ISSN 2504-5687

EUREKA: LIFE SCIENCES

- Agricultural
- Biological Sciences
- Biochemistry, Genetics
- Molecular Biology
- Environmental Science
- Immunology
- Microbiology
- Neuroscience

Volume 1(7)
2017

SCIENTIFIC JOURNAL

EUREKA: Life Sciences covers interdisciplinary areas of research in biology as the life science. Therefore, the authors in their materials should ***emphasize areas of application*** of their research, always ***emphasizing the ability to attract knowledge from related scientific fields to the knowledge of living objects.***

The problems of the following areas:

- Agricultural and Biological Sciences
- Biochemistry, Genetics and Molecular Biology
- Environmental Science
- Immunology and Microbiology
- Neuroscience

EUREKA: Life Sciences
publishes 4 types of materials:
– review article,
– progress reports,
– full paper,
– research news: at the forefront of life science

PUBLISHER OÜ «Scientific Route»
European Union
Editorial office
«EUREKA: Life Sciences»
Narva mnt 7-634, Tallinn, Eesti
Harju maakond, 10117
Tel. + 372 602-7570
e-mail: info@eu-jr.eu
Website: http://eu-jr.eu

EDITORIAL BOARD

CONTENT

THE STUDY OF CHANGES IN THE STRUCTURAL AND MECHANICAL PROPERTIES OF TURKEY FILLET DURING STORAGE

Svetlana Shapoval
Department of Engineering and Technical Disciplines
Kyiv National University of Trade and Economics
19 Kioto str., Kyiv, Ukraine, 02156
Shapovalknteu@gmail.com

Abstract

The method and equipment for the study of changes of structural-mechanical properties of turkey fillet at storage were offered. The improved express-method of the study allows determine the relaxation effort and time at axial deformation and the firmness limit of meat fibers using penetration.

The selection of registering equipment and sensors for the study of structural-mechanical properties of turkey fillet was grounded. The methods of compensation of axial displacement of sensors at the study of penetration and relaxation were described. The principal scheme, appearance and example of work of setting were given.

The changes of firmness and relaxation effort of cooled turkey fillet at different storage terms were characterized.

It was established, that organoleptically perceptible changes of structural-mechanical properties of turkey fillet take place after 46 hours of storage. In first turn, relaxation is decelerated, the firmness limit is lowered that together with less firmness limit of fibers allows recommend it for formation of natural culinary goods of the given form, for example, rolls.

The offered equipment and methodology of getting results of axial relaxation and penetration allows make conclusions about the essence of changes of rheological properties of turkey fillet and also recommend the studied samples for one or another type of culinary processing.

Keywords: penetration, structural-mechanical properties, turkey fillet.

DOI: 10.21303/2504-5695.2017.00284

1. Introduction

The structural-mechanical properties of meat samples are traditionally determined organoleptically. After pressing on the meat sample, the time of previous form restoration is determined visually [1].

But such method has its shortcomings: from subjective human perception of time and necessary experience to unknown structure of sample under the pressed surface.

In technique it is used to study the firm properties of real bodies by deformation graph that reflects the graphic dependency between relative deformation and mechanical tension, received experimentally [2, 3].

The structural-mechanical properties of the raw material determine the quality of culinary products of poultry, but it is difficult to detect them organoleptically. The mechanical properties of meat depend on forage, age, sex of poultry and essentially differ in different sets [4, 5]. That is why the elaboration of universal methods and equipment for the study of structural-mechanical properties of the meat raw material under condition of mini-productions is an urgent problem.

The main aim was to elaborate the methods of the study of structural-mechanical properties of turkey fillet for recommendation of optimal type of culinary processing.

The task of research was to improve the methods of the study of poultry meat structure that allow fix insignificant changes of rheological properties that are difficult to detect organoleptically.

2. Materials and Methods

The series of experiments was carried out at the laboratory of Kyiv National university of Trade and Economics (KEUTE), Ukraine.

For fixing effort in the process of relaxation and penetration the series of dynamometers ITM Load-5.3/3s of Ukrainian production with measuring limit 2,6…6 N was used. Dynamome-

ters, used at relaxation and penetration are equipped with triaxial stabilization of indenter to diminish its deviation from vertical line [6].

The digital measuring bloc UMCD, made by "ITM" LTD (Kharkiv, Ukraine) was used for fixing indications of dynamometers [7]. The electric bloc was produced in metal body. On the upper surface of the body are situated four inputs of DB-15 type, intended for connection of external devices (analogous and digital sensors). On the right side surface of the body is the input of USB-B type for connection with computer. The exchange of data between computer and electric bloc and also the power supply of electric bloc and sensor is realized through connecting flex USB A-B.

The registration of sensors indicators takes place in the program "Multimedia laboratory MIG-1.3" (Kyiv, Ukraine) that is a frame of program "Teaching laboratory ITM" (Kharkiv, Ukraine). The frame was created to facilitate the study of physical properties of the raw material and food products.

The photo of elaborated setting is given on the **Fig. 1.**

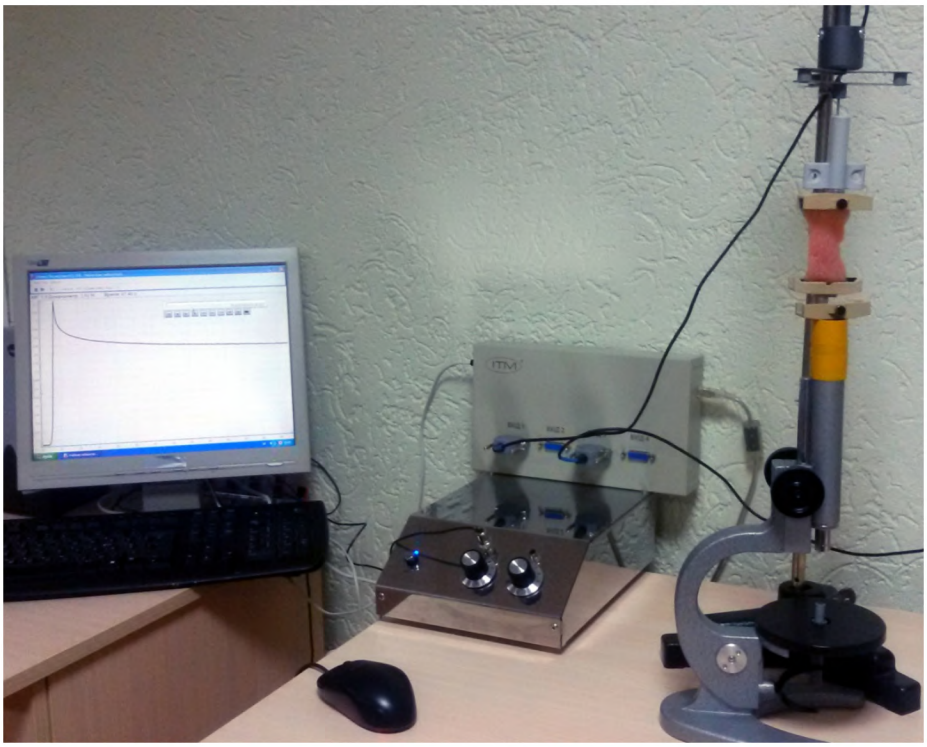

Fig. 1. The setting for study of dynamics of changes of relaxation effort of turkey fillet at extension deformation

The software was used for dependency modeling: office package ApacheOpenOffice 4.1.0 (USA), Delaware, especially the table processor Calc, made by ApacheSoftwareFoundation (USA), the program for formation and analysis of graphs AdvancedGrapher 2.2 by AlentumSoftware (Israel).

The studies were carried out with threefold (penetration with twelvefold) repeat, the processing of results was realized with reliability 0,95. The measurement was carried out with exactness, optimal for each problem.

At the study of relaxation the data volume was up to three thousand points that is why the influence of casual errors is minimal. Because of irreversible changes each sample underwent axial deformation only once. Under such conditions, the large number of experiments needs big unreasonable expenditures. That is why it was decided to limit the study to three experiments for each fillet of certain storage term.

At penetration the essential casual error appears because of unevenness of product structure, that is why it was decided to increase the multiplicity of measurements to the maximal number

on one sample under condition of non-hitting of the same place by indenter twice. Depending on sample, there were received 16–12 reliable results. At the next stage the most and least values of indenter resistance force were rejected to receive 12 results for each sample.

For testing the methods there were used the samples of cooled turkey fillet, produced by "Turkey" LTD (Sumy, Ukraine) in vacuum packing with storage term 7 days at temperature 0…+6 °C.

The possibility to realize the first experiment appeared in 6 hours of storage after the packing time, indicated by producer. The second and third experiments were carried out in 22 and 46 hours after production. The unevenness of interval is explained by the laboratory working hours.

The results of the study of the structure of samples, stored during 70 and 94 hours essentially differed from the data, given in article that is explained by the processes of partial autolysis of tissues.

Because of partial deformation of structure, the relaxation effort at axial extension deformation was carried out only once for one sample, that is why the study for each storage time was carried out on three samples. The samples were extended along the fibers by 15–20 % of initial length.

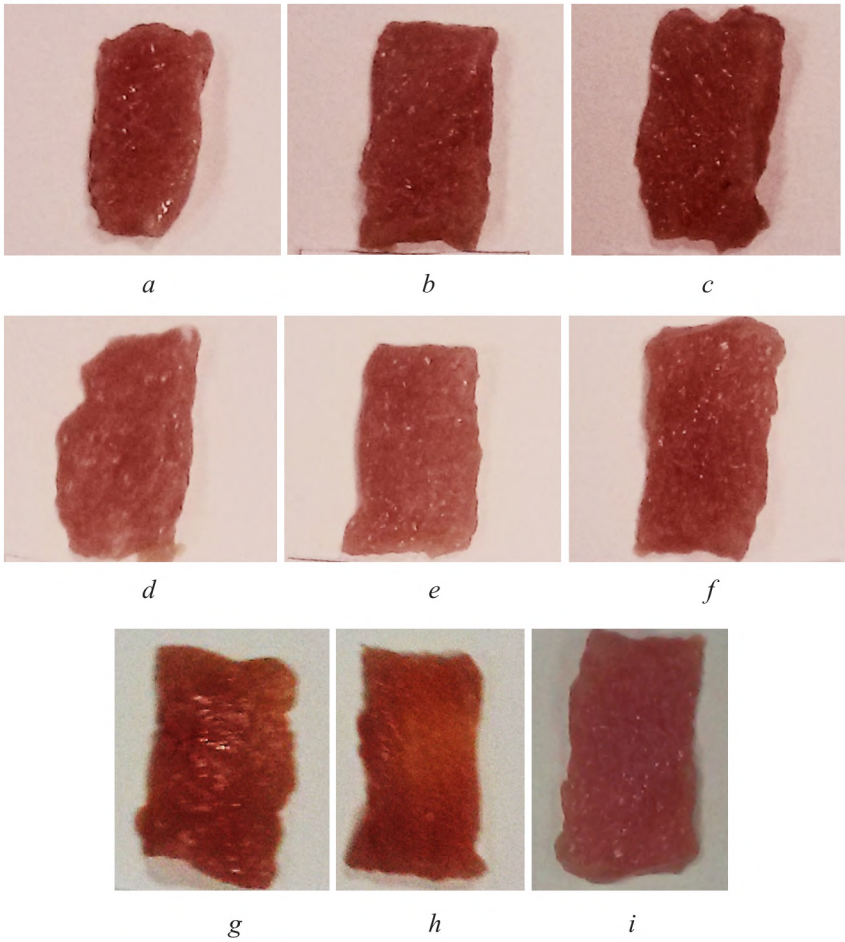

Fig. 2. The turkey samples before axial extension deformation: *a*–c – samples 1 (6 hours of storage); *d*–f – зsamples 2 (22 hours of storage); *g*–*i* – samples 3 (46 hours of storage)

The mean arithmetical value of relaxation speed was taken for further processing of the results of relaxation effort.

The penetration was carried out 6…14 time on one sample, each time in new place at angle 85..95° to muscular fibers with permanent speed of indenter immersion in product. The indenter of cylindrical form with diameter 1,5 mm was used.

All experiments were carried out at maximal temperature of storage +7±0,5 °C.

As far as at formation of natural culinary products the crucial role is played by deformation of extension but not compression, there was elaborated the equipment, which allows detect the relaxation parameters at axial extension deformation.

For studying the relaxation of turkey fillet the setting of original construction MIG 1.3, elaborated at laboratory of KNUTE together with "ITM" LTD (city Kharkiv, Ukraine), was used. The scheme of setting is presented on the **Fig. 3.**

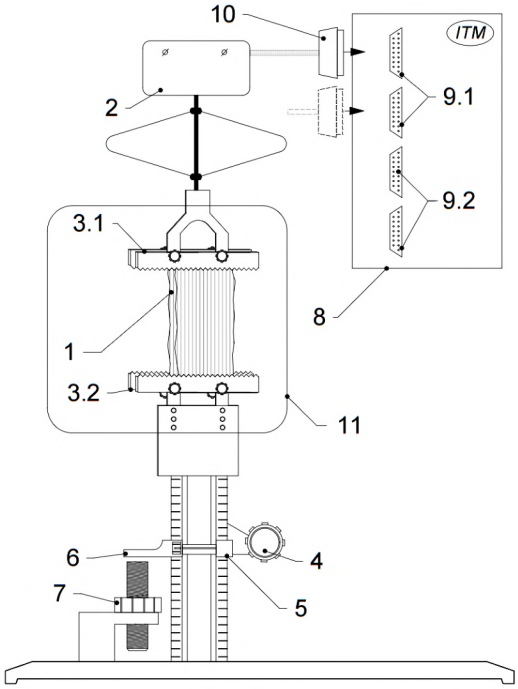

Fig. 3. The scheme of "Rheology" module of setting MIG 1.3:
1 – studied sample, 2 – digital dynamometer, 3.1 – upper stationary fixer,
3.2 – low movable fixer, 4 – handle for lifting and lowering of low fixer, 5 – stand with rule,
6 – height limiter, 7 – screw of height limiter, 8 – measuring cell, 9.1 – digital inputs,
9.2 – analogous inputs, 10 – socket of measuring module, 11 – measuring cell

The studied sample (1) was clamped by the upper (3.1) fixer and hung down freely during 5–10 s for smoothing the form. After that it was connected to the low (3.2) fixer and the length of the part of sample between fixers was detected.

The 1/5 size of sample length was inset by the screw of height limiter (7). The platform together with low fixer was evenly lowered by turning the handle (4) along the stand (5), till the height limiter (6) is stopped by screw (7).

The experiment was carried out during 60–120 s. The fixing of indications was stopped, if during 10 s the sample resistance effort did not change by 1 % from the current value. For visible comparison of the results the data volumes were limited to 60 seconds.

The sample was in extended state during aforesaid time (during experiment). During the whole time of relaxation the digital dynamometer fixed the values of sample resistance force with measuring period 0,005 s. The received data were kept in memory of MIG 1.3 and exported to the table processor for further processing.

The scheme of measuring cell of sample with marking the vector of forces, acting in studied sample, is presented on the **Fig. 4.**

The experiment is carried out under compulsory condition of absence of vibration of working surfaces and floor of laboratory.

The lowering of the low part of construction, as opposite to the upper one, prevents the transmission of vibration from handle turning on dynamometer and also diminishes the vibration of movable part of dynamometer itself.

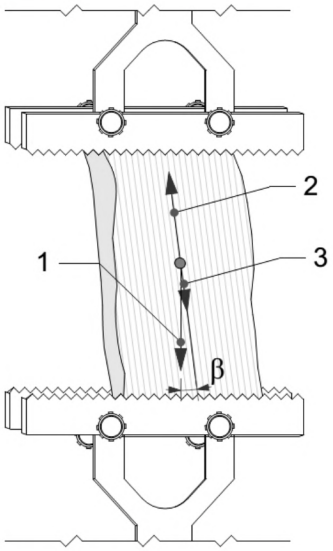

Fig. 4. The measuring cell of "Rheology module" of setting MIG 1.3.
The scheme of interaction of forces at extension of meat samples along fibers: 1 – load ($\vec{F}_{sp}$); 2 – relaxation effort ($\vec{F}_{rel}$); 3 – weight of sample ($\vec{P}_{smpl}$)

Mechanical ways of extinguishing vibrations and digital damping were not used because of inevitable disfiguration of data.

Before the beginning of experiment the sample is clamped in measuring cell, in given direction of fibers, but as far as muscular tissue has uneven structure, it is not possible to inset sample at precisely determined angle. The three-point system of dynamometer stabilization allows compensate deviation (β) between load vector ($\vec{F}_{sp}$) and resistance force vector ($\vec{F}_{rel}$) up to 5° at load up to 6 N.

After extension of sample by certain value (15–20 % of initial length), it is immovable. The processes of changes and deformation of structure that take place in it lead to the decrease of resistance force ($\vec{F}_{rel}$). The sample is also influenced by its weight ($\vec{P}_{smpl}$) – on the **Fig. 4**, for facilitation of perception, the point of sample weight application is presented in the center of body masses, although the point of sample weight application must be in the center of upper fixer (**Fig. 1**, (3.1)). But as far as the sample weight ($\vec{P}_{smpl}$) is a stable value and is essentially less that resistance and load force, the sample weight can be neglected.

The resultant of forces after extension of sample is equal to null. At diminishing of resistance force (relaxation effort, $\vec{F}_{rel}$), the load is diminished too ($\vec{F}_{sp}$), the load is fixed by digital dynamometer in real time.

The scheme of setting for the study of limit of meat surface firmness by penetration method is given on the **Fig. 5.**

As far as meat structure is uneven and at hitting the connective tissue by indenter, the value of resistance force can differ in times from the value of resistance force of muscular tissue, the penetration of samples was carried out 8–10 times by both indenters. That is 16–20 data points were received.

The inset of thermometers within indenters is conditioned by temperature influence on surface phenomena, especially adhesion between material of indenter and meat. If in the process of measuring the temperature changes more than by 2 °C, such experiment is considered as unreliable one.

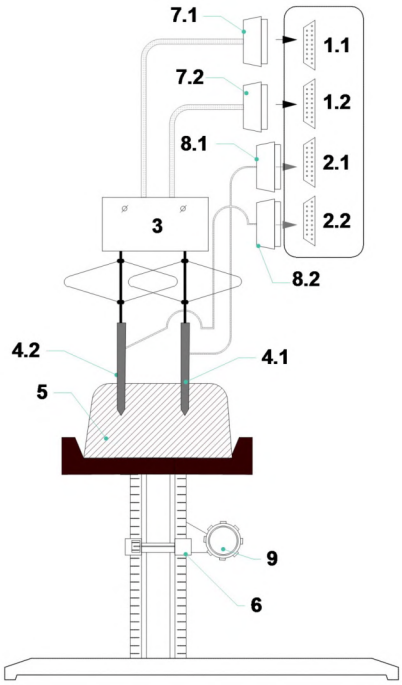

Fig. 5. The scheme of module "Penetration" of setting "MIG C 1.3": 1.1, 1.2 – analogous inputs, 2.1, 2.2 – digital inputs, 3 – dynamometer, 4.1, 4.2 – indenters with thermometers, 5 – studied sample, 6 – stand with rule, 7.1, 7.2, 8.1, 8.2 sockets DB-15, 8 – measuring cell, 9 – handle for lifting and lowering of low fixer, 10 – socket of measuring module, 11 – measuring cell

2. 1. Experimenta lprocedures

After deformation of sample, the software of measuring bloc formed the diagram of muscular tissue relaxation. The example of data of one sensor of elaborated setting at deformation of turkey fillet sample № 1 is presented on the **Fig. 6.**

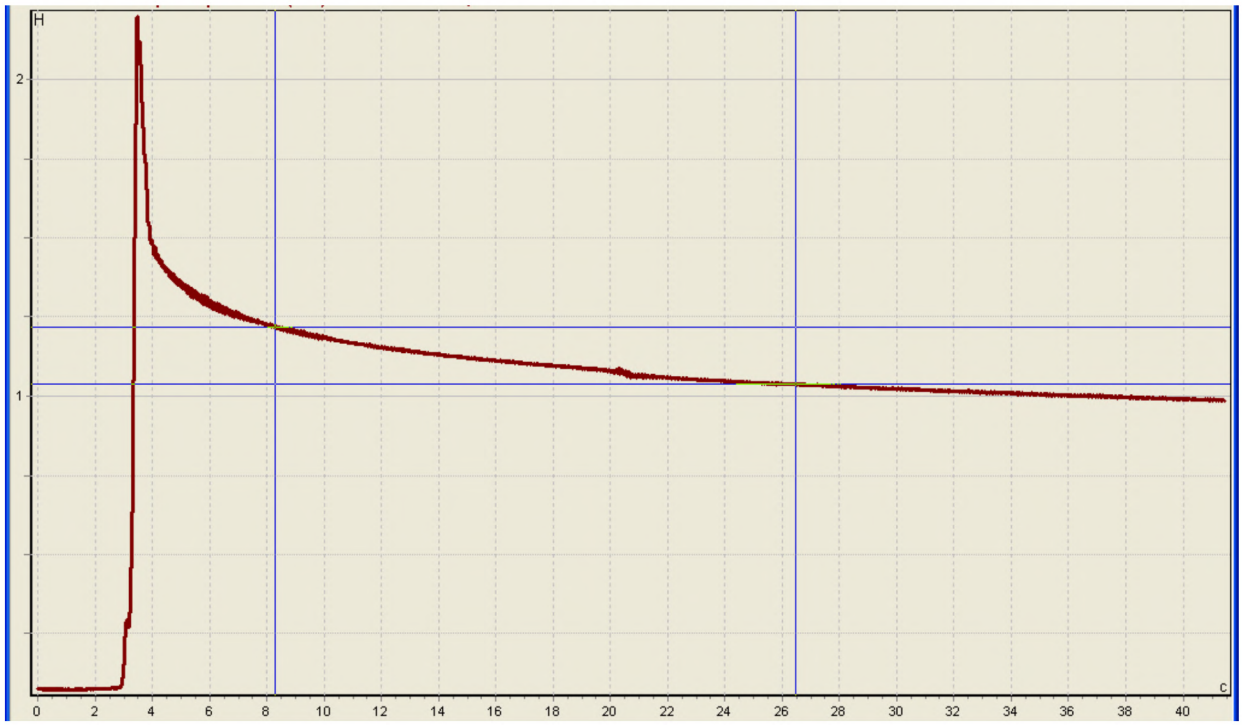

Fig. 6. The window of program "Multimedia laboratory MIG 1.3". The graph of deformation of turkey fillet sample № 1 at extension along the fibers

In further the data were exported to the table processor for processing. The initial graph of deformation is hold for journal or protocol of research.

