Research of the toxicological and pharmacological effects of new blends on the body of biological objects

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Abstract

Introduction. A research aim is a toxicological study to the coupage from a digister that is used for making of low-alcoholic drinks.

Materials and methods. The drink made the method of natural fermentation contains to the 4,0% alcohol and mixture of extract substances of different plants with high antioxidant ability. Studied subsharp toxicness to the coupage and his influence on the prooxidant-antioxidant state of liver of white mise comparatively with solution of alcohol of 4,0% and beer of 4,0%.

Results and discussion. After intragastric introduction to the coupage in a maximal for these animals dose 36,1 g/kg during 14 days of signs of intoxication for animals were not observed. Middle mass of animals (24,33 g) kept indoors outside a physiology norm and did not differ ($p > 0,05$) from the indexes of middle mass of animals of group of intact control (24,33 g).

Mass coefficient of liver (0,1g/10,0g) for the animals of intact control (38,567) and those a coupage (39,467) was entered that, considerably differed from the liver of animals beer (42,867) and solution of ethyl spirit (45,633) was entered that, is beginning of development of scray displays, in particular hypertrophies of organ. A coupage diminishes in general lines toxic influence to the alcohol on the organism of animals that normalizes the coefficient of mass of liver.

Biochemical researches of homogenate fabrics are livers of animals a coupage shown that on content in the liver of diene conjugates (3,633 mkmol/g), TBA-reagents (2,500 mkmol/g), renewed glutathione (2,420 mkmol/g) and catalase (0,163 mkmol/min·g) did not statistically different from the indexes of animals of intact control and been within the limits of physiology norm. In the group of animals beer was entered that, content of renewed glutathione (2,267 mkmol/g) went down and activity of catalase (0,153 mkmol/min·g) diminished. In the group of animals solution of alcohol was entered that, reliable change ($p < 0,05$): content of TBA-reagents (3,033 mkmol/g) grew, content of renewed glutathione (2,333 mmol/g) went down and activity of catalase (0,157 mkmol/min·g) was repressed.

Conclusions. Drinks on the basis of investigated to the coupage due to the presence of the alcoprotector operating on a liver, can be an alternative to the modern beerhouses and low-alcoholic drinks that is produced with the use of alcohol ethyl.

Introduction

Today a consumption of alcohol is a meaningful social, cultural, psychological and medical factor. According to last given of Word Organization of ahealth a leader in the consumption of alcohol is Europe and poisoning an alcohol is reason of death 3,3 million people on a year in the whole world. Middle part of consumption of alcohol in Ukraine presents a 13,9 litre, from what 40% is on beer, beerhouses and low-alcoholic drinks [1].

First of all potential harm from the consumption of beer and some low-alcoholic drinks of brands with high maintenance of alcohol can be caused by that the process of their preparation envisages addition of clean food ethyl spirit without the admixtures of methanol and aldehydes [2]. This alcohol must be got by fermentation of products with high maintenance of glucose (from a beet, potato, feed treacle and other). However cheaper raw material for the receipt of alcohol ethyl are arboreal sawdusts (receipt of alcohol by the method of hydrolysis of cellulose). The alcohol got thus has high maintenance of admixtures: fusel oil, alcohols of C_3-C_6, methanol, that are very toxic for a human organism [3].

The above-mentioned grounds advantages of the drinks got by yeast fermentation without addition the alcohol (beer, wine, cider and other).

It costs to mark that an alcohol in any shape or form finds out the dose-dependent toxic operating on the organism of man. In case of overconsumption of alcohol there is progress of cardiovascular, chronic diseases of organs of digestion, there is an alcoholic psychosis, miorenalny syndrome, cerebral and lungs edema and others like that. Alcohol is particularly dangerous for children, teenagers, pregnant women, seniors [4].

High power value of alcohol together with insufficient content of microelements and vitamins negatively influence on the state of health of man and are reason of origin of serious alimentary problems [5].

