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RESUMO

A presença de ácidos orgânicos de cadeia curta, glicerol e etanol em nove amostras de vinagres comercializados no Brasil foi investigada. Cromatografia líquida por exclusão iônica foi empregada nesse estudo, permitindo a simultânea identificação dos ácidos cítrico, tartárico, málico, malônico, succínico, láctico, acético e propiônico. Estes compostos foram quantificados empregando um detector Ultravioleta (UV), enquanto glicerol e etanol foram quantificados por um detector de índice de refração (IR), baseado nas áreas e alturas dos picos. Em todas as amostras de vinagre o ácido acético foi o composto mais abundante (40-56 g/L) seguido do ácido láctico (0,2-2,2 g/L). O ácido tartárico foi detectado em todos os vinagres de vinho. Todas as amostras apresentaram concentração de etanol abaixo de 1°G.L.. O método empregado proporcionou fácil e rápida operação, sem requerer nenhuma preparação complicada da amostra. Determinação da acidez total foi também realizada nos vinagres por titulação, mostrando valor mínimo de 4 % (m/v).

ABSTRACT

The presence of short-chain organic acids, glycerol and ethanol in nine samples of marketed vinegars in Brazil was investigated. The ion-exclusion liquid chromatography was employed in this study allowing the simultaneous identification of citric, tartaric, malic, malonic, succinic, lactic, acetic and propionic acids. These compounds were quantified using an Ultraviolet (UV) detector, while glycerol and ethanol were quantified employing a refraction index (RI) detector, based on peaks area and height. In all the vinegars samples acetic acid was the most abundant component (40-56 g/L) followed by lactic acid (0.2-2.2 g/L). Tartaric acid was detected in all the wine vinegars. All the samples presented ethanol concentration below 1°G.L.. The employed method proportioned easy and rapid operation, without requiring any complicated preparation of the sample. Determination of the total acidity was also accomplished in the vinegars by titration, showing minimum value of 4% (w/v).

PALAVRAS-CHAVE
KEY WORDS

ácidos orgânicos, etanol, vinagre, cromatografia
líquida / organic acids, ethanol, vinegar,
liquid chromatography

1. INTRODUCTION

The vinegar comes from the french word *vinaigre*, that means sour wine. Originally it was obtained from the spontaneous fermentation of beers and wines. It is a fermented drink obtained by two different processes, consisting of the acetic fermentation of diluted alcoholic solutions, which comes from alcoholic fermentation of sugary or amylaceous musts. The alcoholic fermentation is an anaerobic process accomplished by yeasts, while the acetic fermentation is an aerobic process promoted by bacterias. In the first fermentative process, the culture should be pure (usually *Saccharomyces cerevisiae*), while in the acetification the employment of a mixed culture of *Acetobacter* species is considered more efficient. Bacterias of the kind *Gluconobacter* are also used, but they are less productive due to the oxidation of the formed acetic acid (Tefsaye et al., 2002; Zancanaro, 2001).

After fermentation stage, the vinegar is clarified by filtration or decantation, pasteurized and, depending on the characteristics wanted to the product, aged for improvement of its organoleptics properties (Tefsaye et al., 2002; Zancanaro, 2001). In agreement with the effective legislation in Brazil, the produced vinegar should be diluted at least to 4% (% w/v) in acetic acid and it should not surpass 1°G.L. (% v/v) in ethanol. To determine its origin, and to prevent some adulteration, it is common to analyze the concentrations of tartaric acid in vinegars of grape wines, of malic acid in apple vinegars and amino acids and phenolic compounds in vinegars of fruits and cereals (Zancanaro, 2001).

Some organic acids found in vinegars can be originally from the grape or produced in the alcoholic, acetic or malolactic fermentations. The total acidity is expressed in acetic acid, the major organic acid in vinegars. Tartaric acid is major acid in wines and vinegars of grape wines, because it is original from the own fruit. Its concentration can arrive up to 3 g/L (Morales et al., 1998). The malic acid is more available in apple vinegars and it can be converted into lactic acid during malolactic fermentation, accomplished after alcoholic fermentation (Silva and Muratore, 2003). The lactic acid can be found in concentrations of up to 9 g/L (Yang and Choong, 2001), but its contents can be reduced by the action of the acetic bacterias. The citric acid can be also formed during the fermentative processes (Horiuchi et al., 1999).

The ion-exclusion chromatography technique has been very employed for determination of alcohols and organic acids present in vinegars (Morales et al., 1998; 2001; Cocchi et al., 2002) and in other biological samples (Molnár-Perl, 1999; Aguiar et al., 2002; 2005). It is an appropriate technique for separation of basic, neutral or slightly acid substances from ionic compounds, based on an ion-exclusion mechanism rather than ion-exchange. For the identification of organic acids, this technique makes the separation in agreement with the differences in their pKa values, using a strongly acid ionic exchange resin as stationary phase. Weak acids behave as nonelectrolytes at low pH, and consequently they are strongly retained by the resin and stronger acids are firstly eluted. Thus, the pH of the mobile phase is a factor of great relevance for the separation of the compounds (Morales et al., 1998).

