

Lyudmila Reshetnyak<sup>1</sup>,  
Oksana Vitriak<sup>2</sup>,  
Anton Serenko<sup>3</sup>

## IMPROVEMENT OF THERMAL TREATMENT TO ENHANCE BIOLOGICAL RESISTANCE OF FERMENTED BEVERAGES

<sup>1</sup>National Aviation University  
1, Kosmonavta Komarova ave., Kyiv, 03580, Ukraine  
<sup>2,3</sup>Kyiv National University of Trade and Economics  
19, Kyoto Street, Kyiv, 02156, Ukraine  
E-mail: <sup>1</sup>isr@ukr.net; <sup>2</sup>vitrox@ukr.net; <sup>3</sup>anton\_serenko@ukr.net

### Abstract

**Purpose:** The perfection the modes of thermal treatment for the increase of biological resistance of the fermented drinks that were got with the use of culture *Medusomyces gisevii*. **Methods:** We investigated the effect of thermal treatment in the range 55...80°C for 10-60 minutes to change the physicochemical and organoleptic characteristics of fermented beverages during storage and storage conditions of beverages in glass bottles (temperature, filling factor). **Results:** It is established that to increase the shelf life of the beverage in the optimal processing conditions are the temperature ranges and duration, respectively, of 63°C and 40 min to 75°C and 15 min. The optimum fill factor for drink based on the culture of *Medusomyces gisevii* in glass bottles is 0.90-0.96. **Discussion:** The stability of non-alcoholic fermented beverages obtained using *Medusomyces gisevii* culture, treated with pasteurization under conditions of two treatment regimes: 63°C and 40 min. and 75°C and 15 min was investigated. Taking into account the rather mild conditions of thermal treatment and achieved a bacteriostatic effect during 60 days of storage at 30°C, the 1th mode can be recommended as acceptable to the increase of biological firmness of drink. Storage of beverages at temperatures up to 12°C can stabilize the microbiological state up to 180 days. In the 2nd mode of heat treatment the stability of the beverage remained stable at a storage temperature of 30°C for 180 days, and at 12°C – not less than 210 days. The use of modes of thermal processing of beverage within the specified limits allows guaranteed shelf life of 2 to 6 months without significant reduction of organoleptic characteristics and content of biologically active substances compared to unprocessed beverage.

**Keywords:** biological resistance; culture *Medusomyces gisevii*; expiration date; fermented drink; fill factor of bottles; tea mushroom; thermal treatment.

### 1. Introduction

Mineral water, juices and soft drinks, belong to the products of everyday demand and daily consumption. The tendencies of healthy feed that was folded in society lately influence on market of soft drinks development in Ukraine. The segments of sweet water, power drinks grow short due to the increase of consumption of functional drinks from natural raw material. Producers react on it by expansion of the assortment line by functional drinks that contain juice, extracts of spice-aromatic herbs and others like that. Also, at the market of soft drinks of Ukraine the marked tendency to high demand on products that has the comfortable

packing and high firmness at storage without addition of synthetic preservative [1].

### 2. Analysis of recent research and publications, problem statement

In recent years in Ukraine are highly popular non-alcoholic fermented drinks (or fermented beverages). These include drinks such as kvass, honey drinks, fermented drinks based on sugar sorghum, as well as beverages such as "tea mushroom", "rice mushroom" and others. Drinks of this group contain a balance of amino acids, enzymes, vitamins, minerals, due to which they have a positive biomedical effect on the human body [2, 3].

Fermented beverages (even filtered) contain residual cells of the mixed culture of microorganisms used for fermentation. Therefore, an important indicator of this group of drinks is their stability during storage. For unpasteurized fermented beverages, this index is governed by the term of not less than 7 days, and for pasteurized – not less than 30 days [4]. The existing technology of fermented non-alcoholic beverages provides a period of storage at a temperature not higher than 12°C no more than 2 days, or include heat treatment at high temperatures, which leads to a decrease in the content of biologically active substances in the finished beverage and deterioration of its taste [5]. Thus, the study of and search for optimal ways to increase the biological stability of the fermented beverages is an important task and has both scientific and practical importance.

