TECHNICAL UNIVERSITY OF MOLDOVA

As a manuscript C.Z.U.: 663,242(478)

BOISTEAN ALINA

OPTIMIZATION OF THE TECHNOLOGY AND CHARACTERIZATION OF THE QUALITY OF VINEGAR FROM LOCAL WINE

253. 01 - Technology of food products of vegetable origin

Summary of the doctoral thesis in engineering sciences

CHISINAU, 2022

The thesis was developed within the Department of Food and Nutrition, Faculty of Food Technology, Technical University of Moldova.

Scientific supervisor :
CHIRSANOVA Aurica, doctor in biology, university lecturer, TUM
Scientific consultant :
GAINA Boris, PhD, hab. in technical sciences, university professor, academician, Academy of Sciences of Moldova

The Specialized Scientific Council D 253.01-22-11 , authorized with the right to organize the defense of the doctoral thesis, was approved by the Management Council of ANACEC by decision no. 2 of September 30, 2022 in the following composition:

Chairman: GENDOV-MOŞANU Aliona, PhD, hab. in engineering sciences, associate professor, TUM, Republic of Moldova.
Scientific secretary: NETREBA Natalia, PhD in technical sciences, associate professor, TUM, RM.

Member: STURZA Rodica, PhD, hab. in technical sciences, university professor, TUM, RM.
Member: ȚISLINSCAIA Natalia, PhD in technical sciences, associate professor, TUM, RM.
Member: SCUTARU Iurie, PhD in chemistry, associate professor, TUM, RM.
Member: CHISELIȚA Oleg, PhD in biology, lecturer researcher, IMB, RM.

Official references :
1 ALEXANDROV Eugeniu, PhD, hab. in biological sciences, Institute of Genetics, Physiology and Plant Protection, Republic of Moldova.
2 MACARI Artur, PhD in technical sciences, associate professor, head of the Department of Food Technology, TUM, Republic of Moldova.

The defense will take place on 2022.11.04, at 2.00 pm in the meeting of the Doctoral Committee within the Technical University of Moldova, FFT, 9/9 Studentilor Street, study block no. 5, aud. 120, MD-2045, Chisinau, Republic of Moldova.

The doctoral thesis and the abstract can be consulted at the library of the Technical University of Moldova and on the ANACEC website (www.anacec.md).

The summary was sent on 2022.10.05.

Scientific Secretary of the Doctoral Committee:	**NETREBA Natalia** , PhD, associate professor
Scientific supervisor:	**CHIRSANOVA Aurica** , PhD , associate professor
Scientific consultant :	**GAINA Boris**, PhD, hab., university professor, acad.
Author:	**BOISTEAN Alina**

CONTENTS

CONCEPTUAL ELEMENTS OF THE RESEARCH

The motivation for choosing the subject. Viticulture is one of the key sectors of the economy of the Republic of Moldova (RM). According to the data provided by the International Organization of Vine and Wine, the area of vineyards constitutes 140 thousand ha in the territory of the RM, representing 1,9% of the total area of vineyard plantations worldwide. The largest share of the plantations is held by the technical varieties intended for the production of wine and juice. The RM contributed to the production of wine in the world in 2018 with 2 million hectoliters [1]. The varieties grown in the country have a genetic potential determined by high productivity. The realization of this potential is negatively influenced by the following factors: monoculture and periodic climatic stresses [2]. Another problem is related to the political relations between the countries, which stop the sale of wines.

The COVID-19 pandemic has affected the world industry, including the wine industry - a priority and strategic branch for the RM. According to statistics, exports of wine products from Moldova decreased by 9% in March 2020 compared to the same period in 2019. The main reasons for the decline include logistical constraints due to quarantine measures, reduced demand in traditional markets and the postponement or cancellation of promotional actions wine [3].

One of the main tasks of the RM policy in the field of healthy food is to expand the range and improve the quality of products through the fuller use of local raw materials and the improvement of processing technologies [4]. The rational processing of local raw materials, including the production of natural vinegar demanded by consumers at HORECA enterprises and the food industry, is of major importance to ensure high-quality products.

Currently, natural products are increasingly in demand among the population. Natural vinegar, thanks to the metabolic processes caused by acetic bacteria, is rich in minerals, trace elements, organic acids, a number of enzymes and amino acids, a small amount of esters, aldehydes and other organic compounds, which give it a special taste and a pleasant aroma. Synthetic vinegar for food use is usually produced with the addition of a variety of flavors (identical to natural and synthetic). In some countries (USA, France, Bulgaria) the production of vinegar for food purposes from synthetic acetic acid is prohibited [5].

It should be noted that a good part of the vinegars offered on the RM market come from imports. The low volume of domestic vinegar production is determined by some impediments, such as technical instructions, some outdated regulations and the lack of starter culture based on native acetic bacteria. The problems related to the optimization of some technological stages, widening the assortment and improving the quality of vinegar are elucidated in the works of national and international scientists, including: B.Gaina [6], D. Beceanu [7], M. Begea [8], J. Horiuchi [9], C. Vegas [10] and al.

A scientific research was carried out regarding the optimization of some technological parameters for obtaining vinegar by natural fermentation, using concentrated juice from white grapes and white wine, raw materials accessible in our country.

One of the problems many domestic producers face is procuring imported acetic bacteria cultures and adapting them to local raw materials. From an economic point of view, the use of these crops is not efficient, as they directly affect the cost of the finished products [11]. The presence of strains of acetic bacteria isolated from local raw materials with increased productivity isolated from the products used in the manufacture of vinegar allows producers to obtain a higher quality product in a shorter time.

Another problem is the sulfiting of wines, a necessary technique for wine production. Sulfur dioxide is introduced into juice, must or wine, even organic wines, but in smaller doses. Many producers refuse to use industrial wine in the production of vinegar, because sulfur dioxide inhibits the activity of acetic bacteria, and the maximum admissible amount for carrying out fermentation by different methods is not known [12].

It is known that different substrates are used to obtain vinegar, such as: wood chips or sawdust, corn cobs, sugar cane stalks and others [13]. For the first time, native raw materials were used as substrate for acetic fermentation: walnut (*Juglans regia L.*) and hazelnut (*Corylus L.*) shells. Approximately 49-50% of the walnut's mass is shell, which is not exploited at its fair value, only sometimes being used in the manufacture of briquettes or as fertilizers.

Based on the above, it is obvious that the development and application of new, optimized technology and the improvement of the quality of wine vinegar by capitalizing on local raw materials with the involvement of local acetic bacteria are evident.

The purpose of the research presented in this paper is to optimize the technology for obtaining vinegar from wine using the strain of acetic bacteria isolated from local wine products, inoculated on the substrate from local raw materials and its utilization for obtaining non-alcoholic beverages.

Research objectives were established:

1. Isolation of pure cultures of acetic bacteria from local wine products and their identification according to morphological, cultural, physiological, biochemical and molecular characteristics;
2. The study regarding the existing producers and assortment of vinegars in the RM;
3. Evaluation of the influence of different factors on acetic fermentation;
4. Optimizing the technology for obtaining vinegar from wine;
5. Utilization of vinegar from wine to obtain non-alcoholic beverages.

The research hypothesis result from the analysis of the situation in the field and consist of the following:

1. The efficiency of wine vinegar manufacturing technologies can be increased by using native acetic bacteria strains with advanced technological properties, adapted to the quality of the raw material and the specific production conditions.
2. The valorization of low-quality wine, isolated acetic cultures and ecological substrates of domestic origin in the process of manufacturing wine vinegar by ensuring a double positive effect - technological and hygienic.

Synthesis of the research methodology and justification of the chosen research methods. Classical and high-performance methods were used to carry out the work. To isolate and identify the strain of acetic bacteria from native raw materials, the following were used: morphological, physiological and biochemical analysis of the culture; confirming the belonging of the isolated bacteria to the genus Acetobacter by comparing the DNA of the bacteria, using the RT-PCR method. To control and confirm the quality of the fermentation process, microbiological and physico-chemical methods were applied. The effect of the substrate on the color indices was evaluated by UV/Vis spectroscopy and the CIELab system. Methods were used to determine the quality of processed foods (sensory, physico-chemical and microbiological), as well as their glycemic index.

Theoretical importance and scientific innovation of the work. For the first time, the isolation and identification of an autochthonous strain of acetic bacteria *Acetobacter aceti* CNMN-AcB-01 from artisanal white wine vinegar obtained from the Noah grape variety was performed. Limit doses of sulfur dioxide and starter culture were established to ensure the efficient progress of acetic fermentation. For the first time, the shell of walnuts and hazelnuts was used as a substrate for the acetic bacteria, thus reducing the fermentation period and increasing the organoleptic qualities of the obtained vinegar. The evolution of blood sugar after consumption of non-alcoholic drinks based on fruits, berries, aromatic plants, white wine vinegar, etc. was examined, finding that the drinks are classified in the group of products with a low glycemic index and can be recommended as a healthy alternative to commercial drinks .

Theoretical significance. The application of molecular biology techniques for the isolation and identification of the studied strain by using the real-time PCR method. It was demonstrated the possibility of improving the chromatic parameters of white wine vinegar by using walnut shells as a substrate for the development of acetic bacteria, increasing the contact surface with the product, thus making the vinegar manufacturing process more efficient.

Summary of thesis chapters. The thesis consists of an introduction, 5 chapters, conclusions and recommendations, bibliography (205 titles) inserted in 110 pages of basic content, including 43 figures, 39 tables (except those indicated in the appendices), 13 appendices. The results were reflected in 14 scientific papers and 2 patents.

Applicative value of the work: it consists in establishing the optimal conditions for acetic fermentation of local wine and developing technical instructions for obtaining vinegar from wine. The technological procedures recommended on the basis of the study can be applied to specialized enterprises, and the use of walnut shells solves the problem of agri-food waste. The 2 invention patents were obtained.

Approval of the work at national and international scientific forums. The main results of the thesis were communicated and discussed at a number of national and international scientific conferences and symposia: "Euro-Aliment" International Symposium, Galati, 2019; The International Conference "Days of the Academy of Technical Sciences from Romania", 2019, the XIVth edition with the theme "Creativity in the development of the Knowledge Society" organized by the Academy of Technical Sciences from Romania and from Chisinau together with the Technical University of Moldova on October 17-18 2019; Technical-Scientific Conference of Collaborators, PhD Students and Students, UTM, Chisinau, 2020; International conference for students, masters and doctoral students "Student in Bucovina", December 18, 2020; National scientific-practical conference "Innovation: factor of social-economic development", 5th edition, Cahul, December 17, 2020; The national scientific symposium with international participation: Modern biotechnologies - solutions for the challenges of the contemporary world, Chisinau, 2021, May 20-21; 16th International Conference on constructive design and technological optimization in the field of machine construction, Bacău, May 25-27, 2021; EUROINVENT International Innovative Research Conference, May 20-21, 2021, Iasi, Romania; Doctoral School Scientific Conference, SCDS-UDJG 9th edition, Galați, June 10-11, 2021; EURO-ALIMENT 2021, the 10th International Symposium , 7-8 October , Galati, Romania; PROINVENT Scientific Research, Innovation and Invention Fair, 19th edition, October 20-22, 2021, Cluj-Napoca, Romania; International Specialized Exhibition INFOINVENT, XVII edition from November 17-20, 2021 ; International Scientific Symposium "Agrifood Sector - Achievements and Perspectives" November 19-20, 2021, Chisinau, Moldova.

Publications on the topic of the thesis. The basic content of the doctoral thesis is presented in a chapter of an international monograph; in 4 articles published in scientific journals indexed in BDI; 1 paper without co-authors; two invention patents in the Republic of Moldova; 9 theses in collections and abstracts at national and international scientific events.

Keywords: vinegar, acetic bacteria, alcoholic fermentation, acetic fermentation, substrate, wine.

THESIS CONTENT

1. PHYSICAL-CHEMICAL AND MICROBIOLOGICAL ASPECTS OF PRODUCING VINEGAR FROM WINE

The first chapter represents a brief comparative analysis of the existing situation in the field, of the scientific materials related to the acetic fermentation process and to the slow and fast biotechnological processes for obtaining acetic acid.

The bibliographic study carried out regarding the physico-chemical and microbiological aspects of obtaining vinegar from wine allowed the formulation of the following conclusions:

- In the RM, vinegar is obtained by diluting ethyl alcohol and fermenting it. The obtained product is devoid of macro- and micronutrients and does not possess an important nutritional value. Thus, this product can only be recommended for industrial use.
- Natural vinegar, thanks to the metabolic processes caused by acetic bacteria, is rich in minerals, trace elements, organic acids, etc. The important nutritional value contributes to the increasing demand of natural vinegar by consumers for use in healthy food as well as by economic agents for industrial use in the manufacture of organic products.
- The production of vinegar from wine in a natural way with the use of the characteristic RM substrate presents one of the basic problems of enterprises and researchers in the field.
- According to the studies carried out, an important role in the manufacture of natural vinegar is played by acetic bacteria, which through their vital activity ensure specific properties of the finished product. In order to direct the biochemical processes and obtain natural vinegar with superior organoleptic properties, pure cultures are used, which ensure a double technological and hygienic effect. The utilization of strains of acetic bacteria isolated from their natural habitat allow the selection of microorganisms adapted to the quality of raw materials and have enhanced, safe and stable biotechnological properties. This fact allows obtaining natural vinegar of high quality.
- Optimizing the technology for obtaining vinegar from wine through natural fermentation with the use of strains isolated from accessible raw materials, developed on a natural substrate, represents an effective possibility of capitalizing on wines of average quality or poorly competitive on the market.

