for the process of homogenization. And based on this, a large number of existing theories determined the most effective, which is used for the production of emulsions. Much attention is paid to the use of different stabilizers receipt of test data required for the calculation formulas finished products, and technological design process of emulsions. To assign these methods are used two stabilizers: gum arabic and modified starch, which when used for stability during storage of emulsions yield different results.*

*Of great importance for the stability of these products is the size of the particles. The diameter of the emulsion depends on a process of manufacturing technology. More detail the process of homogenization of emulsions. For the features of this process are a few examples that will visually see the results emulsion stability during storage. Based on the processed foreign sources give modern technology and types of equipment for the homogenization process, with reference to the drawings, which will assimilate the information produced. We consider the design features of these devices, their advantages and disadvantages.*

**Key Words:** emulsion, particle size, phase, stability, stabilizer

### **I. Introduction**

The article is devoted to the important issue of improving production technology emulsions, which are widely used in various sectors of the food industry.

The issue of improving the production technology of aromatic emulsions closely related to features using hydro colloids. Constant attention to the researchers hydrocolloids due to their importance for food technology. Despite the large number of studies on the physicochemical properties of hydrocolloids, there is no scientifically based data on their use in food emulsions [1].

Therefore, current and future issues is the definition of certain laws (based on the recommendations of the manufacturers of) on the influence of the main factors in the production of emulsions: selection of hydrocolloids; the ratio of oil and water phases; (sequence making components, mixing time and temperature parameters of each ingredient in order to ensure their complete solubility); selection of process parameters homogenization. This will facilitate the development of new formulations and optimization processes.

There is a theory about the mechanism of emulsification [1]. The first stage of this process lies in the tension drops of liquid dispersion in a field

environment. Pulling drops in thread accompanied by an increase of the surface and flow of work to overcome the molecular forces of surface tension. This extended liquid drop becomes so unstable that spontaneously breaks into small spherical droplets. This is the second stage of the formation of emulsions, which is accompanied by a decrease in surface and spontaneous process. Then comes the next, third stage, when formed droplets on one hand, coagulated in collisions, and on the other - again stretching into smaller parts to equilibrium. The basis of increasing dispersion emulsion is spontaneous decay drops learned to unstable size [2–4].

Found that emulsions are closely associated with the mechanism of dispersion and depends on many factors, such as oil content, type and concentration of emulsifier, the route of administration phases, time and intensity and degree of dispersion and temperature. Study of factors that ensure stability of emulsion, led to the conclusion that the critical degree of dispersion [5–9].

Experiments found that each type of emulsifier has its own optimum concentration that provides the highest resistance obtained emulsions [7]. For an introduction to emulsify oils (for each concentration of emulsifier) there is also optimum in which the most stable emulsion is obtained, that are

determining the optimal ratio between the aqueous and oil phases. Introduction of excess oil is causing separation. Thus for each emulsifier is its optimum concentration, the corresponding amount of oil in the emulsion [8].

The process of destruction of the emulsion described rate of destabilization (V) by Stokes' law:

$$V = \frac{2 \cdot r^2 (d_1 - d_2) \cdot g}{9 \cdot q}$$

where: *V* – speed destabilization of the emulsion;  
*d*<sub>1</sub> and *d*<sub>2</sub> – density of the dispersed phase and the dispersion, respectively;  
*q* – the viscosity of the medium;  
*r* – radius of the globule of fat;  
*g* – acceleration due to gravity.

To reduce the *V*, you must use oil with a high density (about 1.0) or increase the density of light oil (such as citrus, for which *d* ~ 0,80) by making authorized for use in foodstuffs agents such as sucrose acetate isobutyrate (SAIB).

To reduce the fat globules range 0.4–1.0 microns are used to mixing with a high shear stress and homogenisation of emulsions pressure 100–300 kg/cm<sup>2</sup>. With this amount of fat globule coalescence is minimized, and the dissolution is a strong turbidity.

The optimum concentrations of emulsifiers for certain ratios of the phases in obtaining stable emulsions are not fixed and depend on the degree of dispersion. Using of high-speed mixing, and especially increasing pressure homogenizer leads to increased dispersion, viscosity and the formation of more stable emulsions [9].