Adding to the alcoholic beverages of extracts of plants, that have in the composition a complex of antioxidants, vitamins and microelements, can decrease the toxic action of alcoholic products and give the last certain useful properties [6].

The aim of the study. A toxicological and pharmacological study became the aim of this research to the coupage from a digister, that offers for making of low-alcoholic drinks, comparatively with solution of alcohol of 4,0% and beer of 4,0% (intragastric introduction to the white mise during 14 days one one time per days). Pharmacological research of drinks included the study of influence on prooxidant-antioxidant status of liver of animals.

Study is undertaken an on the base of the Central research laboratory of the National pharmaceutical university, that is certificated State Expert Centre of Ministry of Health of Ukraine, as a base of researches from experimental pharmacology. Before the beginning of experiment animals passed acclimatization in a room for testing during seven days; mise passed a quarantine and corresponding acclimatization according to operating norms.

Materials and methods

Composition and technology of preparation to the coupage from a digister for low-alcoholic drinks (BPMD) were worked out in the Kharkiv state university of feed and trade on the department of merchandizing on custom business. The marked drink is made the method of fermentation, he contains to the 4,0% alcohol and mixture of extract substances of different plants with high maintenance of antioxidants, mass %:

chokeberry 35,0–37,0;
dry hop cones 2,0–4,0;
pine needles 1,0–2,0;
ginger 0,3–0,5;
stevioside 0,3–0,5;
wine yeast 0,2–0,4;
water and whey (from the cheese dairy) – rest.

Chokeberry has in the composition: vitamins and provitamins (A, C, E, K, P, vitamins of group B), microelements (phosphorus, magnesium, manganese, iron), organic acids, tanning and pectin substances. These bioactive substances (BAS) positively influence to work of thyroid, cardiovascular and nervous system, show the bracing operating on the organism of man, increasing resistance to the unfavorable factors of environment [7].

The cones of hop contain dry flavonoids, essential oil and organic acids. Marked higher BAS able to find out седативну and antimicrobial action, to improve an appetite and general of organism of man [8-10].

Among BAS, that enter in the complement of pine-needle of pine-tree, also distinguished vitamins (A, E, C), microelements (zinc, cobalt, copper, calcium), resins, fat and organic acids, essential oil, glycosides and phenic connections that are antioxidant characteristics. The BAS of pine-needle of pine-tree bracing, antimicrobial and antiinflammatory properties find out [11].

A ginger root is antiseptic and restorative characteristics, high antioxidant potential due to high maintenance of essential oil able to inactivate free radicals of oxygen and improve circulation of blood of brain [12].

Stevioside (glycoside that is contained in the plants of sort of Stevia), that is included in composition to the coupage as a proof-reader of taste, capable to normalize a piesis, diminish the symptoms of heartburn, level of urinary acid and glucose in blood [13].

A lactoserum is a source squirrel, мікро- and macronutrients, a most value from that has a calcium. Laktoalbuminy and lactoglobulins (in composition serum proteins) contain most irreplaceable amino acids, also these albuminous factions are antioxidant characteristics [14].

A study of toxicness is the obligatory stage of research of new medical and food products, that allows to estimate the ununconcern of substances for the health of man. In this experiment studied subsharp toxicness that envisages the receipt of data in relation to toxic property of substance as a result of introduction of her during a limit time. BPMD entered to the animals during 14 days that answers two months of application for people.

Subsharp toxicness of BPMD of investigated comparatively with beer («Lviv light», the company «Carlsberg Ukraine», by volume part of alcohol of 4,0%, party № 16.06.15.08.30) and solution of alcohol of ethyl 4,0%. Conducted experiment on mise of both sexes at intragastric introduction that is envisaged for application of drink in practice and is expedient, taking into account possibility of casual situations that cause accidents, suicidal and criminal poisoning or cases of abuse of alcoholic beverages [15].

Study was undertaken an on 48 white nonlinear mise–males and females – with body weight 20,0–25,0 g. Before research animals were up-diffused after groups, for 6 animals in each.

