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The action of polygalacturonase, pectin-esterase and high temperatures to increase yield in apple juice (*Malus domestica*)

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Abstract

The effects of temperature and the action of the polygalacturonase and pectin esterase enzyme have been studied as ways to increase the yield of the apple juice made from apple varieties harvested in the orchard of *Mălâncrav, Romania*. Quality parameters such as the soluble solids content, the density, the total acidity, the pH, the soluble solids content/total acidity ratio, the kinematic viscosity, the turbidity, and the color of these juices have been determined. In the apple juice production, the best yield was obtained in the sample which was thermally treated, namely 61.1 – 62 % with a specific consumption of 1.5-1.63 kg/kg. The best values for the analyzed quality indicators were obtained for the juice made from apples of the *Jonathan* variety and subjected to an enzymatic treatment with polygalacturonase and pectin esterase enzyme. In terms of sensory, the best score was obtained by the control sample of apple juice made from apples of the *Jonathan* variety. In terms of apple varieties, it is recommended to use the *Jonathan* one. This research offers the possibility of choosing a model for increasing the juice yield in the BIO/organic variant(with heat treatment) and a database on the *Jonathan* and *Starkrimson* apple juice from an orchard in Transylvania.

Keywords: apple juice, pectinases, heat treatment, yield in juice, quality indicators

1.Introduction

The apple (*Malus domestica*) is one of the main fruits grown worldwide (93 million tons in 2020) [1]. A considerable amount of this production is processed as apple juice, which is the second most consumed fruit juice, after the orange juice and the consumption of juice and nectar in the EU is 9.067 billion liters [2] due to flavor, nutritional values given by the vitamins, minerals, antioxidants and phytochemical compounds. Because the appearance of the juice is a decisive factor for consumers, the juice industry invests in methods that optimize this characteristic [3]. The most used method of improving juice yield is the enzyme addition [4]. The first commercial preparation was used precisely on apples as early as 1930 [5].

The enzyme preparations used nowadays are approved by the FAO/WHO Expert Committee on Food Additives (JECFA) and Food Chemicals Codex (FCC) as food-grade-enzymes. The used enzymes can be immobilization by covalent bonding to the support matrix [6,7], the adsorption, the captation [8]. The commercial pectinases for the fruit juice industry come from selected strains of *Aspergillus niger*, *Trichoderma reesei* and others. When added to the crushed fruit, the pectinases rapidly decrease the viscosity of the hydrolysis of the pectin, the demethylation of the soluble pectin which forms the insoluble pectate that disintegrates the ground fruit. The endo-polygalacturonase acts on the substrate 1,4-*O*-*α*-Dgalacuonate + H_2O and produces the random hydrolysis of 1,4-α-D galactosiduronic linkages in the pectate and other galacturonans, the hydrolysis of *O*-glycosyl and reduces the viscosity.

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The exo polygalacturonase acts on the 1.4-α-D galacturonide + H_2O substrate and catalyzes the degradation of the terminal D-galacturonans, releasing the galacturonic acid [5]. They facilitate the extraction of juice from pulpy fruits, increase the load of the pressing and the juice production, the yield, by 1 to 2%. The residual waste, such as apple pomace, is smaller in quantity and lower in moisture. The enzymes increase the overall productivity of the processing plant. The rapid juice processing with enzymes lowers the risk of microbial spoilage, reduces the oxidation and extends the shelf-life of the juice. The hydrolysis of the pectin in the cell walls weakens the structure and thus maximizes the extraction of their components, such as pigments, essential oils, flavor substances and phenolic antioxidants [5].

The morphological structure of the apples is a factor that maximizes the juice yield. The variability of harvest in terms of apple types, growth, picking, handling, and storage conditions are influencing factors in juice extraction along with different methods of technology adjustment. Fruit softening during ripening involves various tissue deconstructions such as those responsible for mealy and juicy textures. In particular, the juicy textures are distinguished by the way the cellular tissue is, by the amount of water retained in the cell wall and the degree of pectin solubilization [9]. In the case of apples, the cell wall contains cellulose, pectin, hemicellulose and the way in which they are arranged and how the water is compartmentalized determine the firmness and the mealiness of the apple pulp [10].

Around 7.500 varieties of apples are cultivated in the world, out of which 60 varieties in Romania, including *Jonathan* and *Starkrimson* [11]. Among the methods of analysis used in the investigation of the obtained juices are: the pH, the soluble solids content (SSC) [12], the titratable acidity (TTA), the color investigation [13], the turbidity, and the density [14].

The main objective of this study was to compare two methods of increasing the yield of apple juice using an organic variant and one with enzyme preparations, two varieties of apples - *Jonathan* and *Starkrimson* - and a common extraction method by cold centrifugal pressing.

The collateral objectives of this study were to obtain the values of some quality indicators of the obtained cloudy apple juices, with the possibility of choosing the best variant for the processor and for the consumer.

