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THE TOTAL CONTENT OF POLYPHENOLS AND THE ANTIOXIDANT PROPERTIES OF SEVERAL BERRY VINEGARS

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Abstract: *Berry fruits represent a valuable source of natural aroma and antioxidants for vinegars. The aim of this research was the evaluation of the total polyphenol content (TPC), total anthocyanin content (TAC) and antioxidant properties (by ABTS and DPPH assay) of several vinegars produced by an acetous fermentation process, using substrates that contain berry (raspberries, blueberries, blackberries) juice (40%; 60%) inoculated with acetous bacteria (0.5%; 1%). Experimental results indicated that blueberry vinegar samples obtained using 0.5% acetous bacteria and 60% berry juices lead to the highest mean values for TPC, TAC and levels of antioxidant activity, but all these variants had significantly the lowest values of acetic acid content.*

Key words: *berries vinegars, antioxidant capacity, polyphenols, anthocyanin.*

1. Introduction

Vinegar is an acidic and special food ingredient (preservative and flavouring). Because of vitamins, various phenols compounds and acids in its composition, vinegar has beneficial health effects (especially appetite stimulation, blood

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pressure regulation, digestive, antimicrobial, antidiabetic and lipid lowering effects) [6], [7], [15-18]. In certain situations, vinegars can reduce food consumption (thus indirectly facilitating weight loss). Some of the compounds present in vinegar also contribute to calcium absorption [13].

Vinegars are obtained by scalar fermentation carried out using several microorganisms acting in different phases. Generally, the first phase is represented by an alcoholic fermentation carried out using yeasts (*Saccharomyces cerevisiae*). After alcoholic fermentation, at the stage of acetic fermentation, acetic acid bacteria (*Acetobacter pasteurianus, A. aceti, A. xylinum and Gluconobacter* spp.) oxidizes ethanol into acetic acid [1], [10], [21], [22].

Vinegar is often a product obtained after the fruit juices fermentation and it contains 4–10% acetic acid. Fruits (in our case the berry) used for vinegar production contain many health-associated substances and valuable antioxidants [4]. Phenolic substances from the fermentable substrates used and the technology applied affect the properties of the vinegar (including the antioxidant and antimicrobial potential) [11]. These bio active compounds could protect molecules from damage caused by the free radicals, reactive species that can cause degenerative diseases, especially cardiovascular diseases [4]. Because of its excellent sensorial properties and nutritional compositions having health-promoting properties (most from them due to antioxidant activities) [14], berries are appealing ingredients for the production of vinegar.

This study was carried out to compare the level of total polyphenols, the total anthocyanin contents and the antioxidant capacity of several vinegar variants obtained using different percentages of raspberry, blueberry and blackberry juices in the substrates and two concentrations of acetic bacteria.

2. Materials and Methods

2.1. Obtaining Berry Vinegars

Berry fruits of three species: blackberries (*Rubus fruticosus* L.), raspberry (*Rubus idaeus* L.), and blueberries (*Vaccinium myrtillus* L.) from the commercial market were used as raw material for the production of berry vinegars. The berries were sorted, the defective ones were set aside and the healthy fruits were crushed mechanically with a beater and the juice was obtained using a hand juicer. In the processing of vinegar, first, the berry juices were mixed with water and apple vinegars using the following percentages of the substrates: 60%juice/20%water/20%apple vinegar (for variants B1, Bc1, R1) and 40%juice/ 40%water/20%apple vinegar (for the other variants), depending on the ID of each sample (Table 1). These substrates were mixed with ethanol (7%) and glucose (4%). Glucose was utilized during the production for obtaining more alcohol, and so more acetic acid. During the alcoholic fermentation, hexose sugars are metabolized (by glycolytic pathway) to pyruvate. After that, this is decarboxylated to acetaldehyde and the acetaldehyde is reduced to ethanol [10]. Commercial yeast (*Saccharomyces cerevisiae*) at a ratio of 0.4% (v/v) (2.12×109 cell/mL) was inoculated to the samples. After that, these substrates having enough alcohol for the acetous fermentation were inoculated with a natural culture of

acetous bacteria (*Acetobacter aceti*) at a ratio of 0.5% and 1% (v/v).

