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ORIGINAL RESEARCH PAPER

IMPLEMENTATION OF APPLE ACIDIFIER IN CANNED CUCUMBERS PRODUCTION

Diana Crucirescu[1,2*]

[1]Public Institution of the Scientific-Practical Institute of Horticulture and Food Technologies, Laboratory of Food Quality Verification, Vierului str., 59, MD-2011, Codru, Chisinau, Republic of Moldova
[2]Technical University of Moldova, Doctoral School of TUM, 168 Ştefan cel Mare blvd., MD-2004, Chisinau, Republic of Moldova

*Corresponding author: diana.crucirescu@saiem.utm.md; dcrucirescu@mail.ru

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Abstract: The present study analyzes the acidifier from thinned unripe apples, in order to obtain a source of natural acidity. The technological scheme for obtaining the natural acidifier was developed, which was characterized by high acidity (2.21 ± 0.1 %) and significant amount of total soluble solids (7.93 ± 0.2 %). The contents of organic acids and carbohydrates were analyzed. The main organic acid was malic acid with 26.43 ± 0.003 $g \cdot dm^{-3}$, and the main carbohydrate was fructose with an amount of 35.17 ± 0.095 $g \cdot dm^{-3}$, glucose being 2 times less. The acidifier has been proposed as an alternative to acetic acid in the preservation of cucumbers (*Cucumis sativus*). Thus, 2 series of experimental samples were produced, using the substitution of 50 % (PA50) and 100 % (PA100) of acetic acid with apple acidifier, compared to the control sample (according to the classic recipe). All samples were characterized physico-chemically and subjected to organoleptic evaluation. The developed products showed very good results compared to the control. However, the most relevant and valuable was the PA100, having the best quality indices. The production and use of apple acidifier allows the efficient utilization of raw materials by increasing sustainability of horticultural sector and leads to obtaining healthy and ecological products.

Keywords: *apple acidifier, canned cucumbers, physicochemical parameters, organoleptic evaluation, unripe apples*

INTRODUCTION

Currently the demand for natural sources of acidity used in the food industry is increasing, because consumers are more and more aware of its health. The nutritional aspects related to the ecology and naturalness of the food products they consume has become increasingly important and necessary. This fact is now considered a key factor in the development of new healthy foods.

Few natural acidifiers and their implementation in food are known. Recently, a liquid food product derived from unripe apples called apple acidifier has been discovered, which is characterized by high acidity and sour taste [1, 2]. This product represents the unfermented juice obtained from unripe, thinned or physiologically fallen apples which, as a rule, are not used for food, but are left as waste. Being a source of acidity, it can be used to preserve vegetables as an alternative to acetic acid. This research would be particularly interesting because apple acidifier does not contain acetic acid and its acidity is predominantly due to malic acid.

Experimental level attempts to preserve vegetables with natural sources of acidity, such as verjuice and grape acidifier, have been carried out by some researchers [3 – 5].

Canned cucumbers (*Cucumis sativus*) are popular among consumers due to the sensory aspects of a favorable flavor and texture, as well as firmness and a crunchy consistency [6]. Cucumbers can be eaten both fresh and canned. Being rich in water (90 - 95 %) and low in calories (12-15 kcal per 100 g fresh weight), consumption of cucumbers can be beneficial in boosting immunity and stimulating metabolism [7, 8]. Their processing consists in the preservation of vegetables with minimal damage and may vary depending on the type of vinegar, salt content, *p*H value, stabilizing additives, optimization of heat treatment during pasteurization or storage temperature [6]. Cucumbers are preserved by direct acidification, which involves the addition of salt and an acidic liquid as the primary source of preservation, followed by their pasteurization. Preservation can improve the quality, affect the organoleptic properties and improve the general appearance of cucumbers [4, 9].

Due to the increasing need for the implementation in the food industry of natural sources of acidity, the aim of this work was to evaluate the quality indices of canned cucumber products using apple acidifier as a substitute for acetic acid in the classic recipe.

MATERIAL AND METHODS

Raw materials and materials

Raw materials for obtaining apple acidifier in this study served unripe apples, in the early ripening phase, of the variety *Rewena* (Figure 1). The fruits were picked between June 1 and July 20, 2020 from the experimental lots of the Scientific-Practical Institute of Horticulture and Food Technologies, Chisinau, Republic of Moldova. The fruits were harvested at 71st day after full bloom.

