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CONTENT

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RESEARCH OF SENSOMOTOR REACTION, MEMORY AND ATTENTION INDICIES UNDER SENSORY DEPRIVATION

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Abstract

The article considers the influence of sensory (visual and auditory) deprivation on a sensorimotor response, memory, and attention among 8–11 years old children. The literature review concludes that sensory deprivation causes discomfort and problems in perceiving of reality. Also, under the influence of various factors that damage body structures, compensatory reactions are launched to compensate for impaired functions. The processes that provide the body with the restoration of lost structures and impaired functions of the pathology are called «compensatory-adaptive processes». Having conducted statistical analysis of the obtained data of latent periods of different sensomotor responses among children with sensory deprivation and the control group, it is seen, that the level of stimuli in the form of figures was higher among children with hearing impairments, and in the form of sounds – in the group of children with visual impairments. According to the results of our study, we can assume that in groups of children with sensory deprivation the process of compensating the impaired function of a particular analyzer takes place at the expense of another one.

Keywords: sensomotor response, latency period, sensory deprivation, compensatory reactions, memory, attention.

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1. Introduction

In Ukraine, the health of children and young people cannot be considered satisfactory. Among the negative factors, affecting the health of preschool and school-age children, are hearing and visual impairment, infections, traumas, and overuse of modern electronic devices.

Nowadays, children with hearing and visual impairments take a significant place among children with disturbances of development. According to the world statistics, in progressive countries, every twentieth preschool child and every fourth schoolchild has visual impairments. The most common childhood diseases are myopia, hyperopia, and astigmatism. As for hearing, according to the statistics, in Ukraine, there are more half a million children with hearing impairments. More than 6 % of the population has severe hearing impairments, so the problem of deafness and hearing loss is as important as vision problems [1].

The phenomenon of sensory deprivation attracted the interest of people for a long time. In some scientific works the arrest of a child's response is explained as a result of the deprivation of contact with the outside world and the environment. Some works indicate that children's long-term sensory deprivation causes disturbance of the mechanisms of the analytical system of the brain, leads to deviations in the development of its activating and regulating systems and their interaction. Also, the limited receipt of sensory information leads to the formation of emotional stress (Solntseva L., 2000) and creates unusual conditions for the development of the child's psyche [2, 3].

At the end of the twentieth century, active research began concerning a sensory deprivation's influence on the child's psychophysiological development. Most data do not give a complete picture of the effect of deprivation on the overall functional state, so our study aims to obtain and analyze scientific data on the specificity of the effects of visual and auditory deprivation on sensomotor response, memory, and attention [4–6].

2. Materials and Method

2. 1. Organization of the research

The study was conducted in compliance with the basic biotic provisions of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the World Health Association's Helsinki Declaration on Ethical Principles for Conducting Scientific Medical Research with Human Participation (1964-2008), and the Order Ministry of Health of Ukraine No. 690 dated September 23, 2009.

We have officially formalized contracts with schools No. 01-27/1918 dated September 21, 2015; 05-13/53 dated October 01, 2015 and No. 01-27/2237 dated November 04, 2015. We have obtained the informational consent from the parents/guardians of patients.

The survey involved 155 individuals aged 8–11 years. The students were divided into 3 groups: the 1st group – control (children with normal vision and hearing); the 2nd – schoolchildren with visual deprivation; the 3rd group– students with auditory deprivation. Each group, in turn, was divided into two subgroups by age: 1st subgroup included students aged 8–9 years; 2nd subgroup – 10–11 years ones.

A group of students with visual sensory deprivation consisted of children, who had refractive abnormalities: inborn myopia, astigmatism, myopia. Based on of studying medical records, 51 students were selected (26 –8–9 years old, and 25 individuals aged 10 to 11 years).

The group of students with auditory sensory deprivation consisted of children, who had inborn or early acquired pairedsensoneural hearing loss of II–III degrees. Based on the analysis of medical records and audiogram data, 53 students were selected (8–9 years – 26; 10–11 years – 27). Children with traumatic brain injury and asymmetric hearing were not included in the examination.

The control group consisted of 51 persons (8–9 years – 25; 10–11 years – 26), who have no visual or hearing impairment.

The study took place during 2016–2018, based on the Kherson boarding schools of the Kherson regional council, Kherson educational complex No. 11, and No. 48, as well as at the comprehensive school№31 in Kherson.

Taking into account fluctuations in mental capacity during the working day and week, all studies were conducted on days of high mental capacity: on Tuesday – Thursday from 9.00 till 13.00 [7, 8]. The total amount of experimental study for each observable was no more than 30–40 minutes per examination.

The order of the research for the whole contingent of the subjects was carried out according to the same scheme and was as follows: first, the sensorimotor response to stimuli of different complexity was studied (figures and sounds), which was developed on the device. The reliability of this technique was proved by the data of a number of experimental works, performed on adults and children [9–11]. Subsequently, a set of techniques for determining memory and attention functions was used.

2. 2. Methods of research of sensomotor response

In the study of visual/auditory motor reactions of different degrees of difficulty a computer system «Diagnost-1M» was used, which was created in the laboratory of physiology of a human's higher nervous activity at the Bohomolets Institute of Physiology (Ukraine).This technique allows to evaluate individual speed features of a person, to identify their ability to perform effectively and adequately under the conditions of processing information of varying degrees of complexity [12].

The studies started with the determination of the latent period of the simple visual/auditory motor response (LP SVAMR). The task was to react as quickly as possible by pressing and releas-

ing the button with the right hand, when a stimulus – any geometric figure (sounds of different tones) appeared on the screen. The observable was given 30 signals. Exposure time was 0.9 sec, and the pause duration was changed randomly, which was included in the program and did not depend on the response rate of the subject. After the presentation of stimuli, average time latency in milliseconds of SVAMR (M_{av}), standard deviation (σ), coefficient of variation (CV), and error of arithmetic mean value (m ±) appeared on the screen.

After determination of SVAMR, a latent reaction period of one of three stimuli choice (LRPSC 1–3) was detected. The examined were presented with the same signals in the same amount as under the terms of the SVAMR determination, but taking into account their differentiation. The examined had to press and release the right button with the right hand as soon as possible, when the «square» appeared on the screen (high-pitched sound) and take no action, when a «triangle» or «circle» (low- and mid-tone) appears. Signal exposure was also 0.9 s.

The determination of the latency period of two of three stimuli choice (LRPSC 2–3) differed from the previous test in that the subject was offered, except for the right-hand response to the figure «Square» (high pitched sound) to respond as soon as possible to the appearance of a figure «Circle» (low tone) by clicking the left button with the left hand. If a triangle appears on the screen (middle tone), the examined had to press no button, as it was a brake. The tempo, duration of exposure, and pause between stimuli were the same as in the previous study.

2. 3. Methods of memory and attention research

Various types of material (geometric shapes, numbers, and words) were suggested for the study of short-term visual memory of schoolchildren, presented in the form of tables of 10 depicted elements.

30 seconds were given to memorize cards with geometric shapes, numbers, and words. The examined had 30 seconds to retain the material he/she had to memorize. Then, within 1 minute, the student had to randomly reproduce what he/she had seen in writing. We had calculated the number of correctly reproduced material.

The performance (volume) index of short-term visual memory depended on the number of correctly reproduced elements of each table (the bigger the number of elements, the higher the memory capacity).

We used correction tables by Anfimov and «red and black» Schulte tables to study attention.

The corrective method allows to obtain data on the mental capacity of the child, the level of mental capacity (the speed of the fulfilling the task and its accuracy), to study the speed and the nature of the conditioned reflex. Proofreading tables were used in the study of performance, speed, and volume of attention. Anfimov's tables are presented in the form of printed 1600 characters in the form of 40 lines (40 characters in each row). These characters are represented by eight letters of the Russian alphabet: А, В, Е, И, К, Н, С, Х in a chaotic order. The subject should, in the allotted time (4 minutes), cross out as many specific letters as possible.

To evaluate the completed task, it is necessary to take into account the total number of characters processed (attention span), the number of crossed out characters, mistakes made, omitted characters.

Volume, speed, stability, performance, and accuracy of attention were calculated by the formulas:

– volume of attention (Q):

$$Q=N/1600, \quad (1)$$

where N is the number of characters, processed in 4 minutes; 1600 is the total number of characters.

– speed of the processed information (H):

$$H=N/t, \text{ bit/s}, \quad (2)$$

where N is the number of characters, processed in 4 minutes; t – time of the task.

– indicators of stability (A) and performance (E) of attention were calculated by the Whipple formulas:

$$A=\frac{C-O}{C+M}, \quad (3)$$

where C is the number of crossed out characters; O –the number of omitted characters that should have been crossed out;

M is the number of made mistakes.

$$E=A\cdot N, \quad (4)$$

where A is an indicator of the stability of attention; N –the number of characters, processed in 4 minutes.

– attention accuracy (S):

$$S=C/n\cdot 100\ \%, \quad (5)$$

where C is the number of crossed out characters; n is the number of characters to be crossed out.

The «red and black» Schulte table technique is intended to evaluate the switching of attention. The examined were offered a table, divided into 49 cells (7×7) with red and black numbers. They need to find red and black numbers in turn, with reds in the descending order (from 24 to 1) and blacks in the increasing order (from 1 to 24). Only the letters that are near the numbers are needed to be written wind. Working time – 5 minutes. The faster the subject performed the task, the higher the level of shifting attention.

2. 4. Statistical Analysis

Statistical processing of the obtained experimental material was performed by parametric and non-parametric statistics using Microsoft Excel and Statistica for Windows 6.0. The reliability of changes and differences between the studied values was evaluated by the criterion of difference (t) according to the Student's table, the nonparametric criterion «U» Wilcoxon-Mann-Whitney [13].

3. Results

Having conducted the statistical analysis of the obtained data of latent periods of different sensomotor responses among children with sensory deprivation and the control group, it is seen, that the level of stimuli in the form of figures was higher among children with hearing impairments, and in the form of sounds – in the group of children with visual impairments. This was observed in both age subgroups [14].

It has been revealed that the latent period of simple visual-motor reactions of children with auditory sensory deprivation to figure statistically almost does not differ from similar indicators of the control group children. This is observed in both age subgroups. Thus, in the group of children with auditory sensory deprivation at the age of 8-9 years, the average group indicator of SVAMR is 347.3±5.5 msec, and in the control group, the comparatively shorter latency periods are 331.2±5.7 msec. The indicator of the group of children with visual deprivation was longer and totaled 457.6±6.2 ms.

In the group of children with auditory sensory deprivation at the age of 10–11 years, the average group index of SVAMR is 271.7±4.9 msec, and the control group has comparatively longer latency periods – 284.5±5.7 msec. The indicator of the group of children with visual deprivation was 363.7±6.6 msec longer (**Table 1, Fig. 1**).

Table 1

Average indicators of sensorimotor reactions among children to figures

Index	Control group		Group of children with visual deprivation		Group of children with hearing deprivation	
	8–9 years (n=25)	10–11 years (n=26)	8–9 years (n=26)	10–11 years (n=25)	8–9 years (n=26)	10–11 years (n=27)
LP SVAMR	331.2±5.7	284.5±5.6	457.6±6.2**	363.7±6.6**	347.3±5.5	271.7±4.9
LRPSC 1–3	505.3±7.3	443.3±7.8	546.2±7.2*	481.5±5.9*	475.8±7.9*	407.5±6.3*
LRPSC 2–3	603.1±6.5	528.2±7.4	624.3±5.7*	576.7±5.1*	578.2±6.5*	479.4±7.5*

*Note: LPSVAMR (msec) is the latent period of simple visual-motor reactions; LRPSC 1–3 (msec) is the latent reaction period for selecting one of three stimuli; LRPSC 2–3 (msec) is the latent reaction period of choice of 2–3 stimuli. The probability of difference between groups * – $p<0.05$;** – $p<0.01$ – the difference is significant with respect to the children of the control group.*

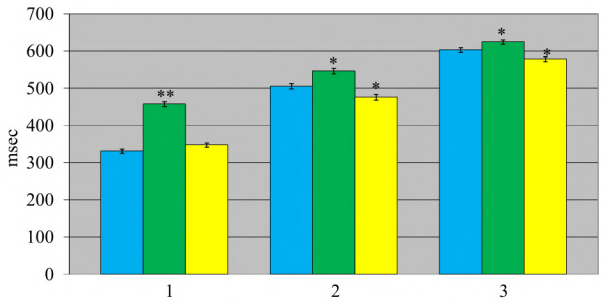

Fig. 1. Indicators of the latent periods of different visual and motor reactions among children of 8–9 years of age on the figures: 1 – LPSVAMR; 2 – LRPSC1–3; 3 – LRPSC 2–3; – Control group; – Group with visual impairments; – Group with hearing impairments

Mean values of LRPSC 1–3 among children with auditory sensory deprivation were better and was equal in the subgroup 8–9 years – 475.8±7.9 ms (10–11 years – 407.5±6.3), for children of the control group – 505.3±7.2 ms (10–11 years – 443.3±7.8), and in the group of children with visual impairments – 546.2±7.2 mess (10–11 years – 481.5±5.9 ms). When analyzing – LRPSC 1-3 indicators, using Student's test, we found significant differences in the survey groups (**Table 1, Fig. 1; 2).**

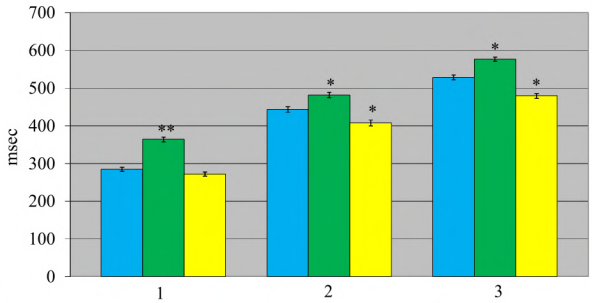

Fig. 2. Indicators of the latent periods of different visual and motor reactions among children of 10-11 years of age on the figures: 1 – LPSVAMR; 2 – LRPSC 1–3; 3 – LRPSC 2–3; – Control group; – Group with visual impairments; – Group with hearing impairments

The mean values of LRPSC 2–3 among children with visual sensory deprivation were longer ($p<0.001$) and were equal to 624.3±5.7 ms (10–11 years – 576.7±5.1 ms), for children in the control group of 8–9 years – 603.1±6.5 (10–11 years – 528.2±7.4) ms, and in students with hearing impairments the average group indicator was the best – 578.2±6.5 ms (10–11years – 479.4±7.5ms).

We conducted and obtained the results of the study of sensomotor reactions among children with sensory deprivation and the control group for sound stimuli (3 sounds with a different tone: low, middle and high tone). The results are presented in **Table 2.**

After conducting a statistical analysis of the obtained data of the latent periods of different sensomotor responses in the experimental and control groups, the level was higher in the group of pupils with visual deprivation (**Table 2, Fig. 3, 4**).

The latent periods of simple auditory motor responses among children with visual impairments to sounds were found to be statistically better than those of children in the control group and the group of students with hearing impairments. Thus, in the group of children with visual sensory deprivation mean LP SVAMR value in the subgroup aged 8–9 years is 351.1±5.3 ms (10–11 years – 346.7±6.3 ms), the control group had slightly longer latency periods – 366.8±5.3 ms (10–11 years – 352.1±6.1 ms). In the group of schoolchildren of 8–9 years with hearing impairment, the LP SVAMR was worse and was 536.2±6.3 ms (10–11 years – 511.7±5.1). This is explained by the existing hearing aid problems among children with hearing impairments and the high level of spatial hearing development in people with visual impairments.

Table 2

Average indicators of sensorimotor reactions among children to sounds

Indicator	Control group		Group of children with visual impairments		Group of children with hearing impairment	
	8–9 years (n=25)	10–11 years (n=26)	8–9 years (n=26)	10–11 years (n=25)	8–9 years (n=26)	10–11 years (n=27)
LP SVAMR	366.8±5.3	352.1±6.1	351.1±5.3	346.7±6.3	536.2±6.3***	511.7±5.1***
LRPSC 1–3	415.7±7.6	381.2±5.8	409.5±6.2	375.3±6.9	593.4±7.2***	579.3±5.6***
LRPSC 2–3	498.5±5.8	479.3±6.4	480.1±5.5*	461.8±6.1*	596.2±5.5**	566.1±7.2**

Note: LP SVAMR (msec) is the latent period of simple visual-motor reactions; LRPSC 1-3 (msec) is the latent reaction period for selecting one of three stimuli; LRPSC 2-3 (msec) is the latent reaction period of choice of 2–3 stimuli. The probability of difference between groups– p <0.05;** – p <0.01;*** – p<0,001 – the difference is significant with respect to the children of the control group*

The mean values of CRT 1-3 among children with auditory sensory deprivation at the age of 8–9 years were longer ($p<0.001$) and equaled 593.4±7.2 ms (10–11 years –579.3±5.6 ms), for control children –415.7±7.6 ms (10–11 years–381.2±5,8), and in the group with visual impairments – 409.5±6.2 ms (10–11 years – 375.3±6.9). The very large difference between healthy and impaired students can be explained by the fact that children with hearing problems respond better to low-key stimuli than high-key stimuli. When analyzing LRPSC 1–3 indicators, using Student's t-test, we found significant differences in the survey groups (**Table 2, Fig. 3, 4**).

The mean values of LRPSC 2–3 among children 8–9 years with auditory sensory deprivation were longer ($p<0.001$) and equaled 596.2±5.5 ms (566.1±7.2 ms), for control children – 498.5±5.8 ms (10–11 years –479.3±6.4). The best results were recorded in students with visual problems – 480.1±5.5 (10–11 years – 461.8±6.1) ms. This is because the visually impaired have better developed auditory memory, they are quicker to understand and determine the source of the sound.

Therefore, the best performance of sensorimotor functions for sounds is in the visually impaired children as opposed to healthy and visually impaired. This is explained by the present hearing problems among children with hearing impairments and high levels of spatial hearing development in people with impaired vision due to the need to navigate in a diverse sound field. Significant differences are observed between the indicators of LRPSC 1–3 and LRPSC 2–3 in sensory-deprived children with hearing impairment as opposed to healthy ones. This means that hearing impaired students are more likely to perceive low-pitched sounds than high-pitched ones.

In addition to neurodynamic and sensorimotor functions, we also studied the memory and attention features of children with visual and hearing impairments.

When conducting a study on the amount of short-term visual memory, significant differences were found in the indicators of short-term visual mechanical memory between children with sensory deprivation, and the control group.

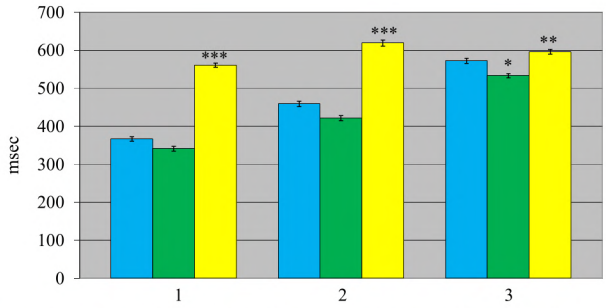

Fig. 3. Indicators of the latent periods of auditory-motor reactions of different complexity among children of 8–9 years for sounds: 1 – LPSVAMR; 2 – LRPSC 1–3; 3 – LRPSC 2–3; – Control group; – Group with visual impairments; – Group with hearing impairments

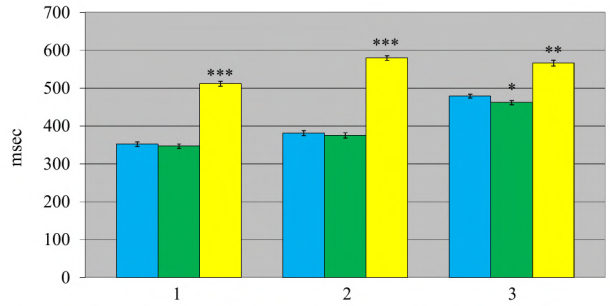

Fig. 4. Indicators of the latent periods of auditory-motor reactions of different complexity among children of 10–11 years for sounds: 1 – LPSVAMR; 2 – LRPSC 1–3; 3 – LRPSC 2–3; – Control group; – Group with visual impairments; – Group with hearing impairments

Students in the control group, in contrast to sensory-deprived children, have higher memory scores for numbers and one-/two-syllables words. So, in the control group of children aged 8–9 years the index of number memorizing reached 6.35±0.18 points (10–11 years 6.56±0.19), among children with hearing impairments – 5.62±0.18 (10–11 years – 5.83±0.12), and in the group with visual impairments – 4.69±0.14 points (4.87±0.21). The indicator for one- and two-syllables words in healthy students of 8–9 years – 5.41±0.21 points (10–11 years – 5.66±0.22), group with visualdeprivation – 4.15±0.17 (4.29±0.11), and auditory – 5.18±0.13 (5.47±0.16) (**Table 3, Fig. 5, 6**).

When comparing the short-term memory on geometric figures, it was founded that in students with hearing deprivation of both age subgroups indices are higher in contrast to the control group. In the group with visual impairment of 8–9 years, it is 6.65±0.15 points, and in students of 10–11 years – 6.81±0.20 (**Table 3**). A better indicator of imaginative memory (remembering figures) can be explained by the different way of formation of the second system (an image – a gesture).

Table 3

Average rates of memorization of different material among children 8–11 years

Memory volume, points	Control group		Group of children with visual impairments		Group of hearing impaired children	
	8–9 years (n=25)	10–11 years (n=26)	8–9 years (n=26)	10–11 years (n=25)	8–9 years (n=26)	10–11 years (n=27)
geometricshapes	6.51±0.13	6.76±0.15	4.97±0.12**	5.09±0.18**	6.65±0.15	6.81±0.20
numbers	6.35±0.18	6.56±0.19	4.69±0.14**	4.87±0.21**	5.62±0.18*	5.83±0.12*
words	5.41±0.21	5.66±0.22	4.15±0.17**	4.29±0.11**	5.18±0.13	5.47±0.16

*Note: * – p<0.05;** – p<0.01 – the difference is significant with respect to the children of the control group*

The results of the study revealed that children with visual deprivation of 8–11 years are worse in remembering the material, unlike the control group and students with auditory depri-

vation. This indicates that visually impaired students' memory is related to the visual analyzer, developed at a low level. For problems with eyesight impose its imprint on our received findings.

Also, the presented results give grounds to claim that the older the child, the higher the rates of short-term visual memory.

Attention is a dynamic characteristic of activity, as it activates the necessary and inhibits unnecessary mental processes, promotes a purposeful selection of received information, regulates and controls the course of activity. Attention is characterized by such features as volume, selectivity, stability, distribution, switching. All these properties are formed among children gradually in preschool and younger school age [16, 17].

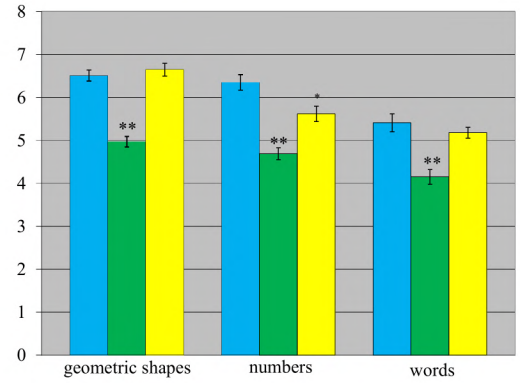

Fig. 5. Indicators of short-term visual memory among the children 8–9 years:
– Control group; – Group with visual impairments; – Group with hearing impairments

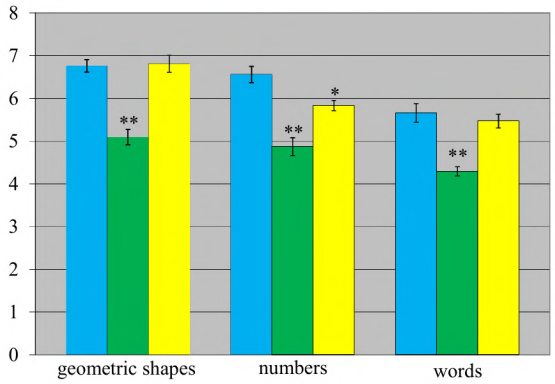

Fig. 6. Indicators of short-term visual memory among children 10–11 years:
– Control group; – Group with visual impairments; – Group with hearing impairments

Table 4

Mean values of attention features of students of 8–11 years

Indexes	Control group		Group of children with visual impairments		Group of hearing impaired children	
	8–9 years (n=25)	10–11 years (n=26)	8–9 years (n=26)	10–11 years (n=25)	8–9 years (n=26)	10–11 years (n=27)
Volume	618±17.2	621±19.3	619±15.4	623±18.3	622±15.7	628±16.1
Productivity	532±18.7	541±16.8	472±14.6*	488±15.3*	478±14.5*	491±15.6*
Stability	0.91±0.01	0,93±0,01	0.85±0.01*	0.87±0.01*	0.85±0.01*	0.86±0.01*
Switching	294±8.5	291±8.4	324±10.7**	320.5±7.6**	320±9.7**	318±10.9*
Speed	5.4±0.12	5.7±0.15	4.9±0.16*	5.1±0.11*	4.8±0.12*	5.1±0.18*

Note: – $p < 0.05$;** – $p < 0.01$ – the difference is significant with respect to the children of the control group*

Significant differences between the indicators are observed between sensory deprivation students and controls when conducting the survey and obtaining the results of attention traits.