The example of computation of firmness limit of muscular fibers by penetration method is given on the **Fig. 7**

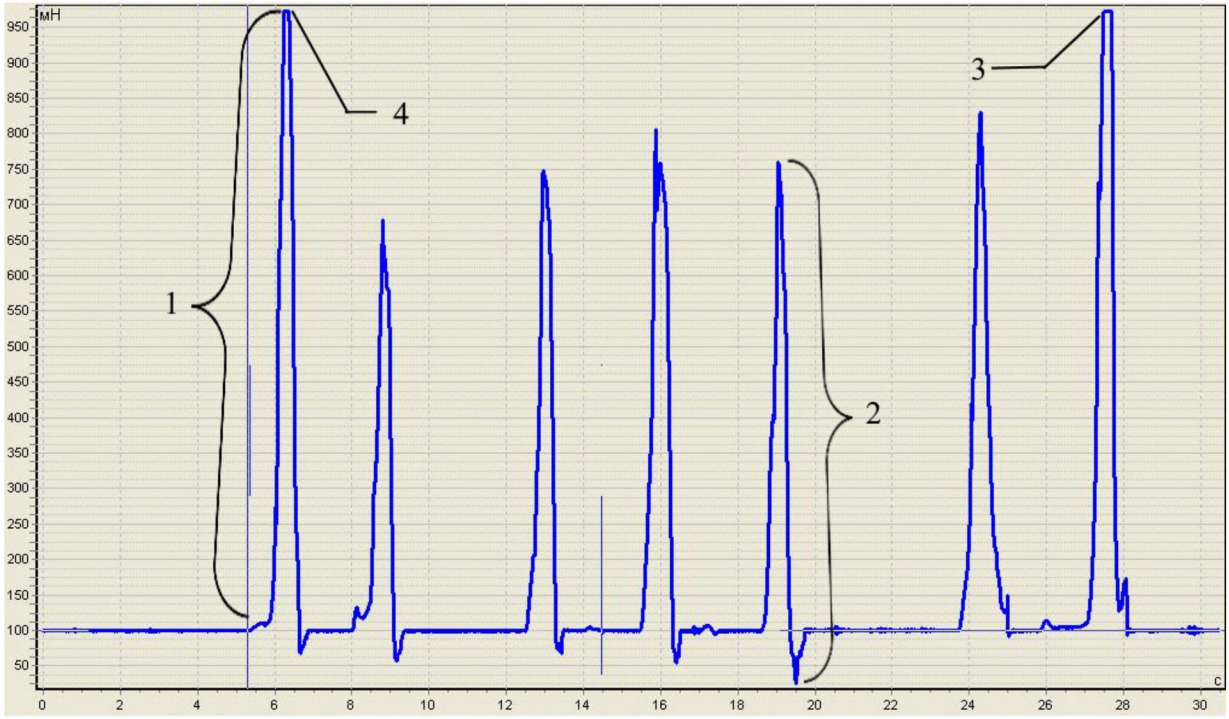

Fig. 7. The window of program "Multimedia laboratory MIG 1.3". The computation of firmness limit of turkey fillet sample 1 by penetration method at angle 85–95° to the fibers: 1 – sector of resistance force growing; 2 – sector of resistance force diminishing, intensified by adhesion of product, 3 – penetrating effort at unsuccessful experiment (indications of dynamometer are beyond the measuring scale of device), 4 – penetrating effort

The structure firmness is usually assessed by the special parameter – value that characterizes the degree of indenter penetration within the studied sample of material under the effect of permanent load. This parameter characterizes the structure firmness of material at little deformation speeds. For assessing the structure of such complicated systems as meat it is not expedient to use it [8] that is why it was offered to use the cylindrical indenter and fix the resistance force of product, dipping indenter at the permanent speed up to the permanent depth [9]. At dipping the resistance force increases fast **(Fig. 7,** sector 1), but after rupture of the sample surface (in our case, the muscular fibers in surface layer), the muscular tissues resistance abruptly decreases (**Fig. 7,** sector 2). The graph of change of resistance force in time creates peak (**Fig. 7**, mark 4), which measuring helps to calculate the firmness limit of product surface.

3. Results

After analysis of relaxation diagrams we can get equations and angles of inclination of tangents (**Table 1**) to the graph of change of resistance effort in time. The most difference of inclination angles of tangents was observed at 4 s of relaxation that is why these very data are given for analysis of curves.

The difference between inclination angles of tangents of the samples 1 and 2 are within statistical error. The difference between the angle of tangent of the sample 3 and inclination angles of tangents of other samples is reliable. It indicates the essentially slower restoration of turkey fillet structure after application of deforming force during 4 s. This difference is imperceptible organo-

leptically, but after 46 hours of storage, autolytic processes essentially influence the firm properties of turkey fillet [1].

In technological aspect, fillet, stored more than 46 hours, is expedient to be used for product of special form, rolls or twisted cakes.

The relative penetrating strain ($\Theta_п$) and firmness limit of muscular tissue surface (Θ_{og}) were calculated by the method [10]. The results of penetration of turkey fillet are given in the **Table 2.**

Table 1

The analysis of tangents to the graph of relaxation of turkey fillet samples at 4 s of relaxation

Sample № (storage term)	Derivative of relaxation equation	Equation of tangent	Inclination angle of tangent, ^{0}
1 (6 hours)	$y'(x)=-0.63x^{-1}$	–0.158x+7.205	–8.970
2 (22 hours)	$y'(x)=-0.67x^{-1}$	–0.147x+6.996	–8.388
3 (46 hours)	$y'(x)=8.28x^{-0.0921}-0.0921x^{-1}$	–0.167x+7.308	–9.477

Table 2

The results of penetration of turkey fillet samples of different storage term

Sample №	Penetrating effort, mN			Relative penetrating load ($\Theta_п$), Pa	Limit of surface firmness (Θ_{og}), Pa
	P_{max}	P_{min}	$\bar{P}$		
1	955	534	734	3262.22	3849.42± 137.12
2	948	432	692	3146.64	3713.07± 87.18
3	946	352	627	2786.67	3288.27± 116.05

Note: P_{max} – maximal resistance effort, P_{min} – minimal resistance effort, $\bar{P}$ – mean arithmetical resistance effort

Having analyzed the **Table 2**, it can be noted, that any essential changes of fibers firmness were not observed during 22 hours of storage. But after 46 of storage of turkey fillet, the firmness limit of surface changed by 15 % at pricking by cylindrical intender across fibers.

4. Conclusions

The setting MIG 1.3 of original construction was used at the study of structural-mechanical changes of turkey fillet properties. It was established, that during 22 of storage the significant changes of structural-mechanical properties took place, thus, for example, the relaxation time increased by 18,4 % and the firmness limit of surface decreased by 0,9 %. During the following 24 hours of turkey fillet storage, the relaxation energy decreased by 11 % (by 29 % after the beginning of research) and the firmness limit also decreased that together with less firmness limit of fibers (by 15 %), allowed recommend it for formation of natural culinary product of given form, for example, rolls or twisted cakes.

The elaborated method was tested at warehouse of learning-producing institution KNUTE. It also can be used at mini-productions, culinary departments or laboratories of wholesale institutions for evaluation of raw material quality.

In further it is planned to improve the setting by introduction of managed movable platform for samples to automate experiments and eliminate the human factor influence on the results of experiment.

The authors form the data base of rheological properties of turkey meat that allows approximately establish the age of turkey, fat, storage term by determination of rheological properties of sample, and recommend the optimal type of culinary processing of studied set of poultry.

References

[1] Pearson, A. M., Dutson, T. R. (2013). Production and processing of healthy meat, poultry and fish products. Springer Science & Business Media, 367.

[2] Pearson, A. M. (2013). Quality attributes and their measurement in meat, poultry and fish products. Springer, 505.

[3] Zochowska, J., Lachowicz, K., Sobczak, M., Gajowiecki, L. (2007). Effects of Carcass Weight and Muscle on Texture, Structure, Rheological Properties and Myofibre Characteristics of Roe Deer. American Journal of Animal and Veterinary Sciences, 2 (4), 114–120. doi: 10.3844/ajavsp.2007.114.120

[4] García-Segovia, P., Andrés-Bello, A., Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle. Journal of Food Engineering, 80 (3), 813–821. doi: 10.1016/j.jfoodeng.2006.07.010

[5] Damez, J. L., Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. Meat Science, 80 (1), 132–149. doi: 10.1016/j.meatsci.2008.05.039

[6] Shapoval, S., Forostyana, N., Litvinov, U., Romanenko, R. (2013). Rekomendatsiyi do vikonannya naukovo-doslidnih robit na UVKP. Kyiv: KNTEY, 92.

[7] Datchiki. TOV "Firma ITM". Avaible at: http://itm.com.ua/datchiki/

[8] Panea, B., Ripoll, G., Gonzalez, J., Fernandez-Cuello, A., Albertí, P. (2014). Effect of nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat quality. Journal of Food Engineering, 123, 104–112. doi: 10.1016/j.jfoodeng.2013.09.029

[9] Romanenko, O. (2013). Metod viznachennya strukturno-mehanichnih vlastivostey ribnih preserviv. Tovari i rinki, 2, 58–65.

[10] Sems, A. (2007). Pererabotka myasa ptitsyi. Saint Petersburg: Professiya, 432.

[11] Savinok, O., Kosoy, V., Garbuz, V. (2015). Vyibor ratsionalnyih parametrov izmereniya reologicheskih harakteristik tselnomyishechnoy tkani govyadinyi metodom penetratsii. Naukovi pratsi ONAHT, 2 (46), 160–163.

THE METHOD OF DETERMINATION OF THE SORPTION CAPACITY OF ACTIVATED CARBON BY GAS CHROMATOGRAPHY

Ksenia Naumenko
Department of Food Expertise
National University of Food Technologies
68 Volodymyrska str., Kyiv, Ukraine, 01601
ksenianaumenko@ukr.net

Natalia Frolova
Department of Technology Health Products
National University of Food Technologies
68 Volodymyrska str., Kyiv, Ukraine, 01601
nef1956@mail.ru

Oksana Petrusha
Department of Foodstuff Expertise
National University of Food Technologies
68 Volodymyrska str., Kyiv, Ukraine, 01601
petrushaoo@ukr.net

Natalia Chepel
Department of Technology of Milk and Dairy Products
National University of Food Technologies
68 Volodymyrska str., Kyiv, Ukraine, 01601
natachepel@yandex.ua

Abstract

The article analyzes the possibility of gas chromatography use for determination of sorption capacity of adsorbents on the example of activated carbon BAC-A. The offered method provides the use of gas chromatograph with flame-ionizing detector and with nozzle geyser that is filled with studied adsorbent. At that the isotherms of absorption of substance are constructed by manifestation curve – desorption branch of substance peak on chromatogram.

As a result the isotherms of absorption of isoamylol and camphor on activated carbon were constructed and the values of specific sorption capacity for these substances were calculated.

This method allows receive fast and precisely the data about absorption characteristics of adsorbent and also adapt the conditions of the study using gas chromatography to the real conditions of adsorption of substances by studied adsorbent (temperature, adsorbent concentration in vapor phase and so on).

Keywords: gas chromatography, isotherm of adsorption, adsorption capacity, curve of adsorbate manifestation, isoamylol.

DOI: 10.21303/2504-5695.2017.00290

1. Introduction

The important parameter of exploitation properties of adsorbents, used in the catchers of aromatic substances of secondary vapor of juices and their extracts, is their sorption capacity. The absorption capacity is established for the concrete substances and expressed in units of mass or volume that can be maximally kept by adsorbent under certain conditions [1].

The static methods of constructing isotherms of adsorption by the weight (by increment of adsorbent mass after adsorption) or volumetric method (by the decrease of adsorbate amount in vapor phase after adsorption) are mainly used to determine the capacity of adsorbents [2]. Besides the static methods, the dynamic ones are widely used, and they allow assess the sorption capacity of adsorbent in time at permanent passing of absorbate flow through it [3, 4]. The dynamic ones include the method of determination of sorption capacity using gas chromatography. This method

comparing with aforementioned ones allows establish the necessary conditions of the system "substance-adsorbent" – adsorption temperature, adsorbate concentration, speed of vapors movement through adsorbent and so on. That is why the method of gas chromatography was described and used for constructing isotherms of adsorption for the components that are in certain concentration in vapor phase [5, 6].

2. Materials and Methods

The method of determination of sorption capacity of activated carbon was based on the modeling of adsorption process by placing of studied adsorbent in nozzle geyser of gas chromatograph [6]. The metal nozzle geyser-concentrator with diameter 5 mm, length 100 mm was used to construct isotherms of adsorption of gas chromatographic data and to optimize the process.

As it is known, the isotherm of adsorption a=f(C) is a dependence of adsorption value a on balanced concentration of the substance C. That is why the isotherms of adsorption are constructed on the base of integration for all kept volume of substance that corresponds to desorption branch of manifestation curve – peak of substance on chromatogram, presented on [7] (**Fig. 1**).

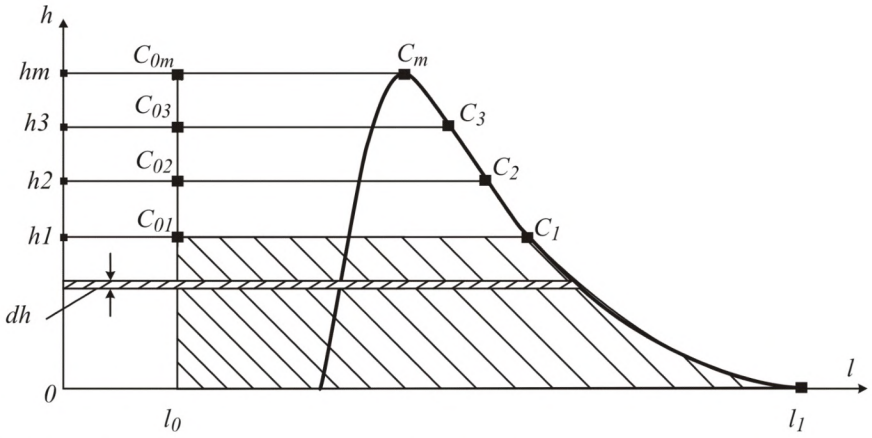

Fig. 1. The curve of manifestation of adsorbed substance, used for constructing isotherm of adsorption: C – concentration of substance in gas medium; h – response of detector signal, mV or cm; t – time of keeping substance, s or cm

The concentration of substance in gas medium corresponds to certain point on chromatographic peak, is proportional to the response of detector signal and is calculated by formula (1):

$$C=K\cdot h, \tag{1}$$

where K – constant of chromatograph, mmol/cm^3·cm, h – response of detector signal, cm.

Determination of constant of chromatograph K was carried out using calibration on the base of dependence of peak area S on chromatogram on concentration of the substance q (2) [7]:

$$q=\int_{V_1}^{V_2} c\cdot dw=\int_{t_1}^{t_2} K\cdot h\cdot\frac{a}{u}\cdot dt=\frac{K\cdot a}{u}\int_{t_1}^{t_2} h\cdot dt=\frac{K\cdot a}{u}\cdot S, \tag{2}$$

where S – peak area, cm^2, α – gas-carrier speed, cm/min, t – absolute time of keeping substance, cm, w – absolute volume of keeping substance, cm^3; u – constant that takes into account chromatogram scale, cm/min.

The constant of chromatograph at gas-carrier speed 27 cm^3/min and constant u 0,4 cm/min is determined by (2):

$$K=\frac{q\cdot u}{\alpha\cdot S}=\frac{13,2\cdot 10^{-6}\cdot 0,4}{27\cdot 43,4}=4,4\cdot 10^{-9}.$$

The mathematical description of equation of material balance of gas chromatographic process [7, 8] was used for determination of the amount of adsorbed substance

$$-\alpha\cdot\frac{\partial C}{\partial x}+D\cdot\chi\cdot\frac{\partial^2 C}{\partial x^2}=\frac{\partial a}{\partial t}+\chi\cdot\frac{\partial C}{\partial t}, \quad (3)$$

where D – coefficient of longitudinal diffusion; χ – share of free cut of adsorbent – ratio of volume of intervals between seeds and the general volume of adsorbent in geyser; C – concentration of substance in flow, mg/cm^3; a – amount of adsorbed substance, mg/cm^3 by adsorbent; t – absolute time of keeping, min; α – speed of gas-carrier flow, cm^3/min; x – distance from the beginning of adsorbed geyser-concentrator for the direction of gas-carrier flow, cm.

Neglecting the diffuse erosion, that is accepting of the instantaneity of establishing balance of adsorption and desorption of substance, and having dependencies a=f(C) and c=f(t), the main equation (4) of chromatography was used in calculations in general form:

$$\frac{\partial x}{\partial t}=\frac{\alpha}{\frac{\partial a}{\partial C}+\alpha}, \quad (4)$$

$$a=\frac{1}{L}\int_0^C\left(VdC-L\cdot\chi dC\right), \quad (5)$$

where

$$\frac{\partial x}{\partial t}\approx\frac{x}{t}\approx\frac{L}{t},$$

$$\chi=\frac{V_0}{L},$$

where V_0 – volume of intervals between the seeds of adsorbent in geyser, L – length of geyser-concentrator [7].

At internal diameter of geyser-concentrator 5 mm takes place the general case of determination of amount of adsorbed substance a for 1 g of adsorbent according to (6):

$$a=\frac{\Delta}{g}\int_0^C\left(V^s-V_0^s\right)dC, \quad (6)$$

where V^s – gas-carrier volume at cross-cut S, g – adsorbent batch, Δ – poured specific weight, g/cm^3.