Rewena is a new resistant variety of apple obtained in Germany. It is considered one of the best varieties of apples in the world. The trees are demanding to the soil, have high winter hardiness. It has stable scab resistance and high resistance to powdery mildew. The fruit is of medium to large size, a mass of 160 - 230 g, with a globular or conicalglobular shape. It bears fruit in abundance early; the flowering season is medium and requires the thinning [10].

Figure 1. *Image of apple fruits of the variety Rewena [10]*

Cornichon variety extra quality cucumbers served as raw material for the production of canned cucumbers and were purchased from the local market (HG no. 929, 2009). At the same time, materials such as table salt (HG no. 596, 2011), fresh garlic (HG no. 929, 2009), greens (parsley, dill, celery) (HG no. 1078, 2008), acetic acid (HG no.1403, 2008), glass jars with a volume of 580 cm^3 and Twist-off lids, were used to obtain preserves. The HG symbols mean the Government Decision that represents the normative documentation in force in the Republic of Moldova.

Location of research

All the technological, physico-chemical and organoleptic research was carried out in the Food Products Quality Verification Laboratory of the Scientific-Practical Institute of Horticulture and Food Technologies, Chisinau, Republic of Moldova.

Technology of apple acidifier production

Unripe apples of variety *Rewena* harvested around day 71 from the full flowering phenophase were transported in wooden crates to the research laboratory. They were washed under a stream of water, shredded-crushed and then the shredded mass was heated to the temperature of 50 ºC with subsequent enzyme treatment for 1 hour. After that, the mass was pressed, clarified and filtered. The green juice obtained was thermally treated at a temperature of 60 ºC for 20 min (pasteurized), packed, sealed and stored. The production of apple acidifier was carried out according to patent no. 1286 BOPI [11].
Laboratory-scale processing of unripe apple to obtain apple acidifier is presented in the Figure 2.

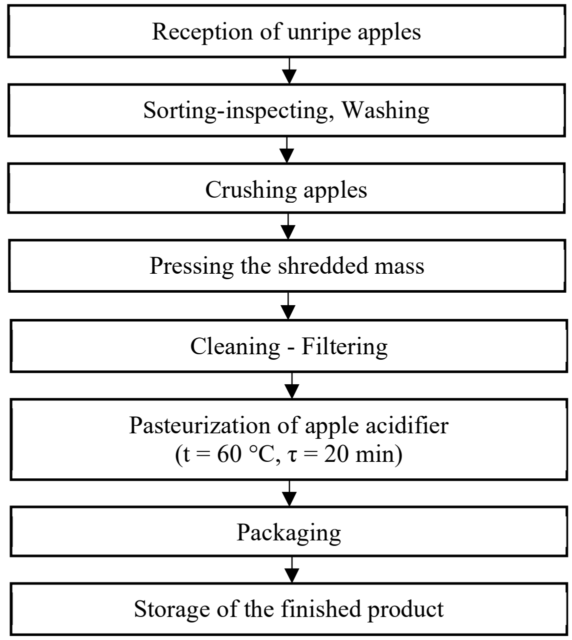

Figure 2. *Lab-scale processing of apple acidifier from unripe apple [11]*

Technology of canned cucumbers production

The canned cucumber samples were prepared under laboratory conditions according to the production recipes shown in Table 1 [12, 13]. Cans of 3 types were obtained: no. 1 - sample according to the classic recipe (control); no. 2 and no. 3 - replacing 50 % and 100 %, respectively, of acetic acid with apple acidifier. The control sample was coded PC, and samples no. 2 and no. 3 - were marked as PA50 and PA100.