2.Materials and methods

2.1 Materials

The materials used in this study were apples harvested from Malâncrav orchards, Romania, *Jonathan* and *Starkrimson* variety, 2022 production. The highly concentrate fungal (*Trichoderma reesei*) enzyme preparation ROHAPECT MA Plus T(PF), comprising solely polygalacturonase and pectin esterase enzyme. Enzymatic activity is 1100 PGX/g with dosage 100 ppm and 1h mash treatment, offered by AB *Enzymes*, Germany. For analyzes and technological studies, an average apple sample was made according to the standard [15].

2.2. Apple juice preparation

The amount of apples was divided by three. The centrifugal juicer was used to prepare the juices. The technological schemes are shown in figure 1. The peculiarities were in the preparation of the sample after crushing, namely: for the variant with heat treatment after shredding, the mixture was kept at a constant temperature of 60°C for 15 minutes using Termomix TM 6 (Switzerland) and the enzymatic variants using a Thermostat Laboratory-IT-25 type thermostat incubator (Romania) at 25 °C.

2.3 Technological studies

Losses were calculated using the formula:

$$l_t(\%) = (W_i - W_{out}) \times 100 / W_i$$

and for calculating the production yield:

$$Y_p (\%) = (W_{juice} \times 100)/W_{apple\ reception}$$

and for extraction the formula was used:

$$Y_e (\%) = (W_{juice} \times 100)/W_{apple\ crushing}.$$

The specific raw material consumption was calculated using the formula:

$$c_{sapple} = W_{apple} / W_{apple\ juice}\ (kg/kg).$$

in which: lt-technological losses (extraction or cleanning-elimination inedible part); W_i-weight entered; W_{out}- output weight; Y_p- production yield; Y_e- extraction yield; W_{juice}-weight juice; $W_{apple\ in\ reception}$- weight apple reception; $W_{apple\ crushing}$ - weight apple crushing; c_{sapple}-specific consumption of apple

2.4 Physical and chemical analyses

The collateral objectives consist in determining the values of the quality indicators. The following methods are used for this: morphological characteristics of apples, grading, amount of retentate/permeate, humidity [16], relative density [17] (pycnometer, DMA-35 and areometer), pH-value [18] (pH-meter type Orion 2-STAR-England), titratable acidity (TTA) (expressed as malic acid g/100g) [19]; soluble solid content (SSC)(ºBrix), refractive index at 20ºC (refractometer Krüss, Germany, connected to a bath room ultrathermostated Brookfield, with the outer circulation, Germany) [20], kinematic viscosity (cSt) (Ubbelhode viscometer with 3 tube) [21], turbidity (NTU) (TB 100 Portable Turbidity Meter, China) [22], SSC/TTA(soluble solid content/titratable acidity).

2.5.Determination of color characteristics

Color properties were measured using Chroma Meter-CR-400/410 (HunterLab, Japan) and measured: L* CIE lightness coordinate, a* CIE red (+)/green (-) color attributes, b* CIE yellow (+)/ blue (-) color attributes.

The following were calculated:

C*- chroma using the equation [23]:

$$C* = \sqrt{a*^2 + b*^2}$$

h^*-hue angle, using the equation:

$$h* = \tan^{-1}\left(\frac{b*}{a*}\right)$$

The color difference ΔE* was determined between the samples analyzed and obtained by enzymatic and thermal treatment of the raw material, two by two [24]. The formula is used:

$\Delta E^* = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$, where the meaning of the terms is the same as that set forth above.

2.6 Sensory analyses

The organoleptic analysis method was used with unitary scoring comparison scales The organoleptic characteristics evaluated were: appearance, color, taste, smell, aroma. It is compared with the score scale from 0 to 5 for each characteristic. The interpretation of the collected results is done by calculating the average score for each characteristic and then the weighted average score using the formula**:**

$M_{sw} = M_s \times f_i \times f_c$,

In which: M_s- mean score; M_{sw} -Weighted mean score f_i – factor of importance or appearance it is 0.1, color is 0.1, smell is 0.3, taste is 0.3, aroma is 0.2; f_c is the conversion factor from 5 to 20 points f_c=4. There is a basis for assessing the organoleptic characteristics, questionnaires were drawn up and filled in by the panelists. It was used a group of panelists with ages between 19-24 yers old, girls and boys, students of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection of the "*Lucian Blaga*" University of Sibiu. They have technological knowledge, application methodology in sensory analysis and have been trained for three weeks. They are also consumers of apple juice and fresh apples, which is a very important aspect.

The samples analyzed were coded: 736-CJ apple juice variety *Jonathan*-control; 763-TJ *Jonathan* variety apple juice - with heat-treated apples; 637-EJ apple juice *Jonathan* variety-with apples treated with enzymes; 183-CS apple juice variety *Starkrimson*-control; 187-TS *Starkrimson* variety apple juice with heat-treated apples; 873-ES apple juice of the *Starkrimson* variety with apples treated with enzymes.

2.7 Statistical analysis

The samples had triplicates. The mean value, deviation from the mean, squared probable error, mean squared error, mean squared error of the selection mean, confidence interval were calculated, tabular ”*t*” students was used for a 0.05 significance levels and two degrees of freedom and then the actual value of the indicator and performed using Microsoft Excel.