Thus, the value of correspondent concentration of substance C is calculated by the indication of detector by formula (3). Taking into account the previous equations, the amount of adsorbed substance a was calculated by (7), (8):

$$a=f(C)=\frac{\alpha\cdot K\cdot\Delta}{u\cdot g}\int_0^h(1-l_0)dh=\frac{\alpha\cdot K\cdot\Delta}{u\cdot g}\cdot S, \quad (7)$$

$$a_m=\frac{\alpha\cdot K\cdot\Delta}{u\cdot g}\cdot S_m, \quad (8)$$

where the area of peak fragment was calculated by (9):

$$S_m=0h_mC_ml_l-0h_mC_{0m}l_0. \quad (9)$$

The reliable determination of adsorbents capacity was realized at temperature regime, correspondent to adsorption of aromatic substances at residual pressure 0,03…0,07 mPa. The special

nomogram UOP [9] was used for recalculation of temperature regime of absorption from the residual pressure 0,037 mPa on atmospheric one. Since the stability of ratio between temperatures of boiling in vacuum and at atmospheric pressure is realized under condition of equal amount of vapors. According to the equation (10) the temperature of adsorption is calculated from the lowered pressure on atmospheric one:

$$\frac{T_i}{T_{boil}} = 1 + 0,21 \cdot \left(\lg \frac{P_i}{P} \right) + 0,41 \left(\lg \frac{P_i}{P} \right)^2 + 0,004 \left(\lg \frac{P_i}{P} \right)^3, \quad (10)$$

where T_i, T_{boil} – the temperatures of boiling of equal amount of AS according to TTB curves at the low P_i atmospheric P pressure, K.

Thus, the parameters of experiment on determination of activated carbon capacity for aromatic substances are presented in the **Table 1.**

Table 1

The parameters of experiment on determination of BAC-A activated carbon capacity

Parameter	Value
Temperature of evaporator of chromatograph	200 ºC
Temperature of adsorption (thermostat of geyser)	100 ºC
Consumption of gas-carrier	27 cm^3/min
Sensitivity of detector	$10 \cdot 10^{-10}$
Amount of AS input	0,1 mg

2. 1. Experimental procedures

The object of research is the activated carbon BAC-A, that has an ability to adsorb the aromatic substances from water vapors, doesn't cause the chemical transformations of aromatic substances at adsorption and desorption, has developed active surface of pores [2, 10].

The physical-technical characteristics of activated carbon BAC-A are presented in the **Table 2.**

Table 2

The physical-technical parameters of activated carbon BAC-A

Parameter	Characteristic
Specific surface, m^2/g	700…800
Mean diameter of pores, nm	≤200
Poured density, g/cm^3	0,161
Size of seeds, mm	0,5…0,9
Temperature limit, °C	300

The isotherms of adsorption on activated carbon were determined for such adsorbates as camphor with molecular mass 152,2 g/mol and isoamylol with molecular mass 88 g/mol.

The experiments were carried out on gas chromatograph Selmichrom-2003 (Ukraine) with flame-ionizing detector. The gas carrier was nitrogen.

The measuring was carried out in following way: the nozzle geyser-concentrator was filled with activated carbon BAC-A, and placed in thermostat of chromatograph, one end was connected with injector and other one – with detector, using connecting elements. The aromatic substance was inserted in injector of chromatograph by micro-syringe. The speed of nitrogen flow was established at the level 27 cm^3/min. As a result the partial pressure of substance in mixture "nitrogen-substance" decreases, its quantity is registered by detector and is recorded as the peak – adsorption. After balanced satiation at fixed concentration of adsorbate, the transmission of nitrogen through the geyser-concentrator continued. At that the emission of adsobate from the surface of adsorbent took place and its amount was recorded as the desorption peak. (**Fig. 1**).

The established parameters of experiment (**Table 1**) allowed receive the curves of manifestation of camphor and isoamylol at passing through the nozzle geyser, filled with activated carbon BAC-A. The value of concentration of substance in gas medium C, μ/ml, and correspondent amount of adsorbed substance a on adsorbentμ/ml were calculated by the points on manifestation curves.

3. Results

On the **Fig. 2, 3** are presented the isotherms of monomolecular adsorption of isoamylol and camphor on activated carbon BAC-A.

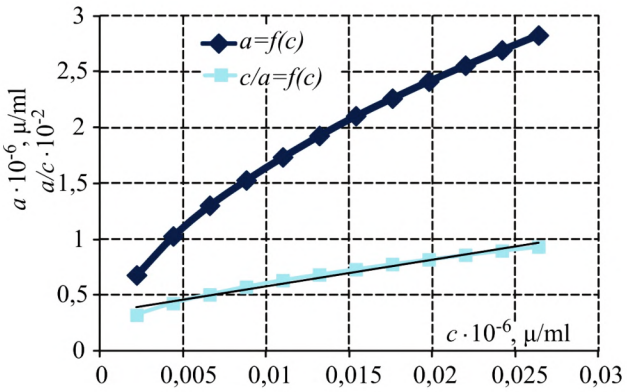

Fig. 2. Isotherm of adsorption of isoamylol on activated carbon BAC-A

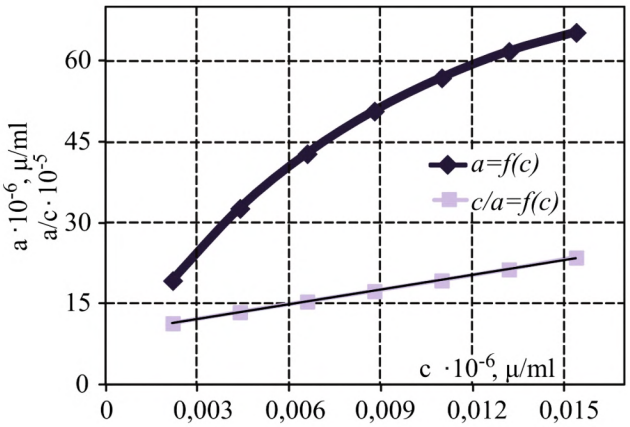

Fig. 3. Isotherm of adsorption of camphor on activated carbon BAC-A

The constructed isotherms allowed to determine their typicality for Lengmuir's monomolecular adsorption at isoamylol and camphor quantity in gas phase up to 0,1 mg.

The isotherm of adsorption is described by Lengmuir's equation according to (11):

$$a = a_0 \cdot \frac{c}{K_1 + c} = a_0 \cdot \frac{p}{K_1 + p}, \quad (11)$$

where a_0 – capacity of mono-layer; p – partial pressure of adsorbate; c- concentration of adsorbate in vapor phase; K_1 – constant that depends on temperature and type of adsorbent and is a constant value for couple adsorbent-adsorbate, numerically equal to adsorbate concentration, at which the half of active centers of adsorbent is occupied [11].

The graphic solution of Lengmuir's equation (11) by transformation of isotherm of adsorption of the type a=f(c) into the direct one of the type c/a=f(c) determine cotangent of inclination angle that is equal to the capacity of mono-layer of activated carbon for isoamylol and camphor.

The results of calculation of specific weight of adsorption capacity of activated carbon are presented in the **Table 3.**

Table 3

The values of specific adsorption capacity of activated carbon BAC-A

Adsorbate	Specific weight of mono-layer	
	$\times 10^{-6}$ mmol/cm^3	Mg/g
Isoamylol	3,96	1,97
Camphor	109,45	103,47

The relative error at measurement and calculation of the value of specific adsorption capacity of adsorbent was no more than 2,5…2,8 %.

The received data of calculations indicate the difference of specific sorption capacity of activated carbon for camphor and isoamylol that is explained by the differences at "adsorbent-absorbate", interaction seizes and polarity of molecules of camphor and isoamylol.

4. Conclusions

The results of research allowed to receive the value of specific sorption capacity of activated carbon BAC-A for isoamylol and camphor that has practical importance in calculation of the optimal quantity of adsorbent for adsorption of aromatic substances of extracts, juices. At the same time depending on the fractional composition of aromatic substances and taking into account the selectivity of adsorbents their quantity can be optimized. Thus, the activated carbon is more selective to camphor (103,47 mg/g) than to isoamylol (1,97 mg/g).

Analyzing the realized studies, the following advantages of using gas chromatography for determination of sorption capacity of adsorbents must be noted:

– exactness of method, proved by the high sensitivity of detector of chromatograph;

– flexibility of conditions of researches: possibility to change the temperature of adsorption, adsorbent, adsorbate and its concentration in vapor phase.

The disadvantages include:

– complexity of calculations for construction of isotherms of adsorption:

– complication of the study of adsorption of AS with high boiling temperature by the "erosion" of desorption curve.

Taking into account the aforesaid, the further studies can be directed on widening of spectrum of studied adsorbents and their aromatic substances.

References

[1] Ekymova, Y. A., Mynakova, T. S. (2013). Adsorbtsyonnye yssledovanyia parov vody na oksydakh y ftorydakh shchelochnozemelnykh metallov y mahnyia. Natsyonalnyi yssledovatelskyi Vestnyk THASU, 2, 263–275.

[2] Andryiantseva, S. A., Bondarenko, A. V., Petukhova, H. A. (2012). Ekspress-metod yssledovanyia yzotermy adsorbtsyy benzola uhlerodnymy hydrofobnymy materyalamy. Sorbtsyonnye y khromatohrafycheskye protsessy, 12 (1), 114–118.

[3] Vagner, C., Finqueneisel, G., Zimny, T., Weber, J. (2002). Water vapour adsorption on activated carbons: comparison and modelling of the isotherms in static and dynamic flow conditions. Fuel Processing Technology, 77-78, 409–414. doi: 10.1016/s0378-3820(02)00090-5

[4] Ivanova, E., Karsheva, M. (2007). Ethanol vapours adsorption by natural clynoptilolitee. Journal of the University of Chemical Technology and Metallurgy, 42, 391–398.

[5] Kondor, A., Dallos, A. (2014). Adsorption isotherms of some alkyl aromatic hydrocarbons and surface energies on partially dealuminated Y faujasite zeolite by inverse gas chromatography. Journal of Chromatography A, 1362, 250–261. doi: 10.1016/j.chroma.2014.08.047

[6] Viacheslavov, A. S., Efremova, M. (2011). Opredelenye ploshchady poverkhnosty y porystosty materyalov metodom sorbtsyy hazov. Moscow: MHU ym. M. V. Lomonosova, 65.

[7] Viakhyrev, D. A., Shushunova, A. F. (1987). Rukovodstvo po hazovoi khromatohrafyy. Moscow: Vysshaia shkola, 335.

[8] Scott, R. (2003). Principles and practice of chromatography. Library for science, 106.

[9] Khafyzov, F. Sh., Krasnov, A. V. (2012). Davlenye nasyshchennykh parov dlia nefteproduktov. Elektronnyi nauchnyi zhurnal «Neftehazovoe delo», 3, 406–412.

[10] Naumenko, K. A., Frolova, N. E., Ukrainets, A. I., Usenko, V. O. (2010). Selection and estimation of adsorbents for catching of aromatic substances during the concentration of juices and extracts. Naukovi pratsi NUKhT, 33, 8–16.

THE ELABORATION OF CHEESE MASSES OF THERAPEUTIC AND PROPHYLACTIC DIRECTION WITH CRYOADDITIVE "PUMPKIN"

Bogdan Gutyj
Department of Pharmacology and Toxicology
Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyi
50 Pekarska str., Lviv, Ukraine, 79010
bvh@ukr.net

Yuriy Hachak
Department of Technology of milk and milk products
Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyi
50 Pekarska str., Lviv, Ukraine, 79010
gachak61@mail.ru

Jaroslava Vavrysevych
Department of Biological and General Chemistry
Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyi
50 Pekarska str., Lviv, Ukraine, 79010
GutaZor@gmail.com

Volodimyra Nagovska
Department of Technology of milk and milk products
Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyi
50 Pekarska str., Lviv, Ukraine, 79010
gachak61@mail.ru

Abstract

There was grounded the expedience of using cryopowder "Pumpkin" in the technology of sweet and salt cheese masses. The possibility of using cryopowder "Pumpkin" as a component of therapeutic and prophylactic cheeses masses was studied. The main factors of introduction of cryoadditive "Pumpkin" are: normative organoleptic properties of product and its daily norm. The production of cheese masses with cryoadditive "Pumpkin" provides their combination with sugar or salt.

The use of cryoadditive "Pumpkin" needs preliminary comminution and mixing them with sugar-sand or salt. The receipts of 4 types of cheese masses with cryoadditive "Pumpkin" (two fatless and two semi-fat ones) were elaborated. At introduction of cryoadditive "Pumpkin" in cheese masses their food value increased. The organoleptic, technological and commodity characteristics of these cheese masses were studied. It was established, that the color of sweet cheese masses was cream with separate yellow dots of comminuted powder-like cryoadditive and the color of salt cheese masses was, correspondingly, yellow. In sweet cheese masses the distinct smell of cryoadditive was perceptible, whereas in salt cheese masses it was fresh, sour-milk. The flavor of studied samples was more expressed in sweet cheese masses. The offered cheese masses had pleasant, original commodity look, normative physical-chemical characteristics.

The titrated acidity of studied samples of salt cheese masses was 124–130 °T, moisture ms – 62–60 % and dry substances – 40–38 %, and titrated acidity of studied samples of sweet cheese masses was 126–134 °T, moisture ms 63–66 % and dry substances – 34–37 %.

The offered production widens the assortment of milk products of therapeutic and prophylactic direction.

Keywords: cheese masses, bioadditives, phytoadditives, pumpkin, therapeutic and prophylactic products, food technologies.

DOI: 10.21303/2504-5695.2017.00306

1. Introduction

Taking into account the modern ecological condition, there is an acute need in improvement of the nutrition structure of population at the expanse of raising quality, biological value and gustatory characteristics of products [1–3]. The important direction at that is their enrichment with

vitamins, mineral and immune substances, especially with ones on natural base [4, 5]. The skillful combination of cryoadditives (as bioadditives) with "milk" base has great prospects in both bio-technological and social aspects. The cryopowders are wholesome for both children and adults [1]. It seems more attractive to use the natural vegetable bioadditives that include significant number of deficit microelements and other biologically active substances in cheese masses technology [1, 6].

It remains extremely important to use the component of vegetable origin in milk production [7, 8]. The combination of vegetable and animal raw material allows maximally correct the content and properties of products [9]. The use of such additives allows refill the deficit of essential food substances and increase the non-specific resistance of organism to the effect of unfavorable environmental factors [10].

The elaboration and production of food goods of therapeutic and prophylactic direction including milk ones is extremely urgent. In such connection there was offered the research as to the study of the possibility of using cryopowder "Pumpkin" as phytoadditive in technology of salt and sweet cheese masses of therapeutic and prophylactic direction.

2. Materials and Methods

The experimental part was carried out under conditions of the laboratory of production workshops of "Prometey" LTD ("Lviv milk factory", Ukraine) and the laboratory of the department of milk and milk products technology of Lviv national university of veterinary medicine and biotechnologies, named after C. Z. Gzhytskyi (**Fig. 1**).

Fig. 1. Laboratory of the department of milk and milk products technology of Lviv national university of veterinary medicine and biotechnologies, named after C. Z. Gzhytskyi

The unified bioadditive "Pumpkin" (CSC PA "Gummy") (**Fig. 2**), taken and calculated, based on its prophylactic and therapeutic doses for 100–150 g of cheese mass was used for the study. The way of cheese masses production includes the receiving of cheese base – normative sour-milk fatless cheese or with fms 5 %, its cooling, placing in mixing machine and adding of biologically active pumpkin additive, preliminary prepared, according to the receipt with sugar or salt at continuous mixing, their packing and storage.

The receipt of cheese masses was recalculated for industrial, namely: with calculation for 1000 kg of ready product.

Cryoadditive "Pumpkin" (TC 9184-017-51784815-09) is a unified bioadditive. It is recommended for people with liver, gallbladder, cardio-vascular diseases, at gastritis, colic pains, obesity, sleep disorders. This bioadditive is useful at anemia and nervous system disorder. It is recommended for pregnant women as a remedy for toxicosis. Pumpkin strengthens the immune system and activates the processes of stomach ulcers healing.

The experiments included the revelation of optimal ratio of cryopowder and components of "milk bases". At the same time the organoleptic, technological and laboratory parameters of milk bases were studied.

Fig. 2. Cryopowder "Pumpkin" and cheese mass

The determinative factor at using Cryoadditive "Pumpkin" was preservation of the normative characteristics of sweet and salt cheese additives (**Fig. 3**).

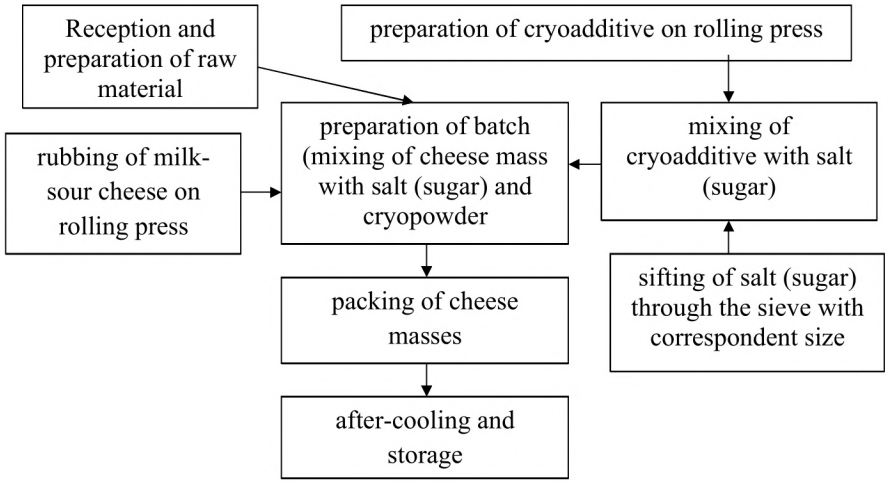

Fig. 3. Technological scheme of production of salt (sweet) cheese masses with microelements

The qualitative assessment of studied cheese products was carried out according to conventional methods, normative documents. The taking of samples of ready products was carried out according to SST 26809-86 "Milk and milk products. Rules of reception. Methods of taking and preparation of samples for analysis" and SSTC ISO 707-2002 "Milk and milk products". Regulations for taking of samples SSTC ISO 5538:2004 "Milk and milk products. Taking of samples. Control of quality parameters".

The selection of point samples of cheese masses is realized by lowering probe to the package bottom. Three point samples can be separated: one from the center, other ones at the distance

2–3 cm from the side wall of package. The mass of combined sample is 500 g, sample for research 100, and the one of products with stuffs is 150 g. The mass of combined sample in consumer package is 100 or 150 g.

The estimation of cheese masses acidity by titration method:

1. To rub the batch of sour-milk cheese (5 g) in porcelain pestle and grind it, to add 50 ml of distilled water with temperature 35–40 °C.

2. To add 3 drops of phenolphthalein and to titrate by 0,1 N solution of alkali to the faint rose coloration that doesn't disappear during 1 min.

3. To calculate the acidity of sour-milk cheese, to multiply the alkali quantity (ml), spent for titration, by 20. The difference between parallel calculations must not be more than 4 °T.

The study of moisture in sour-milk cheese by express-method:

1. To place the porcelain cup with glass stick and 20–25 g of sand in drying cupboard for 1 hour. At temperature 102–105 °C.

2. To weigh out in cup 5 g of sour-milk cheese, to mix it with sand and to place for 20 min. in cupboard at temperature 160–165 °C. To weigh it again.

3. The moisture quantity was calculated by the formula:

$$M = \frac{m - ml}{5} \cdot 100,$$

where M – moisture content in sour-milk cheese (%); m – mass of cup with sand and cheese before drying (g); m1 – mass of cup after drying (g).

The estimation of fat mass share in cheese products

The fat quantity in milk products is estimated using cream or milk fat butyrometers.

The technique of estimation of fat mass share (fms) in cream butyrometer:

1. To balance butyrometer on technochemical scales and to weigh 5 g of cheese mass in it.

2. To add 5 ml of water, 10 ml of sulfuric acid and 1 ml of isoamyl alcohol.

3. To cover butyrometer with rubber cork, to place in water bath at water temperature 65±2 °C, periodically shaking for solving protein.

To center it for 5 min. with speed no less than 1000 turn/min.

4. To place butyrometer in water bath for 5 min. at temperature 65±2 °C cork down.

5. To calculate the fat content by scale. The butyrometer indicates the fat content in sour-milk cheese in percents. The difference between parallel calculations of microbiological control at sour-milk cheese production must not be more than 0,5 %.

Microbiological studies of product are carried out no later than in 4 hours from the moment of taking samples. The preparation of samples and solutions of cheese masses for microbiological studies were carried out according to SSTC "IDF 122C:2003". The samples of cheese mass were taken according to SSTC 73-57: 2013 "Milk and milk products. The methods of microbiological studies".

Microbiological preparations were studied according to BS 49113-77. BCBG were estimated by inoculation of product solution on Kessler medium.