Table 1. *Recipes for making canned cucumbers with and without apple acidifier*

No.	Raw material and materials	Container type (jar volume 0.58 liters), sample		
		no. 1 (PC)	**no. 2 (PA50)**	**no. 3 (PA100)**
1	Cucumbers [g]	348 – 360	348 – 360	348 – 360
2	Black peppercorns [pcs]	0.5 – 1	0.5 – 1	0.5 – 1
3	Greens [g]	8 – 10	8 – 10	8 – 10
4	Peeled garlic cut [pcs]	1 – 2	1 – 2	1 – 2
	Composition of the preservation solution			
5	Water [g]	200 – 210	200 – 205	185 – 200
6	Salt [g]	15 – 18	9 – 12	8 – 10
7	Acetic acid 5 % [g]	12 - 15	6 – 8	–
8	Apple acidifier [g]	–	12 – 15	24 – 30

*Note: [12, 13]; *packing ratio in container: vegetables – 50-55 %, preservation solution – 45-50 %; PC - control sample; PA50 - the sample in which 50 % of the acetic acid is substituted with apple acidifier; PA100 - the sample in which 100 % of the acetic acid is substituted with apple acidifier.*

Extra quality *Cornichon* variety cucumbers were received, sorted, calibrated, washed and left in cold water for 30 - 60 min. To obtain the preservation solution, the amount of salt and the required volume of acetic acid and/or apple acidifier were added to the water boiled and cooled to 60 ºC. Greens, black peppercorns, garlic and cucumbers were placed in washed and conditioned glass jars, according to the production recipe. The vegetables were covered with the preservation solution, having a temperature of 60 ºC. The jars filled and closed with Twist-off lids were sterilized at 100 ºC for 8.2 min, followed by 20 min of cooling. The cans were stored at a temperature of 18 - 20 ºC and a maximum relative humidity of 75 % for 9 months.

Physicochemical analysis

Preparation of samples for analysis
The apple acidifier was homogenized with a glass rod. Equal amounts of liquid part and solid part (canned cucumber) were taken from the cans of cucumbers and homogenized with a homogenizer. The obtained mass was diluted with distilled water (1:5), heated in a water bath (80 ºC, 30 min), cooled to 20 ºC and filtered. The obtained supernatant was used for the necessary determinations.

Physicochemical analysis
The amount of total soluble solids was obtained using the electronic refractometer ATAGO PAL-3 (Japan). The titratable acidity was determined by titrating the analyzed supernatant with the sodium hydroxide solution in the presence of the phenolphthalein indicator (ISO 750:1998), and the ionic acidity with a HANNA 211 *p*H-meter (Germany) the methods described in ISO 1842:1991.

HPLC analysis
Determination of organic acids (malic, citric) was analyzed by high-performance liquid chromatography (HPLC) on the Agilent 7100 chromatograph (Agilent Technologies, USA). The separation was achieved using a C_{18} column (250 × 4.6 mm), with a particle size diameter of 5 µm and a guard column (Agilent Technologies, USA). All separations were maintained at 25 ºC. The detection wavelength was 210 nm. The mobile phase was composed of KH_2PO_4 buffer solution adjusted to pH = 2.8, with a flow rate of 0.7 $mL \cdot min^{-1}$. The injection volume of each sample was 10 µL.

CE analysis
The determination of carbohydrates (fructose, glucose, sucrose) was performed by the capillary electrophoresis (CE) method at the Capel 105M facility (Lumex, Russia). Separation conditions: background electrolyte based on dipicolinic acid with the addition of tetradecyltrimethylammonium bromide (TTAB); capillary L_{efect}/L_{total} = 65/75 cm, ID = 50 мкm; voltage - 25 kV; detection wavelength 230 nm. All separations were maintained at 20 ºC. The data were processed using the Elforun program.

Organoleptic evaluation of canned cucumbers

The glass containers were opened just before the organoleptic evaluation. One cucumber was taken from each jar, sliced and transferred with the proper preservation liquid, to avoid curdling, in three separate bowls, corresponding to the number of the sample. The samples were evaluated at room temperature.

The tasting committee consisted of 8 evaluators trained in the field of food technologies aged between 35 and 75 years. The indices of both the preserved vegetables and the preserving liquid were evaluated. The appearance, color, smell, taste and consistency were evaluated both by the descriptive and the scoring method, using the 5-point system, which includes the following scores: 5 - very good; 4 - good; 3 - satisfactory; 2 - unpleasant; 1- bad and 0 - very bad (ISO 6658:2017).