The scientific research carried out today allowed the formulation **of the research problem** which consists in the development and scientific substantiation of biotechnological regimes for obtaining vinegar from wine through natural fermentation using the strain isolated from native raw materials, developed on a natural substrate.

2. MATERIALS AND RESEARCH METHODS

The study was carried out in the scientific laboratories of the Faculty of Food Technology (Technical University of Moldova), as well as in industrial conditions at the specialized enterprise SRL "V.DEVELOP".

For the isolation of acetic bacteria, the native viticultural material was used: white grapes and white wine of the Noah variety, harvest year 2019; lab-grown Noah white grape vinegar and commercial untreated white wine vinegar. For the microbiological examinations and isolation of acetic bacteria, were used usual and special media such as: RAE, GYC and Hoyer, prepared by the Institute of Microbiology and Biotechnology of the Republic of Moldova.

For the identification and detection of isolated bacteria, various biochemical tests were used, such as: Gram stain, Catalase test, KOH test. Gene amplification was performed using Real-Time PCR CFX96 Deep Well (Bio-Rad, USA). The experiment was performed on each isolated strain separately, on RAE culture medium. For the identification of acetic bacteria, the kit "For everyone Detection Kit B Acetics Screening" (PIKA Weihenstephan GmbH, Germany). The acetic acid bacteria DNA extraction and detection reaction was performed according to the manufacturer's protocol [14]. When configuring the amplifier, FAM waves - which have an emission of 520 nm and HEX -550 nm - were chosen as detectors.

The main raw material used in the optimization of the vinegar production technology was KINETA grape concentrate with 65% sugar concentration from the domestic producer. Yeasts were used to carry out the alcoholic fermentation ENARTIS FERM SC, NUTRIFERM SPECIAL and NUTRIFERM ADVANCED, producer country Italy, were used as nutrients to activate the process.

The biotechnological line for the manufacture of vinegar was made for the company SRL "V. DEVELOP" by the domestic company SRL "URI ENGINEERING", the equipment is assembled according to the projects and standards of the Austrian company VOGELBUSCH. Agents were used to clarify the vinegar: Maxibent P (activated sodium bentonite); Maxibent G (sodium bentonite); Gelatin Vinigel ORO; PVVIN (polyvinylpyrrolidone) and bentonite POWDER Oro.

For the preparation of non-alcoholic beverages with white wine vinegar, the basic and auxiliary raw material was used: plums (*Prúnus domestic*); peaches (*Prúnus peach*); apples (*Malus domestica Jonathan*); strawberry (*Fragária pineapple*); raspberry (*Rúbus idáeus ruby*); lavender (*Lavandula L.*); spearmint (*Mentha L.*); green basil (*Ocimum L* .); cinnamon; vanilla; sugar cough; white wine vinegar produced in the research.

The conducted research also required a series of chemical reagents and laboratory materials that were of good quality and corresponded to a degree of analytical purity, procured from the local supplier " Ecochimie " SRL.

The multifactorial ANOVA analysis (Two-way ANOVA) with multiple comparison techniques was performed , which allowed the highlighting of the samples that differ from the average values. Fisher's exact test was used to determine the degree of correlation between the data obtained for pH, total titratable acidity and density versus fermentation time. This was performed automatically using the XLSTAT software package (Addinsoft, Paris, France). All experiments were performed in duplicate or triplicate. The statistical significance threshold chosen: $p \leq 0.05$.

3. SELECTION OF ACETIC BACTERIA STRAINS FROM INDIGENOUS RAW MATERIALS

The isolation of a culture of pure acetic bacteria went through 3 steps:

Stage 1 - the study of the initial microflora of the selected materials, using selective nutrient media; detection and isolation of acetic bacteria from them.

Stage 2 - the selected strain of acetic bacteria was exposed to morphological, physiological and biochemical analysis.

Stage 3 - confirming the belonging of the isolated bacteria to the *Acetobacter genus* by comparing the DNA of the obtained acetic bacteria, using the RT-PCR method.

3.1. Isolation of pure cultures of acetic bacteria

It was found that grape bunches have a rich and diverse microflora, which complicates the process of isolating acetic bacteria. Wine also is not a perfect source of acetic bacteria, as their presence was in very small amounts on one (GYC) of the three media. Based on the morphological characteristics of the cells, the isolated bacteria can be assigned to the genus *Acetobacter*. It is assumed that the presence of a large number of other microorganisms in wine prevents their normal development. Wine with increased acidity is a more suitable source of acetic bacteria because their numbers dominate. Different species of colonies grew on all three nutrient media, of which two strains, according to cell morphology, can be attributed to acetic bacteria.

Fig. 1. Identification of colonies on GYC medium by clear zones

A small number of microorganisms have been isolated from unfiltered and unpasteurized commercial vinegar. It is assumed that at the end of fermentation, the acetic bacteria lose their activity under the influence of the lack of alcohol. However, the vinegar has also undergone the filtration process. From the colonies of microorganisms in wine vinegar obtained under laboratory conditions, acetic bacteria were isolated on two nutrient media (RAE, GYC).

3.2. Morphological and physiological characteristics of the isolated strains

When studying colonies grown on GYC medium, characteristic transparent halos can be observed around the colonies. This is characteristic of acetic bacteria, because some of them consume calcium during their life cycle, and this element is one of the ingredients of the GYC nutrient medium (fig.1). Microscopy of the isolated acetic bacteria confirmed their belonging to the *Acetobacter genus*. This fact is confirmed by their morphological characteristics (small and medium sticks).

3.3. Detection and identification of isolated strains

In order to study the ability of isolated acetic bacteria to ferment ethyl alcohol to acetic acid, it was decided to introduce them into some wine substrates. During the fermentation, on the 3rd day, a cloudy film appeared on the surface of the liquid, dislocating to the wall (fig. 2). This indicates that the acetic bacteria are sufficiently active.

Fig. 2. Bacterial film

The next step was to identify the acetic bacteria, using various biochemical tests. The first test identifies the enzyme catalase, which is a component of acetic acid-producing bacteria. The positive result confirms the nature of the microorganisms and their belonging to the bacteria of the *Acetobacter genus*. The KOH test and the Gram stain provide information about the physiology of the cells, namely, the structure of the cell wall. The test results are inserted in Table 1.

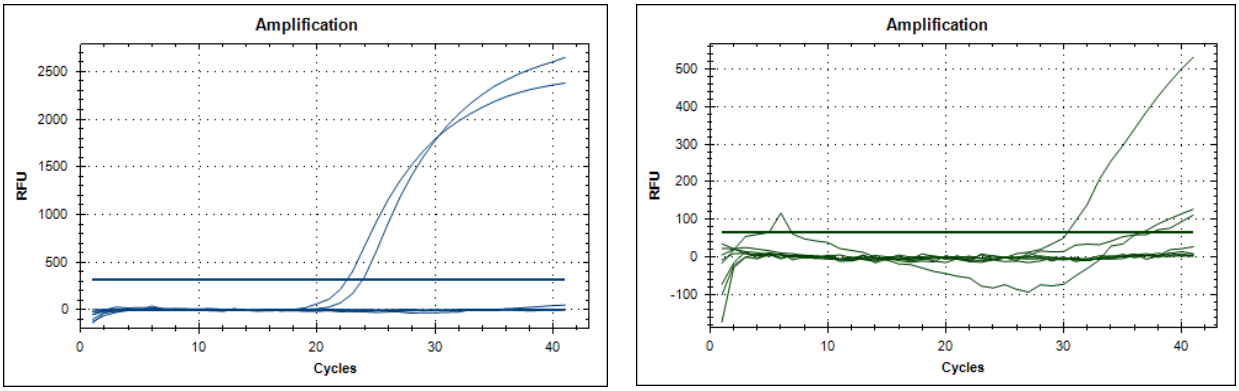
Table 1. Qualitative reactions for the identification of isolated bacteria

№	Source of bacteria	Gram staining	KOH test	The catalase test
1	*White wine with increased acidity*	-	-	-
2	*Vinegar obtained in the laboratory*	-	++	+

Note. *"++" - intensively positive reaction, "+" - positive reaction, "-" - negative reaction*

Bacteria isolated from white wine with increased acidity on RAE medium were found to fulfill only one requirement with reference to Gram staining. Thus , it was found that the bacteria in the study are Gram- · Qualitative determination of catalase activity was negative. Likewise, the KOH interaction test was negative. These responses can have two reasons: the cultures lost their activity during the research stages or they are not part of the *Acetobacter family.*

Two samples with bacteria were subjected to amplification, to be able to definitely confirm the belonging of the isolated bacteria to the *Acetobacter genus*. The following samples were analyzed: DNA purified directly from the vinegar being in the fermentation process and DNA purified from colonies inoculated from this vinegar on RAE medium, using the enzyme mixture included in the kit. The results of this analysis are shown in figure 3a. and 3b.



a) **b)**

Fig. 3. DNA amplification of acetic bacteria on FAM (a) and HEX (b) channel

Purified DNA from bacterial colonies grown on RAE medium showed positive amplification. The three curves crossing the baseline represent the amplification plots of the negative control sample (from the reference kit) and DNA isolated from the bacterial colonies that gave the positive signal on HEX.

It was found that from all the samples that were simultaneously amplified, only the bacteria isolated from the untreated vinegar, cultivated on the RAE medium showed a positive reaction, which confirms the belonging to the *Acetobacter genus*. Despite the fact that a sample was taken from the vinegar during the fermentation period, selected during the exponential growth phase did not give a positive result. Finally, we can conclude, first of all - that the bacteria isolated from untreated vinegar definitely belong to the *Acetobacter genus*; secondly - the use of vinegar in the phase of maximum accumulation of active microorganisms, does not ensure detection by RT-PCR of acetic bacteria [15].

4. OPTIMIZATION OF THE TECHNOLOGY FOR OBTAINING VINEGAR FROM WINE

4.1. Obtaining vinegar from concentrated juice

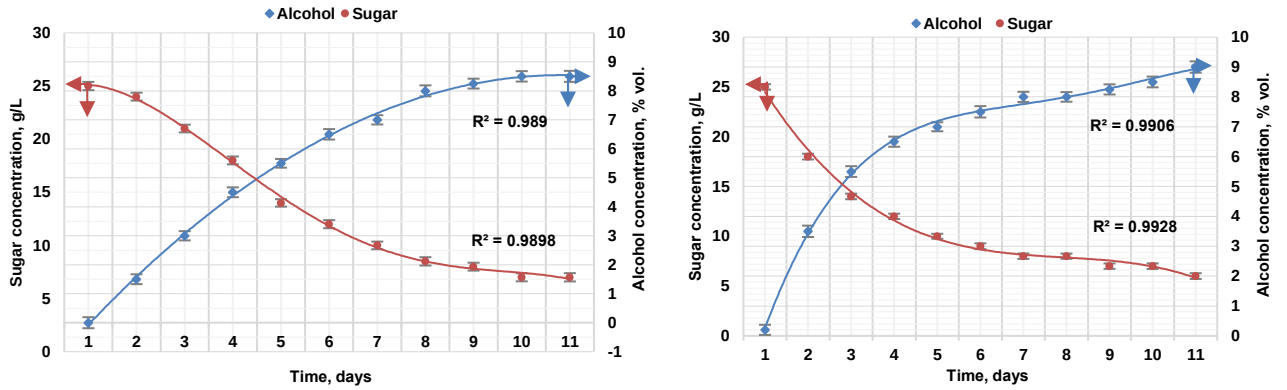
The experimental part was focused on the study of the processes of alcoholic fermentation of grape juice to wine, respectively, and the subsequent acetic fermentation of wine to obtain vinegar. The concentrated juice from white grapes was used, the characteristics of which are inserted in table 2. The quality of the concentrated juice was in accordance with the norms provided in the technical regulations HG no. 1111 [16].

Table 2. Characteristics of white grape concentrate*

Quality indices	KINETA grape concentrate
Composition: basic ingredients	100% white grape juice
Physical description	Viscous, dark yellow color
Physico-chemical characteristics	Brix ^{0}-65; pH - 2.57; Density-1260 kg/m^{3} at T=20°C
Organoleptic characteristic	The taste, aroma specific to grape juice
Expiration date	12 months at a temperature of 10-15°C

**Elaborated by the author based on the manufacturer's data SA "ALFA-NISTRU".*

Alcoholic fermentation of the juices was carried out after diluting them with drinking water up to a sugar concentration of 25g/L using ENARATIS FERM SC yeasts (0,3g yeasts/L of juice) with/and without the addition of nutrients (complete fermentation activators alcoholic) in the fermentation medium: NUTRIFERM SPECIAL 35 g/hL at the beginning of fermentation and NUTRIFERM ADVANCE 30 g/hL in the middle of the fermentation process. The evolution of the sugar and ethyl alcohol content during the alcoholic fermentation of grape juice with/and without the addition of nutrients is presented in figure 4a. and 4b.



a) **b)**

Fig. 4. Evolution of sugar and alcohol content during alcoholic fermentation of grape juice without addition (a) and with addition of nutrients (b). Results are presented as mean ± standard deviation; $p \leq 0.05$

The physico-chemical parameters of the products resulting from the alcoholic fermentation of grape juices are included in table 3.

