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M.Y. SPIVAK¹, N.M. DMYTRUKHA², I.L. GARMASHEVA^{1*},
T.Y. GROMOVOY³, O.G. VASYLENKO¹, D.K. ZADOROZHNA², O.M. KARMAN²,
M.V. NAUMENKO², O.V. HERASIMOVA², B.S. BOZHUK², T.V. ZHYLIKHOVSKA¹

¹ D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Akademika Zabolotnoho Str., Kyiv, 03143, Ukraine

² State Institution «Kundiiev Institute of Occupational Health
of the National Academy of Medical Science of Ukraine»,
75 Saksaganskogo Str., Kyiv, 01033 Ukraine

³ Chuiko Institute of Surface Chemistry NAS Ukraine,
17 Oleh Mudrak Street, Kyiv, 03164, Ukraine

* Author for correspondence; e-mail: garmasheva.il@gmail.com

THE USE OF *LACTOBACILLUS PLANTARUM* STRAINS FOR PREPARATION OF «BEETROOT DRINK» AND ITS PROTECTIVE EFFECT AGAINST TRANSIENT DRUG-INDUCED LIVER STRESS

Transient sub-molecular stress on the liver induced by medications represents an early stage in the potential development of drug-induced liver injury (DILI). Among natural protective agents, fermented red beetroot juice has recently attracted special attention. The aim of the work was to improve the composition of the starter for the «Beetroot Drink» to shorten the processing time and to study its protective effect on the liver function of patients undergoing rehabilitation in an occupational disease clinic and taking medications by the standard protocol. The study involved 15 male patients per group, experiencing transient increases in the liver biomarkers due to medication, with some exceeding normal ranges but insufficient for a DILI diagnosis. In the group consuming the drink prepared with a new Lactobacillus plantarum starter combination, biomarkers normalized after 14 days. Methods. «Beetroot Drink» was produced by fermentation of beetroot juice with a new composition of Lactobacillus plantarum strains that exhibits probiotic properties. Viable microorganism count, pH, titrable acidity, and betalains content were determined before and after fermentation and storage. The protective activity of the «Beetroot Drink» was evaluated in 15 volunteers who were treated at the Clinic of Occupational Diseases of the Kundiev Institute of Occupational Medicine of the NAMS of Ukraine. The «Beetroot

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Drink» was consumed at a dose of 50 ml daily for 14 days. Hematological and biochemical parameters were determined at the beginning of the experiment and after 14 days. Statistical significance was assessed using *t*-tests and Mann-Whitney *U*-tests ($p \leq 0.05$). **Results.** The use of the new composition based on the *L. plantarum* strain of the original «Beetroot drink» starter and the *L. plantarum* strain of the «Lactocap» significantly shortened the time of obtaining the beverage compared to the original one. The pH value of 4.0–4.2 corresponded to the required for «Beetroot drink» after 6 hours, and the titrable acidity was 24 ± 2 °T. Inoculation of the beetroot juice led to significant inhibition of autochthonous bacteria growth compared to non-inoculated spontaneously fermented juice. After pasteurization, the concentrations of betacyanins and betaxanthins decreased by 24.5% and 13.7%, respectively. In the fermented «Beetroot drink», the number of betalains remained unchanged for 14 days of storage. Consumption of «Beetroot Drink» reduced transient elevations in the liver biomarkers and improved lipid metabolism. **Conclusions.** The new *L. plantarum* strain combination can be used for «Beetroot Drink» production with a protective effect against transient drug-induced liver stress. A positive effect was observed in 80.0% of cases, with all biomarkers returning to normal ones in the study group, suggesting its potential as a preventive agent for individuals under drug-induced stress.

Keywords: fermented red beet juice, lactic acid bacteria, probiotics, functional activity, hepatoprotective effect.

Over the past decades, medications have been associated with transient sub-molecular stress on the liver, characterized by temporary elevations in biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, which represent an early stage in the potential development of drug-induced liver injury (DILI) (Williams & Hoofnagle, 1988). Statistical data show hepatotoxicity accounts for 10% of the total side effects of taking medications (Zimmerman, 2000; Aithal & Kulkarni, 2023). In Ukraine, drug-induced liver damage is the main cause of acute liver failure and accounts for 50% of the total structure of acute jaundice (Nedashkivskiy, 2018). To prevent the development of drug-induced liver injury, studies are being conducted to find means of prevention, which include the development of new safety guidelines and methods of drug management, identification of risk factors, and the use of natural effective hepatoprotectors (Zhuravlyova & Shekhovtsova, 2018; Osyodlo et al., 2022). One of these hepatoprotectors is functional foods, which not only have nutritional properties but also have a targeted effect on the functional activity of organs and systems of the human body. Functional foods include red beetroot (*Beta vulgaris*), which is the most popular traditional vegetable grown and widely consumed in Ukraine, containing high amounts of biologically active substances including microelements (Na, K, Ca, Mg, Zn, Mn, Fe,

and Cu), dietary fibres, proteins, carbohydrates, vitamins (A, K, C, E, B5, B6, B12), betalains, and inorganic nitrates (Gliszczynska-Swiglo et al., 2006; Wruss et al., 2015; Clifford et al., 2015; Pohl et al., 2019; Babarykin et al., 2019a; Janiszewska-Turak et al., 2023). Among functional foods, fresh and fermented vegetable juices have recently attracted special attention (Peter, 2002; Kazimierczak et al., 2014; Casciano et al., 2022). The mechanisms of the positive impact on human health of beetroot juice are being intensively studied (Webb et al., 2008; Babarykin et al., 2019b). The role of red beetroot juice nitrates in the regulation of blood pressure and the effect on iron metabolism, cholesterol reduction, and blood clotting regulation have been described (Webb et al., 2008). A positive effect of beetroot juice on the gastrointestinal tract and cardiovascular system, as well as the ability to increase the body's endurance to physical activity, has been shown (Babarykin et al., 2019a; Babarykin et al., 2019b). Fermentation increases the usefulness of beetroot juice by enriching it with lactic acid bacteria metabolites, extending the range of its biological activity. It was shown that fermented beetroot juice has potent antioxidant, antihypertensive, hepatoprotective, anticancer, antimicrobial, antidepressant, hypocholesterolemic, immunomodulatory, anti-inflammatory, and probiotic properties (Kazimierczak et al., 2014; Kim et al., 2021; Kumari et al., 2022).