Four hours prior to introduction of the investigated substances of animals did not feed. Intragastric introduction was carried out in the morning on an empty stomach. The investigated substances entered slowly by means of special metallic зонда, whereupon animals were held two hours without a meal, but with free access to water.

During the choice of doses for the study of subsharp toxicness at the terms of intragastric introduction introduction of maximally possible volume became a limiting factor for this type of introduction. In accordance with methodical recommendations for mise he presented 0,8 ml on an animal mass 25,0 g, that 32,0 ml/kg (Stefanov O.V., 2001) equal. The also entered amount of substance was enumerated on content of alcohol ethyl, an amount of that for non-permanent introduction 7,5 time had less than, than middle lethal dose. According to literary given, for the alcohol of the ethyl rectified high degree of cleaning a middle lethal dose presents 9,5 ml/kg (7,71 g/kg) [16]. The corresponding volume of the cleared water (table 1) was entered intact animals.

The term of watching animals for the study of subsharp toxicness according to methodical recommendations presented two weeks. Registered the displays of violations of the physiology state of animals, survivability, dynamics of body weight.

After completion a term the supervisions of animals killed by a counteretch, conducted a section and macroscopic inspection of internal organs (heart, liver, brain, kidneys, lungs, spleen, thymus, adrenals, gonads), mass coefficients (MC) expected them.

Table 1

Design of research of subsharp toxicness for BPMD, beer and solution of alcohol of 4,0%

Group	The dosage form substances ml/g (g/kg)	The dose for the active substance (calculated on the ethyl alcohol 96%) ml/kg (g/kg) at a temperature of 20°C	Number of animals in the group	
			males	females
Intragastric route of administration				
Intact control (purified water)	32,0 (32,0)	0 (0)	6	6
KPCCH	32,0 (36,1)	1,28 (1,02)	6	6
Beer	32,0 (34,4)	1,28 (1,02)	6	6
A solution of ethyl	32,0 (31,8)	1,28 (1,02)	6	6

The study of subsharp toxicness included for itself the biochemical analysis of indexes functioning of separate таргетних organs, to that the toxic action of substance is sent. As an alcohol the ethyl in the first turn strikes liver, violates antioxidant balance of hepatocytes and accelerates the processes of oxidation of peroxide of lipids for them, then in this research measured prooxidative and antioxidant markers at homogenate fabrics of liver of animals.

For the evaluation of the state of the antioxidant system of animals in homogenate livers determined: content of products of oxidationof peroxide of lipids, id est diene conjugates (DC) and products that react with 2-thiobarbituric acid (TBA-reactants); markers of activity of the antioxidant system, lutation (VH) is namely renewed and activity of catalase.

The markers of prooxidants balance of cages testify to activity of free-radical processes, antioxidants markers – about activity of enzymatic chain of antiradical defence of cages. The classic markers of prooxidants-antioxidants equilibrium are DC, TBA-reactants VH and catalase, here high value of VH and catalase and subzero value of DC,

TBA-reactants testify to normal status of cage, in another case - about activating of oxidation of peroxide of lipids and membrane destruction [17].

The content of diene conjugates in the liver tissue homogenate was determined by the formula

$$C(\text{mkmol/g}) = 227,27 \cdot Å\text{sample}, \quad (1)$$

where C – the contents of control;

E sample – absorbance sample studied.

The content of TBA-active products in homogenate of liver tissue studied animals determined by the formula

$$\tilde{N}(\text{mkmol/g}) = \frac{Å\text{sample}}{1,56 \cdot 10^5} \cdot 2 \cdot 10^6, \quad (2)$$

where C – the content of TBA-active products;

E sample – absorbance sample studied.

Calculation of reduced glutathione in the liver tissue homogenate was performed by the formula

$$C(\text{mkmol/g}) = Å\text{sample} \cdot 1094\text{ì g\%} \quad (3)$$

where C – glutathione content;

E sample – absorbance sample studied.

