OPTIMIZATION OF PROCESS PARAMETERS FOR VINEGAR PRODUCTION USING CONCENTRATED APPLE OR GRAPE JUICE

OPTIMIZAREA PARAMETRILOR PROCESULUI DE PRODUCȚIA OȚETULUI CU UTILIZAREA SUCULUI CONCENTRAT DE MERE SAU DE STRUGURI

Alina BOISTEAN
Technical University of Moldova
E-mail: alina.boistean@toap.utm.md
ORCID ID: 0000-0002-5374-5853

Aurica CHIRSANOVA
Technical University of Moldova
E-mail: aurica.chirsanova@toap.utm.md
ORCID ID: 0000-0002-1172-9900

Rodica STURZA
Technical University of Moldova
E-mail: rodica.sturza@chim.utm.md
ORCID ID: 0000-0002-9552-1671

Boris GAINA
Academy of Sciences of Moldova
E-mail: alina.boistean@toap.utm.md
ORCID ID: 0000-0002-3536-1477

Abstract: *Vinegar production is a small industry in the general economy of industrialized countries, but no less important. It is important to emphasize that the raw materials used for the production of vinegar are also a very important part of the industrial economy. Within the framework of this study, the technology of obtaining vinegar from grape and apple concentrates was analyzed in order to unify production. As well as obtaining vinegar from concentrates with the same physical and chemical parameters all year round. The influence of nutrients and salts on the fermentation process has also been studied. In this study, considered the problem associated with obtaining vinegars from concentrated juices with the maximum identical physical and chemical parameters, obtained without deviations and unnecessary costs and with better organoleptic characteristics. And also, the study of the influence of nutrients on the processes of alcoholic and acetic fermentation.*
Keywords: *fermentation, vinegar, grape concentrate, apple concentrate, acetic acid*

Rezumat: *Producția de oțet este o mică industrie în economia generală a țărilor industrializate, dar nu mai puțin însemnată. Este important de subliniat faptul că materiile prime utilizate pentru producerea oțetului reprezintă o parte foarte importantă a economiei industriale. În cadrul acestui studiu a fost analizată tehnologia de obținere a oțetului din concentrate de struguri și mere în vederea unificării producției. Precum și obținerea de oțet din concentrate cu aceiași parametri fizico-chimici pe tot parcursul anului. S-a studiat și influența nutrienților și a sărurilor asupra procesului de fermentație. În acest studiu s-a luat în considerare problema asociată obținerii oțeturilor din sucuri concentrate cu parametrii fizico-chimici identici maximi, obținute fără abateri și costuri inutile și cu caracteristici organoleptice mai bune. Și, de asemenea, studiul influenței nutrienților asupra proceselor de fermentație alcoolică și acetică.*
Cuvinte cheie:: *fermentație, oțet, concentrat de struguri, concentrat de mere, acid acetic*

Introduction

Vinegar consumption dates at least from 5000 BC, when Babylonians used it as a condiment and as a preservative [1]. Before the advent of sulfuric acid, acetic acid was the strongest known acid, so vinegar was used as a preservative due to its antimicrobial activity. This antimicrobial activity was used to heal wounds and as a general antiseptic, and was even used for treating wounds during World War I. The preservative properties of vinegar laid the foundation for the development of the process of pickling vegetables [2, 3].

Every year, large amounts of vegetables and fruits are produced and wasted since the excess cannot be consumed or because they are considered of a second or third quality category. According to the FAO 21.6% of the fruit produced in the world is wasted, starting from the post-harvest stage until its distribution [4]. Very often, fruit is rejected simply because of its “imperfect” appearance or inadequate size, even if the fruit is perfectly edible. These actions lead to both ecological and economic problems; therefore, environmental pollution and rising prices can be the consequence of fruit and vegetables overproduction [5].

Thus, the alternatives that these products can use are extremely valuable. One of the options for using these second-rate products may be processing them into purees, juices, concentrates, etc. And also, options are possible associated with the production of vinegar, its enrichment or use for the production of functional drinking vinegar drinks [6, 7].

In recent years, new vinegar varieties have been developed to diversify the offer and cover the new requirements from consumers. Attending to its official definition, vinegar is a suitable for human intake liquid obtained from double fermentation (alcoholic and acetic) based on agricultural raw materials that contain starch and/or sugars (*Codex Alimentarius).* Therefore, any fruit juice could be susceptible to be transformed into vinegar [8-10]. The lasts research has focused on obtaining vinegar not only from fruits, for example, from guava and banana [11], kiwi [12], orange [13] or of cajá (Spondias mombin L.) [14] but also from vegetables, such as onion [15] or tomatos [16].

Figure 1 shows the number of scientific articles by Scopus on fruit vinegar published between 1990 and 2020. As you can see, there has been an exponential growth in recent years, which will demonstrate the growing interest of the scientific community in these products [17].