3. Results and discussion

Table 1 contains the results of the assessment of the quality of the two varieties of apples, *Jonathan* and *Starkrimson*, based on the organoleptic and morphological analysis and certain physicochemical measurements bearing importance for the technological calculations. For the analysis, average samples were taken from the apple production of the orchard of Mălâncrav, Romania. The obtained values are representative of the analyzed batches. They are real and specific to the varieties harvested at technological maturity and known to be suitable for juice extraction. Other studies led to similar values [25, 26].

The SSC/TTA ratio is greater than 15, which means that these apple varieties have well-balanced taste characteristics and are good for consumption and juicing. The moisture content of the fruit ranges between 81.5 and 81.6 % w/w while in other studies between 52.3 and 70.2 % (w/w) and is influenced by the apple variety, the harvest period, the storage duration and conditions [27].

These morphological characteristics influence the fruit processing. Thus, at the plant tissue level, the size, the shape and the distribution of the cell affect the mechanical properties of the pulp. The cell wall and the water compartmentalization play an interconnected role in the hydrostatic pressure, which is the main determinant of the fruit firmness and the pulp juiciness [28].

3.1 Technological studies

The stages shown in the technological diagram in figure 1, were used in processing the samples to be studied. The peculiarity of obtaining the raw juice is observed, this consists in the method of preparing the fruit for extraction. The thermal treatment was used as a physical procedure for 15 minutes at 60 ^oC, using the Thermomix, followed by the centrifugal extraction and hot pressing. Another variant of preparing the pulp was mincing it as a puree and treating it with an enzyme preparation. The enzyme addition operation was carried out at a temperature of 25 ^oC, for 1 hour, using a laboratory thermostat. The juice was extracted by centrifugation and cold pressing. The values of the important technological indicators are presented in table 2. They are shown: the technological losses (l_t) as: peduncle, seminal house, seeds, calyxes; extraction losses (l_{ex}); production yield (Y_p) and extraction yield (Y_{ex}) and specific apple consumption (c_s). When processing the apples in order to obtain a cloudy raw juice, we have noted a yield increase of the juice extracted from the samples of apples of the *Jonathan* variety and a decrease of the yield in the samples using the *Starkrimson* variety. Compared to other juice yield values from other studies which reached 92.3%; 95.3%; 81.5 % [29], those obtained in this study are lower and influenced by the characteristics of the extractor. However, as expected, the temperature destabilized the internal structure of the plant tissue and the amount of juice released was higher especially in TJ.

The pectinolytic enzymes that were used contributed to the degradation of the pectin in the matrix of the primary cell wall and in the middle lamella and thus more juice was released into the environment [30]. However, this theory does not apply to the *Starkrimson* variety apples where a 7.8% decrease in yield was recorded compared to the control sample. The samples that have been thermally treated proved a juice yield increase when compared to those with an enzyme preparation addition, and this aspect could help maintain the characteristic of organic product of the apple juice. The variation of apple juice yield can also be analyzed in terms of the mechanical properties of the pulp and the plasmolysis, the water distribution, the polysaccharide composition of the cell wall and the pectin hydration, or the apple variety [27].

3.2. Physico-chemical analysis of the juice

In addition to fulfilling our main objective, the collateral objectives were also achieved. This is because not only the yield is important for the juice industry, but also the quality of the juice and the possibility of further processing

3.2.1 Calculation of the amount of permeate and retentate

The coarse particles were separated by centrifugation. The parameters of the centrifugation operation were: 4.000 x g and the duration of 15 minutes. The permeate is considered to be the amount of clear liquid and the retentate is the amount deposited at the bottom of the capsules used in centrifugation (50mL). The results are shown in table 2. In case the retentate is separated in order to be preserved by concentration, these calculations are necessary when clarifying the juice by centrifugation. Table 2 shows that the action of the enzyme preparation containing polygalacturonase and pectinesterase led to a significant decrease in the amount of retentate by 91.54% in EJ and by 76.6% in ES. Therefore, the pectinolytic enzymes influence the destabilization of the protopectin in the cell walls more than in the thermal treatment: 9.6% (TJ) and 18.9% (TS).

3.2.2. Density

The relative density is an important quality indicator that is influenced by the temperature and the composition of the juice and that influences the values of the other quality indicators such as: the acidity, the kinematic viscosity, the color, the pH.

The standard determination (pycnometric and areometric) method is used, but also the method that uses the DMA 35 electronic density meter. The obtained values have a level of variation of $p < 0.05$, therefore a 95% probability of obtaining these values. The graph in figure 2a contains linear regression equations and correlation indices with values below 50%, respectively $R^2 = 0.4121$ for the pycnometer, $R^2 = 0.4447$ for the hydrometric method and $R^2 = 0.4635$ for DMA 35, which were generated by representing the values of the relative densities of the juices studied using instruments that are based on different physical measurement principles. Although these methods are acknowledged as being able to characterize liquid samples, in this study the density values are different due to the methods of processing the raw material, namely the apples, regardless of variety. The presence of pulp particles of different sizes was an influencing factor on the employed devices.

