

TECHNICAL UNIVERSITY OF MOLDOVA



As a manuscript
C.Z.U.: 664.863; 664.843.5; 664.858 (043)

CRUCIRESCU DIANA

USE OF UNRIPE APPLES TO OBTAIN THE NATURAL ACIDIFIER

253.01 PLANT BASED FOOD TECHNOLOGY

Abstract of the PhD in engineering sciences dissertation

CHISINAU, 2024

The thesis was developed in the Department of Food Technologies, Food Products Quality Verification laboratory, Scientific-Practical Institute of Horticulture and Food Technologies (IȘPHTA), Chisinau, Republic of Moldova.

PhD supervisor:

IORGA Eugen, PhD, scientific associate

Guidance committee:

STURZA Rodica, Dr. Hab., m.c. ASM, professor, UTM

BAERLE Alexei, PhD, associate professor, UTM

MACARI Artur, PhD, associate professor, UTM

Official Reviewers:

STURZA Rodica, Dr. Hab., m.c. ASM, professor, UTM

VRABIE Elvira, PhD, research associate, Institute of Applied Physics, USM

CECLU Liliana, PhD, associate professor, USCh

Composition of the Doctoral Thesis Examination Committee (DTEC):

1. GHENDOV-MOȘANU Aliona, President, Dr. Hab., associate professor, UTM

2. GUREV Angela, Scientific secretary, PhD, associate professor, UTM

3. IORGA Eugen, Member, PhD, scientific associate, IȘPHTA

4. SAVA Parascovia, Member, Dr. Hab., scientific associate, IȘPHTA

5. STURZA Rodica, Referent, Dr. Hab., m.c. ASM, professor, UTM

6. VRABIE Elvira, Referent, PhD, research associate, Institute of Applied Physics, USM

7. CECLU Liliana, Referent, PhD, associate professor, USCh

Public defense will take place on the 22.03.2024, at 14:00, in the Meeting of the Doctoral Thesis Examination Committee within the Technical University of Moldova, 9/9 Studentilor street, academic building nr. 5, Faculty of Food Technology, lecture hall 5-1, MD-2045, Chisinau, Republic of Moldova.

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

The summary was sent on February 21, 2024.

Scientific Secretary of the Doctoral Commission:

GUREV Angela, PhD, associate professor

Scientific supervisor:

IORGA Eugen, PhD, scientific associate

Author:

CRUCIRESCU Diana

© Crucirescu Diana, 2024

CONTENTS

CONCEPTUAL RESEARCH GUIDELINES	4
THESIS CONTENT	8
1 Acidifiers used in the food industry and biochemical changes in apples during development	8
2 Materials and methods	8
3 Research on the influence of unripe apple parameters on the quality of natural acidifier	8
3.1 Physicochemical characteristics of unripe apples during development	9
3.2 Dependence of the juice yield value on the treatment of unripe apples	11
3.3 Influence of parameters determined in unripe apples on juice yield	12
4 Elaboration of the manufacturing technology of the apples acidifier, its implementation in obtaining preserved fruits and vegetables and studying the quality of the elaborated products	13
4.1 Technology of obtaining the apples acidifier	13
4.2 Evaluation of the quality indicators of the acidifiers in the studied apples	14
4.2.1 Analysis of physicochemical indicators	14
4.2.1.1 Content of organic acids	14
4.2.1.2 Content of simple carbohydrates	16
4.2.1.3 Total polyphenol content	17
4.2.1.4 Antioxidant activity	18
4.2.2 Microbiological indicators in apple acidifiers	18
4.2.3 Organoleptic evaluation of apple acidifiers	19
4.2.4 Shelf life of apple acidifiers	19
4.3 Use of apple acidifier in fruit and vegetable preservation	21
4.3.1 Pickled tomatoes	21
4.3.2 Canned cucumbers	22
4.3.3 Vegetable stew of type „Zacusca”	23
4.3.4 Canned hot peppers	24
4.3.5 Sour Cherry Jam and Canned Strawberry	25
4.4 Feasibility study of the apple acidifier manufacture and some elaborate canned products	26
GENERAL CONCLUSIONS AND RECOMMENDATIONS	27
SELECTIVE BIBLIOGRAPHY	30
LIST OF THE AUTHOR'S PUBLICATIONS ON THE SUBJECT OF THE PhD THESIS	32
ANNOTATION	34

CONCEPTUAL RESEARCH GUIDELINES

The actuality of the investigated topic. The insufficiency of sources of natural acidity, used in the food industry, and the increased interest of consumers for healthier foods require scientists to find the possibilities of replacing synthetic organic acids in production recipes with natural components. A safe and advantageous way would be to capitalize on the natural compounds from unripe apples, obtained as a result of thinning or physiological falls, between 40 - 65 days after blooming (DAB).

Thinning of undeveloped fruit is carried out in order to balance the amount of fruit left on the tree with the surface of the leaves and to minimize the assimilation competition between apples (Peșteanu et al., 2017; Assirelli et al., 2018). Thus, in the plantations, approx. 25 - 30% of the expected fruit end up being removed, especially in years with insufficient soil moisture (Chen et al., 2015). Significant volumes of unripe apples are not used for food purposes and, being horticultural waste, represent sources of environmental pollution that cannot be neglected. At the same time, unripe apples contain impressive amounts of valuable substances, such as organic acids, carbohydrates, phenolic substances, minerals, etc. (Yang et al., 2021; Geleta et al., 2023). Total content of organic acids is high at the beginning of ripening and decreases during fruit development, malic acid being the main (Alberti et al., 2016; Ma et al., 2018; Bandic et al., 2019).

The analysis of bibliographic sources and normative documentation (OMAIA153/2010) shows that the preservation recipes (especially of fruits and vegetables) contain synthetic acidifiers as a source of acidity. Chemical and quality management issues regarding synthetic food additives are currently widely discussed, as reports of food poisoning have led to a search for safe and effective preservatives, mostly of plant origin. (Fiorino et al., 2019; Anyasi et al., 2017). The addition of natural compounds with functional properties to new food formulas provides one of the main challenges of the contemporary food industry. International producers are oriented towards the use of organic acids of natural origin (verjuice, acidifier from grapes, fruits and concentrated juice from corks), with the aim of manufacturing healthy products with a high nutritional value (Nilgun et al., 2019; Dupas de Matos, 2019; Golubi, 2019).

Motivation for choosing the research topic. Healthy eating is becoming more and more popular and necessary worldwide. Consumer's intolerance to synthetic food additives increases the need for their substitution with natural compounds, derived from natural sources of plant origin, stable and safe for human consumption.

Natural acidifiers can be obtained by harnessing unripe apples, thus encouraging the promotion of the transition to sustainable food systems, mainly the development of products with

"zero impact" on the environment, reducing climate change and increasing access to healthy and sustainable nutritious food.

Research hypothesis: Unripe apples, being horticultural waste, can be used to obtain the natural acidifier, favoring the reduction of raw material losses and substituting synthetic organic acids in food. This perspective could help supplement natural additives in the food industry, according to the trend to meet consumer demand for healthy and sustainable products.

Aim of the research: utilization of unripe apples to obtain a source of natural acidity, called apple acidifier, and its use in the food industry, especially in the preservation of fruits and vegetables, substituting acids in classic production recipes.

To achieve the proposed goal and verify the formulated scientific hypothesis, the following **research objectives** were established:

1. Studying the biochemical processes in apple fruits during growth and development to establish the optimal harvesting period for obtaining the natural acidifier.
2. Application of mathematical modeling to determine the influence of enzyme preparations, temperature and system parameters on juice yield from unripe apples.
3. Elaboration of the technological flow for obtaining apple acidifiers and producing their experimental batches.
4. Determination of the content of organic acids, carbohydrates, total phenolic substances and antioxidant activity in acidifier samples.
5. Analysis of the microbiological and sensory characteristics of apple acidifiers; establishing shelf life.
6. Implementation of the apple acidifier in obtaining preserved fruits and vegetables to substitute acetic and citric acids from the classic production recipes; studying the quality of the products developed.
7. Carrying out the feasibility study of the manufacture of apple acidifier and canned products with its application.

Synthesis of research methodology and justification of chosen research methods. To carry out the present work, a series of both classic and modern research methods were applied, such as: analysis by high-performance liquid chromatography (HPLC), for the determination of organic acids; capillary electrophoresis (CE) – of carbohydrates; DPPH free radical test was applied to determine the antioxidant activity; UV/Vis method for calculating the total content of polyphenols. Sensory, physico-chemical and microbiological methods were used to determine the quality indicators in the studied unripe apples, in the acidifiers obtained from these fruits and in

the food products developed with the application of apple acidifier. At the same time, statistical and mathematical modeling methods were used in the given study.

The theoretical importance and scientific innovation of the work consist in arguing the need to exploit the biomass of unripe apple fruits to obtain a source of natural acidity, necessary for the food industry, and in the possibility of using it for the manufacture of new food products. In order to solve the proposed problem such as researches were carried out:

- for the first time in the R. Moldova, the parameters of unripe apple fruits were studied and the optimal period for their harvesting was established in order to obtain a source of natural acidity;
- influence of enzymes and temperature on the shredded mass during the production of the acidifier was determined, regarding the increase of the juice yield; mathematical model was applied based on the parameters determined from the system;
- possibility of applying an optimized pasteurization regimen in the process of obtaining the acidifier from apples was argued theoretically and experimentally;
- production technology of the acidifier from apples was argued and elaborated with the obtaining of experimental batches;
- organic acids, carbohydrates, antioxidant activity, total polyphenol content, quality indicators, microbiological stability and shelf life of apple acidifiers were qualitatively and quantitatively determined;
- possibility and necessity of substituting acetic and citric acids, from the traditional recipes for preserving fruits and vegetables, with the acidifier from apples was argued theoretically and experimentally; manufacturing technologies of new food products were developed;
- advantage of producing the apple acidifier and the products made with its use was theoretically argued and economically demonstrated.

Applicative value of the work:

- according to the obtained experimental results, the procedures for obtaining the apple acidifier and the acidifier blended from apples and grapes were developed and patented;
- technologies for the production of canned vegetables and fruits with application of apple acidifier were developed (pickled tomatoes, canned cucumbers, vegetable stew, canned hot peppers, sour cherry jam and canned strawberries);
- the impact of unripe apple parameters on quality indicators, sensory characteristics, microbiological stability and shelf life of apple acidifier and food products using it was elucidated;
- it has been proven that the manufacture of the acidifier and elaborated products will ensure an increase in profitability by 0.9-3.1%, compared to analogue products manufactured according to classic recipes.

Approval of results. The results obtained within the thesis were communicated and discussed at **international and national symposiums and conferences:** International Conference „Modern Technologies in the Food Industry”, Chisinau (2018); Technical-scientific conference of students, masters and PhD-students TUM, Chisinau (2019–2022); National Scientific-Practical Conference "Innovation: Factor of social-economic development", Cahul (2020); Conference CASEE „Sustainable agriculture in the context of climate change and digitalization”, Chisinau (2022); International scientific conference "Prospects and problems of integration in the European Research and Education Area", Cahul (2022); International Conference „Ecological & Environmental Chemistry”, Chisinau (2022); International Symposium „Euro-Aliment”, Galati (2021); International Scientific Symposium "Agrifood Sector - Achievements and Perspectives", Chisinau (2021–2022); Scientific-Practical Conference with international participation “Technologies for processing waste to produce new products”, Kyrov, Russia (2022); **national and international salons of inventions:** Innovation and Research Salon „UGAL INVENT-2019”, Galati, Romania; International exhibition of inventions „INFOINVENT-2019”, Chisinau, Moldova; European Exhibition of Creativity and Innovation „EURO INVENT-2018”, Iasi, Romania; „INVENTICA-2018”, Iasi, Romania; **round tables:** "Innovative processing technologies of agri-food raw material" (2019), and "Quality through research. From soil to product. Improving product quality through innovation and collaboration between research and industry" (2023), Specialized International Exhibitions „Food & Drinks”, MoldExpo, Chisinau; **international project reports** „Use of apple acidifier in preserving fruit and vegetables” (2022-2023) FMS „National Scholarship Programme of the World Federation of Scientists”, Switzerland. The research results were published in national magazines „Journal of Engineering Science” (DOAJ – cat. B+), TUM, Chisinau (2021; 2023) and international „Scientific Study & Research – Chemistry & Chemical Engineering, Biotechnology, Food Industry” (WoS – ESCI) Bacau, Romania (2023). Work done during studies and research was appreciated by the Government of the Republic of Moldova by approving the Government Excellence Scholarship (2021-2022).

Publications on the thesis. Research results and the issues addressed have been published in 16 scientific papers, including 3 single-author scientific articles with reviewers, 2 invention patents, 11 articles in collections and abstracts at national and international scientific events.

Summary of thesis chapters. The PhD thesis includes annotation (RO, RU and EN), introduction, 4 chapters, general conclusions, practical recommendations, 318 bibliographic sources and 3 appendices. The basic text contains 115 pages, including 50 tables and 44 figures.

Key words: unripe apples, apple acidifiers, organic acids, carbohydrates, phenolic substances, antioxidant activity, canned fruit and vegetables with the apple acidifier application.

THESIS CONTENT

1 Acidifiers used in the food industry and biochemical changes in apples during development

The first chapter represents the current knowledge study on acidifiers used in the food industry, including those of natural origin implemented in experimental level food. At the same time, this chapter describes the dynamics of the accumulation and transformation of the main biochemical compounds in apples during development, the benefit of apple consumption on human health and the technology of the apple juice production.

The bibliographic analysis shows that unripe apples contain, depending on the ripening phase, significant amounts of organic acids and other valuable substances such as carbohydrates, phenolic compounds, polysaccharides, minerals and vitamins, and the total antioxidant activity of unripe apples is higher than of ripe fruit. At the same time, the substitution of synthetic additives with natural ones in the production of healthy foods is very current.

The study carried out raises the level of knowledge regarding the biochemical compounds in unripe apples, which represent horticultural waste following thinning or physiological drops, intending to exploit them to obtain the natural acidifier.