Table 3. Physico-chemical parameters of products resulting from alcoholic fermentation*

No. do	Indices	Values after alcoholic fermentation	
		without nutrients	with nutrients
1	pH	3,20±0,05	3,13±0,05
2	Sugar concentration, g/L	7,0±0,1	6,0±0,1
3	Alcohol, %vol.	8,5±0,4	9,0±0,5
4	Density, kg/m^3	1025±10	1005±10

**Elaborated by the author, and the results are presented as mean ± standard deviation; p<0,05.*

The process of acetic fermentation of products resulting from alcoholic fermentation includes the following main stages:

- preparing the wine to obtain the vinegar with the desired acidity;

- the actual acetic oxidation of alcohol and obtaining vinegar.

The progress of the acetic oxidation process was followed by determining the content of acetic acid formed and the residual ethyl alcohol in the yeast. The controlled parameters, which determined the development of the acetic oxidation process under optimal conditions under the action of acetic bacteria, were the following:

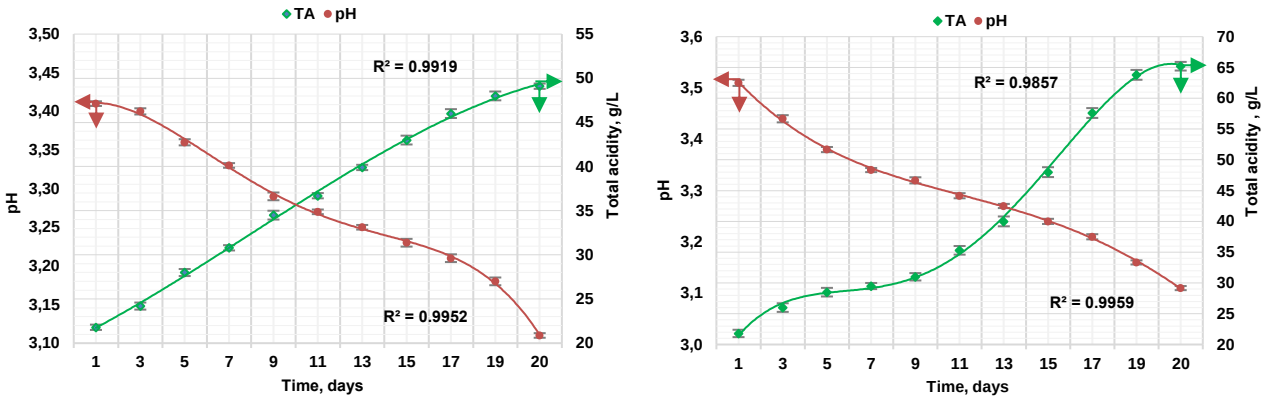
- the amount of air that ensures the viability and multiplication of bacteria and implicitly the oxidation of alcohol into acetic acid (the optimal value being about 5 liters of air/liter);

- constant and uninterrupted aeration;

- the temperature of 28±2^{0}C-this value was constant, because its variations prevent the activity of bacteria;

- to speed up the process, the following nutrients were used: ammonium sulfate $(NH_4)_2SO_4$-0,135 g/L, sucrose $C_{12}H_{22}O_{11}$-2,7g/L, potassium carbonate K_2CO_3 -0,005g/L.

The evolution of the acetic fermentation process was followed by the total acidity and pH of the fermentation medium. The evolution of these parameters during the acetic fermentation of the samples with/and without the addition of nutrients is represented in figure 5a. and 5b.



a) b)

Fig. 5. Evolution of TA and pH during the acetic fermentation of samples without addition (a) and with addition of nutrients (b). Results are presented as mean ± standard deviation; $p \leq 0,05$

The physico-chemical parameters of the products resulting from acetic fermentation are inserted in table 4.

Table 4. Physico-chemical parameters of products resulting from acetic fermentation*

No. do	Indices	Values after acetic fermentation	
		without nutrients	with nutrients
1	Total acidity, g acetic acid/L	49,1±0,6	65,0±0,3
2	pH	3,11±0,05	3,10±0,04
3	Density, kg/m^3	1040±15	1042±20

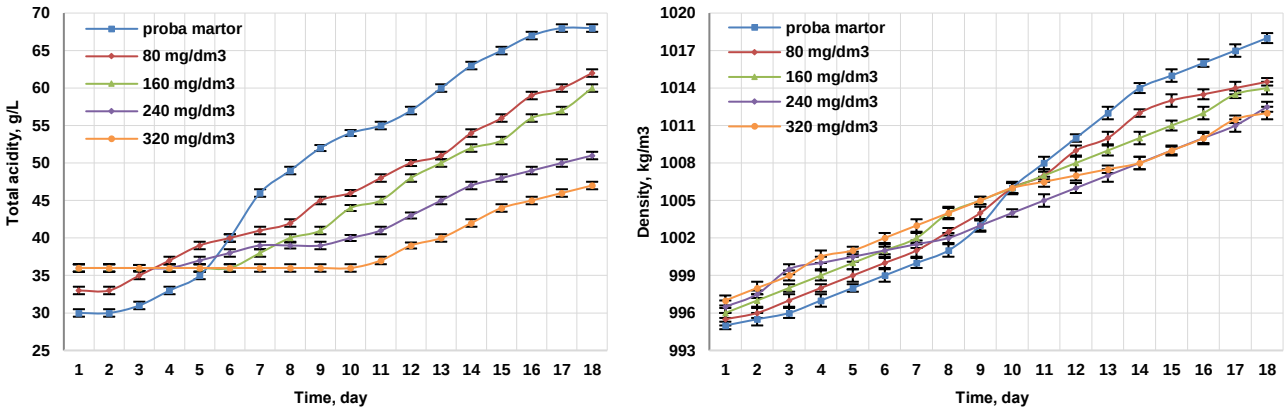
**Elaborated by the author, and the results are presented as mean ± standard deviation.*

After obtaining the vinegar from the concentrated juice of white grapes, it was found that nutrients do not play an important role in the alcoholic fermentation process, because the difference between the samples of the alcohol content is only 0,5%. At the same time, during acetic fermentation, 33% more acetic acid was recorded in the sample with nutrients compared to the control. To increase acetic fermentation in industrial conditions, when obtaining vinegar from white grape concentrate, we recommend the use of nutrients in quantities of $(NH_4)_2SO_4$ - 0,135g, K_2CO_3 - 0,005g and sucrose $C_{12}H_{22}O_{11}$ – 2,7g.

4.2. The impact of sulfur dioxide

Currently, sulfur dioxide is one of the safest and most immediate means that can be used to preserve wine. Therefore, the addition of SO_2 inhibits the development of acetic bacteria and respectively blocks the production of vinegar [17].

The influence of the dose of sulfur dioxide, which varied from 80 to 320 mg/dm^3 SO_2, on the acetic fermentation process of white wine and the changes in pH values, density and total acidity was investigated. In Figure 6 a and b, changes in TA and density during acetic fermentation are shown.



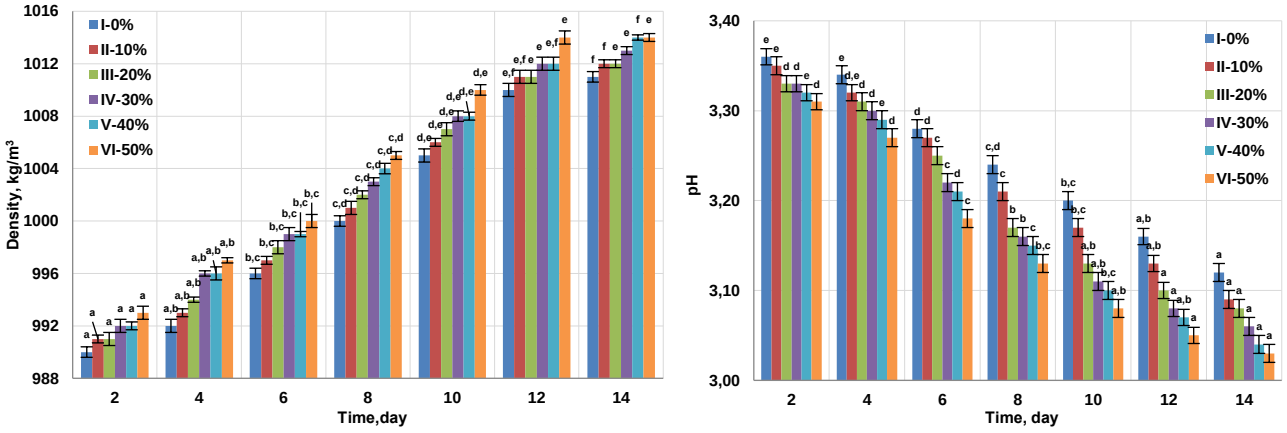
a) **b)**

Fig. 6. Evolution of AT (a) and density (b) of wine with different concentrations SO_2 during acetic fermentation. Results are presented as mean ± standard deviation; $p \leq 0,05$

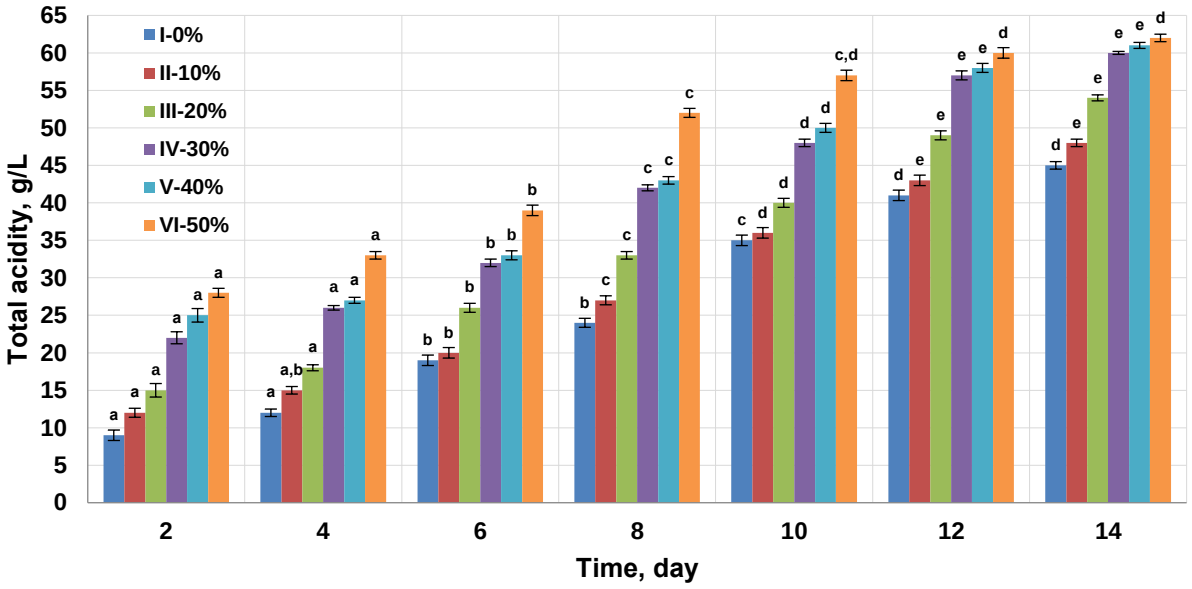
With the increase in the dose of SO_2 , a stoppage of the acetic fermentation process is observed. For example, in the sample with the dose of SO_2 320 mg/ dm^3, the initial value of acidity is maintained until the 10th day, then it increases insignificantly. The increase in the value of total acidity is only 11 g/L, which is two times lower compared to sample II (dose of SO_2 80 mg/dm^3). However, during the 18 days, for the control sample, the process proceeded very actively, the value of total acidity increasing from 30 g/L to 68 g/L, which is 2 times more. The admissible values of the acetic acid content (minimum of 60g/L) are certified on the 13th day [18] .

4.3. Establishing the optimal dose of the starter culture

Was analyzed the changes in physico-chemical parameters (TA, pH, density) during the acetic fermentation process of white wine with different amounts of starter culture (figure 7), which varied from 10% to 50% white wine vinegar yeast obtained from the Noah variety.



a) **b)**



c)

Fig. 7. Evolution of density (a), pH (b) and TA (c) of samples with different amounts of yeast (unfiltered white wine vinegar) during acetic fermentation. Different letters a-f indicate significant differences between samples ($p < 0,05$).

From figure 7c. it is observed that the control sample (without the addition of yeast) minimum TA was not reached during fermentation of 14 days. TA of 60g/L (minimum required for vinegar) was reached in the 50% yeast sample on the 12th day, and in the 30 and 40% yeast samples it was reached on the 14th day. This confirms that the studied vinegar has an average value of acetic acid and can be used in the food industry, according to normative documents [18, 19, 20].