3. Results

The optimal receipt of salt and sweet cheese masses of different fattiness using cryopowder "Pumpkin" is presented in the **Table 1.** It was established, that with increase of fat mass share of milk raw material the quantity of cryoadditive "Pumpkin" in the receipt increases too. Thus, for 1000 kg of ready product for salt cheese masses the quantity of cryopowder increase from 9,75 to 13,59 kg, whereas for sweet cheese masses the quantity of cryopowder was a bit higher and varied correspondingly from 17,23 to 33,61 kg.

Such change of amount of cryopowder "Pumpkin" in cheese masses is connected with introduction of more sugar in fatter cheese mass and with fat of milk base itself that essentially intensifies the gustatory perception of the offered bioadditive.

The analysis of organoleptic characteristics of cheese masses with cryopowder "Pumpkin" shows that they don't essentially change and mainly completely correspond to the normative requirements. Thus, the color of sweet cheese masses was light-cream and cream, whereas the color of salt samples with cryoadditive was light-yellow and yellow.

Table 1

The recommended receipts of cheese masses with addition of cryoadditive "Pumpkin"

Cheese masses content	Cheese masses	
	Fatless with cryopowder	Semi-fat (4,8 %) with cryopowder
	Sweet cheese masses	
Fatless cheese	862,07	–
s/m cheese with fms 5 %	–	840,34
Sugar-sand	120,69	126,05
Cryopowder	17,23	33,61
Totally	1000	1000
	Salt cheese masses	
Fatless cheese	974,66	–
s/m cheese with fms 5 %	–	970,87
Table salt	15,59	15,54
Cryopowder	9,75	13,59
Totally	1000	1000

The smell of cheese masses remained fresh, sour-milk. But in sweet samples the distinctly expressed smack and smell of pumpkin was perceived. The flavor of studied samples was sweet or salt. The pumpkin smack was more expressed in sweet samples. The consistence of studied samples was homogenous, pasty, the separate dots of cryopowder were present.

The other important group of parameters for description of cheese masses is their physical-chemical characteristics. The physical-chemical parameters of studied samples of sweet and salt cheese masses with cryopowder are presented in the **Table 2**.

The titrated acidity of studied samples of salt cheese masses was 124–130 °T, ms of moisture 62–60 % and dry substances – 40–38 %, and titrated acidity of studied samples of sweet cheese masses had 126–134 °T, ms of moisture 63–66 % and dry substances – 34–37 %.

The analysis of figure material of the **Table 3** testifies that the addition of cryopowder has certain influence also on physical-chemical characteristics.

The increase or decrease of microorganism quantity can take place in the process of production and storage of cheese masses. The microorganisms come with output raw material from the surface of technological equipment and communications. At breaking of sanitary-hygienic conditions of production the development of pathogenic microflora is possible that results in creation of toxic substances that causes food intoxications. That is why the study of microflora at storage of cheese masses is important at their production. The dynamics of microbiological parameters of cheese masses at storage is presented in the **Tables 3, 4**.

Table 2

The main physical-chemical parameters of cheese masses with cryopowder "Pumpkin"

Cheese mass name	Acidity (°T)	Mass chare			Food value (kcal/100g)
		Moisture	Dry substances	Fat, %	
Normative values of cheese masses	120–140	60–70	–	s/f; 4–6	120–180
Salt cheese masses: fatless and semi/fat with cryopowder "Pumpkin"	130/124	60/62	40/38	s/f; 4,8	118/164
Sweet cheese masses: fatless and semi/fat with cryopowder "Pumpkin"	134/126	63/66	37/34	s/f; 4,6	128/174

Table 3

The results of microbiological studies of sweet cheese masses with cryopowder at their storage

Day	The studied microbiological parameters at storage of product	Sweet cheese mass	
		Fatless with cryopowder	Semi/fat with cryopowder
1	Acidity, °T	130	124
	BCBG	Solution 10^{-5} were not detected	
	Microbic landscape	Sour-milk streptococci	
2	Acidity, °T	134	130
	BCBG	Solution 10^{-4} were not detected	
	Microbic landscape	Sour-milk streptococci	
3	Acidity, °T	138	134
	BCBG	Solution 10^{-3} were not detected	
	Microbic landscape	Sour-milk streptococci	
4	Acidity, °T	144	138
	BCBG	Solution 10^{-3} were not detected	
	Microbic landscape	Sour-milk streptococci 3–4 micrococci; 1–2 bands	Sour-milk streptococci 1–2 micfrococci

Thus, on the base of the studies there was grounded the expedience of using cryopowder "Pumpkin" in technology of cheese masses of different fat and type that increases their biological value.

Table 4

The results of microbiological studies of salt cheese masses with cryopowder at their storage

Day	The studied microbiological parameters at storage of product	Salt cheese mass	
		Fatless with cryopowder	Semi/fat with cryopowder
1	Acidity, °T	134	126
	BCBG	Solution 10^{-5} were not detected	
	Microbic landscape	Sour-milk streptococci	
2	Acidity, °T	136	130
	BCBG	Solution 10^{-4} were not detected	
	Microbic landscape	Sour-milk streptococci	
3	Acidity, °T	138	136
	BCBG	Solution 10^{-3} were not detected	
	Microbic landscape	Sour-milk streptococci 1–2 micrococci	
4	Acidity, °T	142	140
	BCBG	Solution 10^{-3} were not detected	
	Microbic landscape	Sour-milk streptococci 3–4 micrococci	Sour-milk streptococci

4. Conclusions

There was grounded the expedience of using cryopowder "Pumpkin" in technology of sweet and salt cheese masses. The optimal dose of cryoadditive "Pumpkin" as a component of therapeutic-prophylactic cheese masses was offered.

Some receipt differences between sweet and salt cheese masses were established. At the study of organoleptic, physical-chemical and biological characteristics it was established, that the color of salt cheese masses with cryoadditive was yellow, whereas the color of sweet ones was cream with separate yellow dots of comminuted powder-like cryoadditive. The smell of salt cheese masses remained fresh, sour-milk, whereas in sweet ones the distinctly expressed smell of cryoadditive was perceptible. The flavor of studied samples was more expressed in sweet cheese masses. The consistence of cheese masses with addition of cryoadditive "Pumpkin" was homogenous, pasty and tender.

At the study of organoleptic parameters of cheese masses using cryoadditive "Pumpkin" it was established, that the color of sweet cheese masses was cream with separate yellow dots of comminuted powder-like cryoadditive, whereas the color of salt cheese masses was correspondingly yellow. In sweet cheese masses the distinctly expressed smell of cryoadditive was perceived, whereas in salt ones it remained fresh, sour-milk.

The offered cheese masses had pleasant original commodity look, normative physical-chemical characteristics.

So, the use of vegetable components as bioadditives in technological process of cheese masses is important from both theoretical and practical points of view. The experiments in this direction are prospective and interesting; they contain practical recommendations for production. The result is the new product, useful for potential consumers.

References

[1] Hachak, U. R., Vavrysevych, J. (2016). The use of cryopowder «pumpkin» in the technology of cheese masses with different fat content. Scientific Messenger of LNU of Veterinary Medicine and Biotechnology, 18 (2 (68)), 41–46. doi: 10.15421/nvlvet6808

[2] Mamone, G., Picariello, G., Caira, S., Addeo, F., Ferranti, P. (2009). Analysis of food proteins and peptides by mass spectrometry-based techniques. Journal of Chromatography A, 1216 (43), 7130–7142. doi: 10.1016/j.chroma.2009.07.052

[3] Piska, I., Stetina, J. (2004). Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. Journal of Food Engineering, 61 (4), 551–555. doi: 10.1016/s0260-8774(03)00217-6

[4] Barbano, D. M., Margolies, B. (2016). Critical factors for evaluation of cheese yield performance and fat loss in large cheese factories. Journal of Animal Science, 94 (supplement 5), 269–270. doi: 10.2527/jam2016-0566

[5] Bejarano Toro, E. E., Sepulveda Valencia, J. U., Restrepo Molina, D. A. (2016). Characterization of a processed cheese spread produced from fresh cheese (quesito antioqueño). Revista Facultad Nacional de Agronomía, 69 (2). doi: 10.15446/rfna.v69n2.59146

[6] Fiol, C., Prado, D., Mora, M., Alava, J. I. (2016). Nettle cheese: Using nettle leaves (Urtica dioica) to coagulate milk in the fresh cheese making process. International Journal of Gastronomy and Food Science, 4, 19–24. doi: 10.1016/j.ijgfs.2016.05.001

[7] Shabani, J., Sarfarazi, M., Mirzaei, H., Jafari, S. M. (2016). Influence of the sunflower oil content, cooking temperature and cooking time on the physical and sensory properties of spreadable cheese analogues based on UF white-brined cheese. International Journal of Dairy Technology, 69 (4), 576–584. doi: 10.1111/1471-0307.12305

[8] Smith, G. P. S., Holroyd, S. E., Reid, D. C. W., Gordon, K. C. (2016). Raman imaging processed cheese and its components. Journal of Raman Spectroscopy, 48 (3), 374–383. doi: 10.1002/jrs.5054

[9] Katz, G., Merin, U., Bezman, D., Lavie, S., Lemberskiy-Kuzin, L., Leitner, G. (2016). Real-time evaluation of individual cow milk for higher cheese-milk quality with increased cheese yield. Journal of Dairy Science, 99 (6), 4178–4187. doi: 10.3168/jds.2015-10599

[10] Ferrao, L. L., Silva, E. B., Silva, H. L. A., Silva, R., Mollakhalili, N., Granato, D., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Padilha, M. C., Zacarchenco, P. B., Barbosa, M. I. M. J., Mortazavian, A. M., Cruz, A. G. (2016). Strategies to develop healthier processed cheeses: Reduction of sodium and fat contents and use of prebiotics. Food Research International, 86, 93–102. doi: 10.1016/j.foodres.2016.04.034

THE STUDY OF INFLUENCE OF ARONIA ADDITIVES ON FUNCTIONAL-TECHNOLOGICAL PROPERTIES OF WHEAT FLOUR

Yurii Korenets
Department of technology in a restaurant economy and hotel and restaurant business
Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky
16 Tramvaina str., Kryvyi Rih, Ukraine, 50005
yurii.korenets@gmail.com

Iuliia Goriainova
Department of technology in a restaurant economy and hotel and restaurant business
Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky
16 Tramvaina str., Kryvyi Rih, Ukraine, 50005
kaum@dkt.dn.ua

Radion Nykyforov
Department of technology in a restaurant economy and hotel and restaurant business
Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky
16 Tramvaina str., Kryvyi Rih, Ukraine, 50005
nikradion@yandex.ua

Iryna Nazarenko
Department of technology in a restaurant economy and hotel and restaurant business
Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky
16 Tramvaina str., Kryvyi Rih, Ukraine, 50005
nazarenko5555@mail.ru

Olga Simakova
Department of technology in a restaurant economy and hotel and restaurant business
Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky
16 Tramvaina str., Kryvyi Rih, Ukraine, 50005
simakovaolgaal@gmail.com

Abstract

The expedience of the use of aronia in technology of short dough products as a source of vitamins, mineral, pectin substances, phenol compounds, easily assimilated sugars, organic acids and so on was substantiated. To study the influence of aronia additives on the main receipt component of short dough (wheat flour) there was elaborated an algorithm of the study that includes theoretical analysis and physical-chemical experiment. The expedience of introduction of aronia as a powder directly into wheat flour was theoretically grounded. The methods of estimation of the content of polyphenol compounds, influence of aronia additives on amylolytic and proteolytic activity of wheat flour, its sugar-creating ability, were selected. The methods of experiment planning and mathematical processing of experimental data were realized using computer program MS Excel 97 2003.

In was established, that the aronia additive weakens the gluten of wheat flour and prevents the swelling of gluten proteins. It positively influences the process of short dough formation and provides a possibility of its storage during the long time until baking. The use of aronia additives in technology of short dough products allows not only raise their food value but also improve the quality of short dough.

Keywords: aronia, short dough, wheat flour, anthocyanins, amylolytic activity.

DOI: 10.21303/2504-5695.2017.00299

1. Introduction

The unconventional vegetable raw material is widely used for improving the food value of flour confectionary [1]. It is prospective to use aronia as a source of vitamins, mineral substances, phenol compounds and so on [2].

The studies in vitro and in vivo testify that the polyphenols of plants, including anthocyanins play the important role in protection of human health [3]. It was established, that just aronia is characterized with high content of anthocyanins (4341 mg/kg^{-1}) and their high antioxidant activity [4]. Due to the high content of anthocyanins aronia fruits positively influence the cardio-vascular system activity, digestive organs and so on [5].

It is expedient to use aronia as a powder. The anthocyanins of aronia fruits were released from dried raw material by extraction by the solutions of hydrochloric acid in water or ethanol with ω=1 % [6]. Their content in water and alcoholic extract is almost equal – 1,98 and 2,12 % respectively. At the same time it is well-known, that the general content of bioflavonoids in fruits is near 5 %. It is proved [7] that at using ultrasound the significant acceleration of production process and increase of the main product output comparing with other methods are observed.

The studies, directed on the increase of food value of short dough products adding aronia directly to the wheat flour are practically absent. Such method of the use of this unconventional raw material can open new ways of improvement of short dough products quality.

The aim of the work is to study the influence of physiologically active compounds of aronia additives (AA) on functional-technological properties of wheat flour.

For attaining the set aim it is necessary to solve the following problems: to study AA influence on the state of protein complex of wheat flour; to determine AA influence of amylolytic activity of wheat flour.

2. Materials and Methods

The powder from aronia dry fruits was gotten by their refinement using micro-shredder of tissues RT-1 (**Fig. 1, *a***), produced by PQG "GRANAT", Saint Petersburg city, (Russia). The refinement was realized by knives, which rotation speed is 4000 turn/min. The drying of aronia fruits was carried out at temperature 80...90 ºC during 2...3 hours up to the constant mass of sample. The alcoholic and water extracts from aronia fruits were prepared both according to well-known methods [8], and using ultrasound technologies (UST) [7].

For estimating the content of polyphenol compounds [9] the sample of well-refined vegetable raw material with mass 2 g was put in chemical glass, 70 ml of distilled water were added and it was heated up to 80 ºC [10].

The cooled mixture was quantitatively transferred in measuring flask with volume 250 ml, glass was rinsed 2...3 times with distilled water and the content of flask was brought to the mark. The gotten mixture was left for 30 min, at that time it was energetically shaken 5–6 times then filtered through paper folded filter. 10 ml of filtrate was measured in big porcelain cup with volume 1 l, 750 ml of distilled water, 25 ml of indigo carmine solution and 10 ml of diluted H_2SO_4 (1:4) were added. The mixture was titrated by 0,05 N solution of $KMnO_4$, being energetically mixed by glassy bacillus. Titration was realized with speed 1 drop a second up to faintly rose color. The color of mixture in cup gradually changes: from blue it becomes deep-green, green-yellow then golden-yellow. For correcting data for other oxidized components of material, there was realized the control experience, during which the polyphenols were eliminated by their adsorption from extract by activated carbon. The content of phenol substances was calculated according to the formula (1):

$$X = \frac{(a - в) \cdot 0,004157 \cdot V \cdot 100}{G \cdot 2 \cdot V_1}, \quad (1)$$

where X – the content of phenol compounds in sample, %; (a–c) – the difference between volumes of 0,05 N solution of $KMnO_4$, spent for titration in working and control experiences, ml; 0,004157 – coefficient of recalculation of the result of titration in phenol compounds (1 ml of 0,1 N solution of $KMnO_4$ corresponds to 0,004157 g of phenols, for example, tannin); V – general volume of extraction, ml; V_1 – volume of filtrate, taken for analysis, ml; G – sample of studied product, g.

The estimation of summary activity of amylases of aronia fruits was realized by Bendelow method. For that 2 ml of distilled water and 3 ml of citrate buffer were added to 5 ml of enzyme extract (ω=5 %). To prepare this reagent 21,008 g of citric acid and 200 ml of 1 N solution of NaOH

were mixed in measuring flask with volume 1 l, were added with distilled water up to the mark. 700 ml of this solution were mixed with 300 ml of 0,1 N solution of NaOH. Then 10 ml of starch solution (ω=2 %), heated to 40 ºC, were added to reactive mixture. The mixture was heated at this temperature during 15 minutes, the sample was taken away and analyzed for maltose content by well-known methodology using Felling reagent [9].

The estimation of sugar-creating ability of flour at AA was carried out as following:

– 10 g of flour were weighed (9,5 g of flour and 0,5 g of additive are weighed at using additives in dry form);

– it was put in dry measuring flask for 100 ml;

– kept on water bath at temperature 27 ºC during 15 minutes.

Such methodology of estimation of sugar-creating ability of the flour is realized by the quantity of milligrams of maltose, created from 10 g of flour for 1 hour of keeping from 50 cm^3 of water at temperature 27 °C.

The technique of estimation – 10 g of flour, weighed with error no more than 0,05 g, is quantitatively transferred in dry measuring flask with capacity 100 cm^3. The flask with sample is placed on water bath or thermostat with temperature 27 °C for 15 min for hitting. Then 50 cm^3 of distilled water with temperature 27 °C are added in flask by pipette, fast and accurately mixed up to homogenous state (without clots) and thermostated at the same temperature during 1 hour, shaking mixture each 15 min. In this period hydrolysis of flour starch takes place under influence of own amylolytic enzymes.

After 1 hour, inactivation of enzymes is realized, adding in flask 15 cm^3 of 15 % $ZnS0_4$ and 15 cm^3 of 1 N solution of NaOH by cylinder, continuously stirring mixture. Then it is added with water to the mark, mixed during 3 min, kept for 3–5 min and filtered through the paper folded filter in dry flask.

The amount of created sugar is estimated in transparent filtrate using different methods.

For estimating proteolytic activity of the raw material 5 ml of additive extract (ω=5 %) were added to 20 ml of gelatin solution (ω=2 %), reactive mixture was kept in thermostat at temperature 25 ºC during 2 hours [11].

After that the reactive mixture was filtered and kinematic coefficient of filtrate viscosity was found by capillary viscosimeter VTL-2 (“Oilchemigroup, LTD, laboratory equipment, Kyiv city, Ukraine) with capillary diameter 0,56 in water thermostat **(Fig. 1, *b*)** [12].

a *b*

Fig. 1. The types of equipment, used during the study: *a* – micro-shredder of tissues RT-1; *b* – viscosimeter VTL-2 with capillary diameter 0,56 in water thermostat

Thermostating of the solutions was carried out with exactness up to +0,1 ºC. The system was kept no less than 15 min before starting measuring. Before experiment the solutions were filtered through Shotta filter. The kinematic coefficient of viscosity was calculated according to the formula (2):

$$\eta = t_{solution} / t_{solvent}, \quad (2)$$

where η – kinematic coefficient of viscosity; $t_{solution}$ – time of solution outflow, s; $t_{solvent}$ – time of solvent outflow, s.

The methods of experiment planning and mathematical processing of experimental data were realized using computer program MS Excel 97 2003. For statistical reliability all experiments were fivefold repeated.

2. 1. Experimental procedure

AA influence on protein-proteinase complex of wheat flour was estimated. To reveal this influence the qualitative characteristics of gluten were studied. The one was washed from the dough, prepared of extra wheat flour (TM "Chutorok", Ukraine) with AA in quantity 5 % of wheat mass. Another – from the dough, prepared on water extract of aronia with mass share 5 %. The results of AA influence on the quality of wheat flour gluten are presented in the **Table 1**.

Table 1

The influence of aronia additives on the quality of wheat flour gluten

Studied sample of dough	Output of raw gluten, %	Output of dry gluten, %	Gluten ability to stretching, cm	Gluten ability to spreading, mm
Without additive	34,2±2,4	14,0±0,8	3,5±0,2	41,0±3,3
With AA	33,4±2,3	14,9±0,6	4,3±0,2	45,0±3,2
On AA water extract	32,0±1,9	15,5±1,1	3,8±0,2	42,0±3,4

For quantitative assessment of acidic properties of compound there was carried out electrometric titration of water extract of aronia by 0,01 N solution of sodium hydroxide, which results are presented on the **Fig. 2.**

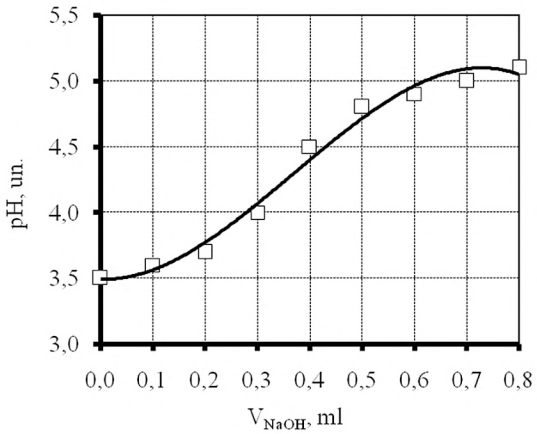

Fig. 2. Electrometric titration of water extract of aronia by 0,01N solution of NaOH

At the assessment of acidic properties of anthocyanins pH was considered as its value in the point of semi-neutralization. The data of **Fig. 2** testify that it is 3,7. This cipher characterizes the aronia anthocyanins as rather strong acids.