2 Materials and methods of the research

Chapter two describes the raw, secondary and auxiliary materials for research; reagents, devices and installations used in the study; standardized methods of determination, as well as modern methods of analysis (high-performance chromatography - HPLC, capillary electrophoresis - CE, UV-VIS spectrophotometry), technological research methods; the methodology for determining the quality indicators of the analyzed raw materials and the elaborated products.

The main objects of research served the unripe apples of 4 perspective varieties: Coreana, Golden Reizistent, Rewena and Reglindis, harvested during development on days 45, 58, 71, 84 and 97 days after blooming (DAB) (the months of June - July) of years 2018 – 2020 from the experimental lots of the Scientific-Practical Institute of Horticulture and Food Technologies (IŞPHTA). The experimental samples of apple acidifiers and developed food products with their application were obtained and analyzed.

3 Research on the influence of unripe apple parameters on the quality of natural acidifier

In this chapter, the study on unripe apples of 4 varieties selected for research is described and the physicochemical indicators are determined. At the same time, methods to increase the yield of juice from these fruits were applied and studied.

3.1 Physicochemical characteristics of unripe apples during development

Knowing the physico-chemical characteristics of thinned unripe apples is a promising way of efficient management of horticultural waste, regarding the rational use of plant raw materials by increasing sustainability.

The physicochemical indicators were determined in the studied unripe apples, and the obtained results are presented in Table 3.1.

Table 3.1. Physicochemical indicators of studied apple fruits during development

Variety	DAB*	W, %	AT**, %	SUH, °Brix	Total sugar, %	pH
Coredana	45	83,84 ± 0,02	1,97 ± 0,01	6,55 ± 0,01	3,35 ± 0,04	3,05 ± 0,012
	58	86,39 ± 0,04	1,89 ± 0,00	7,80 ± 0,13	5,27 ± 0,08	3,16 ± 0,035
	71	84,18 ± 0,02	1,76 ± 0,00	8,65 ± 0,07	6,54 ± 0,03	3,14 ± 0,004
	84	85,32 ± 0,01	1,53 ± 0,03	10,58 ± 0,01	8,29 ± 0,07	3,07 ± 0,008
	97	84,28 ± 0,04	1,35 ± 0,04	12,35 ± 0,01	9,76 ± 0,13	3,19 ± 0,002
Golden Rezistent	45	86,16 ± 0,03	2,22 ± 0,04	6,65 ± 0,01	5,18 ± 0,01	2,95 ± 0,016
	58	85,45 ± 0,01	2,12 ± 0,01	7,06 ± 0,07	5,62 ± 0,07	2,98 ± 0,023
	71	86,62 ± 0,07	1,97 ± 0,01	8,47 ± 0,11	7,44 ± 0,11	3,01 ± 0,005
	84	86,48 ± 0,03	1,88 ± 0,02	9,95 ± 0,08	8,82 ± 0,08	3,06 ± 0,001
	97	87,08 ± 0,03	1,72 ± 0,03	11,43 ± 0,05	10,02 ± 0,05	3,15 ± 0,002
Reglindis	45	84,73 ± 0,08	2,63 ± 0,08	6,60 ± 0,07	4,09 ± 0,07	3,07 ± 0,017
	58	83,20 ± 0,10	2,50 ± 0,10	7,85 ± 0,14	5,27 ± 0,02	3,17 ± 0,008
	71	85,04 ± 0,07	2,40 ± 0,01	8,70 ± 0,05	6,01 ± 0,05	3,05 ± 0,003
	84	84,38 ± 0,03	2,12 ± 0,09	10,60 ± 0,02	7,73 ± 0,01	3,20 ± 0,002
	97	85,03 ± 0,02	2,00 ± 0,01	11,85 ± 0,01	9,33 ± 0,13	3,13 ± 0,023
Rewena	45	86,90 ± 0,07	2,79 ± 0,08	6,50 ± 0,12	4,29 ± 0,14	2,50 ± 0,023
	58	85,66 ± 0,20	2,73 ± 0,07	7,65 ± 0,07	5,20 ± 0,09	2,63 ± 0,002
	71	87,10 ± 0,08	2,63 ± 0,00	8,50 ± 0,03	6,15 ± 0,05	2,70 ± 0,002
	84	84,63 ± 0,07	2,30 ± 0,01	10,75 ± 0,00	7,83 ± 0,01	2,94 ± 0,021
	97	83,88 ± 0,03	2,09 ± 0,02	12,10 ± 0,02	9,69 ± 0,02	2,81 ± 0,019

Note: * DAB – days after blooming; W – moisture; AT** – titratable acidity, expressed in malic acid; SUH – water-soluble dry substances; pH – active acidity; results represent average ± SD.

During the growth and development of apples, the moisture content varied between 83.20 % and 87.10 % and no significant differences were observed between the varieties tested. Moisture is an important parameter, because it influences the yield of the juice (Zheng et. al, 2012).

The content of water-soluble dry matter (SUH) increased considerably with the growth and development of apple fruits. The highest concentrations of this indicator were recorded in the Coredana varieties, with values between 6.55 Brix and 12.35 Brix, and Golden Rezistent, between 6.65 Brix and 11.43 Brix; followed by the varieties Rewena (6.60 Brix – 11.85 Brix) and Reglindis (6.50 Brix – 12.10 Brix). A sudden increase in SUH is observed around the 84th DAB, followed by a slow increase thereafter.

The total carbohydrate content increases significantly during fruit development, with values between 3.35 % and 9.76 % for the Coredana variety, between 5.18 % and 10.02 % for the Golden Rezistent variety, between 4.09 % and 9.33 % for the Reglindis variety and between 4.29

% and 9.69 % for the Rewena variety. As in the case of SUH, a sudden increase in total sugar content was observed around the 84th days after blooming (DAB), as these two indicators are in a directly proportional correlation. The increase in SUH and total sugar content is due to the hydrolysis of starch contained in unripe apples. Some researchers have shown that starch accumulation occurs between the 35th and 85th DAB, peaking around the 85th day (Bart et al., 2008; Zheng et al., 2012; Mureșan et al., 2015).

Aciditatea în toate soiurile de mere studiate a scăzut pe parcursul creșterii fructelor. Astfel au fost înregistrate valori cuprinse între 1,97 % și 1,35 % pentru soiul Coredana, între 2,22 % și 1,72 % pentru soiul Golden Rezistent, între 2,63 % și 2,00 % pentru soiul Reglindis și între 2,79 % și 2,09 % pentru soiul Rewena. Merele soi Rewena au avut un conținut de aciditate cel mai mare, urmat de soiul Reglindis, apoi Golden Rezistent și Coredana. Concentrațiile cele mai mari au fost înregistrate la fructele recoltate în a 45-a zi după înflorirea deplină, scăzând ușor spre ziua a 97-a, similar la toate soiurile studiate. Scăderea ușoară a conținutului de aciditate pe parcursul coacerii s-ar putea datora degradării acidului citric, transformării acizilor în timpul respirației, conversiei acestora în zaharuri și utilizarea ulterioară în procesul metabolic din fruct (Roshan et al., 2012; Bizjac et al., 2013). At the same time, the processes involved in the metabolism and accumulation of malic and citric acids in mesocarp cells are under both genetic and environmental control (Etienne et al., 2013). Malic acid concentrations represent about 90% of the total content of organic acids (Bandic et al., 2019). Many agronomic studies have shown the impact of cultivation practices, including irrigation, mineral fertilization, temperature, thinning, on apple components (Wu et al., 2002; Burdon et al., 2007; Thakur et al., 2012).

The active acidity (pH) of the fruit depends mainly on the organic acid contained in it. The pH values of the apple pulp do not change essentially, however, a small increase of this indicator was recorded during the growth and development of the fruit. The values showed from 3.05 to 3.19 in the case of the Coredana variety, from 2.95 to 3.06 for the Golden Rezistent variety, from 3.05 to 3.20 for Reglindis and from 2.50 to 3.13 for Rewena. Zheng et al reported similar results in their studies for the Fuji variety (Zheng et al. 2012).

Based on the results obtained (tab. 3.1) and those presented above, it can be concluded that apple fruits harvested 71th DAB of all 4 studied varieties are the most suitable for processing in order to obtain natural acidifiers.

The SUH/TA ratio represents an industrial processing indicator. In this paper, the modification of this index was studied for all 4 varieties of apples and presented in Figure 3.2.

According to the obtained calculations (fig. 3.2), the SUH/AT ratio between days 45th and 97th DAB of the studied apples presented the following values: Coredana – 3.32-9.15; Golden

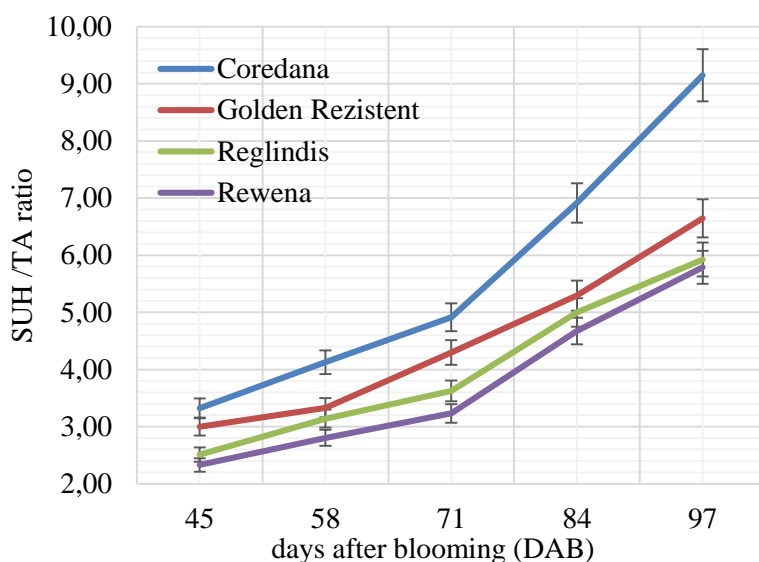


Fig. 3.2. Dynamics of the SUH/TA ratio during apple fruit development in the varieties Coredana, Golden Reizistent, Reglindis and Rewena

Reizistent – 3,00-6,65; Reglindis – 2,51-5,93; Rewena – 2,33-5,79. From the 45th day after full flowering to the 71st day, a slow increase of this index was recorded, approx. by 1.30 units on average, after which a sudden increase follows, on average by 3.36 units. The increase in values is due to the increase in carbohydrate content and the decrease in the amount of acidity during fruit development. Similar

results were presented by Golubi (2019) in his study on unripe grapes, where it was recommended to direct the berries to the production of the acidifier until the glucoacidometric index did not exceed the limit of 10 units. Albert et al. (2016) demonstrated in his work that unripe apple fruits presented a ratio lower than 20, which is suitable for industrial processing.

3.2 Dependence of the juice yield value on the treatment of unripe apples

Unripe apples contain protopectin, which is insoluble, and a large amount of starch (Zheng et al., 2012; Mureşan et al., 2019), the fact that leads to a relatively low extraction yield. In the present study, the action of temperature and enzymes on the crushed mass of the studied fruits before the pressing process were studied in order to increase the juice yield. Thus, 10 kg of unripe apples of each studied variety were harvested and shredded by crushing. The obtained volume of crushed mass was divided into three parts. The first part was passed directly to the press. The second part was heat treated at 50 °C for 30 min, then pressed. The third part of the crushed mass was treated with pectolytic preparation Pectinex® Ultra AFP (0.3 mL/kg) and amyolytic preparation Amylase® AG 300 L (0.3 mL/kg). The enzymatically treated mass was incubated at 50 °C for 30 min. The pressing operation was carried out at the screw press, and the results are presented in Table 3.2.

From the resulting dates (tab. 3.2) it shows that the thermal treatment of the crushed mass favored the increase of the juice yield after pressing in the medium by 7-10 %, which is due to the partial solubilization of the pectic substances, favoring the partial plasmolysis of the fruit cells and even breaking the walls cellular. However, after the simultaneous enzymatic and thermal

treatment, the yield of the juice increased in the medium by about 24 %. This is due to the degradation of pectic substances and starch under the action of enzymes. The juice obtained after treatment with pectic enzymes is clear, due to the low degree of viscosity (Srivastava et al., 2013).

Table 3.2. Yield of the juices from unripe apples with/without pretreatment

Variety	DAB*	Yield after pressing, % of raw material mass:		
		without pretreatment	thermal treatment 50°C/30min	enzymatic + thermal treatment 50°C/30min
Coredana	45	27,14±0,21	29,09±0,83	35,73±0,50
	58	30,62±0,60	32,70±0,32	39,84±0,26
	71	39,85±0,13	42,80±0,40	47,92±0,34
	84	46,19±0,45	52,24±0,28	59,70±0,23
	97	55,35±0,34	61,72±0,49	65,56±0,35
Golden Resistent	45	30,94±0,31	33,22±0,60	39,50±0,41
	58	36,40±0,41	38,84±0,72	45,22±0,63
	71	40,73±0,23	46,37±0,23	52,17±0,52
	84	49,52±0,46	54,89±0,30	59,65±0,74
	97	55,86±0,42	60,35±0,51	64,91±0,53
Reglindis	45	27,67±0,51	31,20±0,23	33,57±0,41
	58	33,12±0,31	37,56±0,22	41,50±0,35
	71	36,24±0,20	42,33±0,43	47,29±0,21
	84	46,62±0,61	51,92±0,51	54,38±0,20
	97	56,41±0,54	60,30±0,32	63,46±0,70
Rewena	45	26,13±0,70	29,87±0,44	33,28±0,25
	58	31,83±0,42	35,13±0,32	39,84±0,53
	71	38,72±0,35	43,51±0,21	47,82±0,41
	84	42,99±0,52	47,74±0,73	53,50±0,54
	97	54,34±0,41	61,70±0,50	65,18±0,35

Note: * DAB – days after blooming

3.3 Influence of parameters determined in unripe apples on juice yield

Applying the least squares method, mathematical modeling was performed regarding the influence of the factors determined in the system (titratable acidity **AT**, water-soluble dry substances **SUH**, total sugar **Z_t** and **pH**) on the value of juice yield from unripe apples after pressing (**η**). The regressions (3.1 - 3.3) were presented, characterizing the influence of the factors on the mixture of the varieties studied, for different methods of treatment of the crushed mass (without pretreatment **FPT**, thermal treatment **TT**, enzymatic and thermal treatment **TETT**).

$$\eta_{(FPT)} = -8,7AT - 3,0SUH + 6,8Z_t + 13,9pH : R^2 = 0,999, \quad (3.1)$$

$$\eta_{(TT)} = -3,6AT - 3,2SUH + 8,0Z_t + 9,9pH : R^2 = 0,999, \quad (3.2)$$

$$\eta_{(TETT)} = 7,6AT - 1,0SUH + 6,9Z_t + 1,1pH : R^2 = 0,999, \quad (3.3)$$

It was hypothesized that due to these regressions, valid applicability and predictive ability for multiple apple varieties would occur.