At the Zabolotny Institute of Microbiology and Virology, researchers from the Department of Physiology of Industrial Microorganisms have developed a starter based on lactic acid bacteria strains for the production of fermented beet juice, «Beetroot Drink». The production process was carried out in two stages and took 24–30 hours, due to the rate of carbohydrate fermentation and acidity accumulation in beet juice by the strains of the original starter (*Lactobacillus plantarum* 11/16 (UKM B-2694) and *Enterococcus faecium* K-50 (UKM B-2535)). In 1982–1992, many studies showed that «Beetroot Drink» had a positive effect on the human gastrointestinal tract and cardiovascular system. Based on the results obtained, «Beetroot Drink» was recommended for therapeutic and preventive consumption, especially for people working in harmful conditions (Kovalenko et al., 1990).

The work aimed to improve the composition of the starter for the «Beetroot Drink» to shorten the processing time and to study its protective action on the liver function in patients experiencing transient drug-induced liver stress during rehabilitation in an occupational disease clinic.

Materials and Methods. *Bacterial strains.* The *Lactobacillus plantarum* 11/16 (UKM B-2694) strain of the original «Beetroot drink» starter (Kovalenko et al., 1990) and the *L. plantarum* LMB B7565 strain of the starter for vegetable fermentation «Lactocap» (Vasyliuk et al., 2020) were used in this work. Overnight, bacterial cultures growing in MRS broth were mixed at a ratio of 1:1 (v/v) and used as inoculum.

Beetroot juice fermentation. «Beetroot drink» was produced under laboratory conditions. Fresh beetroot was purchased at a local market, washed, and peeled. Juice was squeezed from the vegetables with a juice extractor. Beetroot juice was filtered, diluted with distillate water (1:1 v/v), and pasteurized for 20 min at 80 °C. After pasteurization, the juice was cooled to room temperature, inoculated by *L. plantarum* strains (5% v/v), and incubated at 37 °C to rich

pH 4.0–4.2. After fermentation, the «Beetroot drink» was stored at a temperature of 4 °C. Non-pasteurized and non-inoculated juices were used as control. The pH of the juice samples was measured by pH meter, and the total titrable acidity (TTA) was determined by titration with 0.1 N NaOH until the pH reached 8.5.

Microbiological analysis. Serial dilutions of juice samples were spread on a plate with Sabouraud agar for a total count of yeast and mold determination, Meat–Pepton agar — for a total count of mesophilic and aerobic bacteria, and MRS agar — for a total count of LAB and cultivated at appropriate temperature for 24–72 h. Viable microorganism counts were determined before and after fermentation. The total LAB count was also determined after storage.

Betalaine concentration determination. Betalain pigments were quantified spectrophotometrically as previously reported (Fernández-López et al., 2002). UV/Vis absorption spectra of beetroot juice in the range from 220 to 700 nm were recorded using a DeNovix DS-11 FX+ spectrophotometer (DeNovix, USA). Betacyanins and betaxanthins (C_B) were expressed as betanin and vulgaxanthin I content, respectively, and calculated according to the formula:

$$C_B = A \times DF \times MM \times 1000 / (\varepsilon \times L),$$

where A is the absorption (A_{538} for betanin and A_{476} for vulgaxanthin I), DF — dilution factor, MM — molar mass (MM of betanin — 550 g/mol, vulgaxanthin I — 339 g/mol), ε — a molar extinction coefficient (60.000 l/(mol×cm) for betanin, 48.000 l/(mol×cm) for vulgaxanthin I), and L — path length (cm) (Wruss et al., 2015). Betalain content was assessed as the sum of the concentrations of betacyanins and betaxanthins (Skalicky et al., 2020).

Protective effect evaluation. The protective activity was assessed in 15 male volunteers (study group) who consumed 50 mL of «Beetroot Drink» prepared with the new combination of *L. plantarum* stains daily for 14 days. The con-

trol group (15 men) received the same treatment protocol without the drink. Both groups, aged 35–45 years, were undergoing rehabilitation and taking medications (antibiotics, NSAIDs) causing transient elevations in liver biomarkers (ALT, AST, and bilirubin). In some participants of both groups, biomarkers exceeded normal ranges, but these elevations were insufficient to meet diagnostic criteria for DILI, indicating transient sub-molecular stress rather than established pathology. Chronic liver diseases (alcoholic, viral, NAFLD) were excluded via HBV/HCV serology, alcohol history, and metabolic syndrome screening. Transient stress was characterized by a hepatocellular pattern (ALT/ALP ratio, $R > 2$) (Lee et al., 2015). The male-only cohort reflects the clinic's demographics (industrial workers), but women, who are more prone to drug-induced stress, should be included in future studies (Lee et al., 2015). The study complied with bioethical requirements, with informed consent obtained. Measurements were taken at the baseline and after 14 days. The sample size (15 per group) is sufficient for a pilot study, but larger studies (≥ 20 per group) are needed for 80% power ($\alpha=0.05$).