The inoculum was obtained in 250 Erlenmeyer flasks using 10 mL acetic bacteria culture (bacteria isolated from grape vinegar and cultivated in a medium containing 100 g/L glucose and 10 g/L yeast extract) and 50mL apple cider. This mixture was incubated at 30°C for 48 h (orbital shaker, 100 rpm) for adaptation, growth and improvement of the quality of cells.

For the fermentation process, glass jars (0.5 L) were used, containing 225 mL substrates inoculated with a culture of *Acetobacter aceti* prepared as presented above.

The fermentation was stopped when the content of acetic acid was unchanged (for 7 days) and until the ethanol content was between 0.5% and 1%. The acetic fermentation process was completed after 65 days from the inoculation. Sampling was performed at given time points to collect the berry vinegars. These experimental variants were preserved at 4°C in sterile tubs until analysis.

2.2. Acidity, pH and Total Soluble Solids

The pH, acidity (% acetic acid) and total soluble solids values were determined for the substrates (before fermentation) and for the vinegar samples. So, the pH was measured using a pH meter (Consort C5020T, Consort, Belgium), the acidity (% acetic acid) method is to titrate with a solution of 1N NaOH in the presence of phenolphthalein as an indicator and the total soluble solids content (TTS, °Brix) was analysed using a refractometer (OE Germany/ OE Swiss/MASS) at room temperature.

2.3. Total Phenolic Content

Total phenolic content (TPC) of the vinegar variants was determined using the Folin – Ciocalteu method [20]. The samples absorbance was measured at 750 nm using a spectrophotometer DR2800 (Hach Lange, USA). The total phenolic content was expressed as milligrams GAE (gallic acid equivalents) per liter of vinegar.

2.4. Total Anthocyanin Content

The total monomeric anthocyanins content (TAC) was determined using a pH differential method [9]. Two dilutions of the same sample were prepared, the first dilution in buffer potassium chloride (0.025 M, pH 1.0) and the second dilution in buffer sodium acetate (0.4 M, pH 4.5). After incubation at room temperature (15 min), the absorbance values of the two dilutions was read at 510nm and 700nm using a plate reader (Tecan, SunRise™, software Magellan™ Switzerland). The total monomeric anthocyanins content was expressed as mg per liter of vinegar.

2.5. ABTS Assay

The antioxidant potential of vinegar variants in reaction with the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical (ABTS·+) was analysed according to a method described by Re et al. [19]. The vinegar sample (20 µL) was mixed with 980 µL of ABTS+ solutions (at a dilution 1:50 in water). After 15 min reaction time, the absorbance was measured at 734 nm using a spectrophotometer reader (Tecan, SunRise™, software Magellan™). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-

carboxylic acid) standards were used for the calibration curve. Results are expressed in mmol Trolox Equivalents per liter of vinegar (mmol TE/L).

2.6. DPPH Assay

For the DPPH (2,2-diphenyl-1-picryl hydrazyl) assay a method described by Kalita et al. [12] was used. 20 µL samples diluted with 20 µL of water were added in a microplate. After that, 200 µL of 120mg/L DPPH alcoholic solution were added and mixed. After keeping the plates for 30 min in the dark, the absorbance values at 515nm were measured (reader Tecan SunRise™, software Magellan™). A control (20 µL ethanol) was used. The DPPH scavenging activity was calculated with the following formula [12]:

DPPH scavenging activity (%) =
= [(A control – A sample)/ A control]x100

where A is the absorbance at 515 nm.

2.7. Statistical Analysis

Data were performed using the SPSS software and analyzed by the ANOVA and Duncan's multiple range test (results considered significant if $p < 0.05$). The Pearson correlation coefficients were used for the estimation of the correlation between the variables. The analyses were made in triplicate and the results were reported as mean value ± standard deviation.