4 Elaboration of the manufacturing technology of the apples acidifier, its implementation in obtaining preserved fruits and vegetables and studying the quality of the elaborated products

In the given chapter, the technology for obtaining the apple acidifier is described; its quality indicators are evaluated with the determination of antioxidant activity and shelf life; the technologies for obtaining canned fruit and vegetable products with the application of apple acidifier are described; the quality indicators of processed foods are analyzed; the feasibility study of the acidifier and the products developed with its application is presented.

4.1 Technology of obtaining the apples acidifier

The production technology of apples acidifiers constituted the stages shown in figure 4.1.

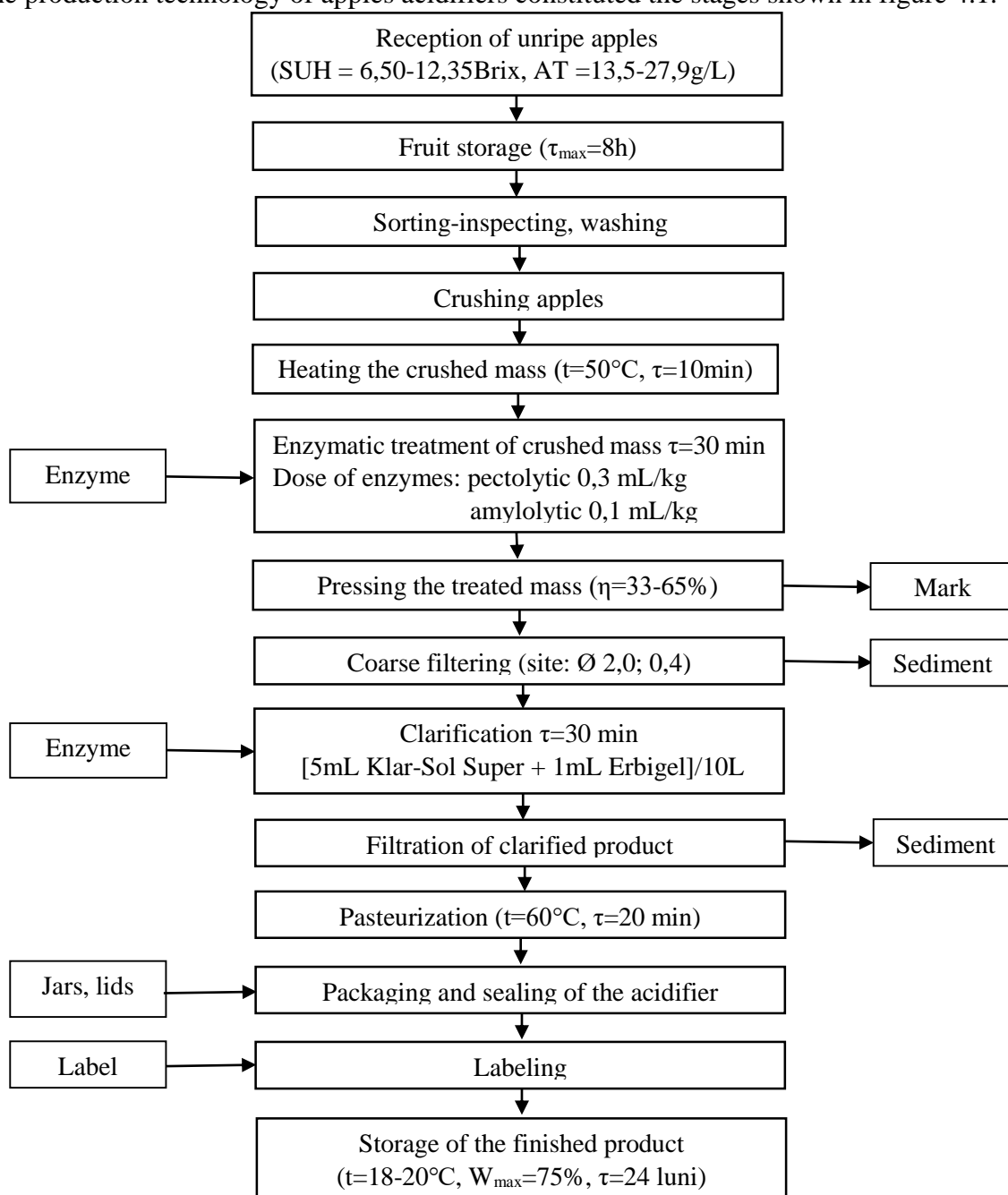


Fig. 4.1. Scheme of the technological flow for obtaining apple acidifier

The technological flow of production of the acidifier had some specific peculiarities, which presented the scientific novelty compared to the manufacturing technology of apple juice, being the closest analog product:

- ✓ Following the study of unripe apples of 4 varieties established between days 45th and 97th DAB, the parameters of the raw material for obtaining the acidifier were established: the SUH content had values of 6.50 – 12.35 Brix and TA of 13.50 – 27.90 g/L, expressed in malic acid.
- ✓ The technological process included the stage of treatment with enzymes (pectolytic and amylolytic) of the crushed mass from unripe apples before pressing with thermal treatment (50 °C, 30 min). The purpose of this stage was to increase the juice yield, at the same time, acting positively on the extraction of total polyphenolic substances.
- ✓ Based on the experimental data, the optimized pasteurization regime of the acidifier at 60° C for 20 min was implemented. Due to the moderate content of carbohydrates, high organic acids and acidic environment (pH=2.9-3.2), this thermal treatment ensures a sufficient conservation effect (Golubi, 2019).

4.2 Evaluation of the quality indicators of acidifiers in the studied apples

Following research, the content of organic acids, simple carbohydrates, total polyphenols, antioxidant activity, organoleptic evaluation and microbiological stability were determined in apple acidifiers. The shelf life of these products has also been established.

4.2.1 Analysis of physicochemical indicators

4.2.1.1 Content of organic acids

The content of six organic acids was determined in the analyzed apple acidifiers (tab. 4.1). The results show that the amount of detected acids tends to decrease continuously, starting with the samples obtained from the fruits harvested from the 45th to the 97th day, the only exception being tartaric acid, which increases insignificantly during the given harvest times.

Table 4.1. Concentration of organic acids determined in acidifiers from unripe apples by varieties Coredana, Golden Rezistent, Reglindis and Rewena

Acids	Acidifiers from apple varieties	DAB (days after blooming) **				
		45	58	71	84	97
		Concentration of organic acids (g/L)				
1	2	3	4	5	6	7
Malic	Coredana	25,35±0,01	22,54±0,02	20,68±0,01	18,08±0,01	17,97±0,02
	G. Rezistent*	35,79±0,01	33,37±0,01	32,95±0,01	19,55±0,01	17,22±0,05
	Reglindis	38,08±0,02	36,74±0,03	36,51±0,01	26,43±0,01	20,50±0,03
	Rewena	33,17±0,01	32,25±0,04	30,31±0,01	17,61±0,02	16,90±0,01
Citric	Coredana	0,23±0,02	0,19±0,01	0,15±0,01	0,17±0,01	0,20±0,01
	G. Rezistent*	0,34±0,01	0,29±0,01	0,22±0,03	0,16±0,02	0,15±0,06
	Reglindis	0,35±0,01	0,32±0,04	0,25±0,02	0,24±0,07	0,24±0,01
	Rewena	0,44±0,01	0,42±0,01	0,39±0,01	0,36±0,06	0,36±0,02

Continued table 4.1.

1	2	3	4	5	6	7
Succinic	Coredana	0,10±0,01	0,06±0,01	0,05±0,07	0,05±0,03	0,03±0,01
	G. Rezistent*	0,09±0,02	0,08±0,01	0,04±0,09	0,11±0,01	0,07±0,01
	Reglindis	0,19±0,01	0,15±0,01	0,09±0,06	0,04±0,02	0,05±0,03
	Rewena	0,08±0,01	0,08±0,02	0,06±0,04	0,07±0,02	0,04±0,07
Acetic	Coredana	0,21±0,01	0,22±0,01	0,21±0,01	0,15±0,03	0,14±0,05
	G. Rezistent*	0,10±0,01	0,11±0,02	0,11±0,01	0,10±0,07	0,09±0,01
	Reglindis	0,20±0,02	0,18±0,01	0,19±0,02	0,101±0,01	0,11±0,01
	Rewena	0,20±0,02	0,21±0,01	0,20±0,01	0,16±0,02	0,12±0,02
Lactic	Coredana	0,24±0,01	0,22±0,01	0,21±0,01	0,10±0,03	0,10±0,02
	G. Rezistent*	0,20±0,01	0,18±0,01	0,16±0,02	0,13±0,04	0,12±0,01
	Reglindis	0,22±0,01	0,22±0,03	0,20±0,01	0,19±0,02	0,17±0,03
	Rewena	0,21±0,01	0,21±0,02	0,20±0,02	0,18±0,02	0,16±0,01
Tartric	Coredana	0,20±0,01	0,21±0,01	0,22±0,01	0,22±0,01	0,24±0,01
	G. Rezistent*	0,25±0,02	0,25±0,03	0,25±0,01	0,26±0,02	0,27±0,02
	Reglindis	0,27±0,02	0,27±0,04	0,28±0,01	0,29±0,01	0,30±0,01
	Rewena	0,19±0,01	0,18±0,01	0,19±0,01	0,21±0,04	0,21±0,02

Note: *- variety Golden Rezistent; **- samples of acidifiers corresponding to the harvesting days of unripe apples of the varieties studied

Of the total organic acids detected (tab 4.1), malic acid predominated, representing values from 94.99 % to 97.61 %, similar to the statements of other researchers (Zhang et al., 2010; Etienne et al., 2013; Ma et al., 2018). The content of malic acid in apple acidifiers was reduced in those obtained from the Coredana variety from 25.34 g/L, harvested on the 45th DAB, to 17.97 g/L, towards the 97th DAB. Analogously, it also decreased in samples from the Golden Rezistent varieties (from 33.79 g/L to 17.22 g/L) and Reglindis (from 38.03 g/L to 20.50 g/L), followed by Rewena (from 33.17 g/L to 16.90 g/L). Most of the malic acid in apples is in the vacuole of the parenchyma cells, and its concentration reaches its maximum up to 6 weeks (about 45 days) after flowering, followed by a continuous decrease until ripening (Zhang et al., 2010).

Citric acid had values between 0.65 % and 2.02 %. It had a concentration thousands of times lower than that of malic acid, and was also reduced in all four samples of acidifiers from the studied apple varieties and harvest times: Coredana from 0.230 g/L to 0.198 g/L, Golden Rezistent from 0.335 g/L to 0.150 g/L, Reglindis from 0.353 g/L to 0.237 g/L and Rewena from 0.438 g/L to 0.360 g/L, respectively (tab. 4.1). Citric acid was predominantly detected in wild apple species (Ma et al., 2018).

Succinic acid represented the lowest content value, varying between 0.13% and 0.56%. Its concentration in the studied samples had the following values: in those obtained from the Coredana variety – 0.370 g/L - 0.130 g/L; Golden Rezistent – 0.235 g/L - 0.410 g/L; Reglindis – 0.493 g/L - 0.227 g/L and Rewena – 0.240 g/L - 0.260 g/L, respectively (tab. 4.1). Some researchers have demonstrated that in unripe apples the concentrations of malate and other organic acids from the Krebs cycle (for ex.: succinic and citric acids) are significant (Zhang et al., 2010; Xu et al., 2020).

Acetic and lactic acids are produced by the corresponding bacteria, found on the fruit surface in small quantities, or abundance in the case of spoiled fruit (Worobo et al., 2005). Their quantity in the studied acidifiers was minimal, with values of 0.27 % - 0.95 % (acetic acid) and 0.49 % - 0.98 % (lactic acid), indicating that they harvested healthy and undamaged fruit to obtain the acidifiers. The concentrations of the acids given in the acidifiers of analyzed apple varieties are the following values: 0.212 g/L - 0.136 g/L and 0.235 g/L - 0.100 g/L for Coredana; 0.101 g/L - 0.091 g/L and 0.196 g/L - 0.123 g/L for Golden Rezistent; 0.197 g/L - 0.107 g/L and 0.224 g/L - 0.165 g/L for Reglindis; 0.198 g/L - 0.123 g/L and 0.210 g/L - 0.158 g/L for Rewena, respectively.

Tartaric acid accumulates in apples at an advanced stage of maturity (Zhang et al., 2010; Xu et al., 2020). In the present study, its amount was 0.60 % - 1.15 %, which represented 0.196 g/L - 0.235 g/L for the acidifiers obtained from Coredana apples; 0.241 g/L - 0.269 g/L for Golden Resistent; 0.268 g/L - 0.299 g/L for Reglindis and 0.186 g/L - 0.209 g/L for Rewena, respectively (tab. 4.1).

The impressive amounts of organic acids in apple acidifiers represent one of the promising ways to supplement the need for natural sources of acidity in the food industry.

4.2.1.2 Content of simple carbohydrates

The concentration of each carbohydrate for each acidifier of a certain apple variety and harvesting time is shown in Table 4.2.