In this work the contents of different short-chain organic acids and alcohols were analyzed in samples of

commercial vinegars from different raw materials. Titration method was used for determination of the total acidity and the specific analysis of organic acids and alcohols was done by high performance liquid chromatography (HPLC).

2. MATERIALS AND METHODS

For determination of organic acids in vinegars, nine commercially available samples were chosen, being two originated from red wine and three from white wine, two from apple vinegar, one sample from alcohol vinegar and one from rice vinegar. Table 1 shows the description of the studied vinegars.

Table 1 Samples of vinegars analyzed for the determination of total acidity, pH and alcohols and organic acids.

Vinegars	Description of label
V1	White Wine and Alcohol Vinegar A
V2	Red Wine and Alcohol Vinegar A
V3	White Wine (10%) and Alcohol (90%) Vinegar B
V4	White Wine and Alcohol Vinegar C
V5	Apple Vinegar C
V6	Apple Vinegar D
V7	Red Wine and Alcohol Vinegar C
V8	Rice Vinegar E
V9	Alcohol Vinegar C

2.1 Determination of total acidity and pH

Total acidity was evaluated by titration with standardized solution of 0.1 N sodium hydroxide, using phenolphthalein as indicator and the results were expressed as acetic acid content. The pH values of all the vinegars were determined through a pH-meter Metrohm-632 previously calibrated with buffers at pH 4 and 7.

2.2 Determination and quantification of alcohols and organic acids by HPLC

In order to determine the organic acids, a liquid chromatography Shimadzu model CTO-6A was used, with the aid of an Ultraviolet detector (UV) Shimadzu, model SPD-10AV, operated at 210 nm, and of a refraction index detector (RI) Shimadzu model RID-10A for determination of alcohols. The samples were diluted (five-fold) in triplicate, filtered in membranes Millipore 0.45 mm and injected into a column of ionic exchange Aminex HPX-87H (300 x 7.8mm, Bio-Rad Laboratories Ltda) coupled to a pre-column of cationic exchange (Bio-Rad Laboratories Ltda). Mobile phase was a 0.005 mol.L⁻¹ H₂SO₄ solution (pH 2.1), with flow-rate of 0.6

mL/min at 45°C. 20 mL of each sample were put into a injection loop Rheodyne with automatic system.

Solutions of ethanol, glycerol and malic, malonic, lactic, tartaric, succinic, acetic, citric acids were prepared using reagents of analytic degree for obtainment of the calibration curves.

3. RESULTS AND DISCUSSION

3.1 Determination of the total acidity and pH of the vinegars

Through the analyses of total acidity by titration, it can be verified that all the samples presented minimum acidity of 4%, value that is demanded by the brazilian legislation and confirmed in the labels of each vinegar.

Table 2 Values of total acidity and pH of the vinegar samples.

Vinegars	pH	Total acidity (% w/v)
V1	2.62	4.62 ± 0.03
V2	2.69	4.52 ± 0.05
V3	2.74	4.43 ± 0.05
V4	2.73	4.25 ± 0.03
V5	3.19	4.23 ± 0.05
V6	3.28	4.27 ± 0.05
V7	2.71	4.30 ± 0.06
V8	2.91	4.67 ± 0.05
V9	2.69	4.32 ± 0.05

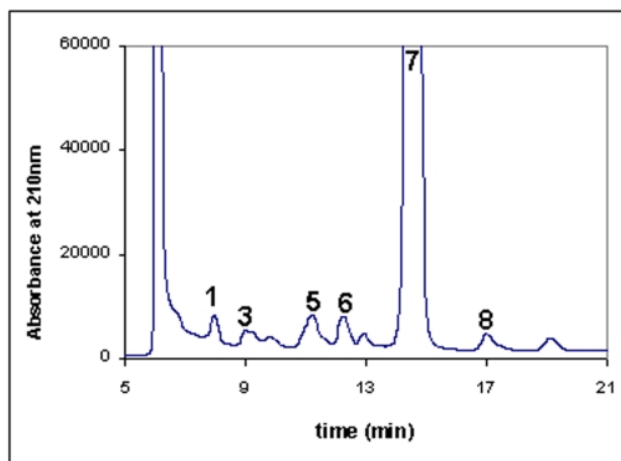
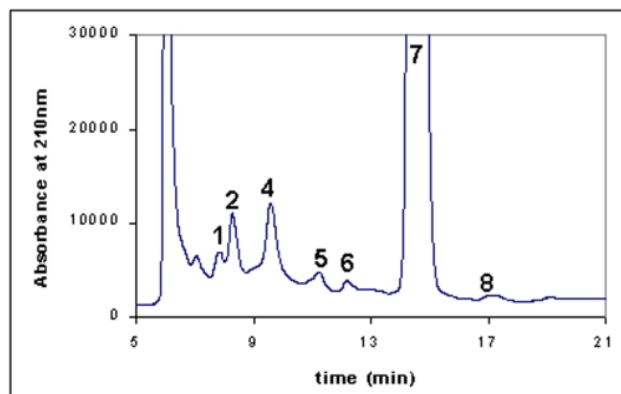
Comparing the characteristics of commercial apple vinegars with those produced experimentally in biorreator airlift and by the classic process, Pedroso et al. (2003) verified that all the samples presented minimum acidity of 4%. The pH of the samples presented values around 3.2, which is similar to the two apple vinegars showed in the Table 2. The higher pH value it can be attributed to the presence of vitamins, amino acids, organic acids and proteins in fruits vinegars. Except for the apple vinegars, the other samples presented lower pH values (< 2.9).