3. Results and Discussion

3.1. Row Material (Fruits) Physicochemical Parameters

Several physicochemical quality parameters of the berry fruits that were used as raw material for obtaining vinegar are presented in Table 1.

Table 1

Total phenolic content, total anthocyanin content and antioxidant potential (expressed as ABTS and DPPH assay) of the berry fruits (raw material)

Row material (fruits)	Acidity [g citric acid/100g]	TPC [mg GAE/100g]	TAC [mg/100g]	ABTS [mM TE/100g]	DPPH [% inh]
Blueberry	1.32±0.02	642.37±0.24	80.28±0.82	12.53±0.74	72.47±5.94
Blackberry	1.79±0.01	392.46±0.65	58.24±0.15	9.23±0.52	58.84±7.03
Raspberry	1.58±0.02	195.38±0.18	46.84±1.43	6.93±0.16	40.29±3.83

Note: TPC is the total phenolic content; TAC - total monomer anthocyanin contents; GAE - gallic acid equivalent; DPPH - 2,2-diphenyl-1-picrylhydrazyl; ABTS - 2,2'-azino-bis (3-thylbenzothiazoline-6-sulfonic acid); TE - Trolox equivalent.

3.2. Vinegar Variants (pH, Acidity, TTS)

The mean values of pH, titratable acidity and TSS of the vinegars obtained in this study are presented in Table 2. Generally, the acidity values were much lower than those obtained in a previous study in which an acetic acid content of 5.5% was detected in the strawberry vinegar after 80 days of acetous fermentation [10], but similar with the data presented by Boonsupa et al. [5] for raspberry and blackberry vinegars (obtained after 15 fermentation days). The maximum value of acetic acid content (4.94 ± 0.12) and the minimum value of the pH value (2.78 ±

0.01) were obtained for variant R3 (40%raspberry juice/1%*AcB*) (Table 2).

Regardless of the berry fruits used, in the variants with 40% juice in substrates, the values of acidity were significantly higher as compared to the vinegars obtained using 60% juices.

These variants stood out due to the highest difference between the sample acidity before and after fermentation.

The increase of these acidity values was significantly higher compared with those for the variants that used 60% juices (Table 2, Figure 1).

Acidity, pH and total soluble solids of the berry vinegars obtained[1] Table 2

Berry fruits (row material)	% berry juices / % *AcB*	ID samples	pH	Acidity [%acetic acid][2]	Difference between acidity[2]	TSS [^{o}Bx]
Blueberries	60%/0.5%	B1	3.10 ± 0.06 ab	3.56 ± 0.06 d	2,39 c	15.17 ± 0.29 a
	40%/0.5%	B2	2.99 ± 0.02 b	4.28 ± 0.01 c	3,18 b	14.5 ± 0.30 abc
	40%/1.0%	B3	2.97 ± 0.02 b	4.20 ± 0.01 c	3,12 b	13.57 ± 0.10 cd
Blackberries	60%/0.5%	Bc1	3.27 ± 0.12 a	3.90 ± 0.17 cd	2,76 c	14.67 ± 0.15 ab
	40%/0.5%	Bc2	2.90 ± 0.10 bc	4.53 ± 0.06 b	3,40 a	14.57 ± 0.51 ab
	40%/1.0%	Bc3	3.07 ± 0.06 ab	4.44 ± 0.07 b	3,44 a	14.17 ± 0.29 bc
Raspberries	60%/0.5%	R1	3.30 ± 0.01 a	3.83 ± 0.12 cd	1,75 d	13.93 ± 0.12 bc
	40%/0.5%	R2	2.86 ± 0.01 b	4.65 ± 0.12 b	3,10 a	13.73 ± 0.12 cd
	40%/1.0%	R3	2.78 ± 0.01 c	4.95 ± 0.12 a	3,43 b	13.10 ±0 .10 d
MEAN			3.12 ± 0.11	4.28 ± 0.41	-	13.54 ± 2.11

[1] Data were expressed as mean of 3 experiments ± standard deviation. Values with different letters differ significantly by ANOVA and Duncan's test ($p<0.05$);

[2]The values represent the difference between the mean acidity before and after fermentation. Abbreviations: *AcB* - acetous bacteria; TSS - total soluble solids; Bx - Brix.

