Organic Acids Chemical Profiling in Food Items

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A fast separation method for simultaneous determination of eleven organic acids was validated and applied to different commercial food items to evaluate their organic acids content. The present method gives detection limits between 0.04 and 4.65 μg mL^{-1}, recovery values in real samples between 78.2 and 97.3% and relative standard deviation values for precision lower than 5%. All validation data were in acceptable range and prove the method's fit for purpose. The advantages of the method are the short runtime analysis (15 min), no preparation step for the samples before the injection combined with good sensitivity which recommends it for routine control analysis in food industries. Moreover, this methodology has high potential in drinks industry but can by further extended to other types of food items.

Keywords: organic acids, commercial food, HPLC-PDA, tea infusions

Organic acids represent the third important class of chemical compounds that give the organoleptic properties on foodstuffs. They result from biochemical processes or are produced by various bacteria and molds. Fruits and vegetables are important sources of dietary micronutrients, well-known for health promoting properties, which include among the antioxidants, vitamins and minerals also certain organic acids [1-3]. Since organic acids are relatively stable compounds, the changes in their concentration in food can affect chemical and sensory qualities [4,5] and as consequence, their assessment is considered essential for food experts in issues of concern as food safety (storage conditions and processing) and food quality by providing information on authenticity and technological processes that take place.

A variety of techniques have been used for the determination of organic acids in food and clinical samples based on gas chromatography, electrochemistry, capillary electrophoresis but liquid chromatography (LC) was by far the most employed one due to so much possible separation mechanisms and detection options [6-12]. Another advantage of using HPLC for identification of organic acids is the rapid and simultaneous analysis with minimum sample preparation steps. Some organic acids are used as natural preservatives, inhibitors of microbial growth and for changes in the taste and aroma characteristics of a product due to their chemical properties [13-15].

The beverage industry (juices and alcoholic drinks) is one of the most controlled and regulated food branches in terms of composition, stability, microbiological control and authenticity due to their high level of consumption. The reasons for quantifying organic acids in this type of drinks include monitoring the fermentation processes, product stability and hygiene control, authenticity confirmation. Thus, analytical methods capable of identification of a high number of compounds within a short time and in a convenient price is desired.

The aim of this research work was to describe an HPLC method which detects a number of eleven organic acids: oxalic, tartaric, formic, malic, malonic, ascorbic, lactic, acetic, citric, succinic and propionic acids, from different food items. The investigated samples were hole-packed ready to drink fruits juices for child consumption, two types of wine and medicinal plant tea infusions that are on the market in various shapes, packaging, and flavors. This method would be of most interest for industry laboratories where a reliable fast and simple analytical method would help the quality control processes.

Experimental part

Materials

The standards of organic acids were all of analytical grade (purity >98 %) except for acetic acid which had 96% purity. L-(+)- tartaric acid, malic acid monosodium salt, malonic acid, citric acid, succinic acid, propionic acid and potassium phosphate monobasic (ACS reagent) were purchased from Sigma-Aldrich (St. Louis, MO, USA), ascorbic acid , L-lactic acid sodium salt from Fluka (Buchs, Switzerland), acetic acid from Riedel-de-Haen (Germany) and formic acid from Roth (Karlsruhe, Germany). Phosphoric acid 85% and oxalic acid were obtained from Merck (Darmstadt, Germany). Methanol (LiChrosolve) was gradient grade for liquid chromatography and deionised water was obtained using a Milli-Q water purification system, Elix 3 (Millipore Co., USA). Solvents and solutions were filtered and degassed prior to use. Stock solutions of individual compounds were prepared by dissolving the appropriate amount of substance in deionised water at a concentration level of 1 mg.mL^{-1} and kept at 4^{0}C over a period of maximum one month. Further, we prepared the working standard solutions daily by diluting the stocks according to the calibration levels and each standard mixture solution was analyzed in triplicate.