A detailed analysis of the results of the study showed that the volume of attention among children with visual and hearing impairments was significantly higher than in the control group. Thus, in the group of schoolchildren with visual deprivation of 8–9 years, the volume of attention was 619±15.4 (10–11 years – 623±18.3), with auditory deprivation – 622±15.7 (628±16.1) and 618±17.2 (621±19.3) in the control group.

Analyzing the data in **Table 4**, we can observe the low speed of information processing in sensory-deprived children of 8–11 years. The focus is on increased perceptual loading, which requires deeper focus and resilience. Due to the high tension, children are more tired. This leads to a slower pace of tasks and an increase in the number of errors. It can be assumed, that this is due to abnormalities of the central nervous system that can cause deprivation.

Performance, resilience, and shifting performance are better in the children from the control group as opposed to students with visual and hearing impairments. Schoolchildren with visual and hearing impairments have difficulty switching attention, they need more time, which leads to a decrease in the speed of the task fulfilling and increase the number of mistakes.

Analyzing literary sources, we can assume that virtually all qualities of attention, such as activity, focus, latitude (volume, distribution), switching ability, intensity, or concentration, resilience are affected by visual impairment, but children with visual impairments, who are capable of high development, reach, and sometimes exceed the level of development of these qualities in people, who don't have sight deprivation.

4. Conclusions

The study of sensorimotor response to the burden of varying degrees of complexity revealed that:

– latent periods of simple visual-motor responses among children with auditory sensory deprivation to figures are almost indistinguishable from those of children in the control group. This is observed in both age subgroups;

– latent periods of complex visual-motor reactions (SC 1-3, SC 2-3) among children with auditory sensory deprivation to figures are better than the same indicators in the control group of children;

– significantly worse rates of latent periods of different complexity of reactions to figures are shown by the group of children with visual sensory deprivation. This is explained by the existing problems with the visual analyzer;

– significantly worse rates of latent periods of different responses to sounds are in the group of children with auditory sensory deprivation. This is due to existing hearing aid problems among children with hearing impairments;

– children of the experimental group (with impairment of hearing) have better indicators of sensorimotor response to low-pitched sound stimuli than to high-pitched stimuli;

– significantly better indicators of latent periods of different responses to sounds are shown in the group of children with visual sensory deprivation. This is because the visually impaired have better developed auditory memory, they are quicker to understand and determine the source of the sound;

– based on the analysis of the results of the sensorimotor response study, it can be assumed, that in groups of students with sensory deprivation occurs compensatory-adaptive processes (the process of compensating the lost function of a particular analyzer at the expense of another).

Having analyzed the results of the study of short-term memory and attention it was revealed that:

– indicators of short-term memory for geometric figures of visually impaired students of both age subgroups are higher than in the control group. The best indicator of figurative memory (memory of figures) can be explained by the fact that the formation of the second signal system is fundamentally different;

– children with visual deprivation of 8–11 years are worse in memorizing the material in contrast to the control group and students with auditory deprivation. This indicates that visually impaired students have low memory that connected with the visual analyzer;

– the presented results give grounds to state that the older the child, the higher the short-term visual memory;

– the volume of attention among children with visual and hearing impairments was significantly higher than in the control group;

– there is a low rate of information processing among sensory-deprived children of 8–11 years. It can be assumed, that this is due to the abnormality of the central nervous system that can cause deprivation;

– performance, resilience, and shifting indices are better among children in the control group as opposed to students with visual and hearing impairments. In schoolchildren with impaired vision and hearing difficulties there was marked an attention shift, they need more time, leading to the decrease in speed of fulfilling a particular task and increased errors.

In the future, we plan to study the strength and functional mobility of nerve processes, sensorimotor response to a moving object and muscular endurance through the computer system «Diagnost-1M».

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INVESTIGATION OF SALICYLIC ACID-INDUCED CHANGE ON FLAVONOIDS PRODUCTION UNDER CADMIUM TOXICITY IN BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH) PLANTS

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Abstract

Salicylic acid (SA) is an imperative endogenous plant hormone. It is considered as one of the most important signaling molecule, involved in both abiotic and biotic stress tolerance. Application of optimal concentrations (0,05 mM) of SA enhances plants tolerance to cadmium stress by modulating levels of several metabolites, including components of antioxidative defense, osmolytes, secondary metabolites, and metal-chelating compounds. We showed that when SA and Cd were applied simultaneously, the damage was less pronounced than without SA. SA treatment itself also caused the oxidative stress, but decreased flavonoids content, regulated phenolic synthesis and lignin formation. Thus, the main purpose was to investigate how SA treatment, used prior the Cd stress, prevented the damaging heavy metal effects in buckwheat plants. And show that regulation of flavonoids and lignin formation are an important indicator of stability and stress resistance. The obtained data will expand the knowledge about the role of phenolic compounds and the action of salicylate under the cadmium chloride conditions. Also data with this type of buckwheat – *Fagopyrum esculentum* Moench, Rubra variety under the action of cadmium chloride and salicylic acid not found.

Keywords: buckwheat – Rubra variety, flavonoids production, lignin formation, salicylic acid, cadmium chloride.

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1. Introduction

Salicylic acid (SA) may act as a component of the signal transduction system; it has promoting effects on various physiological processes, related to plant growth and development [1, 2]. As a phenolic compound it is important in defense against pathogen attack [3], certain abiotic stresses, in particular, heat and heavy metals stress. Heavy metal contamination issues are becoming of increase in Ukraine and worldwide, with many documented cases of metal toxicity in mining industries areas, foundries, smelters, coal-burning power plants and agriculture. The heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food safety and marketability, crop yields and environmental health of soil organisms. Cd is one of the most aggressive heavy metals that induce oxidative stress in plants [4]. Cadmium has a high impact on plants and consequently it affects an ecosystem, where plants form an integral component.

Salicylic acid (SA) is an imperative endogenous plant hormone. It is considered as one of the most important signaling molecule, involved in both abiotic and biotic stress tolerance. Application of optimal concentrations (0.05 mM) of SA enhances plants tolerance to the cadmium stress by modulating levels of metabolites, including components of secondary metabolites.

Flavonoids, the important secondary metabolites with many and diverse key functions that belong to a largest class of substances, produced by plants – phenylpropanoids. These substances are of interest of plant and animal biochemists, plant pathologists, geneticists and biotechnologists.

They are involved in major processes, such as cell wall formation, photosynthesis, respiration, allelopathic interactions between plants, protection against pathogens and herbivores [5]. They are produced by plants in response to biotic or abiotic stresses, such as wounding, UV-radiation, exposure to pollutants, ozone, and other adverse environmental conditions [6, 7]. It was reported, that 0.05 mM exogenous SA before the reproductive stage resulted in a higher seed germination.

Buckwheat (*Fagopyrum esculentum* Moench) belongs to crops of secondary importance in many countries. The main producers are China, Ukraine, Kazakhstan and Russian Federation, but generally it is consumed or traded locally. Except for the wide spread of this variety, Rubra variety – *Fagopyrum esculentum* Moench., characterized a high initial content of phenolic compounds, and can be investigated the effect of salicylate and cadmium, as well as changes under these conditions.

The aim of the present study was to investigate the effect of SA on cadmium stress in buckwheat, changes of flavonoids contents, lignin formation, which will allow to see external (lignification) and internal (flavonoid formation) under stress conditions. Because the lignification and formation of flavonoids are an important process in the chelation of heavy metals and their retention in the compartments of the plant cell.

2. Materials and Methods

Buckwheat (*Fagopyrum esculentum* Moench.) Rubra variety was used in the laboratory experiments. Growing conditions: 16 h – photoperiod, 15 °C – night temperature, 20 °C – day temperature. Previously sterilized seeds were soaked for 5 h (experimentally selected for buckwheat) in the 0.05 mM SA solution (experiment) and distilled water (control). Then seeds were germinated on a filter paper in Petri dishes at 23±1 °C for three days. Uniform seedlings were transferred to pots, filled with 1.5 kg washed and inciderated sand, artificially contaminated with Cd as $CdCl_2 \cdot 2.5H_20$ at levels of 0 and 25 mg Cd kg sand. The pots were watered to 60 % water holding capacity of the sand and fertilized twice a week with 25 ml modified Hoagland's nutrient solution. The concentrations of flavonoids and lignin were determined in shoots on the 14th and 21st days of plant growth. The experimental plants were in the phase of the second (14 days) or the third leaf (21 days).

2. 1. Flavonoids estimation

The plant material was fixed at 105 °C for 15 min and dried at 40 °C for dry matter obtaining. Dry samples (50–100 mg) were homogenized with a 0.2 g glass powder and transferred to the test-tube with 2 ml methanol for 1 hour for extraction. Then, the mixture was centrifuged at 3000 g for 5 min. The supernatant was used for the next steps of flavonoids analysis. Series of standard solutions of rutin and quercetin (concentrations 0.5, 1, 2, 4 mg/ml) and 0.5 µl of the extract were dropped on a chromatography thin plate with silicagel Sorbfil (Ukraine). The solvent system for the separation of flavonoid compounds was ethyl acetate – acetonitrile – 35 % formic acid (13:5:2, v/v). Visualization was performed by sprinkling with a 0.1 % $TiOSO_4$, chromatograms were analyzed at wavelength 450 nm with spectrophotometer ULAB 101 (China) [8].

2. 2. Lignin determination

We used the Wiesner's reaction, based on phloroglucinol condensation with cinnamic aldehydes (coniferyl aldehyde) in the acidic environment and formation of cherry red product [9]. A thin stem sections after washing in the distilled water were placed on a glass slide and stained by 5 % alcohol solution of phloroglucine, followed by 25 % H_2SO_4 as described in [10]. After 10 mins, the sections were viewed into a microscope. The aperture in each variant of the lignin test was measured, using a program Image Tool (USA).

2. 3. Statistical analysis

Each experiment was performed in five replications. The means and standard deviations were calculated by the JMP Pro 14 and Microsoft Office Excel 11. Statistical significance of difference was evaluated with Student's t-test (P<0.05).

3. Results and Discussion

SA plays a crucial role in the regulation of physiological and biochemical processes during the plants entire lifespan. It is known that the content of endogenous SA can be increased by exogenous applications [11]. In our experiments exogenous 0.05 mM SA in dosage 10 ml per g of seeds influenced a seed germination rate (**Table 1**).

Table 1

Effect of 0.05 mM SA on the germination of buckwheat seeds Rubra variety in the laboratory conditions, Petri dishes, 20.0±1.0 °C

Variants	Germination rate, %			Percent of germination, %
	First day	Second day	Third day	
Control (H_2O)	78.0±3.0	87.0±6.0	91.0±3.0	92.0±3.0
SA (0.05 mM)	85.0±3.0	88.0±5.0	91.0±5.0	92.0±3.0

Exogenous SA also promoted root growth on the initial stages, compared with the control treatment (**Fig. 1**).

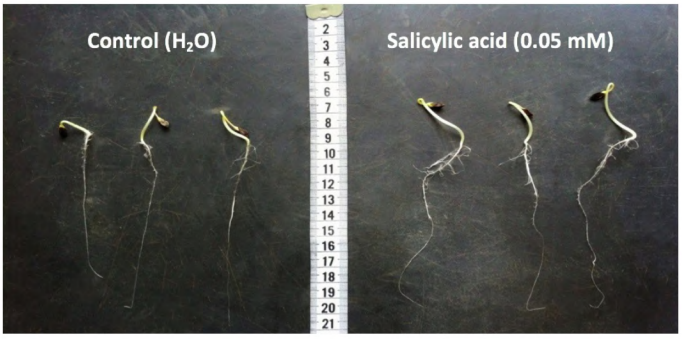

Fig. 1. The effect of the SA (0.05 mM) on early growth of buckwheat Rubra var. plants (7^{th} day of germination, Petri dishes)

The endogenous SA content could be increased exogenously and this action can induce plant stress [12].

Any biotic and abiotic stress effects can intensify the biosynthesis of flavonoids in different anatomical parts of the plant [15]. A plant cell wall is the first barrier against external hazards, one of the general reactions of plants under biotic and abiotic stresses is the accumulation of reactive oxygen species, accompanied by an increase in lignin accumulation [16–18]. Flavonoids possess antioxidant properties and realize a protective effect and barrier function due to the formation of lignin [13].

The result of our investigation showed that flavonoids level was higher in buckwheat shoots (**Fig. 2, 3**). Under the SA treatment an increase of the flavonoids content on a 14^{th} and 21^{st} days of experiment was observed. Cadmium (25 mg/kg) induced a slight increase in flavonoids concentration, compared to the control (**Fig. 3**). Under the combined action of SA and cadmium on 21^{st} day of experiment a nearly 3-fold decrease in flavonoids was observed (**Fig. 2**).

The results obtained are confirmed by literature data for minor changes in the flavonoids pool due to the cadmium stress, which may indicate their conversion to coumarins or lignin [13, 14].

In the roots we observed a slight increase in flavonoids (**Fig. 3**). On 14-day of growth with SA it could be caused by changes in common synthetic phenylpropanoid pathways. Cadmium led to 8 % decrease of flavonoids concentration in the roots on 14-days plants, and its 3-fold increase on 21 days (**Fig. 3**). However, in the shoots it did not change during the experiment (**Fig. 2**).

Lignin biosynthesis is also closely related to plant heavy metals absorption, transport and tolerance. It is reported, that lignification of xylem in roots and shoots can reduce the transport of Cd to the grain [19].

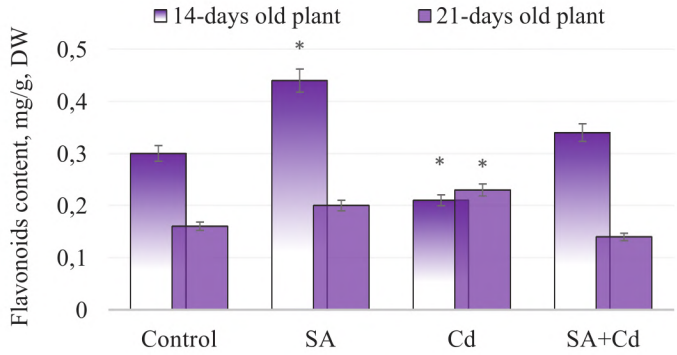

Fig. 2. The SA (0.05 mM) and $CdCl_2$ (25 mg/kg) effects on flavonoids content in a shoots of 14- and 21-days buckwheat (*Fagopyrum esculentum* Moench, Rubra var.) plants; *– $P<0.05$

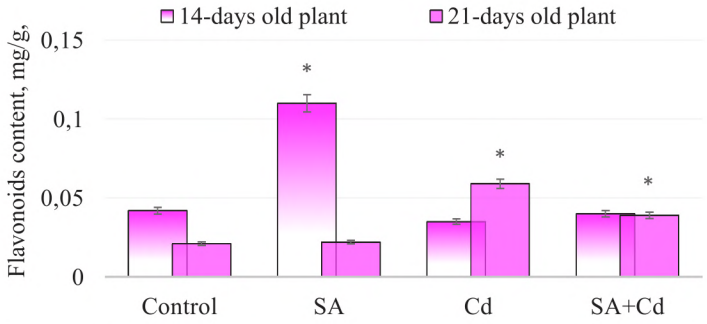

Fig. 3. The SA and $CdCl_2$ effect on flavonoids content in roots of 14 and 21-days buckwheat (*Fagopyrum esculentum* Moench, Rubra var.) plants; *– $P<0.05$

Our results show the differences in lignin accumulation in the shoots under the SA and Cd influence. It should be noted, that for the detection of lignin there were selected different parts of the plant – leaf, stem, node. Lignin formation was not noticed in the control condition, no in the longitudinal **(Fig. 4)** or in the cross sections **(Fig. 5)** of the shoots.

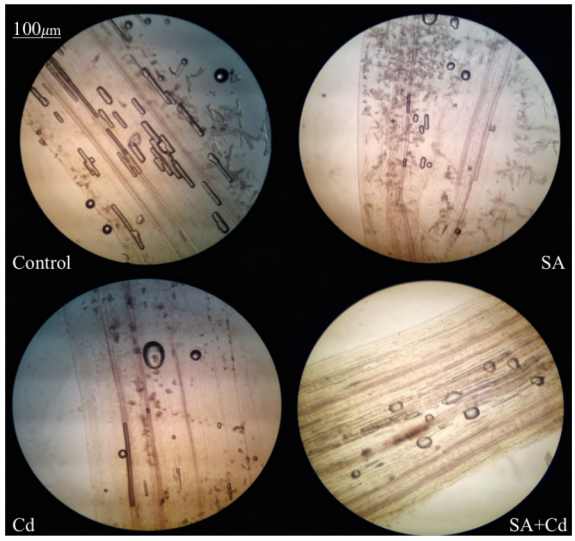

Fig. 4. Localization of lignin (longitudinal section) in a shoots of buckwheat (*Fagopyrum esculentum* Moench, Rubra var.) plants under the action of cadmium chloride and salicylic acid

It was shown, that different parts of plants have different content and distribution of lignin. The stem node had a significantly higher density than the internode. The reason for this may be the

high content of phenolic acids [13]. We assume SA as a substance of phenolic origin that also had an influence on the process of lignification in buckwheat plants, the similar results were obtained on mutants and transgenic plants and they proved the metabolic plasticity of lignin biosynthesis (**Fig. 4, 5**).

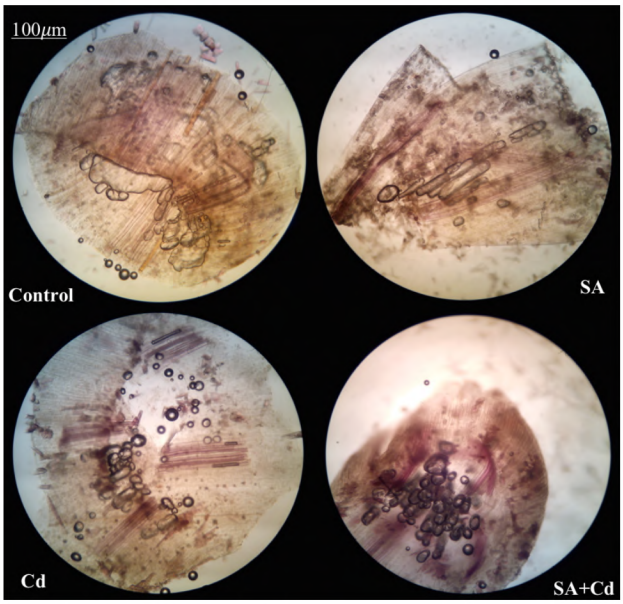

Fig. 5. Localization of lignin (cross section) in a shoots of buckwheat (*Fagopyrum esculentum* Moench, Rubra var.) plants under the action of cadmium chloride and salicylic acid

4. Conclusions

We can assume that SA in concentration 0,05 mM has the positive effect on seed germination. Pre-soaking with this concentration had more beneficial effect on the plant than the treatment with distilled water. SA treatments revealed a stimulative effect on flavonoids accumulation in buckwheat plants. The obtained results indicate that lignin formation correlated with flavonoids content. Moreover, cadmium and SA acid increase lignin formation, but without any change in flavonoids content. But, when the SA treatment used prior the Cd stress, it prevented the damaging heavy metal effect.The treatment with salicylic acid can regulate the content of phenolic compounds, which in turn leads to chelation of metals and prevents entry into the stem or grain of the plant. Our results can be implemented in agronomy and horticulture of cereal plants.

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FERMENTATION OF MULTIGRAIN DOUGH – AN APPROACH TO REDUCE GLYCEMIC INDEX FOR HEALTHY BREAD

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Abstract

The use of sourdough as the starter culture for bread making is one of the oldest processes in food fermentation and is very much prevalent in being used for the manufacture of various multigrain breads. The fermentation process of breads from mixed flours is one way, reported to reduce the glycemic index as compared to white bread. In this paper, we have discussed the use of (autochthonous) native culture vs pure culture use, in fermentation to prepare a starter culture sourdough by propagative fermentation. Since such a dough is incorporated in the sourdough bread making process (1:3), by the initial process of intermittent back-slopping (at intervals of 3.5 and 7 days) to propagate sourdough with a starter culture, as a part of the process, we observed the reduction in glycaemic index of the sourdough itself to as low as *GI*=40, at 3rd day of fermentation when the pure consortium and at 5th day of fermentation *GI*=43, when the native consortium was used. The sourdough process is thus an essential tool, aimed to make healthy breads, as it is incorporated as an ingredient in the process, to make sourdough bread.

Keywords: sourdough, multigrain bread, acidity, glycaemic index, hydrolysis index, starter culture.

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1. Introduction

Healthy diets with preferences on multigrain breads, can contribute to low glycaemic indices. Since the glycaemic index (GI) is a measure, used to classify foods, according to their potential to raise blood glucose levels [1], low GI foods are preferred in diets. High glycaemic foods are responsible for weight gain and obesity [2–4] and high consumption of such products has often been associated to an increased risk of type 2 diabetes [5, 6] and cardiovascular diseases [4].

Preferences towards healthy diets can be possible, if they are culturally relevant, actionable and accessible to all that can help to promote robust health systems [7]. Thus, an important issue by adopting healthy food systems to halt the rise of obesity, prevalence of raised blood pressure and Type 2 diabetes, generated indirectly by consumption of high GI foods, can help to reduce such non-communicable diseases [8]. The nutritive, sensory, textural, and shelf life

advantages of the sourdough technology in manufacture of baked products has been reported in baked goods as wheat and rye breads, crackers, pizza, multigrain and gluten-free products also [9–11].

Fermentation of dough, using an initially prepared sourdough (starter), allows biochemical changes that occur in the carbohydrate components of the flour with the use of starter, due to microbial fermentative metabolism of combined metabolic activity of lactic acid bacteria and yeasts [12], can decrease the glycaemic index in such baked goods. Under this context, the flat breads from dough fermented in age old ways, 'sourdough' are also available in India, known colloquially by traditional names such as *pao*, *nan*, *tandoori roti*, *kulcha*, *khamiri roti, moghlai roti*. They are also available in many other Asiatic parts of the world. In Germany sourdough bread as german vollkornbrot, is awholegrain seed bread [13, 14] from whole wheat flour, with characteristic taste and flavor. Even cinnamon rolls (sweet), and various other sourdough breads [15], and brown breads (Kurbiskernbrot), have a mention of the use of whole wheat flour and flours that are locally available. Sourdough breads involve a fermentation step with a starter culture. The starter can be available or propagated in the flour to as old as 100 years, as used fresh/dry as starters cultures or even propagated finally as dried starter cultures, for the purpose [15]. Such techniques can play a role in modification and improvement of bread quality so prepared [16] with sourdough. Also some brown breads that are available in the Indian market are sprinkled with grains on the bread, instead of using multigrain flour to bake the bread. There are known limitations to the use of whole wheat flour in breads (brown breads), which tend to make it crusty and hard [17]. Little is known about predicted glycaemic indices of the sourdoughs due to fermentation of the dough to be used for bread making. However the use of starter culture(s) to make the bread dough, improves its final texture [17], taste and flavor [18]. Most importantly these starters can provide resistance to contamination by other microorganisms and starters can also be maintained for decades [19]. The use of either a native flour microflora or known pure culture in flour, as starter culture, can allow differences in fermentation of dough, due to their capacity to utilize the available carbohydrates present in the flour. Since the process of leavening sourdough, consists of adding an active microbial consortium [yeast and lactic acid bacteria (LAB)] in the flour, it helps in its acidification [20]. The use of starter cultures in this study, provided information on the spontaneous vs known culture use, in sourdough bread making. The resulting sourdough was used to formulate multigrain sourdough bread. Thus the present work aimed to evaluate the use of two different starter cultures (native microflora or pure culture in fermented dough), that may change the glycaemic indices of sourdough at different stages of the fermentation. Also fermentation allowed biochemical changes that favorably worked towards low sugar release also. The results have generated interest to further this study in making it a profitable enterprise for sourdough bread, in a market demand that prefers healthy diet products. The work thus explored fermented dough (sourdough), as a possibility to formulate the multigrain bread, with an acceptable taste. Such components are also known to bring down gluten contents in bread. Bread fermenting with sourdough technology is not very new to Indian markets with available flatbreads. Hence, if this technology gets adopted it can very well be brought into healthy diet list in an Indian market.

