ICSD
INTERNATIONAL CONFERENCE ON SUSTAINABLE DEVELOPMENT
12 - 15 November 2015, Belgrade
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Antimicrobial and Antioxidative Activity of Commercial versus Traditional Apple Vinegar

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Abstract

Four samples of commercially produced apple vinegars, by different producers, and one sample obtained traditionally or homemade were analyzed. Their dry mass, total acids, pH value as well as antioxidant DPPH activity and antimicrobial capacity against Salmonella typhimurium, Escherichia coli, Staphylococcus aureus and Candida albicans were determined and compared. In commercially obtained vinegars dry mass varied from 2 to 3.1%, but in traditional was about 8.2%. Content of total acids in commercially products was in the range of 1.5% to 4.6% and about 18.6% in traditionally obtained vinegar. pH varying in the range of 2.7 – 3.5 in commercially and 2.8 in homemade vinegar. One of the commercial sample was with higher DPPH antioxidant activity than homemade vinegar, while two samples were with very weak activity. Traditionally obtained vinegar was the most effective to Salmonella typhimurium, but the coommercially vinegars to Candida albicans.

Keywords: Antimicrobial, Antiradical Activity, Apple Vinegar, Commercial, Traditional.

1. INTRODUCTION

The word vinegar comes from the French "vinaigre", that means sour wine (vin=wine, aigre=sour) [1]. The definition of vinegar itself differs from country to country. The available definition from the Codex Alimentarius [2] indicates that vinegar is "a liquid, fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars, or starch and sugars, by the process of double fermentation, first alcoholic and then acetous". Vinegar was first made from wine, as its name indicates, however, today it is a product of mash prepared from cereals or a wide varieties of fruits. It is a clear aqueous liquid, which may be colorless, with the color of its raw material, or additionally colored, with pH value of about 2.0-3.5, and enough level of acetic acid [3]. Vinegar has long been used worldwide as an additive in a wide variety of foods which it preserves due to the properties of acetic acid. However, recent research has shown that, in addition to its well-known anti-bacterial activity, vinegar when consumed as a drink, confers considerable health benefits and provide refreshment after exercise [4]. Therapeutic effects of vinegar arising from consuming the inherent bioactive components including acetic acid and phenolic compounds like gallic acid, catechin, ephicatechin, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid cause antioxidative, antidiabetic, antimicrobial, antitumor, antiobesity, antihypertensive, and cholesterol-lowering responses [5]. In the last decades there is a growing demand for fruit vinegars, which are sold as a health food [6]. Vinegar can be produced by different methods ranges from traditional employing wood casks and surface culture to submerged fermentation in acetators, and from various raw materials (apple, grape, orange, peach, pear, pineapple, apricot and banana, honey, rice, malted barley, etc.) [7]. Traditional methods include slow processing of raw material, while industrial quick processing. Traditionally obtained vinegar became as much as popular among the consumer, because of their green technology. Because vinegar is generally an inexpensive product, its production requires low-cost raw materials, such as sub-standard fruit and seasonal agricultural surpluses [8]. Some knowledge about the nutritional and health benefits as well as organoleptic

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properties of apple fruit made apple vinegar very popular among the consumers, worldwide and especially in the region of West Balkan [9]. However, usually stay dilemma, what product is better traditional or commercial vinegar? Aiming to get more relevant information in this study we compare two most important properties, antimicrobial and antioxidant activity of some commercial (industrial) and one sample of traditionally obtained vinegar.

2. MATERIALS AND METHODS

Four samples of apple vinegars (COM1, COM2, COM3 and COM4) commercially produced by different producers, purchased from local markets, and one sample of traditionally/ homemade vinegar (TRD) were analyzed. The procedure of traditionally obtaining of vinegar is shematic given on the Figure 1. Apples (*Malus domestica*), characteristic product of Maleshevia region of R.Macedonia (1.4 kg) were used.