The samples were obtained at different supermarkets from Bucharest, Romania. The evaluated samples consisted of six fruit juices packed in boxes for children, (J1-J6, 0.2 L Tetra Pak package), two samples of red wine from Valea Calugareasca (Feteasca Neagra-FN and Negru Aromat-NA), apple vinegar-AV and balsamic vinegar-BV, as well as powdered tea bags of medicinal plants. The samples description is presented in table 1.

All samples were obtained by homogenizing the total content of three packages/bottles/bags from three

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Table 1
SAMPLES DESCRIPTION

Code	*Label Information*
J1	Banana (11%), Apple (8%), Raspberry (1%), citric acid
J2	Apple (10%), Peach (10%), citric acid
J3	Banana (15%), Pineapple (5%), citric acid
J4	20% mix of orange, apple, pineapple, lime and coconut, citric acid
J5	Mix of fruity cherry, orange and sweet banana (12%), citric acid
J6	Apple min. 12 %, citric acid
FN	Red wine
NA	Red wine
BV	Cooked must of selected grapes and vintage wine vinegar
AV	Apple vinegar, Potassium metabisulfite
T1	St. John's wort infusion
T2	Forest fruits infusion
T3	Linden infusion
T4	Rooibos infusion
T5	Dandelion infusion
T6	Chamomile infusion
T7	Mint infusion
T8	Marigold infusion

different batches; for the drinks, a suitable volume was filtered through PVDF membranes of 0.22 μm porosity (Millipore, Bedford, MA, USA) diluted proper and directly injected in the HPLC system. To prepare the tea infusions, approximately 2g of powder (from the mixed content of three bags) was weighted and added to 100 mL of boiling distilled water and left to stand at laboratory temperature for 15 minutes, and then filtered through 0.2 μm membranes and injected into HPLC system. All samples were injected using a 1:5 (v: v) dilution, except for tea infusions, which were injected undiluted.

Instrumentation

The chromatographic separation was performed based on a method of Ding et al. [25] with slight modifications using a Shimadzu instrument (Shimadzu Corporation, Kyoto, Japan) equipped with LC-20AD SP solvent delivery system, an LC 20AC autosampler, CTO-20AC Column Oven thermostat, DGU-20A5-Degasser and an SPD-M20A Diode Array Detector. A Kromasil C18 (250 x 4.6 mm length, 5μm particle size) column served as stationary phase at laboratory temperature. The mobile phase consisted of 10 mM phosphate buffer (*p*H 2.57±0.01) (A) and methanol (B). The elution of organic acids was performed using an isocratic method of 90% solvent A at a flow rate of 0.8 mL min^{-1} as a compromise between optimum retention times and baseline stability. The values for pH and the flow rate of the mobile phase were found to be optimum from a studied range of 2.0-2.9 and 0.6-1 mL min^{-1} (Supplementary data). The injection volume was 20 μL. The UV–Vis absorbance of the peaks was monitored at 245 nm for ascorbic acid and 210 nm for the rest of organic acids.

Validation

The developed method was validated in terms of linearity, precision, recovery and limits of detection and quantification. Calibration curves were constructed by plotting the peak area as a function of the concentration introduced and the sample peak purity was checked using the LabSolution system software. The precision of the method involved repeatability (six successive injections of a mix solution during the same day, n=6) and intermediate precision (six successive injections in three consecutive days, n=18) ascertained at two concentration levels from the calibration curves and were expressed as relative standard deviation (RSD %) of both the retention time and peak area. The limits of detection (LOD) and quantitation (LOQ) for each analyte were calculated as three times, ten times respectively, the standard error of the linear regression equation against the slope of the linear regression equation. For recovery studies, each sample was fortified with two concentration levels for each standard respectively. The samples were then analyzed adopting the method described above and the recovery of each analyte was calculated as percent recovery (R %) of the mean value for three analyses.