The activity of catalase in liver tissue homogenate calculated by the formula

$$Å\text{càt}(\text{mÌ } /1 \cdot \text{min}) = \frac{(À\text{êontr} - À\text{dosl})}{K \cdot t} \cdot V \cdot 10^6, \quad (4)$$

where E – catalase activity;

A_{kontr} and A_{dosl} – optical density (extinction) and idle studied samples;

V – volume samples (3,02 ml);

t – incubation time (10 min);

K – millimolar extinction ratio of hydrogen peroxide, 22,2 10^3 mM^{-1} sm^{-1}.

The obtained experimental data statistically processed the method of variation statistics. Experimental data were worked out by the methods of variation statistics with the use of standard package of softwares «Statistica 6.0» by means of t-criterion of Styudenta for independent selections, U-criterion of Manna–Whitney and transformation of Fisher. Reliable a difference was considered at the level of meaningfulness of $p < 0,05$ (calculated mean arithmetic and him standard error).

Results and discussions

After intragastric introduction of BPMD to the maximal for the marked animals dose – 36,1 g/kg during 14 days of signs of intoxication for animals were not observed: animals were trim, active, had a satisfactory appetite, reacted on voice and light irritants, processes

of urine and defecation were in a norm, violation of breathing and cramps was not. Reflex excitability all animals had stored. During watching animals during two weeks not a single animal perished from this experimental group. Comparison of behavior of animals, consumption of water and meal of experience and intact animals showed absence of no differences.

For animals that during 14 days gave beer of 4,0% in a maximally possible dose, also there were not signs of general intoxication, however agile activity was some less than, than for animals BPMD was entered that, and excretions to urine and defecation took place considerably more often. For 14 days not a single animal perished, but on a 13th day some females had cramps and change of behavior. For some animals the consumption of meal diminished and the consumption of water increased, the excited was marked, increase reaction on voice and auditory irritants.

In the group of animals, where solution of alcohol of ethyl 4,0% was entered mise in a maximally possible volume, there was the registered death of one animal (females) on the 10th day of introduction of substance. Beginning from 8 days of introduction of alcohol for some animals the changes of tint of wool (gray), oppression of motive activity (the states of oppression alternated with the states of an increase excitation) were marked, something excessive selection to urine, increase of consumption of water. Beginning from 10 twenty-four hours some animals had an inadequate reaction on introduction of drink through a probe, to the rumor and light irritants, violations of rhythms of dream and cheerfulness.

Changes in the index of body of animals weight registered on 4, 7 and 14th day introduction of drinks, that answered standard methodology. The masses of body of males and females changed proportionally, but did not differ after a dynamics, that is why it was expedient to compare the general middle masses of animals after groups.

In a group, where to the animals intragastric entered beer, on the 14th day of experience the increase of body weight was noticed, for certain higher than value in the group of intact control. Middle mass of animals solution of alcohol of 4% was entered that opposite, was for certain less than, than analogical index is in the groupof intact control. Middle mass of animals BPMD was entered that during all supervision kept indoors outside a physiology norm and did not differ ($p > 0,05$) from the indexes of middle mass of animals of intact control (table 2).