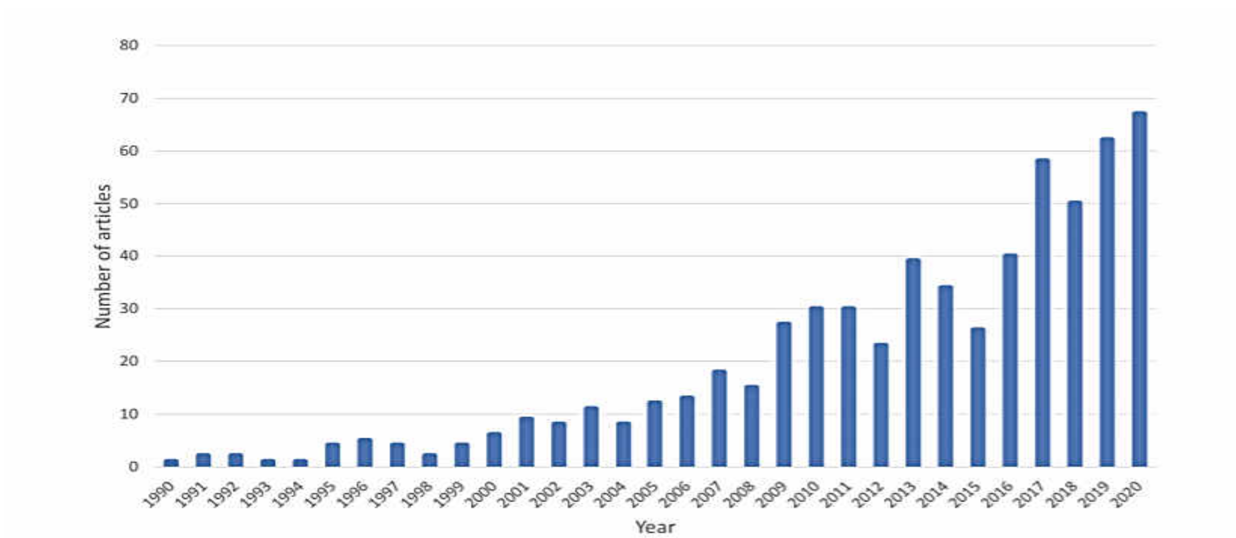

Figure 1. Number of scientific articles on fruit vinegar published per year [16].

The raw material employed for vinegar production plays an important role in the final characteristics of the developed product. This is why the production of fruit vinegar has its own problems. Firstly, for the production of fruit vinegar, quality raw materials are needed, and fruit is one of the most perishable products [18]. Therefore, the production of vinegar must be close to the place of fruit collection or have special refrigerated chambers for their proper storage, these costs affect the cost of the final product. Secondly, the quality of raw materials varies from year to year, which makes it difficult to obtain a standard product or requires additional steps to fit the standard. All these problems can be solved by using a stabilized juice concentrate as a raw material, which can be stored and used more conveniently in the production of both juices and vinegar [19].

The Republic of Moldova has a high potential for the production of apples (about 300 thousand tons per year) and grapes for industrial processing (over 350 thousand tons). For fresh consumption, no more than 50% of the fruit harvest is harvested and exported, so over 150 thousand tons of apples are directed to industrial processing [20, 21]. Given that the market of the Republic of Moldova is saturated with apple juice and concentrate, and their export prices decrease from year to year, due to high competition with producers in Ukraine, China and the European Union, it is appropriate that part of the juice apples and grapes should be directed to the production of a new biologically active product such as natural vinegar [22, 23].

Research Methodology

The research was carried out in two stages: the first stage consists of alcoholic fermentation and the second stage acetic fermentation. In this study, two types of fruit concentrates were used: apple and wine, which were purchased from the local manufacturer S.A. "ALFA-NISTRU" with the provision of all sanitary documents [23]. The physico-chemical characteristics of the juice concentrates are presented in table 1.

Table 1. Characteristic of concentrates

Quality indices	**Grape concentrate**	**Apple concentrate**
Composition: basic ingredients	100% white grape juice	100% white apple juice
Physical description	Viscous, yellow	Viscous, dark yellow
Physico-chemical characteristics	Brix0 55 ± 0.5 at T 20± 1°C pH 2.57± 0.2 at T 20± 1°C Density = 1260± 0.5 kg/m^3	Brix0 70 ± 0.3 at T 20± 1°C pH 2,93 ± 0.5 at T 20± 1°C Density = 1 370± 0.1 kg/m^3
Organoleptic characteristic	Taste, aroma specific to grape juice	Taste, aroma specific to apple juice
Period of storage	12 months at T=10-15± 1°C	18 months at T= max +15°C

To carry out the first stage of the study, that is, obtaining wine and cider by alcoholic fermentation, the yeast ENARTIS FERM SC was used. Parameters of yeast ENARTIS FERM SC (Esseco Srl - Enology Division, Italia) are presented in Table 2.

Table 2. Parameters yeast ENARTIS FERM SC

Parameters	**Value**
Fermentation temperature	15-30°C
Fermentation speed	high
Alcohol tolerance	≤ 13%
SO_2 resistance	50 mg
Sugar / alcohol ratio	16.5 g of sugar to 1% alcohol

Two types of nutrients were used to accelerate the fermentation process investigate the significance of their influence: NUTRIFERM SPECIAL (diammonium phosphate (DAP) 50%, autolyzed yeast 49.9%, thiamine-0.1%) and NUTRIFERM ADVANCE (inactive yeast 50%, DAP 40 %, cellulose 10%), also produced by the company Esseco s.r.l. – Enartis Division, Italia. To obtain vinegar from the intermediate, we used vinegar mother-culture (Brouwland bv, Belgium) for starting up acetic fermentation.

In both fermentation periods, the quality parameters were monitored: pH-using pH meter Hanna HI 9124; total soluble solids (ºBrix)-using ATC refractometer, model RHB-32; density by densitometer, temperature, alcohol concentration and sugar concentration (for alcoholic fermentation), total acidity and alcohol concentration (for acetic fermentation) [24].

Vinegar is produced through a two-stage fermentation process, the first being the conversion of fermentable sugars into ethanol by yeasts- Saccharomyces, and the second being the oxidation of ethanol by bacteria- Acetobacter. Fermentation process is importance in the production of vinegars, during which many volatile compounds, polyphenols, and organic acids, are modified through chemical and microbial actions.

For the first stage of the study, we used concentrated juice diluted with distilled water to a sugar content of 25%. Before the beginning of alcoholic fermentation, the diluted juice was brought to a boil, to reduce microbiological contamination, the level of sulfur dioxide, which suppresses the activity of yeast to achieve alcoholic fermentation and for the purity of the experiment. Further, the study proceeded according to scheme 2a and b for two samples, with and without the addition of nutrients, at a temperature of 22 ± 1°C, isolated from sunlight and without oxygen.