2. Materials and Methods

2. 1. Raw material and dough preparation

Flour used: The flour mix used was from different grains, namely, whole wheat, barley, ragi and sorghum were mixed in the proportion 80:10:5:5 (w/w) respectively. The proportions of these flours were decided, based on preliminary trials (not reported herein) suitable for bread making [21]. This multigrain mix flour was pasteurized for two consecutive days and used in two dough preparations. Two kinds of dough were prepared, based on two different starter culture doughs, used.

The starter culture preparation: Starter culture (used as fresh dough starter) was prepared by Sourdough Type II process [22], for propagating sourdough with starter culture from:

1) native microflora containing, sourdough starter, that was propagated in the flour naturally;

2) a sourdough starter, made and propagated with an added starter flour (dry, contained pure cultures for San Francisco bread), supplied by Sourdough International, Idaho, USA, as comple-

mentary packets. The pure culture in flour, when supplied by Sourdough Int., was communicated as being the original culture [20, 23], that was used for sanfrancisco bread, and used here in this study.

These two fermented doughs, were the starter culture doughs and also used for propagating mixed flour to higher volumes for bread. Thus, the starter dough and sourdough for bread were scaled up simultaneously. The mix that was propagated to higher volume starter culture dough contained starter dough and fresh dough in ratio 1:3 (starter culture dough: flour mix with water).

Process to propagate the sourdough by fermentation to a higher volume:

The propagation/up-scaling of the dough followed same ratio (1:3), each time the dough was needed in a higher volume, and left to ferment under room temperature (21±3 °C), to a fermented dough mix, with back slopping technique after every 72 h intervals. Both the doughs were thus propagated by back-slopping technique on 0^{th} day, 3^{rd} day, 5^{th} day and 7^{th} day to reach a final volume of 1200 g dough (**Fig. 1**) in each case, for making sourdough bread [15] (**Fig. 2**). This was thus done to suit the requirement of making bread using the sourdough technology.

Fig. 1. Final sourdoughs for sourdough bread

a *b* *c*

Fig. 2. Processing the sourdough to bread after kneading: *a* – Dough shaping; *b* – Loaf proofed; *c* – Brushing oil on a baked bread loaf, to shine and prevent a loss of moisture (optional)

2. 2. Biochemical Analysis

At each period of propagation, a sample (20 g) from the fermented dough was harvested and frozen at 0 ºC for further analysis. The pH, titratable acidity, total solid content of such fermented/ propagated dough (with native/pure starters) was determined by standard procedures. The pH was determined with a pH meter (Labman Scientific Instruments Model No LMPH 10) that was previously standardized. The total acidity was indicated from a titer value of a 10 g of sample that was diluted to 100 mL with distilled water and titrated with 0.1 N NaOH with an indicator (phenolphthalein), to an endpoint of titration as a permanent light pink color that persisted for 30 sec., usually obtained at pH of 8.3 [24]. The acidity was expressed as a titer volume of alkali to neutralize the total acidity present.

The total solid contents were determined by heating a sample in an oven at 105 °C until a constant weight was obtained and calculated for percent solids, present in a sample, and calculated as below (1).

$$\% \text{ Solids} = \frac{\text{Wt of dry sample} \times 100}{\text{Wt of fresh sample}}. \quad (1)$$

Since the propagated fermented dough was used to bake bread, we studied the glycaemic index (GI) of fermenting dough on 0, 3, 5 and 7 days of fermentation. For this the reducing sugars

[Total reducing sugars (TRS)] and total carbohydrates (TC) that were released in dough (0, 3, 5 and 7 days) were estimated [25, 26]. An *in-vitro* dialysis procedure was followed [27], where a 5 g sample was homogenized in 20 mL of 0.1 M potassium phosphate buffer solution (pH 6.9) at 37.8 °C in 100 mL Erlenmeyer flask. The system simulated the digestive tract environmental temperature. The sample pH was lowered to pH 1.5 with 8 M HCl, and digested with 1 mL pepsin enzyme (115 units) (Sigma-Aldrich). The sample was then placed in a water bath at 37.8 °C for 30 min incubation with stirring with glass ball beads, to simulate peristalsis. Each sample was then buffered back to pH 6.9 with 10 % NaOH solution and 1 mL α-amylase enzyme (16 units) (Sigma-Aldrich) added and left to digest for 10 min in the same water bath. All such digested sample contents in the flask were then transferred into a dialysis tubing (MWCO 14,000) (250×20 mm strips). The ends of the tubing bags were clipped and placed individually in flasks, containing 500 mL of phosphate buffer solution pH 6.9, under continuous and slow stirring (115 rpm), emulating intestinal movements. A dialysate volume of 30 mL was drawn out from the buffer solution, at every 30 minutes interval in the 4 hrs of dialysis and the volume was replaced with fresh buffer. Each of the so withdrawn dialyzed samples was placed in a boiling water bath for 5 minutes, quickly cooled and kept frozen until further analysis for total reducing sugars and total carbohydrates. Thus, these individually dialyzed samples of sourdough (that were sampled in different periods of being fermented), were analyzed for glucoses (as TRS) [25] and TC [26], using glucose as a standard and buffer (phosphate buffer solution pH 6.9), as a negative control of fermentation. The concentration of glucose (moles/L) vs time (0–180 min) was graphically analyzed for an area under curve (AUC) for each of the fermented samples. The rate of carbohydrate hydrolysis for hydrolysis index (HI) values was calculated as:

$$HI = \frac{AUC\ (\text{ferm sample})}{AUC\ (\text{sample at 0 day})} \times 100.$$

The glycaemic index (GI), was then calculated from equation [28]:

$$GI = 0{\cdot}862HI + 8{\cdot}189.$$

The fermented samples were compared to the 0 day fermented starter dough for calculated GI.

2. 3. Enumeration of bacterial and yeast colonies

The total counts of the pure dough starter cultures were enumerated by standard procedure [29] on Nutrient agar. The MRS agar and GPY medium (Hi-media) were used for enumeration by dilution plating of the sourdough samples for lactic bacteria and yeasts respectively. The native sourdough and pure culture samples were enumerated.

To isolate cultures from the pure culture, containing flour, used in the study, de- Mann Rogosa Sharpe agar (MRS agar) [30] and GPY media were used. The plates were incubated for 5 days and re-streaked if necessary to obtain pure culture isolates. The pure isolates were sub-cultured and stored for any further mass culture requirement.

3. Results

3. 1. Flours as an ingredient

Blending wheat flour with other flours can bring additional advantages during SD preparations. As with sorghum as a flour in the blend for SD held significance during fermentation. This flour has a kafirin-rich protein matrix, usually not affected by proteolytic degradations. But during fermentations, soluble peptides are released from hydrolysed proteins that are taken up for the bacterial growth, from sorghum flour [31] which ultimately leads to the souring action in fermenting dough. On the other hand, on boiling/cooking of this flour (as in pasta), kafirins are converted into almost insoluble protein aggregates that are not accessible to disulphide-reducing agents. This reflects its importance in fermentation when present in sourdough versus unfermented flour (extruded) when used for pasta making. Nevertheless, each product has its own advantages,

but still fermentation, plays a role in the dough, by releasing starch granules from such compact (kafirin-rich protein) matrices. On the other hand, the use of barley flour can help to give a rise in dough, by preventing a collapse of the loaf at proofing stage and also helps to give a good texture to the bread loaf [32].

3. 2. Sourdough starter inoculums/ cultures

In our study, the doughs from both the fermented samples showed countable colonies after 10^6 times dilution of sample. The native culture fermented dough showed 5×10^8 cfu/g with 5–6 different morphological colony types. The pure dough however showed a gummy yeast culture on the glucose peptone yeast extract agar (GPY) medium that lost the gumminess after repeated streaking. Lactic acid bacteria, isolated on MRS agar medium [30], and yeast culture, isolated on GPY medium (by incubation at 30 °C), from the dough. They have not been taken up for detailed studies. The pure cultures are reported to be as used in bakery previously [20; 23]. Previously it was reported [33] that total bacterial counts of fourteen sourdough samples to be in the range of 5.97 to 9.57 log cfu g^{-1}.

Since sourdough that has constant properties (e. g., acidification, leavening capacity) is mature, we considered the propagated sourdough (**Fig. 3, *a***) as mature. This dough had a pleasing flavor and odor and was thus used as a starter for making multigrain bread (**Fig. 3, *b***).

a *b*

Fig. 3. Dough proof and loaf proof: *a* – Flattening dough in shaping step, observing mature fermented sourdough; *b* – Multigrain bread made from the sourdough showing evenly distributed holes and a soft texture

The total counts of this pure sourdough starter were 7×10^7cfu. g^{-1} on the nutrient agar medium. At this stage, lactic acid bacteria (on MRS agar) reached values as high as 53×10^8 cfu. g^{-1}. Similar values have also reported earlier for mature dough 9.0 log cfu. g^{-1} [6].

3. 3. Changes in the pH, titratable acidity and total solid content of the fermented dough

Changes in pH, titratable acidity (%) and total solid (%) content with respect to fermentation time of the leavened doughs using two different starters are shown (**Table 1**). As is evident, there was a continuing decline in the pH of the dough, fermented with the native microflora, till 7^{th} day of propagation, showing the lowest pH value of 5.04.

In contrast, fermentation of dough with the pure starter culture led to maintenance of pH throughout the fermentation period and a gradual increase in titratable acidity. There seemed to be a pH buffering stability that persisted at pH 6.2, along with the increasing titratable acidity level in the dough, at each propagation stage (**Table 1**).

A microbiota development for sourdough is known to be a deciding factor in fermentation, especially when using pure culture dough starter [34]. The starter dough favours the growth of lactobacilli over the yeast growth, a characteristic feature, when pH values are observed to be >4.5 in the final sourdough, used for bread, and *L. sanfranciscensis,* in particular, does not grow below pH 3.8 [22]. Such a pH environment of sourdoughs, formed after 7^{th} day, indicated predominance of lactic bacterial activity. The dough, prepared with the native starter culture, also showed comparable pH values. Thus, a dough environment can be a decisive growth- limiting factor, for the organisms in sourdough.

A similar reduction in pH of sourdoughs has been reported by others (35), in wheat–legume sourdough, chickpea sourdough and lentil sourdough during the 10 days back-slopping technique,

as also with a corresponding increase in titratable acidity. Microbiota that develops in the changing pH of sourdough may be a reason to influence the final dough of a different starter culture. The mature Italian sourdoughs have pH from 3.70 to 4.28 in bread [6]. This technology still requires better insights into the genetic and phenotypic diversity of strains, to exploit them further. In previous studies, it has been shown that sourdough fermentations, carried out in the laboratory with flour as the sole non-sterile ingredient, harbor more different species diversity than artisan sourdoughs, prepared in a bakery, with respect to both LAB and yeast species [36–38]. Thus, preparing sourdough with the pure culture consortium with favorable metabolism can help to reduce the sugar release as required for a low GI, perhaps when the sourdough as a constituent, is used for baking it into bread.

Table 1

Changes in pH, Titer for neutralizing acid and total solids (%) of the sourdoughs after three, five and seven days of propagation

Sourdough sample (days after propagation)	pH	Vol of NaOH (0.1 N) (mL/10g dough)	Total solids (%)
Native			
0 day	6.61	14.48	43.360
3rd day	5.71	15.12	39.264
5th day	5.70	14.42	42.504
7th day	5.04	14.75	41.032
Pure			
0 day	6.90	2.97	49.522
3rd day	5.98	8.71	54.795
5th day	6.21	9.87	42.280
7th day	6.20	9.82	45.286

Now observing the acidity in fermentation of dough with the pure starter culture, an increase at 3rd day, intermediate phase, is an evidence of the presence of yeasts and lactobacilli that increased in propagation of the dough. They are metabolically active [39] during propagation and can hydrolyze carbohydrates (HI, **Table 2**) in the fermenting dough. Such changes in the sourdoughs were similar to those, reported in earlier studies [12, 28].

Table 2

Release of reducing sugars and carbohydrates from sourdough samples after in vitro digestion with pepsin and amylase

S. No. (Days of fermentation after inoculum added*)	Maximal release of TRS$ (mg %) (in digestion time, h)	Hydro lysis index	Glyce mic index
Native SD as Inoculum propagated in flour mix			
1 (0)	53.28 (3.5)	100.00	94.4
2 (3)	46.64 (3)	90.33	86.1
3 (5)	26.68 (3)	40.84	43.4
4 (7)	18.22 (3)	31.12	35.0
Pure SD as Inoculum propagated in flour mix			
5 (0)	32.55 (4)	100.00	94.4
6 (3)	14.48 (3.5)	36.74	39.9
7 (5)	11.66 (4)	35.16	38.5
8 (7)	11.66 (4)	27.86	32.2

*Note: * – Inoculum was also propagated for a period of 3 days initially before adding in to flour mixes for further propagation; $ – Total reducing sugars are expressed as glucose*

Comparing the pure culture to native culture dough fermentations, all the sourdoughs, developed by pure culture fermentations, showed much lower titratable acidity, as compared to the native culture fermented counterparts till the last period, under propagation. To note, the sample propagated by pure cultures, had lower final acidity (9.82 %) than that, propagated by the native culture dough (14.75 %), on the 7th day.

The solid content in the samples, fermented with the native culture dough and pure culture dough, was 41 % and 48 %, respectively. Thus, the native micro-flora and pure culture (of lactic acid bacteria and yeast), propagated in the dough, were the reason for such varying levels of pH and acidity in the fermentations. Hence, pure culture fermentations are always a preference to spontaneous fermentations, wherein we can obtain fermented conditions more suitable to a taste (less sour), more liked in a product like bread. Since this was sourdough fermentation, the acidity and pH, observed in dough, fermented with the native culture, showed pH and TA changes towards making the dough more acidic and perhaps sour, fermentation with the pure culture would be far much a preferred starter for such products (bread).

The other advantages of sourdough fermentations are for the lactic and acetic acids, produced in the medium to resist contamination by other microorganisms [19] and also for improved flavors, as in wheat breads [38]. The use of the pure culture in the multigrain sourdough yielded characteristically pleasant and fruity aromas in the dough as compared to native culture sourdough. According to previous report [40], whole wheat flour sourdough bread, enriched with oat fibre, showed more pronounced acidulous smell and taste, and an intense and more appreciated aroma with respect to normal breads, owing to the lactic acid bacteria, growing in the sourdough.

The analysis showed an overall status of fermentable ingredients on GI of dough which may have an influence on the ultimate low sugar diet requirement from consuming bread. The sourdough mix when propagated and fermented till a 7 day period showed maximum total reducing sugars (TRS) at 0 day in sourdough (53 mg %) that reduced with fermentation to 47 mg %, 27 mg % & 18.22 mg % at 3rd and 5th & 7th day after propagated fermentation with the native flora respectively (**Table 2**). All these fermented samples showed the maximal release of these sugars after 3 h of enzymatic digestion (in vitro). Interestingly, in contrast to the higher release of reducing sugars in case of the samples, fermented with the natively present micro-flora of flour, there was a steady release of reducing sugars in the samples, fermented with pure cultures and known sourdough inoculums, that released 14 mg % (3 day) to 12 mg % (5th and 7th day) from 33 mg % of TRS, present initially in the inoculated sample at 0 day. Again, digestion of carbohydrates showed less release into the solution when the flour was fermented with sourdough of known pure cultures as compared to the samples, fermented with the native microflora (**Table 4**). The reduction in hydrolysis indices of the samples was also directly related to incremental days of fermentation. This pointed to a potential lowering of glycaemic index due to fermentation. The sourdough from a pure culture as inoculum was a better potential starter in reducing glycaemic index of sourdough in 3 days only as compared to 5 days of fermentation with the native microflora.

4. In vitro digestibility of carbohydrates in fermented dough

Sourdough, propagated with the native culture, showed randomness in breakdown of the complex polysaccharides during the 3h digestion period (**Fig. 4**) in the propagated samples at different time intervals. The release of reducing sugars was not uniform as monitored over 30 minutes regular intervals of *invitro* digestion.

On the other hand, the dough propagated using the pure culture, sampled at the different propagation periods (0, 3, 5 and 7 days), showed a gradual and progressive increase in the reducing sugars with the increase in digestion period (**Fig. 5**). The maximal sugars released were 46 mg % in the native sourdough sample (3rd day, 3 h digestion period) as compared to 14.48 mg % in the pure culture sourdough sample (3rd day, 3.5 h digestion period) (**Table 2**).

In the sourdough, fermented by native cultures, the reducing sugars (3 days after fermentation), released after 3h digestion, were about 80 % higher in dough as compared to sourdough fermented with pure cultures. The pure culture propagated sourdough showed a sharp 36 % drop in the total reducing sugars, released post 3 days fermentation (**Fig. 3, 4**). At the 7th day of fermen-

tation the sourdough with the native microflora released about 58 % higher total reducing sugar content as compared to the pure culture propagated dough. The higher titratable acidity and also the presence of enzymes maybe attributed to such an observation [41]. A low release of sugars is the preference for dietary GI. Thus, the pure culture propagated sourdough that showed the slow release of sugars (TRS) is the dough, preferred for use in making multigrain bread (**Fig. 2, *b***), indicating the low GI that would also be present in bread.

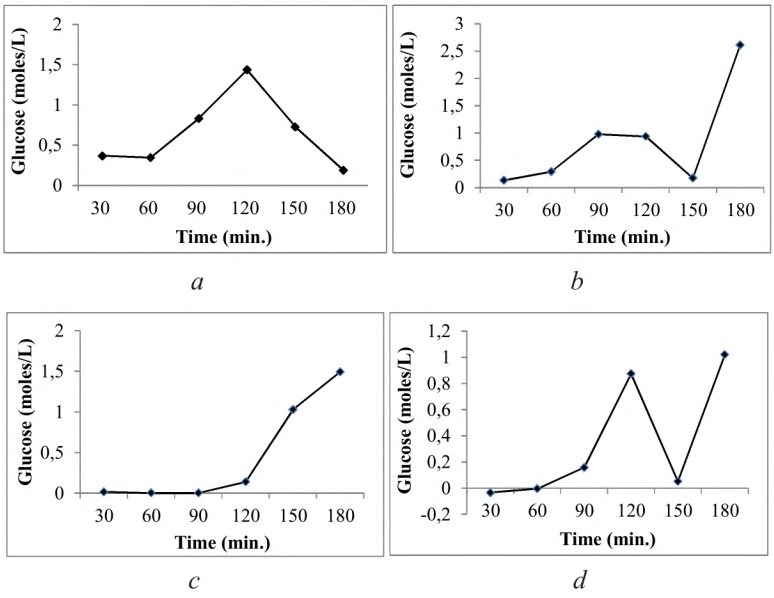

Fig. 4. Total reducing sugars (moles/L) released in sour dough propagated by native cultures for different intervals: *a* – 0 day; *b* – 3 days; *c* – 5 days; *d* – 7 days

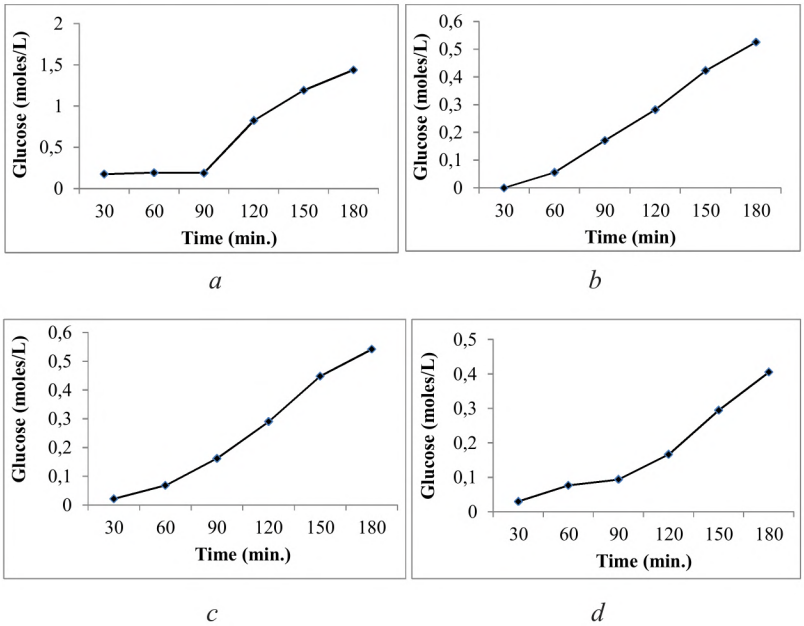

Fig. 5. Total reducing released in sour dough propagated by pure cultures for different intervals: *a* – 0 day; *b* – 3 days; *c* – 5 days; *d* – 7 days

The hydrolysis indices (of carbohydrates) and predicted GI of sourdough at different intervals of propagation, using the native and pure microflora, are shown in **Table 2**. The rate of *in vitro* carbohydrate hydrolysis was used for GI as it is considered to be a presumptive measure of the GI

in healthy subjects [42]. The hydrolysis index (HI) is dependent on the total release of sugars (TRS) in the full period of digestion. A higher HI indicates higher AUC for sugars in dialysates. An overall low rate of hydrolysis up to 3 days could be a reason, explained by the presence of the native microflora (spontaneous fermentation), whereas pure culture fermentations helped in higher rate of hydrolysis at 3 days. The corresponding, GI values were much lowered in the dough, developed by pure culture propagation, after 3 days of fermentation. In case of native culture propagation, the low GI values were obtained only after 5 days of fermentation in the dough (**Table 2**).

5. Conclusion

Dough fermentation creates a matrix, formed by the entrapment of gas bubbles, due to yeasts in fermentative stage along with dough expansion (leavening) and acidity development due to added microbial inocula. The leavening step enhances the surface, exposed to enzymatic activity. *L. sanfranciensis*, the lactic culture with yeast strain, in the pure starter culture consortium, as supplied, was used in this study. *L. sanfranciscensis* is also known for its ability to produce an enzyme, glutathione reductase (GshR). This enzyme can reduce the sulfhydryl compounds, present in wheat flour. This culture then helps to maintain a required redox homeostasis in fermentation due to its GshR activity and serves in disulphide exchange reactions in wheat doughs. The sulfhydryl compounds, which undergo a disulfide-sulfhydryl interchange with other low-molecular-weight thiol compounds, can cause cleavage or reformations of disulfide bonds in wheat dough. Such interactions lead to formation of glutenin macropolymers in wheat doughs that then determine a dough rheology, and its gas retention. All such changes influence the resulting consistency of dough [43]. The presence of such lactic starters, thus contribute to beneficial effects in sourdough fermentation and in bread texture. Thus, bread volume and texture is hence determined both by the flour and starter culture used.

Now considering the aspect of healthy diet, the sourdough was a source of reduced sugars in fermented flour (sourdough) itself. Also that the fermentable carbohydrates that remain in the bread even after baking also can affect the food's GI and can also have a potential to regulate postprandial responses to a second meal effect, by reducing non-esterified fatty acids (NEFA) competition for glucose disposal and, to a minor extent, they also affect the intestinal motility [44]. Low postprandial blood glucose is associated with a low risk of metabolic diseases (diabetes).

Thus, the sourdough technology has beneficial effects and can provide healthy diet food in the form of sourdough multigrain bread.