Thus, we can conclude that both in the sample with a high content of acetic acid and in the one with a low content, the process of qualitative acetic fermentation took place with the achievement of the minimum value of acetic acid of 60 g/L took place in the samples at adding yeast by 30, 40 and 50%.

4.4. The influence of different types of substrate

During the research, the following substrates were chosen from local raw materials considered as waste from the agro-food industry:

1. Chips of apple wood - AM (*Malus domestica*) size 6x12x3 mm. Manufacturer Smart Energy Solutions SRL.

2. Walnut shell - CN (*Juglans regia L.*) harvested in 2019, with the size that does not pass through the metal sieve with the diameter of the holes 4 mm.

3. Hazelnut shell - CA (*Corylus avellana*) harvested in 2019, with the size that does not pass through the metal sieve with the diameter of the holes 4 mm.

4. Tescovina from the grape variety -TM (*Vitis vinifera Muscat*) in dry form, fruit of the year 2020, Cimișlia district, Javgur winery.

The use of various substrate materials, with the exception of tussock, was subjected to primary processing which consisted of the following:

1. Washing under running water at a temperature of 80±1°C, for 10 minutes.
2. Drying in an oven at a temperature of 32±1°C, 48 hours.
3. Aging with maya (unfiltered white wine vinegar) for 72 hours, in a ratio of 1:4.

Tescovina was subjected to the oven drying procedure at 32±1°C, for 5-6 days until the moisture level reached 9±1%. It was then subjected to the maturation process described above in point 3.

Before using the substrate, as a surface source for the development of acetic bacteria, each type of substrate was introduced into leaven, which consisted of untreated vinegar in the ratio 1:4 (50 g of substrate and 200 mL of leaven). The samples were kept for 72 hours, at a temperature of 25±1°C (table 5).

Table 5. Evolution of the volume of samples with different substrates*

Substrate type	Ratio leaven: substrate , mL /g	$V_{initially}$, mL	Volume after 72 h, mL	Volume difference, mL
Nutshell	200:50	250	234.30 ±1.33	-15.60±0.86
Hazelnut shell	200:50	250	234.33 ±0.67	-15.66±0.34
Apple chips	200:50	250	240.16 ± 0.83	-9.83±0.53
Marc Muscat	200:50	250	255.13±0.24	+5.13±0.24

**Elaborated by the author, and the results are presented as mean ± standard deviation.*

From table 5 it can be seen that the volume of vinegar with walnut and hazelnut shells decreased by 16 mL , and with apple chips only by 10 mL. This difference is due to the absorption properties of the peel. In the case of the pomace substrate, the result was the opposite, after 72 hours this volume increased by 5 mL. The fact is due to the content in its composition of cellulose and soluble fibers.

The number of acetic bacteria in the used must was investigated, before and after maceration, to confirm the necessity of planting and its development on the substrate. The possibility of using apple wood shavings, which are known to be an effective substrate for the development of acetic bacteria, was tried. They were macerated in leaven for 72 hours, in a ratio of 1:4. According to the obtained data, it is confirmed that after placing on the surface of the substrate, the acetic bacteria develop intensively and after 3 days their number is higher compared to the initial amount.

Since the process of acetic fermentation by the classical method takes place slowly, the purpose of using the substrate is to increase the speed of acetic fermentation. The evolution of the physico-chemical parameters of vinegar is presented in figure 8.

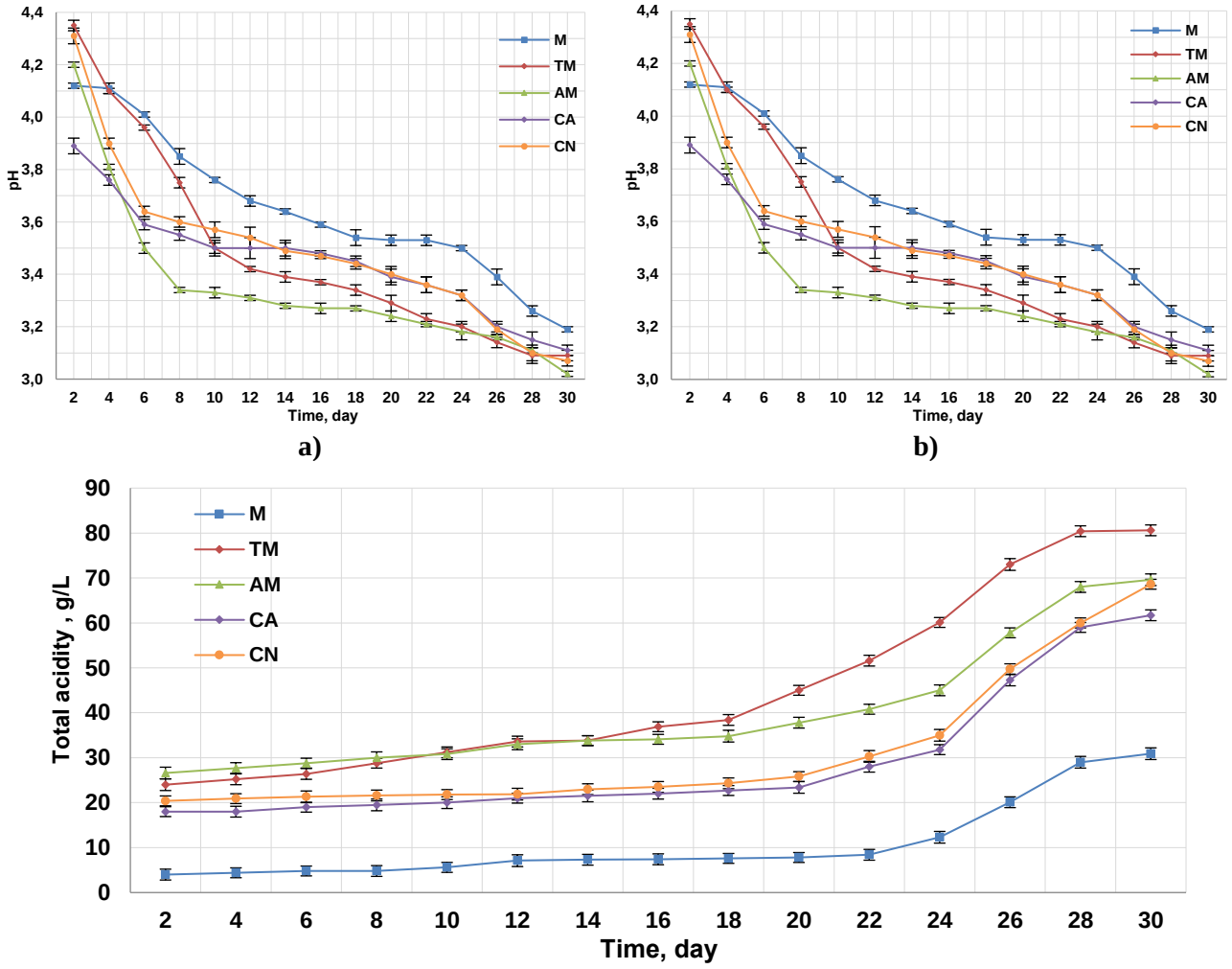


Fig. 8. Evolution of density (a), pH (b) and TA (c) of samples with substrate: M - control; TM – marc muscat; AM - chips from apple wood; CN - nut shell; CA - hazelnut shell . Results are presented as mean ± standard deviation; $p \leq 0,05$

It is found that the substrate plays a significant role in the acetic fermentation process. Thus, in the sample with apple wood chips this process evolves faster, because compared to the other samples, the structure of the chips allows a better adhesion of acetic bacteria on their surface, increasing the probability of obtaining vinegar in a shorter time. The use of substarts increased the total acidity of the obtained vinegar by about 2 times during 30 days. It is worth noting that the walnut shell equally with the apple chips positively influenced the accumulation of acetic acid in the finished product.

The chromatic changes of the vinegar depending on the substrate were evaluated, according to the color difference between the control sample (M) and those with different substrate. According to the chromatic parameters inserted in Table 6, the change in intensity (Ic) and color shade (Nc) is indicated in all samples, but the greatest change in Ic occurs in the CN sample, which increased 30 times compared to the control. At the end of the fermentation period, Nc had a maximum value of 2,59 in the sample with apple shavings.

Table 6. Chromatic parameters of samples with different types of substrate*

Sample	L*	a*	b*	ΔE*	C*	H°	Ic	Nc
M	17,07±0,10	6,55±0,12	10,57±0,15	-	12,43±0,05	58,21±0,11	0,69±0,07	1,96±0,10
TM	18,83±0,05	7,29±0,01	11,61±0,04	2,17±0,04	13,71±0,01	57,88±0,13	1,48±0,20	1,94±0,18
AM	30,51±0,04	6,54±0,02	29,79±0,04	23,45±0,03	29,60±0,01	77,24±0,08	1,67±0,53	2,59±0,46
CA	40,45±0,03	-0,56±0,05	16,55±0,04	25,15±0,02	16,56±0,02	91,94±0,05	1,55±0,36	1,65±0,67
CN	37,51±0,31	2,69±0,02	27,64±0,10	26,90±0,23	27,77±0,01	84,44±0,14	20,70±0,29	1,68±0,32

** Results are presented as mean ± standard deviation; p≤0,05.*

The data in table 6 demonstrated that in samples AM, CA and CN the brightness values are the highest, 30,51, 40,45 and 37,51 in which the white color predominates, and in the case of samples M and TM the L* values are more small, constituting 17,07 and 18,83 respectively. The representation of the colors of the vinegar samples obtained according to the CIELab system attests that the tonality of the yellow color predominates (figure 9). The data for the a* parameter in most samples are positive, which denotes the presence of red pigments. In the case of the CA sample, the a* component was shifted towards the green color. The negative a* value -0,56 indicates the insignificant presence of green pigments (chlorophyll).

The results show the biggest change in the walnut shell sample compared to the control , where the L* value increases about 2 times, the a* component changes its values from 6,55 to 2,69, and the b* value doubles. The ΔE* value demonstrates that the color change for the TM sample is insignificant compared to the control (2,17). In the CN sample, the ΔE* value denotes important color changes (26,90).

Also, AM and CN samples have more intense color because the C* chromaticity values are further from the origin of the coordinate system, being 29,60 and 27,77. In the case of samples M, TM and CA, the C* values are 12,43, 13,71 and 16,56 respectively, demonstrating that the color intensity is reduced due to the presence of shades of gray. The values of the hue angle H* denote that the samples M and TM are in the trigonometric quadrant I (58,21° and 57,88°), in which the orange color predominates, and the samples AM, CA and CN are in the trigonometric quadrant II, the values being 77,24°, 91,94° and 84,44° respectively, in which the yellow color is dominant (figure 9).

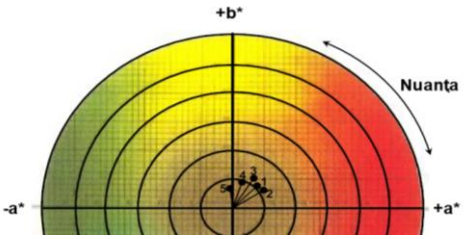


Fig. 9. Representation of sample colors according to the CIELab system: 1-M; 2-TM; 3-AM; 4- CA; 5- CN

4.5. Vinegar finishing with different clarifying agents

Clarified vinegar evolves normally and maintains its constant quality for a long time, having a finer bouquet and aroma compared to the cloudy one [21]. For these reasons, the process of clarifying wine vinegar with five types of agents, which can be found on the RM market, was studied.

Industry recommendations state that the doses of bentonite used should be within the limits of 30-80g/hL. Taking into account the manufacturer's recommendations for the use of the selected

clarifiers , concentrations of 1,5g/L, 2,5g/L, 3,5g/L, 5g/L and 7,5g/L were used. The clearing capacity of the different agents was determined by reading the absorbance in the spectrophotometer at a wavelength of 420 nm (Fig. 10).

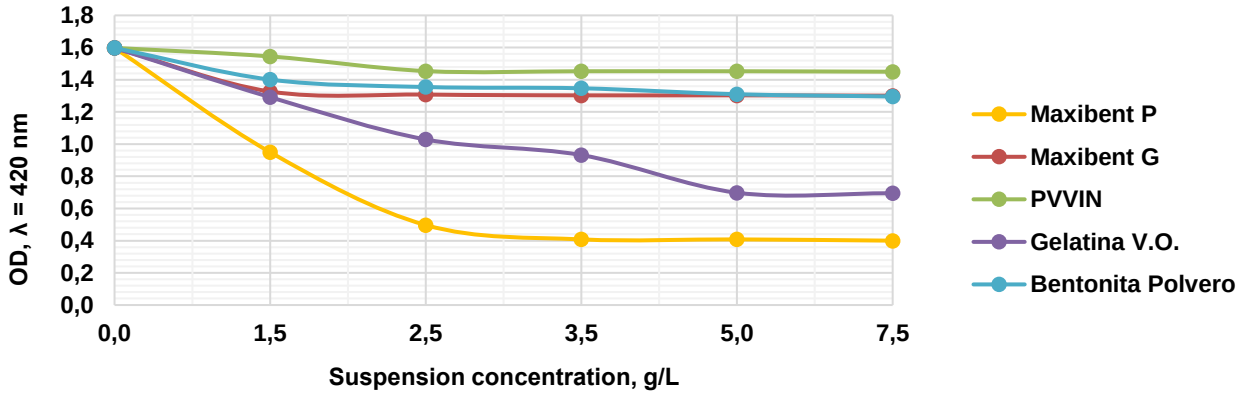


Fig. 10. Influence of the concentration of clarifying agents on the optical density of vinegar

From the analysis of the data presented in figure 14, it is observed that for most agents the effect on the optical density of vinegar is manifested starting with the concentration of the suspension of 1,5 g/L, after which the absorption continues, but proceeds more slowly. The agent Maxibent P has the most pronounced clarifying effects on wine vinegar, followed by Gelatin V.O.