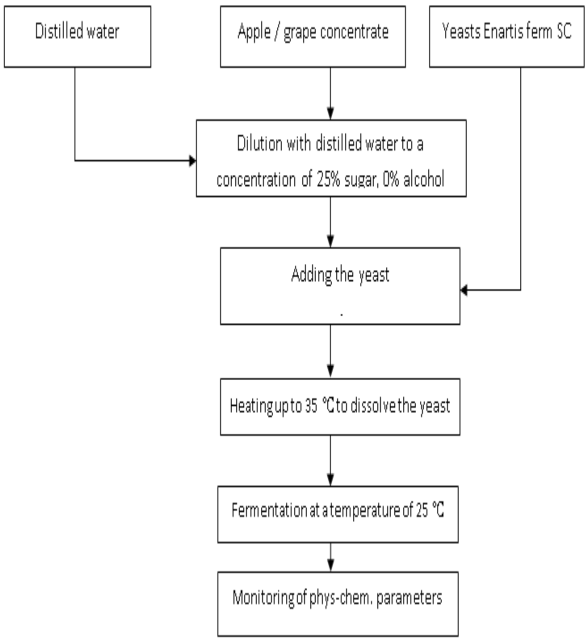

Figure 2a. Technological scheme for obtaining wine/cider from concentrated juice.

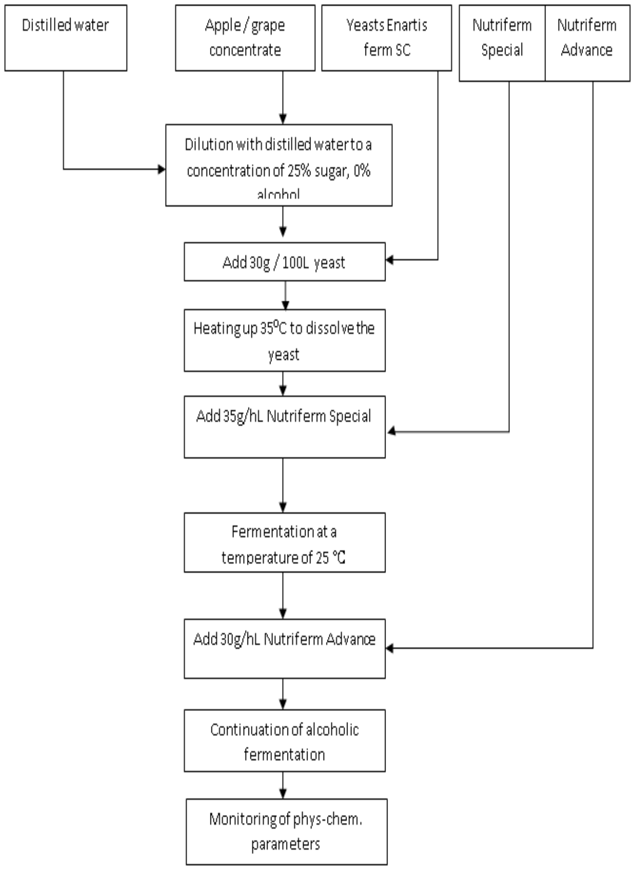

Figure 2b. Technological scheme for obtaining wine/cider from concentrated juice with the addition of nutrients.

Once the sugar has been transformed into ethanol, the next fermentation that takes place in the process to elaborate fruit vinegars is the acetic fermentation, which consists of the oxidation of the alcohol into acetic acid. This is an oxygen-dependent reaction, and, therefore, the oxygen concentration must be increased for the acetic fermentation to take place. Despite the fact that the metabolism of acetic acid bacteria is aerobic, aeration is not used for research. However, they can survive under anaerobic conditions or with very low oxygen concentrations since they have the possibility to use quinones instead of oxygen as the final electron acceptor [18]. To speed up the process, the following substances were used as nutrients: ammonium sulfate $(NH_4)_2SO_4$-0,135 g/L, sucrose $C_{12}H_{22}O_{11}$-2,7 g/L, potassium carbonate K_2CO_3-0,005 g/L.

Discussions

Results of alcoholic fermentation of grape juice

The results obtained show that the alcohol concentration of the juice from grapes increased from 0 g/L to 8,5 g/L and the sugar content decreased from 25 g/L to 7 g/L reflected in Figure 3a, so it was observed that alcoholic fermentation of the juice from grapes without nutrients for 11 days, a wine with an alcoholic concentration of 8.50% was obtained.