3.2 Determination and quantification of alcohols and organic acids by HPLC

The determination of acetic acid and other compounds present in the vinegar samples by HPLC was made by comparison between the retention times of reagents of analytic degree. For the quantification of the identified compounds, the areas and heights of each peak were analyzed. All the calibration curves presented correlation coefficient higher than 0.9994.

With the aid of the UV detector, eight short-chain organic acids were determined, such as citric (7.8 min), tartaric

Figures 1 and 2 Chromatograms of the red wine and alcohol vinegar V2 and apple vinegar V6: 1 - citric acid; 2 - tartaric acid; 3 malic acid; 4 - malonic acid; 5 - succinic acid; 6 - lactic acid; 7 - acetic acid; 8 - propionic acid.



(8.2 min), malic (9.2 min), malonic (9.5 min), succinic (11.2 min), lactic (12.2 min), acetic (14.5 min) and propionic (17.0 min) acids. The values in parentheses correspond to the retention times of each compound. The oxalic, formic, fumaric, levulinic and butyric acids were not found. In the Figure 1 and 2 the chromatograms of the V2 and V6 vinegars are presented, respectively.

In the two chromatograms a peak can be observed with retention time of 6.0 min, which was not identified, inclusive in the other samples. Morales et al. (1998) also observed a similar peak during analyses of organic acids in several samples of vinegars, attributing it to the caramel presence. Using the same column and the same detector, these authors identified the citric, tartaric, malic, lactic and acetic acids in different samples of vinegars.

The Table 3 presents the concentrations of the organic acids based on the areas of the chromatographic peaks, and the Table 4 the concentrations based on the heights. In all the vinegar samples the acetic acid was the predominant compound. Higher concentrations of this compound were observed analyzing the height of the chromatographic peaks. The lactic acid was the second acid present in larger quantity.

Yang and Choong (2001) identified the acetic, isovaleric, lauric, capric, lactic and levulinic acids in some samples of vinegars and other drinks. The acetic and lactic acids were also the most abundant.

The citric, lactic and acetic acids were identified in all the samples. The succinic acid was not found only in the alcohol vinegar. In the rice vinegar only the tartaric and malic acids were not detected.

The malic acid was found in the apple vinegar V6, but not in the sample V5. The lactic acid concentration of this sample was quite superior compared to the apple vinegar V6, demonstrating that during the fermentative process practically the whole malic acid of the vinegar V5 must have suffered malolactic fermentation.

The propionic acid was not found only in the white wine and alcohol vinegar V1. In all the samples of wine vinegar the presence of tartaric acid was observed, mainly in the V1 and V2 samples, indicating that the presence of this compound

serves to discriminate the origin of the vinegar (Zancanaro, 2001). Higher concentrations of malonic acid were also observed in the samples of vinegars V1 and V2.

The concentrations based on areas and heights of the chromatographic peaks were similar for the analyzed acids, except for the acetic acid. The concentration values of this acid based on the area of the peaks were very similar to the values of total acidity, calculated by titrimetry, showing that the quantification based on the area is more accurate for these compound.

Using the RI detector, it was possible to identify the ethanol and the glycerol (Table 5). All the analyzed samples presented ethanol concentration below 1°G.L.. Higher ethanol content was observed in the samples V1 and V2.

For the glycerol, higher concentrations were observed in the apple vinegar V6 and rice vinegar V8, based on the areas of the chromatographic peaks. In the alcohol vinegar (V9) this polyalcohol was not found.

Table 3A Content of organic acids in the vinegars in g/L based on the areas of the peaks.