It can be observed in figure 2.b; c; d that the relative density values that were determined with the pycnometric method and using the portable electronic density meter (DMA 35) $R^2= 0.9068$ were best linearly correlated. Considering the composition of these juices and the method of measuring a very precisely measured volume and the "*U*" tube principle, we consider that the pycnometric method and the portable electronic density meter can be used to characterize the density of the cloudy apple juices. The value obtained for the relative density of the cloudy apple juices is d^{20}_{20} = 1.05-1.06 and is influenced by the apple variety, the juice extraction technology and the physical principle underlying the achievement of the values of this quality index.

Tabel 1. Morphological and physico-chemicals characteristics, measured of apple varieties. The student "*t*" test was used and each experiment was observed 3 times (n = 3) as result given us the mean ± standard deviation.

Characteristics	**Description of organoleptic characteristics**	
	***Jonathan* variety apple**	***Starkrimson* variety apple**
Pulp consistency	crunchy, compact, fondant, slightly softened	floury with stony texture, slightly coarse
The juiciness of the pulp	juicy, pleasant	dry, a little juicy
Taste	the sweet taste is well harmonized with the acidity	the sweet taste is well harmonized with the acidity, at most with a fine astringency
Flavour	the pulp has a pronounced or fine, pleasant flavor specific to the variety	the pulp has fine, pleasant flavor specific to the variety
Morphological part determinations		
Weight, g/buc.	120(±0.4)	139(±0.1)
Volume, cm^3	110(±0.1)	130(±0.3)
Density, g/cm^3	1.09(±0.0)	1.069(±0.01)
Form	globular conical	globular conical
Size, mm	70(±0.2)	75(±0.1)
Peel, %	10.45(±0.8)	9.35(±0.5)
Pulp, %	81.5(±0.7)	82(±0.6)
Peduncle, %	0.065(±0.001)	0.13(±0.02)
The seminal house, %	5.62(±0.3)	5.48(±0.29)
Seeds, %	0.28(±0.09)	0.10(±0.3)
Calyx, %	0.16(±0.1)	0.5(±0.4)
The primary quality indicators of the apple varieties		
Moisture % w/w	81.5(±0.00)	81.6(±0.00)
Total dry matter, % w/w	18.5(±0.00)	18.4(±0.00)
Solid soluble content (SSC), ºBrix	14.5(±0.1)	13.5(±0.1)
Refraction index (nD)	1.3574(±0.1)	1.3570(±0.1)
pH	3-3.5(±0.00)	3.5-4(±0.00)
Titratable total acidity (TTA), g/100 g expressed in malic acid	0.78(±0.01)	0.60(±0,02)
SSC/TTA	18.58(±0.00)	22.5(±0.00)