To the best of our knowledge, the application of sourdough to the fermentation of wheat-sorghum-barley mix sourdough in bread making has never been investigated. This study gives a comprehensive and comparative view of the effect of fermentation on the development of sourdough, by a spontaneous fermentation (native flora in dough) as compared to fermentation with known starter (pure microbiota) culture dough. Sourdough, prepared in making bread, can also be expected to have reduced GI values and are yet to be confirmed though. However, such GI values are an interesting part of this investigation that has never been explored earlier. An acceptable taste and softer texture (details not reported here) (**Fig. 6, *a***) as compared to similar brown bread, available in the market of Austria, prepared by sourdough fermentation (**Fig. 6, *b, c***) had a very hard inner texture.

a *b* *c*

Fig. 6. Sourdough breads showing color and inner texture of bread: *a* – Sourdough multigrain bread (this study); *b* – Brown bread (available in Vienna, Austria), see the hard outer crust; *c* – harder inner texture

Reports are available on the different kinds of flours that can reduce GI values of gluten free breads [27]. The GIs so reported were in the range of 69 to 95 which are high, but lower than white

breads. Low GI bread from sourdough, made from such low GI sourdough starters, is the next important component to be accomplished for further inclusions in healthy diets. The use of the pure starter culture in propagation of sourdough is thus emphasized by this study. This technique can also be used to enrich the functional quality with bioactive compounds, for health (anti- inflammatory activity, from black carrot color extracts substitution in wheat flour as 7–8 %, for bread [45–47]. By adding such ingredients at sourdough stage, we can also increase digestibility of bioactive anthocyanin compounds in black carrots [48]. The results of this study will be helpful to choose specific starter cultures and specific fermentation time of sourdough starter, to develop low glycaemic multigrain breads.

Multigrain breads, prepared by the use of the sourdough process, can help to improve the bread volume, crumb structure [49–52], flavour [18], nutritional values [49, 53, 54] and shelf-life [49, 55–58].

Henceforth this technology can have multifaceted advantages towards bread, with better textural properties along with health benefits towards lifestyles disorder diseases, as type 2 diabetes, that is widespread in the present world. To have a sourdough bread-making technology in place with its benefits as above, will be a direction towards listing healthy diet foods.

One of the limitations in this technology is the mass multiplication of the starter and availability of a pure starter culture, especially in India. The feasibility of using a starter culture from an already available manufacturing/production unit, as supplied by Sourdough Int. (in this study), shows that this technology is an adoptable technology. The technology must have hygiene certifications (HACCP), necessary both for a starter culture production unit as well as for the bread-making production unit. Attention also needs to be drawn on the use of appropriate starters. Some of the lactic bacteria are known to produce biogenic amines, that can result into toxicological problems, if such foods contain relatively high levels of these compounds (especially tyramine and histamine), and consumed [59]. However, the starters, used in this study, produced none or negligible levels of biogenic amines, as checked, *in vitro* for histamine and putrescine [60].

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APPLICATION OF CO-BIOPROCESSING TECHNIQUES (ENZYMATIC HYDROLYSIS AND FERMANTATION) FOR IMPROVING THE NUTRITIONAL VALUE OF WHEAT BRAN AS FOOD FUNCTIONAL INGREDIENS

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Abstract

Last time the food industry pays the great attention to questions, connected with changing existing technologies for raising the efficacy of the raw materials complex processing and increasing the output of high-quality products and food ingredients with a minimal amount of waste. Cereal crops are the most reach source of functional ingredients and main component in the human food ration. The technological process of cereal crops processing at enterprises is closely connected with creating a great number of secondary raw material resources and its further utilization.

For confirming the efficacy of using secondary products of grain processing as cheap raw material resources of dietary fiber and physiologically functional ingredients, there is characterized the accessibility of their biotransformation that gives a possibility to get biologically active substances of different chemical nature with a wide spectrum of physiological effects.

Secondary products of cereal crops processing (bran) are multi-component substrates, formed of different histological layers of wheat grains after comminution, consisted of (external pericarp, internal pericarp, grain coat, hyaline and aleurone layer of a grain coat).

Wheat bran is rich in dietary fiber, nutritive and phytochemical substances, that is why, it is most often used for feeding animals. But for today there are important proofs of using it in the food industry.

The development of new innovative technologies, modern achievements in microbiology and biotechnology have an important value for secondary products of grain processing, because they allow to conduct directed technological processes at the qualitatively new level that provides using soft regimes of vegetable raw materials processing, allowing to preserve natural biologically active substances and nutrients.

The modeling of the combined complex processing that includes enzymatic hydrolysis and fermentation by microorganisms improves technological, sensor and also nutritive and physiologically functional properties of wheat bran at the expanse of: bioavailability increase of phenol compounds, vitamins and minerals, assimilability of proteins and decrease of the content of anti-nutritive compounds.

Enzymatic preparations allow to use vegetable raw materials rationally, to intensify technological processes, in such a way increasing the output of biologically active substances and to widen the assortment of created products. The process of wheat bran formation results in increasing the nutritional value, enriching the biopolymeric complex with probiotic microorganisms and prebiotic substances.

Based on the structural peculiarities and multicomponent composition of wheat bran, presented and studied in the article, it has been established, that the use of the directed modification allows to get functional ingredients and products with set properties that influence the human health favorably. So, wheat bran must be used not only in agriculture as a cattle fodder, but also in the food industry.

Keywords: wheat bran, microstructure of wheat bran, fermentation, lactic acid bacteria, enzymatic hydrolyses, dietary fiber, bioactive compounds, antioxidant activity.

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1. Introduction

Wheat is a grassy plant that is the second by prevalence and consumption after rice. The main direction of its use is bakery and macaroni production [1–3]. Wheat grains consist of a series of anatomic parts: embryo, endosperm, containing starch grains for providing energy at germination; thick layer of aleurone cells, encircling an endosperm; and pericarp. Bran fractions consist of a pericarp, hyaline and aleurone layers. In the total mass of wheat grains a bran fraction is (14–16 %), embryo (2–3 %) and endosperm (mainly starch): 81–84 % [4]. The traditional technology of wheat grains processing is based on separating an endosperm from the bran and embryo layers. Cells of the aleurone layer together with other pericarp layers and embryos are eliminated with forming a bran fraction [5, 6].

The chemical composition of wheat bran is remarkable for the high content of not assimilated polysaccharides and their complexes (dietary fiber): cellulose, hemicelluloses, pectin substances and lignin (near 43–44 %). Dietary fiber favor the decrease of risks of several chronic diseases, such as cardiovascular ones (CVD), metabolic syndrome, diabetes 2 type and several types of cancer diseases [7–10]. Many countries of the world have dietary recommendations for consumption norms of dietary fiber. Today the Ministry of health protection of Ukraine has the accepted physiological norm of dietary fiber that is 15 g for 1000 kcal of the food ration. The norm of dietary fiber is divided in soluble 2–6 g and insoluble 25–30 g for day. The medical consumption norm is no more 40–45 g for day, the maximal day dose must not exceed 60 g [11, 12].

Alongside with dietary fiber, bran contains also bioactive compounds (or phytosubstances), such as alkylresorcinols, lignans, phenol acids, phytosterols, tocopherols, tocotrienols, folates [13, 14].

Fig. 1 schematically presents the location of the main food substances and phytocomponents of wheat grains, conventionally divided by anatomic layers [15–18].

The analysis of the wheat bran microstructure shows that they may be considered as a perfect raw material for developing optimized products and ingredients of the functional directionality [19–22].

Scientific studies, directed on revealing a possible connection between nutrition and degenerative human diseases started in the middle of the previous century. Health nutrition is provided by the presence of correspondent food products. Healthy products must contain enough and balanced quantities of different ingredients: proteins, lipids, carbohydrates, mineral substances,

vitamins and other biologically active substances, manifesting the specific physiological activity, adding sensor and food properties of products.

The history of creating healthy food products, then named physiologically functional or shortly functional ones (PFP) started from 80-ies of the previous century. For the first time the conception of PFP and the correspondent term were proposed in 1984 in Japan. In Europe and USA this conception found its wide spread in 1990-ies and still developing actively till today [23].

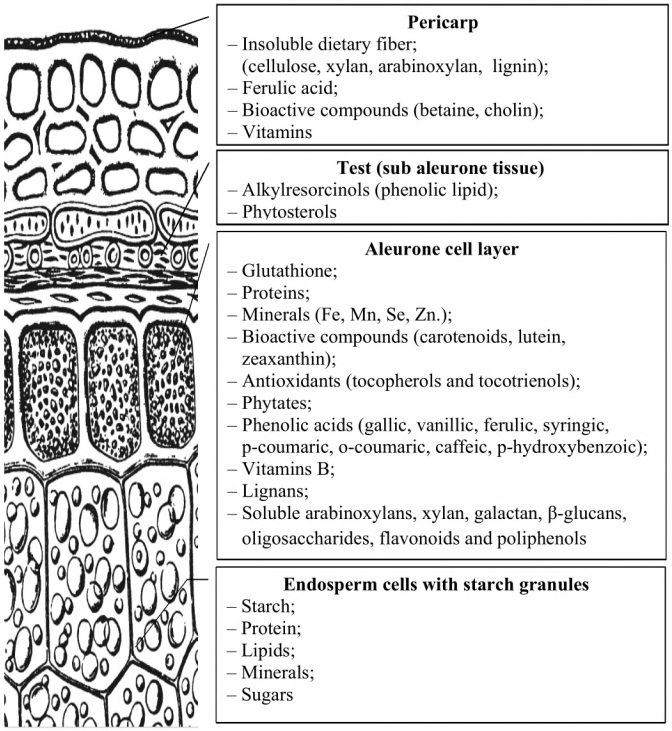

Fig. 1. Microstructure of wheat bran and the chemical composition of the individual layers

PFP are defined as products that, due to the presence of physiologically active components, provide advantages for health and pay an important role in decreasing or minimizing a risk of diseases, including prophylaxis and treatment of different ones [24].

Functional-physiological properties of wheat bran that allow to relate them to the category of functional products are conditioned mainly by the presence of biologically active substances in them: dietary fiber, phenol compounds of phytic acid, vitamins of B group, alkylresorcinols and other phytochemical substances [25, 26]. They have a great potential for applying in the composition of food products.

The promising direction of increasing bran consumption is to create new biotechnological approaches of the complex raw material processing by biotransformation of the polymer complex of cellular walls, using enzymatic preparations, as the most effective way of getting functional ingredients and widening the market assortment of functional food products that may have an independent physiological effect on the human organism.

2. Physiological effects of wheat bran and grains

Bran fractions, obtained by dry comminution, are rich in cellulose, minerals, vitamins of B group, thiamine, folates-vitamin E and several phytochemical substances, exceptionally, antioxidants, such as phenol compounds. Nevertheless, the bioavailability of food substances of bran depends on processing type and conditions. Bran is used in production of brown and wholegrain flour, in such a way preserving several valuable food components that diminish at their elimination and refinement of white flour. The main physiological effects of wheat bran are presented on **Fig. 2** [27–32].

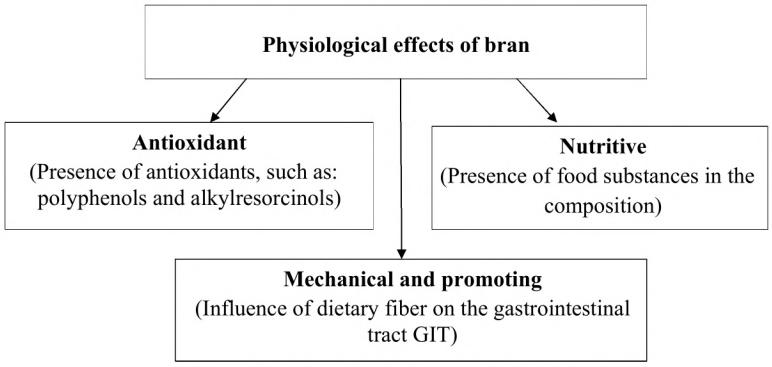

Fig. 2. Physiological effects of wheat bran

The base of bran is polymers of carbohydrate nature – cellulose, hemicelluloses, pectin substances, xylans, at the same time it contains starch, proteins and low-molecular organic substances. The chemical composition of bran varies depending on wheat variety, cultivation conditions and processing methods (bran separation). **Table 1** presents main nutrients and biologically active compounds, found in wheat bran [5, 34, 35].

The results of experimental studies testify to the positive influence of antioxidant phytochemical substances from a whole wheat grain. It is known, that antioxidants are presented in all bran fractions, where their content reaches 83 % from the total amount of phenol substances in wheat grains. As a result, a bran fraction has a higher antioxidant activity than other ones, obtained at wheat grain milling [36–38].

Phytic acid is an organic compound, present in crops, usually as hexaphosphate myoninositol. It is concentrated in the external layer of a pericarp and covers the aleurone layer of a grain. 90 % of phytic acid in a grain is in the aleurone layer and 10 % in the embryo. So, an amount of phytic acid essentially varies in different fractions, eliminated at grain milling [39, 40].

Table 1

Chemical composition of wheat bran (% abs. Dry substances)

Parameters	Content	Parameters	Content
Moisture	12.5–13.1	Vitamins E, mg/g	0.01–0.02
Protein	13.1–13.8	B_1, mg/g	15.5–16.3
Lipids	3.7–4.0	B_2, mg/g	12.2–13.0
Carbohydrates	57.0–58.5	B_5, mg/g	12.2–12.7
Starch	20.3–22.5	Flavonoids	0.03–0.4
Hemicelluloses	18.2–18.9	Alkylresorcinol	0.29–0.32
Oligosaccharides	3.7– 4.0	Phytosterols	0.15–0.17
Cellulose	19.5–20.3	Phytic acid	4.2–4.4
Lignin	5.4–6.1	Ferulic acid, mcg/g	100–220
Phenol acids	1.1–1.6	Mineral substances	5.1– 5.7
Polyphenols	1.1–1.2	Dietary fiber	41.4–54.2

Wheat contains near 1.13 % of phytate. The content of phytic acid varies from 200 to 400 mg/100 g in refined flour and 600–1000 mg/100 g in wholegrain flour. Its content in wheat bran varies from 3116 to 5839 mg/100 g of dry weight. Most mineral substances are present in wheat bran as complexes with phytic acid [27, 41, 42].

A mature wheat grain has a high phytase activity that favors hydrolysis of phytates and obtaining of mineral substances, well assimilated at digestion. Nevertheless, phytates are considered as inassimilable substances for humans, because of their influence on the bioavailability of iron,

magnesium, zinc and calcium. Although this mechanism has not been discovered, it is supposed that phytic acid creates a strong connection with mineral cations, creating phytate, forming mineral complexes that change their solubility [43–45].

Novel sources of dietary fiber, such as those, generated from by-products of food processing have received much attention recently. Such a by-product is wheat bran, which is generated during the milling process. It has predominantly been used for animal feed, however there is a currently strong argument for its use for human consumption as it is rich in dietary fiber, protein and phytichemicals [46, 47].

A diet, abundant in dietary fiber, has been shown to decrease the risk of obesity, type II diabetes and coronary heart disease. Wheat bran, which has a fiber content of about 50 %, is a suitable source of insoluble fiber for foods [48].

The content of dietary fiber (cellulose, hemicelluloses, pectin substances and lignin) in wheat bran of different wheat varieties, cultivated in the South of Ukraine, varies from 38.9 to 55.1 g/100 g, and include both soluble and insoluble forms of dietary fiber (**Table 2**) [49].

Table 2

Content of dietary fiber in wheat bran of different wheat varieties, cultivated in the South of Ukraine (g/100g)

Total content	Insoluble	Soluble
41.4–54.2	39.1–48.9	2.4–5.2
38.9–55.1	33.7– 49.7	5.2–5.4
40.5–52.6	37.7–44.5	2.7–8.1

The content of cellulose in wholegrain flour varies from 11.6–12.7 %. One of most rich cellulose sources is non-starchy polysaccharides (NSP) of bran, near 46 %. Main NSPs of bran are arabinoxylans, cellulose and β-glucan, their content is 70 %, 24 % and 6 % respectively. The mass share of soluble cellulose in wheat is essentially less, near 1 %, than in other crops, for example, its content in barley is 3–11 %, oat – 3–7 % [50–52].

Bioprocessing of bran with microorganisms (yeast, lactobacteria fermentation) or/with cell wall degrading enzymes (xylanase, cellulase, pectinase) is known to increase the soluble part of dietary fiber and there are physiological functional and rheological properties [53–55].

3. Wheat bran and its influence on the human health

In the last years experimental studies contain more and more data about the positive influence of wheat bran on the prophylaxis of different diseases [56].

Due to their antioxidant properties, wheat bran can prevent or brake the formation of certain cancer tumors, to intensify the motor-secretory function of the intestine, to prevent constipations, to have a positive influence at hypokinetic disorders of the biliary system, to influence positively the prophylaxis of different diseases of the gastrointestinal tract, including the diverticular stomach disease and the irritated intestine syndrome, CVD [57–60].

The colon microflora has a deep influence on health. Intestinal flora components can be modified, using dietary means, such as the increase of prebiotics consumption.

Prebiotics are considered as indigestible food ingredients that positively influence the master's health, selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon. There are more and more proofs, confirming the theory about the favorable influence of prebiotics on the intestine health and the decrease of the risk of colon cancer and CVD. Prebiotic components of dietary fiber in wheat bran (including β-glucans) can be fermented by the colon microflora, in which result physiological changes of a colon content take place that influence swelling, water-retaining capacity and viscosity [61–64].

Butyric acid is one of products of prebiotics fermentation, acknowledged as a fuel for coloncytes, it also favors normalization of feces pH, influencing the colon function. Changes in the colon microflora after bran consumption and metabolic output (including free ferulic acid) increased [65].

4. Fermentation biotechnology in wheat bran processing

Cereal crops are one of main food sources in the human ration and many by-products are created in their production chain [66].

Secondary products of grain processing are a rich source of physiologically functional ingredients, which biotransformation gives a possibility to get biologically active substances of different chemical nature with the wide spectrum of physiological effects [67–70].

Just that is why the biocatalytic processing of traditional types of grain raw materials, such as bran, has an important value for the further complex use at developing and making new commercially competitive functional products and ingredients.

Biotechnology, namely the use of enzymatic preparations and fermentation, is an important direction in realizing this idea, allows to improve technological, sensor and especially nutritive and functional properties of bran [71].

As a result of the bioavailability increase of mineral and phenol substances, vitamins, assimilability of proteins and different nutritive substances of the complicated matrix of grain coats, it is possible to get new products and physiologically functional ingredients by biotransformation with the participation of hydrolytic enzymes and further fermentation by selected yeast cultures. The complex biotechnological approach to bran processing will allow to widen their use in food essentially [72, 73].

Fermented grain by-products intensify their food profile and can act as a carrier of correctors of the gastrointestinal tract GIT microbiota (prebiotics and probiotics) [74].

Biotechnological solutions of bran processing are the complex effect of biological objects on the complicated matrix of cellular walls of bran coats.

The components of bran cellular walls, such as cellulose, hemicelluloses, proteins and phenol compounds are connected with each other by both covalent and non-covalent connections, creating a structure of the complex matrix that is rather resistant to the effect of different physical-chemical and biological factors. Modifying effects of both exogenous and endogenous enzymes can partially change a structure of the complex of biopolymers of the bran matrix and increase their biological activity, bioavailability and nutritional value. Successive fermentation or one, associated with fermentolysis by sour-milk bacteria (and other ones) allows to get products with improved physiological and functional (healthy) properties [59, 75].

Wheat bran is multiple external wheat layers (external and internal pericarp, grain coat, nuclear epidermis), aleurone layer. Different types of bran milling (rough or usual bran, fine bran, thin or middle bran) can be divided, depending on particles size and endosperm content in them [5, 6, 29].

Most spread polysaccharides of the bran layers – arabinoxylans and β-glucans. Nevertheless, such bioactive compounds of bran as dietary fiber and phenol acids, forming a cellular wall, are little accessible, because of the low bioavailability at penetrating the human organism [72, 76].

The additional fractioning and comminution of bran with its further enzymatic processing and fermentation are directed on the partial destruction of the multi-component complex of biopolymers of the matrix of bran cellular walls and allow to raise the food potential, to give new physiological properties.

Main groups of microorganisms, most often used in the composition of probiotic products and medical preparations, are sour-milk bacteria *p. Lactobacillus* and ones of the actinomycete group *p. Bifidobacterium*. Just these representatives under normal optimal conditions form the main part of the constant human microflora and play the key physiological role in its functioning.

We offer the biotechnological processing of bran, including two stages:

1) hydrolytic splitting by enzymes (xylanase, endoglucanase, cellulase);

2) further fermentation of the forming product by microorganisms *Lactobacilus acidophilus, Bifidobacterium bifidum*.

Such biotechnological effect has an essential influence on the bran microstructure, causing the partial destruction of structural components of cellular walls, resulting first of all in the solubility increase of arabinoxylans (in more than 10 times, comparing with native bran), that is the content of soluble dietary fiber and formation of oligomers of them with polymerization degree 5–8 that are carbohydrate high-effective prebiotic substances [77].

The studies, conducted with certain fractions of wheat bran by their additional processing by amylolytic enzymes (α-amylase and glucoamylase), allowed to modify (destruct) the matrix structure deeper at the expanse of hydrolytic splitting of starch polysaccharide fractions. Destarched wheat bran had the essential sorption and water-absorbing capacity that created products of the complex character, positively influenced probiotic microorganisms, protecting from the GIT aggressive medium, and also prebiotic components as arabinoxylan oligosaccharides, in the further chain of biotechnological transformations [49, 77].

Alongside with dietary fiber, wheat bran are a rich source of protein and may be considered as a replacement of animal protein (more 18 %) in the food and fodder industry. The protein bioavailability is influenced by different factors, including the stratified structure of wheat bran. The last studies show that the bioprocessing of wheat bran raises the protein bioavailability at the expanse of releasing them from the structural matrix and increasing the solubility [54]. Due to the proteolytic activity of sour-milk bacteria and endogenous proteases, activated by the low pH value at fermentation, the wheat bran bioavailability increased. It is known, that the digestibility of proteins *in vitro* (pepsin+trypsin) increases by 35 % in fermented bran [69, 78].

The processing by different biotechnological ways influences the release and transformation of wheat bran protein. Under acid conditions the protein solubility essentially increases, reaching its maximum at endogenous enzyme activation. Using enzymatic preparations of the directed effect and controlling the process of enzymatic hydrolysis, it is possible to get the maximal output of protein [73]. The results of this study discover new possibilities of biotechnology that increase the bioavailability of wheat bran proteins.

For releasing soluble proteins at dehydration of a cellular wall of wheat bran, there was applied hydrolysis of carbohydrates, and proteolytic enzymatic preparations were used. The study showed that enzymes with the exceptionally carbohydrate hydrolyzing activity increased the level of water-soluble pentosan and decreased the sugar content, but didn't increase the protein water-solubility and its release from cells of the aleurone layer [73]. Enzymes with the proteolytic activity essentially increased protein solubilization to 58.2 % in 4 hours after the beginning of fermentation. The protein increase took place at the processing by protease (during 3 hours, at temperature 35 °C and enzyme specific activity 550 [nkat/g]), protein solubilization was (>48 %), and content of amino nitrogen free (<45 mg/l). It has been established, that the protein intensive solubilization in the aleurone layer of wheat bran is possible at using exogenous enzymes with the proteolytic activity [79].

The use of microorganisms in fermentation of vegetable raw materials results in increasing the nutritional value, enrichment of the biopolymeric complex with probiotic microorganisms and prebiotic substances. For today there is a tendency of increasing the demand for food products, made by the natural way, without adding chemical supplements, nutritive and safe.

For producing multi-functional ingredients by fermentation of the medium on the grain base in the bakery industry, there were studied mixed cultures of sour-milk and propionic acid microorganisms and exopolysaccharides for the additional carbohydrate metabolism. Sour-milk bacteria produce lactate, and propionic acid ones metabolize lactate to acetate and propionate. The conducted screening of cultures of sour-milk and propionic acid bacteria demonstrated that the high potential was inherent to strains of *L. plantarum SM39* microorganisms (with the high content of folate) *P freudenreichii DF16* and three strains of *Weissella*, producing organic acids for the anti-microbial effect; folate and vitamin B_{12} for nutrition; exopolysaccharides for improving the texture and storage term [80].