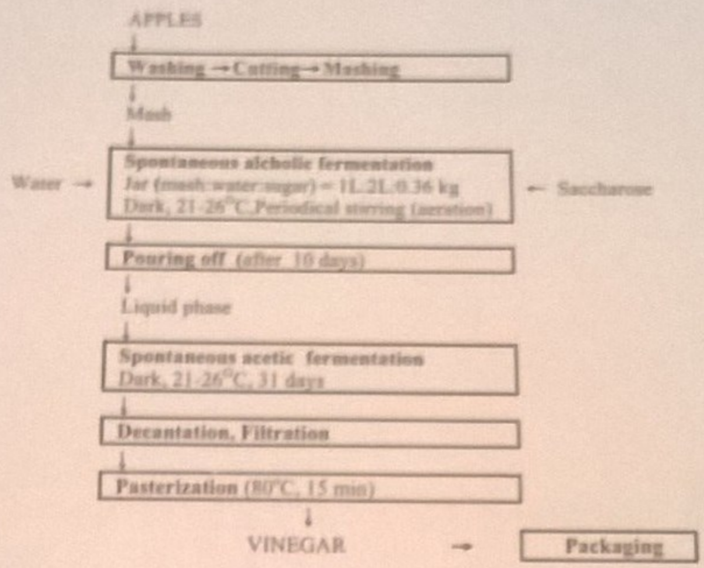

Figure 1. Schematic presentation of apple vinegar obtaining by traditional procedure

The chemical analyses were performed using chemicals with p.a. quality, purchased from Alkaloid Ltd., Skopje, Republic of Macedonia, and Merck KGaA, Darmstadt, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was product from Sigma Aldrich (Germany). Content of soluble solids was measured with refractometer. Total acidity was evaluated by titration with standardized solution of 0.1N sodium hydroxide, using phenolphthalein as indicator and the results were expressed as acetic acid content. The pH values of all vinegars were determined through a pH-meter Metrohm-632 previously calibrated with buffers at pH 4 and 7. To determine the antioxidant activity of the vinegars the DPPH radical scavenging capacity assay described in [10] was used. Here, 5 μL of sample was added to 995 μL of DPPH solution (0.035 g/L in ethanol). The absorbance of control sample and the mixtures was measured at 517 nm, after 30 minutes, using a cuvette filled with ethanol as a blank. A Varian Cary 50 (Netherlands) UV/Vis spectrophotometer with SPS3 autosampler was used. Calibration curve was constructed with six different concentrations of vitamin C (0.2, 0.4, 0.8, 1, 2 and 3 mg/L) used in the same sample conditions. The percentage inhibition of DPPH radical by the samples was calculated according to the formula [11]:

$$\% \text{ inhibition of DPPH} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \cdot 100 \quad (1)$$

The final antioxidant capacity (AOX) was expressed as mg vitamin C equivalents /ml of vinegar sample. All the determinations were realized at least in triplicate.

The gram positive bacterial culture *Staphylococcus aureus* (ATCC 49444), and gram negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) and the fungal yeast *Candida albicans* were used in assaying antimicrobial activity of the vinegars using agar diffusion on solid media (Muelleller-Hinton). Muelleller-Hinton Agar (Oxford, UK) was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate in 12x12 cm Petri dishes. The inoculums were spread on to agar plates using sterile swabs. The solid agar was punched and 5 ml of the vinegar sample were applicated on the agar walls. The plates were then incubated at 37°C for 24h. After incubation, zone of growth inhibition for each sample of vinegar was measured.

3. RESULTS AND DISCUSSION

3.1. Chemical characteristics of the vinegars

Comparing the main characteristics of commercial apple vinegars with those produced traditionally (Table 1) an obvious differences were obseved in all of analyzed parameters. Dry matter ranged from 2.0 to 3.1 in commercial vinegars, but 8.2% were determined in traditionally obtained vinegar. These contents are with ageement with Codex Allimentarius Commission [2] recommendation, not to be less than 2.0 g/L. High content of solids (7.87 to 8.70%) were obtained in vinegar from sweet orange peels [12].