The complicated complex of different chemical substances of additive probably blocks the effect of flour proteolytic enzymes acting as their inhibitors. To prove the process of inhibition of proteolytic enzymes of wheat flour, the process of hydrolysis of wheat flour gluten proteins was studied under the effect of its own proteolytic enzymes and with AA. It is well-known, that proteolytic activity of enzymes can be estimated also by the character of protein substances disintegration and by the quantity of created products of hydrolysis that is free amino acids [13]. The quantity of created amino acids is estimated by the content of nitrogen of amino groups, analyzed by formol or electrometric titration. The study of autolytic activity of wheat flour was carried out without additives and with AA (its water extract with ω=5 % and dry powder in concentration 5 % relative to the flour mass). The quantity of amino acids, created as a result of enzymatic hydrolysis was estimated by formol titration. The results of the study of AA influence on the content of free amino acids in the products of wheat flour autolysis are presented in the **Table 2.**

Table 2

The influence of aronia additives on free amino acids content in the products of wheat flour autolysis

Substrate	Amino acids content of hydrolyzate, mg/100 g
Water suspension of wheat flour	352,3±28,2
Wheat flour suspension in aronia water extract	212,6±14,9
Water suspension of wheat flour with AA	155,1±13,9

It was established, that AA doesn't only manifest any proteolytic activity but also rather effectively decelerate the process of protein molecules disintegration under the effect of proteolytic enzymes of wheat flour.

Such active process of inhibition of proteolytic enzymes of wheat flour allows presume that other hydrolytic enzymes of flour will be also paralyzed by AA effect. That is why there was studied the influence of aronia additive of sugar-creating ability of wheat flour that is the activity of its enzymes-amylases. The process was controlled by estimation of maltose quantity in reactive mixture. The results of the study of AA influence on amylolytic activity of wheat flour are presented on the **Table 3**.

The mechanism of inhibition of enzymes-amylases by aronia preparations can by interpreted in different ways. Optimal pH effect of enzymes-amylases is 5,6 [9], but acid extracts of aronia fruits decrease pH of the medium and in such a way decrease the amylolytic activity of enzymes. To verify this mechanism the amylolytic activity of wheat flour was estimated at presence of aronia preparations. pH of reactive medium was kept within 5,5...6,0. The mixture was neutralized by the solution of sodium hydrocarbonate. The received parameters of maltose content in reactive mixture are presented in the **Table 3**.

Obviously, there is an interaction of metal cation with two different molecules of anthocyanidins with creation of compounds with twice more molecular mass. As a result of this process, the cations of metals-activators are removed from reactive mixture and become inaccessible for activation of enzyme-substrate complex. That, in its turn, leads to inhibition of hydrolytic processes in wheat flour (the effect of enzymes-amylases of wheat flour is inhibited by 29...82 %).

To verify this suggestion the amylolytic activity of wheat flour was estimated at presence of acknowledged amylases activator – Ca^{2+} cation. The results of the study of AA influence at presence of Ca^{2+} cation on amylolytic activity of wheat flour are given in the **Table 4**.

It was established, that AA at presence of calcium ions practically completely paralyzes the effect of wheat flour enzymes, bounding all ions of reactive mixture in strong complex compounds.

Table 3

The influence of aronia additives on amylolytic activity of wheat flour

Substrate	Maltose value, % of maltose	Maltose value, % of maltose (pH of medium – 5,5...6,0)
Water suspension of wheat flour	4,10±0,37	4,10±0,37
Suspension of wheat flour in aronia water extract	0,72±0,04	0,93±0,07
Water extraction of wheat flour with AA	2,91±0,17	3,21±0,13
Suspension of wheat flour in aronia fresh juice	–	–

Table 4

The Aronia additives influence at presence of Ca^{2+} cation (C^{2+}_{Ca} = 0,1 %) on amylolytic activity of wheat flour

Substrate	Maltose value, % of maltose
Water suspension of wheat flour + Ca^{2+}	8,56±0,34
Suspension of wheat flour in aronia water extract + Ca^{2+}	1,53±0,09
Water extract of wheat flour with AA + Ca^{2+}	3,94±0,28

3. Results

It was established (**Table 1**), that AA weakens the flour gluten (gluten ability to stretching increases by 9...23 %). It is a positive factor for short dough formation. It becomes more plastic and doesn't need the starch, often added to the receipt just for increasing the receipt mixture plasticity. Due to the presence of phenol compounds of hydroxyl groups in molecules and due to the features of electronic structure of benzene ring, they have unique properties. The main one is an ability to reverse oxidation that is to the transfer of phenol forms in quinoid forms. That is why practically all phenol substances have brightly expressed anti-oxidant activity [14, 15].

The powder of aronia has more active influence on this process comparing with extract that can be explained by more polyphenol concentration in dough semi-finished product at using dry preparation. The raw gluten output at using AA decreases by 3...6 %. It testifies to the decrease of hydrogen bonds in protein macromolecules and partial loss of ability to bound water and to keep it.

AA use prevents the swelling of wheat flour gluten proteins due the decrease of ability to keep water. It allows increase the duration of short semi-finished product manufacturing and provides a possibility of its storage during the long time till baking.

It was established (**Table 3**), that aronia preparations are the strong inhibitors of enzymes-amylases of wheat flour. At that the inhibition regularity is quite other than in case with enzymes-proteinases of flour. The water extract from fruits inhibits amylases significantly stronger than the powder of dry fruits, in the case of proteolytic enzymes it was vice versa.

The gotten data practically coincide with earlier ones, received at using aronia preparations without neutralization of their acidic properties (**Table 3**). There was set practically invisible but stable growth of maltose value that can be explained by acidic inhibition of enzymes, but the contri-

stable growth of maltose value that can be explained by acidic inhibition of enzymes, but the contribution of such inhibition way is absolutely insignificant. It is proved, that the change of pH medium practically doesn't influence the elements of aronia compounds structure that are responsible for enzymes inhibition.

The results of the study of AA influence at presence of Ca^{2+} cation of amylolytic activity of wheat flour (**Table 4**) testify to more high degree of amylases inhibition by the extract or juice of aronia comparing with powder of dry fruits. It can be explained by the fact that water-soluble phenol compounds of aronia in reactive mixture at once begin to interact with metal cations that are present there and bound them in stable complexes. At using the dry powder of aronia, certain time is necessary to extraction of polyphenol compounds by the water that makes them accessible for reaction with metal cations. During this time the flour amylases can partially realize their hydrolytic function.

The received experimental data allows manage the hydrolytic processes in dough semi-finished product.

Thus, AA usage in technology of short dough products favors not only the increase of their food value but also allows increase the short dough quality.

4. Conclusions

1. The influence of physiologically active AA compounds on the wheat flour gluten quality was studied. It was established, that AA as a powder and water extract weakens the flour gluten that is a positive factor for short dough formation. The gluten ability to stretching increases by 9...23 %. The dough becomes more plastic. It doesn't need starch, often added to the receipts just for increasing the receipt mixture plasticity.

It was established, that AA polyphenol substances prevent the swelling of wheat flour gluten proteins due to the decrease of ability to keep water that provides a possibility of its storage during the long time till baking.

2. The higher degree of amylases inhibition in wheat flour by extract of aronia fruits or its juice comparing with powder of dry fruits was revealed. The water-soluble aronia phenol compounds in reactive mixture begin to interact with metal cations that are present there and bound them in strong complexes. The received experimental data allow not only manage hydrolytic processes in dough semi-finished product but also widen the possibilities of using wheat flour of lower grades, neutralizing the harmful enzyme effect in it.

The prospects of further research are the study of AA influence on the state of fatty component of short dough, substantiation of AA rational concentration to wheat flour and elaboration of technology of short dough with AA.

References

[1] Matveeva, T. V., Koriachkina, S. Ya. (2016). Muchnye konditerskie izdeliia funktsionalnogo naznacheniia. Nauchnye osnovy, tekhnologii, retseptury. St. Petersburg: GIORD, 360.

[2] Simakhina, G., Naumenko, N., Khalapsina, S. (2012). Biological value of aronia berries. Ukrainian food journal, 1, 8–11.

[3] Poracova, J., Sedlak, V., Posivakova, T., Mirutenko, V., Gruova, D., Mydlarova-Blascakova, M., Kotosova, J. (2013). Measurement of antioxidant activity in chokeberry (Aronia melanocarpa WILD.) and black elderberry (Sambucus nigra L.) using the DPPH method. Materialy vtoroi Mezhdunarodnoi nauchno-prakticheskoi internet-konferentsii "Lekarstvennoe rastenievodtvo: ot opyta proshlogo k sovremennym tekhnologiiam". Poltava, 132–136.

[4] Jakobek, L., Seruga, M., Medvidovic-Kosanovic, M., Novak, I. (2007). Antioxidant Activity and Polyphenols of Aronia in Comparison to other Berry Species. Agriculturae Conspectus Scientificus, 72 (4), 301–306.

[5] Olas, B., Wachowicz, B., Nowak, P., Kedzierska, M., Tomczak, A., Stochmal, A., Oleszek, W., Jeziorski, A., Piekarski, J. (2008). Studies on antioxidant properties of polyphenol – rich

extract from berries of Aronia melanocarpa in blood platelets. Journal of Physiology and Pharmacology, 59 (4), 823–835.

[6] Mariichuk, R., Feier, J., Eliashova, A., Grulova, D., Shalamon, I. (2014). Ekstraktsciia antotsianiniv iz roslynnoi syrovyny. Naukovij visnik Uzgorods'kogo universitetu. Seria Himia, 2 (32), 35–37.

[7] Hmelev, V. N., Slivin, A. N., Barsukov, R. V., Tsyganok, S. N., Shalunov, A. V. (2010). Primenenie ultrazvuka vysokoi intensivnosti v promyshlennosti. Biisk: Altai State University, 203.

[8] Melnychuk, D. O., Vovkotrub, M. P., Melnikova, N. M., Melnychuk, S. D., Bukhtiiarov, V. K., Yakubovych, T. M., Krotenko, V. V. (2010). Praktykum z organichnoi ta biologichnoi himii. Kyiv, 300.

[9] Krasilnikova, L. O., Avksentieva, O. O., Zhmurko, V. V. (2007). Biohimiia roslyn. Kharkiv: Koloryt, 191.

[10] Ermakov, A. I. (Ed.) (1987). Metody biohimicheskogo issledovanii rastenii. Leningrad: Agropromizdat, 430.

[11] Aivazov, B. V. (1973). Praktikum po himii poverhnostnyh iavlenii i adsorbtsii. Moscow: Vysshaia shkola, 206.

[12] Starosta, V. I., Yanchuk, O. M. (2014). Koloidna himiia. Praktykum. Lutsk: Lesya Ukrainka Eastern European National University, 360.

[13] Prosekov, A. Yu., Ulrikh, E. V., Noskova, S. Yu., Budrik, V. G., Botina, S. G., Agarkova, E. Yu., Melnikova, E. I. (2013). Poluchenie fermentnykh gidrolizatov belkov molochnoi syvorotki s ispolzovaniem proteoleticheskikh fermentov. Fundamentalnye issledovaniia, 6, 1089–1093.

[14] Katalinic, V., Milos, M., Kulisic, T., Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry, 94 (4), 550–557. doi: 10.1016/j.foodchem.2004.12.004

[15] Laguta, I. V., Stavinskaya, O. N., Dzyuba, O. I., Ivannikov, R. V. (2015). Analysis of antioxidant properties of plants extracts. Reports of the National Academy of Sciences of Ukraine, 5, 130–137. doi: 10.15407/dopovidi2015.05.130

THE STUDY OF BEER QUALITY WITH THE REDUCED TOXIC EFFECT

Nataliya Penkina
Department of merchandise in customs
Kharkiv State University of Food Technology and Trade
333 Klochkivska str., Kharkiv, Ukraine, 61051
penkina-nataliya@rambler.ru

Larisa Tatar
Department of Commodity, quality management and environmental safety
Kharkiv State University of Food Technology and Trade
333 Klochkivska str., Kharkiv, Ukraine, 61051
tornado.1972@mail.ru

Victoria Kolesnyk
Department of Commodities in customs
Kharkiv State University of Food Technology and Trade
333 Klochkivska str., Kharkiv, Ukraine, 61051
vkol240584@gmail.com

Tetiana Karbivnycha
Department of Commodity, quality management and environmental safety
Kharkov State University of Food Technology and Trade
333 Klochkivska str., Kharkiv, Ukraine, 61051
tanya280372@rambler.ru

Tetiana Letuta
Department of Commodity and expertise of goods
Kharkov State University of Food Technology and Trade
333 Klochkivska str., Kharkiv, Ukraine, 61051
Lettanya@ukr.net

Abstract

There was grounded the expedience of using unconventional vegetable raw material, namely Pinus sylvestris needle that partially replaced hop for beer enrichment. The optimal parameters of extraction of Pinus sylvestris needle relative to anti-oxidant activity of extract: hydromodulus 1:20, temperature 60 ºC, extraction 30 min. The needle extract has pure smell, harmonic refreshing taste with needle note. The content of ascorbic acid in extraction is 0,275 mg/100 g, antioxidant activity – 202,3 C/100 g. The quantitative ratio of hop and pine needle in beer receipt was determined by the way of mathematical modeling. Quantitative content of Pinus sylvestris needle is no more than 20 % by mass from the rated norm of hop that is enough for preserving hop bitterness and smell. The receipt of beer, including Pinus sylvestris needle, was elaborated. The quality parameters of ready drink were studied. The addition of needle increases the beer gustatory properties, decreases methanol content. The content of ascorbic acid in ready drink is 3,52 mg/100 g. Antioxidant activity of elaborated beer is 178,1 C/100 g that determines its high biological value.

The influence of beer with needle extract on antioxidant system of organism of biological objects was assessed. Under conditions of acute pathological condition the beer with needle extract decreases oxidative influence of drink on brain of biological objects. The beer "Smaragd" decreases toxic effect on organism of living creature due to its antioxidant properties.

The introduction of needle extract that includes vegetable antioxidants in beer is the one of the ways to increase antioxidant capacity of ready drink. It opens the prospect of studies, directed on elaboration of arrangements of stabilization of different sorts of beer. The studies may be introduced in production that has positive effect on ready product quality.

Keywords: beer, pine needle, antioxidant capacity, oxidative stress, toxicity, liver homogenate, pro-antioxidant markers.

DOI: 10.21303/2504-5695.2017.00303

1. Introduction

The addition of antioxidants in beer allows decrease oxidative and toxic effect of alcohol on human organism [1]. The leaves of conifers are the source of natural antioxidants, have the high food, biological value and may be used as hop alternative. Beer, produced with addition of vegetable raw material has its advantages: functional directive effect, improved organoleptic and physical-chemical parameters, more storage life [2, 3]. The elaboration of beer with addition of needle extract gives a possibility to get new original sorts of beer and widen the assortment of beer branch.

The realized studies were aimed at estimation of influence of Pinus sylvestris needle extract on formation of the ready beer quality. It allows get a drink with high antioxidant properties and decrease the negative effect of alcohol on human organism.

2. Materials and Methods

The water extract of unconventional vegetable raw material – Pinus sylvestris needle was chosen for partial replacement of hop and beer enrichment with biologically active substances.

For realization of experiment the samples of pine needle were gathered in early winter period, because in this time the maximal accumulation of biologically active substances in them takes place [4]. The raw material corresponded to the requirements TC U 15.8-31062507-022:2009 "Vegetable natural raw material for production of diet additives".

The simplest way of extraction was selected to get the needle extract – maceration. The material, subjected to extraction – freshly gathered needle of Pinus sylvestris; extractant – water. Hydromodulus, temperature and duration of extraction process were set experimentally using mathematical modeling.

The gotten water extract of Pinus sylvestris needle is presented on the **Fig. 1**.

Fig. 1. Water extract of Pinus sylvestris needle

2. 1. The study of the quality parameters of needle extract and beer, elaborated with its addition

The organoleptic parameters were estimated by the method of sensor analysis [5].

The mass share of dry substances in initial wort and mass share of alcohol in beer were estimated by aerometric and refractometric methods [6]. The acidity was estimated by direct titration of sample [7], color – by colorimetric method [8]. Mass share of carbon dioxide was studied by

measuring of pressure in gas space above the sample in corked utensil, stability – by visual observation of turbidity or sediment [9].

The antioxidant capacity, based on coulometric titration of studied sample by bromine, was estimated in water extract and ready beer. The content of ascorbic acid was estimated by iodometric method [10].

The identification of micro-components in beer was estimated by the standard gasochromatographic method, standardized for vodka, ethyl alcohol and water-alcoholic mixtures and possible for beer samples [11, 12].

For express-method of methanol revelation the colored screening-sample by oxidation reaction of methanol to formaldehyde is recommended. The negative reaction testifies to falsification of beer, because methanol is a product of yeast fermentation. The positive one needs estimation of methanol concentration by chromatographic method together with other micro-components.

The chromatograms of beer samples were gotten on chromatograph "Crystal-2000M" (Russia) using flaming-ionizing detector.

The experimental data were processed by the methods of variational statistics using the standard package of computer programs "Statistica 6.0" (England) [13] with Student t-criterion and MathCAD.

2. 2. The study of quality parameters of needle extract and ready beer, elaborated with its addition, on the organism of biological objects

The frequent taking of alcohol results in intensification of processes of free radicals and prooxidants creation. Thus, it was expedient to study the influence of beer product with pine needle under conditions of increased oxidation.

The subchronic disorder of antioxidant system of organism was reproduced on the model of oxidative stress. The experiment was carried out on 36 white non-linear rats with mass 220–250 g of both sexes, divided in six groups (**Table 1**). For relevant interpretation of the results the experiment included groups, in which the influence of refined water, beer, produced by classic technology, and beer with Pinus sylvestris needle was compared under ordinary conditions and under conditions of oxidative stress.

Table 1

Exposition of experimental groups

Group №	Introduced substance	Dose g/kg	Term, days
	Animals of intact control		
1	Refined water		
2	Beer, produced by classic technology	15	14
3	Beer with Pinus sylvestris needle extract		
	Animals with experimentally reproduced oxidative stress		
4	Refined water		
5	Beer, produced by classic technology	15	14
6	Beer with Pinus sylvestris needle extract		

Oxidative stress was modeled according to the standard methodology (glucocorticoid-induced oxidative stress) by everyday intra-abdominal administration of prednisolone in dose 50 mg/kg during 14 days. In three hours after administration of preparation the studied drinks were adminis-

tered in dose 15 g/kg, equivalent to the mean quantity of beer, taken by human at a time. The intact animals were administered with correspondent quantity of refined water. It was expedient to study the influence of aforesaid drinks on prooxidant balance of liver under conditions of worsened state of organism. At 14-th day the animals were excluded from experiment and their liver homogenate was studied for quantitative content of antioxidant (RG, catalase) and prooxidant (DC, TBA-reactants) markers according to the standard methods:

– the estimation of diene conjugates (DC) level was carried out according to the method of Stalna I. D. in modification of Skornyakov V. I. [14, 15];

– the estimation of TBA-reactants level was carried out according to Uchiyma M. & Michara M. Methd in modification of Volchegorsky I. A. by the test with thiobarbituric acid (TBA) [16, 17];

– the content of restored glutathione (RG) in skin was estimated by spectrophotometric method with Ellman reagent [18];

– the catalase activity was estimated by the method, based on hydrogen peroxide (H_2O_2) ability to create the stable colored complex with molybdenum salts [19, 20].

The markers of prooxidant balance of cells testify to the activity of free radical processes, antioxidant markers – to the activity of enzymatic chain of antiradical protection of cells. The classic markers of prooxidant-antioxidant balance are DC, TBA-reactants, RG and catalase, at the same time the high values of RG and catalase and low values of DC, TBA-reactants testify to the normal status of the cell, in opposite case – to activation of peroxide oxidation of lipids and membranodestruction.

The content of diene conjugates in homogenate of liver tissues was calculated by the formula:

$$C(\text{тcmol} / g) = 227,27 \times \text{Esample}, \quad (1)$$

where C – DC content; Esample – optic density of studied sample.