The optimized stages of two-stage fermentation with co-cultivation (3 days – aerobic and 4 days – anaerobic) increase the output of vitamins, exopolysaccharides and antimicrobial activity. The authors have established the optimal parameters, at which the maximal output and synchronously production of natural folate (5-formyl tetrahydrofolate acid), cyanocobalamin near 6.1 mg/l and near 20 g/l of exopolysaccharides were observed. The antimicrobial effect of biologically active substances (added in dough at the equivalent propionate levels from 0.1 to 0.3 %) were demonstrated and studied in a test for baking bread, adding different spores of mould or bacilli. The high stability (near 60 %) of folate, acetate and propionate retention at baking was observed with mini-

mal losses at storage; adding exopolysaccharides resulted in the improvement of the bread texture. Really, new polyfunctional biologically active ingredients, developed in this project, discover new possibilities for raising the value of natural bread and bakery products and for prolonging their storage life [81].

The special attention to classic propionic acid bacteria as to potential probiotics is conditioned by their properties. They are oriented on producing antibiotics and bifidogenous substances of different nature. There has been proved the antibiotic activity of propionic acid bacteria against gram positive and gram negative bacteria, yeast, fungal mycelium. Today it is considered, that polypeptides, activating proteases, are very similar by structure to peptides, separated from *Lactobacillus lactis*. Their gen is contained in plasmids and is characterized by the horizontal transfer. The antibiotic activity is inherent to *Propionibacterium jenseniiSM11* and different strains of *Lactobacillus paracasei subsp. paracasei* to yeast, causing spoilage of sour-milk products [81]. The bifidogenous activity of propionic acid bacteria is conditioned by production of 1,4-hydroxy-2-naphthoic acid, 2-amino3-carboxy-1,4-naphthoquinone and several short chain fatty acids that are inhibitors of gram negative facultative and obligate anaerobes. Main producers of bifidogenous factors are *Propionibacterium freudenreichii subsp. freudenreichii* and *P. freudenreichii subsp. Shermanii* bacteria [82–84].

At revealing synthesis of bifidogenous factors of *P. acidipropionici* under aerobic conditions, there was studied the cultural liquid, obtained by cultivating propionic acid bacteria. For co-cultivation, cultures of sour-milk and propionic acid bacteria were selected for the additional metabolism on sugars. The study showed that new polyfunctional biologically active ingredients may be used for getting natural high-quality supplements with antifungal active propionate. It has been also established, that the use of *L. acidophilus* leaven results in releasing ingredients that may be used for producing high-quality supplements and active concentration of antifungal propionate [20].

At studying pripionic bacteria and factors, connected with food matrixes that influence synthesis of the vitamin B_{12}, it has been established, that the mean output of the vitamin B_{12} in matrixes of cereal crops by *P. shermanii* was 2.5 mcg/100 g. Propionic bacteria can produce the active vitamin B_{12} in cereal culture models, at that fermentation of wheat bran may have an essential importance for enriching food products with the vitamin B_{12} [85].

The use of *L. acidophilus* leaven results in releasing bound phenolic acids (ferulic, hydroxycinnamic), that are a structural component of cellular walls of the aleurone layer of bran and are mainly etherified by arabinoxylans **Fig. 3.**

The release of ferulic acid allows to increase the content of free phenolic acids (by 80 %), and to raise the antioxidant activity of a product essentially [86, 87].

The influence of bioprocessing on the structure and chemical properties of wheat and rye bran *in vitro* and microbial (*Lactobacillus acidophilus*) release and conversion of phenolic acids of bran were studied. Wheat bran was bioprocessed with hydrolytic enzymes (Viscozyme L) and with *L. acidophilus*. The chemical composition of dietary fiber (DF), arabinoxylans (AX), protein, starch, fat, phenolic acids, ash, xylooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS) and physiological properties of the fermented products of wheat and rye bran were analyzed. Co-bioprocessing of bran improve the technological and nutritional profile, thereby changing modification of carbohydrates and proteins. Fermentation is a tool to improve the nutritional profile of bran: releases proteins and bioactives from the cell wall bran matrix. The results suggest that bioprocessed wheat and rye bran are "stable" functional ingredients for food products that can be used to improve their nutritional and technological properties [77, 88].

It is known, that the bioavailability of mineral substances of bran strongly depends on the content of phytic acid that is considered as an antinutritive factor. At fermentation by sour-milk bacteria there is observed the increase of phytase activity, especially at using hydrolase enzymes for bioprocessing bran that favor the decrease of the phytic acid mass share.

The main parts of minerals in cereals are complexly bound to phytic acid as phytate, a phosphor-rich component, used by all cereal grains as mineral store. Phytate are insoluble at psychological pH, and, therefore, minerals are unavailable for absorption in the human intestine. The reduction of phytate in bran by cutting off the phosphate groups causes an increased absorption of

the minerals. The reduction of phytate can be achieved by enzymatic degradation during food processing activity of phytases from the cereals or by addition of phytase-active *lactic acid bacteria* or yeasts. The identification of high phytase-active microorganisms is necessary in order to find prominent candidates for the production of bran with high content of bioavailable minerals. High phytase-active yeasts and/or *lactic acid bacteria,* adapted in bran matrixes, might be a good choice for the modification bran. The high extracellular phytase activity was found in isolates of *S. cerevisiae*, followed by *C. humilis* and *L. acidophilus* [79, 89].

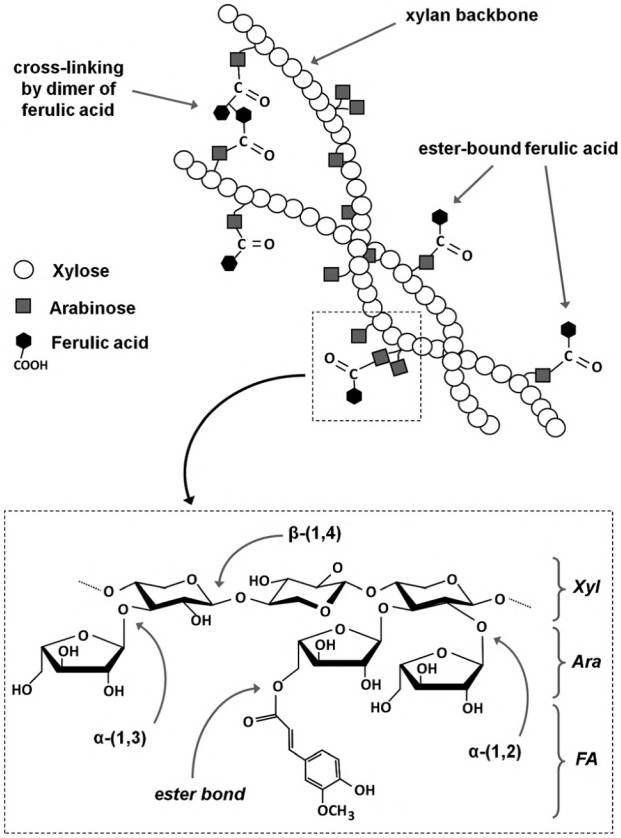

Fig. 3. Scheme of ferulic acid binding with arabinoxynan of the cellular wall of wheat bran

It is necessary to note, that the enzyme-fermentative biotechnology of wheat bran bioprocessing allows to get bioproducts (dietary supplements) with the complex carbohydrate composition of inassimilable dietary fiber of the double-oriented prebiotic effect: low-molecular (xyloarabinooligomers) and high-molecular polysaccharides (hemicelluloses). The schematic presentation of the biotechnological processes, taking place in wheat bran at obtaining functional food ingredients, is given on **Fig**. **4**.

Prebiotic complex preparations of low- and high-molecular inassimilable food fiber can create a great potential in preventing and treating dysbioses, because at fermentation in the lower sections of the intestine, they are substrates for creating such short chain fatty acids, important for the human organism, as propionate and butyrate. The offered model of metabolic activity of high-molecular and low-molecular carbohydrate prebiotics is formed on creation of short chain fatty acids (SCFA) at their fermentation in GIT.

The series of works demonstrate that compositions of low-molecular and high-molecular carbohydrate prebiotics differ by healthy effects for GIT and for preventing a series of diseases [78, 90–92].

Carbohydrate complex prebiotics, fermented in the low section of the intestine, can change microbiome much more effectively, taking into account the fact that such fermentation is realized by sour-milk bacteria.

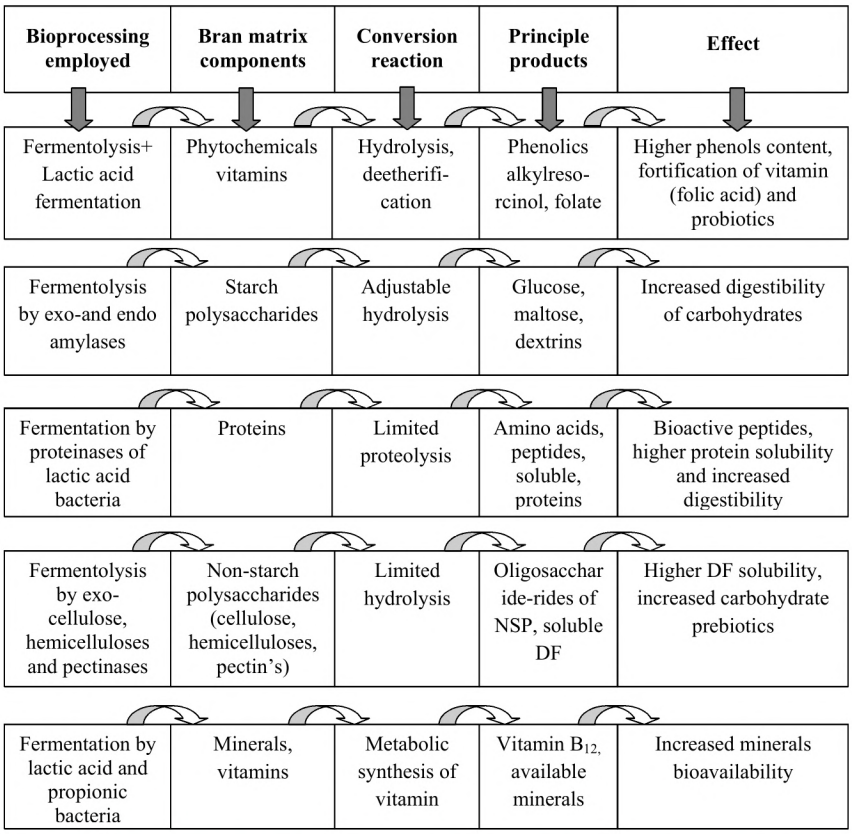

Fig. 4. Schematic representation of biotechnological processes by wheat bran for obtaining functional food ingredients

5. Conclusions

Wheat is the most important food component around the globe. Ukraine produces 25 ml tons of wheat each year. It is commonly used in the refined form, excluding its outer husk waste, in the cereal industry, currently considered as valuable resources for conversion to value added products such as functional physiologically ingredients. Each year millions and tons of bran has been produced as a by-product of the wheat milling industry. Bran is one of the most important by-products of the cereal industry. Bran comprises the outer layers of grain, separated in the milling process during the production of flours. The high nutritional quality, especially the high content of dietary fiber, protein and phytochemicals, makes bran an interesting raw material for food products, but it is currently under-utilized as a food ingredient due to its technological and sensory challenges.

The high bioavailability of vitamins, minerals, protein, dietary fiber, low-molecular and high-molecular carbohydrate prebiotics and phenol compounds makes bran a unique and exclusive product in the food industry.

Treating-prophylactic properties of bran use the directed biomodification, giving a possibility to separate different biologically active supplements and ingredients with a great diapason of physiological effects that have the positive influence on the human health.

Today creation of new approaches to wheat bran processing for getting biologically active ingredients and functional products is urgent and needs scientifically substantiated solutions.

The study of different extraction methods showed that the use of rigid methods for destructing the combined complex of biopolymers of cellular walls (as a result of the effect of organic solvents and high temperatures) essentially decreases the biological value and also the output of purpose-oriented products (polyphenols, hydrolysates, hemicelluloses, carbohydrate-protein concentrates, dietary fiber).

Biocatalytic processing is the full use of secondary raw resources, at which the increase of the output of purpose-oriented products with determined and beforehand given functional properties takes place.

Enzymatic and fermentation conversions have a principal role in increasing the efficacy of the production processes and utilization rate of the cereal raw material, and improving the sensory quality of the end product. Hydrolases are the most commonly used enzymes in industrial processes, and depending on the process, the aim is either complete or partial degradation of the cereal substrate. Solubilization, oxidation and degradation of cell wall components of bran by bioprocessing with hydrolytic enzymes such as xylanases, cellulases, pectinases, proteases has shown the potential as a means to improve the technological and nutritional properties of the bran in food applications.

The key factor of a successful use of wheat bran in food applications is the use of a co-bioprocessing technology, including enzymatic treatment and fermentation with selected microorganisms. In the journey toward a more efficient food chain, biotechnological approaches for the valorization of cereal side streams can be considered a very valuable help. Wheat bran can serve as a potential nutritious and cheap raw material for production of food and could further improve the nutritional impact of the functional food.

Based on the studied data, we have developed the biotechnology for processing secondary products of grain processing, including hydrolytic splitting by enzymatic preparations (xylanase, endoglucase, cellulase) with further fermentation of the product by *Lactobacilus acidophilus, Bifidobacterium bifidum* microorganisms. Such biotechnological effect has the positive influence on the bran microstructure, causing partial destruction of cellular walls, resulting in the solubility increase of arabinoxylans that are carbohydrate high-effective prebiotic components. Such approach allows to get food products with treating and prophylactic properties.

This direction of studies potentially has the wide practical importance at making food products of the functional and prophylactic directionality in different branches on the food industry, for example, in the meat industry, milk production and bakery.

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INVESTIGATION OF THE INFLUENCE OF FLAXSEED MEAL ON THE BIOCHEMICAL PROCESSES OF THE WHEAT TEST

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Abstract

One of the well-known tasks in the organization of nutrition is the development of new bakery products with high nutritional and biological value and wellness properties.

Linen meal is an important source of dietary fiber, high protein, unsaturated fatty acids, minerals and vitamins.

The purpose of the research was to determine the effect of this meal on the depth of the processes occurring in the dough during kneading and maturing.

A linseed meal LLC «Zhytomyrbioproduct» (Ukraine) with a certain chemical composition was used in the research.

The research was conducted to study the effect of flaxseed meal on the kinetics of sugars in the dough during its maturation. The study was performed using an iodometric method, based on the determination of the amount of oxidized copper before and after recovery of the alkaline copper solution with sugar.

It has been established, that the addition of flax seed meal to the dough reduces the amount of sugars, formed during the maturation of the dough, which adversely affects the activity of the dough microflora. This is confirmed by the lower consumption of sugars for fermentation.

The viscosity of water-flour slurry with a flaxseed meal has been investigated. The starch grains are infinitely swollen in the process of increasing temperature of the slurry in the amylograph, and a starch paste is formed, the viscosity of which increases with temperature. At the same time, under the action of flour enzymes, there is a decrease in viscosity due to the hydrolysis of starch.

The results of the researches testify about the effectiveness of the offered methods of studying the rules and depth of processes in the dough with a meal of flax seed during its preparation and maturing, which will allow to increase the yield of the dough and the output of high quality finished products.

Keywords: flax meal, sugar kinetics, viscosity, functional products, dough quality.

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1. Introduction

Organization of nutrition for consumers is a priority function of the restaurant industry. Nutrition is an important factor what helps the human body resist the adverse effects of the environment and the diseases of civilization.

The actual task in the organization of nutrition is the development of new types of products with high nutritional and biological value and health properties.

Bakery products have always been considered to be the basis of diets that are available to all segments of the population, therefore, professionals of food and restaurants industry have a task to make these products maximum functional [1].

In conditions of hard competition in the fight for the consumers, the urgent task is to use innovative approaches to nutrition, thus creating new formats in the concepts of restaurant industry.

Innovation is to improve and further develop food technologies that are enriched with wellness. This goal can be achieved by adding natural plant products to the formulations that are rich in biologically active substances and functional properties.

For enriching the physiological value of food products, scientists conduct researches about use of raw materials of oilseeds that have functional properties, in particular, flax and its products of processing [2–5]. Studies of recent years increasingly reveal the chemical composition of flax seeds, its biological value, technological properties, medical and hygienic value [6–8].

The meal contains 33.6 % protein, 9–10 % lipids, 37.6 % dietary fiber. After interacting with water, the meal will swell and form mucus, giving it antibacterial and anti-sclerotic properties. Flax meal is the source of most vitamins such as B1, B2, B6, niacin (PP), pantothenic (B3) and folic acid (B9), biotin (B7), tocopherol (vitamin E).Very important is the content of thiamine (B1). This product is a natural source of selenium. The research conducted by scientists at the University of South Dakota (USA) have found that the content of selenium in flax meal varies from 0.13 to 3.06 mg/kg or an average of more than 1 mg per 1 kg of product [9].

Reasoning, the establishment of optimal technological parameters of bread preparation, which ensure high quality of finished products, requires exploring of the course of these processes. From the practical point of view, adding a meal of flax seeds to bakery products causes a change in the organoleptic properties [8] of finished products and the structural and mechanical properties of dough and finished products [9].

The specificity of the chemical composition of flax meal, namely the high content of proteins, lipids, dietary fibers requires reasoning of the influence of the inclusion of flax meal in the preparation process of dough. Thus, the research of scientists [8, 10] found that the addition of more than 5 % of flax seeds in bakery products causes a change of the organoleptic properties of finished products and structural and mechanical properties of dough and finished products. Adding 2.5–3 % of flax meal to the flour does not affect the quality of bread, but such amount is not enough to enrich the bread with biologically active substances. Dosing more than 10 % of flax meal significantly impairs the technological process and reduces the quality of bread. The reason for these changes may be the influence of the components of the flax seed meal on the course of the fermentation process of the dough. This leads to studying the effects of flax meal on biochemical processes in the dough.

During the maturation of the dough due to the course of interrelated biochemical, microbiological, colloidal and other processes, significant changes occur in the carbohydrate-amylase and protein-proteinase complex of flour, which accumulate sugars, nitrogen and other substances in the dough and provide alcohol and lactic acid fermentation and are precursors of flavoring in dough semi-finished products and finished products.

The depth of these processes define accumulation and fermentation of sugars, the dynamics of formation and excretion of carbon dioxide during the maturation of test semi-finished products, increase of their titrated acidity, pH, organic acids, volatile acids content, changes in the fractional composition of proteins, accumulation of water-soluble nitrogen, activity of a fermenting microflora of dough.

Starch plays an important role in the formation of bread crumbs in the baking process. If starch is able to bind a lot of water, a dry hard crumb forms. If starch binds too much water, a wet blob is formed because some water, released by protein coagulation, remains in the free condition. After analyzing this process, examining the behavior of the water-slurry when heated on an amylograph by changing the viscosity of this slurry in the process of increasing the temperature of warming, you can predict the condition of the bread crumb.

Dough is a multi-component system, so other components are involved in forming the viscosity of the water-flour system, along with starch [8]. Linseed meal has more water absorption capacity than flour, it contains a significant amount of dietary fiber that actively binds water, so it was necessary to investigate the properties of a water-flour suspension, containing linseed meal.

Therefore, the production of bakery products with functional properties is ensured by the introduction of flax seed meal as a raw material with the valuable chemical composition and biologically active substances, but provided the study of the influence of this meal on the course of biochemical processes in the dough and finished products.

The aim of this research is to study the effect of flaxseed meal on the quality of wheat flour dough. It allows to study more deeply the influence of this unconventional raw material on the technological processes of preparation of dough semi-finished products for bread. Considering the specificity of the chemical composition of flax meal, this exploring will help in the selection of technological methods to improve the structural and mechanical properties of dough and finished products, the results of which will be published in subsequent periodical editions.

2. Materials and methods

2. 1. The materials used in the experiment

The meal of seeds of flax-mezheumka variety Nizhynsky, obtained by the method of "cold pressing" production of LLC "Zhitomirbioproduct" (Zhytomyr region, Ukraine) with a chemical composition containing proteins 32.6 %, dietary fibers 37.6 %, lipids 10 %, was used in the research.

When determining the sugar content, two types of dough are prepared: yeast and yeastless. Preparation of the control sample of yeast dough was carried out in accordance with the recipe for "Bread of wheat" SSTC 7517: 2014 in the following proportions:

– wheat flour first grade – 100 %;

– Yeast bakery pressed –3 %;

– kitchen salt – 1/5 %.

During the exploring, the yeast dough was prepared by the one-phase method without flax seed meal (control) and with the addition of 2.5; 5.0; 7.5 and 10 % of flax seed meal to flour. The moisture content of the dough was 44 %. The yeastless dough was prepared according to the same formulation without the use of yeast.

For sedimentation of proteins, 15 % zinc sulfate and 4 % sodium hydroxide was used.

The studied flax meal had the following organoleptic characteristics: a dark brown color, a pleasant nutty smell, and the taste was a little bit bitter-spicy. The groats were felt to the touch.

The size of its particles is not provided in the regulatory documentation about meal. In fact, the market meal has a size of 1000 microns, which is much larger than the size of wheat flour. Therefore, before using it was crushed on a laboratory mill and sieved through a sieve with cells of 0.52 mm. Grinding was carried out because of the size affects the technological properties of the raw material, because if a degree grinding is high, then the contact surface of the raw material with water is bigger and then physical-chemical processes occur more actively. Meal was added to the appropriate samples at the kneading stage of the dough.

2. 2. Methods for determining biochemical parameters

Appropriate techniques were used («Iodometric method for determining sugars (semi-micromethods)», «Method for determining the viscosity of a water-flour suspension on amylographs».

These techniques were used to study the effects of flax meal on biochemical processes occurring in the dough.

The intensity of the fermentation processes and the formation of coloring of the crust of bread due to the Mayer reaction depend on accumulation of sugars in the dough during its maturation.

And analyzing the behavior of the water-flour suspension during heating on an amylograph by changing the viscosity of the suspension in the process of increasing the temperature of heating assumes the condition of the bread crumb.

The research was conducted in the laboratory of clinical and biological research of the State Research Institute of Veterinary Drugs and Feed Additives (Lviv, Ukraine), the laboratories of the Department of Baking and Confectionery Technology of the National University of Food Technologies (Kyiv, Ukraine), the biochemical laboratory in University of physical culture, named after I. Bobersky (Lviv, Ukraine).

2. 2. 1. Determination of the influence of flaxseed meal on the kinetics of sugars during maturation

The meaning of the method is to determine the amount of oxidized copper before and after the recovery of the alkaline copper solution with sugar [11].

During the research, the yeast dough is prepared in a one-phase way without flax seed meal (control) and with the addition of 2.5; 5.0; 7.5 and 10 % of flax seed meal to flour. Such proportions in the limit of 10 % were taken to determine the more accurate regularity of the influence of the flax seed meal on the test specimens, because, according to established data [8–10], the dosage of flax meal more than 10 % significantly worsens the technological process and reduces the quality of bread. The duration of fermentation of the dough was 4 hours with the temperature of 30 °C.

To determine the sugar content of the prepared samples, 10 g of the semi-finished product is taken, ground with 100 cm^3 of distilled water and quantitatively transferred to a 200-ml volumetric flask and mixed. For sedimentation of proteins, 10 cm^3 of 15 % zinc sulfate is poured, and during stirring – 10 cm^3 of 4 % sodium hydroxide also is poured. The contents of the flask were well shaken and remained for 15 minutes. The precipitated liquid is filtered into a dry flask. In filtrate the mass fraction of directly reducing sugars is determined. The sugar content is determined by the iodometric (accelerated) method.

The amount of sugars, formed during the maturation of the dough, was determined by the difference between their content in the yeastless dough after mixing and after 4 h of its fermentation.

The amount of fermented sugars was determined by difference between the amount of sugars in the yeast dough after mixing and the amount of sugars, formed in the yeastless dough, and the amount of sugars, contained in the yeast dough after 4 h of fermentation.

2. 2. 2. Investigation of the viscosity of a water-flour suspension with a flax seed meal on amylograph

The research of viscosity of the water-flour suspension with a flax seed meal were carried out at Amylograph-E of Brabender company(Germany)

In the course of the research, a suspension was prepared according to the instruction in the device [11, 12]. Flax seed meal was added to flour in the suspension in the amount of 2.5; 5.0; 7.5 and 10 %.The control was a suspension without meal. For the research, a suspension of 80 g of flour and 450 cm^3 of water is prepared in a special vessel, stirring it with a hand stirrer, which is added to the device, during 1.5 minutes.