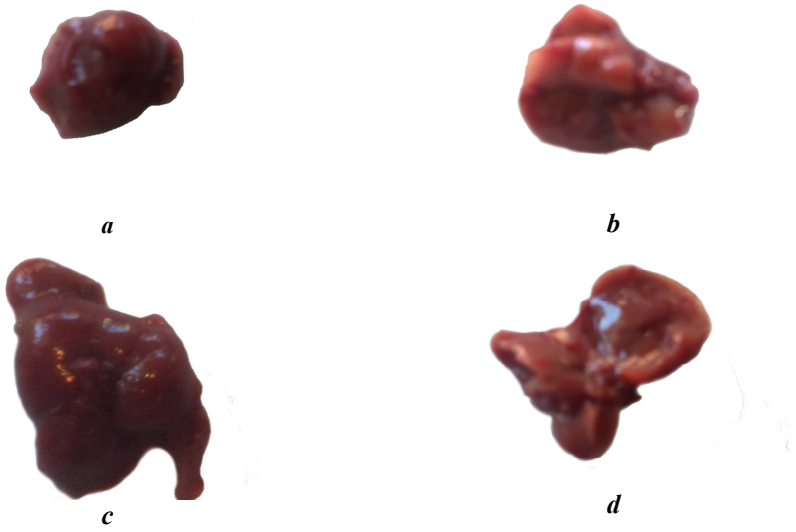
Autopsy and microscopic examination of internal organs of animals spent 14 days after the experiment beginning. The color, texture, finding organs of both sexes of mice control and study groups did not go beyond the physiological norm and did not differ among themselves. Liver of animals injected alcohol and beer were larger in intact mice and liver of animals which was administrated BPMD (Fid. 1).

Table 2

Dynamics of body weight of mice after administration BPMD compared with beer 4,0% solution of ethyl alcohol and 4,0% (g, M ± m)

Day experiment	The tested object			
	Intact control (n = 6)	BPMD (n = 6)	Beer (n = 6)	A solution of ethyl 4,0% (n = 6)
The initial values	19,83±0,87	20,33±0,84	20,17±1,01	20,2±0,66
4th day	21,00±0,97	21,17±0,60	21,83±0,87	21,4±0,51
7th day	22,67±0,67	22,67±0,42	23,67±0,56	22,2±0,66
14th day	24,33±0,67	24,33±0,33	26,00±0,26*	22,4±0,40*

* The change is likely on the values of intact control animals ($p < 0,05$).



Fid. 1. Appearance of livers of animals on day 14 of the experiment:
a – intact mouse liver;
b – mouse liver which was administered BPMD;
c – mouse liver which was administered beer of 4,0%;
d – mouse liver which was administered the solution of alcohol of ethyl 4,0%

After the calculation of MC of internal organs set, that this index does not differ for animals of both sexes intact control and groups of animals, BPMD (table 4, 5) was entered that. In the group of animals beer (for males and females) was entered that the masses of liver and kidneys increased for certain. Besides MC of gonads for males was for certain less than, than analogical index is in the group of intact control. For animals of both sexes, what 14 during days intragastric entered solution of alcohol of ethyl 4,0%, for certain MC of liver increased comparatively with a physiology norm. Also for males of this group MC spleens was anymore ($p < 0,05$) after MC of this organ for intact animals (table 3, 4).

The brought results over of researches from the study of subsharp toxicness to the coupage from a digister for low-alcoholic drinks showed that due to ingredients that is included in his composition, drink diminishes negative generaltoxic influence to the alcohol on the organism of animals, that normalizes the coefficient of mass of liver first of all. Biometrical index of relative mass of liver of animals, that used beer and solution of alcohol, more than for animals that used the investigated drink, is testifies to beginning of development of scray displays, in particular hypertrophies of organ, although maintenance of alcohol in all investigated drinks was identical.

The results of biochemical researches of homogenate fabrics of liver showed that content of DC, TBA-reagents, VH and catalase in the liver of animals BPMD was entered that, for certain did not differ from the indexes of animals of intact control and was within the limits of physiology norm.

For animals that consumed beer, the level of antioxidant markers (VH, catalase) diminished for certain, comparatively with the indexes of group of intact animals. Introduction solution of alcohol also negatively influenced on proantioxidant balance

tissues of liver of animals. Comparatively with the indexes of intact animals, maintenance of TBA–reagents grew for certain, content of VH went down and activity of catalase (table 5) was repressed.