For further research, related to the establishment of the technological parameters of the clarification process (temperature, duration, centrifugation regime, etc.), the concentrations of the clarification agents were selected: Maxibent P - 3,5g/L; Maxibent G - 2,5 g/L; PVVIN-3,5 g/L; Gelatin Vinigel Oro - 5 g/L; Polvero bentonite - 2,5 g/L.

Next, the influence of temperature on the degree of clarification of vinegar was investigated. Thus, the vinegar samples with the necessary clarifying agent (optimal concentrations were mentioned above) were placed in spaces that ensured the temperature regime 4±2ºC, 40±2ºC, 20±2ºC.

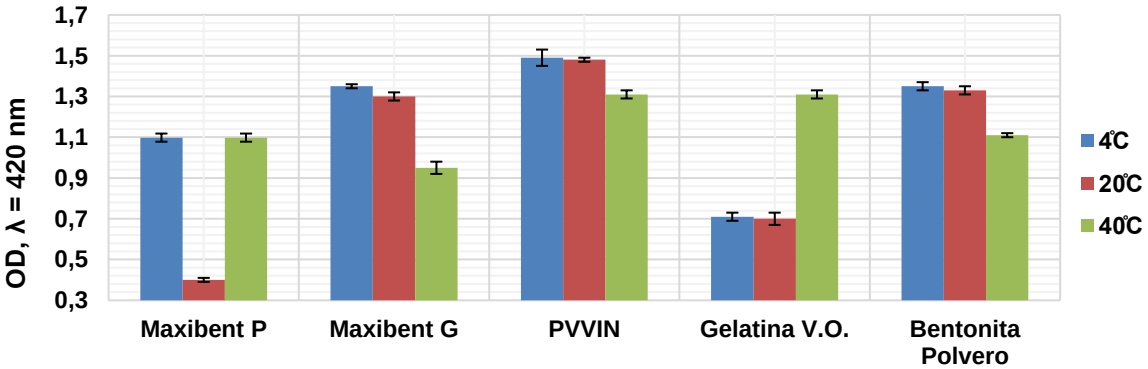


Fig. 11. Influence of temperature on the clarification process

The results obtained (fig. 11) show that the suspension of the agent Maxibent P showed the best behavior, followed by Gelatin V.O. The suspensions of these clarifying agents had the best results at a temperature of 20±2ºC. Thus, as far as regarding the economic aspect (creating a certain temperature

regime requires energy expenditure), these clarifying agents will be the most effective. Maxibent P and Gelatin V.O. have the ability to reduce the optical density below the values of 1, respectively giving the vinegar a higher degree of clarity.

As a technological parameter of the vinegar clarification process, the duration of contact of the vinegar with the clarification agent suspension was selected. The mixture samples of the vinegar with the suspensions were analyzed after the first hour of contact, 2 hours, 3 hours and 24 hours. The optical density values for the given periods are represented in figure 12.

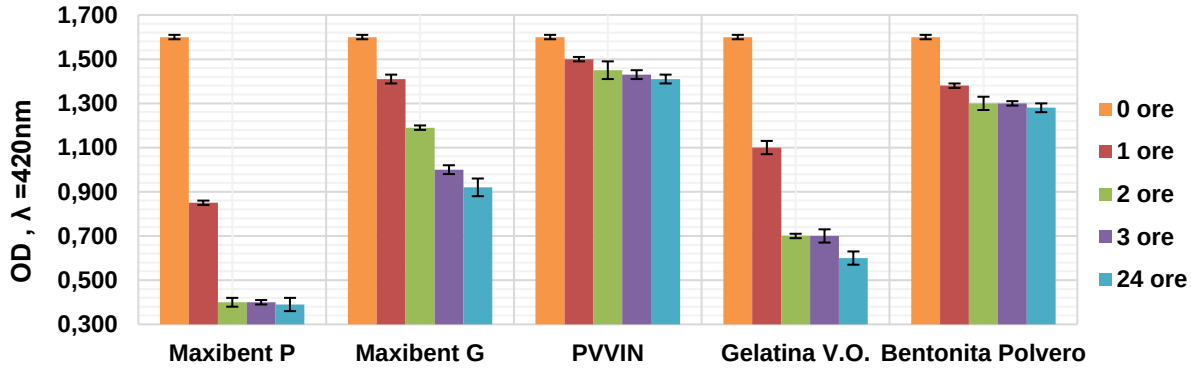


Fig. 12. Influence of contact time on the clearing process. Results are presented as mean ± standard deviation; $p \leq 0,05$

The data show that the contact between the vinegar and the clarifying agent manifests itself in the first hours, thus, for Maxibent P and Gelatin V.O., the optimal contact period is 2 hours. After 2 hours the optical density has a tendency to decrease which proceeds slowly. At the same time, the optical density of the vinegar treated with Bentonite Polvero, Maxibent G and PVVIN stabilized after 3 hours. In all samples, the 24-hour contact period does not cause significant changes compared to 3-hour contact.

The obtained results demonstrated that the best clarifier property was manifested by the agent Maxibent P, where after 2 hours the absorbance reached the value of 0,400, while with other agents this value was not reached even after 24 hours.

A clearer vinegar also implies a higher degree of purity, respectively higher acidity and higher density. Based on these considerations, the evolution of these parameters was determined depending on the duration of the vinegar treatment.

Table 7. Changes in pH, TA and density of vinegar after clarification depending on the duration of the clarification process*

Time	Maxibent P	Maxibent G	PVVIN	Gelatin V.O.	Bentonite Polvero
1	2	3	4	5	6
	pH values				
$_{initial}$ pH	2,89±0,03	2,89±0,02	2,89±0,05	2,89±0,02	2,89±0,02
1 hour	2,84±0,02	2,86±0,07	2,87±0,02	2,88±0,01	2,86±0,04
2 hours	2,80 ±0,06	2,84 ±0,02	2,85 ±0,07	2,86 ±0,03	2,85 ±0,01
3 hours	2,80±0,07	2,83 ±0,01	2,83 ±0,02	2,84±0,05	2,84 ±0,02

Continuation of the table 7.

1	2	3	4	5	6
24 hours	2,79 ±0,04	2,83±0,01	2,81±0,01	2,84 ±0,07	2,83±0,03
	TA, g acetic acid/L				
$_{Initial}$ TA	56,2±0,8	56,2±0,2	56,2±0,9	56,2±0,8	56,2±0,5
1 hour	58,1±0,3	57,4±0,6	57,9±0,4	57,8±0,1	57,5±0,1
2 hours	58,5±0,1	58,2±0,5	58,2±0,7	58,3±0,3	58,2±0,8
3 hours	58,8±0,2	58,4±0,3	58,2±0,6	58,5±0,1	58,4±0,3
24 hours	58,8±0,4	58,5±0,1	58,3±0,3	58,6±0,8	58,5±0,9
	Density, kg/m^3				
$_{initial}$ ρ	1020±10	1020±18	1020±12	1020±13	1020±14
1 hour	1024±16	1023±13	1021±11	1022±11	1023±19
2 hours	1027±12	1024±17	1023±11	1023±18	1024±12
3 hours	1027±13	1026±15	1025±13	1023±17	1026±11
24 hours	1027±12	1026±11	1026±15	1024±14	1026±15

**Elaborated by the author, and the results are presented as mean ± standard deviation.*

From the data inserted in table 7, it can be seen that the relationship between the duration of vinegar treatment with clarifying agents and the physico-chemical parameters of vinegar after clarification is directly proportional. However, some agents have a greater ability to reduce the pH or increase the density and acidity of vinegar than others. Following the treatment of vinegar with Maxibent P, the lowest pH value (2,79) and, respectively, the highest acidity (5,88 g/L) were obtained. The lowest values of these parameters were recorded in the case of vinegar treatment with PVVIN and Gelatin Vinigel Oro.

The parameters of the obtained vinegar were compared with the parameters of the Decision of the Government of the RM, no.1403 of 09.12.2008, regarding the approval of the Technical Regulation "Vinegars and acetic acid for food use" [18]. All obtained parameters correspond to the norms and no deviations were recorded.

4.6. Optimization of wine vinegar manufacturing technology

In the research was:

✓ developed the technological instruction IT MD 67-41184408-01:2021 regarding the manufacture of vinegar from wine, from fruits and from forest fruits, according to the requirements of the technical regulations "Vinegars and acetic acid for food use" approved by GD no.1403 of 09.12.2008 to the group of authors: Gaina B. dr.hab., university professor, acad., AȘM; Boiștean A. university lecturer, FTA, UTM and Baciu V. head of SRL"V.DEVELOP";

✓ tests of the culture of acetic bacteria *Acetobacter aceti were carried out CNMN-AcB-01* , isolated from grapes of the local Noah variety in the industrial process of manufacturing wine vinegar in accordance with the technological instructions IT MD 67-41184408-01:2021.

✓ applied as a prototype the technological scheme described by Budak et al. and subsequently optimized by substituting the substrate, and respectively, the processes required for its preparation [22]. The optimized block diagram for obtaining vinegar from white wine is represented in figure 13.

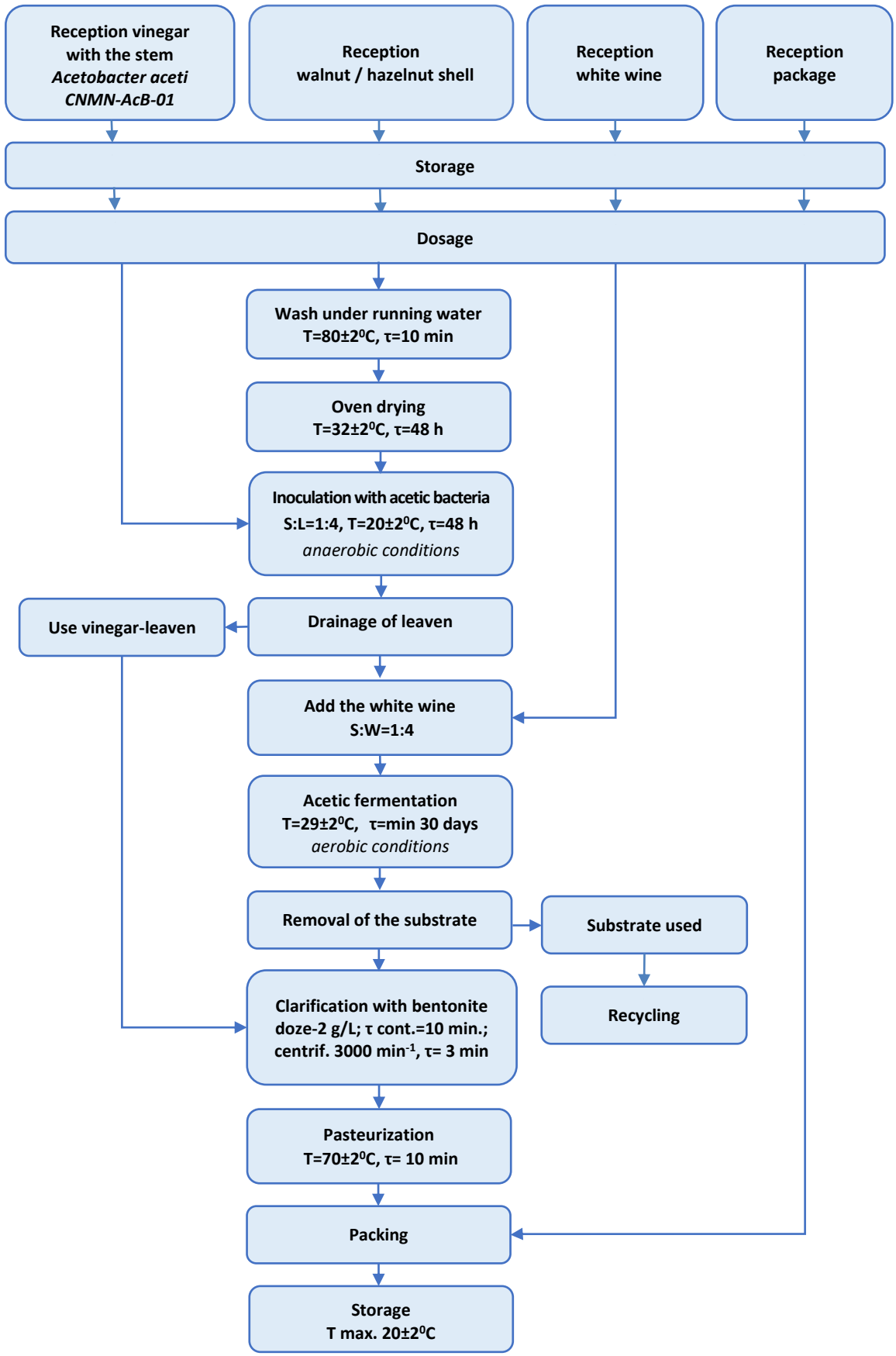


Fig. 13. Optimized block diagram for obtaining vinegar from white wine

The use of the substrate from native material (walnut shells and hazelnuts) led to the increased development of the *Acetobacter aceti CNMN-AcB-01* bacteria strain, isolated from local wine products, and contributed to the accumulation of acetic acid in a 2-fold time shorter compared to the classic fermentation technology, which led to the optimization of the technological scheme for obtaining vinegar from white wine.