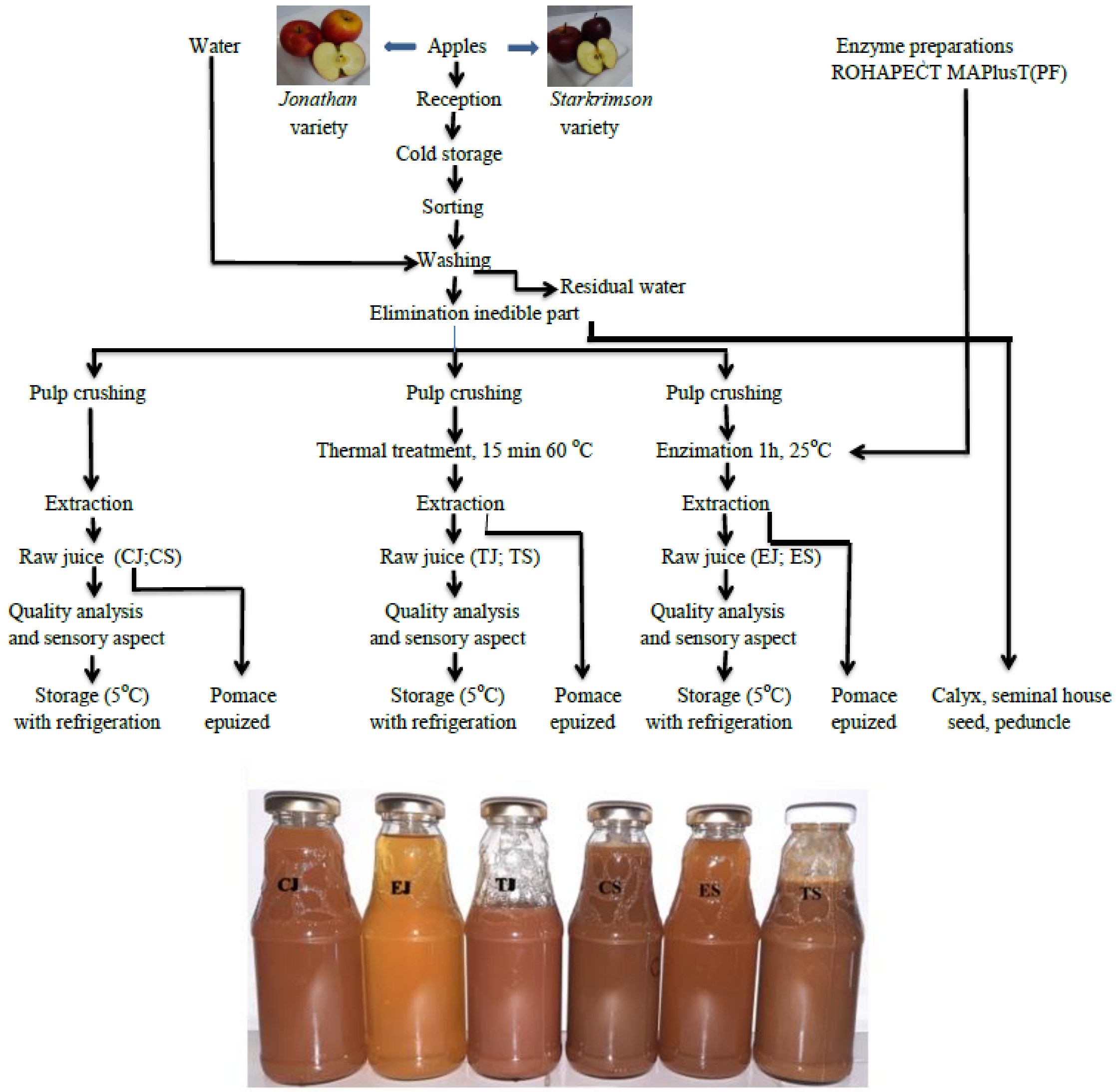

Figura 1. The production technological scheme of raw, cloudy apple juice, using: simple extraction (control-C), heat treatment (for crushed pulp- T) and enzymation (for crushed pulp- E)

Table 2. Technological elements for obtaining the raw, cloudy apple juice by different auxiliary processes (thermally, enzymatically)

Varieties of apples	Treatment type	I_c, %	I_{ex}, %	Y_p, %	Y_e, %	c_s kg/kg	Permeate %	Retentate %	Permeat/ retentate
Jonathan	CJ	7.47 (±*0.04)	67.9 (±0.01)	59 (±0.00)	64.2 (±0.0)	1.55 (±0.00)	74.1 (±0.06)	25.89 (±0,06)	2.86
	TJ	7.25 (±0.1)	66.4 (±0.09)	62 (±0.00)	66.36 (±0.0)	1.5 (±0.00)	76.6 (±0.21)	23.38 (±0.22)	3.27
	EJ	7.30 (±0.06)	63.2 (±0,1)	62 (±0.00)	65.04 (±0.0)	1.54 (±0.00)	95.42 (±0.07)	2.19 (±0.06)	20.85
Starkrimson	CS	10.08 (±0.03)	32.5 (±0.05)	60.6 (±0.00)	67.4 ±0.0	1.64 ±0.00	45.46 ±0.33	54.53 ±0,33	0.83
	TS	9.96 (±0.2)	32,1 (±0.07)	61.1 (±0.00)	67.8 (±0.0)	1.63 (±0.00)	55.78 (±0.1)	44.21 (±0.09)	1.26
	ES	10.26 (±0.07)	37.8 (±0.2)	55.7 (±0.00)	62.1 (±0.0)	1.79 (±0.00)	85.16 (±0.05)	12.76 (±0.06)	6.67

*The student "*t*" test was used and each experiment was observed 3 times (n = 3) as result given us the mean ± standard deviation.

3.2.3.Soluble solid content (SSC), pH, titratable acidity (TTA)

The soluble solids content in the apple juices indicates the sugar content and is the most important quality indicator. The variation of the SSC, pH, TTA values are shown in figure 2. These quality indicators are the most important and are used for grading the juices. The SSC value ranges between 13.4 and 14.63 ºBx, the pH varies between 3.36 and 3.93 and the total acidity has values between 0.53 and 1.04 g/100g expressed in malic acid. These values are comparable to those in the literature [29]. The most important influencing factors are the thermal treatment followed by the enzyme activity compared to the control sample.

The same behavior is recorded regardless of the variety used and is comparable to other studies on the juice of fresh apples from the Izmir area (Turkey) for which the values of a few determined quality indicators are the following: SSC=12.17 ºBx (20ºC) – 14.8 ºBx (60ºC); pH=3.82 (20ºC) - 4.02 (60ºC); TTA = 0.34 g/100g expressed in malic acid (20ºC) – 0.47 g/100g expressed in malic acid (60 ºC) and these values are strongly influenced by the increase in temperature, the action of the pectinolytic enzymes and the sonication [25]. In the case of the fresh apple juice from South Africa, the values of these parameters are as follows: SSC = 8 ºBx, TTA = 0.06 g/100g expressed in malic acid and pH = 3.57 [26]. Therefore, we conclude that another factor that influenced these values was the temperature.