Statistical analysis

The analysis of variance of the results was performed using one-way analysis of variance and Student's test. The software application Microsoft Office Excel version 2010 was used for statistical analysis. All determinations were performed in triplicate, with a maximum error of less than 5 %. The obtained results were expressed as mean ± SD.

RESULTS AND DISCUSSION

Apple acidifier is a liquid that represents the unfermented juice obtained from unripe apples, thinned or physiological fruit drop and, typically, not used for food, but normally thrown away [14 - 16].
The apple acidifier of *Rewena* variety harvested on day 71 after full flowering (which coincided with June 27, 2020) was studied to be implemented in the production of canned cucumbers. The acidifier presented a clear juice with sediment (less than 3 %) of a dark yellow color with an intensely acidic taste, pleasant and agreeable, with a slight taste of green apple. The yield was 38 - 48 % after the pressing process to obtain the acidifier. The determined physicochemical indices are presented in Table 2.

Table 2. *The physicochemical indices determined in the apple acidifier*

Characteristics	Results
Total soluble solids [%]	7.93±0.2
Titratable acidity, expressed in malic acid [%]	2.21±0.1
*p*H	3.24±0.1
Content of organic acids [$g\cdot dm^{-3}$]:	
Malic	26.43±0.003
Citric	0.25±0.001
Content of simple carbohydrates [$g\cdot dm^{-3}$]:	
Fructose	35.17±0.095
Glucose	14.68±0.070
Sucrose	0.19±0.043

The results show that the acidifier from apples of *Rewena* variety is characterized by an impressive amount of titratable acidity (2.21 ± 0.1 %) and a low *p*H (3.24 ± 0.1). The content of malic acid was 26.4 ± 0.003 $g\cdot dm^{-3}$, being a predominant acid, and citric acid was hundreds of times less. Acidity is an important property for assessing food quality, as it directly contributes to the formation of taste. At the same time, it was determined that the main simple carbohydrate represents fructose in the amount of 35.17 ± 0.095 $g\cdot dm^{-3}$, and glucose constituted a concentration 2.4 times lower. Sucrose, however, had very small amounts, registering 0.19 ± 0.043 $g\cdot dm^{-3}$.

During cell division in apples, in the mesophyll of the leaves, sucrose and sorbitol are formed in the photosynthesis process [17], which are then translocated to the immature, unripe and undeveloped fruits. These are sources of carbon needed in fruit metabolism [18, 19].

Marques *et al.* in her study performed a complete physicochemical characterization of malic acid, emphasizing its potential as a food ingredient and its application in pectin gels [20]. Due to the ability of organic acids to penetrate the microbial cell wall, thus exhibiting antimicrobial activity, malic acid has been applied to obtain food packaging, extending their shelf life and protecting them from microbial contamination [21].

According to the results obtained (Table 2), following the analyzes carried out, it can be concluded that apple acidifier represents an acidic food ingredient, having in its composition functional compounds, such as organic acids (especially malic acid) and simple carbohydrates. This fact encouraged us to use the apple acidifier in obtaining new functional products.

The studied acidifier was applied as a substitute for acetic acid, as a source of acidity, when obtaining preserved cucumbers (*Cucumis sativus*), which are considered some of the most popular preserved vegetables. The preservation was carried out by direct acidification, obtaining two experimental samples (no. 2 - PA50, no. 3 - PA100, respectively) and one control (no. 1 - PC), according to the production recipes (Table 1).

Table 3. *The physicochemical indices determined in the canned cucumber samples*

Characteristics	Norm	Samples of canned cucumbers		
		no. 1 (PC)	no. 2 (PA50)	no. 3 (PA100)
Total soluble solids [%], not less	4.0	4.20±0.01	4.80±0.02	5.60±0.03
Titratable acidity [%]	#0.5 – 0.6*	*0.57±0.03	*0.46±0.05	**0.24±0.04
Content of chlorides [%]	2.5 – 3.0	2.70±0.05	2.49±0.03	2.40±0.07
*p*H	–	3.43±0.01	3.95±0.02	4.88±0.02
Impurities, including mineral	n/a	–	–	–

*Note: according [22, 23]; # for slightly acidic preserves; *titratable acidity, recalculated to acetic acid; **titratable acidity, recalculated to malic acid; PC-control sample; PA50-the sample in which 50% of the acetic acid is substituted with apple acidifier; PA100-the sample in which 100% of the acetic acid is substituted with apple acidifier; n/a - not admitted.*

The results show that the substitution of acetic acid with a larger amount of acidifier contributes to the increase of the soluble solids content compared to the control sample (4.20 %), as follows: 4.80 % for PA50 and 5.60 % for PA100. This can be explained by the fact that the apple acidifier contains its own soluble solids (Table 2) which increase the soluble solids content of the finished product.