Table 4.2. Concentration of simple carbohydrates determined in acidifiers from unripe apples by varieties Coredana, Golden Rezistent, Reglindis and Rewena

Carbohydrates	Acidifiers from apple varieties	DAB (days after blooming) **				
		45	58	71	84	97
		Concentration of simple carbohydrates (g/L)				
Fructose	Coredana	26,54±0,11	34,82±0,02	41,15±0,07	64,64±0,12	73,42±0,11
	G. Rezistent*	32,79±0,09	37,23±0,13	45,55±0,10	64,44±0,11	75,73±0,10
	Reglindis	22,68±0,09	29,14±0,10	38,76±0,09	63,84±0,13	74,56±0,11
	Rewena	25,17±0,14	30,73±0,11	37,45±0,09	55,88±0,10	67,79±0,11
Glucose	Coredana	13,26±0,14	18,16±0,14	21,69±0,12	28,89±0,13	29,15±0,13
	G. Rezistent*	15,38±0,12	18,73±0,11	23,51±0,13	26,29±0,15	26,26±0,01
	Reglindis	11,74±0,11	15,54±0,10	18,72±0,13	22,50±0,13	25,47±0,11
	Rewena	12,15±0,04	16,23±0,12	17,63±0,14	19,62±0,14	23,36±0,10
Sucrose	Coredana	0,19±0,03	0,21±0,03	0,24±0,02	0,05±0,03	0,14±0,01
	G. Rezistent*	0,10±0,01	0,12±0,03	0,08±0,01	0,05±0,02	0,09±0,02
	Reglindis	0,12±0,04	0,13±0,01	0,18±0,03	0,08±0,02	0,11±0,04
	Rewena	0,11±0,02	0,13±0,02	0,18±0,03	0,03±0,01	0,13±0,02

Note: * - variety Golden Rezistent; ** - samples of acidifiers corresponding to the harvesting days of unripe apples of the varieties studied

The amounts of fructose and glucose in the studied samples had a content of 26.54 g/L – 73.42 g/L and 13.26 g/L – 29.15 g/L for the acidifiers obtained from Coredana variety apples; 32.79 g/L – 75.73 g/L and 15.38 g/L – 26.23 g/L for Golden Resistent; 22.68 g/L – 74.56 g/L and 11.74 g/L – 25.47 g/L for Reglindis; 25.17 g/L – 67.79 g/L and 12.15 g/L – 23.36 g/L for Rewena,

respectively (tab. 4.2). The fructose content was from 65.68 % to 74.36 % of the total carbohydrates determined, and the glucose content was twice as low and showed 25.62 % - 34.68 %. Sucrose was detected in very small amounts, constituting 0.04 % - 0.48 % (tab. 4.2).

The amount of carbohydrates determined had continuously increasing values in all samples of acidifiers starting from 45th DAB of fruit harvest until 97th day. The exception was the acidifiers obtained from apples harvested on the 84th day in all 4 studied varieties which showed a sudden increase in the amount of fructose and a sudden decrease in sucrose compared to the other samples, followed by a slow increase. The sudden changes may be due to the hydrolysis of starch contained in unripe apples and reaching a maximum amount around this period (Zheng et al., 2012; Mureşan et al., 2015) and the fact that more than half of the sucrose turns into fructose (Zhang et al., 2010; Li et al., 2012).

The results (tab. 4.2) show that the acidifiers, obtained from apples at the early stage of ripening, contain significant amounts of soluble carbohydrates.

4.2.1.3 Total polyphenol content

The total content of polyphenols was determined in the apple acidifiers of 4 studied varieties, which showed a tendency to decrease phenolic substances in all acidifiers obtained from

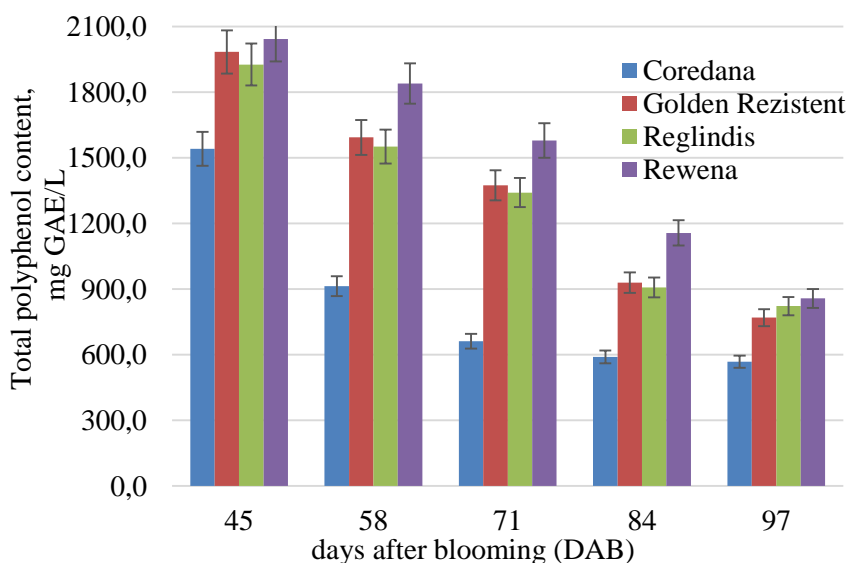


Fig. 4.2. Total polyphenol content in acidifiers from unripe apples by varieties Coredana, Golden Rezistent, Reglindis and Rewena

apples harvested during ripening at the studied times and varieties (fig. 4.2). However, the concentrations in all samples are quite significant. Acidifiers from apples Coredana variety had the lowest level of polyphenols (568.7 - 1541.3 mg GAE/L), while the highest values were detected in Rewena variety samples (857.3 - 2042.8 mg GAE/L).

The acidifiers obtained from Golden Rezistent and Reglindis cultivars had similar amounts of polyphenols: 769.7 - 1983.3 mg GAE/L and 822.1 - 1926.5 mg GAE/L, respectively. Some studies have reported that the action of enzymes on unripe apples increases not only the yield juice, but significantly improves the extraction of total phenolic content (approx. 3 times), antioxidant activity (approx. 2 times), reducing sugars (approx. 1.5 times) (Zheng et al., 2009; 2014).

4.2.1.4 Antioxidant activity

To evaluate the antioxidant potential of the acidifiers in the studied apples, the determination of the antioxidant activity was carried out using the free radical DPPH. The results obtained were related to ascorbic acid, which is included in the antioxidant category and provides a 50 % inhibition of the DPPH radical (IC_{50}) (Pérez-Lamela et al., 2021). The antioxidant activity of the samples was expressed in μg equivalent of ascorbic acid (AA) per mL of product (fig. 4.3).

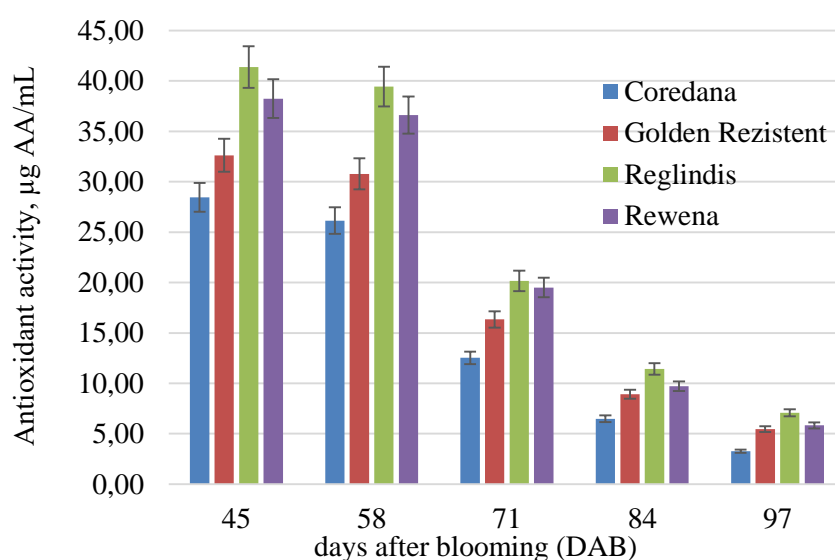


Fig. 4.3. Antioxidant activity determined in acidifiers from unripe apples by varieties Coredana, Golden Reizistent, Reglindis and Rewena

Analyzing the results in Figure 4.3, it was established that the studied acidifiers are characterized by high values of antioxidant activity in the samples produced from apples of all 4 varieties harvested on days 45th and 58th DAB, and in the samples on days 71st, 84th and 97th the values they were practically in half compared to those of the previous dates.

So, the acidifiers from apples Coredana variety had antioxidant capacity between 28.46 and 3.27 $\mu\text{g AA/mL}$; Golden Reizistent – 32.63 and 5.46 $\mu\text{g AA/mL}$; Reglindis – 41.37 and 7.08 $\mu\text{g AA/mL}$; Rewena – 38.25 and 5.83 $\mu\text{g AA/mL}$. Given the fact that total phenolic substances decrease slowly during apple development (fig 4.2), the sudden decrease in antioxidant activity around the 71st day of harvest may be due to the decrease in the content of other categories of antioxidant substances. The high levels of antioxidant capacity in the researched acidifiers are explained by the high amount of biologically active compounds with an antioxidant character, especially phenolic compounds. Thinned unripe apples are a good source of bioactive compounds, which is clearly reflected in the high values of antioxidant properties (Wojdyło et al., 2020).

4.2.2 Microbiological indicators in apple acidifiers

The investigation of the microbiological indicators of the studied acidifiers was carried out after 3 months of storage from their production, according to the requirements and rules of the normative documentation regarding the microbiological criteria for food products (HG no. 221, 2009). The results obtained show that all studied acidifiers showed microbiological stability and correspond to the requirements of the normative documentation in force. Thus, yeasts and molds

were not detected in them, and the number of aerobic and facultatively anaerobic mesophilic microorganisms was much lower than the norm ($< 4.5 \times 10^1$), which can be attributed to the high acidity and low pH. Malic acid, as a food safety reagent, has been reported as the most potent acid with antimicrobial activity evaluated on various pathogen strains (Eswaranandam et al., 2006).

4.2.3 Organoleptic evaluation of apple acidifiers

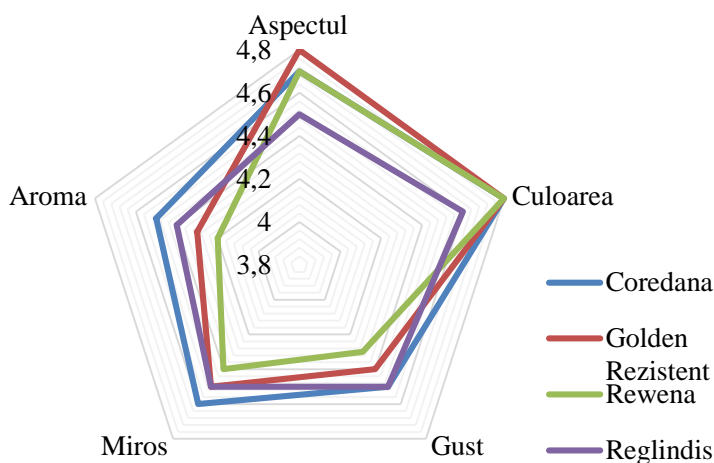


Fig. 4.4. Sensory profile diagram of acidifiers from unripe apples by varieties Coredana, Golden Delicios, Reglindis, Rewena

Apple acidifiers were analyzed sensory by assessing 5 basic criteria, namely: appearance, color, taste, smell and aroma. The evaluation of each index was appreciated from 0 to 5 points. Based on the scores recorded by the evaluators, a diagram of the organoleptic characteristics of acidifiers from unripe apples of the 4 studied varieties was drawn up (fig. 4.4). The samples

showed relatively clear juices with the sediment up to about 0.2 cm, straw-yellow to light-brown in color. The taste was intensely acidic and pleasant, slightly sweet, without extraneous nuances in taste and smell. The aroma was pleasant, typical of unripe apples, moderately expressed, characteristic of apple varieties.

4.2.3 Shelf life of apple acidifier

To establish the shelf life of acidifiers, apple juice was presented as a reference, being the closest analog food product, which has a maximum shelf life of 2 years, according to the Technical Regulation "Juices and certain similar products intended for human consumption", (HG RM no. 1111 of 06.12.2010).

In order to establish the storage period, the quality indicators (physicochemical, microbiological and organoleptic) of the apple acidifiers were determined after 3 months from production (the year 2019), which represented the beginning of storage, and after 36 months (the year 2022), representing the end of "reserve" storage, according to the above. The most representative samples were selected, which constituted the acidifiers obtained from all 4 studied apple varieties (Coredana, Golden Rezigent, Reglindis and Rewena) harvested on days 71th and 84th DAB. Thus, samples of two dates of production of each name were presented. Their storage took place in the warehouse at a temperature of 18-20 °C and a relative air humidity of 75 %.

Results of the physicochemical and microbiological determinations of the studied acidifiers at the beginning and end of "reserve" storage are presented in Tables 4.3 and 4.4, respectively.

Table 4.3. Results of the physicochemical indicators determinations of apple acidifiers studied at the beginning and end storage of "reserve"

Acidifiers from apple varieties	DAB*	3 months (the year 2019)			36 months (the year 2022)		
		AT, %	SUH, °Brix	pH	AT, %	SUH, °Brix	pH
Coredana	71	1,60±0,05	8,71±0,01	3,09±0,03	1,45±0,03	10,21±0,07	3,24±0,01
	84	1,54±0,03	10,81±0,07	3,06±0,08	1,39±0,07	12,60±0,03	3,19±0,09
Golden Rezistent	71	1,82±0,14	9,20±0,01	2,95±0,01	1,67±0,09	10,42±0,07	3,12±0,06
	84	1,77±0,02	11,05±0,01	3,03±0,01	1,54±0,04	11,71±0,05	3,07±0,01
Reglindis	71	2,35±0,12	8,40±0,05	3,00±0,03	2,15±0,08	9,43±0,05	3,19±0,03
	84	2,07±0,06	10,43±0,02	3,07±0,02	2,00±0,05	11,91±0,05	3,15±0,04
Rewena	71	2,75±0,01	8,36±0,03	2,70±0,05	2,28±0,03	9,85±0,09	3,27±0,05
	84	2,60±0,01	10,51±0,07	2,77±0,21	2,14±0,06	12,22±0,01	3,12±0,01

Nota: DAB* – days after blooming; AT - titratable acidity, expressed in malic acid; SUH – water-soluble dry substances.