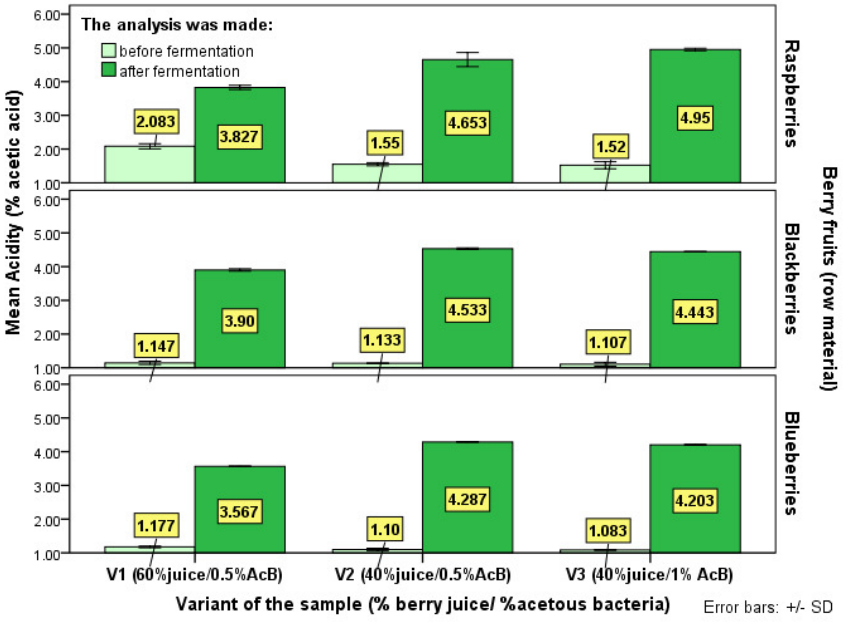

Fig.1. *The acidity (% acetic acid) of the samples before and after fermentation*

As illustrated in Figure 1, all the berry vinegars showed a significant increase in acetic acid by fermentation, because the alcohol from the substrate was converted to acetic acid by acetous bacteria.

3.2. TPC of Vinegar Variants

Table 3 integrates the TPC of vinegar samples. Between the variants, there were significant differences, the variant B1 (60%juice/0.5%AcB) having the highest TPC values (180.83±1.96 mg GAE/L).

The most diminished TPC value was identified in the samples obtained using substrates with 40% blackberry and 40% raspberry juices (inoculated with 0,5% and 1% *AcB*) (48,10 ±5,14 mg GAE/L for variant Bc3, 35,87±5,89 mg GAE/L for variant R3), these samples having the highest values of acidity (content of acetic acid). As shown in Table 3 the composition of the fermentable substrates had a major impact on the TPC, especially upon the level of juice percentages.

Table 3

Total phenolic content, total anthocyanin content and antioxidant potential (expressed as ABTS and DPPH assay) of the berry vinegar

Berry fruits (row material)	% berry juices / % *AcB*	ID samples	TPC [mg GAE/L]	TAC [mg/L]	ABTS [mM TE/L]	DPPH [% inhibition]
Blueberries	60%/0.5%	B1	180.83 ±1.96 a	96.87±3.12 a	1.52±0.07 a	49.90±2.12 a
	40%/0.5%	B2	124.30±15.07 b	47.51±2.10 b	0.93±0.01 c	40.62±2.15 b
	40%/1.0%	B3	90.97 ±5.35 cd	44.24±3.33 bc	0.87±0.10 c	36.4±1.11 bc
Blackberries	60%/0.5%	Bc1	108.90 ±7.69 bc	38.80±3.54 cd	1.09±0.00 b	39.65±2.70 b
	40%/0.5%	Bc2	56.37 ±16.19 de	35.10±2.66 de	0.64±0.07 de	28.13±0.36 d
	40%/1.0%	Bc3	48.10 ±5.14 f	27.89±2.51 ef	0.73±0.05 d	25.26±4.98 ef
Raspberries	60%/0.5%	R1	67.67 ±19.26 e	32.48±9.69 de	0.63±0.04 e	31.35±2.77 e
	40%/0.5%	R2	36.60 ±3.41 f	20.86±5.95 f	0.44±0.02 f	17.81±1.91 h
	40%/1.0%	R3	32.53±4.44 f	15.10±3.57 g	0.49±0.06 f	18.01±2.21 g
MEAN			82.01±53.52	39.87±23.17	0.82±0.33	31.90±6.37