Vinegars	Malic acid	Acetic acid	Propionic acid	Malonic acid
V1	0.17 ± 0.02	46.4 ± 0.7	nd	0.33 ± 0.02
V2	nd	45 ± 2	nq	0.54 ± 0.09
V3	nd	44.9 ± 0.6	nq	nq
V4	nq	42.9 ± 0.8	nq	nq
V5	nd	41 ± 2	0.45 ± 0.02	0.23 ± 0.09
V6	0.129 ± 0.009	42.4 ± 0.6	0.326 ± 0.006	nd
V7	nq	42 ± 2	nq	nq
V8	nd	45.5 ± 0.4	0.14 ± 0.05	nq
V9	nd	43.1 ± 0.7	nq	nd

nd- not detected; nq- identified, but not quantified

Table 3B Content of organic acids in the vinegars in g/L based on the areas of the peaks.

Vinegars	Lactic acid	Tartaric acid	Citric acid	Succinic acid
V1	nq	0.24 ± 0.04	0.12 ± 0.02	nq
V2	0.20 ± 0.03	0.218 ± 0.006	0.127 ± 0.002	nq
V3	1.62 ± 0.06	nq	nq	0.191 ± 0.004
V4	0.19 ± 0.01	0.134 ± 0.003	nq	nq
V5	2.2 ± 0.2	nq	0.55 ± 0.04	1.07 ± 0.08
V6	0.63 ± 0.03	nd	0.163 ± 0.002	0.74 ± 0.02
V7	0.150 ± 0.005	0.126 ± 0.003	nq	nq
V8	0.45 ± 0.01	nd	nq	0.217 ± 0.006
V9	nq	nq	nq	nd

nd- not detected; nq- identified, but not quantified

Table 4A Content of organic acids in the vinegars in g/L based on the heights of the peaks.

Vinegars	Malic acid	Acetic acid	Propionic acid	Malonic acid
V1	0.20 ± 0.02	56.2 ± 0.8	nd	0.30 ± 0.01
V2	nd	49 ± 2	nq	0.41 ± 0.02
V3	nd	54.2 ± 0.8	nq	nq
V4	nq	52 ± 1	nq	nq
V5	nd	50 ± 2	0.54 ± 0.01	0.17 ± 0.05
V6	0.150 ± 0.009	50.6 ± 0.8	0.319 ± 0.004	nd
V7	nq	44 ± 1	nq	nq
V8	nd	52 ± 2	0.28 ± 0.07	nq
V9	nd	52.0 ± 0.8	nq	nd

nd- not detected; nq- identified, but not quantified

Table 4.b - Content of organic acids in the vinegars in g/L based on the heights of the peaks.

Vinegars	Lactic acid	Tartaric acid	Citric acid	Succinic acid
V1	nq	0.23 ± 0.02	nq	nq
V2	0.20 ± 0.02	0.211 ± 0.005	nq	nq
V3	1.88 ± 0.07	nq	nq	nq
V4	0.18 ± 0.01	nq	nq	nq
V5	1.75 ± 0.06	nq	0.56 ± 0.02	0.94 ± 0.04
V6	0.635 ± 0.004	nd	0.173 ± 0.003	0.440 ± 0.007
V7	nq	nq	nq	nq
V8	0.44 ± 0.02	nd	nq	0.208 ± 0.005
V9	nq	nq	nq	nd

nd- not detected; nq- identified, but not quantified

Table 5 Content of ethanol (°G.L.) and glycerol (g/L) found in the vinegars.

Vinegars	Ethanol (°G.L.)		Glycerol (g/L)	
	Height	Area	Height	Area
V1	0.304 ± 0.007	0.302 ± 0.008	nq	nq
V2	0.316 ± 0.008	0.317 ± 0.002	nq	nq
V3	0.174 ± 0.002	0.180 ± 0.002	0.333 ± 0.006	0.38 ± 0.01
V4	0.174 ± 0.001	0.182 ± 0.001	nq	nq
V5	0.200 ± 0.005	0.228 ± 0.003	0.80 ± 0.02	1.03 ± 0.02
V6	0.151 ± 0.001	0.178 ± 0.001	1.442 ± 0.005	2.04 ± 0.02
V7	0.164 ± 0.007	0.163 ± 0.007	nq	nq
V8	0.130 ± 0.004	0.170 ± 0.005	0.92 ± 0.03	1.31 ± 0.04
V9	0.15 ± 0.03	0.18 ± 0.01	nd	nd

4. CONCLUSIONS

Through the ion-exclusion liquid chromatography technique, it was possible to identify several organic acids in samples of vinegars, besides ethanol and glycerol. All the samples presented values of alcoholic content and total acidity acceptable by the Brazilian legislation. The tartaric acid was found in all samples of wine vinegars, serving to discriminate the origin of the vinegar. The employed technique showed easiness of operation, excellent linearity, without needing complicated preparations of the samples, serving to analyze a variety of biological samples.

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