The process of manufacturing emulsions involves creating optimal conditions that allow for uniform, homogeneous and stable system with virtually insoluble in each other components (oil - water) by adding emulsifiers (stabilizers), the relative density of the equalizer.

## II. Materials and methods

The aim is to study particle size effects on the stability of emulsions during storage and use in the manufacture of beverages and their stability during 180 days. As materials for research are prepared samples of emulsions with various stabilizers (gum arabic, modified starch). Stability of emulsions depends of viscosity, particle size, muddy turbidity depends on the ratio of water and oil phases.

For studies prepared sample emulsions of varying oil phase and a constant amount of stabilizer:

- gum arabic (table 1)
- starch (table 3);

and samples of emulsions (at constant oil phase) different amount of stabilizer:

- gum arabic (table 2)
- starch (table 4).

Formulations of emulsion of varying oil phase and a constant quantity of gum arabic

Table 1

The ingredients of the emulsion	Content ingredient, g/kg				
	Number of emulsion				
	1	2	3	4	5
Citrus oil	60	60	60	60	70
Rezynogum (E 445)	20	40	50	60	70
Gum arabic (E 414)	50	50	50	50	50
Citric acid (E 330)	5	5	5	5	5
Sodium benzoate (E211)	2,5	2,5	2,5	2,5	2,5
Colorant (E124)	1,5	1,5	1,5	1,5	1,5
Colorant (E110)	14	14	14	14	14
Antioxidant (E320, E321)	0,025	0,025	0,025	0,025	0,025
Water	846,975	826,975	816,975	806,975	786,975
Total	1000	1000	1000	1000	1000

Formulations of emulsions with constant quantity of fat phase and a variable quantity of gum arabic

Table 2

The ingredients of the emulsion	Content ingredient, g/kg				
	Number of emulsion				
	6	7	8	9	10
Citrus oil	60	60	60	60	60
Rezynogum (E 445)	40	40	40	40	40
Gum arabic (E 414)	40	50	55	60	70
Citric acid (E 330)	5	5	5	5	5
Sodium benzoate (E211) 211211)	2,5	2,5	2,5	2,5	2,5
Colorant (E124)	1,5	1,5	1,5	1,5	1,5
Colorant (E110)	14	14	14	14	14
Antioxidant (E320, E321)	0,025	0,025	0,025	0,025	0,025
Water	836,975	826,975	821,975	816,975	806,975
Total	1000	1000	1000	1000	1000

Formulations of emulsion of varying oil phase and a constant quantity of starch

Table 3



The ingredients of the emulsion	Content ingredient, g/kg				
	Number of emulsion				
	1	2	3	4	5
Citrus oil	40	50	55	60	70
Rezynogum (E 445)	40	50	55	60	70
Starch (E 1450)	120	120	120	120	120
Citric acid (E 330)	5	5	5	5	5
Sodium benzoate (E211)	2,5	2,5	2,5	2,5	2,5
Colorant (E124)	1,5	1,5	1,5	1,5	1,5
Colorant (E110)	14	14	14	14	14
Antioxidant (E320, E321)	0,025	0,025	0,025	0,025	0,025
Water	776,975	756,975	746,975	736,975	716,975
Total	1000	1000	1000	1000	1000

Formulations of emulsions with constant quantity of fat phase and a variable quantity of starch

Table 4

The ingredients of the emulsion	Content ingredient, g/kg				
	Number of emulsion				
	6	7	8	9	10
Citrus oil	55	55	55	55	55
Rezynogum (E 445)	55	55	55	55	55
Starch (E 1450)	80	100	110	120	140
Citric acid (E 330)	5	5	5	5	5
Sodium benzoate (E211)	2,5	2,5	2,5	2,5	2,5
Colorant (E124)	1,5	1,5	1,5	1,5	1,5
Colorant (E110)	14	14	14	14	14
Antioxidant (E320, E321)	0,025	0,025	0,025	0,025	0,025
Water	786,9	766,975	756,975	746,9	726,975
Total	1000	1000	1000	1000	1000

*Preparation of emulsions*

*1. Preparation of oil phase.*

Weigh the required amount of flavor; add Esther scales in stirrer at room temperature until Esther scales completely dissolved.