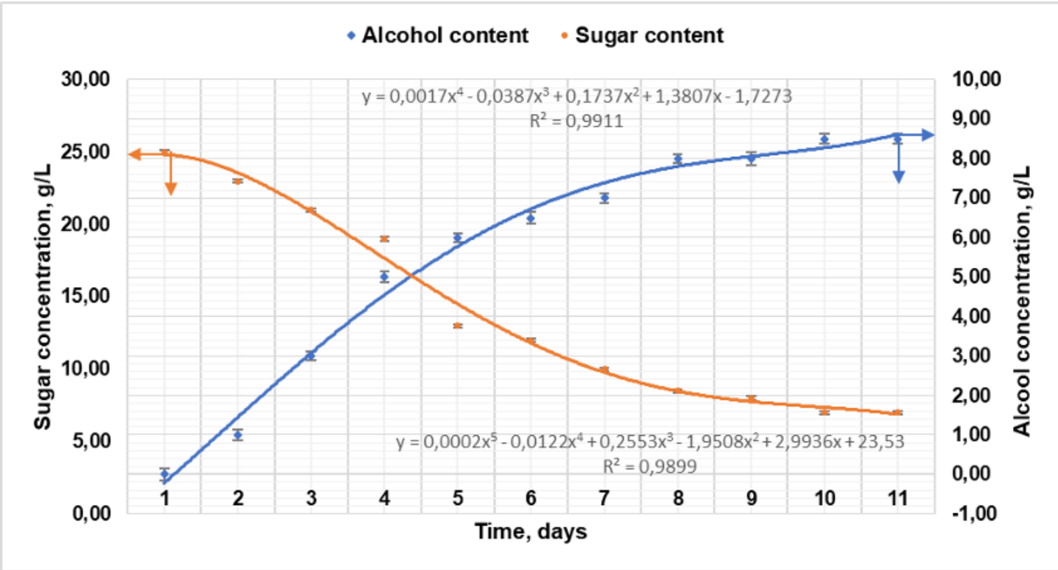

Fig.3a Evolution of sugar and alcohol concentration during alcoholic fermentation of grape juice

The results of samples with nutrients are shown in the figures 3b. As you can see, the alcohol concentration increased from 0 g/L to 9 g/L, which is 1.3 times more than in the samples without nutrients. The sugar content accordingly decreased from 25 g/L to 6 g/L, therefore, as a result of alcoholic fermentation of juice from grapes with nutrients for 11 days, a wine with an alcohol concentration of 9% was obtained.

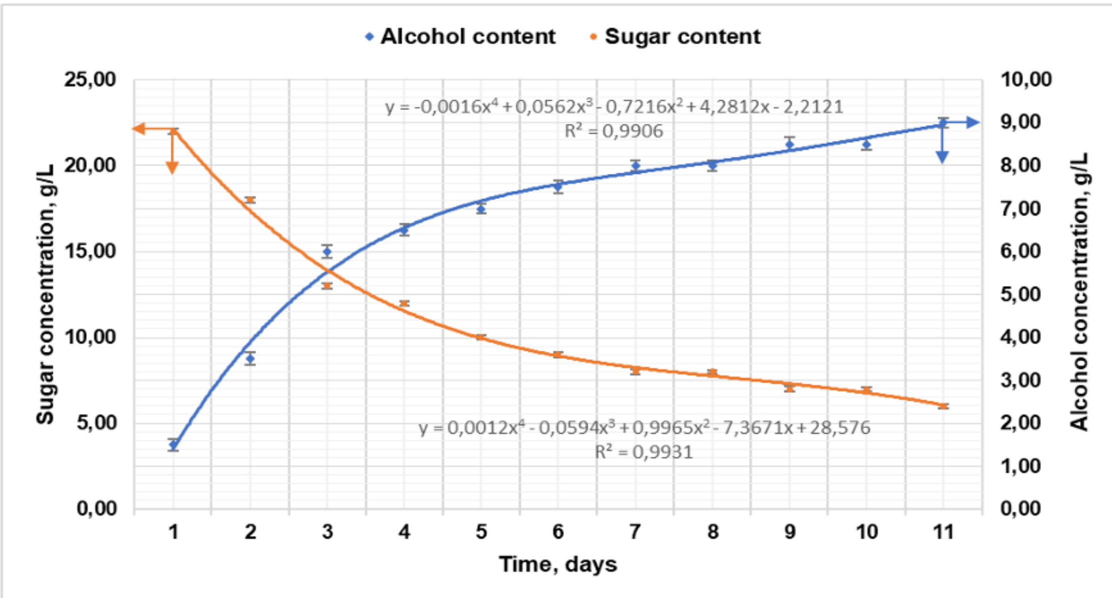

Fig.3b Evolution of sugar and alcohol concentration during alcoholic fermentation of grape juice with nutrients

Based on the results for grape juice with and without nutriments, it can be concluded that the influence of nutrients is not so important in alcoholic fermentation, since the difference between the samples is only 0.5% alcohol. It can be assumed that the juice contains all the nutrients for the fermentation process and the yeast does not need additional feeding.

Results of alcoholic fermentation of apple juice.
The results obtained in the process of alcoholic fermentation of apple juice without nutriments are presented in figure 4a, and show an increase in alcohol concentration from 0 g/L to 8.5 g/L, and a decrease in sugar from 25 g/L to 7 g/L. That is, after fermentation of apple juice without nutrients for 11 days, a cider with an alcohol concentration of 8.50% was obtained.

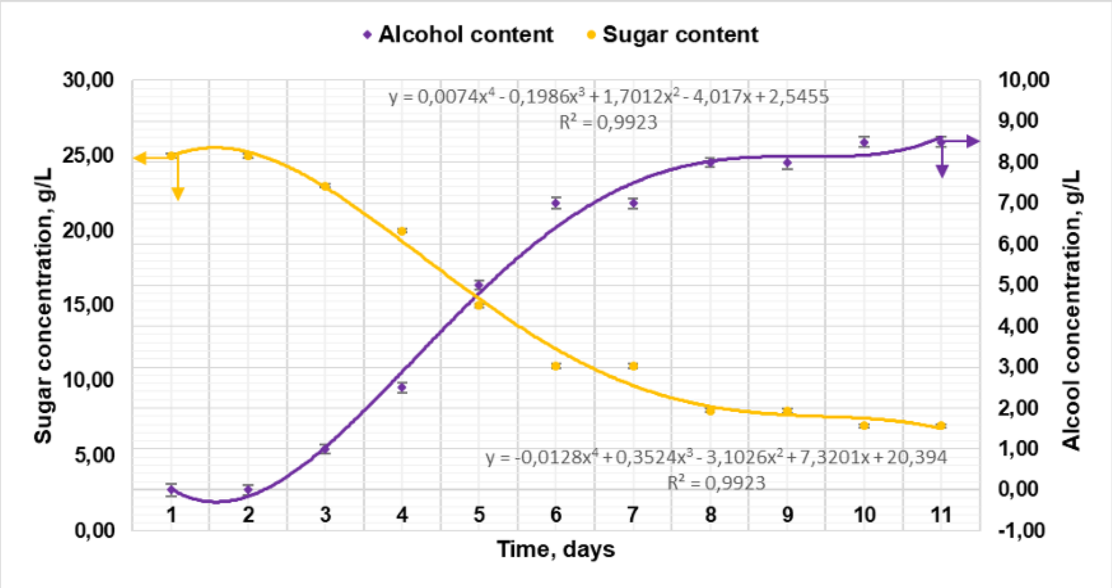

Fig.4a Evolution of sugar and alcohol concentration during alcoholic fermentation of apple juice