Results and discussions

The separation of eleven organic acids was achieved in less than 15 minutes with the following elution order: oxalic acid, tartaric acid, formic acid, malic acid, ascorbic acid, malonic acid, lactic acid, acetic acid, citric acid, succinic acid, propionic acid. The identification was based on a comparison of their retention times with those of the standards. Figure 1 represents the overlaid HPLC chromatograms of a mixture of standards and one sample of tea infusion.

From the linear calibration curves, the R^2 (coefficient of determination) values were found to be ≥ 0.999 for all the investigated acids except for malic acid which presented a value of 0.994. The values for LOD ranged from 0.04 μg mL^{-1} (tartaric acid) to 4.65 μg mL^{-1} (malonic acid). The HPLC method developed and validated herein was compared with other data from literature and our LOD results are comparable or even lower than values reported using advanced techniques. Several methods used for quantifying organic acids from food samples are presented in table 2 along with their limits of detection.

The validation data are summarized in table 3. All the analytes presented a relative standard deviation (RSD %) lower than 5% for both the retention time and peak areas. The intermediate precision RSD values for all the organic acids ranged from 0.06 to 0.55% for the retention times and from 0.88 to 4.48% for peak areas. The repeatability

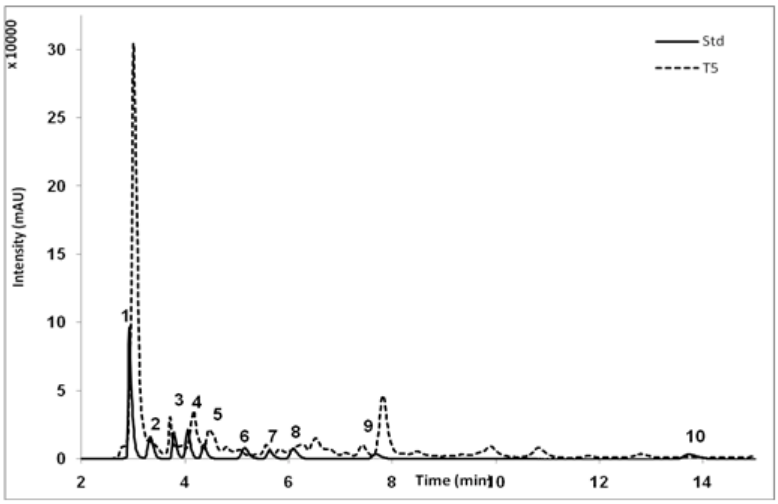

Fig. 1. HPLC chromatograms of a standard of ten organic acids (Std) at 50 μg mL^{-1} and a sample of tea infusion (T5); peak identification: 1-oxalic, 2-tartaric, 3-formic, 4-malic, 5-malonic, 6-lactic, 7- acetic, 8-citric, 9-succinic, 10-propionic acids; PDA detection at 210 nm

Table 2
LITERATURE DATA FOR COMPARISON ON THE CONTENT OF ORGANIC ACIDS IN FOOD ITEMS

Sample	*Analytes*	*Method*	*LOD(μg mL^{-1})*	*Reference*
Peach fruit	5	LC-ESI-MS	0.05 - 0.83	[9]
Juices, wine and beer	9	CZE -UV	2-10	[26]
Brasilian sugarcane spirits	10	CE	0.69 – 2.70	[6]
Sour cassava starch wastewater	4	HPLC-UV	1.0-3.7	[8]
Alcoholic and non-alcoholic drinks	11	HPLC-PDA	0.04 - 4.65	Present study