Table 3

Odds mass of internal organs of male white mice after 14 days intragastric administration beverages studied, M ± m

Mass ratios of 0,1g/10,0g	Experimental group			
	Intact control (n = 6)	BPMD (n = 6)	Beer (n = 6)	A solution of ethyl 4,0% (n = 6)
Liver	38,567±0,173	39,467±0,931	42,867±1,252*	45,633±0,626*
Heart	3,333±0,021	3,333±0,021	3,400±0,110	3,333±0,165
Brain	16,567±0,148	16,567±0,152	16,500±0,073	16,500±0,159
Kidneys	9,667±0,042	9,633±0,165	10,233±0,056*	9,633±0,165
Adrenals	0,183±0,002	0,183±0,013	0,190±0,000	0,190±0,000
Spleen	3,813± 0,008	3,700±0,110	3,813±0,008	4,200±0,110*
Lights	6,633± 0,056	6,667±0,259	6,733±0,021	6,767±0,201
Thumus	0,933± 0,003	0,933±0,003	0,957±0,004	0,933±0,016
Testes	4,100 ± 0,037	4,033±0,076	3,767±0,021*	4,133±0,128

* The change is likely on the values of intact control animals ($p < 0,05$).

Table 4

Odds mass internal male white mice after 14th day intragastric administration beverages studied, M ± m

Mass ratios of 0,1g/10,0g	Experimental group			
	Intact control (n = 6)	BPMD (n = 6)	Beer (n = 6)	A solution of ethyl 4,0% (n = 6)
Liver	37,467±0,201	37,867± 0,152	39,967±0,595*	40,933±0,138*
Heart	3,500±0,146	3,733±0,112	3,600±0,073	3,800±0,073
Brain	16,100±0,123	16,133±0,220	16,167±0,259	16,233±0,148
Kidneys	9,533±0,076	9,367±0,092	10,267±0,117*	9,667±0,056
Adrenals	0,193±0,004	0,183±0,002	0,183±0,006	0,197±0,002
Spleen	3,767±0,092	3,800±0,110	3,800±0,037	3,867±0,056
Lights	6,200±0,110	6,067±0,076	6,167±0,128	6,200±0,037
Thumus	0,960±0,011	0,950±0,022	0,980±0,038	0,967±0,012
Ovaries	0,263±0,006	0,273±0,011	0,277±0,012	0,263±0,002

* The change is likely on the values of intact control animals ($p < 0,05$).

Table 5

The content of the antioxidant-markers in mice liver homogenate after 14th days intragastric administration beverages studied, n = 6

Indicator	Intact control	BPMD	Beer	A solution of ethyl 4,0%
Diene conjugates (mkmol/g)	3,700±0,132	3,633±0,056	3,833±0,092	4,033±0,105
TBA–reagents (mkmol/g)	2,733±0,092	2,500±0,058	2,800±0,126	3,033±0,084*
Glutathione (mkmol/g)	2,527±0,064	2,420±0,025	2,267±0,076*	2,333±0,021*
The activity of catalase (mkmol/g·min)	0,203±0,015	0,163±0,013	0,153±0,012*	0,157±0,008*

* The change is likely on the values of intact control animals ($p < 0,05$).

That BPMD in case of intragastric introduction during 14th days did not change proantioxidant balance of liver of animals it is in the first turn related to his composition. Vegetable and serum antioxidants that level harmful influence of alcohol on a liver enter in the complement of drink, promote activity of enzymatic chain of antiradical defence and activating of processes of окиснення of peroxide of lipids in hepatocytes, that is caused by the protracted use of alcohol, prevent.

Conclusions

The investigated product is a coupage for low-alcoholic drinks - did not have a toxic action during inwardly gastric introduction to the biological objects during 14 days in a maximally possible volume. Drink did not influence on macroscopic descriptions of internal organs of animals and their coefficients of mass, comparatively with beer and solution of alcohol of ethyl 4,0%.

From data of biochemical researches of homohenate liver of the mise shown out of experiment after research of subsharp toxicness, a coupage from a digister for low-alcoholic drinks did not change prooxidant-antioxidant balance of fabrics of liver and kept it within the limits of physiology norm. Beer and solution of alcohol of ethyl 4,0% changed this balance in direction of activating of processes of peroxidation.