[1] Data were presented as mean of three experiments ± standard deviation. Values with different letters differ significantly by ANOVA and Duncan's test (p<0.05). Abbreviations: *AcB* is the acetous bacteria; TPC - total phenolic content; TAC - total monomer anthocyanin contents; GAE - gallic acid equivalent; DPPH - 2,2-diphenyl-1-picrylhydrazyl; ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TE - Trolox equivalent.

Vinegars obtained from fruit juices conserve just a part of these health-associated compounds, because the commonly used methods during vinegar production affect the content of these bioactive substances. Andlauer et al. [2] reported that the acetic acid fermentation could reduce the TPC of vinegars obtained using traditional methods.

As seen in Figure 2, in our study, the levels of TPC and TAC decreased significantly after fermentation, especially in the most acidic samples.

Despite its broad utilization, the Folin-Ciocalteu method is significantly affected by various obstructing substances such as sugar, ascorbic acid, proteins and non-organic substances that could interact with the Folin-Ciocalteu reagent [3], [20]. It is important to specify that we have used for all variants the similar fermentation process, the only factors that could influence the TPC level were the berry variety, the percentage of berry juices and acetous bacteria of the substrates.

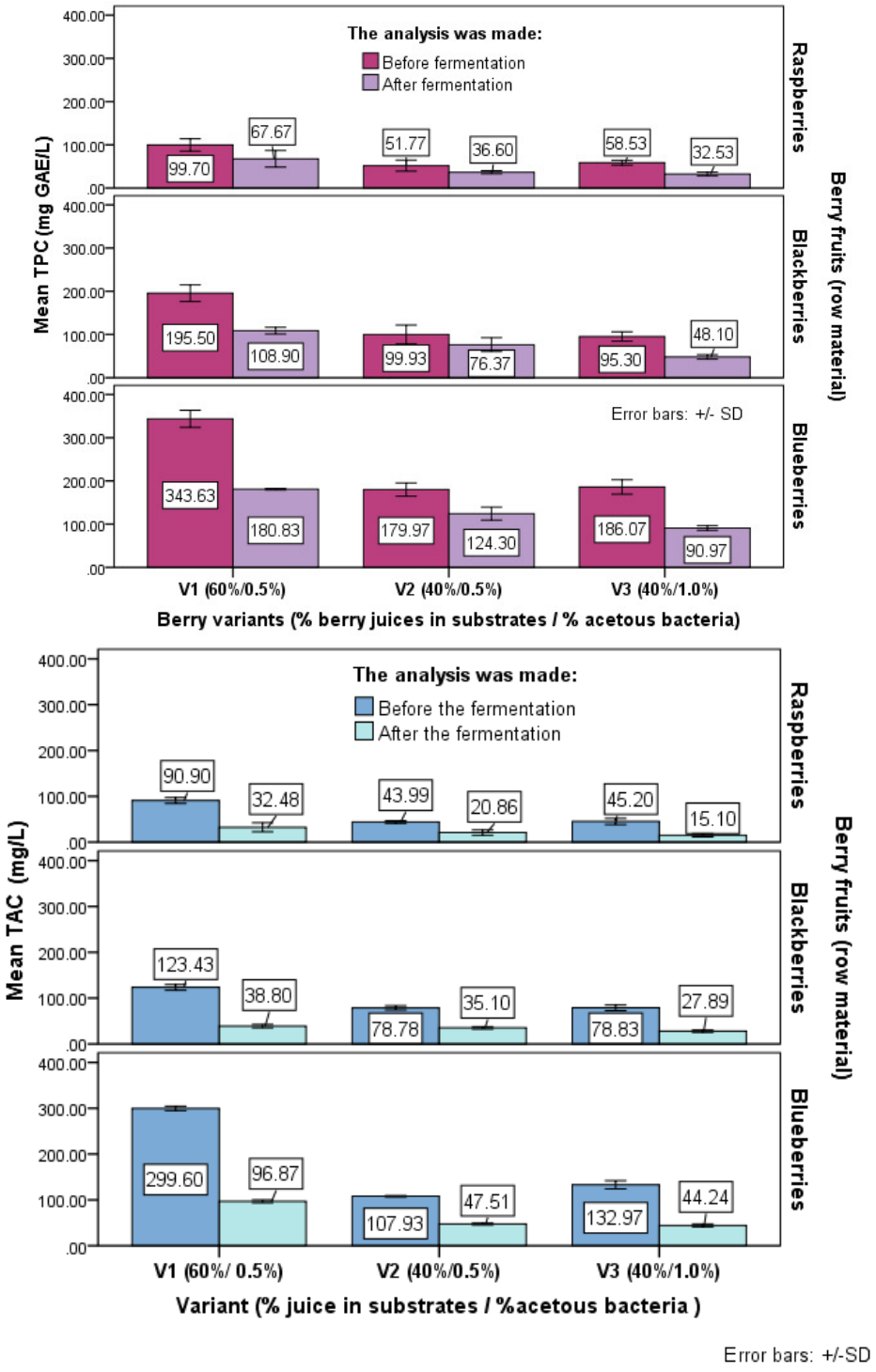

Fig. 2. *TPC and TAC values of the samples before and after fermentation*

Regarding the influence of the berry on vinegar TPC, the use of blueberries led to significantly higher values of this indicator as compared with the other berry fruits used (Table 3). The percentage of berry juices and acetous bacteria had significant influence on this indicator.

3.3. TAC of the Vinegars