Table 3
VALIDATION PARAMETERS FOR THE HPLC SEPARATION METHOD OF ORGANIC ACIDS EXPRESSED AS RSD%

Acid	R^2	*Precision[a]*				*Precision[b]*				*LOD*	*LOQ*
		Level 1		*Level 2*		*Level 1*		*Level 2*		*(μgmL^{-1})*	*(μgmL^{-1})*
oxalic	0.999	0.13	0.78	0.11	2.07	0.13	2.78	0.09	0.88	0.87	2.89
tartaric	0.999	0.12	1.85	0.13	2.01	0.57	4.06	0.11	3.22	0.04	0.13
formic	0.999	0.10	1.31	0.12	2.66	0.12	4.18	0.10	2.11	1.74	5.79
malic	0.994	0.10	1.24	0.24	1.76	0.09	3.32	0.24	3.18	1.78	5.88
ascorbic	0.999	0.11	1.25	0.26	1.81	0.11	2.97	0.27	4.48	0.11	0.35
malonic	0.998	0.20	1.07	0.32	1.51	0.17	2.80	0.28	2.89	4.65	15.51
lactic	0.999	0.09	3.98	0.23	3.87	0.04	3.93	0.25	2.87	1.57	5.22
acetic	0.999	0.07	3.39	0.25	2.86	0.06	4.17	0.26	2.37	0.79	2.64
citric	0.999	0.09	0.42	0.46	2.71	0.29	3.85	0.55	2.19	0.36	1.18
succinic	0.999	0.08	4.14	0.45	2.19	0.24	4.46	0.53	2.14	0.60	2.00
propionic	0.999	0.11	2.70	0.45	1.36	0.14	2.48	0.50	1.57	2.00	6.67

[a] Repeatability and [b] Intermediate precision for retention time (left) and peak area (right)

RSDs for the retention time and peak areas were below 0.46 and 4.14%, respectively. Recoveries ranged from 78.2 to 97.3% for all the organic acids. The validation parameters confirmed that the proposed method was reliable and sensitive for the selected organic acids and can be further applied to commercial samples.

Table 4 summarizes the content of organic acids in the juices, wines and vineyard samples. The label on the boxes of fruit juices declared the addition of citric acid as acidifier but without stating the values. The results are within the range of values described in the literature but with some obvious variations depending on the origin and type of food item. All the investigated samples presented recent fabrication dates.

The commercial box packed fruit juices showed a varied content in organic acid, especially the J1-J3 samples, and malic acid was the predominant organic acid in all the investigated samples. In J1 and J2 samples, coming from the same producer, we found the highest concentrations of citric acid, 2527.49μg mL^{-1} and 1850.02 μg mL^{-1} respectively, but we knew that some supplementary addition, as acidifier, of this acid was already done by the producer. The rest of samples presented values from 6.06

Table 4

THE ORGANIC ACID CONTENT IN READY-TO-DRINK JUICE SAMPLES, TWO TYPES OF WINE AND VINEGAR SAMPLES

Acid	J1	J2	J3	J4	J5	J6	FN	NA	BV	AV
oxalic	1.16	27.18	n.d	1.95	5.92	3.04	10.33	5.66	14.82	1.86
tartaric	44.15	58.74	95.82	20.44	20.01	11.98	429.00	547.34	36.71	75.16
formic	23.23	24.16	123.50	70.63	22.01	20.02	481.71	539.11	36.56	n.d
malic	590.71	1.83*	648.45	550.91	528.36	811.91	25.49	n.d	2.00	13.47*
ascorbic	20.44	216.45	7.81	6.48	6.23	9.49	7.04	0.35	2.84	4.42
malonic	28.45	20.10	7.80	n.d	7.60	6.08	332.13	225.70	7.65	n.d
lactic	1.78*	1.47*	27.51	32.83	8.21	51.91	132.25	304.88	107.46	115.22
acetic	890.05	43.59	1.03*	10.11	213.59	37.49	136.23	276.23	21.95	12.02
citric	2.53*	1.85*	11.30	6.06	4.29	11.12	963.20	3.37*	12.32	62.42
succinic	17.22	12.25	34.17	n.d	21.38	9.25	234.86	140.45	n.d	n.d
propionic	257.56	9.43	16.68	78.65	162.21	18.23	12.04	159.56	39.32	n.d
Total*	6.18	5.57	2.01	0.78	0.99	0.99	2.76	5.57	0.28	13.74