Low-alcoholic drinks on the basis of investigated to the coupage due to the presence of the алкопротекторної operating on a liver can be an alternative to the modern beerhouses and low-alcoholic drinks that have an ethyl spirit in the composition.

Taking into account all brought indexes over, the drinks created on the basis of worked out to the coupage will be able to lay down a competition to modern low-alcoholic drinks and extend their assortment.

References

1. Global status report on alcohol and health (2014), *WHO Library Cataloguing-in-Publication Data*, Geneva.
2. C.Lucy, W.Jane, S.Claire (2008), Soft drink and «desire to drink» in preschoolers, *International Journal of Behavioral Nutrition and Physical Activity*, 5(1), pp. 60.
3. Miskevich D., Borodinskij A., Petushok N., Konovalenko O., Lelevich V. (2006), Preryvistaja alkogolizacija i pechen': svobodnoradikal'nyj gomeostaz, oksid azota, adaptacionnye mehanizmy, Biomedicinskaja himija, 5, pp. 489-495.
4. Watson R. (2014), *In soft drink marketing a funny thing happened on the way to market,* Authorhouse, Salt Lake City and Los Angeles.
5. Deitrich R., Zimatkin S., Pronko S. (2006), Oxidation of Ethanol in the Brain and Its Consequences, *Alcohol Research and Health*, 29(4), pp. 66-73.
6. Gorelikova G., Vasil'eva S., Adaeva A. (2009), Biotehnologicheskie aspekty poluchenija plodovyh vin iz mestnogo syr'ja, *Tehnika i tehnologija pishhevyh proizvodstv*, 3, pp. 19-22.
7. Najda A., Kabuda H. (2013), Content of phenolic compounds and antioxidant properties of fruits of selected orchard shrub species, *Modern Phytomorphology,* 3, pp. 105-109.
8. Ashurst P.P. (2008), *Chemistry and Technology of soft drink and fruit juice*, Wiley & Sons Ltd, Hardcover.
9. Holubkova A., Mosovska S., Sturdik E., Bologhova B. (2013), Hop pellets as an interesting source of antioxidant active compounds, *Scientific journal for food industry*, 7(1), pp. 53-57.
10. Shiva Shanker A., Kodaparthi A., Kumar Pindi P. (2012), Microbial diversity in soft drinks, *Journal of Pharmaceutical and Scientific Innovation*, 54(0), pp. 23-26.
11. Cheng M., Chang W., Chen C., Li W. (2015), Antioxidant properties ofessential oil extracted from pinus morrisonicola hay needles by supercritical fluid and identification of possible active compounds by GC/MS, *Molecules,* 20(10), pp. 19051-19065.
12. Eleaxu K.C., Eleaxu C.O. (2012), Physico-chemical Properties and Potential of 6 new varieties of ginger, *American journal of food technology,* 7(4), pp. 214-221.
13. Ashurst P., Hargitt R. (2009), *Soft drinks and fruit juice – problems solved,* Woodhead Publishing, Boston.
14. Suskovic J., Kirin S.,Frece J.(2009), Production of fermented probiotic beverages from milk permeate enriched with whey retentate and identification of present lactic acid bacteria, *Mijekarstvo,* 59(1), pp. 11-19.
15. Sigrid G., Susan M. (2013), Beveragesconsumptions habits"24/7" among British adults:association with total water intake and energy intake, *Nutrition Journal*, 12(1), pp. 9-12.
16. Babak O. (2006), Alkogol'naja bolezn' pecheni. Nauchnye dostizhenija i klinicheskie perspektivy, *Suchasna gastroentirogolija*, 6, pp. 4-9.
17. Krasnovskij A. (2004), Fotodinamicheskoe dejstvie i singletnyj kislorod, *Biofizika*, 2, pp. 305-321.