It is known that such a fermented drink as a "tea mushroom" is produced by leaven - the culture of *Medusomyces gisevii*, which is a mixed population of microorganisms and consists of yeast and acetic acid bacteria. In the process of vital functions cultures produces carbon dioxide, a small amount of ethyl alcohol, enzymes, vitamins (C, group B, and others), organic acids (acetic, lactic, gluconic, malic, etc.), amino acids and other biologically active substances. Due to the fermentation of the tea-sugar solution by this consortium of microorganisms, a pleasant carbonated, refreshing drink with a sweet and sour taste is obtained which has functional properties and can be used as a preventive drink [6-9]. One of the problems of soft drinks technology is their low biological resistance. The specific of the technology of such beverages is to suspend the fermentation process at a certain point in reaching certain physic and chemical indicators. Not stopped the activity of microorganisms will lead to the appearance of the precipitate, formation of acetaldehydes, esters, which give the taste and aroma of the finished drink.

To increase the stability of such beverages it is necessary to remove microorganisms from them or to stop their livelihoods. A number of studies have been carried out to increase the biological stability of the beverage obtained through the culture of *Medusomyces gisevii*, by ultrasound (US) and ultrahigh-frequency (microwave) processing of fermented wort and with the addition of clarifiers and preservatives.

US-treatment of a fermented drink "tea mushroom" was carried out under the following conditions: the frequency of oscillations is 22 kHz, the power of oscillations is 0.1 kW, the temperature is 20°C, the processing time 40 minutes. It was established that this type of processing allows virtually stop

microorganisms that create a mixed population *Medusomyces gisevii*, without changing the organoleptic characteristics and achieve biological stability drink for 14-15 days [10].

Microwave processing of a finished fermented drink "tea mushroom" was carried out at the installation of "Artemis" under conditions: power 3 kW, frequency 2450 MHz, irradiation duration 10-15 min. It was determined that in these conditions the development and the livelihoods of the mixed culture of microorganisms *Medusomyces gisevii* suspended, thereby increasing the biological stability of the beverage to 16 days, in 2 times exceeds the regulated resistance to unpasteurized fermented beverages. At the same physical, chemical and organoleptic properties of fermented beverage "tea mushroom" met the regulatory requirements for the quality of the finished beverage [11].

Investigated the use of illuminant for such as isinglass, bentonite and "biofine". It is established that the best effect of the increase in transparency of fermented beverage "tea mushroom" obtained using the culture *Medusomyces gisevii*, achieved by using an integrated illuminator, which contains isinglass : bentonite : biofine in the ratio of 1.2 : 80 : 1, respectively.

This suggests that the use of the integrated illuminator makes it possible to remove also colloidal substances, partly microbial cells. Thus, the increased biological stability of the drink, processed integrated illuminator, regulated 7 days [12].

With aim comparisons of results investigated influence of such preservatives, as a benzoate of sodium, sorbic acid and juglone, on biological firmness of drink. The marked stability of organoleptic and physical and chemical indexes of the fermented drink is a "tea mushroom" at storage during 4-6 days [12].

It should be noted that studies to develop an effective way of improving the stability of a fermented beverage produced using a consortium of microorganisms *Medusomyces gisevii*, allow the following conclusions: the use of lighting, including fish glue and bentonite " biofine " colloidal increases and partly biological stability of drink so it may be appropriate for their prior use efficiency filter and heat treatment. Concomitant use of lighting and preservatives has increased the stability of the beverage to 10 days. Ultrasound and microwave processing modes studied at allowing almost completely rid of viable yeast and 8-10 times reduce the content of acetic acid bacteria [10-12].

Thus, there is a need to continue research beverage prepared from culture *Medusomyces*

gisevii, to develop efficient mode of heat treatment that would achieve bactericidal or bacteriostatic effect longer while maintaining high as possible organoleptic characteristics and content of biologically active substances

### 3. The purpose and objectives of research

The aim of this work was to improve modes of heat treatment fermented beverages prepared from culture Medusomyces gisevii, to enhance their biological stability during storage.

During the studies were delivered the following tasks: to investigate the effect of heat treatment on microorganisms culture Medusomyces gisevii during storage of the beverage produced using a consortium of microorganisms Medusomyces gisevii; The optimum processing modes such drinks (temperature, duration); determine the storage fermented beverages in glass bottles (temperature, filling factor).