Results show that the acidifiers studied were characterized by a high acidity content (1.39 % - 2.28 %), even after 36 months of storage. The titratable acidity decreased in all samples at the end of "reserve" storage, compared to the beginning: in the apple acidifiers Coredana variety by 9.56 %; Golden Rezistent by 10.62 %; Reglindis by 5.95 % and Rewena by 17.39 %. Whereas, the content of water-soluble dry substances, on the contrary, increased: in apple acidifiers Coredana variety by 16.96 %; Golden Resistent by 9.70 %; Reglindis and Rewena by 13.16 % and 13.17 % respectively. After 3 years of storage, the pH values increased in all samples in the medium by 6.43 %. Quantitative physicochemical changes can occur due to the conversion processes of organic acids and carbohydrates during storage. These data are similar to the study conducted on the storage of clarified apple juice (Cai et al., 2020).

Table 4.4. Results of the microbiological indicators determinations of apple acidifiers studied at the beginning and end storage of "reserve"

Acidifiers from apple varieties	DAB*	3 luni (anul 2019)			36 months (the year 2022)		
		MMAFAn, UFC/mL	Yeasts, UFC/mL	Molds, UFC/mL	MMAFAn, UFC/mL	Drojdi, UFC/mL	Mucegaiuri, UFC/mL
Norm		¹ 5,0×10 ³	² 2,0×10 ³	³ 5,0×10 ²	¹ 5,0×10 ³	² 2,0×10 ³	³ 5,0×10 ²
Coredana	71	1,0 × 10 ¹	n/d	n/d	3,2 × 10 ¹	n/d	n/d
	84	3,2 × 10 ¹	n/d	n/d	4,2 × 10 ²	1,0 × 10 ²	n/d
Golden Rezistent	71	1,0 × 10 ¹	n/d	n/d	3,2 × 10 ¹	n/d	n/d
	84	4,2 × 10 ¹	n/d	n/d	4,2 × 10 ²	2,0 × 10 ²	n/d
Reglindis	71	1,0 × 10 ¹	n/d	n/d	3,0 × 10 ¹	n/d	2,0 10 ¹
	84	4,0 × 10 ¹	n/d	n/d	4,5 × 10 ²	n/d	2,0 x 10 ²
Rewena	71	2,1 × 10 ¹	n/d	n/d	4,0 × 10 ²	2,0 × 10 ²	n/d
	84	4,0 × 10 ¹	n/d	n/d	2,0 × 10 ²	1,0 × 10 ²	n/d

Nota: MMAFAn – number of aerobic and facultatively anaerobic mesophilic microorganisms; DAB* - days after blooming; n/d – undetected; UFC – colony-forming units of microorganisms; ¹- according SM SR EN ISO 4833-1:2014; ^{2,3}- according SM SR ISO 21527-2:2014.

The microbiological indicators of the researched samples fall within the admissible limits presented and correspond to the normative, which can be attributed to the high acidity and low pH.

According to the investigations carried out, *the shelf life* of acidifiers from unripe apples of the Coredana, Golden Rezistent, Reglindis and Rewena varieties from the day of manufacture in light and dark coreless glass packaging at a temperature of 18-20 0C and a maximum relative humidity of 75 % *can be set for 2 years*.

4.3 Use of apple acidifier in fruit and vegetable preservation

In this subchapter, after 9 months of storage, the canned products from vegetables and fruits with the application of apple acidifier, substituting the acids from the classic recipes, were developed and studied.

4.3.1 Canned Tomatoes

Red tomatoes were preserved by pickling using apple acidifier. The recipe for their manufacture is presented in Table 4.7.

Table 4.7. Recipe for making pickled tomatoes using apple acidifier

No.	Raw materials and materials	Manufacturing recipe for 100 kg finished product
1	Red tomatoes, kg	59,0
2	Black peppercorns, kg	0,03
3	Greens (dill, celery, parsley), kg	1,0
4	Onion chopped, kg	0,2
5	Garlik chopped, kg	0,25
Composition of the preservation solution		
6	Water, L	23,5
7	Salt, kg	1,0
8	Sugar, kg	0,5
9	Apple acidifier**, L	4,0

Note: (Yastrebov, 1980; Crucirescu, 2022b); * packing ratio in container: vegetables – 50-55 %, preservation solution – 45-50 %; **acidifier from unripe apple Rewena variety 71 DAB 2019 (AT=2,75 %; SUH=8,5 °Brix; pH=2,70).

For a deeper evaluation, the physicochemical (tab. 4.8), microbiological and sensory (fig. 4.5) indicators of pickled tomatoes were determined.

Tabelul 4.8. Physicochemical indicators of pickled tomatoes with apple acidifier

No.	Characteristics	Norm	Results
1	Total soluble solids, %, not less	4,0	6,10±0,03
2	Titrateable acidity, %	#0,5 – 0,7*	0,36**±0,07
3	Content of chlorides, %	1,5 – 2,0	1,50±0,04
4	pH	n/n	4,09±0,06
5	Impurities, including mineral	n/a	n/d

Notă: (Crucirescu 2022b); norm, according GOST 1633-73E (valid, according OMAIA153/2010); # for weak pickles; * titrateable acidity, recalculated to acetic acid; ** titrateable acidity, recalculated to malic acid; n/n – not normed; n/a – not admitted; n/d – not detected.

The obtained data show that the pickled tomatoes preserved with apple acidifier had a lower titrateable acidity value (0.36±0.07 %), than the norm for classic weak pickles (0.5 -0.7 %). The amount of salt was 1.50±0.04 %, which is the lower limit, according to the norm, and the pH – 4.09±0.06. The dry matter content was 6.1±0.03 %, which is higher than the norm, and is probably

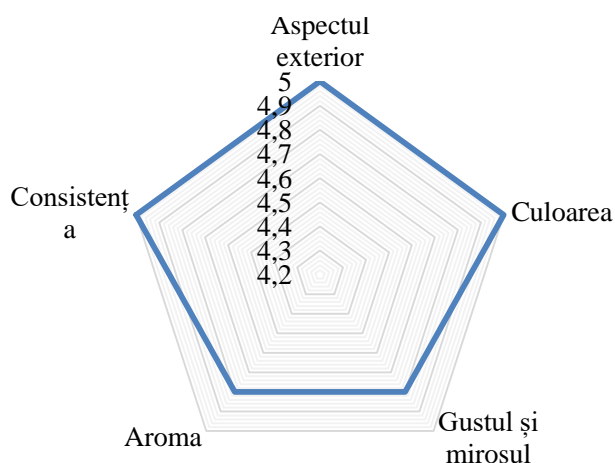


Fig. 4.9. Sensory profile diagram of pickled tomatoes using apple acidifier

sweet-sour, weak salty, without extraneous nuances. It has a pleasant aroma with a slight shade of green apple (fig 4.9).

4.3.2 Canned cucumbers

Preservation of cucumbers with the application of apple acidifier was tried and studied in the thesis. The production recipe is presented in Table 4.9.

Table 4.9. Recipe for making canned cucumbers using apple acidifier

No.	Raw materials and materials	Manufacturing recipe for 100 kg finished product
1	Cucumbers, kg	57,0
2	Black peppercorns, kg	0,04
3	Greens (dill, celery, parsley), kg	1,5
4	Garlik chopped, kg	0,25
Composition of the preservation solution		
5	Water, L	29
6	Salt, kg	1,2
7	Apple acidifier**, L	4,0

Note: (Yastrebov, 1980; Crucirescu, 2023b); * packing ratio in container: vegetables – 50-55 %, preservation solution – 45-50 %; **acidifier from unripe apple Rewena variety 71 DAB 2019 (AT=2,75 %; SUH=8,3 °Brix; pH=2,70).

The analysis of the physicochemical indicators (tab. 4.10) was carried out according to Banu et al. (2007) and the normative documentation in force GOST 20144-74E (valid, according to OMAIA153/2010).

Table 4.10. Physicochemical indicators of canned cucumbers with apple acidifier

No.	Characteristics	Norm	Results
1	Total soluble solids, %, not less	4,0	5,60±0,05
2	Titrateable acidity, %	#0,5 – 0,6*	0,24**±0,04
3	Content of chlorides, %	2,5 – 3,0	2,40±0,07
4	pH	n/n	4,11±0,02
5	Impurities, including mineral	n/a	n/d

Note: (Banu et al., 2007; Crucirescu, 2023b); norm, according GOST 20144-74E (valid, according OMAIA153/2010); # for weak cans; * titrateable acidity, recalculated to acetic acid; ** titrateable acidity, recalculated to malic acid; n/n – not normed; n/a – not admitted; n/d – not detected.

Physicochemical indicators in canned cucumbers reported very good results, with acidity of 0.24 ± 0.04 %, and the amount of salt 2.40 ± 0.07 %, which are values lower than the norm in the

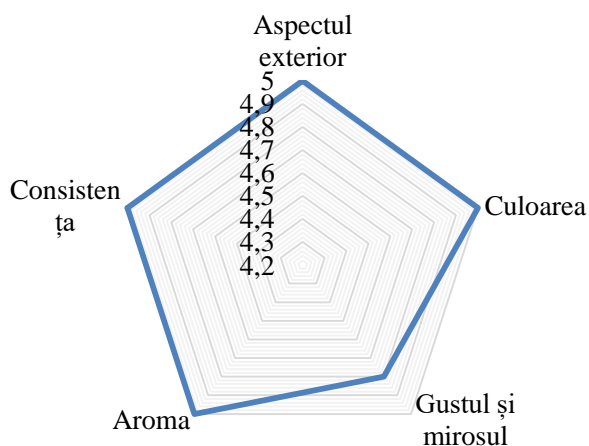


Fig. 4.10. Sensory profile diagram of canned cucumbers using apple acidifier

normative documentation according to classic recipes. The content of dry substances was higher than the norm and showed 5.60 ± 0.05 %, and the pH value was 4.11 ± 0.02 . The microbiological results demonstrated that the product corresponded to industrial sterility.

Sensory evaluation was performed according to ISO 6658:2017 and Banu et al. (2007). The cans presented whole cucumbers about 60 mm long, olive-green in color, and the coating liquid was clear. Pleasant sour taste and smell, without extraneous nuances, with a slight aroma of green apple. The cucumbers were crunchy with a hard core, well soaked with brine (fig 4.10).

4.3.3 Vegetable stew of type „Zacusca”

Samples of vegetable stew of type "Zacusca" with small changes in the traditional recipe were obtained. For the production of stew (recipe tab. 4.11) and the evaluation of quality indicators (tab. 4.12; fig. 4.11), as a reference were the similar stew products from the normative documentation in force GOST 2654-98 (valid, according to OMAIA 153/ 2010).

Tabelul 4.11. Recipe for making vegetable stew of type „Zacusca” using apple acidifier

No.	Raw materials and materials	Manufacturing recipe for 100 kg finished product
1	Eggplants, kg	64,0
2	Bell peppers red, kg	40,0
3	Tomatoes red, kg	11,8
4	Salt, kg	1,0
5	Apple acidifier *, L	3,0

Note: (Crucirescu, 2023a); * acidifier from unripe apples variety Golden Rezistent 71 DAB 2019 (AT=2,05 %; SUH=8,3 °Brix; pH=2,95)

Tabelul 4.12. Physicochemical indicators of vegetable stew of type „Zacusca” with apple acidifier

No.	Characteristics	Norma	Rezultate
1	Total soluble solids, %	18,0 – 27,0	27,0±0,05
2	Titrate acidity, recalculated to malic acid, %	n/n	0,42±0,04
3	Content of chlorides, %	1,2 – 1,6	0,94±0,07
4	pH	n/n	4,81±0,02
5	Impurities, including mineral	n/a	n/d

Note: (Crucirescu, 2023a); n/n – not normed; n/a – not admitted; n/d – not detected.

The results obtained (tab. 4.12) show that this type of vegetable stew is very beneficial for consumption by the population. The total acid content, salt content and pH are quite acceptable,

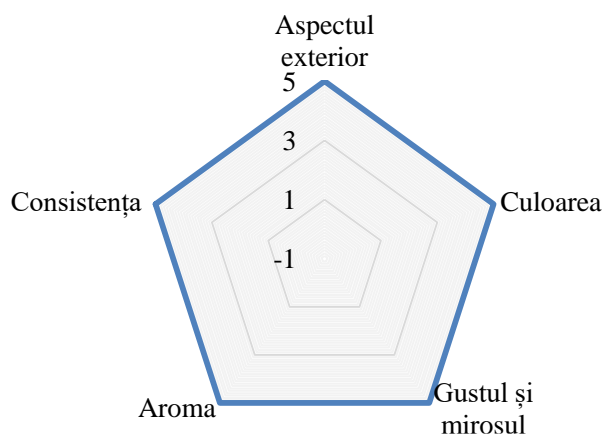


Fig. 4.11. Sensory profile diagram of vegetable stew of type „Zacusca” using apple acidifier

being 0.42 ± 0.04 %, 0.94 ± 0.07 % and 4.81 ± 0.02 , respectively. These values are lower compared to other similar products, according to the normative documentation. The amount of dry substances in the samples of analyzed cans represents 27.0 ± 0.05 %. Microbiological indicators determined show that the developed product met the requirements of industrial sterility.

The appearance of the vegetable stew "Zacusca" was an eggplant paste with inclusions of eggplant pieces, donuts and tomatoes cut almost homogeneously in size without the skin, with soft eggplant seeds evenly distributed throughout the mass and with the characteristic color of canned vegetables. The taste and smell are very pleasant and well expressed, with a grilling of smoke, without bitter taste and extraneous nuances. The tasting committee was pleasantly surprised by all the indications of the stew, especially the aroma and taste of grilling smoke (fig. 4.11).