*2. Preparation of the aqueous phase.*

- Weigh the required amount of water into a glass and heated to 20-50 ° C.
- Attach the required amount of sodium benzoate and completely dissolve. Add citric acid and dissolve completely.
- Attach the required amount of stabilizer and dye solution in warm water (20-50°C).
- Stabilizer* mix and dissolve at a moderate speed mixer until it is completely dissolved. Subject the immediate hydration; leave for a few minutes for aeration.

*3. Preparation of the pre-emulsion*

For the preparation of the pre-emulsion use a high-speed mixer. Slowly adding the oil phase to the aqueous phase, and then stirred at maximum speed.

*4. Preparation of the emulsion by homogenization.*

Homogenization undergoing the parameters

- Pressure is: the first step / second step 200 /50 bar, two numbers of moves for emulsion with starch.
- Pressure is the first step / second step 280 /40 bar, two numbers of moves for emulsion with gum arabic.

5. Measure turbidity, viscosity and average particle size of the emulsion.

**III. Results and discussion**

The results of measurement of each emulsion: Brookfield viscometer - viscosity microscope EASTCOLIGHT 92012 - ES (100x, 250x, 550x, 750h ) - particle size , muddy turbidity meter 2100P, density- lab density meter, pH- lab pHmeter - displayed in Table 5,6

The results of measurement of the finished product (with gum arabic)

Table 5

Number of emulsion	Viscosity Brookfield, cP	Turbidity dilution 0.025 %, NTU	The average diameter of the particles of oil D, μm	Density, g/ cm <sup>3</sup>	pH
1	14	168	0,505	1,03	2,7
2	15	180	0,659	1,06	3,2
3	16	192	0,705	1,07	3,3
4	17	216	0,903	1,09	3,7
5	18	240	1,101	1,1	3,9
6	14	192	0,75	1,04	2,6
7	15	180	0,659	1,06	3,2
8	15,5	174	0,602	1,07	3,3
9	16	168	0,559	1,085	3,4
10	17	154	0,499	1,1	3,8

The results of measurement of the finished product (with starch)

Table 6

Number of emulsion	Viscosity Brookfield, cP	Turbidity dilution 0.025 %, NTU	The average diameter of the particles of oil D, $\mu\text{m}$	Density, $\text{g}/\text{cm}^3$	pH
1	20	143	0,67	1,03	2,6
2	22	156	0,73	1,05	3
3	23	170	0,75	1,07	3,3
4	24	182	0,84	1,09	3,65
5	26	196	0,97	1,1	4
6	19	210	0,98	1,04	2,7
7	21	196	0,91	1,06	3,1
8	22	184	0,83	1,065	3,2
9	23	170	0,75	1,07	3,3
10	25	157	0,68	1,1	3,8

Analyzing the figures emulsions with different stabilizers characterized by an increase in the of oil as a part of the product shows that increasing viscosity, density, particle size of emulsion and turbidity.

By continuing other components of the emulsion, the smaller is the particle size, the lower is the turbidity of the emulsion (but higher storage stability).

If the particle size is less than 1 micron, the emulsion is highly robust stability and gives some turbidity but less than 1 micron particle size, the less turbidity, if the particle size is not greater than 0.3 micron. The principle of leverage ratio of water and oil phase of emulsions with different stabilizers is the same. In the obtained parameters also affects the nature of emulsions stabilizer.

Investigation of the stability of emulsions was carried out by determining the size of the diameter of the particles by laser granulometry and placement on the stability of soft drink, which was used emulsion for 180 days. During storage of beverages prepared from emulsions studied, there was no formation of oil ring or “creaming” bottled, indicating the stability of emulsion systems.

#### IV. Conclusions

1. The best result of research in emulsions - is to obtain the maximum number of particles of about 1 micron.
2. Technology of preparation of emulsions with gum arabic is different from the technology of emulsifying starch.

3. Dissolve gum arabic is faster and easier than with the dissolution of starch as emulsion obtained using gum arabic, stable in quality and more expensive in value compared with emulsions prepared by using starch.
4. For studies prepared sample emulsions of varying oil phase and a constant amount of stabilizers and samples of emulsions (at constant oil phase) different amount of stabilizers.
5. The results of measurement of each emulsion: viscosity, particle size, muddy turbidity, density depends on the ratio of water and oil phases.
6. The results can be the basis for the technology of production of emulsions as a class of foods.

Creating a stable emulsion system is a pressing issue in the food industry, so these studies are useful and important for the development of new food products.

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