The slurry was transferred to the working capacity of the amylograph, in which, during continuous rotation, it warmed from the initial temperature of 25 °C to 90 °C with speed 1.5 °C/min.

The maximum viscosity of the suspension is determined by height of the viscosity curve (amylogram) of the water-flour suspension which is recorded. The beginning temperature of gelatinization (increasing its viscosity) was also recorded.

3. Results of biochemical studies

3. 1. The results of determining the effect of flaxseed meal on the kinetics of sugars during maturation

The results of studies of the effects of meal on the kinetics of sugars are shown in **Table 1**.

It was found, that in the samples with the addition of flax seed meal during the fermentation, less sugars were formed than in the control sample if more flax seed meal was added into the dough.

Table 1

Accumulation and fermentation of sugars in the process of fermentation of the dough, % on dry substances

Indexes	Control	Flax seed meal (FSM), % by weight of flour			
		2,.5	5.0	7.5	10
Yeastless dough					
Sugar content after mixing	2.22	2.21	2.23	2.22	2.24
After 4 h of fermentation	4.60	4.53	4.51	4.41	4.39
Sugars what were formed	2.38	2.32	2.28	2.19	2.15
Yeast dough					
After mixing	2.24	2.20	2.23	2.21	2.22
After 4 h of fermentation	2.55	2.58	2.70	2.68	2.71
Fermented sugars	2.07	1.94	1.81	1.72	1.66

Thus, compared to the control sample, less sugar was formed in the dough with 2.5 % of flax seed meal on 2.6; from 5.0-on 4.3; 7.5-on 8.1; from 10 – on 9.7 %. This indicates that the addition

of meal to the dough leads to a decrease of ammolysis starch susceptibility due to the formation of complexes of starch grains and mucus.

Along with the decrease in the formation of sugars in the test with FSM, their digestion decreases by the dough microflora by 8.7–19.2 %, depending on the amount of flax seed meal added, what is a consequence of the deterioration of the composition of the nutrient medium for yeast cells and a decrease in their fermentation activity with the presence of mucus of flax meal.

3. 2. The results of the research of the viscosity of the water-flour suspension with a flax seed meal on the amylograph

In the process of increasing the temperature of the suspension in the amylograph starch grains are infinitely swollen, a starch paste is formed, the viscosity of which increases with temperature. At the same time, because of action of flour enzymes, there is a decrease in viscosity due to the hydrolysis of starch.

These opposing processes are affected by the addition of raw material, which is reflected in the shape of the curve of the amylograph.

The results of decoding the curves of the amylogram are presented in **Table 2**.

Table 2

Indicators of amylograms of the investigated suspensions

Water-flour suspensions	Time before starch glutinization (viscous system formation), min	Starting temperature for starch glutinization (viscous system formation), °C	Maximum system viscosity, units of device
Control (without FSM)	12	53.5	505
With the addition of FSM, %to weight of flour:			
2.5	10	50.5	540
5.0	9	49.0	580
7.5	8	47.5	660
10.0	7.5	46.5	720

Flaxseed meal slurries, compared to controls, acquire viscous properties earlier in 2–4.5 min and at a lower temperature by 3–7 °C. The maximum viscosity of these systems is higher by 7–42.5 %.

So the specified indicators of the amylogram vary depending on the amount of flax seeds, introduced into the meal system. This can be explained by the fact that the soluble dietary fibers of flax meal swell faster than the starch glutinization begins, which causes the acceleration of formation of the viscous system.

In the control sample, the viscosity increases due to the deepening of the starch gelatinization with increasing temperature and reaches a maximum when it is completely gelatinized.

In the test specimens, after temperature rises to the maximum viscosity, along with the deepening of the starch gelatinization, affects the swelling of soluble and insoluble dietary fiber, which causes an increase in the viscosity of the slurry with flax seed meal.

Thus, due to the increased absorption of water by the ingredients of the meal and the formation of mucus complexes with starch, while baking starch grains are not able to bind sufficient water, resulting of this is a less elastic crumb, which was reported in studies [8–10].

4. Conclusions

In dough with flax seed meal due to the formation of complexes of starch with mucus, its amylolysis susceptibility deteriorates, which causes a decrease of 2.6–9.7 % of the amount of sugars, formed during the maturation of the dough and adversely affects the activity of the microflora of the dough. This is confirmed by a lower consumption of sugars for fermentation by 8.7–19.2 %.

According to the data, obtained on the amylograph, due to the high water absorption capacity of dietary fiber and flax meal proteins, formation of mucus during heating the water-flour suspension what contains flax seed meal, acquires viscous properties earlier in 2–4.5 minutes and at a lower temperature by 3–7 °C, reaches a maximum of 7–42 % of the maximum viscosity, which is reflected in the elasticity of the bread crumb, enriched with a linseed meal.

Flax seed meal is recommended to be added to food formulations, especially in bakery products, to solve problems of replenishing the diet with functional ingredients that not only provide nutrients, but also contribute to the prevention and treatment of diseases.

But the flax meal, like most non-traditional raw materials, does not provide the preservation of the traditional quality of bakery products, which further requires the use of certain technological methods.

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IMPROVEMENT OF THE METHOD OF COMPARATIVE STUDY OF MILK WHEY PROTEINS ENZYMATIC HYDROLYSIS

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Abstract

Milk whey proteins are valueble nutritional ingredients with a number of health-beneficial properties. Whey proteins are also a source of bioactive peptides that can be released in the process of proteins enzymatic hydrolysis. In this connection, there often is a need to compare their proteolytic action on milk whey proteins. It is important to take into account the specificities of the composition and properties of milk whey proteins. The aim of the research was to improve the method of comparative study of milk whey proteins enzymatic hydrolysis. Casein and whey were obtained from fresh cow skimmed milk. The whey was separated by centrifugation after casein precipitation at the isoelectric point. The following enzyme preparations were used in the research: neutral protease, papain, trypsin, chymotrypsin and pancreatin. To select β-LG, gel filtration of the milk whey on the chromatographic column with Sephadex G-150 (Pharmacia) was used. The homogeneity of the received β-LG preparation was analyzed by express electrophoresis in the polyacrylamide gel plates (PAG). The preparation of general casein was isolated by repeated precipitation at the isoelectric point. The fractional composition of the casein substrate was analyzed by electrophoresis in the anode system of homogeneous PAG in the presence of urea. Quantitative treatment of electrophoregrams of the β-LG preparation was performed using the imread reading function. Determination of proteolytic activity of enzyme preparations was carried out according to the method of V. F. Selemenev [6].

In the course of the research, it was determined, that for the research of proteolysis under conditions of identical total proteolytic activity, the concentration of neutral protease should be increased by 1.02 times, papain – by 4.2 times, trypsin – by 2.8 times, pancreatin – by 2.12 times as compared to chymotrypsin. As a result, it has been shown that the use of β-lactoglobulin instead of serum albumin in spectrophotometric determinations allows obtaining more accurate values of the concentrations of whey protein and proteolytic products. In determining the ratio of enzyme : substrate it is advisable to take into account the general proteolytic activity of various enzyme preparations in comparative studies of whey proteins proteolysis with various enzyme preparations. These will simplify the methodology and reduce the time for objective evaluation of enzymatic preparations for proteolysis of milk whey proteins. In some cases, considering the specificity of proteases it could increase the yield of biologically active peptides.

Keywords: milk whey proteins, proteolysis, β-lactoglobulin, gel filtration.

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1. Introduction

One of the important ways of milk whey proteins processing is their proteolysis [1]. It is used for producing low-allergenic food mixtures, food products for children, athletes, as well as for the production of bioactive peptides [2, 3, 4]. Many proteolytic preparations of animal, plant and microbiological origin are tested in the process of development of the technology for

such products [5]. For this reason, there is often a need to compare their proteolytic effect on milk whey proteins. It is important to take into account these specificities of the composition and properties of milk whey proteins.

In many methods, the dependence of the optical density to the bovine blood albumin (BSA) concentration is used to determine the concentration of proteins and their products of splitting by spectrophotometry in the ultraviolet region [6]. In the case of determining the concentration of milk whey proteins, as well as products of their proteolysis, these results differ a lot from the actual ones. This is due to the significant difference in values of absorption coefficients for total milk whey proteins ($E_{280/1\ \%}$=12.3) and BSA ($E_{280/1\ \%}$=6.3) [7]. Much closer values of the absorption coefficient has the main protein of milk – β-lactoglobulin ($E_{280/1\ \%}$=9.6). A similar situation occurs when determining the concentration of products of milk whey proteins proteolysis. When the BSA calibration is used the results differ from the actual ones by almost 2 times.

Another important aspect when proteolysis of milk whey proteins is carried out is an objective comparison of the various proteolytic preparations activity. In many studies, in this case, only the ratio of the substrate: enzyme preparation is taken as the basis without taking into account their activity [8, 9]. Sometimes it is difficult to compare them due to the use of specific methods for various enzyme preparations [10]. It is obvious that in the comparative analysis it is necessary to use the amount of enzyme preparations, taking into account their total proteolytic activity. Accessible to many proteolytic enzymes, casein can be used as a substrate for this. The casein, prepared by Hammarsten, which is often used as a substrate, is badly soluble in water. More suitable is lyophilized casein, isolated during reprecipitation at the isoelectric point. Also, natural milk proteases may be present in the composition of casein preparations [11]. It is therefore advisable to predict a phase of natural proteases inactivation that may have influence on the course of proteolysis.

These will simplify the methodology and reduce the time for objective evaluation of enzymatic preparations for proteolysis of milk whey proteins. In some cases, considering the specificity of proteases, it could increase the yield of biologically active peptides.

2. Materials and methods

Enzyme preparations: neutral protease and papain from «Barrett industrial limited» company (Great Britain), trypsin and chymotrypsin from «Biozym» (Germany) and pancreatin from «Technolog» (Ukraine) company were used in the work.

For the production of whey and casein, fresh skimmed milk (18 °T) was used. Whey was separated by centrifugation (4000 g, 15 min) after casein precipitation at the isoelectric point (pH 4.6). The lyophilized casein substrate was isolated according to the following scheme (**Fig. 1**).Determination of enzyme preparations proteolytic activity was carried out according to the V. F. Selememev's method [6].

The isolation of β-LG was carried out by gel filtration of the milk whey on a column with the Sephadex G-150 from «Pharmacia» (Sweden) as described earlier [12].The columns (1.5×70 cm) from the «Reanal» (Hungary) company of liquid chromatography were used for gel filtration. The speed of the elution was set at 20 ml/h. The amount of taken fraction was 4 ml.

The homogeneity of the received β-LG preparation was analyzed by expressing electrophoresis in the polyacrylamide gel (PAG) plates. Gel's composition and conditions of electrophoresis are described in [13]. Gels were stained with 0.5 % solution of amido black 10 B and were kept in 7 % acetic acid.

The fractional composition of the casein substrate was analyzed by electrophoresis in the anode system of homogeneous PAG in the presence of urea. The composition of the PAG and the conditions for electrophoresis were described earlier [14].

Quantitative processing of β-LG preparation electrophoregrams was performed using the image reading function *imread* [15].

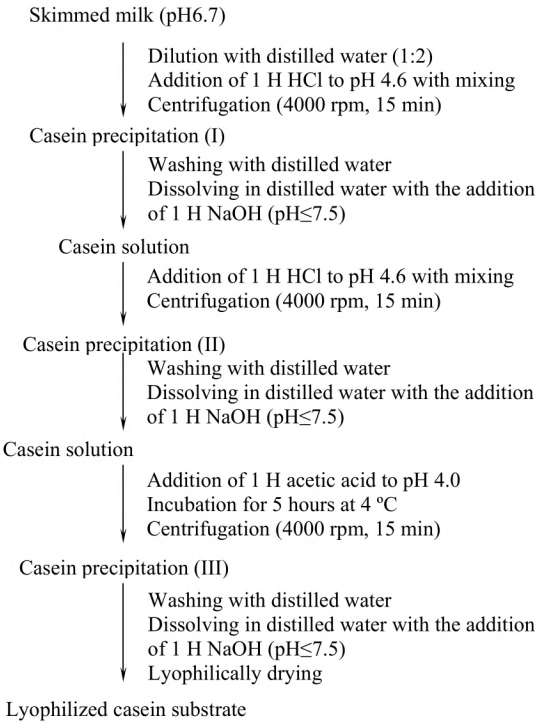

Fig. 1. Scheme of the casein substrate isolation

3. Results

The total casein preparation was isolated by the way of repeated precipitation in the isoelectric point. At the last stage of obtaining, the incubation in acetic acid at pH 4.0 for natural milk proteases inactivation was carried out. The fractional composition of the casein substrate proteins is shown on the electrophoregram (**Fig. 2**). The electrophoregram shows that the isolated substrate has a characteristic fractional composition of the milk casein complex proteins [11]. It is important that the obtained lyophilized preparation is well soluble in water.

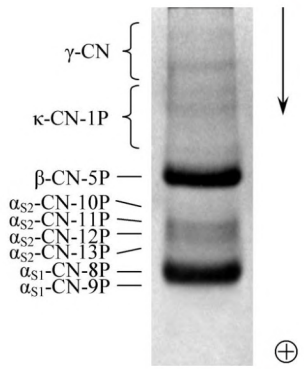

Fig. 2. Electrophoregram of casein substrate, obtained in the anode system of homogeneous PAG in the presence of urea

Taking into account the previously obtained results [12], chromatographic fractions from β-LG after the second gel filtration of milk whey on a column with Sephadex G-150 were combined and selected. The numbers of the combined fractions are shown in **Fig. 3, *a***. The results of the analysis in the combined fractions of β-LG for homogeneity are shown in **Fig. 3, *b***. The electrophoregram shows one band, which corresponds to β-LG in terms of electrophoretic mobility. The results of the densitometry of the obtained electrophoregram indicate a high degree of β-LG preparation homogeneity (**Fig. 3, *c***).

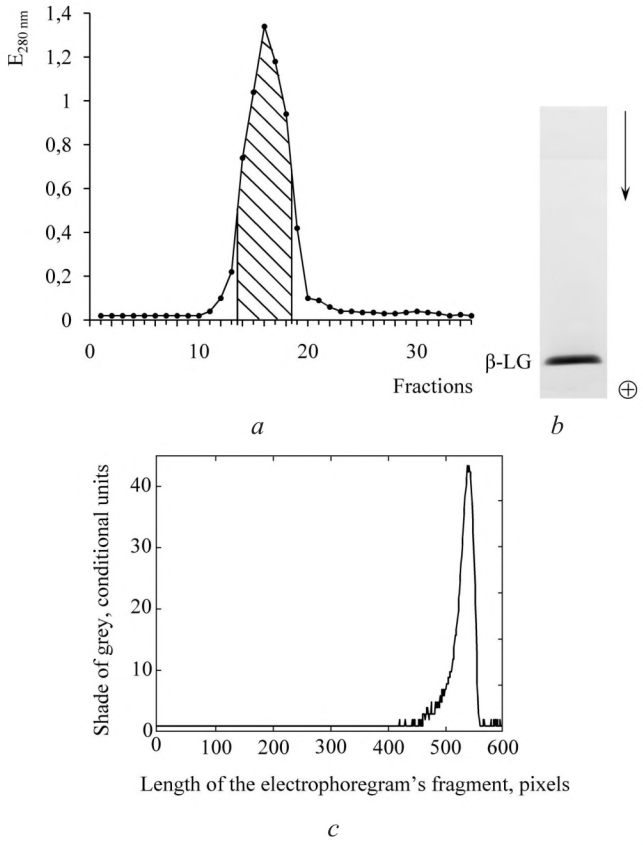

Fig. 3. The combined fractions of β-LG after the second gel filtration (*a*) of milk whey on the sephadex G-150. Electrophoregram (*b*) and densitogram (*c*) of the obtained β-LG preparation

To calculate the concentration of milk whey proteins and proteolytic products, a plot of dependence of optical density on the concentrations of β-LG, BSA and total milk whey protein was constructed (**Fig. 4**). Each point is the average value of three measurements. It can be seen, that the calibration chart of β-LG is much closer to the plot, constructed using a real sample of whey proteins.

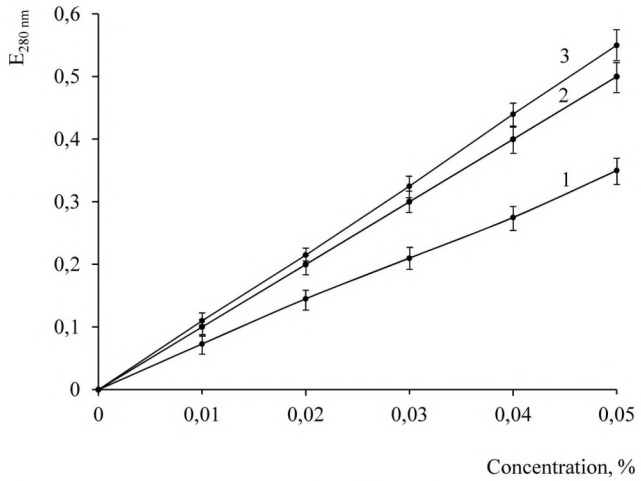

Fig. 4. The dependence of the optical density (λ=280) on the solution of BSA (1), β-LG (2) and total whey protein (3) from their concentration

The results of casein substrate proteolysis products' optical density dependences on the concentration of proteolytic preparations (pancreatin, chymotrypsin, trypsin, papain and neutral

protease) are shown on **Fig. 5**. Then, for each preparation, indicator b which is proportional to the proteolytic activity, according to the method of V. F. Selemenev, was determined [6]. It was as follows: for chymotrypsin – 5.67, for neutral protease – 5.55, for pancreatin – 2.67, for trypsin – 2.02 and for papain – 1.35. That is, the total proteolytic activity of chymotrypsin is greater than that of the neutral protease in 5.67/5.55=1.02 times; for pancreatin in 5.67/2.67=2.12 times; for papain in 5.67/1.35=4.2 times and for trypsin in 5.67/2.02=2.8 times.

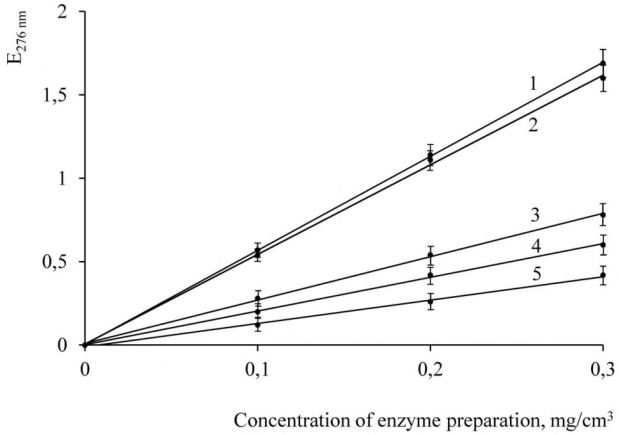

Fig. 5. Dependences of the proteolytic products' optical density (λ=276) on the concentration of enzyme preparations: chymotrypsin (1), neutral protease (2), pancreatin (3), trypsin (4), papain (5)

Thus, for conducting studies of proteolysis in the conditions of identical total proteolytic activity, the concentration of neutral protease should be increased by 1.02 times, papain – by 4.2 times, trypsin – by 2.8 times, pancreatin – by 2.12 times, comparing with chymotrypsin. That is, if the ratio of the enzyme : substrate is 1:50 1 part of chymotrypsin and 50 parts of the substrate should be used. Whereas, to provide a similar ratio for pancreatin the ratio 2.12:50 should be used. Similarly the amount of other enzyme preparations is calculated. The ratio of activity must be set for each match of enzyme preparations.

4. Conclusions

The use of β-lactoglobulin instead of blood serum albumin in spectrophotometric determinations allows obtaining more accurate values of the milk whey proteins concentrations and the products of their proteolysis. β-lactoglobulin, obtained by repeated gel filtration on sephadex G-150, can be used for calibration plot construction.

It is advisable to take into account the total proteolytic activity of enzyme preparations in comparative studies of milk whey proteins proteolysis, when determining the ratio of enzyme: substrate. This will allow obtaining more objective data when choosing a proteolytic preparation. However, this does not give an idea of the hydrolyzed peptide bonds amount. In the future, characterizing the milk whey proteins proteolysis, it is advisable to take into account the total proteolytic activity and the amount of hydrolyzed peptide bonds.

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DECREASE OF REPEATED CONTAMINATION OF PACKED DELICIOUS MEAT PRODUCTS

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Abstract

The study considers a problem of repeated contamination of delicious products, ready for consumption. The aim of the work is to study the repeated thermal pro-cessing of a ready vacuum-packed whole-muscular meat product for inhibiting a surface microbiota.

Today it is urgent for the meat industry, because it influences safety and quality, and also limits a storage term of a product.

After bringing a meat product to culinary readiness by thermal processing, it has an unessential amount of microbiota. Microorganisms, including pathogenic and conventionally pathogenic ones, fall on a product after its cooking at cutting, preparation to package and at the package stage itself. Microbiological contamination of a ready meat product results in fast spoilage and is a serious problem for producers, because the microbiota growth shortens its storage life. In its turn, it results in a refuse of a consumer to buy this product and great economic losses for producers.

The study is directed on a possibility of solving a problem of contamination of a whole-muscular delicious meat product. The solution is in package of a ready product under vacuum and short-term heating at a high temperature.

The work is devoted to the complex study of an influence of repeated pasteurization on safety and quality of a product. There was studied an influence of the repeated thermal processing (post-pasteurization) on microbiological, physical-chemical and also organoleptic parameters of a delicious meat product.

The special attention is paid to an influence of post-pasteurization regimes on a microbiological condition of studied samples. Studies of a total amount of microbiota and also the presence of sanitary-representative microorganisms were conducted.

It has been proven, that the use of post-pasteurization essentially inhibits a number of microorganisms, and also doesn't influence physical-chemical parameters outlook of a product and organoleptic characteristics.

Based on studying an influence of post-pasteurization, it has been established, that inhibition of a microbiota essentially influences safety and prolongs the storage term of a product.

Keywords: post-pasteurization, delicious meat products, microbiota, thermal processing, vacuum package.

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1. Itroduction

Meat and meat products contain valuable nutritive substances for the human growth and heath and are an important part of a ration. Because of a high food value, meat products are ones

of most demanded for consumption. Unfortunately, meat products are fast-spoiling and subjected to microbiological contamination. Semination by microorganisms results in serious problems with safety and quality of a product, and also in economic losses for a producer [1–3].

A question of microbiological safety is important for the meat industry, because spoilage of meat products takes place mainly because of microorganisms [4]. Meat and meat products contain plenty of proteins, enough quantity of water and main nutritive substances with pH, favorable for the microbiota growth [5, 6]. As a result of the life activity of microorganisms, proteins and lipids in a meat product are subjected to denaturation, unfavorable changes of microbiological characteristics, worsening of taste characteristics takes place [2].

A microbiota that causes spoilage of meat and meat products is represented by such mechanisms as Pseudomonas, Acinetobacter, Brochothrixthermosphacta, Moraxella, Enterobacter, Lactobacillus, Leuconostoc and Proteus. As a rule, microorganisms that cause a fact that a product is spoiled, doesn't cause damage to a human, but at their great number can result in disorders of the work of the gastrointestinal tract [7].

For improving a microbiological condition and prolonging a storage term of meat products, it is possible to affect a product after cooking by inhibiting a surface microbiota that fell on a product after its cross contamination [8–11].

One of ways of improving a microbiolgical condition of a meat product and prolonging storage terms may be the use of repeated thermal processing of a vacuum-packed product [12–14].

The aim of this work is to estimate a possibility of inhibiting a surface microbiota of a ready delicious meat product, packed in vacuum by pasteurization, without changing organoleptc characteristics.

The studying procedure is based on the following generalized knowledge:

– an influence of thermal processing on microorganisms and also quality, structural-mechanical and organoleptic parameters of a product [2, 14, 15];

– an influence of vacuum package on the microbiota life activity in a meat product [16, 17];

– thermostable properties of packing films.

2. Materials and methods

Samples of smoked-boiled meat "Balyk vintage" of the highest sort were used in the study. All samples were produced at PE "GARMASH" (Ukraine, Odessa region).