The content of TBA-active products in liver tissues homogenate of studied animals was calculated by the formula:

$$C(\text{mcmol} / g) = \frac{\text{Esample}}{1,56 \cdot 10^5} \times 2 \cdot 10^6, \quad (2)$$

where C – the content of TBA-active products; Esample – optic density of studied sample.

The calculation of restored glutathione in liver tissues homogenate was realized according to the formula:

$$C(\text{mcmol} / g) = \text{Esample} \times 1094 \text{ mg \%}, \quad (3)$$

where C – the content of glutathione; Esample – optic density of studied sample.

The catalase activity in liver tissues homogenate was calculated by the formula:

$$\text{Ecat}(\text{mM} / l \times \text{min}) = \frac{(\text{Acontr} - \text{Astud})}{K \times t} \times V \cdot 10^6, \quad (4)$$

where E – catalase activity; Acontr and Astud – optic density (extinction) of blank and studied sample; V – volume of sample (3,02 ml); t – time of incubation (10 min); K – coefficient of millimolar extinction of hydrogen peroxide, $22,2 \cdot 10^3$ $mM^{-1} \cdot cm^{-1}$.

The influence of studied object on the functional state of organism under conditions of acute pathological state was studied on the model of acute normobaric hypoxia. 18 white non-linear mice with mass 20–30 g of different sex, divided in three experimental groups (six animals in each one) were used in experiment. During 14 days before hypoxia modeling the mice were intra-abdominal-

ly administered with 15 g/kg of water, ordinary beer and beer with pine needle extraction once a day. The research on the model of acute normobaric hypoxia was carried out by keeping animals in close special container with volume 200 cm^3; maximal life duration and thanatogenesis symptoms were registered [21].

The received experimental data were statistically processed by the method of variational statistics using the standard package of programs "Statistica 6.0" with Mann-Whitney U-criterion [13]. The reliable differences between groups were considered as ones with significance level $p<0,05$.

3. Experimental procedures

For receiving the water extract, Pinus sylvestris needle was comminuted in crusher to the particles size 3,0–5,0 mm. The value of antioxidant capacity of water extract was taken as a parameter of technological process. Extraction was realized according to the received optimal conditions: hydromodulus 1:20, temperature 60 °C, time of extraction 30 min. The ready extract was cooled to the temperature 8...10 °C and filtered. Mass share of dry substances in ready needle extract was 3,25 %.

The biological value and organoleptic parameters of needle extract were estimated (**Table 2**).

Table 2

Organoleptic parameters and biological value of water extract of Pinus sylvestris needle

Parameter	Characteristic
Appearance	Transparent liquid with golden color
Smell	Clear, with expressed needle smell
Flavor	Harmonic, refreshing with needle note
Ascorbic acid content, mg/100 g	0,275
Antioxidant activity, C/100 g	202,3

The extract was introduced in wort at the stage of main fermentation, because it gives the minimal loss of aromatic substances.

Technological process consists of the following operations: preparation of jam, its saccharification, filtration, boiling of wort, cooling and introduction of yeast, fermentation of wort, after-fermentation of new beer and bottling of beer "Smaragd". The preparation of beer is realized according to existent "Technological instruction on production of malt and beer" TI 18-6-47-85 and "Technological instruction for production of 10 % lager "Smaragd" TI 14297558-340:2016, elaborated in Kharkov state university of food and trade (Ukraine). The water extract of Pinus sylvestris needle was added in norm 600–620 ml/dal of wort and fermented by brewer's bottom yeast at temperature 8...10 °C. The main fermentation was realized to the content of visible extract 2,5–2,8 %. Fermentation and after-fermentation of beer took place for no less than 25 days.

The clarification (filtration) of beer was carried out at kieselguhr candle filter FK-120 (Destila, Czech Republic). At insufficient beer satiation with carbon dioxide the additional carbonization was carried out at temperature 0...2 °C. After maturation the beer was pumped in forfases for future storage and bottling. Pasteurization is realized in automatic regime according to the technological program of process.

The receipt of 10 % lager Smaragd" is elaborated with addition of water extract of Pinus sylvestris needle (**Table 3**).

The solution about the optimal ratio of ingredients in beer receipt was made by the method of mathematical modeling and organoleptic assessment. The quantitative ratio of hop and Pinus sylvestris needle is no more than 20 % by mass from rated norm of hop that is enough for preserving bitterness and hop smell.

Table 3

The receipt content of 10 % lager "Smaragd", raw material consumption for 1 dal of beer

Ingredients	Content of ingredients
Brewing barley light malt	100 %
Bottom yeast	50–100 g/dal
Hop granules, norm of bitter substances	Gf =0,4–0,7 g/dal
Pinus sylvestris needle extract	600–620 ml/dal
Technological water	Consumption

The quality of ready drink was assessed by organoleptic parameters. It has clear, malt flavor with brightly expressed hop bitterness and refreshing needle note.

The foaming of ready beer: foam height is 30 mm, foam stability – 3,0 min. Food value of beer "Smaragd" is 4,6 g/100 g, energetic value – 42 kcal/100 g.

The results of calculation of chromatograms showed methanol concentration in sample with needle extract addition 2,5 times less than in one, produced according to traditional technology. The addition of needle extract decreases methanol ratio in beer and diminishes its negative effect on organism.

The following stage of the work was the study of physical-chemical parameters and biological value of ready drink (**Table 4**).

Table 4

Physical-chemical parameters and biological value of 10 % lager "Smaragd"

Name of parameter	Value
Mass share of dry substances in initial wort, %	10,3
Mass share of alcohol, %	2,9
Acidity, cm^3, 0,1 mol/dm^3 of sodium hydroxide solution for 100 cm^3 of beer	1,7
Color, cm^3, 0,1 mol/dm^3 of iodine solution for 100 cm^3 of water	1,2
Mass share of carbon dioxide, %	0,3
Stability, days:	
Filtered, pasteurized	35
Unfiltered non-pasteurized, clarified	5
Unfiltered, non-pasteurized, non-clarified	3
Ascorbic acid content, mg/100 g	3,52
Antioxidant activity, C/100 g	178,1

For studying the influence of needle extract on specific properties of beer the antioxidant system of organism of biological object was assessed. The experiments were carried out on animals

of both sexes at intra-abdominal administration that is provided for using beer on practice and is expedient taking into account the casual situations, causing accidents, suicidal and criminal intoxication or abuse of alcoholic drinks [22].

The administration of beer, produced according to classic technology to rats reliably increased the content of prooxidant markers in liver homogenate comparing with animals, received DC placebo, by 20,4 %, TBA-reactant – by 51,6 %. At the same time catalase activity decreased by 40,9 %. The markers of liver homogenate of animal, received beer with needle extract under conditions of oxidative stress modeling, were within physiological norm (**Table 5**).

Table 5

The results of the study of prooxidant markers in liver homogenate of rats after administration of studied drinks, n=6

No group	Administered substance	Diene conjugates (mcmol/g)	TBA-reactants (mcmol/g)	Restored glutathione (mmol/g)	Catalase activity (mcmol/min ·m)
Group of intact control animals					
1	Refined water	9,8±0,8	6,2±0,6	13,2±0,4	6,1±0,8
2	Beer, produced by classic technology	11,8±0,5*	9,4±0,7*	12,4±1,2	3,6±1,1*
3	Beer with Pinus sylvestris needle extract	**9,2±0,9**	**6,9±0,3**	**13,6±1,5**	**5,6±0,8**
Groups of animals with modeled pathology					
4	Refined water	21,4±1,9	16,8±0,9	5,6±0,5	2,1±0,4
5	Beer, produced by classic technology	26,5±1,5*	19,1±1,2*	4,2±0,8*	1,8±0,6
6	Beer with Pinus sylvestris needle extract	22,3±0,7	16,5±1,4	5,3±0,4	2,0±0,5

*Note: * – the change is probable relative to the values of animals in groups, received placebo (water): for intact control animals – index of group No 1, for animals with control pathology – the index of group No 4 ($p<0,05$)*

White non-linear animals, received classic beer on the model of normobaric hypoxia, demonstrated the reliable decrease of mean life duration by 313,1 s at oxygen insufficiency comparing with animals, received the refined water. It can be explained by the fact that on the background of alcohol abuse the negative influence of hypoxia on brain intensifies and the processes of peroxide oxidation of lipids in brain tissues are potentiated. The mean life duration of animals, administered with beer with needle extract, remained on the level of intact indices and had no reliable deviations (**Table 6**).

Table 6

The results of the study of influence of studied drinks on the model of normobaric hypoxia

Experimental group (n=6)	Mean life duration of animals (M±m), s
Intact control (refined water)	1523,5±67,1
Beer, produced by classic technology	1210,4±45,9*
Beer with pine needle extract	1498,6±52,4

*Note: * – the change is probable relative to the values of animals of intact control group ($p<0,05$)*

The assessment of the influence of pine needle extract addition on beer properties under conditions of acute pathological process testifies that taking of this product instead of beer, produced according to classic technology, can decrease the drink oxidative influence on brain.

4. Results

The offered methodology of needle extract can be applied at brewing or low-alcohol enterprises, because its realization doesn't need any additional means. The offered vegetable raw material can be used as an alternative of hop.

The needle extract was introduced in wort at the stage of main fermentation that allows get new original beer with refreshing needle note. The content of pine needle in the receipt of beer "Smaragd" in recalculation on sublimation substance is no more than 20 % by mass from the rated norm of hop, enough for preserving bitterness and smell of hop. The addition of needle extract favors the decrease of methanol content, increases the content of biologically active substances of ready drink.

The elaborated beer due to its antioxidant properties decreases the toxic influence of alcohol on living organism and is the new modern drink of beer market. The drink was administered to animals in dose 15 g/kg, equivalent to the mean quantity of beer, taken by human at a time. The studies were carried out during 14 days that corresponds to 2 months of use in humans.

5. Conclusions

The studies favor the receiving of the new sort of beer and the possibility of widening the assortment of drinks of brewing branch. The use of Pinus sylvestris needle extract at beer production has positive influence on the ready drink quality because it is a source of natural antioxidants, has high food and biological value and may be used as an alternative of hop.

The receipt content and technological instruction for production of 10 % lager "Smaragd" TI 14297558-340:2016 were accepted at the meeting of Specialized branch tasting commission for assessment of quality of beer, non-alcoholic, low-alcoholic drinks, mineral and drinking waters, syrups, Ukrbeer, 15.09.2016.

This scientific research was probated in production conditions at brewing enterprise "OLNA" (Kharkiv city), the patent of Ukraine for effective model No 109200 "The method of brewing beer "Smaragd" was received.

The introduction of needle extract that has vegetable antioxidants in brewing technology is the one of ways of increasing antioxidant capacity of ready drink. It opens the prospect for studies, directed on elaboration of the measures of stabilization of different sorts of beer that may be introduced in production with positive effect on the quality of this food product.

Acknowledgments

The work was supported by "OLNA" LTD, Kharkic city (Ukraine) and Central scientific laboratory of National pharmaceutical university of Kharkiv city (Ukraine).

References

[1] Penkina, N., Tatar, L., Zaychenko, G., Lytkin, D. (2015). Research of the toxicological and pharmacological effects of new blends on the body of biological objects. Ukranian Food Journal, 4 (4), 577–586.

[2] Sydor, V., Koshova, V., Boiarska, O., Lavna, M. (2015). Research of beer quality with the addition of elder sap. Technology Audit and Production Reserves, 4 (4 (24)), 52–55. doi: 10.15587/2312-8372.2015.47701

[3] Danilova, L., Melet'ev, A., Berezka, T., Arutyunyan, T. (2013). Antioxidants from plant materials in the technology of beer stabilization. Eastern-European Journal of Enterprise Technologies, 4 (10 (64)), 23–26. doi: 10.15587/1729-4061.2013.1630

[4] Zubareva, Ye. V. (2013). Sezonnaya izmenchevost' vitamina S v khvoye sosny obyknovennoy v usloviyakh g. Krasnoyarska [Seasonal variability of vitamin C in needles of Scots pine in conditions of Krasnoyarsk]. Vesnik KrasGAU, 4, 70–73.

[5] DSTU 3888:2015. Pyvo. Zahal'ni tekhnichni umovy [Beer. General specifications]. (2015). Kyiv: UkrNDNTS, 14.

[6] DSTU 7104:2009. Pyvo. Metody vyznachannya spyrtu, diysnoho ekstraktu ta rozrakhovuvannya sukhykh rechovyn u pochatkovomu susli [Methods for determination of spirit, actual extract and estimation of dry substances in the initial must]. (2009). Kyiv: Derzhspozhyvstandart, 20.

[7] DSTU 4852:2007. Pyvo. Metody vyznachennya kyslotnosti [Beer. Methods for determination of acidity]. (2007). Kyiv: Derzhspozhyvstandart, 10.

[8] DSTU 4851:2007. Pyvo. Metody vyznachennya kol'oru [Beer. Methods for determination of color]. (2007). Kyiv: Derzhspozhyvstandart, 16.

[9] DSTU 4850:2007. Pyvo. Metody vyznachennya dioksydu vuhletsyu ta stiykosti [Beer. Methods for determining dioxin emissions and sustainability]. (2007). Kyiv: Derzhspozhyvstandart, 16.

[10] Orlova, N. Ya., Mandryka, V. I. (2008). Teoretychni osnovy tovaroznavstva. Prodovol'chi tovary [Theoretical Foundations of merchandise. Groceries]. Kyiv: KNTEU, 145.

[11] DSTU 4222:2003. Horilky, spyrt etylovyy ta vodno-spyrtovi rozchyny. Hazokhromatohrafichnyy metod vyznachennya vmistu mikrokomponentiv [Vodka, ethyl alcohol and water-alcohol solution. Chromatographic method for determination of micro]. (2003). Kyiv: Derzhspozhyvstandart, 14.

[12] DSTU 4646:2006. Spyrt etylovyi, horilky, napoi likero-horilchani. Hazokhromatohrafichnyi metod vyznachannia spravzhnosti [Ethyl alcohol, vodka, drinks liquor. Chromatographic method for determining authenticity]. (2006). Kyiv: Derzhspozhyvstandart, 19.

[13] Halafyan, A. A. (2007). STATISTICA 6. Statisticheskij analiz dannyh [Statistical analysis of the data]. Ed. 3. Moscow: Binom-Press, 512.

[14] Skornyakov, V. I., Kozhemyakin, L. A., Smirnov, V. V. (1988). Produkty perekisnogo okisleniya lipidov u bol'nyh s cherepno-mozgovoj travmoj [The products of lipid peroxidation in patients with craniocerebral trauma]. Laborotornoe delo, 8, 14–16.

[15] Stal'naya, I. D., Garishvili, T. G. (1977). Metod opredeleniya dienovoj kon"yugacii nenasyshchennyh vysshih zhirnyh kislot [Method for determination of diene conjugation of unsaturated higher fatty acids]. Sovremennye metody v biohimii. Moscow: Medicina, 43–44.

[16] In: Karpishchenko, A. I. (1997). Spektorofotometricheskoe opredelenie produktov perekisnogo okisleniya lipidov Medicinskaya laboratornaya diagnostika (programmy i algoritmy) [Spectrophotometric determination of lipid peroxidation products. Medical laboratory diagnostics (programs and algorithms)]. St. Petersburg: Intermedika, 48–52.

[17] Stal'naya, I. D., Garishvili, T. G. (1977). Metod opredeleniya malonovogo dial'degida s pomoshch'yu tiobarbiturovoj kisloty [Method for the determination of malonic dialdehyde with thiobarbituric acid]. Sovremennye metody v biohimii. Moscow: Medicina, 66–68.

[18] In: Severina, S. E., Solov'evoj, T. A. (1989). Praktikum po biohimii Opredelenie chisla sul'fgidril'nyh grupp s reaktivom EHllmana [Determination of the number of sulfhydryl groups with the Ellman reagent]. Moscow: MGU, 160–161.

[19] Baraboj, V. A., Orel, V. E., Karnauh, I. M. (1991). Perekisnoe okislenie i radiaciya. Metodicheskie osobennosti issledovaniya perekisnogo okisleniya [Methodological features of the study of peroxidation]. Kyiv: Naukova dumka, 52–75.

[20] Korolyuk, M. A., Ivanova, L. I., Majorova, I. G., Tokarev, V. E. (1988). Metod opredeleniya aktivnosti katalazy [Method for determination of catalase activity]. Laboratornoe delo, 1, 16–19.

[21] In: Habrieva, R. U. (2005). Rukovodstvo po ehksperimental'nomu (doklinicheskomu) izucheniyu novyh farmakologicheskih veshchestv [Manual on experimental (preclinical) study of new pharmacological substance]. Ed. 2. Moscow: Medicina, 832.

[22] Kovalenko, V. M. (2009). Doklinichni doslidzhennya likars'kykh zasobiv v Ukrayini [Preclinical studies of medicines in Ukraine]. Farmakolohiya ta likars'ka toksykolohiya, 5 (12), 56–61.

Original Research Article: full paper

(2017), «EUREKA: Life Sciences»
Number 1

THE ANALYSIS OF INDICES OF CEREBRAL BLOOD CIRCULATION IN WOMEN-SMOKERS

Lyudmila Aponchuk
Department of Human and Animal Physiology
Lesya Ukrainka Eastern European National University
13 Svobody ave., Lutsk, Ukraine, 43025
aponchykl@yandex.ru

Tatiana Shevchuk
Department of Human and Animal Physiology
Lesya Ukrainka Eastern European National University
9 Potapova str., Lutsk, Ukraine, 43025
tetyana_shevchuk_2013@ukr.net

Abstract

The results of rheoencephalography of female smokers 17–21 years old and control group were studied and analyzed. The aim of this work was to explain and analyze physiological features of smoking effect on functional changes of regional hemodynamics in smoking women.

The study of cerebral hemodynamics was carried out by the method of rheoencephalography (REG) – automated system of complex examination "Askold", intended for automation of medical tasks processing with input of information in "online" regime (insertion of data directly from examined person). The recording of rheogram was carried out in front-mastoid branches that allowed register REG separately in both hemispheres of brain and determine the main amplitude-temporal characteristics of cerebral blood circulation and changes of vascular tone.

Analyzing the main indices of regional hemodynamics in female smokers, the statistically lower values of time of rheowave delay (Ra) were noted. There was also revealed a decrease of volumetric cerebral blood circulation and increase of resistant arteries tone. There were fixed the moderate asymmetry (from 15 to 25 %) of blood filling in vertebral-basilar vascular basin (basin of spinal and internal carotid arteries) and the signs of complicated venous outflow in both hemispheres.

Such changes of indices indicate the decrease of volumetric blood circulation of cerebral hemodynamics, striking volume of blood and increase of tone of distribution arteries that testify to the decrease of blood circulation in main vessels, and also smoking is a cause of hypoxia (oxygen deprivation) of cerebral cells as a result of decrease of blood inflow. In the women of control group all indices are within norm that testifies to the normal course of physiological processes in organism.

Keywords: smoking, women-smokers, brain, vascular tone, blood circulation, rheoencephalography.

DOI: 10.21303/2504-5695.2017.00287

1. Introduction

The one of most important problem of the modern society is the increase of people with different types of addictions, and the most spread of them is smoking. The smoking and its medical-demographic and economic results are the subject of scientific interests of many native and foreign scientists [1–3]. Last time in Ukraine under conditions of social-demographic crisis the question of health protection of young generation, especially the one of student age, became urgent [4].

In most countries of the world the phenomenon of smoking and also the prevalence of diseases and deaths, caused by tobacco consumption are considered as an important medical, social and economic problem [5]. For today Ukraine belongs to the countries with high prevalence of smoking not only among men, but also women. Today near 20 % of women of working age smoke in our country [6].

The little doses of nicotine intensify the excitability of cerebral cortex for a short time, then oppress and exhaust the nerve cells that condition the imaginary increase of working capacity of smokers. Brain becomes inured to the nicotine "doping" and begins to request it, and in the case of lack the anxiety and irritability appear. The balance between excitation and inhibition is broken as a result of overexcitation of nerve cells that decreases the intellectual working capacity of brain, because of being exhausted [7].

The problem of cerebral vascular injuries is the one of urgent problems of health protection. Its medical and socio-economic importance is conditioned by significant specific weight of cerebral vascular pathology in the structure of morbidity and mortality of population, high indices of temporary disability and invalidism. The studies that demonstrate the interconnection between smoking and cerebral blood circulation are almost absent in modern literature, that is why this problem is urgent for today.