4.3.4 Canned hot peppers

Hot peppers are prized for capsaicin, an alkaloid that imparts the pepper's hot, burning taste with anti-inflammatory effects. When preserving hot peppers, in this work, no specific recipe according to normative documentation was used. For preservation, the coating liquid was prepared similarly to that used in the production of canned cucumbers with the use of verjuice (Dupas de Matos 2019). After 9 months of storage in canned hot peppers, the physicochemical (tab. 4.13), microbiological and organoleptic indicators (fig. 4.12) were determined.

Table 4.13. Physicochemical indicators of canned hot peppers with apple acidifier *

No.	Characteristics	Rezultate
1	Total soluble solids, %	$9,70 \pm 0,01$
2	Titrateable acidity, recalculated to malic acid, %	$0,80 \pm 0,03$
3	pH	$3,30 \pm 0,02$
4	Impurities, including mineral	n/d

Note: total content of hot peppers in relation to the net mass – 70 %; * acidifier from unripe apple Reglindis variety 71 DAB 2019 (AT=2,35 %; SUH=8,4 °Brix; pH=3,00); n/d - not detected.

The results show that the developed product is characterized by a low acidity content, constituting 0.80 ± 0.03 %, and a high content of dry substances with 9.70 ± 0.01 %, the pH value was 3.30 ± 0.02 . The microbiological results indicate that the product corresponded to industrial sterility.

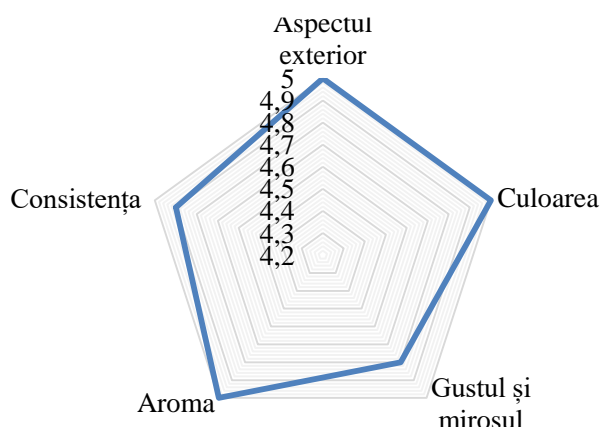


Fig. 4.12. Sensory profile diagram of canned hot peppers using apple acidifier

had a hard, crunchy consistency and were rated with good marks by tasting committee (fig. 4.12).

4.3.5 Sour Cherry Jam and Canned Strawberry

In the framework of the thesis, two ungelled fruit products were developed and studied: cherry jam and preserved strawberries (in their juice), substituting the citric acid in the manufacturing recipe with the apple acidifier. The production recipes are presented in Table 4.14.

Table 4.14. Recipe for making cherry jam and canned strawberries using apple acidifier

No.	Raw materials and materials	Manufacturing recipe for 100 kg finished product	
		Sour Cherry Jam	Canned Strawberry
1	Fructe (vișine, căpșune) kg	65,0	68,0
2	Zahăr, kg	63,0	34,0
3	Acidifiant din mere*, L	20,0	11,0

Note: (Banu, 2009; Băisan, 2018); *acidifier from unripe apples Rewena variety 71 DAB 2019 (AT=2,75 %; SUH=8,3 °Brix; pH=2,70).

The quality indicators in the developed products were evaluated according to the Technical Regulation "Jams, jellies, purees and other similar products" (HG no. 216, 2008) and presented in Table 4.15. The food received high marks from the tasters (fig. 4.13).

Table 4.15. Physicochemical indicators of cherry jam and canned strawberries with apple acidifier

No.	Characteristics	Results	
		Sour Cherry Jam	Canned Strawberry
1	Total soluble solids, %	55,0±0,00	72,0±0,00
2	Titratable acidity, recalculated to malic acid, %	0,80±0,02	0,90±0,02
3	pH	3,90±0,04	3,40±0,05
4	Impurities, including mineral	n/d	n/d

Note: according HG 216/2008 (appendix 3); n/d – not detected.

The physicochemical indicators of cherry sweetness showed the following values: the content of dry substances was 72.0±0.00%; titratable acidity, expressed in malic acid, of 0.8±0.02%; and the pH – 3.30±0.05. The product of canned strawberries with the use of the acidifier was characterized by a high amount of dry matter (55.0±0.00 %) and low titratable acidity (0.9±0.02 %), and the pH was 3.90±0.04. The products corresponded to industrial sterility.

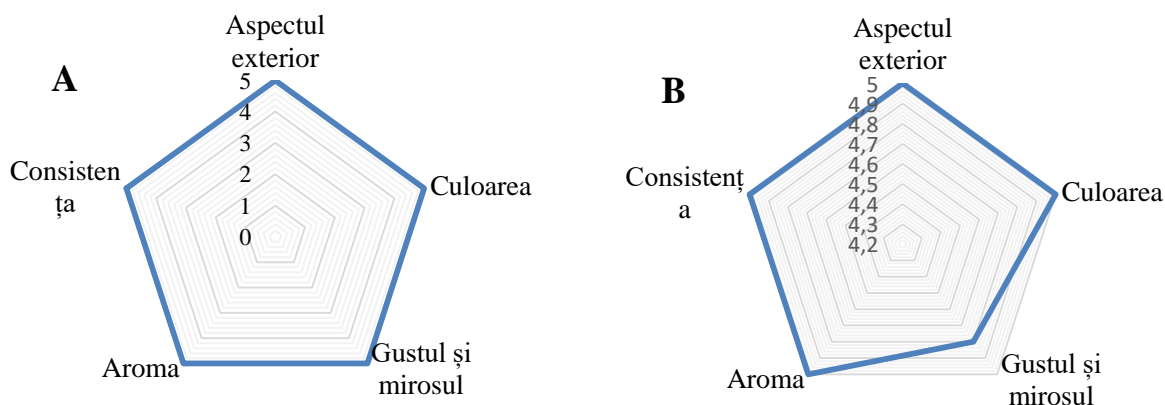


Fig. 4.13. Sensory profile diagram of sour cherry jam (A) and canned strawberry (B) using apple acidifier

For a complex understanding of the developed products, their pictures are presented (fig. 4.14).

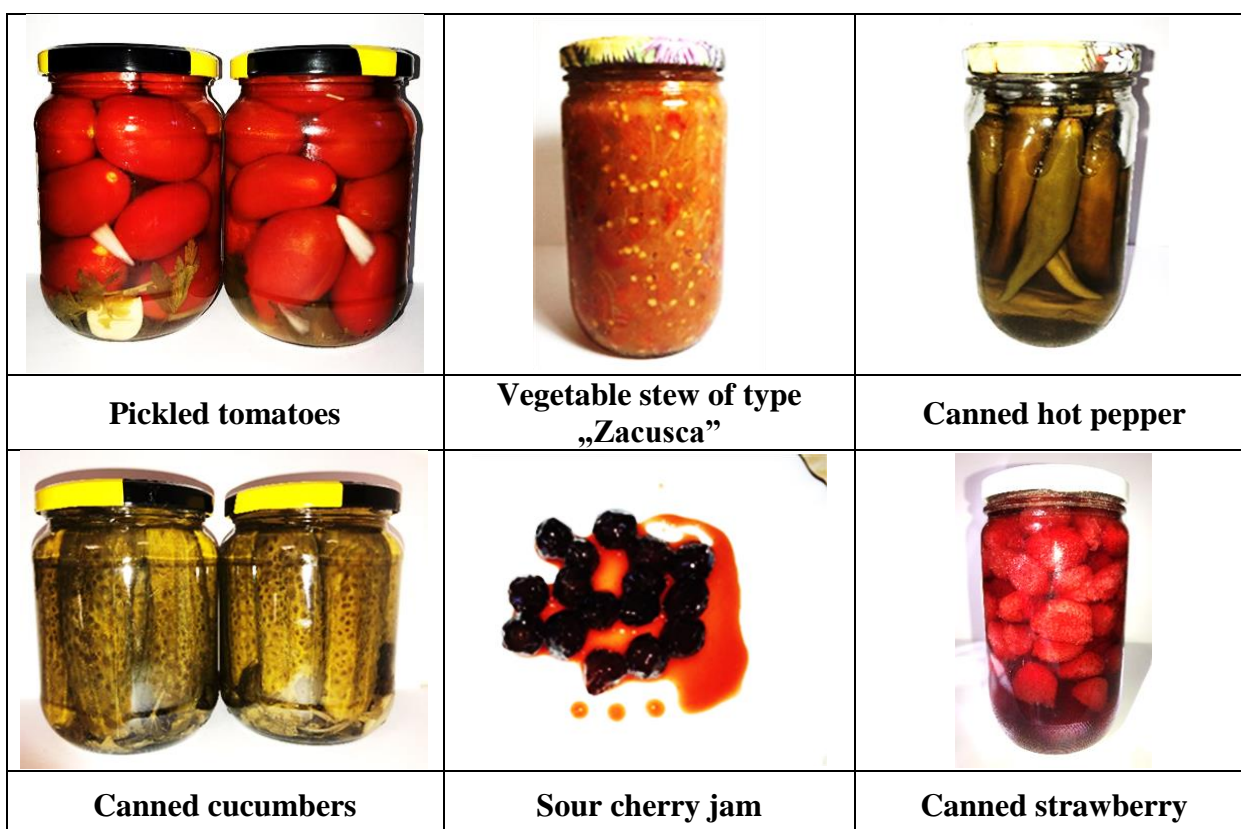


Fig. 4.14. Food products developed in the thesis framework with the use of apple acidifier

4.4 Feasibility study of the apple acidifier manufacture and some elaborate canned products

The total manufacturing costs were calculated and the selling prices of vegetables canned with apple acidifier were determined compared to the prices of canned vegetables produced according to classic recipes, according to the prices of August-September 2021 (tab. 4.15).

Table 4.15. Total costs and estimated prices for the production of canned vegetables according to classic recipes and those with the use of apple acidifier

Economic indices	Products expected for production			
	Pickled tomatoes		Canned cucumbers	
	RC	RAm	RC	RAm
Direct cost, lei/ton	8 609,00	8 320,06	9 965,00	9 874,72
Indirect cost, lei/ton	690,91	690,91	690,91	690,91
Total cost, lei/ton	9 299,91	9 010,97	10 655,91	10 565,63
Profitability, lei/ton	1 859,98	1 802,19	2 131,18	2 113,12
Price total, lei/ton	11 159,89	10 813,16	12 787,09	12 678,76

Note: RC – classic production recipe; RAm – production recipe using apple acidifier

The analysis of the results of Table 4.15 shows that the products preserved with the application of apple acidifier have a lower total price, and the profitability is higher by 0.85 - 3.11 %, than that of the products preserved according to the classic recipe.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The problem addressed in the paper consists of the utilization of unripe apple fruits to obtain the apple acidifier, as a source of natural acidity, and its use for the manufacture of preserved products from fruits and vegetables to replace the synthetic acetic and citric acids from the classic recipes of production.

The study carried out in the thesis framework allows the formulation of the following conclusions:

1. Evaluation of the physicochemical indicators of apple fruits, the varieties Coreana, Golden Reizistent, Reglindis and Rewena during growth and development (45, 58, 71, 84 and 97 days after blooming (DAB)) showed interest in obtaining natural acidifiers. Apples showed high values of titratable acidity (1.35 - 2.79 %), moderate values of water-soluble dry substances (6.50 - 12.35 Brix) and total sugar (3.35 - 10.02 %); low pH values (2.95 - 3.20), the sugar/acidity indicator varying between 2.33 and 9.15 units. Efficient and rational use of these fruits could turn this horticultural waste into a valuable product (Crucirescu, 2019; 2021b; 2022d; subchapters 2.1.1; 3.1).

2. Enzymatic treatment (preparations with pectolytic and amylolytic action) and simultaneous thermal treatment of the shredded mass from unripe apples favored the increase of juice yield after pressing by up to 24% compared to untreated samples. The process was optimized by applying mathematical modeling, with the aim of forecasting and using the process for several varieties of apples. The values of titratable acidity, water-soluble dry substances, total sugar and pH served as parameters for optimization, and the value of juice yield from unripe apples after pressing served as an optimization criterion (Golubi et al., 2019; subchapters 3.2; 3.3).

3. The low pH values and high titratable acidity made it possible to apply an optimized pasteurization regime of the juice from unripe apples, used in the acidifier production process, at a temperature of 60 °C for 20 minutes. These conditions ensure the preservation of the high nutritional value of the product and the reduction of energy consumption, an important factor for industrial application. The optimized technology for obtaining apple acidifiers was developed with the production of experimental samples. The technological process was patented and presented at national and international conferences and exhibitions of inventions (Golubi et al., 2018; Crucirescu, 2021a; Golubi et al., 2019; subchapters 3.4; 4.1).

4. Apple acidifiers (4 varieties) contain important amounts of organic acids, the predominant one being malic acid with values between 16.90 g/L and 38.07 g/L, which constitutes 94.99 % - 97.61 %. The fructose content constituted 65.68 % - 74.36 % of the total carbohydrates, and the glucose content – 25.62 % - 34.68 %; sucrose was detected in very small amounts. Acidifiers have a high content of total phenolic substances (568.7 - 2042.8 mg GAE/L). The antioxidant activity was 28.46 - 41.37 µg AA/mL (acidifiers obtained from fruits harvested on 45th DAB) and 3.27 - 7.08 µg AA/mL (on 97th DAB). The results show that unripe apples can be used to obtain natural acidifiers, rich in nutrients (Crucirescu, 2022a; subchapter 4.2).

5. The recommended shelf life of apple acidifiers from of the Coredana, Golden Rezistent, Reglindis and Rewena varieties, established following the determination of quality indicators and microbiological stability at the beginning and end of "reserve" storage, from the day of manufacture in glass packaging with or without core of light and dark color at the temperature of 18 - 20 °C and relative air humidity maximum 75% can be established 2 years (Golubi et al., 2019; subchapters 4.2.2; 4.2.3).

6. Technologies for the production of canned vegetables and fruits were developed, substituting the acetic and citric acids from the classic production recipes with the apple acidifier. At the same time, the amounts of salt and sugar added in the technological process were reduced. The quality indicators and the microbiological stability of the developed products confirmed the positive influence and benefits of using natural acidifiers in the manufacture of food products (Crucirescu, 2022b; 2023a; 2023b; subchapter 4.3).