The technology for obtaining vinegar from white wine was described in the patent *Process for obtaining vinegar from white wine* [22]. Short-term invention patent with no. MD 1517 Y was requested by SRL Fermented fruts, which concluded a non-exclusive license contract with UTM for its use, for the purpose of marketing the product obtained following the application of the process.

5. TECHNOLOGY OF THE MANUFACTURE OF NON-ALCOHOLIC BEVERAGES WITH WHITE WINE VINEGAR

In chapter 5, the possibility of using white wine vinegar to obtain non-alcoholic beverages was analyzed. As part of the research, non-alcoholic drink recipes were developed that include the addition of vinegar obtained through natural fermentation and sweetening it with sugar. Acetic acid in vinegar acts as a preservative, so the drink remains delicious throughout the year, the recipes of which are presented in table 8.

Table 8. Recipes of non-alcoholic beverages developed*

No. d/o	Naming and coding of beverages	Ingredients ratio (for 1 liter of finished drink)					
		fruits/ apple trees, g**	sugar, g	aromatic plants, g	spices , g	vinegar, ml	water, mL
1	Plums with lavender-PrL	200	200	2	-	100	it bring up to the quota
2	Peaches with cinnamon-PiS	200	200	-	2	100	
3	Apple with vanilla-MV	200	200	-	2	100	
4	Raspberry with mint-ZM	200	200	10	-	100	
5	Strawberry with basil-CB	200	200	10	-	100	

Note. **elaborated by the author, **the rate of fruits/apples is indicated for the net meal.*

During the summer, when water intake is high, gastric acidity decreases, which favors the occurrence of bacterial and viral infections in the digestive tract. During this period, the volume of consumption of soft drinks increases. It is obvious that their demand is increasing in commercial establishments, but they are not recommended due to their high sugar content, high acidity, use of synthetic dyes, preservatives, etc.

It has been shown that processed drinks contain a significant amount of carbohydrates due to sugar, fruits or berries, which have values from 10,38 to 11,50g/100mL. In order to match the carbohydrate content (according to the rules) in the elaborated non-alcoholic drinks, the energy value was compared with those selected from the trade. It was found that the energy value of the elaborated drinks is close to the energy value of the same amount of commercial sweet drinks. The difference is

insignificant, but the advantage lies in the fact that elaborate non-alcoholic drinks are obtained from natural raw materials, while commercial ones contain a series of artificial additives. Therefore, the obtained drinks can be offered as a healthy and natural alternative to commercial non-alcoholic drinks, as well as for serving in the UAP.

The elaborated drinks contain significant amounts of carbohydrates of about 10,2-11,5%, just like the commercial ones. For example, soft drinks Coca-Cola and Fanta contain 10,6% and 11,7% sugar respectively. The mentioned drinks, being consumed frequently and in large quantities, could lead to insulin dependence and diabetes, as well as to coronary, metabolic and obesity diseases [23]. In this sense, the GI of the elaborated drinks was determined. The method was performed according to the international standard ISO 26642 [24]. 9 people in good health participated in the study, who offered themselves as volunteers. Over the course of 7 days, all volunteers consumed each type of elaborated drink in an amount containing 25 g of carbohydrates, powdered glucose as a standard in an amount of 25 g, and a drink without the addition of vinegar. Soft drinks were served before breakfast, on an empty stomach. Capillary blood samples were collected at time intervals 0, 15, 30, 45, 60, 90 and 120 minutes. The results of plasma glucose after consuming one of the drinks are shown in figure 14.

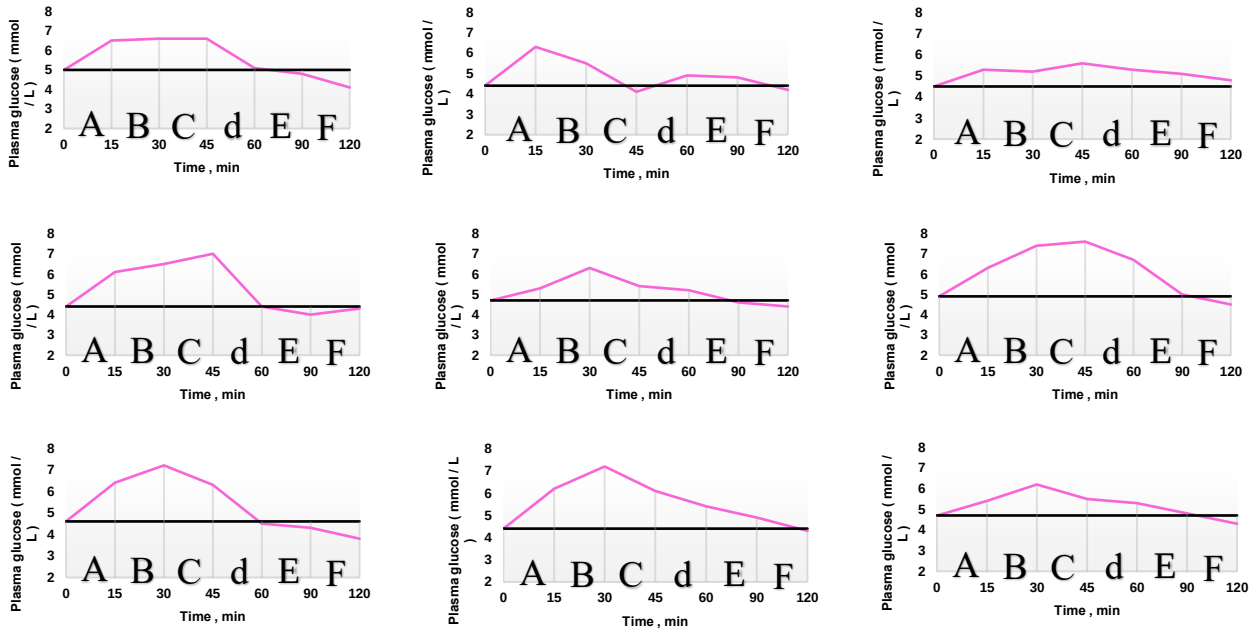


Fig. 14 Graphs of blood sugar versus time after consuming the raspberry mint drink for each participant

It should be noted that after the consumption of elaborate non-alcoholic beverages, the level of plasma glucose in the blood gradually increased from 4,5-4,7 mmol/L to 5,9-6,5 mmol/L in the first 30 minutes, after which it drops rapidly to 4,8-5,1 mmol/L, and after 2 hours it decreased to 4,0-4,4 mmol/L, which denotes that the values reached are lower than before consumption. The glycemic index of the drinks ranged from 36 to 49.

After consuming the sample without vinegar, the plasma glucose level in the blood gradually increases from 4,6 to 6,7 mmol/L in the first 30 minutes, then it drops quickly and after 1,5 hours it has the same value as before consumption. The glycemic index of the drink without vinegar is 112, practically 2 times higher compared to the samples with vinegar.

The results obtained and multiple recent scientific investigations have documented that ingestion of diluted vinegar by healthy adults reduces the glucose response to a carbohydrate load. There is also some evidence that ingesting diluted vinegar increases satiety in the short term [25]. Brighenti et al. determined that the oral administration of diluted acetic acid can have a beneficial effect on the evolution of blood glucose. A small dose of vinegar, in the form of salad dressing consumed at a mixed meal, is sufficient to positively influence the glycemic response and bring it to normal parameters [26].

Based on the results obtained regarding the GI of the elaborated non-alcoholic beverages, it was found that they refer to drinks with low GI (PrL-41 GI, PiS-36 GI, MV-49 GI, ZM-47 GI, CB-49 GI). It was confirmed that the presence of natural vinegar in the amount of 10%/L in the composition of the elaborated non-alcoholic drinks was effective in reducing the postprandial level of glucose and insulin and is considered an effective adjuvant for improving glycemic control.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Carrying out the research and analyzing the obtained results led to the formulation of the following conclusions:

1. Valuable biotechnological properties was isolated from native raw materials. Following the performance of biochemical tests and the application of the RT-PCR method, it was definitely established that the isolated strain belonged to the *Acetobacter genus*. The strain *Acetobacter aceti CNMN-AcB-01* was deposited in the National Collection of Nonpathogenic Microorganisms within the Institute of Microbiology and Biotechnology. Following the testing of the strain *Acetobacter aceti CNMN-AcB-01* in industrial conditions at the company "V. DEVELOP" SRL the practical interest of its use in the production of domestic wine vinegar was found.

2. The possibility was studied obtaining vinegar from concentrated white grape juice and the impact of nutrient addition on alcoholic and acetic fermentation. It was found that the addition of nutrients in the acetic fermentation process in amounts of 0,135g/L-$(NH_4)_2 SO_4$; 0,005g/L-K_2CO_3 and 2,7g/L-$C_{12}H_{22}O_{11}$ contribute to the accumulation of acetic acid by 33%.

3. It was demonstrated the possibility of using sulfite wine (with the content of 80, 160, 240 and 320 mg/dm^3 of SO_2) for the manufacture of vinegar. It was established that the dose up to 160 mg/dm^3 SO_2 allows the acetic fermentation process to take place in sulfite wine without deviations and the accumulation of acetic acid in the minimum admissible amount of 60 g/L.

4. The influence of different amounts (10, 20, 30, 40 and 50%) of vinegar-leaven obtained by exploiting the isolated strain *Acetobacter aceti CNMN-AcB-01* on the physico-chemical parameters of the vinegar was investigated. It has been demonstrated that the amount of 30% vinegar-leaven ensures the minimum value of acetic acid of 60 g/L is reached on the 14th day of fermentation.

5. It has been shown that the use of the substrate reduces the duration of the acetic fermentation process by about 2 times and has an insignificant influence on the physico-chemical parameters of the vinegar. At the same time, walnut shell significantly changes the color of the finished product. Thus the Ic value increased from 0,69 to 20,70, at the same time ΔE*(26,90) demonstrates important color changes.

6. It has been shown that the intensity of the clarification process of white wine vinegar is maximum in the first 10 minutes, followed by an essential decrease in the rate of clarification. The optimal conditions for clarifying white wine vinegar were determined as follows: clarifying agent dose - 2 g/L; stirring time - 3 min.; contact time - 10 min.; separation of the clarifying agent by centrifugation at 3000 min^{-1} for 3 min.

7. Based on the research, the IT MD 67-41184408-01:2021 technological instruction on the manufacture of wine vinegar was developed; carried out tests of the culture of acetic bacteria Acetobacter *aceti CNMN-AcB-01;* optimized block scheme for obtaining vinegar from white wine. The obtained results allowed the process of obtaining vinegar from white wine to be patented.

8. Recipes and the technological scheme were developed for five non-alcoholic beverages with the utilization of the obtained white wine vinegar. The energy value of the processed beverages has been shown to be in the range of 45,93 to 47,98 kcal/100 mL and is close to that of commercial beverages (from 42,00 to 75,60 kcal/100 mL). The advantage of elaborate drinks is argued by the use of local natural raw materials and presents a healthy alternative to commercial drinks.

9. It was found that following the consumption of processed non-alcoholic beverages, they refer to drinks with low GI (PrL-41 GI, PiS-36 GI, MV-49 GI, ZM-47 GI, CB-49 GI). It was confirmed that the presence of natural vinegar in the amount of 10%/L was effective in reducing the postprandial level of glucose and insulin, which confirms its effectiveness in improving glycemic control.

Important scientific problem solved. For the first time a new strain of acetic bacteria *Acetobacter aceti CNMN-AcB-01* was isolated, characterized, identified and passported, which led to the optimization of the technology for obtaining vinegar from white wine with the utilization of walnut shells as a substrate, a fact that allowed the efficiency of the vinegar manufacturing process and its enrichment with mineral substances, chromatic and organoleptic indices.

Based on the results obtained, the following **recommendations** were formulated:

- The manufacture of white wine vinegar in industrial conditions using the amount of min. 30% of the yeast obtained by utilizing the strain *Acetobacter aceti CNMN-AcB-01* isolated from native raw material.
- The use of nutrients in acetic fermentation in the optimal amount of: $(NH_4)_2SO_4$ – 0,135 g/L, K_2CO_3 – 0,005 g/L and $C_{12}H_{22}O_{11}$ – 2,7 g/L.
- Manufacture of commercial wine vinegar with a maximum SO_2 content of 160 mg/dm^3.
- The use of walnut shell (*Juglans Regia L.*) as a substrate for inoculation with the acetic bacteria *Acetobacter aceti CNMN-AcB-01* in a ratio of 1:4 (shell:wine).
- The utilization of domestic white wine vinegar in a quantity of 10%/L, as a natural preservative in the manufacture of non-alcoholic beverages.