2. Aim of research

The aim of this work was to explain and analyze physiological features of smoking effect on functional changes of regional hemodynamics in smoking women.

3. Materials and methods

The research was carried out in laboratory of "Ecological physiology" of the department of human and animal physiology of biological faculty of Eastern European University, named after Lesya Ukrainka. The study of cerebral hemodynamics was carried out by the method of rheoencephalography (REG) [8] on the complex of program and automatic methods of medical examination of children and adults "Askold".

The recording of rheogram was carried out in front-mastoid branches that allowed register REG separately in both hemispheres of brain and determine the main amplitude-temporal characteristics of cerebral blood circulation and changes of vascular tone.

The examined contingent included female persons, 17–21 years old. They were divided in 2 groups (according to Fagerstrom test) [9]:

I group – women with smoking experience more than 3 years, who smoke more than 10 cigarettes a day and have the high level of addiction (30 persons);

II group – no-smoking women (30 persons).

To characterize the functional possibilities of cerebral blood circulation there were used the indications of rheoencephalography that characterize the value of pulse blood filling in vertebral basilar basin (basin of spinal and internal carotid arteries) of the right and left hemispheres, the condition of vascular wall (tone, elasticity), relative speed of blood circulation and also the ratio of arterial inflow and venous outflow.

The following indices of cerebral blood circulation were studied in examined persons: the period of pulse fluctuation (T), time of fast filling (ab), time of maximal filling (ax), time of rheowave delay (Ra), amplitude of fast filling (Ab), rheographic index (Ax), dicrotic index (A1), speed of fast filling (Vmax), mean speed of filling (Vmean), coefficient of asymmetry (CA).

For the analysis of results there were used conventional methods of parametric (for processing of quantitative values – Student t-criterion) and non-parametric (for processing of qualitative values – Wilcoxon W-criterion) statistics (depending on the character of values distribution). The statistical processing of data was carried out using MedStat software [10].

4. Results of research

The indices of two groups of examined were contrasted for comparative analysis. The analysis of rheoencephalorgaphy results demonstrated that the deviations of such indices from the norm were often observed in examined women (**Table 1**).

On the **Table 1** it can be seen, that the index of time of fast filling (ab) in women of both groups was lower than norm (0,111–0,121 s) almost by 50 % ($p>0,05$). It testifies to the decrease of elastic features of vessels and their resilience.

The index of time of rheowave delay (Ra) in female smokers is reliably lower ($p<0,05$), than in the control examined group and is 0,145 s (right hemisphere) and 0,144 s (left hemisphere) (**Table 1, Fig. 1**). The decrease of this interval is a sign of increase of tone of sclerosis of the main vessels.

In female smokers is observed the decrease of the index of time of maximal filling (ax) in the left hemisphere of brain from the norm 0,111–0,127 s (**Table 1**), that indicates the difference in tone and elasticity of arteries and big vessels and also their resilience but the reliable difference was not revealed in any group ($p>0,05$).

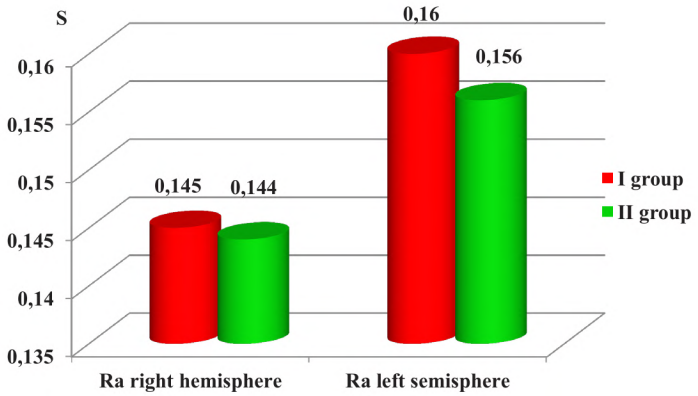

Fig. 1. The time of rheowave delay (Ra) in studied women

The amplitude of fast filling (Ab) in all examined women from both groups was decreased, but the reliable difference was not fixed (p>0,05). In women-smokers this index for right hemisphere was 0,051 Ohm, and in women of control group – 0,064 Ohm. The analogous tendency was traced for the left hemisphere (**Table 2**). The decrease of Ab index almost by 50 % was revealed in all examined women of both groups at the norm 0,124–0,148 Ohm, although in women-smokers, the decrease of this index is more expressed than in the control group (p>0,05) (**Table 2**).

Table 1

The parameters of rheoencephalogram of frontal mastoid branches in examined persons, (n=60)

Index	Hemisphere	Group I		Group II		Reliability of difference between groups
		Median	Error	Median	Error	
Ra, s	right	0,145	0,004483	0,160	0,01378	P<0,05
	left	0,144	0,004125	0,156	0,01681	P<0,05
ab, s	right	0,052	0,04129	0,048	0,01925	P>0,05
	left	0,052	0,1881	0,052	0,002255	P>0,05
ax, s	right	0,128	0,1866	0,128	0,02129	P>0,05
	left	0,126	0,186	0,128	0,01571	P>0,05
Ax, Ohm	right	0,081	0,033	0,0865	0,012	P>0,05
	left	0,088	0,011	0,089	0,010	P>0,05
A1, %	right	91,425	2,511	93,925	4,087	P>0,05
	left	90,62	2,675	93,495	4,451	P>0,05
V_{max}, Ohm/s	right	7,045	2,747	8	0,9264	p>0,05
	left	7,6675	0,5719	7,923	0,7911	p>0,05
V_{mean}, Ohm/s	right	3,3055	0,4392	4,66	0,7051	p>0,05
	left	3,15	0,8662	4,1735	0,7851	p>0,05
CA		17,81	4,614	26,535	11,79	P>0,05
Reliability of difference between hemispheres		P > 0,05		P > 0,05		

Note: <0,05 – data are reliably different

Table 2

The index of amplitude of fast filling (Ab, Ohm) in studied persons, (n=60)

Index	Hemisphere	Group I		Group II		Reliability of difference between groups
		Mean	Error	Mean	Error	
Ab	right	0,051	0,006	0,064	0,007	P>0,05
	left	0,054	0,004	0,056	0,006	P>0,05
Reliability of difference between hemispheres		P > 0,05		P > 0,05		

The decrease of the index of amplitude of fast filling testifies to the disorder of blood filling of cerebral vessels and is also a cause of hypoxia (oxygen deprivation) of cerebral cells as a result of decrease of amount of oxygen that is inhaled and increase of blood satiation with carbonic acid gas.

In two groups was not revealed the reliable difference (p>0,05) between the values of rheographic index (Ax) – an important parameter that gives a possibility to determine the relative value of pulse blood filling of intracranial vessels. Rheographic index is decreased in both groups of examined women comparing with norm (0,129–0,145 Ohm) at p>0,05. The **Table 1** distinctly indicates that the rheographic index less than 1 testifies to the decrease of blood supply of brain and decrease of volume of circulating blood (hypovolemia).

The rheographic parameter that reflects the tone state of mainly small and middle arteries is dicrotic index (A1), that is on average 50 %. It partially depends on peripheral vascular resistance and shows the tendency to increase in all examined persons. The increase of dicrotic index (A1) more than 70 % characterizes the increase of peripheral vascular resistance or hyper-resistivity of the vessels of microcirculatory channel, that can be observed in both groups of examined women at p>0,05 (**Table 1**).

In women-smokers the speed of fast filling (V_{max}) and mean speed of filling (V_{mean}) show the tendency to increase and are by 1 Ohm/s lower than in women of control group at p>0,05 (**Table 1**). It testifies to insufficient filling of small, middle and big arteries and decrease of vascular tone.

The data of rheoencephalogram gave a possibility to reveal the signs of complicated venous outflow (VO) from both hemispheres of brain not only in women-smokers but also in control group of examined women. In women-smokers was observed the complicated venous outflow from both sides – 75 %, for control – 52 %. In insignificant number of persons the venous outflow was complicated only at the right (6 % right hemisphere in women-smokers and the same number in the control group) or at the left (6 % right hemisphere in women-smokers and 13 % in the control group). The norm was registered in women of control group – 29 %, and for women-smokers only 13 %.

The decrease of tone of mean vessels was registered in left hemisphere of brain 30 % in women-smokers and 25 % in the control group of studied persons, at the right – 20 and 15 % of examined persons, respectively. In more than half of women from both groups the tone of middle vessels was increased in right and left hemisphere.

In most examined persons from both groups (women-smokers and control) the tone of small cerebral vessels was increased. It was normal only in insignificant number of examined persons.

The one of most informative and physiologically grounded indices of rheogram is a coefficient of asymmetry (CA) that reflects asymmetry of pulse blood filling of intracerebral vessels for right and left hemispheres. Our data demonstrated the moderate and essential asymmetry of blood filling within the studied basin, which values fluctuated within 15–25 % (women-smokers) and 26 % and more (control group) (**Table 1**).

The type of blood filling asymmetry is defined according to aforesaid parameters. Thus, we observed that in women-smokers S-asymmetry was traced in 24 %, and in control – 30 %. D-asymmetry was inherent to 50 % of women-smokers and 27 % of control group of examined persons. The absence of asymmetry of blood filling of cerebral vessels in women-smokers was diagnosed in 26 %, and in control group by 17 % more often (43 %).

According to the data of rheoencephalography, in 90 % of women-smokers and control group of examined persons the volumetric blood flow was decreased that is conditioned, most probably, by the presence of atherosclerotic changes of arteries of brachiocephalic zone.

The decrease of striking volume of blood is observed in almost all women of both examined group, it is increased only in 10–15 %. The norm is registered in women-smokers at the left and right only in 3 %, the same tendency in control group.

The peripheral resistance (PR) is significantly increased in women-smokers in both hemispheres of brain 94 %, and in the control group – 75 %. In 4 % of smoking women and in 20 % of control group PR is within norm. Separately in the right hemisphere was registered insignificant increase in women-smokers only in 1 %, in control group – 5 %. In 1 % of observed women-smokers PR is increased in the left hemisphere, and it was not registered in the control group.

5. Discussion of the results of research

At the study of cerebral blood circulation we established the reliably lower values of time of rheowave delay (Ra) in the group of women-smokers (1 group) in both hemispheres of brain as opposite to the women from control group at $p<0,05$.

As to the other parameters such as ab and Ax, they are also decreased in women-smokers within physiological norm (ab – 0,111–0,127 s., Ax – 0,129–0,145 Ohm), but the reliable difference between groups was not revealed ($p>0,05$). The decrease of norm indicates the difference in tone and elasticity of arteries and big vessels and also their resilience.

In the process of research was observed the tendency to increase of V_{mean} and V_{max}. Just this fact explains the abrupt fall of pressure in vessels at acute intoxication by nicotine that leads to vertigo, loss of consciousness and other negative results. In 2–3 min. after inhalation of smoke, nicotine already penetrates within cerebral cells and raises their activity for some time together with short-term widening of cerebral vessels and reflex effect of ammonia on nerve endings of respiratory tract that is subjectively perceived by the smoker as refreshing inflow of forces with smoothing action. But in short time the filling of energy inflow and excitation disappear – it is physiologically connected with coming narrowing of cerebral vessels and decrease of brain activity [11].

Computer interpretation of rheoencephalography data gave a possibility to reveal the signs of complicated venous outflow (VO) [12, 13] from both hemispheres of brain not only in women-smokers but also in control examined group.

Depending on the value of asymmetry coefficient several degrees of blood filling asymmetry are distinguished: if coefficient is 7 % and less, there is no essential difference of blood filling, at the value of asymmetry coefficient from 8 to 14 % the blood filling asymmetry is characterized as little. If the asymmetry coefficient is from 15 to 25 %, it testifies to the presence of moderate blood filling asymmetry, at coefficient 26 % and more, it is recognized as significant [14, 15]. Our data showed the moderate (I group) and significant (II group) asymmetry of blood filling within the studied basin.

There were observed changes that testify to the decrease of volumetric pulse blood filling, increase of tone of distribution arteries that is hypertone. That is why it must be noted, that smoking has negative influence on cerebral blood circulation that in further can result in pathologies.

REG data of previous studies [12, 13, 16–18] also revealed the elastic-tonic changes of vessels and presence of signs of complicated venous outflow in smokers.

There is no sufficient information as to reaction of different systems of organism. It is reported about the increase of tone of sympathetic section of VNS that is manifested in growth of frequency of heartbeat (FHB) and arterial pressure (AP) [19, 20].

Thus, we have studied the state of cerebral blood circulation in women at smoking. In further it would be expedient to study more contingent of examined persons and to divide them in several groups by term and number of smoked cigarettes to improve the results. The insufficient sample of examined persons can influence the reliability of results. At the same time the prospect of our research is the study of influence of smoking on central and peripheral hemodynamics that gives a possibility to make deeper analysis of functional state of cardiovascular system just in women-smokers.

6. Conclusions

1. The results of rheoencephalograpgy revealed that women-smokers are characterized with statistically lower values of time of rheowave delay (Ra) at $p<0,05$. The other parameters such as ab and Ax are also decreased in women-smokers within physiological norm, but the reliable difference was not revealed ($p>0,05$).

2. Such change of indices indicates the decrease of volumetric cerebral blood circulation, striking volume of blood, increase of tone of resistance arteries and distribution arteries. The elastic-tonic changes of vessels, manifested in insufficient filling of small, middle and big arteries, increase of peripheral vascular resistance and the signs of complicated venous outflow in smokers that is a cause hypoxia (oxygen deprivation) of cerebral cells as a result of decrease of blood inflow are present.

3. Most indices of the women from control group are within norm that can testify to the normal cerebral blood circulation in examined persons from II group.

References

[1] Ahulova, O. O. (2012). Osobystisni osoblyvosti molodykh zhinok iz nikotynovoiu zalezhnistiu [Personality characteristics of young women with nicotine dependence]. Visnyk Kharkivskoho natsionalnoho pedahohichnoho universytetu imeni H. Skovorody. Psykholohiia, 44 (1), 12–19.

[2] Ng, M., Freeman, M. K., Fleming, T. D., Robinson, M., Dwyer-Lindgren, L., Thomson, B. et. al. (2014). Smoking Prevalence and Cigarette Consumption in 187 Countries, 1980–2012. JAMA, 311 (2), 183. doi: 10.1001/jama.2013.284692

[3] Pirie, K., Peto, R., Reeves, G. K., Green, J., Beral, V. (2013). The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK. The Lancet, 381 (9861), 133–141. doi: 10.1016/s0140-6736(12)61720-6

[4] Belova, N. I., Burtsev, S. P., Vorobtsova, E. A., Martynenko, A. V. (2006). Otnoshenie studentov k zdorovyu i zdorovomu obrazu zhizni [The ratio of students to health and a healthy lifestyle]. Problemy sotsialnoy gigieny, zdravoohraneniya i istorii meditsiny, 1, 14–15.

[5] Preventing Tobacco Use among Youth and Young Adults (2012). US Departament of Health and Human Services. Executive Summary, 11.

[6] Kvasha, O. O., Gorbas, I. M., Smirnova, I. P. (2010). Mediko-sotsíal'niy proyekt «Zdorov'ye bez kureniya». Rekomendatsiyi z profilaktyky i likuvannya tyutyunopalinnya [Medical sotsialny Project «Health without smoking». Recommendations for prevention and treatment of tobacco]. Zdorov'ya Ukrayiny, 2 (231), 34–36.

[7] Chagayda, M. O. (2013). Vpliv palinnya na organizm lyudini [The impact of smoking on the human body]. Vseukrayinskiy naukovo-populyarniy zhurnal. Bezpeka zhittediyalnosti, 4, 5.

[8] Revenko, S. V. (2012). Garmonicheskie perspektivy reografi [Harmonic prospects rheographs]. Nervno-myshechnye bolezni, 4, 8–18.

[9] Smirnova, I. P., Kvasha, O. O., Gorbas, I. M., Davidenko, N. V. (2006). Rekomendatsiyi z profilaktyky i likuvannya tyutyunopalinnya [Recommendations for prevention and treatment of tobacco]. Kyiv, 16.

[10] Lyah, Yu. E., Guryanov, V. G. (2012). Matematicheskoe modelirovanie pri reshenii zadach klasifikatsii v biomeditsine [Mathematical modeling in solving the classification problems in biomedicine]. Ukrains'kiy zhurnal telemeditsini ta medichnoi telematiki, 10 (2), 69–76.

[11] Malko, M. M., Romanenko, K. V. (2012). Osoblyvosti elektrokardiohrafichnykh pokaznykiv pry dykhal′nyy funktsional′nykh probakh [Features electrocardiographic indicators in respiratory function tests]. Aktualni pitannya biologiyi, ekologiyi i himiyi, 1, 46–50.

[12] Vlasova, O. V., Popova, G. A., Kovalnogov, A. V. et. al. (2008). Izmeneniya tsentralnoy gemodinamiki i mozgovogo krovotoka pri kurenii u studentov [The changes of central hemodynamics and cerebral blood flow during smoking in students]. Vestnik NGU. Seriya: Biologiya, klinicheskaya meditsina, 6 (3), 48–55.

[13] Vlasova, O. V., Popova, G. A., Tsirkin, V. I. (2008). Pokazateli tsentral'noy gemodinamiki i mozgovogo krovotoka u zhenshchin pri kurenii [Central hemodynamics and cerebral blood flow in women with smoking]. Voprosy narkologii, 5, 77–86.

[14] Kuznetsov, A. A., Permyakov, S. A. (2012). O estestvennoy normalizatsii diagrammy ritma serdtsa [About the natural normalization of heart rate chart]. Trudy Nizhegorodskogo gosudarstvennogo tehnicheskogo universiteta im. R. E. Alekseeva, 4 (97), 363–368.

[15] Slobodinskiy, A. P., Vasilkivskiy, I. V., Petruk, V. G., Kvaternyuk, S. M. (2014). Vpliv promislovih aerozoliv na dinamiku pnevmokoniozu v Ukrayini [The impact on the dynamics of industrial aerosols pneumoconiosis in Ukraine]. Nauka. Molod. Ekologiya, 194–198.

[16] Korovina, L. D., Zaporozhets, T. M. (2015). Zv'yazki krovopostachannya golovnogo mozku studentiv zi stanom vegetativnoyi nervovoyi sistemi ta faktorami riziku [Links brain blood supply students with the state of the autonomic nervous system and the risk factors]. Vìsn. Dnìpropetr. Unìv. Ser. Biol. Med., 6 (1), 68–73.

[17] Perminov, A. A., Kuvshinov, D. Yu., Rybnikova, O. S. (2008). Gendernye osobennosti vliyaniya kureniya na vegetativnuyu nervnuyu sistemu u lits yunosheskogo vozrasta [Gender-specific effects of smoking on the autonomic nervous system in young people]. Sibirskiy meditsinskiy zhurnal, 3–2, 64–69.

[18] Syvolap, V. V., Poznanskaya, E. A., Nikulina, R. P., Vizir, A. V. (2014). Osobennosti intra-i ekstrakranialnogo krovotoka u bolnyh gipertonicheskoy boleznyu II stadii v zavisimosti ot statusa kurilshchika [Features of intra and extracranial blood flow in patients with essential hypertension stage II, depending on the smoker status]. Patologiya, 1 (30), 25–29.