The results of the nutrient-rich apple juice samples are shown in figure 4b. As seen from the diagram, the alcohol concentration increased from 0 g/L to 9 g/L, which is 1.3 times more than in the samples without nutrients, as well as for grape juice. The sugar content decreased from 25 g/L to 7 g/L, and as a result of alcoholic fermentation of apple juice with nutrients for 11 days, a cider with an alcohol concentration of 9% was obtained, identical to that of the sample from grape juice.

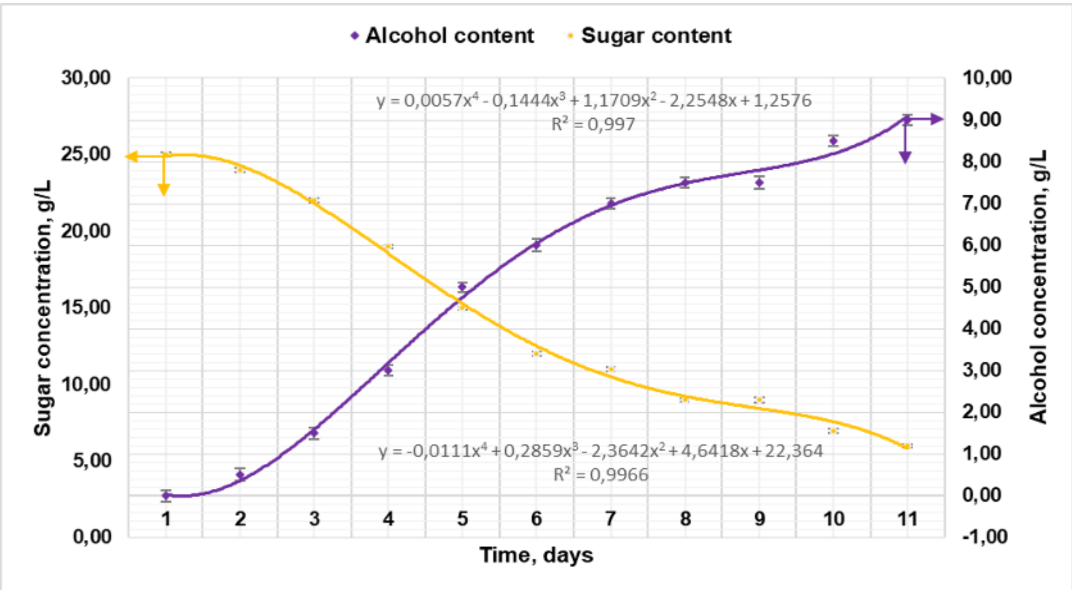

Fig.4b Evolution of sugar and alcohol concentration during alcoholic fermentation of apple juice with nutrients

As in the case of grape juice, a difference of only 0.5% alcohol between the samples was obtained. In this case, it can also be concluded that the introduction of additional nutrients is not so important, since apple juice contains all the nutrients for the fermentation process and perhaps the yeast does not need such an amount of nutrients.

Results of acetic fermentation of grape juice

The results of acidity and pH obtained in the process of acetic fermentation of samples from grape juice without and with nutrients are shown in the figures 5a and 5b. In figures, we can observe the period of adaptation of acetic bacteria to the environment and conditions, this adaptation lasted for 3-5 days.

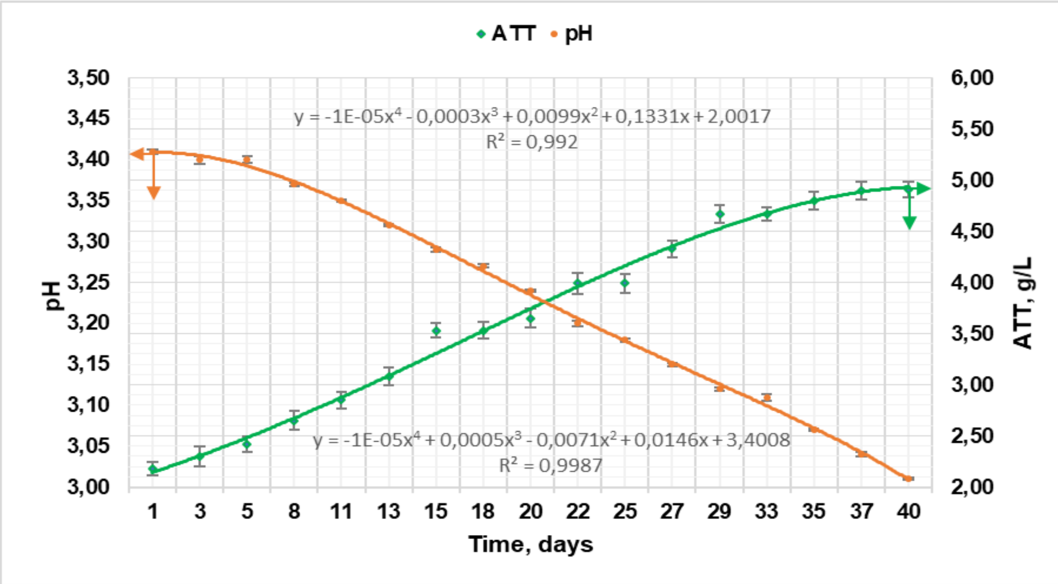

Fig.5a Evolution of pH and titratable acidity during acetic fermentation of grape juice

The acidity values for the sample without nutrients show a rapid increase from 2.18 to 3.53 in the first 15 days after the lag phase. Then, in the remaining 15 days, there is a less rapid increase from 3.53 to 4.91. After 40 days of acetic fermentation, an acidity accumulation of 4.91 g/L is observed. For pH, there is a linear decrease of 12% with a maximum value of 3.41 and a minimum of 3.01.