* Concentration expressed as mg mL^{-1} ; n.d – not detected

to 11.30 μg mL^{-1} for the same acid. The highest concentration of organic acid in all the juice samples was that of malic acid which ranged between 528.36 to 1832.76 μg mL^{-1}. The first two samples of juices (J1 and J2) also exhibited high levels of lactic acid (1784.60 and 1472.98 μg mL^{-1}) and J2 was the richest sample in ascorbic acid (216.45 μg mL^{-1}) from the entire study. Only two organic acids, succinic and malonic, were not detectable in sample J4. Acetic acid was another analyte whose amount varied greatly from 10.11 =μg mL^{-1} to 1035.15 μg mL^{-1}. Lactic acid is used as pH control and flavour donor in food industry which may explain it's presence as a significant amount in juice samples J1 and J2 although it's concentration decreases up to 8.21 μg mL^{-1} in sample J5. The excessive presence of this organic acid in food samples is an indicator of microbial infection [16]. Certain organic acids are added to foods as acidulates or flavour modifiers but some of them may also be produced during fermentation or other processing operations.

The organic acid pattern is fruit specific and the concentration of these acids is also helpful for calculation of juice contents in beverages and estimation of adulteration index in juices. To the best of our knowledge the occurrence and distribution of organic acids in commercial ready to drink box packed fruit juices have never been investigated so far. Our results demonstrate the method's suitability for determining the organic acid composition in various types of beverages, criteria required for evaluation of quality and sensory attributes as well as for authentication.

Reaching the two samples of vinegar, the BV sample exhibited a great variability of organic acids concentration and it was characterized by a significant content of lactic acid (107.46 μg mL^{-1}) which may have been produced during fermentative metabolism of the sugars contained. Among the organic acids expected to be present were tartaric, malic, citric and formic acid which derived directly from the grapes while succinic acid, often reported in the literature, was not detectable [7, 17]. The total concentration of organic acids in balsamic vinegar was 50 times lower compared to the amount of all acids quantified in apple vinegar sample. Malic acid showed the highest concentration (13.47 mg mL^{-1}) in apple vinegar from all the samples analyzed and also this sample presented the highest total content of organic acids.

The two samples of red wine exhibited different composition in organic acids. Surprisingly, malic acid was not detected in NA sample while in FN wine sample the concentration was low (25.49 μg mL^{-1}). These values may indicate that a malolactic fermentation process took place which converted completely the malic acid into lactic acid. Among the most abundant organic acids in both samples of wine were citric, tartaric, formic, lactic, succinic and acetic acids. The presence of a high quantity of citric acid in both samples of wine (963.20 μg mL^{-1} and 3372.09 μg mL^{-1}) suggested a possible supplementary addition of the acid concerned as corrector of the wine's acidity. The obtained citric acid content showed values higher than the specific literature [18] in which the concentrations vary between 30 to 637 μg mL^{-1}. Acetic acid, the most undesirable organic acid in wines presented a significant amount in both samples (136.23 μg mL^{-1} in FN and 276.23 μg mL^{-1} in NA), probably due to a prolong exposure of the wine to oxygen atmosphere. Some authors suggested that the presence of lactic and acetic acid in wine samples may be associated with bacterial contamination due to poor hygiene conditions in fabrication processes [6, 19]. Succinic acid, a by-product of the yeast metabolism was found in low concentrations. Another surprising result was that formic acid concentration was relatively high in our wine samples. This acid has been identified in considerable amounts (from 10 to 201 mg L^{-1}) especially in wines made from raisin or moldy grapes [20]. As can be shown from table 4, the concentration of organic acids found in wines