7. The production of the apple acidifier and its implementation in manufacturing technologies of vegetable and fruit preserves ensures a lower price and a higher economic profit by 0.85% - 3.11%, compared to preserves obtained according to classic production recipes (Golubi et al., 2019; subchapter 4.4).

PRACTICAL RECOMMENDATIONS

Based on the research in the work, technologies were developed for the manufacture of some food products with apple acidifier that are recommended for their implementation in the field of preservation of the food industry:

1. The process of obtaining the apple acidifier, according to the invention patent (Golubi et al., 2019) (fig. A 3.1).
2. The process for obtaining the grape and apple mix acidifier, according to the invention patent (Golubi et al., 2021) (fig. A 3.2).
3. Food manufacturing technologies using apple acidifier, substituting acetic and citric acids in the production recipe: canned vegetables (pickled tomatoes, canned cucumbers, vegetable stew of type "Zacusca", canned hot peppers); canned fruit (sour cherry jam, canned strawberries).

The following prospective research is also recommended:

1. Implementation of apple acidifiers in other areas of the food industry, such as soft drinks, meat, bakery and confectionery; as an alternative to vinegar and lemon juice as a source of acidity and flavoring for many dishes, salads and appetizers; as toppings in the production of desserts; etc.
2. The study of pomace and filter sediment obtained from the processing of unripe apples with the subsequent extraction of valuable substances (ex: polysaccharides, pectic substances, phenolic compounds).
3. The circular approach to the process of obtaining the apple acidifier, provides for "zero waste" (the concept of the Circular Economy).
4. The application of apple and grape mixt acidifiers, according to the invention patent (Golubi et al., 2021), for the manufacture of food products to widen the assortment of healthy foods.

SELECTIVE BIBLIOGRAPHY

- ALBERTI, A., dos SANTOS, T.P.M., ZIELINSKI, A.A.F. et al. Impact on chemical profile in apple juice and cider made from unripe, ripe and senescent dessert varieties. In: *Food Sci Technol-LEB*, 65, 2016: pp. 436–443. <http://dx.doi.org/10.1016/j.lwt.2015.08.045>.
- ANYASI, T. A., JIDEANI, A. I. O., EDOKPAYI, J. N. et al. Application of organic acids in food preservation. In book: *Organic acids: characteristics, properties and synthesis* (pp.45) Chapter: Chapter 1: Application of organic acids in food preservation Pub.: Nova Science Publishers, 2017.
- ASSIRELLI, A., GIOVANNINI, D., CACCHI, M. et al. Evaluation of a New Machine for Flower and Fruit Thinning in Stone Fruits. *Sustainability*, 10, 2018: pp. 4088-4100. DOI: [10.3390/su10114088](https://doi.org/10.3390/su10114088).
- BANDIC, L. M., ŽULJ, M. M., FRUK, G., et al. The profile of organic acids and polyphenols in apple wines fermented with different yeast strains. In: *Journal of Food Science and Technology*, 56(2), 2019: pp. 599–606. <https://doi.org/10.1007%2Fs13197-018-3514-2>.
- BANU, C. *Calitatea și analiza senzorială a produselor alimentare*. București: Ed. ASAB, 2007, 574 p. ISBN: 978-973-720-141-6.
- BART, J.J., THODEY, K., SCHAFFER, R.J. et al. Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. *BMC Plant Biology*, 8(16), 2008: pp. 1-29. <http://dx.doi.org/10.1186/1471-2229-8-16>.
- BĂISAN, I. *Conservarea produselor agroalimentare* (curs pentru studenții anului II master, specializarea. Tehnici nepoluante în industria agroalimentară), 2018. https://mec.tuiasi.ro/ro/images/diverse/Conservarea_produselor_agroalimentare.pdf.
- BIZJAK, J., MIKULIC-PETKOVSEK, M., STAMPAR, F. et al. Changes in Primary Metabolites and Polyphenols in the Peel of "Braeburn" Apples (*Malus domestica* Borkh.) during Advanced Maturation. In: *Journal of Agricultural and Food Chemistry*, 61(43), 2013: pp. 10283-10292. <https://doi.org/10.1021/jf403064p>.
- BURDON, J., LALLU, N., YEARSLEY, C. et al. Postharvest conditioning of Satsuma mandarins for reduction of acidity and skin puffiness. *Postharvest Biology and Technology*, 43, 2007: pp. 102–114. <http://dx.doi.org/10.1016/j.postharvbio.2006.07.014>.
- CHEN, W.Q., GUO, Y.R., ZHANG, J., et al. Effect of different drying processes on the physicochemical and antioxidant properties of thinned unripe apple. *International Journal of Food Engineering*, 11, 2015: pp. 207–219. <https://doi.org/10.1515/ijfe-2014-0211>.
- CRUCIRESCU, D. Application of apple acidifier in vegetable stew of type „Zacusca” production In: *Journal of Engineering Science*, vol. XXX, nr. 3, 2023a: pp. 145 – 154 [https://doi.org/10.52326/jes.utm.2023.30\(3\).10](https://doi.org/10.52326/jes.utm.2023.30(3).10).
- CRUCIRESCU, D. Conținutul acizilor organici în merele imature. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor*. vol. 1, Tehnica-UTM, Chișinău, pp. 462-465, 2022a. ISBN 978-9975-45-828-3.
- CRUCIRESCU, D. Fructele de mere în faza timpurie de coacere - materie primă pentru obținerea acidifiantului natural. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor*. Tehnica-UTM, Chișinău, vol. 1, pp. 505-508, 2019. URI: <http://repository.utm.md/handle/5014/2885>.
- CRUCIRESCU, D. Implementation of apple acidifier in canned cucumbers production. In: *Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry*, vol. 24, nr. 1, 2023b: pp. 061-071. <https://pubs.ub.ro/?pg=revues&rev=csc6&num=202301&vol=1&aid=5533>.
- CRUCIRESCU, D. Indicii de calitate a conservelor de tomate marinate cu acidifiant din mere. În: *Conferința științifică internațională „Perspectivele și Problemele Integrării în Spațiul European al Cercetării și Educației”*, vol. IX, partea 1, pp. 335-340, Cahul: USC, 2022b. https://ibn.idsi.md/vizualizare_articol/166099.
- CRUCIRESCU, D. Physicochemical characteristics in unripe apples. In: *Journal of Engineering Science*, vol. XXVIII, nr. 4, 2021b: pp. 156 – 166. [https://doi.org/10.52326/jes.utm.2021.28\(4\).16](https://doi.org/10.52326/jes.utm.2021.28(4).16).
- DUPAS de MATOS, A. Sensory characterization of cucumbers pickled with verjuice as novel acidifying agent. *Food Chemistry*, v 286, 2019: pp. 78-86. <https://doi.org/10.1016/j.foodchem.2019.01.216>.
- ETIENNE, A., GENARD, M., LOBIT, P. et al. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J. Exp. Bot.*, 64, 2013: pp. 1451–1469. <http://dx.doi.org/10.1093/jxb/ert035>.

- FIORINO, M., BARONE, C., BARONE, M., et al. Chemical Additives for Foods. Impact of Food-Related Quality System Certifications on the Management of Working Flows. In book: *Quality Systems in the Food Industry*, 2019: pp.1-27. https://doi.org/10.1007/978-3-030-22553-7_1.
- GELETA, B.T., LEE, J.-C., HEO, J.-Y. Antioxidant Activity and Mineral Content in Unripe Fruits of 10 Apple Cultivars Growing in the Northern Part of Korea. *Horticulturae*, 9(1), 2023: p. 114. <https://doi.org/10.3390/horticulturae9010114>.
- GOLUBI, R. *Valorificarea strugurilor nematurate la obținerea compozițiilor nutritive. Teză de doctor în științe tehnice*, Chișinău, 2019. 143 p.
- LI, H., SUBBIAH, V., BARROW, C.J., et al. Phenolic profiling of five different Australian grown apples. *Appl. Sci.* 11, 2021: p. 2421. DOI: 10.3390/app11052421.
- MA, B., YUAN, Y., GAO, M. et al. Determination of Predominant Organic Acid Components in *Malus* Species: Correlation with Apple Domestication. *Metabolites.*, 8(4), 2018: p. 74. <https://doi.org/10.1016/j.foodchem.2014.09.032>.
- MUREȘAN, A.E., MUREȘAN, V. *Acumularea, transformarea și caracterizarea unor compuși biochimici din fructele de măr*. Cluj-Napoca: Ed. MEGA, 2019, 98 p.
- MUREȘAN E. A., MUSTE S., VLAIC R. A. et al. The Dynamics of Starch and Total Sugars during Fruit Development for Jonathan, Starkrimson and Golden Delicious Apple. In.: *Bulletin UASVM Food Sci and Technology*, 72(1), 2015: pp. 120-126. <http://dx.doi.org/10.15835/buasvmcn-fst:11140>.
- PEREZ-LAMELA, C., FRANCO, I., FALQUE, E. Impact of High-Pressure Processing on Antioxidant Activity during Storage of Fruits and Fruit Products: A Review. *Molecules*, 26(17), 2021: pp. 5265. doi: [10.3390/molecules26175265](https://doi.org/10.3390/molecules26175265).
- PEȘTEANU, A., CALESTRU, O. Reglarea încărcăturii de rod la pomii de măr de soiul Golden Reinders prin diverse metode de rărire. *Știința agricolă*, 2, 2017: pp. 37-42. ISSN: 1857-0003.
- ROSHAN, S., WONG, W. K., NORAZIAH, M., et al. Chemical composition changes of two water apple *International Food Research Journal*, 19 (1), 2012: pp. 167-174. ID corpus: 73562227.
- SRIVASTAVA, S., TYAGI, S.K. Effect of Enzymatic Hydrolysis on the Juice Yield from Apple Fruit (*Malus Domestica*) Pulp. *International Journal of Biotechnology and Bioengineering Research*, V 4 (4), 2013, pp. 299-306. ISSN 2231-1238.
- THAKUR, A., SINGH, Z. Responses of ‘Spring Bright’ and ‘Summer Bright’ nectarines to deficit irrigation: fruit growth and concentration of sugars and organic acids. *Scientia Horticulturae*, 135, 2012: pp. 112–119. <http://doi.org/10.1016/j.scienta.2011.12.013>.
- WOJDYLO, A., OSZMIANSKI, J. Antioxidant Activity Modulated by Polyphenol Contents in Apple and Leaves during Fruit Development and Ripening. *Antioxidants (Basel)*, 9(7), 2020: p. 567. DOI: [10.3390/antiox9070567](https://doi.org/10.3390/antiox9070567).
- WU, B.H., GENARD, M., LESCOURET, F., et al. Influence of assimilate and water supply on seasonal variation of acids in peach (cv Suncrest). *Journal of the Science of Food and Agriculture*, 82, 2002: pp. 1829–1836. <https://doi.org/10.1002/jsfa.1267>.
- XU, J., YAN, J., LI, W. et al. Integrative Analyses of Widely Targeted Metabolic Profiling and Transcriptome Data Reveals Molecular Insight into Metabolomic variations during apple (*Malus domestica*) fruit development and ripening. *Int. J. Mol. Sci.*, 21, 2020: p. 4797. <https://doi.org/10.3390/ijms21134797>.
- YANG, S., MENG, Z., LI, Y., et al. Evaluation of Physiological Characteristics, Soluble Sugars, Organic Acids and Volatile Compounds in ‘Orin’ Apples (*Malus domestica*) at Different Ripening Stages. *Molecules* 26, 2021: p. 807. <https://doi.org/10.3390/molecules26040807>.
- ZHANG, L., WANG, C., JIA, R. et al. Malate metabolism mediated by the cytoplasmic malate dehydrogenase gene *MdcyMDH* affects sucrose synthesis in apple fruit. *Horticulture Research*, v. 9, 2022: p.194. <https://doi.org/10.1093/hr/uhac194>.
- ZHENG, H.Z., KIM, Y.L., CHUNG, S.K. A profile of physicochemical and antioxidant changes during fruit growth for the utilisation of unripe apples. *Food Chem*, 131, 2012: pp. 106–10. <https://doi.org/10.1016/J.FOODCHEM.2011.08.038>
- ZHENG, H.Z., HWANG, I.W., CHUNG, S.K. Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. *J Zhejiang Univ Sci B.*, 10(12), 2009: pp. 912-9. DOI: 10.1631/jzus.B0920186.
- ZHENG, H.Z., HWANG, I.W., KIM, B.K., et al. Phenolics Enrichment Process from Unripe Apples. *J Korean Soc Appl Biol Chem*, 57(4), 2014: pp. 457–461. DOI: 10.1007/s13765-014-4013-4.