SELECTIVE BIBLIOGRAPHY

1. DAVID, T. *The impact of microelements and biofertilizers on the realization of the productivity and resistance potential of the grapevine* , tz . by Dr. in agricultural sciences, Chisinau 2021. 130 p.
2. BÊLICI, C. *Argumentation of the production and protection of classic sparkling wines with designation of origin "CRICOVA"* , tz . by Dr. in technical sciences, Chisinau 2021. 158p.
3. ARHIP, V., SCUTARU, I. Vine culture in the republic of moldova at the beginning of the third millennium . In: *Journal of social sciences* . 2019, II(2), pp. 87–94. ISSN 2587-3490 [cited 03.09.2022]. Available: http://doi.org/10.5281/zenodo.3235248
4. The Neighborhood Program of the European Union, *Assessment of the situation regarding agriculture and rural development in the Eastern Partnership countries, Republic of Moldova* , FAO [cited 03.09.2022]. Available: https://www.fao.org/3/aq675ro/aq675ro.pdf
5. DAYAN, FE, DUKE, SO Natural Products for Weed Management in Organic Farming in the USA. In: *Outlooks on Pest Management* . 2010, Vol. 21, No. 4, pp. 156-160. ISSN 1743-1026 [cited 03.09.2022]. Available: https://doi.org/10.1564/21aug02
6. GAINA, B. Final scientific report on the execution of the scientific research project *18.80015.5007.222T, The development of biotechnology and the implementation of the modern line for the production of high quality natural spiced vinegar, competitive on the domestic and foreign market 2018-2020*. Chisinau 2019 [cited 03.09.2022]. Available: https://ancd.gov.md/sites/default/files/document/attachments/Raport%20final%20222T.PDF
7. BECEANU, D., ANGHEL, RM Assortment of vinegar of supermarkets in Iasi : qualitative assessment according to my standards . In: *Agronomic research in Moldova (Romania)* . 2010, Vol. XLIII, no. 2 (142) pp. 55–64. Online: ISSN 2784 - 0360 [cited 03.09.2022]. Available: http://www.uaiasi.ro/CERCET_AGROMOLD/CA2-10-07.pdf
8. BEGEA, M., STROIA, I., BEGEA, P. Technology and competitive vinegar varieties on a European level, Scientific papers. In: *Agronomy Series* , USAMV Iasi. 2007, vol. 50, pp. 281–286. ISSN(print): 1454-7414 [cited 03.09.2022]. Available: http://www.uaiasi.ro/revagrois/PDF/2007s_281.pdf
9. HORIUCHI, J., KANNO, T., KOBAYASHI, M. New vinegar production from onions . In: *Journal of bioscience and bioengineering* . 1999, no. 88(1), pp. 107–109. ISSN 1389-1723 [cited 03.09.2022]. Available: https://doi.org/10.1016/s1389-1723(99)80186-8

10. VEGAS, C., MATEO, E., GONZÁLEZ, A. et al. Population dynamics of acetic acid bacteria during traditional wine vinegar production . In: *International journal of food microbiology* . 2010, no. 138(1-2), p.130–136. ISSN 0168-1605 [cited 03.09.2022]. Available: https://doi.org/10.1016/j.ijfoodmicro.2010.01.006
11. BOGDAN, N. *Utilization of microbial strains isolated from goat's milk for industrial application* : tz . by Dr. in biology, Chisinau, 2020. 170p .
12. NARDI, T., BORDIGA, M. Fermentation Process . In Post- Fermentation and-Distillation Technology. CRC Press, 1st Edition , 2017. pp. 1-40. ISBN: 9781315155050 .
13. HUTCHINSON, UF, GQOZO, S., JOLLY, NP et al. Aeration , Agitation and Cella Immobilization on Corncobs and Oak Wood Chips Effects on Balsamic -Styled Vinegar Production , In: Foods. 2020, no.8:303 [cited 03.09.2022]. Available: https://doi.org/10.3390/foods8080303
14. USER GUIDE, Detection of Acetic Acid Bacteria, For research *in vitro* use only, Catalog #2401-15 [cited 03.09.2022]. Available : https://www.pika-weihenstephan.de/wp-content/uploads/2020/03/Manual_2401-15_4everyone_DetectionKit_B_Acetics_Screening2003E.pdf
15. **BOISTEAN, A.,** CHIRSANOVA, A., ZGARDAN, D., MITINA, I., GAINA, B. The methodological aspects of using real-time PCR in acetobacter detection. In: *Journal of Engineering Science*. 2020, vol . V (XXVII), № 3, pp. 232-238. ISSN 2587-3474 [cited 03.09.2022]. Available : http://doi.org/10.5281/zenodo.3949726
16. decision The Government of the Republic of Moldova no. 1111 of 06.12.2010 with regarding approval _ Rulemaking technical " Juices and some products Related for consume human ", published : 17.12.2010 in the screen Official , no. 247–251, art. no. 1234.
17. MATAIX, E., DE CASTRO, ML Determination of total and free sulfur dioxide in wine by pervaporation–flow injection. In the: *Analyst* . 1998, no. 123(7), pp. 1547-1549 [cited 03.09.2022]. Available : https://doi.org/10.1039/A802566E
18. decision Government of the Republic of Moldova no. 1403 of 09.12.2008 with regarding the approval of the Regulation technical Vinegars and acid acetic acid for use food ". Published : 02.11.18 in : *Monitorul Official of the Republic of Moldova* no. 410-415, art. no. 1109.
19. SM SR EN 13188: 2012 Vinegar . Product manufactured from liquidity originating agricultural . Definitions , prescriptions , marking . *catalog sTANDARDS Nation of Republic of Moldova* : [in 2 volumes] / Inst . Nat . of Standardization (INS). – Publication official _ – Chisinau : Institute National Standardization , 2014. – ISBN 978-9975-9526-5-1 .
20. GOST 32097-2013. Vinegars of food raw material. General specifications. Date of introduction: 2014-07-01. *Federal Agency for Technical Regulation and Metrology* : Moscow, Russian, 2014.
21. LAMBRI, M., DORDONI, R., SILVA, A., DE FAVERI, DM Effect of Bentonite Fining on Odor-Active Comăounds in Two Different White Wine Styles. In: *Am. J. Enol. Vine* . 2010, no. 61: 2, pp. 225-233 [cited 03.09.2022]. Available : https://www.dalcin.com/ENG/altridw/pubblicazioni/bentoniti_AJEV_2010.pdf
22. BUDAK, NH, AYKIN, E., SEYDIM, AC et al. Functional properties of vinegar. In: J Food Sci. 2014, no. 79(5), pp. 757-64. PMID: 24811350 [cited 03.09.2022]. Available : https://doi.org/10.1111/1750-3841.12434 .
23. REY-LÓPEZ, JP, GONZALEZ, CA Research partnerships between Coca-Cola and health organizations in Spain. In: *European journal of public health* . 2019, no. 29(5), pp. 810–815 [cited 03.09.2022]. Available: https://doi.org/10.1093/eurpub/cky175
24. ISO 26642:2010 *Food products - Determination of the glycemic index (GI) and recommendation for food classification* . Publication date: 2010-10, Edition: 1, Number of pages: 18 [cited 03.09.2022]. Available : https://www.iso.org/standard/43633.html

25. ÖSTMAN, E., GRANFELDT, Y., PERSSON, L. et al. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. In the: Eur J Clin Nutr . 2005, no. 59, pp. 983–988 [cited 03.09.2022]. Available : https://doi.org/10.1038/sj.ejcn.1602197
26. BRIGHENTI, F., CASTELLANI, G., BENINI, L. et al. Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. In: *Eur J Clin Nutr* . 1995 Apr;49(4):242-7(1995). PMID: 7796781.

LIST OF THE AUTHOR'S PUBLICATIONS ON THE THEME OF THE THESIS

Monographs (chapter in the monograph collective)

1. CHIRSANOVA, A., **BOIȘTEAN, A.** , COVALIOV, E., RESITCA, V. Valorisation of crushed walnut shells in the process of acetic fermentation to obtain vinaigrette, Food waste: management and revalorisation of waste alimentary *publishing house Academic Press* , Cluj-Napoca. 2021, pp. 151-166. ISBN: 978-973-744-886-6. [cited 03.09.2022]. Available : http://cris.utm.md/handle/5014/1046

Articles in magazines scientifically

2. **BOISTEAN, A.,** CHIRSANOVA, A., CIUMAC, J., GAINA, B. The particularities of the clarification process with bentonite of white wine vinegar. In: *Food systems* . 2020, no. 3(1), pp. 25–32. UDC 663.242:666.322.4. DOI: 10.21323/2618–9771–2020–3–1–25–32. [cited 03.09.2022]. Available : https://www.fsjour.com/jour/article/view/65/101

3. **БОИШТЯН, А.В** ., КИРСАНОВА, А.И., РУБЦОВ, С.Л., КИСЕЛИЦА, Н. Finding the optimal source for the isolation of acetic bacteria. In: *Журнал Все о мясе*. 2020, no. 5S, pp. 59-62. ISSN 2071-2499, УДК 579.6 [cited 03.09.2022]. Available : https://www.vniimp.ru/journal/all-about-meat/2071-2499-2020/5s/59.html

4. **BOISTEAN,** A. Aspects of vinegar production and marketing in Moldova. In: *Journal of social sciences.* 2021, no. IV (2). ISSN 2587-3490 Available : https://doi.org/10.52326/jss.utm.2021.4(2).13

5. **BOISTEAN, A** ., CHIRSANOVA, A., ZGARDAN, D., MITINA, I., GAINA, B. The methodological aspects of using real-time PCR in acetobacter detection. In: *Journal of Engineering Science* . 2020, vol . V (XXVII), № 3, pp. 232-238. ISSN 2587-3474. Available : http://doi.org/10.5281/zenodo.3949726

ware and Theses in the collections scientifically

6. **BOIȘTEANA, A** ., POPOVICI, V. Development and optimization of new beverages based on different fruits or berries and white wine vinegar. *SCDS-UDJG 2021 The Ninth Edition, GALAțI, 10th-11th of June 2021* . 243p. Available : http://www.cssd-udjg.ugal.ro/index.php/2020-2/abstracts-2023

7. **BOIȘTEAN,** A. Investigation of obtaining vinegar using concentrated juice. *The 16th International Conference of Constructive Design and Technological Optimization in Machine Building Field, OPROTEH* , Bacău , May 25-27, 2021. 70p. ISSN 2457 – 3388. Available : http://oproteh.ub.ro/assets/abstracts.pdf?v=8439f13s

8. MARCU, O., **BOIȘTEAN,** A. Technology of obtaining wine vinegar from grape concentrate. *The 9th International Conference for Students STUDENT IN BUCOVINA* , December, 18th, 2020. Suceava, Romania. 44 p. ISSN 2068-7648. Available : http://fia-old.usv.ro/fiajournal/Student_in_Bucovina_2020/doc/Abstracte%20SIB%2018%20decembrie%202020.pdf

9. **BOIȘTEAN, A.** , CHIRSANOVA, A., CIUMAC, J. The particularities of the clarification process with bentonite of the wine vinegar. *The 9th international symposium "Euro-Food"* . 5-6 September 2019, Galați , Romania . 60 p. ISSN 1843-5114. Available : https://www.gup.ugal.ro/guppress/index.php/test/catalog/book/859

10. **BOISTEAN, A.** , STURZA, R., CHIRSANOVA A., MITINA I. Comparative analysis of two methods for detection of the DNA of acetic acid bacteria by real-time PCR. *Symposium Scientific International " Sector Agrifood - Achievements and Perspectives"* November 19-20 , 2021. Chisinau , Republic of Moldova. pp. 43-48. CZU: 663.15. ISBN 978-9975-64-329-0. Available : http://dspace.uasm.md/handle/123456789/7308

11. **BOISTEAN, A.** Grapes pomace as a filler in wine vinegar fermentation. *Symposium scientific national with participation international " Modern biotechnologies - solutions for challenges world contemporary "* . Chisinau , 20-21 May 2021. 42p. CZU: 663.252.6:663.242. DOI: https://doi.org/10.52757/imb21.008

12. **BOISTEAN, A.,** CHIRSANOVA, A., CIUMAC, J., GAINA B. The impact fermentation conditions _ on quality wine vinegar . *Conference International days Academy of Sciences technically from Romania with the theme " Creativity in the development company Knowledge ",* October 17-18 , 2019, Chisinau , Republic of Moldova. Available : https :// old . asm . md / gallery / Program %20 ZASTR %202019. pdf

13. **BOISHTYAN , A.** _ The isolation of vinegar bacteria from local wine production. *The scientific- practical conference national " Innovation : factor of social- economic development "* Edition of Va Cahul , Republic of Moldova, December 17, 2020. https :// www . dry _ md / wp - content / uploads /2020/12/ Program - Conference - IFDSE -17.12.2020. pdf

14. **BOISTEAN,** A. the impact added of nutrients on the process of obtaining apple cider vinegar . *The technical-scientific conference of students , master 's students and for PhD students* , April 1-3 , 2020, Chisinau , Republic of Moldova. 2020, vol. I. pp. 441-442. ISBN 978-9975-45-632-6. Available : https://ibn.idsi.md/ro/vizualizare_articol/106516

Patents of inventions and other property objects _ intellectual , materials at the invention salons

15. **BOIȘTEAN,** A., CHIRSANOVA, A., GAINA, B., SIMINIUC, R. Procedure for obtaining vinegar from white wine . *Patent MD 1517(13)Y* . Published in the *: BOPI no. 4/2021.* Available : http://www.agepi.md/sites/default/files/bopi/BOPI_04_2021.pdf

- Silver medal , EUROINVENT 2021, 13th European Exhibition of Creativity and Innovation Iasi, Romania, 20-21 May 2021.
- Silver medal _ and the Diploma of Excellence , PRO INVENT 2021, Salonul international research _ scientific , innovations and inventions-19th edition , Cluj-Napoca, Romania, October 20-22, 2021.
- Silver medal , INFOINVENT 2021, Exhibition International Specialized , Chisinau , Moldova, NOVEMBER 17-20, 2021.