Biochemical and hematological analyses. Aminotransferases (ALT, AST), alkaline phosphatase, glucose, bilirubin, cholesterol, triglycerides, uric acid, urea, and creatinine were measured using a HumaLyzer 2000 analyzer (Germany) with ELITech Group kits (Netherlands). The AST/ALT ratio confirmed a hepatocellular pattern of transient stress (Williams & Hoofnagle, 1988). Complete blood counts were performed on a HORIBA ABX Micros 60 analyzer (France).

Statistical analysis. Data were analyzed using STATISTIKA 6.1. Results are presented as mean \pm standard deviation. Normality was assessed with the Shapiro-Wilk test. Differences between groups were evaluated using the independent t-test (normal distribution) or Mann-Whitney U-test (non-normal distribution), with $p \leq 0.05$.

Results. Fresh beetroot juice had pH 6.2 ± 0.2 , TTA 19.2 ± 0.8 °T, and the total betalain content

1062.67 ± 117.93 mg/L (60.5 % of betacyanins and 39.5% of betaxantins). Betalains are pigments that are specific for plants belonging to the *Centrospermae* order. They can be divided into two structural groups, the yellow betaxanthins and the red-violet betacyanins, both of which have betalamic acid as their basic structure, with the chromophore as the 1,7-diaheptamethinium system, and differ mainly in the radicals associated with the basic structure. Their color is due to the conjugation of the substituted aromatic nucleus with the diaz system, which shifts the absorption maximum from ca. 535 nm in betacyanins to almost 480 nm in betaxanthins (Fernández-López et al., 2002).

The titratable acidity and betalain concentration decreased twice after diluting the fresh juice with water in a 1:1 ratio (v/v). Microbiological analysis of fresh juice revealed the presence of a wide range of microorganisms, including bacteria, yeast, and molds. Non-pasteurized (control) and pasteurized juices were inoculated by a combination of *L. plantarum* strains to evaluate the effect of heat treatment on the fermentation process. The initial LAB counts in unpasteurized and pasteurized juices were 8.29 ± 0.17 and 8.13 ± 0.07 lg CFU/mL, respectively, and increased to 9.53 ± 0.04 and 9.34 ± 0.20 lg CFU/mL, respectively. In the fresh non-inoculated juice, the count of LAB was less than 2 lg CFU/mL.

Incubation of non-pasteurized juice (spontaneous fermentation) for 24 h resulted in significantly increasing the number of autochthonous bacteria, whereas no intensive growth of yeast and mold was observed. It was found that inoculation of fresh juice by a combination of *L. plantarum* strains resulted in the inhibition of beetroot autochthonous bacteria growth after 24 h of incubation but did not affect the content of yeast and mold compared to the spontaneously fermented juice (Fig. 1).

The pasteurized non-inoculated and LAB-inoculated beetroot juices had a count of indigenous microbiota less than 2 lg CFU/mL at the beginning and after 24 h of fermentation. Pasteurization led to a non-significant change in the