An important characteristic of canned products is the titratable acidity, which depends on the microbial stability and the shelf life, as well as the taste and aroma. Acidity is an important aspect for canned vegetables as it contributes to their microbial safety and typical sensory properties. The determinations made demonstrated that the titratable acidity of the products developed with acidifier was lower than the legal norm [23]. But during 9 months of storage, they were shown to be intact. The acidity of PA50 was close to the lower limit of the norm, having 0.46 ± 0.05 % (expressed in acetic acid), and PA100 2 times lower – 0.24 ± 0.04 % (expressed in acid malic); PC with 0.57 ± 0.03 % (expressed in acetic acid) falls within the limits of the norm for weakly acidic canned products. At the same time, the addition of apple acidifier increases the *p*H values of the processed products, the control sample having 3.43 ± 0.01, but PA50 and PA100 – 3.95 ± 0.02 and 4.88 ± 0.02, respectively. It can be mentioned that the *p*H and the total acid content in the final products were dependent on the amount of acidifier added to obtain the preservation solution, similar to the study carried out by Dupas de Matos *et al.* [4].

It was found that the chloride content in the experimental samples had lower values compared to the legal norm and the control sample (2.70 ± 0.05 %), showing the following values: 2.49 ± 0.03 % for PA50 and 2.40 ± 0.07 % for PA100. This phenomenon is due to the fact that a smaller amount of table salt was added to the production recipe (Table 1). With the substitution of acetic acid with 50 % and 100 % acidifier, the amount of salt in the recipe was reduced by 25 % and 50 %, respectively. The decision was made following the study of some papers, in which vegetables preserved with verjuice or grape acidifier had a salty taste compared to the control sample [4, 3], which may be due to the mineral content in the vegetable raw material from which the natural acidifier was obtained [24].

The organoleptic evaluation was carried out by the tasting committee. Descriptive analysis of experimental and control samples is presented (Table 4). Also, Figure 3 presents the diagram of the organoleptic characteristics of canned cucumbers evaluated by the scoring method.

Table 4. *Descriptive analysis of the canned cucumbers samples*

Indices	Sample no. 1* (PC)	Sample no. 2 (PA50)	Sample no. 3 (PA100)
Appearance	Whole, healthy, homogeneous cucumbers with a length of 75-80 mm, which corresponds to extra quality. Length/diameter ratio approximately 4. No mechanical damage.		
Color	Olive-green with various shades, without spots and burns. Clear brine with inclusions of greens, pieces of garlic.		
Smell and taste	Pleasant, sour, aromatic, well expressed, slightly salty, pungent smell, characteristic of cucumbers preserved with acetic acid.	Pleasant, expressed, sour, slightly salty, with a moderate smell of acetic acid and expressed of cucumbers.	Pleasant, well-expressed, balanced, slightly salty, sour, well-expressed smell of cucumbers.
	No bitter taste, no foreign taste and smell		
Aroma	Pungent, characteristic acetic acid, slightly pleasant, with a hint of spices and greens	Pleasant, moderate of acetic acid, slightly unbalanced, with an aroma of spices and greens	Pleasant, with a slight touch of green apple, with an aroma of spices and greens
Consistency	Firm, springy, with a crispy core, tender, with undeveloped seeds.		

*Note: *according* [22, 23]; *PC - control sample; PA50 - the sample in which 50 % of the acetic acid is substituted with apple acidifier; PA100 - the sample in which 100% of the acetic acid is substituted with apple acidifier.*

The results of the organoleptic analysis (Table 4) show that out of the five analyzed indicators, two of them proved to be significantly different between the evaluated samples, which is also demonstrated by the scoring method (Figure 3). As expected, PC was rated with an intensely sour taste, pungent smell and aroma of acetic acid. According to normative documentation [23], canned cucumbers are produced using acetic acid essence, which can lead to pungent characteristics. The flavor of the samples with acidifier was softer and more pleasant. Cucumber flavor was more intense in PA50 and PA100 compared to PC. The perception of salt was not pronounced in the samples, being low-salt canned food products PC and PA50, PA100 – with a reduced amount of table salt (Table 1).