The samples were cooked of 100 % meat of spine-lumbar muscles of pork semi-carcass with adding culinary salt (2.5 % of the meat mass) and pickle preparation (density 1,087 g/cm^3, consisting sodium nitrites 0,05 % and sugar 0.5 %).

All raw and ready materials, used in the study, corresponded to actual standards of Ukraine in the aspect of quality and safety.

2. 1. Experiments

After bringing the product to culinary readiness, it was cooled to temperature 6–8 °C, cut in pieces of 100 g and packed under vacuum in multi-layer polymeric films with width 95 microns, produced by «Orved» (Italy). Package was realized on the vacuum-packing machine, produced by «Cryovac» (Switzerland).

The experimental samples, packed in vacuum, were subjected to post-pasteurization in the electric digester SVC-14, produced by Sammic S. L. (Spain) **Fig. 1.** After post-pasteurization, the samples were cooled to temperature 6–8 °C and kept in the refrigerator Liebherr (Germany) at temperature 4 °C during the whole term. Together with the experimental samples, subjected to post-pasteurization, balyk samples in a vacuum package without post-pasteurization (control samples) were kept under equal conditions in the refrigerator. All samples were kept in the refrigerator during 35 days.

The experimental studies were three- and fivefold repeated. The obtained results were presented in units of the international system CI.

The microbiological studies were conducted in the laboratory of the department of "Biochemistry, microbiology and physiology of nutrition" of the Odessa national academy of food technologies (Ukraine). Microbiological parameters characterize the product safety and also prognosticate and establish its storage term.

Fig. 1. Digester SVC-14

The study was prepared and a batch of the product was taken by sterile instruments under conditions, excluding contamination of a product by microorganisms from the environment. Samples were selected, according to SS 26669-85. The sample batch of 10±0.05 was placed in a sterile bottle with 90 cm^3 of a sterile physiological solution and mixed by round movements during 10–15 minutes. A supernatant liquid is a washout of microorganisms from the product that is in dilution 1:10.

After 14 days of storage the inoculation from dilutions was realized. For preparing dilutions of test samples, 1 ml of the supernatant liquid of the preliminarily prepared sample was taken by a sterile pipette and transferred to a sterile test tube, containing 9 ml of the sterile physiological solution. The content of the test tube was accurately mixed. As a result dilutions 1:100 were obtained.

At 35 and 42 day of storage the inoculation from dilution1:1000 was realized. For such dilutions of test samples, 1 ml of the suspension was taken by a sterile pipette from the test tube with dilution 1:100 and transferred to another test tube, containing 9 ml of the sterile physiological solution and mixed accurately.

A number of mesophilic aerobic and facultative-anaerobic microorganisms was determined, according to SS 8446:2015 Food products. Methods of determination of mesophilic aerobic and facultative-anaerobic microorganisms. This method is based on the ability of microorganisms to multiply on a dense nutritive agar at temperature 30±1 °C during 72 hours.

The inoculation of test samples was realized by taking 1 ml of the suspension of the correspondent solution and introducing it in a Perti dish. After that the heated nutritive medium meat-peptonic agar (MPA) with temperature 45±1 °C in amount 12...15 ml was evenly distributed along the whole Petri dish surface by round movements. After congelation of the nutritive medium, Petri dishes were directed in the thermostat for incubation.

Bacteria of the colon bacillus group (BCBG) were determined, using Kessler medium, according to SS 998-81, because BCBG ferment lactose and as a result acid and gas form during 24 hours at temperature 37±1 °C.

For determining sulfite-reducing clostridia, the method of inoculating 1 cm^3 of the supernatant liquid of the product in the sulfite cyclosulfuric medium (SCS) and Wilson-Blair one, according to SS 998-81, was used. The inoculation was kept in the thermostat at temperature 37±1 °C during 18–24 hours.

Pathogenic microorganisms, including Salmonellaspp., were determined by inoculation on the selective medium, and their serological and enzymatic properties were established, according to SSU ISO 6579 Microbiology of food products and fodders for animals.

Staphylococcusaureus were determined, according to SS 10444.2-94. The batch of the studied product was 25 g. The inoculation was realized in the liquid selective (with preliminary enrichment) and on the dense selective-diagnostic mediums. The inoculations were incubated at temperature 36±1 °C during 24–48 hours. At that the preliminary calculation was conducted in a day, and the final one – after 48 hours.

The physical-chemical and organoleptic studies were conducted at the department of "Technology of meat, fish and seafood" of the Odessa national academy of food technologies

(Ukraine). During the study a concentration of nitrogen ions (pH) was determined. pH index was determined by the potentiometric method, using H-meter Testo 205 (Germany) **Fig. 2**. The methodology provides taking of a sample of comminuted meat with mass 10 g and mixing during 25 min in 100 ml of distilled water. After that, the obtained extract was filtered and pH of the filtrate was determined [18].

Fig. 2. pH-meter Testo 205

The amount of moisture was determined in the experimental and control samples. The mass share of moisture was determined by the accelerated methodology [19], by drying at temperature 150 °C during 1 hour 5 g of the batch of dried meat, rubbed with 6...7 g f sand and preliminarily weighted. Drying was conducted in the drying chamber 2B1-51 (Ukraine). The dried box with the product was placed in the exsiccator. After complete cooling, it was weighted on scales and the mass share of moisture (X_m) was calculated by formula (1):

$$X_m = \frac{(a-b)}{m} \cdot 100\ \%, \tag{1}$$

where a and b – mass of the box with the batch before and after drying, respectively, g; m – batch mass, g.

For checking freshness, quality and safety, presence or absence of changes, the organoleptic studies were conducted after post-pasteurization. The organoleptic studying method is based on analyzing perceptions of the sense organs: sight, hearing, smell, touch and taste. The samples were assessed for establishing correspondence of organoleptic quality parameters to requirements of TC U 15.1-33381354-007:2012. The organoleptic studies were conducted using a five-point scale of assessment. Based on the obtained points, the total point of each sample was calculated. The study was conducted by 6 tasters. During the whole storage term, there were determined such parameters as outlook, consistence, cut look, smell and taste [18].

3. Results

During the study there were determined main microbiological, physical-chemical and organoleptic parameters of the experimental and control samples of whole-muscular delicious meat products.

Meat products of 100 g in a vacuum package were heated at temperature 90 °C during 1 min (sample 1), 2 min (sample 2) and 3 min (sample 3).

Table 1 presents the results of the total number of microorganisms (MAFAnM) in the control sample, and also the number of microorganisms in the experimental samples at 1 day of storage.

Table 1

Index of the total number of mesohilic aerobic and facultative-anaerobic microorganisms at 1 day of storage

Parameter	Control	Sample 1	Sample 2	Sample 3
MAFAnM, CFU in 1 g	$2.0 \cdot 10^2$	$1.4 \cdot 10^2$	$9.5 \cdot 10^1$	<10

Table 1 testifies that the use of pasteurization of the ready delicious meat product, packed in vacuum, essentially inhibits the microbiota.

There were studied the sanitary-representative microorganisms, such as sulfite-reducing clostridia, Salmonella, St. aureus, and also bacteria of the colon bacillus group. The results of these studied demonstrated that no listed microorganisms were found in the product.

The microbiological studies were conducted during the whole storage term in each 7 days. During the storage process there was observed the intensive growth of microorganisms in the control sample, and at 28 day of storage the norm of MAFAnM was essentially exceeded [18].

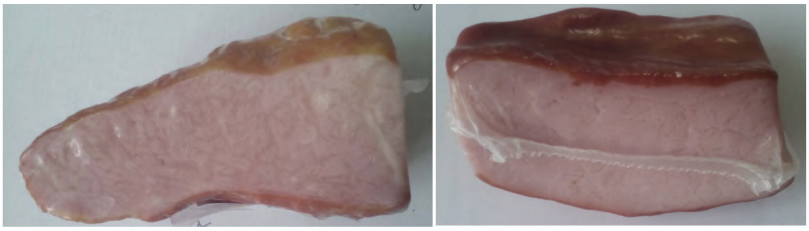
The growth of microorganisms in experimental samples 1 and 2 was less intensive, but at 28 day it exceeded the norm of MAFAnM along with the control. During the storage process sample 3 had the inessential growth of the microbiota and even at 35 day didn't exceed the normative index of total semination.

The sanitary-representative microorganisms were not found during all 35 days of storage in the control and experimental samples.

The effect of thermal processing has the influence on all components of the meat product and can change its outlook and structure [15]. The aim of our work was to diminish the number of microorganisms without changing organoleptic indices of the product. At the same time the storage term of the product is determined by not only microbiological parameters, but also by organoleptic characteristics. The sour-milk bacteria, such as: Lactobacillus, Carnobacterium and Leuconostoc mainly dominate in the product, packed under vacuum. The presented bacteria produce acids: lactic, acetic and formic ones and influence the product taste [20].

The studies of the organoleptic parameters testify to the fact that post-pasteurization doesn't influence the outlook of the product and doesn't cause changes of its organoleptic parameters.

During the process of storage the control sample at 28 day had spoilage signs, namely the sour smell. Control sample 3 didn't have any outlook changes and also taste and smell ones, even at 35 day of storage. **Fig. 3** presents photos of the samples of whole-muscular delicious meat products at 35 day of storage.



a *b*

Fig. 3. Samples of whole-muscular delicious meat products:
a – control; *b* – Sample 3 at 35 day of storage

The studies of pH and mass share of moisture demonstrated that post-pasteurization has no influence on the concentration of nitrogen ions and doesn't cause free moisture release from the product.

4. Conclusions

1. Inhibition of the microbiota of the ready whole-muscular delicious meat product, packed in vacuum, has been proven.

2. It has been established, that post-pasteurization essentially improves the microbiological condition of the product.

3. Temperature-temporal regimes of repeated pasteurization don't cause changes of the product outlook.

4. It has been proven, that the product, subjected to post-pasteurization, has more storage term.

5. The effectiveness of repeated pasteurization at temperature less 90 °C was not verified during the study. Further studies will be directed on searching for optimal post-pasteurization regimes for prolonging storage terms of whole-muscular delicious meat products.

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RESEARCH OF QUALITY INDICATORS OF PROTEIN-FAT MIXTURE FROM FLAX AND SESAME SEEDS FOR NUTRITION OF ATHLETES

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Abstract

The aim of the research is to determine the microbiological stability of a protein-fat mixture of flax and sesame seeds that allows to correct its storage life. A protein-fat mixture has a high content of irreplaceable amino acids BCAA and polyunsaturated fatty acids of ω-3group, so it may be positioned as a component of nutrition for athletes. Flax and sesame seeds, cultivated in Ukraine, were used as research materials. The product was created, based on comminuted flax and sesame seeds in ratio 1:1.

There were determined organoleptic (outlook, taste, smell, color) and physical-chemical (mass share of moisture, ash, protein, fat, acidic, peroxide, anisidine number) parameters of the product. There was determined the microbiological stability of the protein-fat mixture of the increased food value for athletes nutrition after 6 months. It has been proved, that as opposite to the control sample, the protein-fat mixture of the developed composition manifests its microbiological stability by the following

parameters: content of mesophilic aerobic and facultative anaerobic microorganisms, molds, yeast, bacteria of the colon bacillus group and pathogenic microorganisms. The control sample that is comminuted flax seeds doesn't manifest at the end of the storage term any correspondence of microbiological parameters by the content of mesophilic aerobic and facultative anaerobic microorganisms, molds, and bacteria of the colon bacillus group. This regularity is explained by the presence of lignans, sesamol and sesamoline, with preservative properties in the developed product. The obtained data may be used for reasoning recipes of products, based on the protein-fat mixture and correction of the food supplements ratio in them.

Keywords: protein-fat mixture, flax seeds, sesame seeds, oxidative stability, microbiological stability, sesamol, sesamoline.

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1. Introduction

Raw materials and ingredients, included in the composition of products of a special destination, particularly, for athletes' nutrition, must be high-quality, safe and have a high purification degree [1, 2]. Such products must be made, observing requirements of the Alimentarius Code for products of special and child nutrition [3].

It is known, that fat-containing products are disposed to two types of spoilage – chemical (oxidative) and microbiological ones. A spoilage type that limits storage terms of these products is determined by the following factors:

– fat mass share;

– moisture mass share;

– fatty-acid composition of the fatty phase;

– initial indices of oxidation of the fatty phase (acidic, peroxide, anisidine numbers);

– initial microbiological parameters (number of mesophilic aerobic and facultative anaerobic microorganisms, molds, yeast, bacteria of the colon bacillus group and pathogenic microorganisms);

– presence of natural antioxidants and preservatives and so on [4–8].

For increasing oxidative and microbiological stability, the aforesaid factors are affected and also a series of food supplements, preventing oxidative and microbiological spoilage, are applied [9, 10].

Works [11–14] substantiate the composition of the protein-fat mixture of the increased food value for athletes' nutrition, based on oil seeds, investigate its antioxidant properties and also determine technical processing parameters, at which the maximal increase of the biological value of the products is possible.

The aim of the research is to determine organoleptic and physical-chemical parameters and also microbiological stability of a protein-fat mixture of flax and sesame seeds for athletes' nutrition. These data will be applied for reasoning recipes of a series of products, for example, confectionary ones, based on the protein-fat mixture and correction of food supplements (antioxidants and preservatives in them).

2. Materials and Methods

The following materials were used for conducting the studies:

– flax seeds (Ukraine, RI of oil crops of NAAS) by SSU 4967;

– sesame seeds (Ukraine, RI of oil crops of NAAS) by SSU 7012.

The protein-fat mixture of the increased food value for athletes' nutrition is comminuted flax and sesame seeds in ratio 1:1. Comminuted flax seeds are used as a control sample.

Photos of the obtained samples are presented on **Fig. 1**.

Preparation of the protein-fat mixture includes the following stages:

– mixing of flax and sesame seeds;

– comminution of seeds to 150…200 mcm in the knife vertical comminutor *Glasser* (RF);

– moisture-thermal processing, using a source of microwave radiation according to [14].

The protein-fat mixture for athletes' nutrition has the high content of irreplaceable amino acids *BCAA* (*branched-chain amino acids*) – leucine, isoleucine, valine. The ratio of polyunsaturated fatty acids of ω-6 and ω-3 groups is near 3:1. The protein-fat mixture may be used as an in-

dependent product and also in the composition of fat-containing products for athletes in the period of intensive physical loads, for soldiers, workers of hard physical labor under changing climatic conditions and other population layers [11].

a *b*

Fig. 1. Photos of samples of the protein-fat mixture (*a*) and control sample (comminuted flax seeds) (*b*)

Organoleptic parameters of the protein-fat mixture (outlook, taste, smell, color) were determined according to SS 27988 "Oil seeds. Methods of color and smell determination". The moisture mass share was determined according to SSU 4603 "Oils. Methods of moisture mass share and volatile substances determination". The ash mass share was determined according to SS 26226 "Fodders, mixed fodders, mixed fodder raw materials. Methods of raw ash determination". The fat mass share was determined according to SS 10857 "Oil seeds. Methods of oil content determination". The mass share of protein was determined according to SS 7169 "Fodders, mixed fodders, mixed fodder raw materials. Methods nitrogen and raw protein determination". The acidic number of fat in the protein-fat mixture was determined according to SS ISO 660 "Animal fats and vegetable oils. Method of acidic number and acidity determination", peroxide number – according to SSU ISO 3960 "Animal fats and vegetable oils. Method of peroxide number determination", anisidine number – according to SSU ISO 6885 "Animal fats and vegetable oils. Method of anisidine number determination".

Samples for microbiological analyses were taken and prepared according to SS 26669 "Food and taste products. Preparation of samples for microbiological analyses". The number of mesophilic aerobic and facultative-anaerobic microorganisms was determined according to SSU ISO 4833 "Microbiology of food products and animal fodders. Horizontal method of microorganism calculation". The number of bacteria of the colon bacillus group (coliforms) was determined according to SS 30518 "Food products. Methods of revelation and determination of the number colon bacillus group bacteria". The number of bacteria of *Salmonella* genus was determined according to SSU EN 12824 "Microbiology of food products and animal fodders. Horizontal method of Salmonella revelation". The number of molds and yeast fungi was determined according to SSU ISO 7954 "Microbiology of food products and animal fodders. General instructions of yeast and microscopic molds calculation".

3. Experimental procedures

Organoleptic and physical-chemical parameters of samples of the protein-fat mixture (PFM) of the increased food value, based on flax and sesame, and also control sample, based on flax seeds, were determined at the room temperature and presented in **Table 1**.

The studied (sesame and flax seeds) and control (flax seeds) samples of the protein-fat mixture were kept in the closed polyethylene pack in the aerial medium during 6 months at temperature 10 °C. The storage time at the given temperature was chosen, based on data, set in technical conditions for analogous products. At the beginning and at the end of storage the samples were examined for the following microbiological parameters: content of mesophilic aerobic and facultative anaerobic microorganisms (**Fig. 2**), yeast (**Fig. 3**) and molds (**Fig. 4**). There were conducted the studies of samples of the protein-fat mixture for the presence of colon bacillus group bacteria and pathogenic microorganisms, including *Salmonella* genus (**Table 2**). The presence of microorganisms in samples was compared with the maximum permitted value, indicated in normative documents (ND).

Table 1

Organoleptic and physical-chemical parameters of protein-fat mixtures of the increased food value

Parameter name	Parameter characteristic	
	PFM (sesame+flax)	PFM (flax)
Outlook	Homogenous cream-like dense mass	
Taste and smell	Weak, inherent to seeds of used oil crops, without side smells	
Color	cream-beige	Brown-beige
Mass share of moisture, %	5.41	6.13
Mass share of ash, insoluble in 10 % hydrochloric acid, n recalculation for absolute dry substance, %	4.10	3.60
Mass share of fat, %	47.30	44.70
Mass share of protein, %	20.15	21.80
CN of fat, mg KOH/g	2.63	2.75
PN of fat, mmol ½ O kg	1.70	3.85
AN of fat, c.u.	2.30	4.10

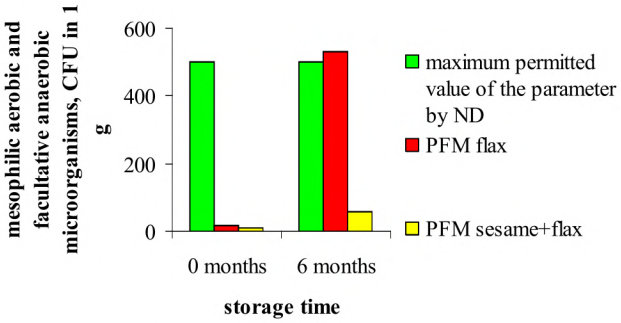

Fig. 2. Dynamics of changes of the content of mesophilic aerobic and facultative anaerobic microorganisms in the studied products

As it is seen from **Fig. 2**, the content of mesophilic aerobic and facultative anaerobic microorganisms in the protein-fat mixture of flax and sesame seeds after 6 months of storage doesn't exceed values, permitted by normative documents. As to the control sample, after 6 months of storage this parameter exceeds the norm. The results testify that components of the protein-fat mixture with flax seeds have preservative properties that allow them to inhibit the growth of mesophilic aerobic and facultative anaerobic microorganisms.

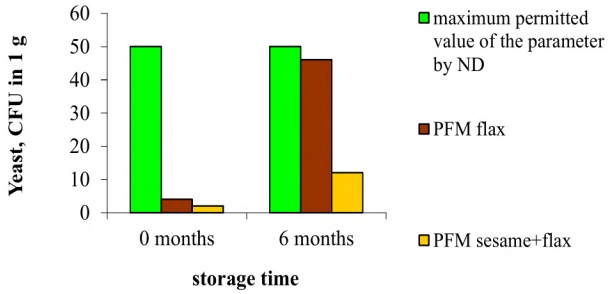

Fig. 3. Dynamics of changes of the yeast content in the studied products

As it is seen on **Fig. 3**, the content of yeast spores in both flax and sesame seeds protein-fat mixture and control sample after 6 months doesn't exceed values, maximally permitted by normative documents. The obtained results testify that the yeast content in samples is not a crucial indicator of the microbiological stability of the product.

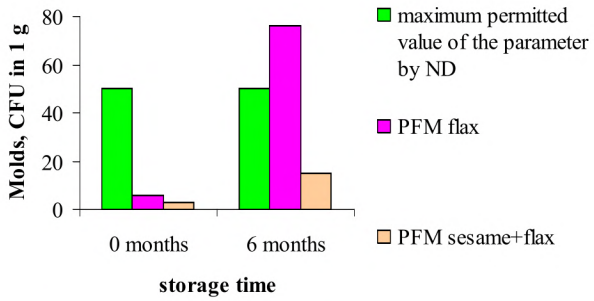

Fig. 4. Dynamics of changes of the mold content in the studied products

As it is seen on **Fig. 4**, the content of mold spores in the flax and sesame seeds protein-fat mixture after 6 months doesn't exceed values, maximally permitted by normative documents. As to the control sample, the content of molds in it after 6 months of storage was 76 CFU in 1 g of the product that exceeds the maximum permitted index (50 CFU in 1 g of the product). The obtained results testify that compounds, included in the composition of the protein-fat mixture with sesame seeds, have preservative properties that allow them to inhibit the growth of molds.

Table 2

Presence of colon bacillus group bacteria and pathogenic microorganisms, including ones of *Salmonella* genus in the studied products

Parameter name, measuring units	Parameter value by ND	Test results			
		0 months		6 months	
		PFM (sesame+flax)		PFM (flax)	
Colon bacillus group bacteria (coliforms) in 0,1 g	Not permitted	Not revealed	Not revealed	Not revealed	6
Pathogenic microorganisms, particularly bacteria of *Salmonella* genus in 25 g		Not revealed	Not revealed	Not revealed	Not revealed

As it is seen from **Table 2**, no colon bacillus group bacteria and pathogenic microorganisms, including bacteria of *Salmonella* genus, were revealed in the protein-fat mixture of flax and sesame seeds after 6 months of storage. As to the control sample, the coliforms, not permitted by normative documents, appear in the product after 6 months of storage. Bacteria of *Salmonella* genus in the control sample are not revealed during the whole storage term. The obtained results testify that components of the protein-fat mixture with flax seeds have preservative properties that allow them to inhibit the growth of colon bacillus group bacteria. As to the content of pathogenic microorganisms, in particular, bacteria of *Salmonella* genus are not a crucial indicator of the microbiological stability of the product.

4. Discussion

There were studied organoleptic and physical-chemical parameters of samples of the protein-fat mixture of the increased food value, based on flax and sesame, and also the control sample – based on flax seeds. The obtained indices (**Table 1**) little differ from each other. An exclusion is only indices of the content of initial (peroxide number) and secondary (anisidine number) oxidation products. The sample, based on flax seeds, has higher oxidation indices that testify to the less lipid stability of the product already at the stage of its obtaining. It may be explained by the high content of polyunsaturated fatty acids in lipids of flax seeds. Comparing with the control, the sample of the developed product is more stable to oxidative spoilage at the expanse of sesame antioxidants (sesamol and sesamoline) presence in it [15–18].

The studies of the microbiological stability of the protein-fat mixture and the control product were conducted (**Fig. 2–4, Table 2**). After 6 months of storage the developed product manifests the higher microbiological stability by the following parameters: content of mesophilic aerobic and

facultative anaerobic microorganisms, molds, yeast, bacteria of the colon bacillus group and pathogenic microorganisms. These parameters don't exceed normative ones after finishing the storage term. As to the control sample, at the end of the storage term it didn't correspond to requirements of normative documents by the content of mesophilic aerobic and facultative anaerobic microorganisms, molds, bacteria of the colon bacillus group and pathogenic microorganisms. This regularity is explained by the presence of lignans of sesame, sesamol and sesamoline with preservative properties, testified by studies [19, 20]. It may be supposed with the high reliability degree, that the preliminary technological processing of the preliminarily moisturized protein-fat mixture (exposition in the field of microwave radiation), offered in [14, not only raises the assimilability degree of proteins in the product at the expanse of inactivating inhibitors of proteolytic enzymes, but favors the increase of microbiological stability of the product.

6. Conclusions

It has been determined, that the protein-fat mixture, based on flax and sesame seeds, has the higher oxidative and microbiological stability, comparing with the control sample. The reserch results proved the microbiologcal stability of the developed product after 6 months of storage. It must be taken into account by developers of recipes and producers of food products, containing the protein-fat mixture, based on flax and sesame seeds, for example, fat-containing confectonary products.