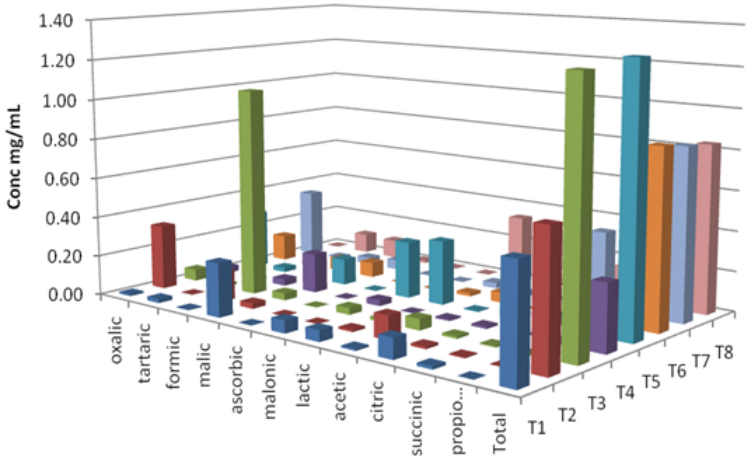

Fig. 2. Concentrations expressed as mg/mL solid state of herbs for the organic acids in tea infusions

varies significantly from one sample to another, suggesting that is strongly dependent on wine nature and making process.

Organic acids are also important constituents in medicinal herbs giving their preparations, like tea infusions or decoct, a certain taste and contributing to health benefits. It is well known that plants are capable of absorbing micro and macronutrients from the soil. There are studies describing the possibility of the migration of organic ions from the soil to different parts of the plant but more research still need to be done to elucidate these pathways [21]. Different amounts of organic acids were quantified as well in our tea infusion samples (fig 2). The concentrations were expresses as mg/mL of dried herb.

The total organic acid content varied from 1.27 mg/mL (linden infusion) to 0.33 mg/mL in rooibos infusion sample. Oxalic acid was the predominant organic compound in most of the tea infusions along with malic, citric, succinic and formic acids. Malic acid is known to be formed in the metabolic cycle of plant but some manufacturers of herbal teas could add it as flavor component. The highest concentration of an organic acid was obtained for succinic acid in chamomile and mint tea infusions, of 0.53 mg/mL and 0.38 mg/mL respectively and formic acid in linden infusion, 1.04 mg/mL. The lowest concentration was obtained for ascorbic acid in most of the tea samples analyzed, due to the way the herbal infusions were prepared (hot water extraction), which involves high temperatures leading to thermal decomposition of the acid. The highest content of oxalic acid was obtained in mint (0.35 mg/mL) and forest fruit (0.33 mg/mL) tea infusions and although some of the organic acids are beneficial for human health, this specific acid may decrease the bioavailability of Ca ions and influence the zinc balance in adults [22]. The presence of low molecular mass organic acids in plants has also been explained by oxidation processes or degradation of monosaccharides. Important chemical reactions occur during thermal treatment of foods producing intermediate compounds. The fragments resulted from sugars cleavage may recombine resulting in the formation of organic acids, such as formic, acetic or propionic acids [23]. There are only a few studies regarding organic acid composition in herbal tea preparations [14, 24, 25] and the results are comparable although differences may appear due to extraction techniques and the sensitivity of the analysis methods. The method combines simplicity and minimum sample preparation in a cost-effective term, with satisfactory speed, sensitivity and precision for food analysis.

Conclusions

In food and beverage industry the most important criteria for quality analysis are simplicity and rapidity of the method, easy preparation steps of the samples and economization. This study significantly offers a fast and low-cost analysis of small molecule organic acids from food samples for routine analysis since the method requires only a common reverse-phase HPLC column and an UV-Vis detector. The samples can be injected directly without any previous treatment except for dilution and filtration. The analysis of organic acids is very important for quality control purposes because it allows verifying the authenticity and possible microbial alteration during storage. The described HPLC method allows the simultaneous determination of eleven organic acids in short time analysis (less than 15 min) and could be applied to a wide range of food items.

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