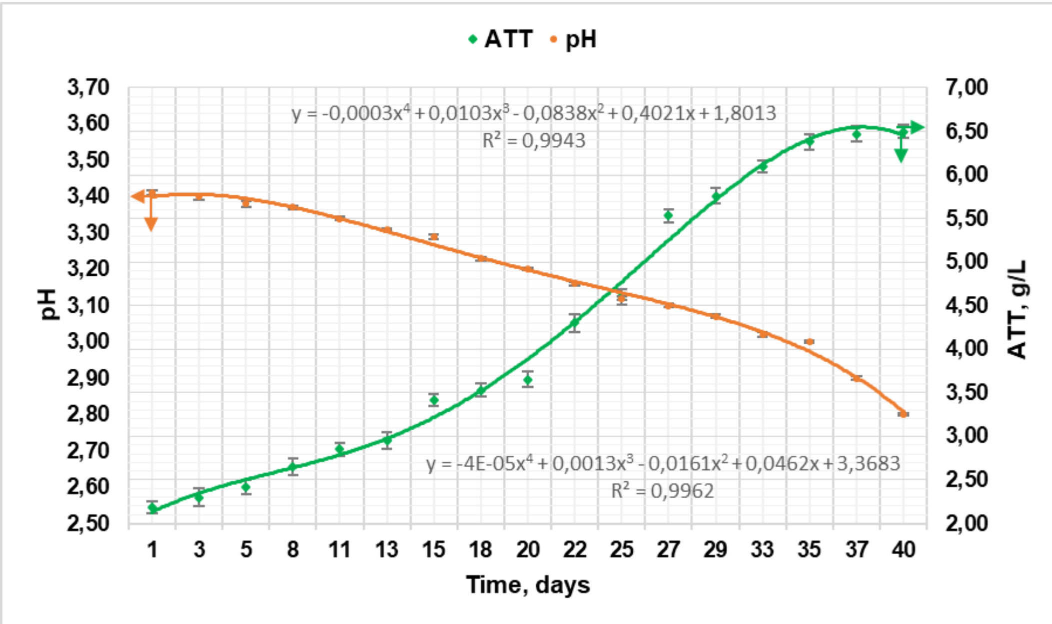

Fig.5b Evolution of pH and titratable acidity during acetic fermentation of grape juice with nutrients

The acidity parameter for the sample with added nutrients in the amount specified in paragraph “Materials and methods-Acetic fermentation” also shows a rapid increase from 2.18 to 3.42 in the first 15 days after the lag phase. But in the remaining 15 days, there is a faster growth from 3.42 to 6.5 g/L. That is, after 40 days of vinegar fermentation for samples with nutrients, there is an accumulation of acidity 33% more than in the sample without nutrients.

Results of acetic fermentation of apple juice

The results of acidity and pH obtained in the process of acetic fermentation of samples from apple juice without and with nutrients are shown in the figures 6a and 6b.

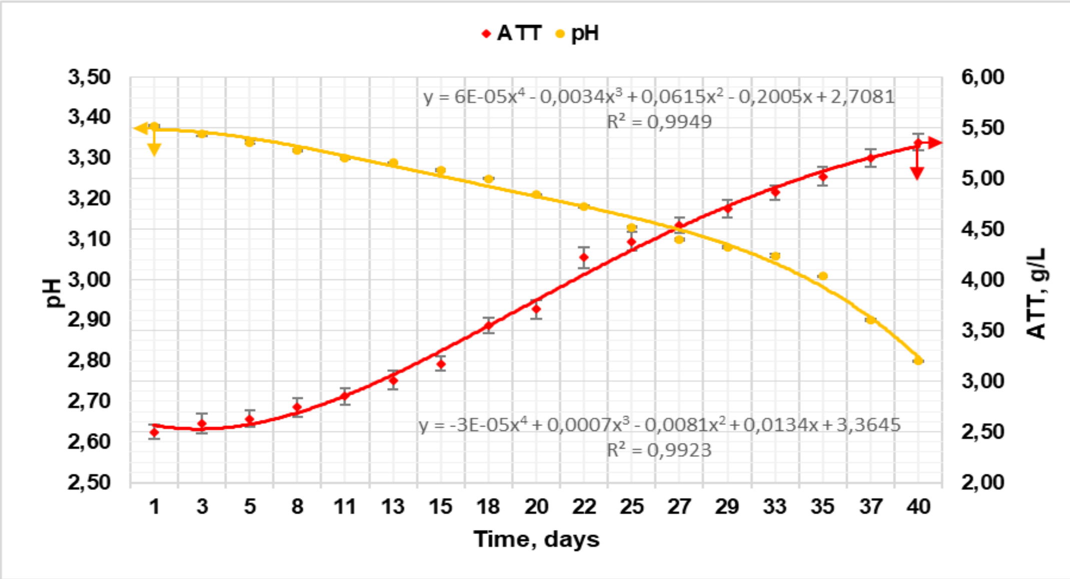

Fig.6a Evolution of pH and titratable acidity during acetic fermentation of apple juice

The acidity values for the sample without nutrients show a rapid increase from 2.5 to 3.17 in the first 15 days after the lag phase. Then, in the remaining 15 days, there is a less rapid increase from 3.17 to 5.36. After 40 days of vinegar fermentation, an acidity accumulation of 5.36 g/L is observed. For pH, there is a linear decrease of 24% with a maximum value of 3.38 and a minimum of 2.84.