An important characteristic for canned cucumbers is texture. In view of this aspect, the firmness of the vegetables and the crispy core did not show differences between the samples. This fact proves that apple acidifier does not worsen the texture of cucumbers compared to acetic acid.

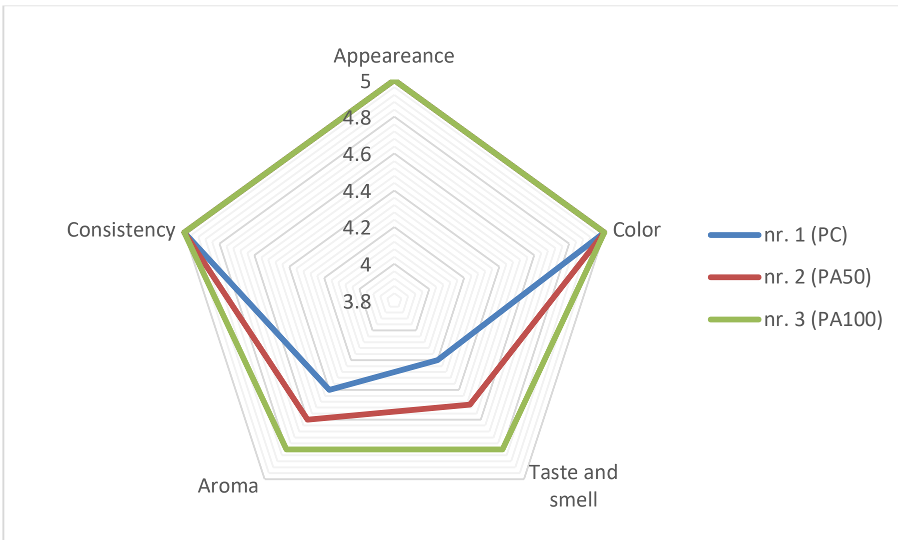

Figure 3. *Diagram of organoleptic characteristics of evaluated canned cucumbers*

According to the results obtained from the physicochemical analyzes and the organoleptic evaluation, it was found that both experimental samples presented better data compared to the control sample. However, the most relevant product was PA100, with the best results. Its image is shown in Figure 4.

Figure 4. *The image of experimental sample PA100*

CONCLUSIONS

The acidifier obtained from unripe apples *Rewena* variety is characterized by a high content of titratable acidity, i.e. 2.21 ± 0.1 %, and a significant content of total soluble solids - 7.93 ± 0.2 %. The main organic acid was malic acid with 26.43 ± 0.003 $g \cdot dm^{-3}$, and the main carbohydrate – fructose with an amount of 35.17 ± 0.095 $g \cdot dm^{-3}$, glucose being 2 times less. The *p*H was 3.24 ± 0.1 %. The acidifier could be used as a source of natural acidity, especially when preserving vegetables.
The experimental samples of preserved cucumbers with the substitution of 50 % and 100 % of the acetic acid in the production recipe had better physicochemical and organoleptic characteristics compared to the control sample. Thus, the titratable acidity of PA50 and PA100 was reduced from the values of 0.5 - 0.6 % for low-acid cans (classic recipe) to 0.46 and 0.24 %, respectively. The amount of soluble solids increased by 14.3 % for PA50 and by 33.3 % for PA100 compared to the control sample, and the amount of salt was decreased by 7.8 % and 11.1 %, respectively. The organoleptic characteristics of the samples produced with the substitution of acetic acid by acidifier were significantly improved compared to PC.
Taking into account that the acidifier comes from thinned unripe apples, being considered a horticultural waste, the presented study allows the efficient utilization of vegetable raw materials by increasing sustainable development. At the same time, its use in the food industry, substituting synthetic additives, leads to the manufacture of ecological products with an improved nutritional value.

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