### 4. Materials and methods of research

As object of researches used the finished fermented beverage obtained using mixed culture of microorganisms Medusomyces gisevii, which consists of three kinds of acetic acid bacteria: Acetobacter xylinum, Acetobacter suboxydans, Acetobacter aceti and two kinds of yeast: Zygospaccharomyces, Torulopsis. The ratio of yeast and acetic acid bacteria in the consortium Medusomyces gisevii is on average 1:100 [10]. The treatment temperature was selected according to the recommended pasteurization of existing fermented beverages with similar composition of microorganisms, that is, the thermal treatment of the studied beverages in glass bottles in the temperature range 55 ... 80 °C within 10 ... 60 minutes [4,5].

The effectiveness of treatment methods studied was determined by aging samples of treated and control temperature 12°C and 30°C for 210 days. During this period (1 every 1-5 days) controlled the solids content of drinks - refractometric method and

titrated acidity value - electrometric titration. Is stable during storage was considered as the samples of beverages, organoleptic and physico-chemical characteristics of which remained unchanged or did not exceed the permissible limits. The main technological parameters are the total acidity of the beverage, which should be in the range of 2.5 to 4.5 cm<sup>3</sup> of NaOH concentration from 1 mol / dm<sup>3</sup> to 100 dm<sup>3</sup> of the beverage.

In order to determine the optimal value of the filling factor of the bottle, the fermentation drink investigated was observed by changing the pressure in bottles with the beverage during their heat treatment.

### 5. Research results

It is established that optimal processing conditions for this type of drink has the temperature ranges and duration, respectively, of 63°C and 40 min. to 75°C and 15 min provided the maximum possible saving high organoleptic characteristics and content of biologically active substances.

The optimal fill factor of the drink based on the culture Medusomyces gisevii in glass bottles can be considered 0,90—0,96.

### 6. Discussion of results

Results previous studies have shown that the recommended pasteurization regimes for the same at the microbiological composition of the fermented beverages (beer, kvass) allowed to achieve a bacteriostatic effect only for 20 days. Complete cessation of vital activity of microorganisms was not detected.

Therefore, for a drink, prepared on the basis of culture Medusomyces gisevii, a study to develop an effective treatment heat treatment that would achieve bactericidal or bacteriostatic effect longer while maintaining high as possible organoleptic characteristics and content of biologically active substances. The research results are presented in the summary table. 1

Table I

#### Summary of determining the effectiveness of heat treatment regimes

Temperature processing, °C	The duration of treatment, min.	The stability of the beverage when stored at 30°C, days	Organoleptic assessment scores
55	60	30	18.5
63	40	60	18.5
70	25	90	18
75	15	180	17
80	10	210	15

It has been established that in order to increase the shelf life of the beverage, the optimum conditions for its processing are temperature ranges and duration, respectively, from 63°C and 40 minutes up to 75°C and 15 minutes.

At the temperature and processing time of more than 75°C and 15 minutes, the organoleptic characteristics of the beverage deteriorate considerably. Under the influence of temperature

less than 63°C and duration less than 40 minutes the shelf life of the drink is significantly reduced.

In table 2 shows the changes of titratable acidity during storage of the beverage at 30°C and 12°C for 210 days for the two limiting regimes of heat treatment, namely, for the I mode with processing conditions 63°C and 40 min. and II modes with processing conditions 75°C and 15 min. Significant changes of dry substance content during the specified shelf life are not observed.

Table 2

**Changing the total acidity during storage of drinks after heat treatment**

Shelf life, days	Total acidity $\text{cm}^3$ of NaOH conc. 1 mol / $\text{dm}^3$ 100 $\text{cm}^3$ drink			
	I heat treatment regime		II heat treatment regime	
	Storage at 30°C	Storage at 12°C	Storage at 30°C	Storage at 12°C
0	3.5	3.5	3.5	3.5
30	3.5	3.5	3.5	3.5
60	3.5	3.5	3.5	3.5
90	4.1	3.5	3.5	3.5
120	4.9	3.5	3.5	3.5
150	5.1	3.5	3.5	3.5
180	5.8	3.6	3.5	3.5
210	7.2	3.7	3.6	3.5

As can be seen from table 2, the increase in storage temperature of a beverage from 12°C to 30°C significantly reduces its biological stability. So, for 90 days, the acidity for the first treatment mode at a storage temperature of 30°C was more by 17% compared with the storage temperature of 12°C. However, given the rather mild conditions of thermal treatment and the bacteriostatic effect achieved during 60 days of storage at 30°C, the first regime can be recommended as acceptable for increasing the biological stability of the beverage. In addition, storage of the beverage at a temperature of up to 12°C allows stabilizing the microbiological state to 180 days. Under the 2-st mode of heat treatment, the stability of the drink remained stable at storage temperature 30°C for 180 days, and at 12°C - not less than 210 days.