LIST OF THE AUTHOR'S PUBLICATIONS ON THE SUBJECT OF THE PhD THESIS

1. Papers in in scientific journals

1.1 in journals from the Web of Science and SCOPUS databases:

- **CRUCIRESCU, D.** Implementation of apple acidifier in canned cucumbers production. *Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry*, vol. 24, nr. 1, 2023: pp. 061-071. CSCC6202301V01S01A0006 [0005533] <https://pubs.ub.ro/?pg=revues&rev=csc6&num=202301&vol=1&aid=5533> (WoS – ESCI)

1.2 în reviste din Registrul Național al revistelor de profil, (cu indicarea categoriei):

- **CRUCIRESCU, D.** Physicochemical characteristics in unripe apples. In: *Journal of Engineering Science*, vol. XXVIII, nr. 4, 2021: pp. 156 – 166. [https://doi.org/10.52326/jes.utm.2021.28\(4\).16](https://doi.org/10.52326/jes.utm.2021.28(4).16) (DOAJ – cat. B+)
- **CRUCIRESCU, D.** Application of apple acidifier in vegetable stew of type „Zacusca” production. In: *Journal of Engineering Science*, vol. 30, nr. 3, 2023: pp. 145 – 154 [https://doi.org/10.52326/jes.utm.2023.30\(3\).10](https://doi.org/10.52326/jes.utm.2023.30(3).10) (DOAJ – cat. B+)

3. Articole în culegeri științifice

3.1. in the proceedings of international scientific conferences (abroad):

- **КРУЧИРЕСКУ, Д.** Рациональное использование незрелых яблок в контексте устойчивого развития. В: *Материалах IV Всероссийской научно-практической конференции с международным участием*, с. 149-152, г. Киров, 2022 г. ISBN 9785-982282576 URI: <http://repository.utm.md/handle/5014/25507>

3.2. in the proceedings of international scientific conferences (Republic of Moldova):

- GOLUBI, R., IORGA, E., ARNAUT, S., **CRUCIRESCU, D.**, et al. Natural acidifier produced from apples in the early ripening phase. In: *MTFI-2018 International Conference*, TUM, pp. 156-158. Chisinau, 2018, ISBN 978-9975-87-428-1 URI: <http://repository.utm.md/handle/5014/5172>
- **CRUCIRESCU, D.** Fructele de mere în faza timpurie de coacere - materie primă pentru obținerea acidifiantului natural. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor*. Tehnica-UTM, Chișinău, v. 1, pp. 505-508, 2019. URI: <http://repository.utm.md/handle/5014/2885>
- **CRUCIRESCU, D.** Utilizarea rațională a merelor imature. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor*. v. 1, Tehnica-UTM, Chișinău, pp. 401-404, 2020. URI: <http://repository.utm.md/handle/5014/8563>
- **CRUCIRESCU, D.** The technological production scheme for tomatoes pickles with apples acidifier. In: *International Scientific Symposium „Agriculture and Food Industry – Achievements and Perspectives”*, State Agrarian University from Moldova, 19-20 november, Chisinau, 2021. pp. 201-203 URI: <http://repository.utm.md/handle/5014/25347>
- **CRUCIRESCU, D.**, VOITCO, E., RABOTNICOVA, L., et al. Organoleptic evaluation of tomatoes pickles with apples acidifier. In: *International Scientific Symposium „Agriculture and Food Industry – Achievements and Perspectives”*, State Agrarian University from Moldova, 19-20 november, Chisinau, 2021. pp. 204-207 URI: <http://repository.utm.md/handle/5014/25348>
- **CRUCIRESCU, D.** Conținutul acizilor organici în merele imature. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor*. v. 1, Tehnica-UTM, Chișinău, pp. 462-465, 2022a. ISBN 978-9975-45-828-3 URI: <http://repository.utm.md/handle/5014/20754>
- **CRUCIRESCU, D.** Argumentarea necesității producerii acidifiantului din mere și obținerea acestuia. În: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și*

Doctoranzilor. v. 1, Tehnica-UTM, Chișinău, pp. 413-416, 2021.
URI: <http://cris.utm.md/handle/5014/979>

- **CRUCIRESCU, D.** Indicii de calitate a conservelor de tomate marinate cu acidifiant din mere. In: *Conferința științifică internațională „Perspectivele și Problemele Integrării în Spațiul European al Cercetării și Educației”*, v IX, partea 1, pp. 335-340, Cahul: USC, 2022b. https://ibn.idsi.md/vizualizare_articol/166099

4. Theses in scientific collections

1. in the proceedings of international scientific conferences (abroad):

- **CRUCIRESCU, D.** Unripe apples – source of natural organic acids. In: *Papers of the international symposium EURO-ALIMENT. Food connects people and shares science in a resilient world.* p. 86, Galati, 2021, p. 10
URI: <http://repository.utm.md/handle/5014/20142>

2. in the proceedings of international scientific conferences (Republic of Moldova):

- **CRUCIRESCU, D.** The acidifier from unripe apples - source of natural acidity. In: *Ecological and environmental chemistry – 2022*, ed. 7, v. 1, USM, pp. 171-173, Chișinău, 2022. ISBN 978-9975-159-06-7
URI: <http://repository.utm.md/handle/5014/19864>

Other scientific works

Intellectual property. Patents:

- GOLUBI, R., IORGA, E., BUCARCIUC, V., ARNĂUT, S., **CRUCIRESCU, D.** Procedeeul de obținere al acidifiantului din mere. Brevet de invenție de scurtă durată MD 1286 Z; 2019.01.31, 2019.
<http://www.db.agepi.md/inventions/Details.aspx?id=s%202018%200026>
- GOLUBI, R., IORGA, E., **CRUCIRESCU, D.** et al., Procedeeul de obținere al acidifiantului de cupaj din struguri și mere. Brevet de invenție de scurtă durată MD 4757 Z; 2021.06.30, 2021.
<http://www.db.agepi.md/Inventions/details/a%202020%200058>

Other specific works (materials for invention salons and participations):

- GOLUBI R., IORGA E., BUCARCIUC V., ARNAUT S., **CRUCIRESCU D.** Process for obtaining the apple acidifier. *Salonul Inovării și Cercetării UGAL INVENT-2019*, Galati, Romania, 16-18 October 2019, p.53. URI: <http://repository.utm.md/handle/5014/25522>
- GOLUBI R., IORGA E., BUCARCIUC V., ARNAUT S., **CRUCIRESCU D.** Process for obtaining the apple acidifier. *Expoziția Internațională Specializată INFOINVENT-2019*, Section D, Chisinau, November 20-23 2019, pp. 151-152. URI: <http://repository.utm.md/handle/5014/25524>
- GOLUBI R., IORGA E., BUCARCIUC V., ARNĂUT S., **CRUCIRESCU D.** Process for obtaining the apple acidifier. *European Exhibition of creativity and Innovation EuroInvent-2018*, Iasi, Romania, Mai 17-19 2018. URI: <http://repository.utm.md/handle/5014/25523>
- GOLUBI R., IORGA E., BUCARCIUC V., ARNĂUT S., **CRUCIRESCU D.** Process for obtaining the apple acidifier. *The XXII-th International Exhibition of Reseach, Innovation and Tehnological Transfer “INVENTICA 2018”* Iasi, Romania, June 27-29 2018, p. 306. URI: <http://repository.utm.md/handle/5014/25365>

ADNOTARE

Crucirescu Diana: „Valorificarea fructelor de mere imature pentru obținerea acidifiantului natural”, teză de doctor în științe ingineresti, Chișinău, 2024.

Structura tezei: teza de doctor constă din introducere, 4 capitole, concluzii generale și recomandări, bibliografia cu 318 titluri, 3 anexe, textul de bază conține 115 pagini, inclusiv 50 tabele, 44 figuri. Rezultatele obținute sunt publicate în 16 lucrări științifice.

Cuvintele-cheie: mere imature, acizi organici, glucide, substanțe fenolice, acidifianții din mere, conserve de fructe și legume cu aplicarea acidifiantului din mere.

Scopul lucrării: valorificarea merelor imature pentru obținerea unei surse de aciditate naturală (acidifiant din mere) și utilizarea acesteia în industria alimentară, în special la conservarea fructelor și legumelor, substituind acizii din rețetele clasice de producere.

Obiectivele cercetării: identificarea soiurilor de mere pentru studiere, stabilirea perioadei optime de recoltare și determinarea indicatorilor fizico-chimici în aceștea; studierea și aplicarea diferitor metode de extragere a sucului din merele imature; obținerea mostrelor experimentale de acidifianți cu evaluarea calității și a termenului de păstrare; aplicarea acidifianților la conservarea fructelor și legumelor; efectuarea studiului de fezabilitate al fabricării produselor elaborate.

Noutatea și originalitatea științifică: pentru prima dată în Republica Moldova au fost stabiliți parametrii merelor imature pentru obținerea acidifianților naturali; au fost încercate metode de majorare a randamentului sucului; aplicat un regim lejer de pasteurizare în obținerea acidifiantului; a fost argumentat științific și demonstrat experimental posibilitatea substituirii acizilor, din rețetele clasice de conservare a fructelor și legumelor, cu acidifiantul din mere.

Problema științifică soluționată: valorificarea fructelor de mere imature cu stabilirea celor mai importante proprietăți fizico-chimice; identificarea condițiilor optime și eficiente de procesare tehnologică și de utilizare a acestora; obținerea unei surse de aciditate naturală cu ulterioara aplicare în producerea alimentelor sănătoase; creșterea durabilității prin utilizarea eficientă a deșeurilor agricole vegetale, utilizându-le în calitate de materie primă.

Semnificația teoretică: definitivarea metodelor de studiere a calității acidifianților din mere; obținerea rezultatelor științifice care denotă posibilitatea utilizării acestora în industria alimentară ca sursă de aciditate naturală; aplicarea metodelor de majorare a randamentului sucului din merele imature și a unui regim mai lejer de pasteurizare în procesul de obținere a acidifiantului.

Valoarea aplicativă: este elaborat fluxul tehnologic de prelucrare a merelor imature pentru obținerea acidifiantului natural și utilizarea acestuia la conservarea fructelor și legumelor.

Implementarea rezultatelor științifice: articole în reviste științifice, culegeri de lucrări ale simpozioanelor și conferințelor internaționale și naționale, brevete de invenții (nr. 1286, nr. 4757).

АННОТАЦИЯ

Кручиреску Диана: «Использование незрелых яблок для получения натурального подкислителя», диссертация на соискание уч. степени док. тех. наук, Кишинёв, 2024.

Структура диссертации: состоит из введения, 4 глав, выводов и рекомендаций, списка цитируемой литературы из 318 ссылок, 3 приложений. Работа изложена на 115 страницах, 44 рисунков и 50 таблицы. Результаты опубликованы в 16 научных работах.

Ключевые слова: незрелые яблоки, органические кислоты, сахара, фенольные вещества, яблочные подкислители, консервы фруктовые и овощные.

Цель работы: исследование незрелых яблок для получения натурального источника кислотности (яблочный подкислитель) и его использование в пищевой промышленности, в частности при консервировании фруктов и овощей, заменяя кислоты в рецептурах.

Задачи исследований: выявление сортов яблок для изучения с установлением оптимальных сроков уборки и определением в них физико-химических показателей; применение различных способов извлечения сока из незрелых яблок; получение опытных образцов подкислителей, с оценкой качества и срока годности, и их применение для консервирования фруктов и овощей; проведение технико-экономического обоснования производства разработанной продукции.

Научная новизна и оригинальность: впервые в Республике Молдова установлены оптимальные параметры незрелых яблок для получения натуральных подкислителей; был увеличен выход сока из незрелых яблок за счет применения ферментов; была научно обоснована и экспериментально продемонстрирована возможность замены кислот в рецептурах, при консервировании фруктов и овощей, яблочным подкислителем.

Решенная научная проблема: использование незрелых яблок с установлением важнейших физико-химических свойств; выявлением оптимальных и эффективных условий технологической обработки и их применения; повышение устойчивости за счет эффективного использования растительных отходов, используя их в качестве сырья.

Теоретическая значимость: совершенствование методов исследования качества яблочных подкислителей; получение научных результатов, демонстрирующих возможность их использования в пищевой промышленности; внедрение пастеризации с пониженным термическим эффектом в процесс получения подкислителя.

Практическая значимость: разработана технолог. схема переработки незрелых яблок с целью получения натурального подкислителя и использования его в продуктах.

Внедрение научных результатов: статьи в научных журналах, сборники докладов международных и национальных симпозиумов и конференций, патенты (№ 1286, № 4757).

ANNOTATION

Crucirescu Diana: „Use of unripe apples to obtain the natural acidifier”, PhD thesis in engineering sciences, Chisinau, 2024.

Thesis structure: thesis consists of an introduction, 4 chapters, general conclusions and recommendations, bibliography from 318 titles, 3 annexes. The basic text includes 115 pages, 44 figures and 50 tables. The obtained results are published in 16 scientific works.

Key words: unripe apples, organic acids, carbohydrates, phenolic substances, apple acidifiers, canned fruits and vegetables with apple acidifier.

The purpose of the work: use of unripe apples to obtain a source of natural acidity (apple acidifier) and use of the in the food industry, especially in the preservation of fruits and vegetables, substituting the acids in classic preservation recipes.

Research objectives: the identification of apple varieties for study; the establishment of the optimal harvesting period, the determination of the physico-chemical indicators in them; study and application of different methods of extracting juice from unripe apples; obtaining experimental samples of acidifiers with evaluation of quality and shelf life; its application to the preservation of fruits and vegetables; the feasibility study of the manufacture of the developed products.

Scientific novelty and originality: for the first time in the Republic of Moldova, the optimal parameters of unripe apples for obtaining natural acidifiers were established; the yield of juice from unripe apples was increased by applying enzymes; the possibility of substituting acetic and citric acids from canned fruit and vegetable recipes with apple acidifier has been scientifically argued and experimentally demonstrated.

The scientific problem solved: valorisation of the fruits of unripe apples with the establishment of the most important physicochemical properties and the identification of the optimal and efficient conditions of technological processing and their use; obtaining a source of natural acidity with subsequent application in the production of healthy foods; increasing sustainability through the efficient use of vegetable agricultural waste, using it as a raw material.

Theoretical significance: defining the methods for studying the quality of apple acidifiers; obtaining scientific results demonstrating the possibility of their use in the food industry (especially in the preservation of fruit and vegetables) as a source of natural acidity; implementation a light pasteurization regime in the process of obtaining of the acidifier.

Applicative value: the technological flow for processing unripe apples has been developed to obtain the natural acidifier and its use in the food industry.

Implementation of scientific results: articles in scientific journals, collections of papers of international and national symposions and conferences, patents (no. 1286, no. 4757).

CRUCIRESCU DIANA

USE OF UNRIPE APPLES TO OBTAIN THE NATURAL ACIDIFIER

253.01 PLANT BASED FOOD TECHNOLOGY

Abstract of the PhD dissertation

Approved for printing: data

Paper size 60x84 1/16

Paper offset. Print offset.

Circulation ___ ex.

Printing sheets: ___

Order no. ___

MD-2004, Chisinau mun., 168 Stefan cel Mare si Sfant str.,

UTM MD-2045, Chisinau mun., 9/9 Studentilor str., Publishing House "Tehnica-UTM"

CHISINAU, 2024