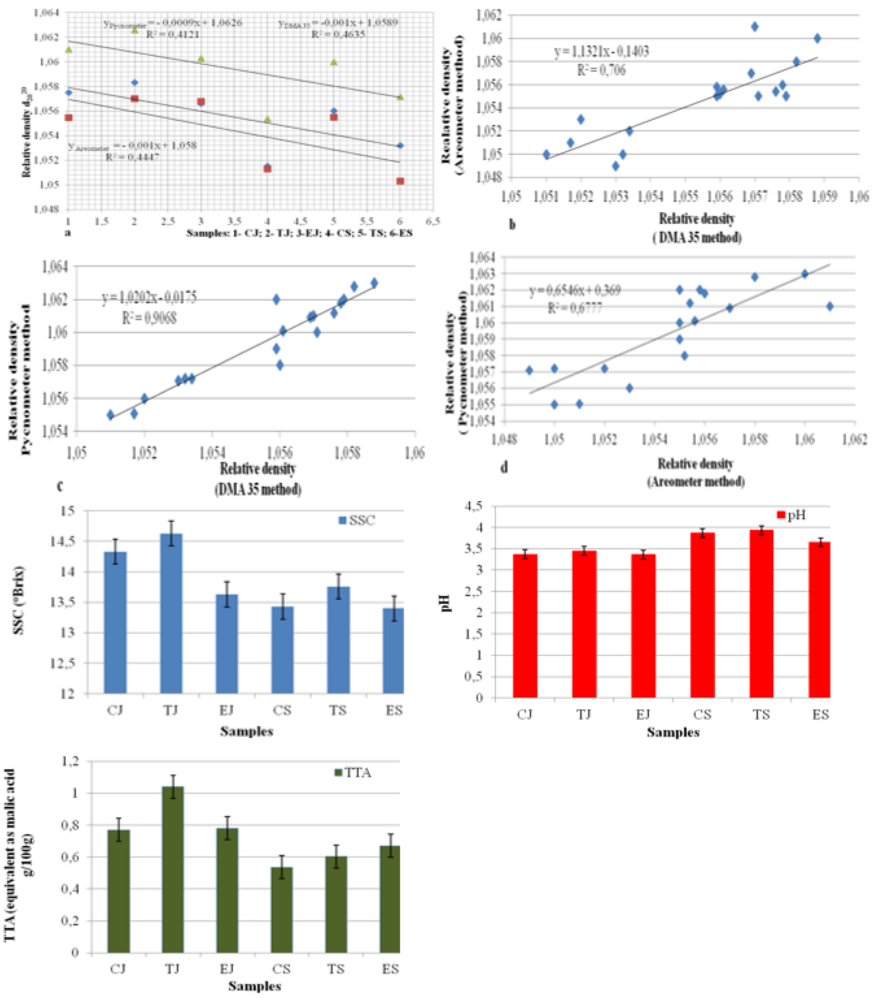

Figure 2. The effect of the working variant on: the variation of the relative density of apple juices (a) and the correlation of the values that were obtained by measurement based on different physical principles (hydrometer–DMA35-b); (Pycnometer–DMA 35-c); (Pycnometer-areometer-d); the variation of the content of SSC, pH and TTA in the cloudy apple juice samples: The student "*t*" test was used and each experiment was observed 3 times (n = 3) as result given us the mean ± standard deviation

3.2.4 Turbidity, viscosity, SSC/TTA and color characteristics

The values of the determined quality indicators: kinematic viscosity, turbidity, SSC/ TTA are shown in table 3.

The high temperature treatment led to increasing the kinematic viscosity and obtaining different values ($p < 0.05$) compared to CJ by 161.4% at TJ. The enzymatic action led to a decrease of the kinematic viscosity compared to CJ by 21% in EJ and by 38.5% in ES. The flow rate of the fluid is hindered by the presence of the solid particles. The pectinases destabilized the plant tissue and, as a result, the kinematic viscosity was much improved both compared to the control sample and to the high temperature sample, lower in the apple juice of the *Jonathan* variety and higher in that of the *Starkrimson* variety.

The turbidity is a measure of juice clarity. The values obtained in this study are significantly different ($p < 0.05$). The apple variety did not greatly influence the obtained values. The temperature led to an increase of the turbidity value by 140.8% (TJ) and 158.29% (TS) and the activity of the pectinolytic enzymes led to a decrease of the turbidity by up to 90% in EJ and 51.99% in ES. This is due to the hydrolysis of the pectin in the cell walls [5]. The student "*t*" test was used and each experiment was observed 3 times ($n = 3$) as result given us the mean ± standard deviation.

In the literature, the turbidity values for the apple juice with apple puree is 3440 NTU and for the apple juice with puree obtained by the action of the pectinases, it is 3660 NTU [31]. In other studies, if immobilized enzymes were used, such as amylases, pectinases and cellulases, they degrade the starch, the pectin and the cellulosic polysaccharides which are found in the composition of the fruits and cause increased turbidity in the fruit juice. This is because the polysaccharide-based macromolecules that cause the turbidity in the fruit juices are broken down [32].

The SSC/TTA ratio values range between 14.06 and 25.05 (table 3). The value of this ratio in the juices preferred by the consumers, regardless of the raw material used, should be around 15, i.e. with balanced taste properties. This study has proven that the high temperature and the action of the enzyme preparation decreased the value of this ratio compared to CJ by 24 % TJ respectively 6% (EJ) and compared to CS by 8.9 % TS respectively 20.5% (ES). This is because the release into the environment of the compounds inside the vacuoles and the cell walls also leads to a change in the ratio of the determined quality indicators.