Shortcomings of this study are the absence of data about sesame lignans content (sesamol and sesamoline) in the studied samples of the protein-fat mixture and their correlation with the microbiological stability indices. In its turn, a weak side of the study is an accent on the one type of raw materials, containing natural components, preventing oxidative and microbiological spoilage of the product. That is why it is expedient to search for similar raw materials and to develop products on their base for increasing the content of synthetic food supplements (antioxidants and preservatives) in products for increasing their food value and, correspondingly, decreasing the potential toxicity from synthetic components.

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RESEARCH OF WHEAT DRYING IN A MICROWAVE AND COMBINED FILTER-MICROWAVE DRYER

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Abstract

The aim of the conducted study is to determine kinetics of the complex effect of microwave energy supply and filter drying of the process of water release from the wheat layer. There is offered a combination of MW and filter drying. A special feature of this combination must be its more effectiveness and high speed of water elimination from surface layers of wet seeds and, as a result, the productivity increase of the drying way, decrease of specific energy consumption.

There was determined the influence of the specific load of the material, radiator power on processes of microwave and filter-microwave drying of wheat seeds. There were compared microwave, filter-microwave and convective drying of seeds by parameters of specific energy consumption, drying speed.

The specific energy consumption at microwave drying of seeds was 4 MJ/kg, at filter-microwave drying 3.8 MJ/kg that is lower than existent convective dryers. The speed of microwave drying changes from 0,5 to 3 %/min, filter-microwave – from 0.3 to 0.7 %/min. The speed is at the level of standard convective dryers.

The conducted studies allow to recommend a new combined way of FMW drying of seeds with low energy consumption.

Revealed features of heating and drying are possible to be used at developing industrial dryers.

The base of experimental data is possible to be used for optimizing and determining effective conditions of MW and FMW drying.

Keywords: microwave drying filter-microwave drying, wheat, drying kinetics.

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1. Introduction

The process of drying is one of main methods of food products preservation. For today there are an essential number of ways and methods of drying initial raw materials and food products. Most effective drying ways are convective, conductive, infrared, microwave, sublimation, acoustic and other ones, and also their combinations.

Great attention is paid to studying combined drying methods, because their use results in increasing the driving force, decreases the time and energy consumption of the process, allows to get a high-quality product.

2. Problem review

A wide diapason of studies of combined drying is considered.

Combined drying processes are applied for different food products: fruits [1–3], vegetables [4–8], in chemical industry [9–13].

There are mainly used combinations of MW radiation and convective drying (CD) [1, 2], MW radiation and vacuum [1], infrared (IR) radiation and CD [4, 5], ultrasound and CD [6], MW, IR radiation and CD [9], discrete IR and CD [5], filter and CD [10–12].

These methods are mainly compared with convective drying. They are compared by kinetics, specific energy consumption of the process and quality of dried products (color, phenol content, vitamin C, rehydration characteristics).

As a result, there are noted: drying speed increase at increasing MW radiation, decreasing pressure [1–3], increasing quality of a product [2, 4, 8, 13], decreasing energy consumption [5].

Filter drying is a combination of CD and air filter by the porous wet material. There are considered results of filter drying of aluminous cake [10], birch veneer in a package [11], peat and coffee slime [12]. Experimental studies of filter drying [10–12] are limited by constructions for chemical industry, variants of energy supply to the material by MW radiation are not also considered.

In methods [1–8] there are used mainly a combination of MW or IR radiation and CD. For grain products the use of combined methods is practically not considered, the studies were conducted only for the static layer of seeds, affected by the MW field [14, 15].

Speed data of wheat seed drying are contradictory. A spread of wheat drying speed in dryers of different types is 0.5…3 %/min. It is connected with the diversity of varieties and properties of wheat, different initial moisture, moisture binding directly in seeds, diversity of drying methods.

Seed dryers are characterized by the high energy consumption as from 5 MJ/kg to 9 MJ/kg [16] and emission of an exhausted coolant in the atmosphere. The heat content of an exhausted coolant is only by 10–15 % lower than one of hot air, supplied to the drying chamber.

The condition of the process of MW wheat seed drying is characterized by an essential amount of moisture, appeared on the surface of processed particles. The presence of such surface moisture worsens conditions of the process of moisture removal and decreases its effectiveness.

It is expedient to consider a possibility of intensifying the process of moisture removal in a MW dryer for wheat seeds at the expanse of creating an intensive air flow, normal to the plane of the wet material layer with speeds, typical for filter drying methods (within 3–8 m/s), and parameters, correspondent to the normal atmosphere condition. At such organization of the moisture removal process it is possible to guarantee a high speed of moisture removal from the surface of particles and invariability (within one drying chamber) of the driving force of the process.

The aim of this study is to investigate wheat drying in microwave and combined filter-microwave dryers. A promising method of wheat seed drying may be a combination of MW and filter drying. A special feature of this combination must be its higher effectiveness and high speed of moisture removal from surface layers of particles of wet seeds and, as a result, the drying method productivity increase and the specific energy consumption decrease.

The tasks of the study:

– to determine the influence of specific load of the material, radiator power of the process of microwave drying of wheat seeds;

– to determine the influence of specific load of the material, radiator power of the process of filter-microwave drying of wheat seeds;

– to compare microwave, filter-microwave ad convective drying of seeds by parameters of specific energy consumption, drying speed.

3. Materials and Methods

The studies of drying were conducted on winter wheat “Podolyanka” with the initial moisture near 20...23 %, that corresponds to the moisture of fresh harvested seeds. The initial moisture of seeds was determined by the digital moisture by Kett PM 600 (Kett Electric Laboratory, Japan). The thickness of the material layer remains stable 20 mm. The initial temperature of seeds is 18...25 °C.

For preliminary assessing the potential of the technology of microwave drying of wheat, there was conducted a series of experiments on the stand with the fixed layer of the product (**Fig. 1**).

The construction of the stand is microwave chamber 1 (chamber is presented without a door), with fixed electronic scales 7, measuring platform of scales on suspension 4, with fixed platform 5 of radiotransparent plastic. The distributed wheat layer is placed on platform 5. A sample was radiated by microwave radiator 2. The seed temperature at its processing was periodically measured by a pyrometer.

The heating chamber was ventilated by staff ventilator 3, the air speed didn’t change. The radiation power (N, W) was set by staff means of control panel 6 of the chamber. For conducting a series of experiments, values 240, 400, 560 and 800 W were selected. The material specific load (G, kg/m^2) on a cartridge was 5.26; 3.95; 2.63; 1.32 kg/m^2.

Before the beginning of a series of experiments the power of magnetron radiation was calorimetered by the method, accepted for microwave heating chambers. 1 liter of pure water was heated during one minute. For calculating the power of magnetron radiation, the following formula was used.

$$P = \frac{C_p \cdot m \cdot \Delta T}{t_h}, \quad (1)$$

where C_p – specific heat of water (4180 J/°C); m – water mass (kg); t_h – time of water heating (s); ΔT – difference between initial and final water temperatures (°C).

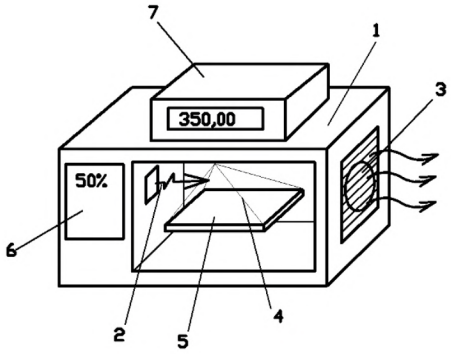

Fig. 1. Scheme of experimental stand for studying the drying process of the immovable wheat layer in the MW field

Two series of experiments with wheat seeds were conducted on the stand. During the first series of experiments the thickness of the wheat layer changed, in the second one the radiation power in the chamber changed.

For studying the moisture removal process at filter-microwave (FMW) drying, the stand was developed (**Fig. 2**).

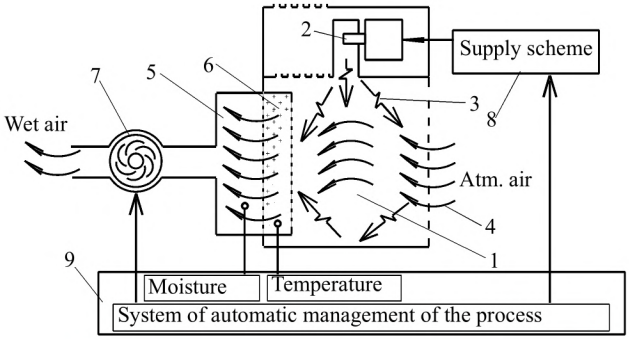

Fig. 2. Scheme of the stand for studying the combined MWF drying method: 1 – microwave chamber, 2 – MW radiator, 3 – Mw radiation, 4 – air flow, 5 – air chamber, 6 – cartridge with the wet material, 7 – ventilator, 8 – supplying system of magnetron, 9 – System of automatic management of the stand

The stand consists of MW chamber 1, with cartridge with the wet material 6. A flow of atmospheric air is blown through the material layer in the cartridge by ventilator 7. Parameters of the moisture removal process are controlled by direct measuring of the cartridge mass and material moisture before and after processing.

The course of the moisture removal process is controlled by indirect parameters – moisture and temperature of the air flow, passed through the cartridge and temperature of the wet material in it.

There was studied a dependence of drying process parameters on a chamber load and, correspondingly, on an energy supply value. The modeling of load changes was realized by choosing three sizes of the cartridge. The material specific load in it was 0.06, 0.08 and 0,085 kg/m^2. The power of MW radiation was 560 W. The MW radiation effect duration was 20 s, blow – 15 s. The air speed at the entrance in the cartridge was 3 m/s. The cartridge was weighted at laboratory scales each 4 minutes.

The managing principle of combined drying in this case was in the successive, standardized in time periodical effect on a test sample (wet material layer in the cartridge) by MW radiation and blow of the layer by a flow of atmospheric air.

Such managing method models the successive heating of the layer by MW radiation at passing drying chambers and intensive blow of the layer at passing filter drying zones.

For realizing the algorithm of management and fixation of measuring results of control parameters of the process, there was developed a system of automatic management of the set. A screen of the human-machine interface of the managing program is presented on **Fig. 3.**

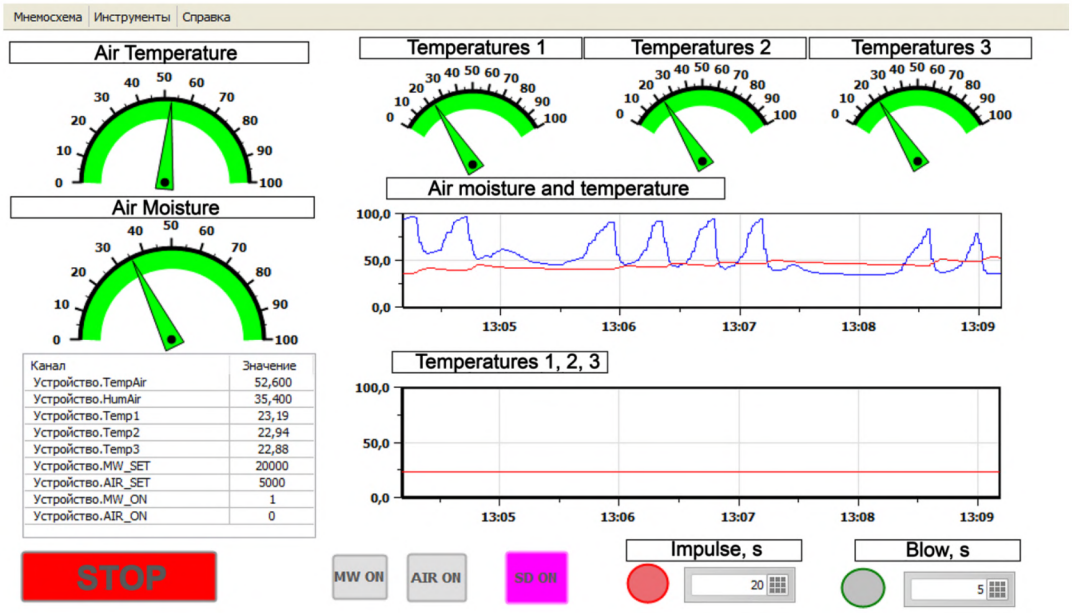

Fig. 3. Screen of the human-machine interface of the managing program

Important parameters of the process are the duration of sample processing by MW radiation and air blow. At the conducted study there were determined main dependencies between the amount of supplied energy and moisture removal intensity.

4. Results

As a result of the conducted study, there were determined dependencies of the speed of seed layer dehydration on the radiation power and load value in the microwave heating chamber.

The first group of experiments, conducted on the stand, presented on **Fig. 1,** allowed to determine a dependence of the speed dehydration on the product mass in the microwave heating chamber.

Graphs of changes of the moisture content of the seed layer at its heating by the microwave field at different power values of microwave radiation and different specific loads are constructed by the obtained data, **Fig. 4, 5.**

The graphs of the temperature curves of the seeds layer in the process of heating by the MW field at the given loads and different radiation power values in the heating chamber are presented on **Fig. 5.**

The thermograms (**Fig. 5**) demonstrate that the seed layer temperature essentially exceeds technologically permitted temperature values at fodder seed drying (50–60 ºC). Such regimes were chosen for widening the base of experimental data, determining stand possibilities.

The dependence of the drying speed on the radiation power in the chamber is obtained by generalizing the experimental results, **Fig. 6.**

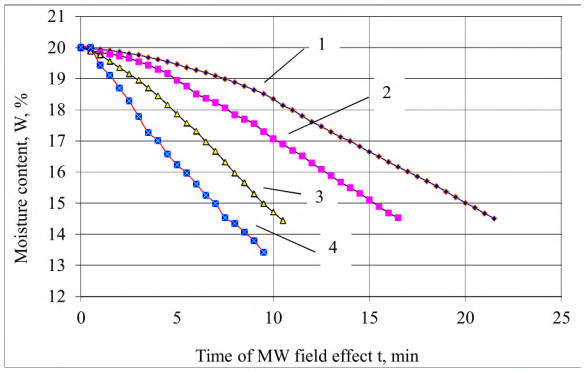

Fig. 4. Change of the moisture content of the seed layer at *N*=240 W, load:
1 – 5.26 kg/m^2; 2 – 3.95 kg/m^2; 3 – 2.63 kg/m^2; 4 – 1.32 kg/m^2

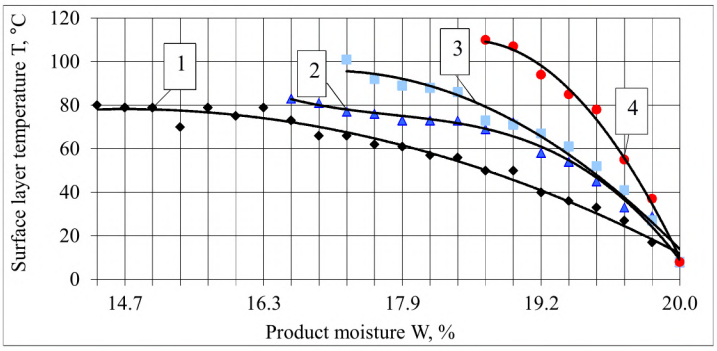

Fig. 5. Thermograms of the seed layer at *G*=1.32 kg/m^2. Power of the MW radiator:
1 – 240 W, 2 – 400 W, 3 – 560 W, 4 – 800 W

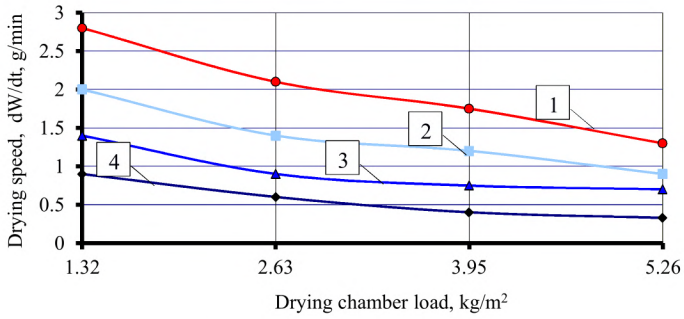

Fig. 6. Dependence of the drying speed on the load value. Power of the MW radiator:
1 – 800 W; 2 – 560 W; 3 – 400 W; 4 – 240 W

The high temperature regimes result in the drying speed increase.

The change of the MW radiator power in 4 times results in 3 times drying speed increase (**Fig. 6**).

As a result of the experiments on the stand (**Fig. 2**), the following data were obtained.

Fig. 7 presents the graphs of changes of the controlled parameters of the process at loading the drying chamber with the layer of wet seeds as 0.08 kg/m^2.

The moisture and temperature of air at the exit from the FMW dryer is of the step type (**Fig. 7**). It is caused by the fact that the material layer was periodically blown. The air moisture successively increased to 95% and remained constant that corresponds to the first drying period. Blow practically doesn't influence the air temperature.

The research results allowed to reveal typical dependencies for the combined drying regime. The main ones are presented on **Fig. 8–10.**

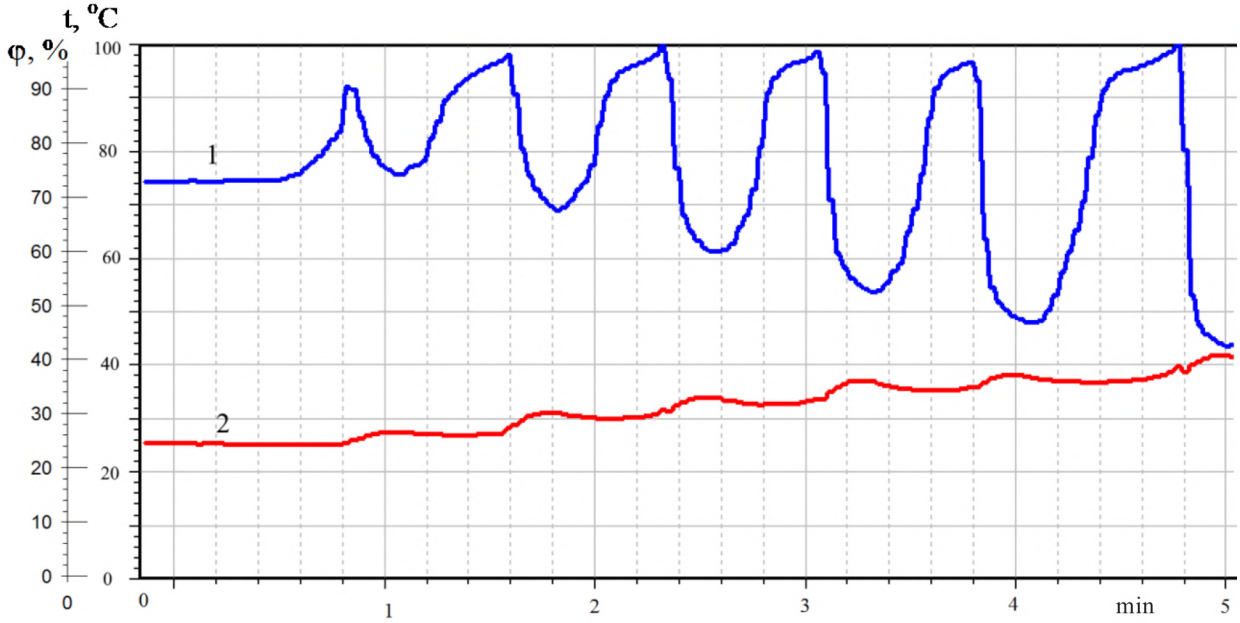

Fig. 7. Changes of the air parameters at the exit from the FMW dryer:
1 – moisture (φ, %), 2 – temperature (t, °C)

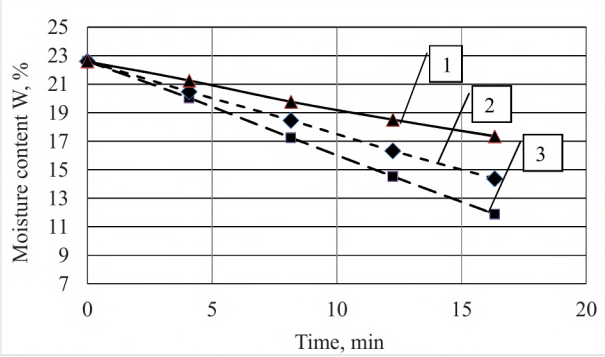

Fig. 8. Change of the moisture content of the seed layer at radiator power 560 W. Specific load:
1 – 0,085 kg/m²; 2 – 0.08 kg/m²; 3 – 0.06 kg/m²

Drying takes place at the first period that is testified by the constant drying speed value, **Fig. 9.** At the first period surface moisture, separated from seeds at the expanse of the MW radiation effect, is removed.

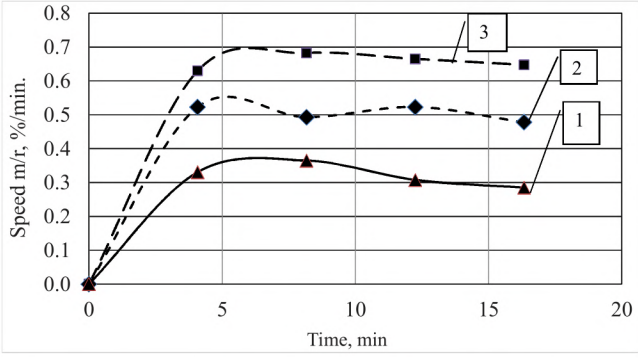

Fig. 9. Dependence of the drying speed on the specific load value:
1 – 0,085 kg/m²; 2 – 0.08 kg/m²; 3 – 0.06 kg/m²

The specific load decrease at FMW drying results in the process speed increase up to 0.7 %/min (**Fig. 9**).

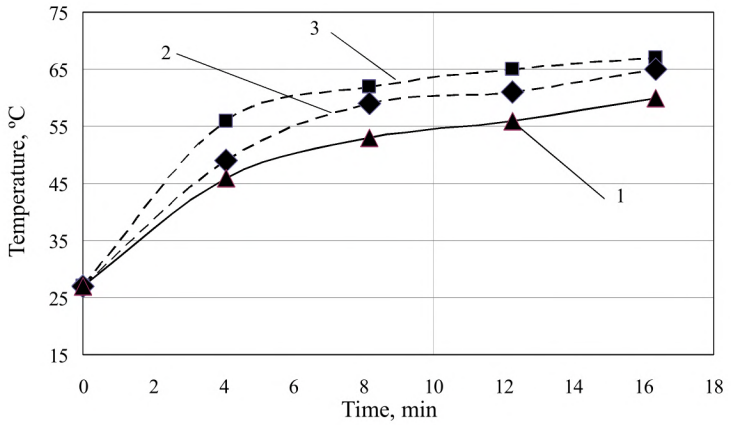

Fig. 10. Thermograms of the seed layer on the specific load value:
1 – 0,085 kg/m^2; 2 – 0.08 kg/m^2; 3 – 0.06 kg/m^2

The drying process in the FMW dryer takes place at the wheat temperatures (curves 1, 2, **Fig. 10**), correspondent to technological requirements for fodder seeds drying.

Specific energy consumption for MW seed drying was 4 MJ/kg, FMW dryer – 3.8 MJ/kg. It is lower than for correspondent convective dryers. The drying speed in MW dryers changes from 0.5 to 3 %/min **(Fig. 6)**, in FMW dryers from 0.3 to 0.7 %/min (**Fig.** 9). The speed is at the level of standard convective dryers.

6. Conclusions

The conducted experiments proves the possibility of intensifying the process of moisture removal in a MW dryer for wheat seeds at the expanse of creating an intensive air flow, normal to the plane of the wet material layer with speeds, typical for filter drying methods (within 3–8 m/s), and parameters, correspondent to the normal atmosphere condition.

The conducted studies allow to recommend producers of the drying equipment the new combined way of FMW drying of seeds with low energy consumption (3.8 MJ/kg).

The revealed features of heating and drying of wheat seeds are expedient to be used at developing industrial MW and FMW dryers.

The base of experimental data is possible to be used for optimizing and determining effective conditions of MW and FMW drying processes.

A shortcoming for using the results for FMW drying is the absence of a product movement that essentially decreases the stand productivity. It must be also noted, that for realizing the process in the industrial scale, it is necessary to develop the specialized microwave equipment. The experimental studies must be continued for this aim.

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