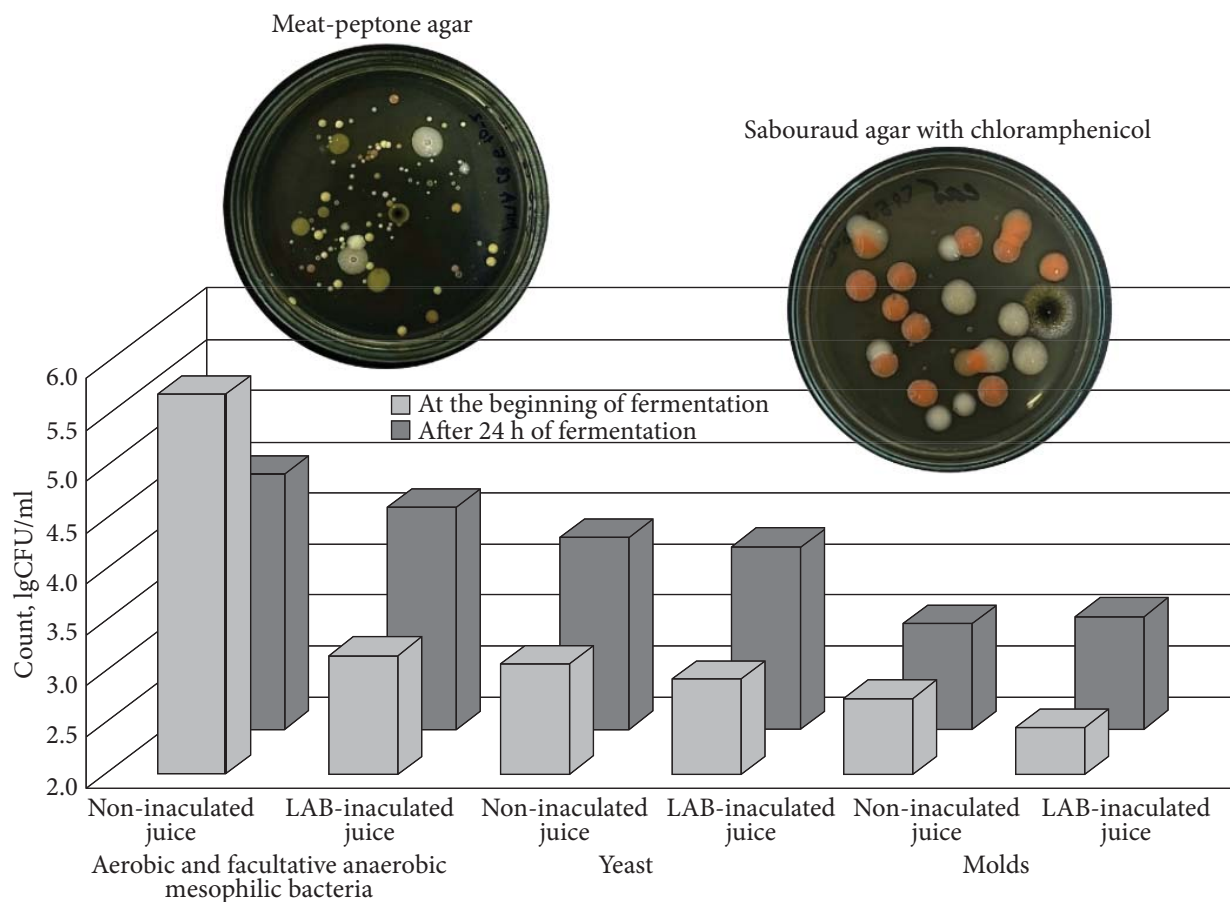


Fig 1. Influence of *L. plantarum* strains on the count of microorganisms in non-pasteurized red beetroot juice during fermentation

pH and TTA values, whereas the concentrations of betacyanins and betaxanthins decreased by 24.5% and 13.7%, respectively (Fig. 2).

Immediately after inoculation, the pH of the diluted juice decreased by an average of 1 unit and after 6 hours already corresponded to the required «Beetroot drink» pH value of 4.0–4.2, and the titrable acidity was 24 ± 2 °T. Further incubation (up to 24 h) resulted in a pH of 3.7 ± 0.2 and a titrable acidity of 66 ± 2 °T, which gave the drink a strong sour taste. At the same time, LAB-inoculation and fermentation for 24 hours did not affect the level of betanin ($p > 0.05$), while the concentration of vulgoxanthin I decreased by 28.7%.

All beetroot juice samples were stored in a refrigerator for 14 days. In non-pasteurized juice,

the pH decreased to 5.3 ± 0.2 , and yeast and spore-forming bacteria remained viable. A slight decrease in the pH of the fermented juice and a decrease in the number of LAB to 8.73 ± 0.12 lg CFU/mL was observed. After storage, the absorption peaks at 538 nm and 476 nm were completely absent in the not-processing juice, and the concentration of betalains in pasteurized juice was 61–66 % lower compared to the initial. At the same time, the concentration of betalains in the fermented «Beetroot drink» was almost unchanged compared to the initial (Fig. 3).

At the baseline, some participants in both groups exhibited elevations in liver biomarkers (ALT, AST, bilirubin) exceeding normal ranges, but these values were insufficient to meet diag-