The results regarding the TAC of the vinegars obtained in this study are presented in Table 3. The use of berry juice in a percentage of 60% exhibited significantly higher total anthocyanin content values as compared to the other variants for all berry varieties.

The highest value for TAC was obtained for variant B1 and B2 (values from 96.87±3.12 mg/L to 47.51±2.10 mg/L). For all the berry juices used, the samples having high values of acidity (variant V2 and V3) had lower values of TAC. Maybe, a high level of acidity could contribute to a degradation of anthocyanin.

3.4 The Antioxidant Capacity of Vinegar

Regarding the antioxidant capacity (Table 3), significant differences were found between the samples, the vinegars from blueberry juices (60%, 0.5% *AcB*) (B1) having the highest values (1,52±0,07 mM TE/L for the ABTS assay, respectively 49,90±2,12% for the DPPH assay). The lowest values of antioxidant potential were identified for the vinegars obtained from raspberry juices (variant R2), (0,44±0,02 TE/L for the ABTS assay, respectively 17,81±1,91% for DPPH).

Blueberries (*Vaccinium myrtillus* L.) contain high levels of polyphenolic phytochemicals, particularly anthocyanin pigments, which give this berry their characteristic colour.

The antioxidant capacity expressed by the ABTS assay determined in this study is similar to those reported by Arvaniti et al. [3] for commercial and homemade Greek vinegars, by Cruz et al. [8] for red wine vinegars and by Li et al. [15] for apple vinegar, persimmon vinegar and kiwifruit vinegar. As seen in Table 3, the DPPH values increased from 17.81±1.91% to 31.35±2.77% for the vinegars obtained from raspberries, from 26.92±0.53% to 39.65±2.70% for the variants obtained from blackberries and from 36.41±1.11% to 49.90±2.12% for the variants obtained from blueberries.

For a good antioxidant potential of the vinegar it is preferable to use 60% juices in substrates and 0.5% acetous bacteria inoculums.