[19] Arosio, E., De Marchi, S., Rigoni, A., Prior, M., Lechi, A. (2006). Effects of smoking on cardiopulmonary baroreceptor activation and peripheral vascular resistance. European Journal of Clinical Investigation, 36 (5), 320–325. doi: 10.1111/j.1365-2362.2006.01628.x

[20] Karakaya, O., Barutcu, I., Kaya, D., Esen, A. M., Saglam, M., Melek, M. et. al. (2007). Acute Effect of Cigarette Smoking on Heart Rate Variability. Angiology, 58 (5), 620–624. doi: 10.1177/0003319706294555

EFFECTS OF 4-THIAZOLIDINONE DERIVATIVES LES-2658 AND LES-1205 ON SLEEP – WAKEFULNESS CYCLE IN KINDLED RATS

Solomija Myronenko
Department of Pharmacology
Danylo Halytsky Lviv National Medical University
69 Pekarska str., Lviv, Ukraine, 79010
myronenko.solomia@gmail.com

Oleh Pinyazhko
Department of Pharmacology
Danylo Halytsky Lviv National Medical University
69 Pekarska str., Lviv, Ukraine, 79010
olehpinyazhko@gmail.com

Roman Lesyk
Department of Pharmaceutical and Bioorganic Chemistry
Danylo Halytsky Lviv National Medical University
69 Pekarska str., Lviv, Ukraine, 79010
roman.lesyk@gmail.com

Abstract

The research is dedicated to in-depth study of neurotrophic and antiepileptic properties of original potential anticonvulsant agents from 4-thiazolidinones – LES-2658 (5-(3-nitro-benzylidene)-2-(thiazol-2-ylimino)-thiazolidin-4-one) and LES-1205 ([2,4-dioxo-5-(thiazol-2-ylcarbamoylmethyl)-thiazolidin-3-yl]-acetic acid ethyl ester), synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry of Danylo Halytsky Lviv National Medical University, Ukraine. Studying of sleep – wakefulness cycle characteristics in animals with chronic epileptic syndrome in conditions of 4-thiazolidinones derivatives LES-2658 and LES-1205 use was performed. The kindling syndrome was induced in Wistar rats via daily pentylenetetrazol (PTZ) (30 mg/kg, i. p.) administrations during three weeks and sleep – wakefulness cycle was studied under conditions of LES-2658 and LES-1205 administrations at doses 25.0 and 100.0 mg/kg i. p.. Total wakefulness, non - rapid eye movement sleep, rapid eye movement sleep, falling asleep latency, REM – onset latency and also number of REM sleep episodes have been determined by behavioral characteristics of experimental animals. It was established that 4-thiazolidinone derivatives Les-1205 and Les-2658 reduce REM sleep fragmentation and increase its duration in PTZ-kindled rats. Les-1205 compound at dose 100.0 mg/kg show a clear correcting influence on kindling – induced sleep disturbances.

Keywords: pentylenetetrazol kindling, sleep – wakefulness cycle, seizure syndrome, 4-thiazolidinones, diazepam.

DOI: 10.21303/2504-5695.2017.00289

1. Introduction

Epilepsy is the most common neurological disorder and its prevalence worldwide is estimated at 0.5–1 % [1]. In most cases, pharmacotherapy is the only one method for satisfactory correction and control of convulsive states [2]. Despite the substantial progress in research and development of efficient anticonvulsants the search of new original anticonvulsant agents remains still open problem, especially in the context of pharmacoresistance and significant side effects under long use [3, 4].

The model of chronic epileptization of brain formed by repeated application of a subthreshold dose of pentylenetetrazol (PTZ) adequately reflects the signs of clinical forms of disease, including the disorders of sleep – wakefulness cycle [5, 6]. The study of the cycle allows estimate the brain mechanisms that control anxiety, to solve which mechanisms ensure its individual phases and to assess the influence of pharmacological agents on brain structure [5, 6]. Despite the well – es-

tablished relation between sleep and epilepsy [7], very few studies are available on the effect of an antiepileptic drug on the sleep – cycle.

4-Thiazolidinone derivatives are promising compounds in modern pharmacology for search of potential neurotropic agents with low acute and neurotoxicity properties [8–12]. Some 4-thiazolidinones demonstrate multi-level anticonvulsant activity with satisfactory toxicity parametrs in conditions of such screening models as pentylentetrazol (PTZ, metrazol) seizures, maximal electroshock test, rotorod test [8–12]. Earlier anticonvulsant activity of original 4-thiazolidinones Les-1205 – [2,4-dioxo-5-(thiazol-2-ylcarbamoylmetyl)-thiazolidin-3-yl]-acetic acid ethyl ester and Les-2658 – 5-(3-nitrobenziliden)-2-(thiazol-2-imino)-4-thiazolidinone (synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University) have been identified in our studies [13–16]. Until recently, the studying of influence on the sleep – wakefulness cycle characteristics in rats with chronic epileptic syndrome formed by PTZ kindling were not used for determination of 4-thiazolidinones anticonvulsant activity mechanisms.

2. Aim of research

The study of 4-thiazolidinone derivatives LES-2658 and LES-1205 influence on the sleep – wakefulness cycle characteristics in rats with chronic epileptic syndrome formed by pentylenetetrazol (PTZ) kindling.

3. Material and methods

All investigative procedures and the animal facilities were conformed to the Guide of Care and Use of Laboratory Animals within European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Stratsburg: Counsil of Europe 18.03.1986) and Law of Ukraine "On protection of animals from cruelty" (21.02.2006). For the study were used 54 male rats in age 5-6 months and weight ~180–220 gr (*Rattus Norvegicus Var. Alba, Wistar*). The animals were divided into 7 groups: one control (intact animals) group (n=6) and six experimental groups. For animals of all experimental groups the kindling syndrome was induced via daily pentylenetetrazol (PTZ) (30 mg/kg, i. p.) administrations during three weeks. Rats that the last three injections caused generalized tonic – clonic seizures were used for the experimental monitoring. The animals of experimental group I (n=9) were administered 0.9 % solution of NaCl; experimental group II (n=9) – animals were administered diazepam ("Gedeon Richter", Hungary), at dose 1.5 mg/kg, i.p., 30 minutes before PTZ; experimental group III (n=7) and IV (n=8) – animals were administered LES-2658 at doses 100.0 and 25.0 mg/kg respectively, i. p., 30 minutes before PTZ; experimental group V (n=7) and VI (n=8) – animals were administered LES-1205 at doses 100.0 and 25.0 mg/kg respectively, I p., 30 minutes before PTZ. The severity of seizures was evaluated according to five-point scale [5]. The tested rats were individually continuously monitored during 4 hours after 24 hours of the last application of PTZ under free behavior conditions and with identical degree of noise and lighting. All recordings were taken between 11.00 a. m. and 3 p. m. for a total recording time of 240 min. Total wakefulness (TW); total non-rapid eye movement sleep (NREM); total rapid eye movement sleep (REM); falling asleep latency (FAL) and REM – onset latency (RL) and also number of REM sleep episodes (RSE) have been determined by behavioral characteristics of experimental animals [6]. The durations of total wakefulness, NREM and REM sleep were given in % compared to the overall experiment duration (100 %); FAL and RL in minutes. Data were analyzed using the statistical criteria – ANOVA + Newmann-Keuls, and Mann-Whitney U-test. All values are expressed as means ± SEM. Differences were considered significant when: * $p \leq 0.05$.

4. Results

The changes were observed in sleep pattern of experimental animals under kindling conditions.

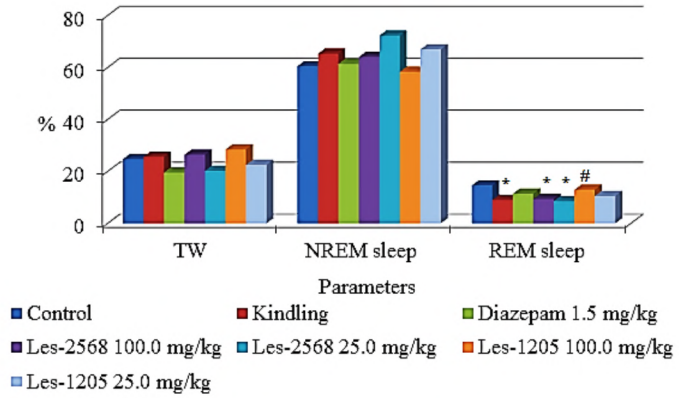

Fig. 1. The durations of total wakefulness, NREM and REM sleep under Les-2568, Les-1205 and diazepam administration in kindled rats. All parameters are given in % compared to the overall experiment duration (100 %). # – P<0.05 compared to the control group and * – P<0.05 compared to the kindled animals

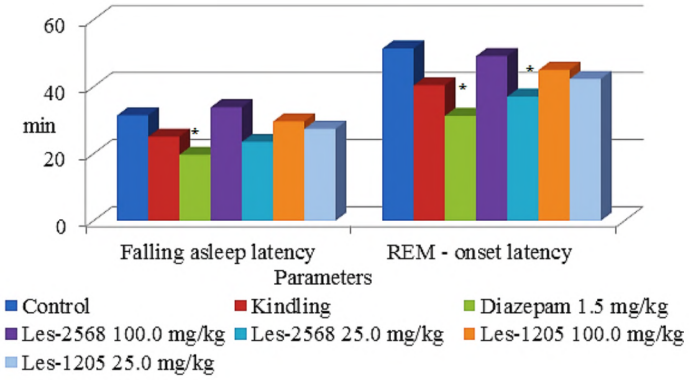

Fig. 2. The characteristics of falling asleep (FAL) and REM – onset (RL) latency under Les-2568, Les-1205 and diazepam administration in kindled rats.
* – P<0.05 compared to the kindled animals

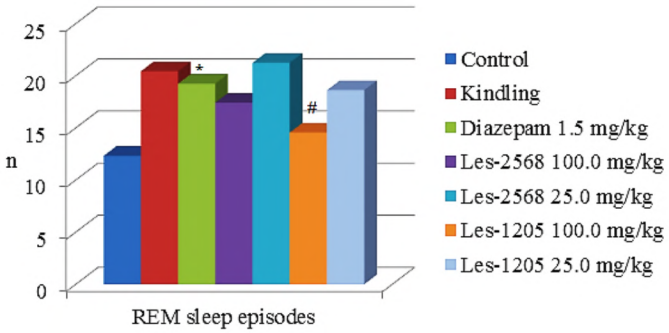

Fig. 3. The number of REM sleep episodes (RSE) under Les-2568, Les-1205 and diazepam administration in kindled rats. # – P<0.05 compared to the control group and
* – P<0.05 compared to the kindled animals

5. Discussion

The tendency to elongation of NREM sleep was observed in kindled rats. Its duration was 65.29±8.79 % and exceeded similar in the control group by 7.9 % (P>0.05) (**Fig. 1**). At the same time, duration of REM sleep decreased by 38.4 % and was 9.04±2.84 % with statistically significant (P=0.015). Furthermore the tendency to decrease of FAL by 20.1 % (25.1±9.48 min) as well as of RL by 20.3 % (40.41±13.1 min) (for all P>0.05) was observed in kindled rats compared to

group I (**Fig. 2**). Also, the number of RSE increased by 65.8 % (to 20.44±5.46) and was statistically significant with P<0.05 (**Fig. 3**).

It was established that administration of referenced drug and tested compounds LES-2658 and LES-1205 provoke changes in the in sleep pattern of kindled rats too.

The TW period was 19.6±5.94 % and decreased by 23.6% (P>0.05) as well as NREM sleep duration was 61.4±7.91 % and increased by 6.05 % (P>0.05) compared to kindled rats in rats when the diazepam was administrated at dose 1.5 mg/kg, i. p.. The duration of REM sleep was 11.45±2.06 % and increased by 26.6 % (P>0.05) but remained lower in comparison of intact rats group by 22.0 % (P>0.05) (**Fig. 1**). Also, FAL and RL were 19.69±6.48 min (P=0.021) and 31.31±8.36 min (P<0.05) and increased by 37.3 % and by 39.1 % respectively compared to the control group (**Fig. 2**). The number of RSE was 1.56 times higher (19.25±4.79) in comparison to the intact rats group (P=0.06) (**Fig. 3**).

When the LES-2658 was administrated at dose 100.0 mg/kg, i. p., duration of TW and REM sleep were 26.46±9.69 % (P>0.05) and 9.43±3.11 % (P<0.05) and increased by 3.1 % and by 26.6 % respectively (**Fig. 1**). While NREM sleep duration was 64.11±7.87 % and decreased by 1.8 % (P>0.05) compared to kindled rats. Also, FAL and RL were 33.86±13.36 min and 49.14±9.82 min and increased by 34.9 % and by 21.6 % (P>0.05 for all) respectively (**Fig. 2**). While the number of RSE was 17.43±3.10 and decreased by 14.7 % (P>0.05) compared to the kindled rats (**Fig. 3**). In case, when the LES-2658 was administrated at dose 25.0 mg/kg, i. p., duration of TW was 20.25±7.43 % and decreased by 21.9 % (P>0.05) in comparison of kindled rats (**Fig. 1**). While the NREM sleep duration was 72.31±6.14 % and increased by 10.7 % (P>0.05) compared to kindled rats and was higher than the same indicator in intact rats by 19.5 % (P = 0.043). Duration of REM sleep was 8.61±2.27 % (P<0.05) and remained lower by 41.4 % compared to the intact rats (**Fig. 1**).

Application of the Les-1205 at dose 100.0 mg/kg caused an increase TW duration (28.51±8.48 %) compared to kindled rats by 11.1 % (P>0.05) and compared to the II experimental group (diazepam used) by 45.5 % (P<0.05) (**Fig. 1**). Herewith the duration of NREM sleep phase was 58.4±9.0 % and slightly decreased by 10.6 % (P>0.05). The duration of the REM sleep was 12.94±2.58 % and increased by 43.1 % (P<0.05) (**Fig. 1**). Under these conditions, FAL and RL were 29.64±7.75 min and 45.0±13.06 min (P>0.05 for all) increased by 18.1 % and by 11.4 % respectively (**Fig. 2**). Also, the number of RSE was 14.57±3.73 decreased significantly by 28.8 % with P<0.05 (**Fig. 3**). When the Les-1205 was administrated at dose 25.0 mg/kg, i. p., duration of REM sleep phase was 10.57±2.51 % and increased by 16.9 % (P>0.05). Also, RL was 42.35±11.29 min decreased by 8.9 %, (P>0.05) in comparison of II experimental group (**Fig. 1, 3**).

For PTZ-induced kindling model such effects as decreasing of total sleep duration and increasing of its fragmentation were characteristic. These experimental results coincide with the research of other authors [4, 17, 18].

The use of 4-tiazolidinone derivative Les-1205 at dose 100.0 mg/kg in above-mentioned model of disorders of sleep – wakefulness cycle provoke opposite effects and is accompanied with increasing of the total sleep duration and decreasing of the number of its fragments. But for the derivative Les-2568 similar action was not observed and that can be explained by its less neurotrophic activity [8, 11].

Comparing of the impact of tested compounds with effects of diazepam showed that the for diazepam action was more typical reduction of FAL and RL in kindled rats and these data coincide with the results of other authors [19, 20]. Herewith, there was not observed an influence of diazepam on the REM sleep characteristics. The total duration of REM sleep was reduced and the number of fragments increased although was not statistically significant compared to the kindled rats group. Hence, this fact is in favor for opposite action of tested compounds and diazepam upon some components of sleep – wakefulness cycle.

So, the obtained and presented results on influence of 4-thiazolidinone derivatives Les-2658 and Les-1205 on sleep – wakefulness cycle in kindled rats cannot pretend to be the full solution of the search for new anticonvulsant agents. It is an attempt of studying and understanding anticon-

vulsant mechanisms and properties of 4-thiazolidinones as possible drug. And these results are promising for in depth-study using other different experimental seizure models.

6. Conclusion

1. Under developed kindling conditions the wakefulness and REM sleep phases were decreased while at one time NREM sleep phase was increased. Moreover there are reducing REM-onset latency and increasing fragmentation of REM sleep phase.

2. 4-Thiazolidinone derivatives Les-1205 and Les-2658 reduce REM sleep fragmentation and increase its duration in PTZ-kindled rats and compound Les-1205 at dose 100.0 mg/kg has been shown a clear correcting influence on kindling – induced sleep disturbances while diazepam at dose 1.5 100.0 mg/kg does not cause any corrective influence on the REM sleep of kindled rats.

3. Such ability of tested 4-thiazolidinones to recover of REM sleep parameters may indicate on importance in the implementation of anticonvulsant activity realization of this class of chemical compounds.

Acknowledgment

The authors would like to thank Dr. John L. Murphy (Anticonvulsant Screening Program, National Institute of Neurological Disordes and Stroke, National Institute of Health, Rockville, MD, USA) and specialists for specified institutions conducted *in vivo* testing anticonvulsant activity of the synthesized compounds. And also authors would like to thank Dr. L. Godlevsky and members of his scientific group (Odessa National Medical University, Ukraine).

References

[1] Berg, A. T., Scheffer, I. E. (2011). New concepts in classification of the epilepsies: Entering the 21st century. Epilepsia, 52 (6), 1058–1062. doi: 10.1111/j.1528-1167.2011.03101.x

[2] Perucca, P., Gilliam, F. G. (2012). Adverse effects of antiepileptic drugs. The Lancet Neurology, 11 (9), 792–802. doi: 10.1016/s1474-4422(12)70153-9

[3] Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Allen Hauser, W., Mathern, G. et. al. (2009). Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia, 51 (6), 1069–1077. doi: 10.1111/j.1528-1167.2009.02397.x

[4] Łuszczki, J. J. (2009). Third-generation antiepileptic drugs: mechanisms of action, pharmacokinetics and interactions. Pharmacological Reports, 61 (2), 197–216. doi: 10.1016/s1734-1140(09)70024-6

[5] Shandra, A. A., Hodlevskyy, L. S., Brusentsov, A. I. (1999). Kindling as a model of epileptic activity. Odessa: Astroprint, 276.

[6] Godlevsky, L. S., Kobolev, E. V., Mustyatsa, V. F., Drozdova, G. A. (2010). Modelling and mechanisms of suppression of epileptic syndrome. Moscow-Odessa, 350.

[7] Raol, Y. H., Meti, B. L. (1998). Sleep-Wakefulness Alterations in Amygdala-Kindled Rats. Epilepsia, 39 (11), 1133–1137. doi: 10.1111/j.1528-1157.1998.tb01303.x

[8] Myronenko, S. I., Kaminskyy, D. V., Nektegayev, I. O., Pinyazko, O. R., Lesyk, R. B. (2012). Search of new anticonvulsant agents among 4-thiazolidinones and relatedheterocyclic systems. Clinical pharmacy, pharmacotherapy and medical standardization, 1-2, 124–131.

[9] Lesyk, R., Zimenkovsky, B. (2004). 4-Thiazolidones: Centenarian History, Current Status and Perspectives for Modern Organic and Medicinal Chemistry. Current Organic Chemistry, 8 (16), 1547–1577. doi: 10.2174/1385272043369773

[10] Verma, A., Saraf, S. K. (2008). 4-Thiazolidinone – A biologically active scaffold. European Journal of Medicinal Chemistry, 43 (5), 897–905. doi: 10.1016/j.ejmech.2007.07.017

[11] Bhaumik, A., Chandra, M. A., Saha, S. (2014). Synthesis, characterization and evaluation of anticonvulsant activity of some novel 4-thiazolidinone derivatives. Sch. Acad. Journal Pharmaceutical, 3 (2), 128–132.

[12] Malik, S., Bahare, R. S., Khan, S. A. (2013). Design, synthesis and anticonvulsant evaluation of N-(benzo[d]thiazol-2-ylcarbamoyl)-2-methyl-4-oxoquinazoline-3(4H)-carbothioamide derivatives: A hybrid pharmacophore approach. European Journal of Medicinal Chemistry, 67, 1–13. doi: 10.1016/j.ejmech.2013.06.026

[13] Myronenko, S. I., Pinyazhko, O. R., Lesyk, R. B. (2015). Screening and studying of anticonvulsant activity of 4-thiazolidinones. Pharmacology and Drug Toxycology, 4-5 (45), 33–38.

[14] Myronenko, S. I., Pinyazhko, O. R. (2015). Dynamics of kindling-provoked seizures in rats under application of 4-thiazolidinones and diazepam. Achievements biology and medicine, 1, 63–67.

[15] Myronenko, S. I. (2016). Features of swimming behavior of pentylenetetrazole kindled rats under of 4-tiazolidinones and aksytynib use. Achievements biology and medicine, 1, 7–12.

[16] Myronenko, S. I., Pinyazko, O. R., Lesyk, R. B. (2016). The influence of original 4- thiazolidinone derivatives: Les-1205 and Les-2658, levetiracetam and diazepam on behaviour conditioned reflex active avoidance in rats. Pharmacology and Drug Toxycology, 4-5 (50), 27–33.

[17] Smirnov, I. V. (2008). Features of wakefulness – sleep cycle in rats in various stages of reproduction korazol kindling. Odessa Medical Journal, 2, 20–22.

[18] Schilling, M., Wetzel, W., Grecksch, G., Becker, A. (2006). Pentylenetetrazole Kindling Affects Sleep in Rats. Epilepsia, 47 (12), 2075–2082. doi: 10.1111/j.1528-1167.2006.00854.x

[19] Rudolph, U., Knoflach, F. (2011). Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. Nature Reviews Drug Discovery, 10 (9), 685–697. doi: 10.1038/nrd3502

[20] Tan, K. R., Rudolph, U., Luscher, C. (2011). Hooked on benzodiazepines: GABAA receptor subtypes and addiction. Trends in Neurosciences, 34 (4), 188–197. doi: 10.1016/j.tins.2011.01.004