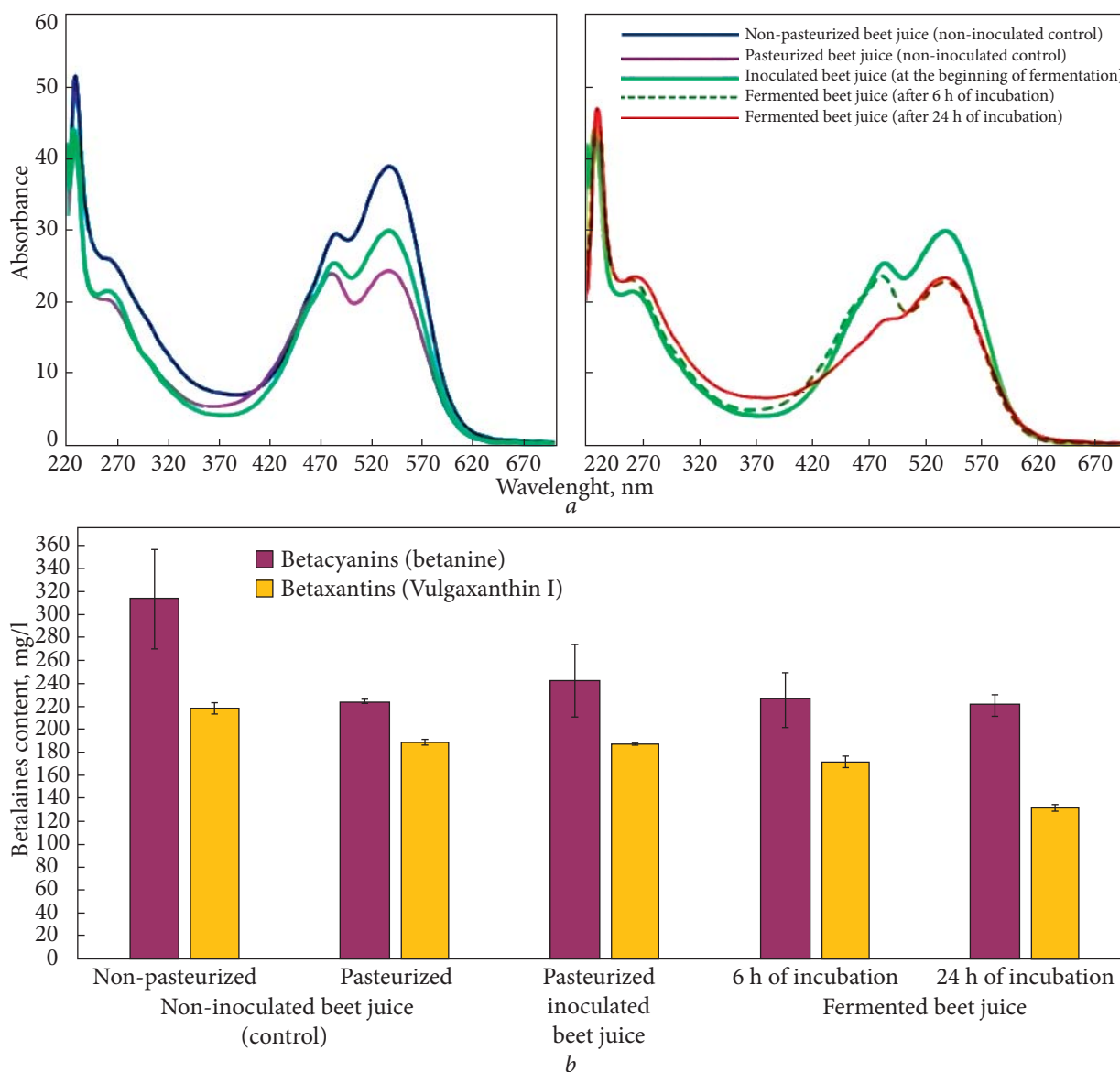


Fig. 2. UV/VIS absorption spectra (A) and betalains concentration (B) during «Beetroot Drink» production

nostic criteria for DILI, indicating transient drug-induced stress. In the study group (15 men), after consuming «Beetroot Drink» for 14 days, all biomarkers returned to normal ranges, with significant reductions in total bilirubin (21.0%, $p=0.03$), ALT (38.6%, $p=0.01$), AST (32.5%, $p=0.02$), cholesterol (11.0%, $p=0.04$), and triglycerides (32.0%, $p=0.01$) compared to baseline, and no significant ALP change ($p=0.45$). In contrast, the control

group (15 men) showed sustained elevations, with increased ALT (56.8%, $p=0.01$), AST (13.5%, $p=0.04$), and ALP (13.4%, $p=0.05$), remaining above normal in some participants. The AST/ALT ratio in the study group remained <1 (mean 0.65 at baseline and 0.71 after 14 days), suggesting a hepatocellular pattern of stress (Williams & Hoofnagle, 1988). The consumption of «Beetroot Drink» improved gastrointestinal function and

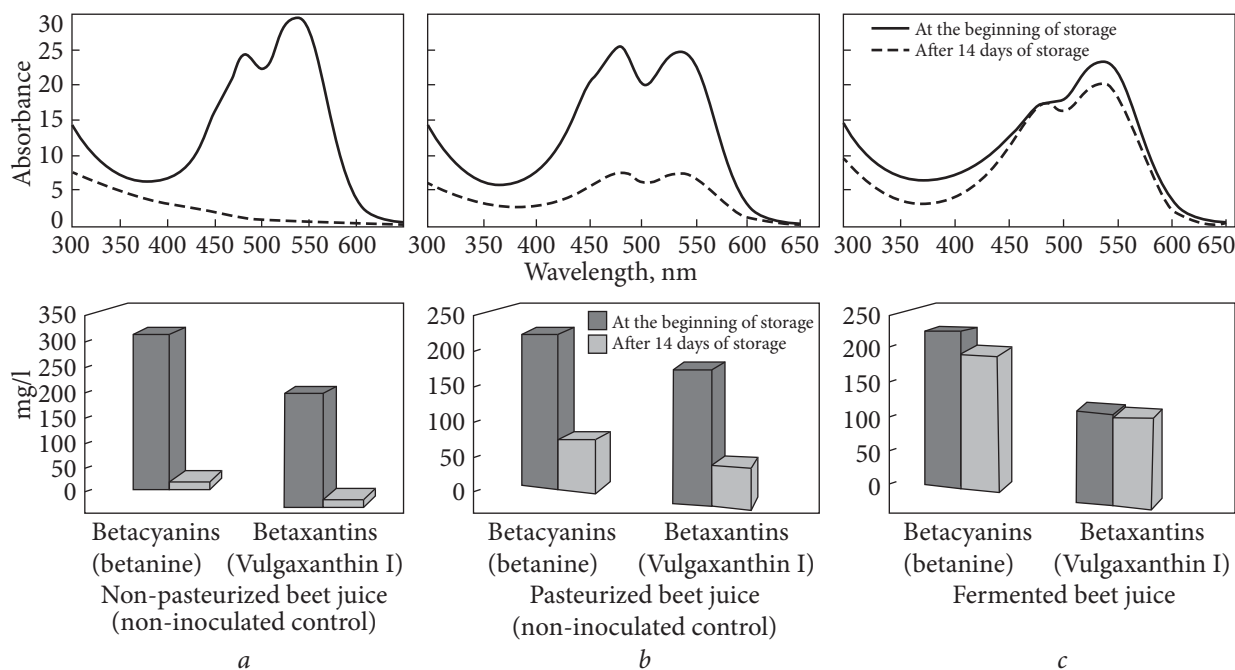


Fig. 3. UV/VIS absorption spectra and betalains content in beetroot juice samples after storage