The values of the parameters that characterize the color can be found in table 3. The L* parameter is the luminance and the closer it gets to 100, the closer it is to the absolute brightness value. In the obtained juices that have been compared, the value of the luminance is different. The lowest value is for the TS sample, namely 29. The highest value was obtained for the CJ sample with a value of 42.9. The increase of the brightness value is a quality of the juice. It is influenced by the method of juice extraction. In the literature, the L* value is 26.9 - control value up to 21.6 for the juice made from sonicated and heat-treated apples (Turkey) and for the juice made from apples treated with orange peel (South Africa) and enzymes, L* = 35.48 – 50.96 [26].

The values for the a* parameter are all positive, affected by the thermal and enzymatic treatment ($p < 0.05$), which means that the juice tends to have a reddish color (brown red-dark brown red). It is observed that compared to the control samples CJ and CS the values are decreasing. Thus, for the sonicated and thermally treated apples from Turkey, the values of a* are from 10.64 to 9.12 decreasing as the temperature increases [13] and a* = - 2.39 to 3.31 in the apples from South Africa treated with enzymes and orange peels [26].

The hue angle (h*), considered the qualitative attribute of color, is the attribute according to which colors were traditionally defined as reddish and greenish, and is used to define the difference of a certain color with reference to the gray color with the same lightness. It has been widely used in the evaluation of color parameters in the analysis of juices obtained by enzymatic clarification and the values are between 76.63 (control sample) to 89-29 (work sample) [26]. In this study the values for h* are not high. They are around 0 (table 3), variable compared to the control sample, tending towards reddish, which means that the reddish characteristic of the studied apple juices increases. ΔE* is very important in this study because significant color differences have been identified (table 3). Values greater than 3 indicate very distinct color differences between samples that can also be assessed from a sensory point of view.

It is observed that the same result was obtained in most cases, except for the comparison between the control samples of the two studied apple varieties for which distinct differences were obtained. Changes in the color of the fruit juice could be attributed to the cavitations caused by the thermosonication at 20 ºC, although the changes can be easily observed with the naked eye. These particles enhance the light scattering effect [33]. These aspects are regulated during the processing of the juice and its preservation and that is why in this study they have higher values because the juice is classified as cloudy. In conclusion, the luminance L* is positive and close to the maximum value (100), which means that there is an intense coloring of the samples perceived by the consumer, oriented towards the reddish shade of the spectrum (brown red). This aspect is influenced by the presence of pigments in the pulp and peel, and the way the sample is prepared for juice extraction. The values of the a* parameter, CIE red (+)/green (-), are positive, therefore tending towards red and decreasing due to the action of temperature and the enzymatic activity of the fungal pectinolytic enzymes. The values of the b* parameter, CIE yellow (+)/blue (-), are positive and therefore oriented towards yellowness, decreasing compared to the control sample. Chroma C* has great, positive values, decreasing compared to the control sample, which means that the color intensity of the sample, perceived by the human eye, is low. The hue angle h* has values around 0 º, which means that, in the studied juices, the reddish character increases. ΔE* is very important in this study because significant color differences were identified due to the suspended particles influencing the light diffusion differently and the influence of the methods of increasing the juice yield.

Table 3. Quality characteristics and CIE color parameters L*, a*, b* measured and C*, h*, ΔE*, calculated for apple juices obtained by different techniques

Apple variety	**Treatment type**	**Kinematic viscosity, (cSt)**	**Turbidity (NTU)**	**SSC/TTA**	
Jonathan	CJ	1.75274 ± 0.001	694.16 ± 0,48	18.6	
	TJ	4.5816 ± 0.0046	1671.6 ± 1,15	14.06	
	EJ	1.38432 ± 0.087	64.81 ± 0,18	17.47	
Starkrimson	CS	2.1347 ± 0.0013	548.5 ± 0,84	25.05	
	TS	*nd	1416.73 ± 1,08	22.8	
	ES	1.311 ± 0.03	263.3 ± 1,02	20	
Chromatic characteristics					
		L*	a*	b*	C*	h*
Jonathan	CJ	42.9 ± 0.15	25.6 ± 0.14	25.6 ± 0.335	36.2	45.0
	TJ	30.5 ± 0.14	27 ± 0.15	25 ± 0.166	37.2	42.8
	EJ	39.9 ± 0.146	25.3 ± 1.34	17.3 ± 0.67	30.7	34.4
Starkrimson	CS	41.3 ± 0.14	24.2 ± 1.346	25.8 ± 0.505	35.4	46.8
	TS	29.0 ± 1.68	21.5 ± 1.013	13.6 ± 1.66	25.4	32.2
	ES	40.6 ± 0.33	17.2 ± 0.715	19.5 ± 0.843	26.0	48.8
Work variants compared	Color difference values and measured parameters and calculated				The significance
	ΔL*	Δa*	Δb*	ΔE*	
CJ-TJ	12.7	-2.6	0.6	12.97	> 3 (very distinct)
CJ-EJ	0.3	8.3	9	8.83	> 3 (very distinct)
TJ-EJ	-9.4	1.4	7.7	12.23	> 3 (very distinct)
CS-TS	19.7	2.7	12.2	23.66	> 3(very distinct)
CS-ES	3.7	7	6.3	10.11	> 3(very distinct)
TS-ES	-11.6	4.3	-5.9	13.7	> 3(very distinct)
CJ-CS	1.96	1.96	0.04	1.98	<1.5; < 3, distinct
TJ-TS	1.1	5.5	11.4	12.74	> 3(very distinct)
EJ-ES	- 0.7	8.1	-2.2	8.42	> 3(very distinct)