Applying heat treatment regimes beverage in the above range enables provide guaranteed shelf life of its 2 to 6 months without a significant reduction in organoleptic characteristics and content of biologically active substances compared with untreated beverage.

The indicated modes are intended for thermal processing of a drink in glass bottles. The content of carbon dioxide in the finished drink is 0.2-0.3%. When heating occurs complex thermophysical processes (increasing the volume of the drink,

changing the solubility of carbon dioxide, etc.). Therefore, in order to prevent the rupture of the bottle with the test beverage, the dependence of the pressure change on the various filling factors of the bottles is determined.

Below are the results of studies to determine the optimal value of the coefficient of filling bottle fermented beverages studied (Fig. 1). Carbon dioxide content was 0.30%, the total volume of the bottle - 520 ml.

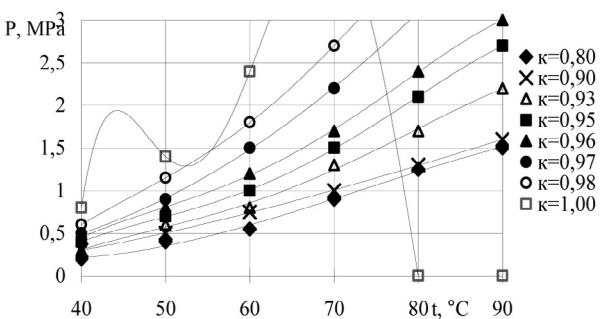


Fig. 1. Effect of temperature on the pressure (P) in a bottle of drink with different fill factor (k)

As can be seen from the graph (Fig. 1), there is a substantial relationship between the filling factor and pressure during thermal processing investigational fermented beverage. For example, already at 65°C Pressure for  $k = 0.98$  was 2.5 times higher than for  $a = 0.90$ . Most significantly for all

values of temperature, pressure increased, since filling factor 0,97.

Taking into account the given results and requirements regarding the minimum allowable coefficient of filling of soft drinks in bottles (in accordance with GOST 28188-89), the optimum value for a beverage on the basis of Medusomyces gisevii culture can be considered as 0.90-0.96.

## 7. Conclusions

It has been established that in order to increase the biological stability of a fermented beverage based on Medusomyces gisevii culture, the optimum conditions for its treatment are temperature ranges and duration 63°C and 40 minutes, respectively, to 75 °C and 15 min.

Applying heat treatment regimes beverage in the above range enables provide guaranteed shelf life of its 2 to 6 months without a significant reduction in organoleptic characteristics and content of biologically active substances compared with untreated beverage provided that the storage temperature of fermented drinks to 12C.

The optimum fill factor for drink based on the culture of Medusomyces gisevii in glass bottles is 0.90-0.96.