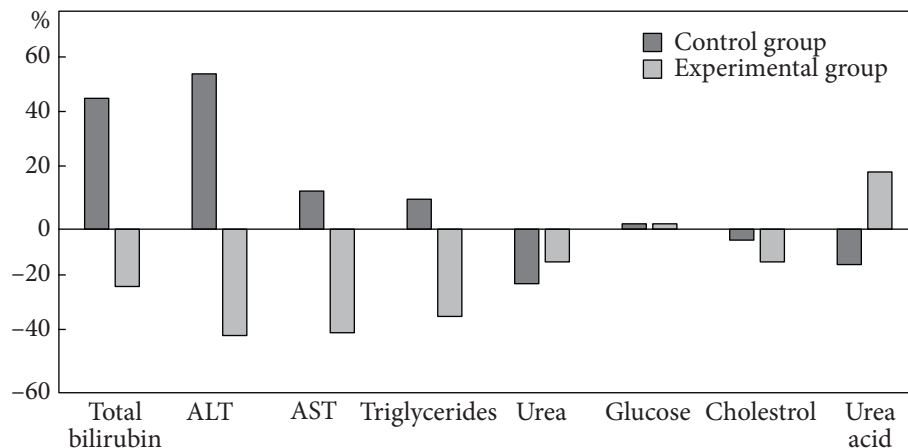


Fig. 4. Differences in biochemical blood parameters of the examined individuals after 14 days of observation, relative to the initial levels, %

lipid metabolism. At baseline, 53.0% of the study group had elevated triglycerides, which normalized post-treatment. In the control group, triglycerides increased in 33.0% of patients (Fig. 4).

Hematological parameters in the control group showed no significant changes, while the study group had increased monocytes by 34.7% ($p=0.03$), reduced platelets by 10.0% ($p=0.04$) and thromb-

ocrits by 14.3% ($p=0.05$) (Table 2). All parameters remained within normal limits (Tables 1 and 2).

Discussion. To improve the composition of the starter for «Beetroot drink» to shorten production time and enhance its functional properties, the possibility of using *Lactobacillus plantarum* strains with probiotic properties of the «Lactocap» starter for the fermentation of beet-

root juice was investigated in this work. A new composition based on the *L. plantarum* strain of the original «Beetroot drink» starter and the *L. plantarum* strain of the «Lactocap» starter was created. The use of the new composition significantly shortens the time of obtaining the finished beverage compared to the original one.

Inoculation of the beetroot juice with *L. plantarum* strains composition led to a significant pH drop and significant inhibition of autochthonous bacteria growth compared to non-inoculated spontaneously fermented juice. These results are consistent with the data obtained by authors who showed that the use of LAB starters has a preser-

Table 1. Changes in biochemical parameters of blood serum of patients who consumed beetroot drink during treatment by the clinical protocol (M ± m)

Parameters	Control group (10 per.)		Experimental group (15 per.)		Norm
	At the beginning	After 14 days	At the beginning	After 14 days	
Total bilirubin, mmol/L	11.18 ± 3.86	16.57 ± 2.99	15.29 ± 3.81	12.11 ± 2.99	up to 21.0
Urea, mmol/L	3.95 ± 0.97	3.18 ± 0.72	3.79 ± 1.85	3.41 ± 0.43	2.14—7.14
ALT, U/L	19.82 ± 10.95	31.15 ± 15.52	35.87 ± 23.77	22.08 ± 9.97	< 45
AST, U/L	16.10 ± 2.50	18.28 ± 3.08	23.30 ± 7.67	15.74 ± 3.44	< 35
Glucose, mmol/L	4.52 ± 0.28	4.63 ± 0.45	5.21 ± 0.49	5.34 ± 0.70	4.1—5.9
Cholesterol, mmol/L	3.69 ± 0.33	3.55 ± 0.58	4.33 ± 0.45	3.88 ± 0.57	up to 5.17
Triglycerides, mmol/L	0.87 ± 0.19	0.96 ± 0.40	1.64 ± 0.51	1.12 ± 0.28	0.7—1.7
Uric acid, μmol/L	372.33 ± 23.83	286.77 ± 51.31	269.69 ± 60.26	327.27 ± 52.67	148.7 ± 45.82

Table 2. Changes in hematological blood parameters of patients who consumed beetroot drink during treatment by the clinical protocol (M ± m)

Parameters	Control group		Experimental group		Norm
	At the beginning	After 14 days	At the beginning	After 14 days	
Leukocytes, 10 ³ /mm ³	5.82 ± 1.46	6.42 ± 1.24	6.75 ± 1.09	6.90 ± 1.16	4—10
Lymphocytes, 10 ³ /mm ³	1.82 ± 0.24	1.87 ± 0.27	1.73 ± 0.32	1.74 ± 0.23	1.2—3.2
Lymphocytes%	34.85 ± 1.05	32.57 ± 1.46	27.51 ± 2.57	26.35 ± 3.39	17—48
Monocytes, 10 ³ /mm ³	0.22 ± 0.06	0.28 ± 0.09	0.23 ± 0.08	0.34 ± 0.13	0.3—0.8
Monocytes%	4.72 ± 0.82	5.05 ± 0.62	4.37 ± 1.30	5.89 ± 1.37	3—10
Granulocytes, 10 ³ /mm ³	3.78 ± 1.31	4.27 ± 1.38	4.79 ± 1.29	4.82 ± 0.94	1.2—6.8
Granulocytes%	60.43 ± 6.88	62.38 ± 10.09	68.12 ± 8.68	67.76 ± 4.17	43—76
Hemoglobin, g/dl	14.08 ± 0.55	14.12 ± 1.05	14.65 ± 1.22	14.02 ± 1.40	11.0—16.5
Eosinophils, %	3.67 ± 0.89	1.50 ± 0.50	2.83 ± 0.22	1.90 ± 0.80	1—4
Band neutrophils, %	3.00 ± 1.00	3.83 ± 0.83	2.89 ± 1.01	3.00 ± 0.60	1—6
Segmented neutrophils, %	58.00 ± 5.00	60.00 ± 7.00	62.89 ± 5.46	64.20 ± 4.60	49—72
Erythrocytes, 10 ⁶ /mm ³	4.41 ± 0.19	4.54 ± 0.28	4.67 ± 0.30	4.71 ± 0.33	3.8—5.8
Hematocrit, %	41.20 ± 1.17	41.65 ± 2.78	43.85 ± 3.60	42.04 ± 4.15	30—50
Platelets, 10 ³ /mm ³	229.67 ± 50.67	224.83 ± 36.50	260.60 ± 59.60	235.40 ± 44.60	180—320
Platelets, %	0.18 ± 0.05	0.17 ± 0.03	0.21 ± 0.05	0.18 ± 0.03	0.4
ESR, mm/h	4.67 ± 2.89	6.17 ± 1.89	8.11 ± 1.51	7.90 ± 1.06	1—10