* nd- undeterminated

The student "*t*" test was used and each experiment was observed 3 times (n = 3) as result given us the mean ± standard deviation

3.2.5 The sensory analysis

A questionnaire developed according to an analytical method, namely the scoring method was used, having a small number of points (5) focusing on materials and methods. The basis of assessment of the organoleptic characteristics was the standard recommended one. The sensory note, as a whole, has different values, with the highest value for work sample 736 (Table 4) and figure 3. The other values are decreasing compared to this sample which is the control sample for the *Jonathan* apple variety. Thus, the apple variety, the thermal treatment of the pulp and its enzymatic treatment with polygalacturonase and pectin esterase enzyme, led to a decrease in acceptability among consumers. The characteristics that decreased the most were the color and the flavor. It seems that the note of freshness of the plain apple juice prevailed, as well as the apple variety, being a known fact that the *Jonathan* variety is preferred by the consumers. The thermal treatment has affected this note of freshness and the consumer prefers these types of juices less.

Table 4. Mean score and weighted mean score of the values of sensory characteristics of *Jonathan* and *Starkrimson* variety apple juice

Code	Appearance		Color		Taste		Smell		Flavour	
	M_s	M_{sw}	M_s	M_{sw}	M_s	M_{sw}	M_s	M_{sw}	M_s	M_{sw}
736	4.31	1.72	4.0	1.6	3.9	4.68	4.45	5.34	4.14	3.31
763	3.13	1.25	3.18	1.27	3.13	3.75	3.4	4.08	3.22	2.57
637	3.72	1.49	3.77	1.508	3.72	4.46	3.9	4.68	3.59	2.87
183	3.36	1.34	3.77	1.508	3.13	3.75	3.59	4.3	3.54	2.83
187	2.4	0.96	2.86	1.14	2.95	3.54	3.59	4.3	2.86	2.29
873	3.09	1.23	3.09	1.23	2.77	3.32	3.59	4.2	2.86	2.29

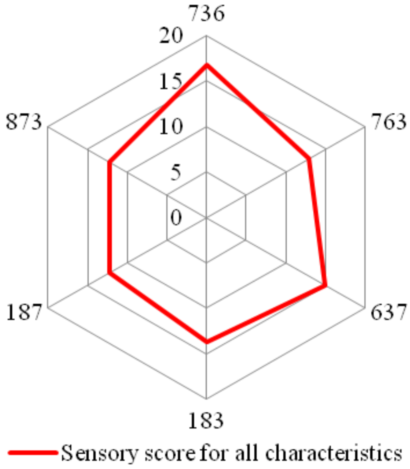

Figure 3. Graphical representation of overall sensory score results for *Jonathan* and *Starkrimson* variety apple juice (736 - CJ apple juice variety *Jonathan*-control; 763-TJ *Jonathan* variety apple juice - with heat-treated; 637-EJ apple juice *Jonathan* variety treated with enzymes; 183-CS apple juice variety *Starkrimson*-control; 187-TS *Starkrimson* variety apple juice with heat-treated; 873-ES apple juice of the *Starkrimson* variety with apples treated with enzymes

4. Conclusions

The comparative assessment of the extraction techniques revealed that the extraction yield was increasing under the action of the temperature and the polygalacturonase and the pectin esterase. The best results were obtained with the work samples where the temperature was set at 60 ^{o}C and enzymes were used on *Jonathan* apples.

It was shown, through the comparative assessment of juices from two varieties of apples, *Jonathan* and *Starkrimson*, that there are differences between the values of the determined and calculated quality indicators. From the point of view of the juice quality, the best results were obtained for the *Jonathan* variety apple juice, following the enzyme addition to the minced pulp using polygalacturonase and pectin esterase.

The permeate/retentate ratio has the highest value for the work sample where enzymes were added to the *Jonathan* variety apples, the soluble solids content is higher for the samples with set restricted temperature, the acidity and the pH are better for the *Jonathan* variety, the kinematic viscosity has the lowest values, therefore the highest flow rate for the samples with enzyme addition. The turbidity was proven to be the best for the work sample with enzyme addition to the *Jonathan* variety apples, as well as the soluble solids content - total acidity ratio. The color characteristics are real in the brown-red range, that is, the range of slightly oxidized juices, but with a color difference > 3, therefore very distinct between the samples. The sensory analysis, however, showed that the consumer's favorite is the cloudy juice, simply extracted from *Jonathan* apples.

Consequently, it is recommended according to this study to use the *Jonathan* apple variety. If it is desired to obtain an organic juice, bearing the BIO characteristic, the recommended technology is to use a temperature of 60 ^{o}C when macerating the pulp before the juice extraction, in order to increase the juice yield. If a high production yield and a juice quality close to the clear version are intended, the enzyme addition technology is recommended.

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