16. **BOIȘTEAN, A.,** CHIRSANOVA , A., RESITCA, V., STURZA, R., DESEATNICOVA, O., CAPCANARI, T. Procedure for obtaining the drink non- alcoholic . *Patent MD 1630 (13) Y* . Published in the *: BOPI no. 7/2022,* p.45. Available : https://agepi.gov.md/sites/default/files/bopi/BOPI_07_2022.pdf

ADNOTARE

Boiștean Alina: ”Optimizarea tehnologiei și caracterizarea calității oțetului de vin autohton”, teza de doctor în științe inginerești, Chișinău, 2022.

Teza constă din introducere, 5 capitole, concluzii și recomandări, bibliografie (204 titluri) înserate în 110 pagini conținut de bază, inclusiv 43 figuri, 39 tabele (cu excepția celor indicate în anexe), 13 anexe. Rezultatele au fost reflectate în 14 lucrări științifice și 2 brevete.

Cuvinte-cheie: oțet, bacterii acetice, fermentare alcoolică, fermentare acetică, substrat, vin.

Scopul lucrării: constă în optimizarea tehnologiei de obținere a oțetului din vin cu utilizarea tulpinii de bacterii acetice izolate din produsele vitivinicole autohtone, inoculate pe substrat din materii prime locale și valorificarea acestuia pentru obținerea băuturilor nealcoolice.

Obiectivele cercetării: izolarea culturilor pure de bacterii acetice din produsele vitivinicole autohtone și identificarea lor după caracteristicile morfologice, culturale, fiziologice, biochimice și moleculare; studiul privind producătorii existenți și sortimentul de oțeturi în RM; evaluarea influenței diferitor factori asupra fermentării acetice; optimizarea tehnologiei de obținere a oțetului din vin; valorificarea oțetului din vin pentru obținerea unor băuturi nealcoolice.

Noutatea și originalitatea științifică. Pentru prima dată a fost efectuată izolarea și identificarea unei tulpini autohtone de bacterii acetice *Acetobacter aceti* CNMN-AcB-01 provenite din oțet artizanal din vin alb obținut din soiul de viță-de-vie Noah. Au fost stabilite dozele-limită de dioxid sulf și de cultură starter pentru asigurarea derulării eficiente a fermentării acetice. În premieră a fost utilizată coaja de nuci grecești și alune în calitate de substrat pentru bacteriile acetice, astfel micșorând perioada de fermentare și sporind calitățile organoleptice a oțetului obținut. A fost examinată evoluția glicemiei după consumarea băuturilor nealcoolice elaborate și s-a constatat că băuturile se clasifică în grupa produselor cu indice glicemic scăzut și pot fi recomandate ca alternativă sănătoasă băuturilor din comerț.

Problema științifică soluționată: dezvoltarea și fundamentarea științifică a regimurilor biotehnologice de obținere a oțetului din vin prin fermentarea naturală cu utilizarea tulpinii de bacterii acetice izolate din materii prime autotone, dezvoltată pe substrat natural.

Semnificația teoretică. Aplicarea tehnicilor de biologie moleculară pentru izolarea și identificarea tulpinii studiate prin utilizarea metodei real-time PCR. A fost demonstrată posibilitatea ameliorării parametrilor cromatici ai oțetului din vin alb prin utilizarea cojii de nuci ca substrat pentru dezvoltarea bacteriilor acetice, mărind suprafața de contact cu produsul, astfel eficientizând procesul de fabricare a oțetului.

Valoarea aplicativă: constă în stabilirea condițiilor optime de fermentare acetică a vinului autohton și elaborarea instrucțiunilor tehnice pentru obținerea oțetului din vin. Procedeele tehnologice recomandate în baza studiului pot fi aplicate la întreprinderile de profil, iar utilizarea cojii de nuci soluționează problema deșeurilor agroalimentare. Au fost obținute 2 brevete de invenție.

Implementarea rezultatelor științifice. A fost elaborată instrucțiunea tehnologică IT MD 67-41184408-01:2021 privind fabricarea oțetului din vin și efectuate testări a tulpinii de bacterii acetice *Acetobacter aceti CNMN-AcB-01* în cadrul întreprinderii SRL V.DEVELOP. Rezultatele obținute au fost reflectate în rapoartele proiectului nr. 18.80015.5007.222T și în 2 brevete de invenție. Brevetul de invenție de scurtă durată cu nr. MD 1517 Y a fost solicitat de către SRL FERMENTED FRUTS care a închiat contract de licență neexclusivă de folosire a acestuia, în scopul comercializării produsului obținut în urma aplicării procedeului descris.

АННОТАЦИЯ

Боиштян Алина: „Оптимизация технологии и характеристики качества местного винного уксуса”, диссертация на соискание ученой степени доктора технических наук, Кишинев, 2022.

Диссертация состоит из введения, 5 глав, заключений и рекомендаций, списка литературы (204 источников), основной текст содержит 110 страницы, в том числе 43 рисунков и 39 таблиц (без учета приведенных в приложениях), 13 приложений. Полученные результаты отражены в 14 научных работах и в 2 патентах.

Ключевые слова: уксус, уксуснокислые бактерии, алкогольная ферментация, уксуснокислая ферментация, субстрат, вино.

Цель работы: заключается в оптимизации технологии получения уксуса из вина с использованием штамма уксуснокислых бактерий, выделенного из местной винодельческой продукции, инокулированного на субстрат из местного сырья и его использование для получения безалкогольных напитков.

Задачи работы: выделение чистых культур уксуснокислых бактерий из местных винодельческих продуктов и их идентификация по морфологическим, культуральным, физиологическим, биохимическим и молекулярным признакам; изучение существующих производителей и ассортимента уксусов в РМ; оценка влияния различных факторов на уксуснокислое брожение; оптимизация технологии получения винного уксуса; использование винного уксуса для получения безалкогольных напитков.

Научная новизна и оригинальность. Впервые проведено выделение и идентификация местного штамма уксуснокислых бактерий *Acetobacter aceti* CNMN-AcB-01 из сырого белого винного уксуса, полученного из винограда сорта Noah. Установлены предельные дозы диоксида серы и заквасочной культуры, обеспечивающие эффективное протекание уксуснокислого брожения. Впервые в качестве субстрата для уксуснокислых бактерий была использована скорлупа грецкого ореха и фундука, что позволило сократить период брожения и повысить органолептические качества полученного уксуса. Было изучено изменение уровня глюкозы в крови после употребления разработанных безалкогольных напитков, и было установлено, что напитки относятся к группе продуктов с низким гликемическим индексом и могут быть рекомендованы в качестве здоровой альтернативы коммерческим напиткам.

Научная проблема: заключается в разработке и научном обосновании биотехнологических режимов получения уксуса из вина путем естественного брожения с использованием штамма уксуснокислых бактерий, выделенных из местного сырья, закрепленного на натуральном субстрате.

Теоретическая значимость. Применение методов молекулярной биологии для выделения и идентификации исследуемого штамма с помощью метода ПЦР в реальном времени. Продемострирована возможность улучшения хроматичсеких параметров белого винного уксуса за счет использования скорлупы грецкого ореха в качестве субстрата для развития уксуснокислых бактерий, увеличение поверхности контакта с продуктом, что делает процесс производства уксуса более эффективным.

Практическая ценность работы: заключается в установлении оптимальных условий уксуснокислого брожения местного вина и разработке технических инструкций по получению винного уксуса. Технологические процессы, рекомендованные на основе исследования, могут быть применены на специализированных предприятиях, а использование скорлупы грецкого ореха решит проблему агропродовольственных отходов. Получено 2 патента на изобретения.

Внедрение научных результатов. Разработана технологическая инструкция IT MD 67-41184408-01:2021 по производству винного уксуса и проведены испытания штамма уксуснокислых бактерий *Acetobacter aceti CNMN-AcB-01* на предприятии SRL V.DEVELOP. Полученные результаты были отражены в отчетах проекта №. 18.80015.5007.222T и в 2 патентах. Краткосрочный патент на изобретение №. MD 1517 Y был запрошен SRL FERMENTED FRUTS, которая заключила неисключительный лицензионный договор на его использование с целью маркетинга продукта, полученного в результате применения описанного процесса.

ABSTRACT

Boistean Alina: "Optimizing the technology and quality characteristics of local wine vinegar", doctoral thesis in engineering sciences. Chisinau, 2022.

The thesis consists of introduction, 5 chapters, general conclusion and recommendations, references (204 bibliographic sources), the basic text contains 110 pages, including 43 figures and 39 tables (except those indicated in the annexes), 13 annexes. The results are reflected in 14 scientific papers and 2 patents.

Keywords: vinegar, acetic bacteria, alcoholic fermentation, acetic fermentation, substratum, wine.

Purpose: consists in optimizing the technology for obtaining vinegar from wine using a strain of acetic acid bacteria isolated from local wine products, inoculated on a substrate from local raw materials and using it to produce soft drinks.

Objectives: isolation of pure cultures of acetic acid bacteria from local wine products and their identification by morphological, cultural, physiological, biochemical and molecular features; study of existing producers and range of vinegars in the Republic of Moldova; assessment of the influence of various factors on acetic acid fermentation; optimization of wine vinegar production technology; using wine vinegar to produce soft drinks.

Scientific novelty and originality. For the first time, a local strain of acetic acid bacteria *Acetobacter aceti CNMN-AcB-01* was isolated and identified from raw white wine vinegar obtained from Noah grapes. The limiting doses of sulfur dioxide and starter culture have been established, which ensure the effective flow of acetic acid fermentation. For the first time, walnut and hazelnut shells were used as a substrate for acetic acid bacteria, which made it possible to shorten the fermentation period and increase the organoleptic qualities of the resulting vinegar. The change in blood glucose levels after consumption of developed soft drinks was studied, and it was found that the drinks belong to the group of products with a low glycemic index and can be recommended as a healthy alternative to commercial drinks.

Solved scientific problem: consists in the development and scientific substantiation of biotechnological regimes for obtaining vinegar from wine by natural fermentation using a strain of acetic acid bacteria isolated from local raw materials, fixed on a natural substrate.

Theoretical significance. Application of molecular biology methods for the isolation and identification of the studied strain using the real-time PCR method. The possibility of improving the chromatic parameters of white wine vinegar by using walnut shell as a substrate for the development of acetic acid bacteria, increasing the contact surface with the product, which makes the vinegar production process more efficient, has been demonstrated.

Applicative value: consists in establishing the optimal conditions for the acetic fermentation of local wine and developing technical instructions for obtaining vinegar from wine. The technological procedures recommended on the basis of the study can be applied in specialized enterprises, and the use of walnut shells will solve the problem of agri-food waste. Received 2 patents for inventions.

Implementation of scientific results. The IT MD 67-41184408-01:2021 technological instruction on the manufacture of wine vinegar was developed and testing of the acetic bacteria strain Acetobacter aceti CNMN-AcB-01 was carried out within the company SRL V.DEVELOP. The results obtained were reflected in the project reports no. 18.80015.5007.222T and in 2 patents. Short-term invention patent with no. MD 1517 Y was requested by SRL FERMENTED FRUTS, which concluded a non-exclusive license contract for its use, for the purpose of marketing the product obtained following the application of the described process.

BOISTEAN ALINA

OPTIMIZATION OF THE TECHNOLOGY AND CHARACTERIZATION OF THE QUALITY OF VINEGAR FROM LOCAL WINE

253. 01 - TECHNOLOGY OF FOOD PRODUCTS OF VEGETABLE ORIGIN

Summary of the doctoral thesis in engineering sciences

Approved for printing: 30.09.2022	Paper format: 60x84 1/16
Offset paper. RISO pattern	Circulation 5 0 ex.
Pattern sheets 2.0	Order no. 75

UTM, 2004, Chisinau , bd. Stephen the Great and Saint , 168.

Drafting and Editing Section of UTM

2068, Chisinau , 9/9 Studentilor str