vative effect on beet juice due to more rapid acidification (Czyżowska et al., 2006). The fresh juice used in this study contained a LAB count of less than 2 lg CFU/mL. The number of lactic acid bacteria in fresh beet juice ranged from 3.0×10^2 to 2.5×10^3 CFU/mL depending on variety and was not sufficient for rapid acid accumulation and pH decrease (Czyżowska et al., 2006). Using the next-generation sequencing techniques, authors showed that the raw juices contained approximately 0.2% *Lactobacillus* species, but in juices inoculated with an LAB culture, the *Lactobacillus* count reached 50% — 97% after fermentation. Inoculation also led to a decrease in the relative abundance of pathogens (Choińska et al., 2022)

After 24 hours of fermentation, the LAB count reached 9.5 lg CFU/mL and after 14 days of storage, a decrease by 0.77 lg CFU/mL (8%) was observed. Similar results were obtained by Klewicka and Czyżowska (2011).

One of the most important valuable components of beetroot is betalains, which are responsible for antioxidant properties. As shown by the authors, high variation in the quantity and quality of betalains can be observed in red beetroot juices extracted from different beetroot cultivars (Wruss et al., 2015). Beetroot juice used in the present study contains 643.4 ± 47.7 mg/L of betacyanins and 419.3 ± 35.7 mg/L of betaxantins. These results are in agreement with the data of the authors for most of the beetroot varieties, who reported betacyanins concentration ranged from 49.40 to 137 mg/100 mL, and that of betaxantins — from 7.21 to 48.27 mg/100 mL (Czapski et al., 2009; Fernández-López et al., 2023). Higher concentrations of betacyanins and relatively low levels of betaxanthins also were found in different beetroot varieties (Wruss et al., 2015; Skalicky et al., 2020; Fernández-López et al., 2023).

It is known that the content of betalains is relatively unstable and influenced by various factors, including temperature and pH (Skalicky et al., 2020). Differences in betalain absorbance values during «Beetroot» production that were observed

in this work may be due to changes in its structure as a result of temperature treatment and changes in pH value. The pasteurization used in this study (80 °C for 20 min) resulted in decreasing the concentration of betanin and vulgoxanthin I by 24.5% and 13.7%, respectively. The results obtained are comparable to those of other authors. In the work of Klewicka and Czyżowska (2011), the content of betalains in beetroot juice decreased by 21% (from 1.27 to 1.01 mg/mL) after pasteurization at 80 °C for 10 min. In other studies, authors showed that thermal treatment of fresh juice at 45 °C and 85 °C for 10 min had no significant effect on the content of betacyanins and betaxantins (Buniowska-Olejnik and Marszałek, 2022). Immediately after inoculation of pasteurized juice with LAB strains, a decrease of pH value by 1 unit and a slight increase in absorbance at 538 nm were observed. As shown by the authors, most of the thermal reactions of the degradation of betanin are reversible, and the acidic environment favors the regeneration of betanin to its original form (Klewicka, 2012). Besides, a recent study has shown that heat treatment of betanin at 99 °C for 60 min, which almost completely degrades betanin, does not affect the biological activity of red beetroot juice, in particular, its antioxidant activity. This may be because the products of betalains degradation also exhibit biological activity (Muramatsu et al., 2023). After 14 days of storage, the color loss was observed in the untreated juice as a result of betalain destruction. According to the literature, the degradation of betalains also may be due the activity of beetroot endogenous enzymes, such as β -glucosidases, polyphenoloxidases, and peroxidase (Liu et al., 2008). It can be speculated that pasteurization partially destroys these enzymes, as about 40% of betalains were maintained in the pasteurized non-inoculated juice. In the fermented «Beetroot drink», the number of betalains remained unchanged for 14 days of storage. By the literature, the fermentation process can have a preservative effect on beet juice pigments (Czyżowska et al., 2006). The studies conducted

by Klewicka and Czyżowska (2011) revealed that betalains in lacto-fermented juice are stable for up to 30 days of refrigerated storage.

Elevated ALT, AST, and bilirubin indicate transient sub-molecular liver stress from medications, an early precursor to DILI (Williams & Hoofnagle, 1988). In this study, some participants in both groups had biomarkers exceeding normal ranges at the baseline, but these elevations were insufficient to diagnose DILI, reflecting transient stress rather than pathology. The AST/ALT ratio (<1) and ALT/ALP ratio (>2) confirmed a hepatocellular pattern (Lee et al., 2015). After 14 days, the study group consuming «Beetroot Drink» showed normalization of all biomarkers, while the control group exhibited sustained elevations above normal in some participants. Similar data were obtained in animal experiments, where orally administered red beetroot extract exhibited a considerable decrease in the activity of serums alanine transaminase, aspartate transaminase, and alkaline phosphatase and a notable increase in albumin serum and total proteins levels in rats chronically exposed to silver nanoparticles (Albrahim & Alonazi, 2020). «Beetroot drink» affected lipid metabolism, normalizing elevated cholesterol and triglyceride levels in 53.0% of patients in the study group. At the same time, «Beetroot drink» did not significantly af-

fect the cellular composition of peripheral blood but promoted the activation of phagocytic cells and reduced the content of platelets. Therefore, we can conclude that «Beetroot drink» activates the formation of phagocytic cells, as well as blood clotting factors synthesis. In our opinion, these changes occurred both due to the activity of LAB probiotic strains of improved starter culture and due to the special composition of beetroot drink (content of free amino acids, including essential, organic acids, and vitamins B, PP, C, E). The hepatoprotective effect of the «Beetroot drink» may be due to the antioxidant properties of betalains, which protect hepatocytes from damage.