Table 1. Proximate chemical composition of vinegars

Vinegar	Dry matter (%)	Total acids (% acetic acid)	pH
COM1	3.1	4.2	3.05
COM2	2.1	2.7	3.30
COM3	2.2	4.3	2.82
COM4	2.0	1.4	3.35
TRD	8.2	18.6	2.78

Soluble solids contribute to vinegar quality. They consist of sugars, disolved acids, amino acids, vitamins, salts or minerals and phenolic compounds. Total acids varied from 1.4% in commercial product COM4 to 4.3% in the vinegar COM3. This values were lower than those proposed by Codex Allimentarius Commission [2], not less than 50 g/liter (calculated as acetic acid). None of the commercial vinegars reached an acid level of 5%, and could thus not strictly be called vinegars [9]. Brazilian legislation demands minimum acidity of 4% [1], but the sample COM4 was with much lower value. This sample was also with the least content of dry matters, indicating on its dilution, and conscious lowering of its quality. However, TRD vinegar was with exceptionally high level (18.6%) of acids, which was very close to that verified in [12]. The pH value which strongly depend of organic acids such as acetic acid, malic acid or lactic acid and also of the presence of many other substances and contribute to vinegar flavor ranged from 2.78 (in TRD vinegar) to 3.35 measured in vinegar COM4 which was characterized with the lowest level of acids and solids.

3.2. Antioxidant capacity of the vinegars

Knowing that apple vinegar is very often used in health protection by contemporary man who is aware about the relation between nutrition and health it is very important to know something more about its antioxidant capacity. As shown on Figure 2, there were very obvious differences in DPPH scavenging ability among the investigated vinegars.

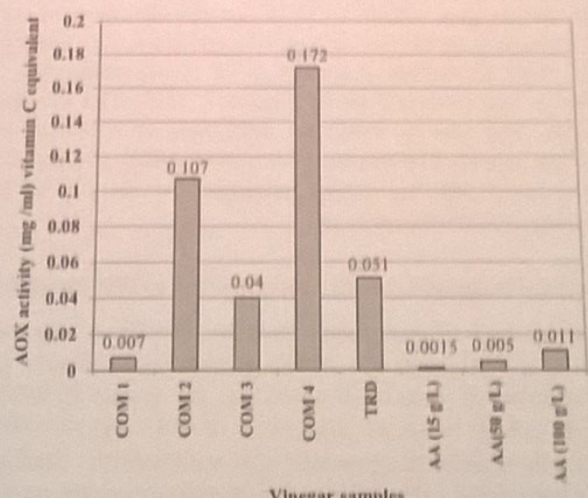

Figure 2. Antioxidant capacity (AOX) of vinegars determined as DPPH reduction ability

All of investigated vinegar samples were with higher antioxidant potential of acetic acid with concentration of 15-100 mg/L. Antioxidant capacity expressed as mg vitamin C/ml of vinegar varied from 0.007 for commercial vinegar COM1 to 0.172 mg vitamin C/ml vinegar COM4. Traditionally obtained vinegar has shown capacity equvalent to 0.051 mg vitamin C/ml, which was higher than for vinegars COM1 and COM3, but half of the capacity of vinegar COM2 and more than three time less than for vinegar COM4. These results sugested that all of commercial vinegars were obtained from different kinds of raw material (apples) and also different procedures contributing to different contents of polyphenolic compounds responsible for antioxidant activity, also observed in [13].

3.3. Antimicrobial activity of the vinegars

According to the ranking of antimicrobial activity on the base of dimensions of inhibition zone (zone o <10 mm indicates on resistant strain, zone between 10 and 15 mm indicates on low antimicrobial activity, zone of 15-20 mm on moderate activity and zone >20 mm on high antimicrobial activity) we could conclude that all of investigated vinegars differ in their antimicrobial activity towards distinct strain.
Our results revealed that *Staphylococcus aureus* and *Escherichia coli* were less sensitive towards the vinegars than gram negative bacteria *Salmonella typhimurium* and fungal yeast *Candida albicans* (Figure 3).