The values of the antioxidant capacity of the samples obtained with the DPPH assay were higher than those determined with the ABTS assay in each of the analyzed variants. The differences in the antioxidant potential could be influenced by the differences between the reaction mechanism involved [8]. In this study, the ABTS values obtained to express the antioxidant potential of the samples were correlated with the TPC and TAC values (Table 4). In all variants, the best correlations were found in the case of TPC values, as compared to TAC values. The vinegar samples could contain several phenolic non antioxidant compounds, provided because of the raw material (Table 1). Maybe this is the reason of the strong correlation between TPC and the antioxidant capacity.

In this study, as can be seen in Table 4, a positive correlation was observed between the antioxidant potential and the total phenol content (0.934** for ABTS,

0.796** for DPPH). A very good correlation was found between TPC and TAC values (Table 4); this aspect indicates that anthocyanin were not the only phenolic compounds in berry vinegars. Arvaniti et al. [3] reported a similar positive correlation between the antioxidant capacity and the TPC values of apple vinegars with those indentified in our study for berry vinegars.

The results reveal that the antioxidant capacity of the vinegar variants was strongly influenced by their total phenol content, followed by the anthocyanin content and by the acidity (% acetic acid) (Table 4). A high reduction in the total phenolic compounds content was noted in the vinegars produced from all the berry juices, which may have been due to the oxidation of phenolics during the fermentation process. A reduction in the anthocyanin content was also found in all the vinegars produced, in comparison with the raw material.

Several scientific studies in the literature described the antioxidant potential of many fruits, but there are only a few reports regarding the antioxidant capacity of fruit vinegars. The results of the antioxidant activity assays (Table 1 and 3) showed that the blueberry fruit and the vinegars produced from them (variant B1), presented the highest antioxidant capacity compared with the other variants obtained in our study. The blueberry vinegars variant B1 showed significant radical scavenging ability against ABTS•+ (1.52±0.07 mmol TE/L) and DPPH inhibition (49.9±2.12%).

Table 4

The Pearson correlation coefficients - the interaction between TPC, TAC and the antioxidant properties of the vinegar samples (by ABTS and DPPH assay)

	TPC [mg GAE/ L]	TAC [mg QE/L]	ABTS [mM TE/L]	DPPH [% inhibition]	Acidity [%acetic acid]
TPC [mg GAE/L]	1	0.930**	0.934**	0.796**	-0.784**
TAC [mg /L]		1	0.911**	0.781**	-0.766**
ABTS [mM TE/L]			1	0.836**	-0.809**
DPPH [% inhibition]				1	-0.692**
Acidity [% acetic acid]					1

** Correlation significant at the 0.01 level. Abbreviations: TPC is the total phenolic content; TAC - total monomer anthocyanin contents; DPPH - 2,2-diphenyl-1-picrylhydrazyl; ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); GAE - gallic acid equivalent; TE - Trolox equivalent.

As presented by other researchers, our study highlights that berry vinegars (obtained using a low level of acetous bacteria) could be rich in bioactive compounds, especially antioxidants. Moreover, a study of the antioxidant potential and TPC of berry vinegars should also present the structure of antioxidants, their contribution, raw material, technology and aging process. This is the reason why we intend to continue this research work and to present in a more complex article these important aspects regarding the aromatic berry vinegars (products with human health benefits on account of their being a source of antioxidants).

4. Conclusions

This study presents the results regarding the total content of phenolic and anthocyanin compounds in correlation with the antioxidant capacity of several vinegars obtained from blueberry, blackberry and raspberry juices used in different percentages.

The ABTS and DPPH antioxidant activity is due to the phenolic and anthocyanin (detected in significant levels in the vinegar samples obtained from substrates having 60% berry juice and inoculated with 0.5% acetous bacteria). The highest levels for TPC and TAC were identified in vinegars from blueberry juices obtained from the substrates with the percentages presented above.

Blueberry and blackberry vinegars obtained in this study represent a good source of phenolic and anthocyanin and could contribute to the daily intake of antioxidants.

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