## References

- [1] Rynok v dvizhenii: osnovnye trendy mirovogo rynka napitkov. (2011) *Produkty & ingredienty*, no 6, pp.76-79 (in Russian).
- [2] Karputina D.D., Frolova N.E., Korolyuk K.E., Melnyk I.V. (2016) Rozroblenna fermentovanyh napoyiv na osnovi czukrovogo sorgo ta koncentratu yabluchnogo soku [Development of fermented beverages on the basis of sugar sorghum and apple juice concentrate]. *Praci TDATU*, issue 15. vol.1, pp.249-257 (in Ukrainian).
- [3] Tsed E. A., Vasilenko Z. V., Koroleva L. M., Volkova S. V., Pribyl'skii V. L., Volchek L. A. (2012) Issledovanie vozmozhnosti ispolzovaniya imbirya pri poluchenii bezalkogol'nykh napitkov brozheniya na osnove risovogo griba [Investigation of the possibility of use of ginger in the preparation of soft drinks based on fermented rice fungus], *Vestnik MGUP*, no. 1 (12), pp. 45-48 (in Russian).
- [4] DSTU 4069-2016. (2016) Napoyi bezalkogolni. Zagalni texnichni umovy. [Vved. 01.01.2016], Kyyiv, DP «UkrNDNCz» (in Ukrainian).
- [5] Domaretskii V.A., Ukrainets A.I., Shubin A.A., Sukmanov V.A., Debelyi V.A. (2006) Tekhnologiya ekstraktov, kontsentratov i napitkov iz rastitelnogo syrya [Technology of extracts, concentrates and drinks from vegetable raw materials]. Vinnitsa, NOVA KNYHA Publ., 368 p. (in Russian).
- [6] Danielyan L.T. Chainyi grib (Kombucha) i ego biologicheskie osobennosti (2005). [Tea mushroom (Kombucha) and its biological characteristics]. Moscow, OOO Meditsina Publ., 176 p. (in Russian).
- [7] Gunter W. F. Kombucha. (1995) Healthy beverage and natural remedy from the Far East. Publ. H. Ennsthaler, F- 4402 Steyr, 160 p.
- [8] Jayabalan R., Malbasa R., Loncar E., Vitas J., Sathishkumar M. (2014) A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus, Comprehensive Reviews. *Food Science and Food Safety*, pp. 4-13.
- [9] Markov S., Jerinic V., Cvetkovic D., Loncar E., Malbasa R. (2003) Kombucha - functional beverage. Composition, characteristics and process of biotransformation. *Hemisiska industrija*, pp. 10-57.
- [10] Zubchenko V.S., Vitryak O.P., Tkachenko L.V. (2008) Vplyv UZ-obrobky na stijkist napoyiv brodinnya. [Influence of ultrasound treatment on the stability of fermentation beverages]. *Xarchova promyslovist. Naukovyj zhurnal*. no 7, pp. 28-30 (in Ukrainian).
- [11] Zubchenko V.S., Vitryak O.P., Tkachenko L.V. (2009) Pidvyshhennya biologichnoi stijkosti fermentovany'x napoyiv shlyaxom NVCh-obrobleniya. [Increase of biological stability of fermented beverages by microwave processing]. *Xarchova promyslovist. Naukovyj zhurnal*, no 8, pp. 20-22 (in Ukrainian).
- [12] Zubchenko V.S., Vitryak O.P., Tkachenko L.V. (2012) Vplyv osvitlyuvachiv na biologichnu stijkist' bezalkogol'ny'x napoyiv brodinnya. [Influence of luminaires on biological stability of non-alcoholic fermentation drinks]. *Xarchova promyslovist. Naukovyj zhurnal*, no. 13, pp. 19-23.

**Л.Р. Решетняк<sup>1</sup>, О.П. Вітряк<sup>2</sup>, А.А. Серенко<sup>3</sup>**

**Удосконалення режимів теплового оброблення для підвищення біологічної стійкості ферментованих напоїв**

<sup>1</sup>Національний авіаційний університет, просп. Космонавта Комарова, 1, Київ, 03058, Україна

<sup>2,3</sup>Київський національний торговельно-економічний університет, вул. Кіото, 19, Київ, 02156, Україна,

E-mail: <sup>1</sup>isr@ukr.net; <sup>2</sup>vitrox@ukr.net; <sup>3</sup>anton\_serenko@ukr.net

**Мета:** удосконалити режими теплового оброблення для підвищення біологічної стійкості ферментованих напоїв, отриманих з використанням культури *Medusomyces gisevii*. **Методи:** досліджували вплив теплового оброблення у діапазоні 55...80°C протягом 10...60 хвилин на зміну фізико-хімічних та органолептичних показників ферментованих напоїв під час зберігання та умови зберігання напоїв в скляних пляшках (температура, коефіцієнт заповнення). **Результати:** встановлено, що для збільшення терміну зберігання напою оптимальними умовами його оброблення є діапазони температур і тривалості відповідно від 63°C і 40 хв. до 75°C і 15 хв. Оптимальним коефіцієнтом заповнення напою на основі культури *Medusomyces gisevii* в скляних пляшках можна вважати 0,90—0,96. **Обговорення:** досліджено стійкість безалкогольних ферментованих напоїв, отриманих з використанням культури *Medusomyces gisevii*, оброблених шляхом пастеризації за умов 2 режимів обробки: 63°C і 40 хв. та 75°C і 15 хв. Враховуючи досить м'які умови термічного оброблення та досягнутий бактериостатичний ефект протягом 60 діб зберігання при 30°C, I режим можна рекомендувати як придатний для підвищення біологічної стійкості напою. Зберігання напою при температурі до 12°C дозволяє стабілізувати мікробіологічний стан до 180 діб. За II режимом теплового оброблення стійкість напою залишалась стабільною при температурі зберігання 30°C протягом 180 діб, а при 12°C – не менше 210 діб. Застосування режимів теплового оброблення напою в наведених межах дозволяє гарантовано забезпечити термін зберігання його від 2 до 6 місяців без значного зниження органолептичних показників і вмісту біологічно активних речовин порівняно з необробленим напоєм.