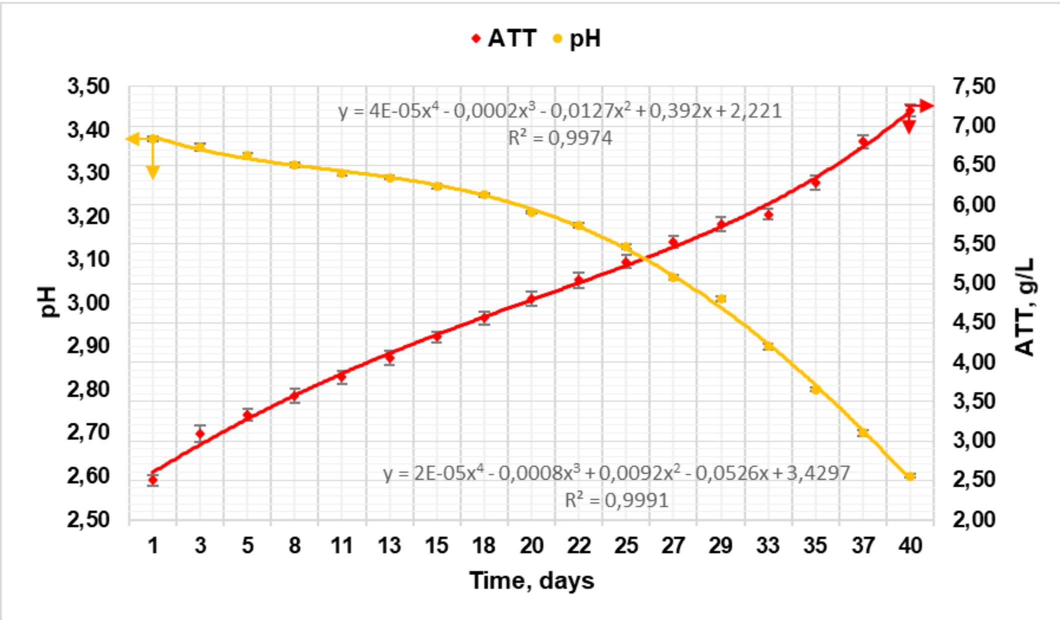

Fig.6b Evolution of pH and titratable acidity during acetic fermentation of apple juice with nutrients

The acidity parameter for the sample with added nutrients also shows a rapid increase from 2.5 to 4.32 in the first 15 days after the lag phase. But in the remaining 15 days, there is a faster growth from 4.32 to 7.2 g/L. That is, after 40 days of vinegar fermentation for samples with nutrients, there is an accumulation of acidity 35% more than in the sample without nutrients.

Conclusions

In the field of food technology, many methods and techniques have been described for obtaining a wide variety of vinegars from various types of raw materials. This article addressed two issues. The first problem is related to obtaining vinegar from concentrated grape and apple juices. In production, it is important to achieve the maximum identity of the physical and chemical indicators of the finished products obtained without deviations and unnecessary costs. And also obtaining fruit vinegar with newer organoleptic characteristics. The second question is related to the assessment of the influence of nutrients on fermentation processes: alcoholic and vinegar. Based on the data obtained, we can conclude that for the first stage, for alcoholic fermentation, the use of nutrients is not necessary, since the difference between the alcohol content in the obtained samples is only 0.5%. This value is insignificant; accordingly, we do not recommend using the studied nutrients and increasing the cost of the finished product. As for the second stage, vinegar fermentation, in this case we recommend the use of nutrients, since the amount used is small, but the effectiveness is much greater. The difference between samples without and with nutrients was about 35% in both types of juice. Therefore, we recommend the use of nutrients in the vinegar fermentation process to shorten the time to make the vinegar. From an economic point of view, the cost of the finished product will increase slightly because very few nutrients are used.

Acknowledgments

The research was funded by State Project 20.80009.5107.09 „Improving of food quality and safety through biotechnology and food engineering”, running at Technical University of Moldova.

Bibliography:

1. Mas, A.M. Troncoso, M.C. García-Parrilla, M.J. Torija, (2016) Vinegar, Editor(s): Benjamin Caballero, Paul M. Finglas, Fidel Toldrá, Encyclopedia of Food and Health, Academic Press, Pages 418-423, ISBN 9780123849533, https://doi.org/10.1016/B978-0-12-384947-2.00726-1.
2. Johnston CS, Gaas CA. Vinegar: medicinal uses and antiglycemic effect. MedGenMed. 2006 May 30;8(2):61. PMID: 16926800; PMCID: PMC1785201. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1785201/
3. Chen, H., Chen, T., Giudici, P. and Chen, F. (2016), Vinegar Functions on Health: Constituents, Sources, and Formation Mechanisms. COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY, 15: 1124-1138. https://doi.org/10.1111/1541-4337.12228
4. Food and Agriculture Organization. The State of Food and Agriculture 2019: Moving Fordward on Food Loss and Waste Reduction; Rome, 2019, http://www.fao.org/3/ca6030en/ca6030en.pdf
5. Eva-Marie Meemken and Matin Qaim, Organic Agriculture, Food Security, and the Environment, Annual Review of Resource Economics 2018 10:1, 39-63; https://doi.org/10.1146/annurev-resource-100517-023252
6. Chirsanova, A. Boiștean, E. Covaliov et V. Reșitca (2021) Valorisation de coquilles de noix broyées dans le processus de fermentation acetique afin d'obtenir du vinaigre, Le gaspillage alimentaire : gestion et revalorisation des déchets alimentaires. ISBN:978-973-744-886-6, Editura AcademicPres, Cluj-Napoca, URI: http://cris.utm.md/handle/5014/1046
7. Kim, SH., Cho, HK. & Shin, HS. Physicochemical properties and antioxidant activities of commercial vinegar drinks in Korea. Food Sci Biotechnol 21, 1729–1734 (2012). https://doi.org/10.1007/s10068-012-0230-y
8. European Commission, VINEGAR Food grade, Pilot project: Proposal for approbation of basic substances, in the context of Regulation (EC) N°1107/2009, Available at: http://www.bicga.org.uk/docs/BSA%20Vinegar%20February%202014.pdf
9. Codex Alimentarius. Regulation of Codex for vinegar (1987), p. 50-71; https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-706-15%252Fal87_19e.pdf
10. Britannica, The Editors of Encyclopaedia. "Vinegar". Encyclopedia Britannica, 31 Aug. 2020, Available at: https://www.britannica.com/topic/vinegar
11. Miskiyah, M., et al. "Inhibition of Escherichia Coli O157:h7 Contamination on Chicken Meat by Natural Vinegar Prepared From Banana Peel and Coconut Water." Journal of Indonesian Tropical Animal Agriculture, vol. 41, no. 1, 1 Mar. 2016, pp. 21-27, doi:10.14710/jitaa.41.1.21-27. https://www.neliti.com/publications/136032/inhibition-of-escherichia-coli-o157h7-contamination-on-chicken-meat-by-natural-v#cite
12. Woo SM, Kim OM, Choi IW, Kim YS, Choi HD, Jeong YJ. Condition of acetic acid fermentation and effect of oligosaccharide addition on kiwi vinegar. Korean J Food Preserv. 2007; 14:100-104. 1738-

7248(pISSN)/2287-7428(eISSN);
https://www.koreascience.or.kr/article/JAKO200712242674221.page

13. Cejudo-Bastante, C., Durán-Guerrero, E., García-Barroso, C. and Castro-Mejías, R. (2018), Comparative study of submerged and surface culture acetification process for orange vinegar. J. Sci. Food Agric, 98: 1052-1060. https://doi.org/10.1002/jsfa.8554
14. Dias, Disney Ribeiro, Schwan, Rosane Freitas, & Lima, Luiz Carlos Oliveira. (2003). Metodologia para elaboração de fermentado de cajá (Spondias mombin L.). Food Science and Technology, 23(3), 342-350. https://doi.org/10.1590/S0101-20612003000300008
15. Hirouchi, J., Yamauchi, N., Osugi, M., Kanno, T., Kobayashi, M., Kuriyama, H., Onion Alcohol Production by Repeated Batch Process Using a Flocculating Yeast. Bioresour. Technol., 75, 153-156 (2000). https://doi.org/10.1016/S0960-8524(00)00044-4
16. Cejudo-Bastante, C., Durán-Guerrero, E., García-Barroso, C., & Castro-Mejías, R. (2017). Volatile compounds, polyphenols and sensory quality in the production of tomato vinegar. J Food Nutr Res, 5, 391-398. DOI:10.12691/jfnr-5-6-5 ; http://pubs.sciepub.com/jfnr/5/6/5/index.html
17. Luzón-Quintana, L.M.; Castro, R.; Durán-Guerrero, E. Biotechnological Processes in Fruit Vinegar Production. Foods 2021, 10, 945. https://doi.org/10.3390/foods10050945
18. Sofia Maina, Aikaterini Papadaki, Vasiliki Kachrimanidou, Effimia Eriotou, Nikolaos Kopsahelis, Chapter-Raw Materials and Pretreatment Methods for Vinegar Production, Book- Advances in Vinegar Production, CRC Press, 2019, p28. ISBN 9781351208475, https://doi.org/10.1201/9781351208475-5
19. Laura Solieri, Paolo Giudici. Acetic Acid Bacteria Taxonomy from Early Descriptions to Molecular Techniques. In Vinegars of the World; Springer: Berlin/Heidelberg, Germany, 2009, P297. ISBN 978-88-470-0866-3; pp. 41–59, https://doi.org/10.1007/978-88-470-0866-3
20. Boiştean, Alina. Aspects of vinegars production and marketing. In: Journal of Social Sciences. 2021, nr. 4(2), pp. 128-138. ISSN 2587-3490. DOI: 10.52326/jss.utm.2021.4(2).13
21. Boistean A., Chirsanova A., Ciumac J., Gaina B. The particularities of the clarification process with bentonite of white wine vinegar. Food systems. 2020;3(1):25-32. https://doi.org/10.21323/2618-9771-2020-3-1-25-32
22. Boiştean, Alina; Chirsanova, Aurica; Zgardan, Dan; Mitina, Irina; Găină, Boris. Methodological aspects of real-time PCR usage in acetobacter detection. In: Journal of Engineering Sciences. 2020, nr. 3, pp. 232-238. ISSN 2587-3474, DOI: 10.5281/zenodo.3949726
23. Boiştean, Alina. Impactul adăugării unor nutrimenţi asupra procesului de obţinere a oţetului de mere. In: Conferinţa tehnico-ştiinţifică a studenţilor, masteranzilor şi doctoranzilor. Vol.1, 1-3 aprilie 2020, Chișinău. Chișinău, Republica Moldova: 2020, pp. 441-442. ISBN 978-9975-45-632-6. https://ibn.idsi.md/en/vizualizare_articol/106516
24. https://www.alfa-nistru.com/ro/items/10.html
25. Association of Official Analytical Chemists—AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists. AOAC Official Method 930.35, vol. 1, 16th ed., AOAC, 1995. http://lib3.dss.go.th/fulltext/scan_ebook/aoac_1995_v78_n3.pdf