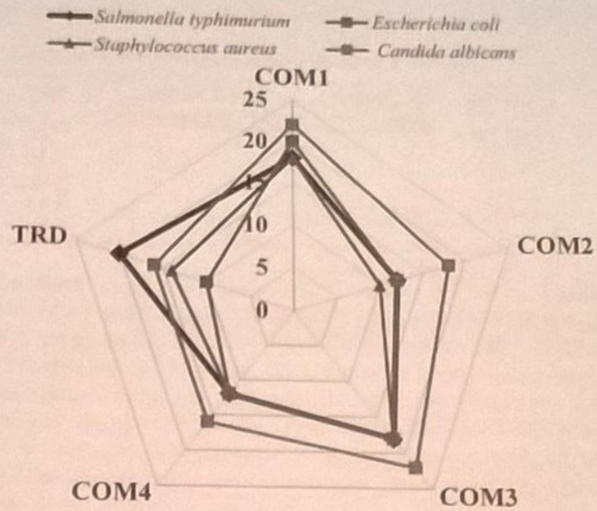

Figure 3. Antimicrobial activities of vinegars against some microorganisms given as zone of inhibition (mm)

This confirmed the facts that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells we expected [14]. Amongst the tested bacteria gram-negative *Salmonella typhimurium* was the most sensitive to traditionally (TRD) obtained vinegar, but much less to all commercial vinegars. The most resistant strain towards TRD vinegar was *Escherichia coli*. *Candida albicans* was the most sensitive strain to the action of all commercial vinegars, except to TRD vinegar. However, if we ranged sensitivity of tested microorganisms to TRD vinegar it would be: *Salmonella typhimurium> Candida albicans> Staphylococcus aureus> Escherichia coli*. High antimicrobial activity to all tested strains had shown vinegar COM1. Very similar antimicrobial activity was observed about vinegar COM3. The vinegar COM4 which exibited the highest antioxidant activity, had shown moderate inhibition of *Candida albicans*, but was with low antimicrobial activity to other investigated strains. Low antimicrobial activity of vinegar COM2 was observed to *Staphylococcus aureus*, but a litle bit higher, althoght low activity to *Salmonella typhimurium* and *Escherichia coli*, and moderate to *Candida albicans*.

3.4. Correlation between investigated quality parameters of the vinegars

Between all of investigated vinegar characteristics Pearson correlation was calculated and presented in the Table 2. Statistically significant correlation was established between chemical quality charactersticts, with the highest positive correlation between content of acids and dry matter (0.9899), and negative between dry matters and content of acids and pH value of vinegars (r=-0.6333 and r=-0.7073, respectively). Antioxidant capacity of vinegars statistically significant correlated only with their pH value (r=0.7542). The content of dry matter and acids positively correlated with vinegar antimicrobial activity to *Salmonella typhimurium*, while pH correlated negatively (r=-0.9482). The last mentioned correlation is reasonable, because acetic acids which possess antimicrobial potential correlated with pH value. The fact that antioxidant activity of vinegars negatively correlated (r=-0.8419) with antimicrobial activity to all of investigated microorganisms suggested that besides acids other substances like phenolics are responsible both for antimicrobial and antioxidant activity. The kind and quantity of each phenolic substance present in vinegar manifested different level of respectively activity.

Table 2. Pearson correlations between vinegars quality parameters

Parameters	**Acids (%)**	**pH**	**AOX***	**Antimicrobial activity- d (mm)**			
				Salmonella typhimurium	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
Dry matter (%)	**0.9899**	**-0.6333**	-0.3345	**0.6801**	-0.4486	0.0464	-0.4126
Acids (%)	1	**-0.7073**	-0.3695	**0.7147**	-0.4332	0.0789	-0.3639
pH		1	**0.7542**	**-0.9482**	-0.2100	**-0.6735**	-0.3190
AOX*(mg vit C/ml)			1	**-0.8419**	**-0.6313**	**-0.7909**	**-0.7105**
Antimicrobial activity - d (mm)							
Salmonella typhimurium				1	0.3082	**0.747**	0.3525
Escherichia coli					1	**0.8251**	**0.9581**
Staphylococcus aureus						1	**0.7925**
Candida albicans							1

*AOX- Antioxidant activity

4. CONCLUSIONS

Apple vinegar is widely used by the population of West Balkan region for its therapeutic benefits and each vinegar is with particular quality characteristics. The results of this research have shown that chemical properties, antimicrobial and antioxidant activities of commercial apple vinegars and obtained traditionally vinegar obviously differed depending on the apple variety and the technique used in its production.

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