**Ключові слова:** біологічна стійкість; коефіцієнт заповнення пляшок; культура *Medusomyces gisevii*; теплове оброблення; термін зберігання; ферментований напій; чайний гриб.

**Л.Р. Решетняк<sup>1</sup>, О.П. Вітряк<sup>2</sup>, А.А. Серенко<sup>3</sup>**

**Совершенствование режимов тепловой обработки для повышения биологической стойкости ферментированных напитков**

<sup>1</sup>Национальный авиационный университет, просп. Космонавта Комарова, 1, Киев, 03058, Украина

<sup>2,3</sup>Киевский национальный торгово-экономический университет, ул. Киото, 19, Киев, 02156, Украина.

E-mail: <sup>1</sup>isr@ukr.net; <sup>2</sup>vitrox@ukr.net; <sup>3</sup>anton\_serenko@ukr.net

**Цель:** усовершенствовать режимы тепловой обработки для повышения биологической стойкости ферментированных напитков, полученных с использованием культуры *Medusomyces gisevii*. **Методы:** исследовали влияние тепловой обработки в диапазоне 55 ... 80 ° С в течение 10 ... 60 минут на изменение физико-химических и органолептических показателей ферментированных напитков во время хранения и условия хранения напитков в стеклянных бутылках (температура, коэффициент заполнения). **Результаты:** установлено, что для увеличения срока хранения напитка оптимальными условиями его обработки является диапазоны температур и продолжительности соответственно от 63°C и 40 мин. до 75°C и 15 мин. Оптимальным коэффициентом заполнения напитка на основе культуры *Medusomyces gisevii* в стеклянных бутылках можно считать 0,90-0,96. **Обсуждение:** исследована стойкость безалкогольных ферментированных напитков, полученных с использованием культуры *Medusomyces gisevii*, обработанных путем пастеризации в условиях 2 режимов обработки: 63°C и 40 мин. и 75°C и 15 мин. Учитывая достаточно мягкие условия термической обработки и достигнут бактериостатический эффект в течение 60 суток хранения при 30°C, и режим можно

рекомендовать как приемлемый для повышения биологической стойкости напитка. Хранение напитка при температуре 12°C позволяет стабилизировать микробиологическое состояние до 180 суток. За II режимом тепловой обработки стойкость напитка оставалась стабильной при температуре хранения 30°C в течение 180 суток, а при 12°C - не менее 210 суток. Применение режимов тепловой обработки напитка в приведенных пределах позволяет гарантированно обеспечить срок хранения его от 2 до 6 месяцев без значительного снижения органолептических показателей и содержания биологически активных веществ по сравнению с необработанным напитком.

**Ключевые слова:** биологическая стойкость; коэффициент заполнения бутылок; культура *Medusomyces gisevii*; тепловая обработка; срок хранения; ферментированный напиток; чайный гриб.

**Reshetniak Lyudmila** (1946). Candidate of Engineering; Associate Professor.

Biotechnology Department Educational and scientific institution of ecological safety; National Aviation University, Kyiv, Ukraine.

Education: Kyiv Technological Institute of the Food Industry, Kiev, Ukraine, (1969).

Research area: biotechnology, microbiology.

Publications: 150.

E-mail: isr@ukr.net

**Vitriak Oksana** (1968). Candidate of Engineering; Associate Professor.

Kyiv National University of Trade and Economics, Kyiv, Ukraine.

Education: Kyiv Technological Institute of the Food Industry, Kyiv, Ukraine, (1990).

Research area: biotechnology, food technologies.

Publications: 75.

E-mail: vitrox@ukr.net

**Serenko Anton** (1995). Student.

Kyiv National University of Trade and Economics, Kyiv, Ukraine.

Research area: food technologies.

Publications: 1.

E-mail: anton\_serenko@ukr.net