Conclusions. The new *L. plantarum* strains combination can be used for faster production of «Beetroot drink» with a protective effect against transient drug-induced liver stress. A positive effect was observed in 80.0% of cases in this pilot study (15 men per group), with all biomarkers returning to normal ones in the study group after 14 days, unlike the control group where elevations persisted. Larger studies, including women, are needed due to their higher susceptibility to drug-induced stress. The «Beetroot drink» shows promise as a preventive agent for individuals under drug-induced stress.

Conflict of Interest. The authors declare that there are no conflicts of interest.

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М.Я. Співак¹, Н.М. Дмитруха², І.Л. Гармашева¹, Т.Ю. Громовий³, О.Г. Василенко¹, Д.К. Задорожна², О.М. Карман², М.В. Науменко², О.В. Герасімова², Б.С. Божук², Т.В. Жилиховська¹

¹ Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

² ДУ «Інститут гігієни праці ім. Ю.І. Кундієва НАМН України», вул. Саксаганського, 75, Київ, 01033, Україна

³ Інститут хімії поверхні ім. О.О. Чуйка НАН України, вул. Олега Мудрака, 17, Київ, 03164, Україна

ВИКОРИСТАННЯ ШТАМІВ *LACTOBACILLUS PLANTARUM* ДЛЯ ПРИГОТУВАННЯ «БУРЯКОВОГО НАПОЮ» ТА ЙОГО ЗАХИСНИЙ ЕФЕКТ ПРОТИ ТРАНЗИТОРНОГО МЕДИКАМЕНТОЗНОГО СТРЕСУ ПЕЧІНКИ

Транзиторний субмолекулярний стрес на печінку, спричинений лікарськими засобами, є ранньою стадією потенційного розвитку медикаментозного ураження печінки (DILI). Серед натуральних ефективних гепатопротекторів ферментований сік червоного буряка останнім часом привертає особливу увагу. **Метою** роботи було удосконалити склад закваски для «Бурякового напою» для скорочення часу його приготування та вивчити профілактичний вплив «Бурякового напою» на функцію печінки пацієнтів, які проходять реабілітацію в умовах клініки профзахворювань та приймають медикаментозні препарати за стандартним протоколом. У дослідженні брали участь 15 пацієнтів чоловічої статі в кожній групі, в яких спостерігалось тимчасове підвищення біомаркерів печінки внаслідок прийому ліків, причому деякі з них перевищували нормальні показники, але були недостатніми для встановлення діагнозу DILI. У групі, яка вживала напій, приготований з новою комбінацією штамів *Lactobacillus plantarum*, біомаркери нормалізувалися через 14 днів. **Методи.** «Буряковий напій» отримували шляхом ферментації бурякового соку новою композицією штамів *Lactobacillus plantarum*, які проявляють пробіотичні властивості. Визначали кількість життєздатних мікроорганізмів, рН, титровану кислотність та вміст беталаїнів до і після ферментації та зберігання. Гепатопротекторну активність «Бурякового напою» оцінювали в 15 добровольців, які перебували на лікуванні в клініці професійних захворювань Інституту медицини праці ім. Ю.І. Кундієва НАМН України. «Буряковий напій» вживали в дозі 50 мл щодня протягом 14 днів. Гематологічні та біохімічні показники визначали за загальноприйнятими методами на початку експерименту та через 14 днів. Статистичну значущість оцінювали за допомогою t-критерію та U-критерію Манна-Уїтні ($p \leq 0.05$). **Результати.** Використання нової композиції на основі штаму *L. plantarum* оригінальної закваски «Буряковий напій» та штаму *L. plantarum* препарату «Лактокап» значно скорочує час отримання готового напою. Необхідне для «Бурякового напою» значення рН 4.0—4.2 досягалось через 6 годин, а титрована кислотність становила 24 ± 2 °Т. Інокуляція бурякового соку привела до значного пригнічення росту автохтонних бактерій порівняно з неінокульованим соком спонтанної ферментації. Після пастеризації концентрація бетаціанінів та бетаксантинів зменшилася відповідно на 24.5% та 13.7%. У ферментованому «Буряковому напої» кількість беталаїнів залишалася незмінною протягом 14 днів зберігання. Вживання «Бурякового напою» позитивно вплинуло на загальний стан здоров'я добровольців, збільшило кількість моноцитів і гранулоцитів у крові, позитивно вплинуло на функцію печінки та ліпідний обмін. **Висновки.** Нова комбінація штамів *L. plantarum* може використовуватися для виробництва «Бурякового напою» із захисною дією проти транзиторного медикаментозного стресу печінки. Позитивний ефект спостерігався у 80.0% випадків, при цьому всі біомаркери поверталися до норми в досліджуваній групі, що свідчить про його потенціал як профілактичного засобу для осіб, які зазнають медикаментозного стресу.

Ключові слова: ферментований сік червоного буряка, молочнокислі бактерії, пробіотики, функціональна активність, гепатопротекторна дія.