Z Lebensm Unters Forsch A (1997) 204: 151–155

ORIGINAL PAPER

Teresa Hernandez · Nieves Ausín · Begoña Bartolomé
Luz Bengoechea · Isabel Estrella
Carmen Gómez-Cordovés

Variations in the phenolic composition of fruit juices with different treatments

Received: 18 March 1996

Abstract In this work we have determined the variations in the composition of phenolic compounds of natural peach and apple juice with different thermal and enzymatic treatments. The following phenolic compounds were identified and quantified in samples of treated and untreated fruit juices: cinnamic acids (caffeic, *p*-coumaric and ferulic acids), cinnamic derivatives (chlorogenic and *p*-coumarylquinic acids and feruloylglucose), flavonols (quercetin glycosides), dihydrochalcones (phloretin glycosides), flavan-3-ols [(+)-catechin and (−)-epicatechin], and procyanidins (dimer B_2, trimer C_1 and tetramer T_4). Furfural derivatives, compounds widely used as indicators of prior thermal treatment, were also studied using these samples. The results indicate that the different processes tested gave rise to a series of changes in composition that may make it possible to identify the type of treatment employed on the basis of the composition of phenolic compounds in the fruit juices.

Key words Fruit juices · Phenolics · Furfural · Pectinases · Thermal treatments

Introduction

Modern analytical methods have made possible the identification of many secondary metabolites from different chemical families. These include phenolic compounds, which encompass a broad range of substances present in all plant tissues and which are usually the most abundant secondary metabolites in fruits and other vegetables [1].

Phenolic compounds are present in variable concentrations. Along with simple soluble phenols, located mainly in the cell vacuoles, the cell walls also contain lignins and tannins [2]. The composition of phenolic compounds in juices and fruits differs according to the amounts of such compounds released through the rupture of the vacuoles and cell walls, depending upon the particular distribution of the phenolic compounds and the technological processes employed.

The composition of phenolic compounds in fruits is characteristic to each species and variety [3], though quantitative differences may occur depending on the maturity stage and environmental growth and storage conditions [4, 5]. Enzymatic and thermal treatments applied during juice making may affect phenolic composition [6]. Pectic enzymes are used in the manufacture of fruit juices to facilitate pressing or juice extraction and to aid in the separation of flocculent precipitate by sedimentation, filtration and centrifugation [7, 8]. The need to use enzymes during pressing or juice expression will depend upon the type of fruit concerned [9].

Furfural derivatives are one of the most studied group of compounds in industrial and commercial fruit juices. The formation of furfural and 5-hydroxymethylfurfural (HMF) from compounds involved in the Maillard reaction during heating has been reported [10–12]. Furfural derivatives are accepted indicators of the deterioration of quality in fruit juices during heating in processes such as concentration and pasteurization as well as during storage. HMF has been related to colour changes in fruit juices [13], while furfural is widely acknowledged to be an indicator of flavour changes [14].

This paper reports the results of applying different processes (thermal and enzymatic treatments) to natural peach and apple juices to determine differences in

T. Hernandez (✉) · N. Ausín · B. Bartolomé[1]
L. Bengoechea · I. Estrella · C. Gómez-Cordovés
Instituto de Fermentaciones Industriales (CSIC), c/Juan de la Cierva, 3, E-28006 Madrid, Spain

Present address:
[1] Department of Food Molecular Biochemistry, Institute of Food Research, Norwich Research Park, Colmey, Norwich NR4 7UA, UK

the composition of phenolic compounds and in the amount of furfural derivatives.

Materials and methods

Sample preparation. Samples were apple and peach juices prepared using a household juicer. Four different processes were applied to each fruit juice: (1) Z_2 – thermal treatment similar to industrial pasteurization; (2) Z_4 – enzymatic treatment + thermal treatment similar to industrial pasteurization; (3) Z_1 – thermal treatment similar to intensive sterilization; (4) Z_3 – enzymatic treatment + thermal treatment similar to intensive sterilization.

For thermal treatment, fruit juices were heated in a microwave (MSD-2000, CEM) at 105 °C for 45 s (industrial pasteurization) or at 120 °C for 20 min (intense sterilization). After heating, samples were cooled to room temperature (20 °C) by immersion in an ice-water bath for 10 min.

For enzymatic treatment, 100 ml of each sample was treated with 15 mg of *pectinase* (P9179-Sigma, Deinsenhofen, Germany) for 12 h.

Control samples of untreated fruit juices (Z) were also analysed.

Sample extraction. Treated and untreated juices (100 ml) were concentrated to 25 ml with a rotatory evaporator, always keeping the bath temperature under 35 °C. The time of the process should not exceed 40 min.

The concentrated juices were extracted twice with 15 ml of diethyl ether and then twice with 15 ml of ethyl acetate; fractions were pooled and evaporated to dryness and the residue was redisolved in 2 ml of methanol/water (1:1, v/v) and filtered by a Millipore filter HV-0.45 μm. Samples were analyzed in duplicate.

HPLC analysis. Samples were analysed by HPLC using a Waters (Milford, Mass., USA) modular chromatograph with a model 600 E pump system controller, a U6K universal injector, and a model 991 photodiode-array detector. The column was a reversed-phase Nova-Pack C_{18} (300 × 3.9 mm I.D.) column; 10 μl was injected. The composition of solvents and the gradient elution conditions are given in Table 1. All solvents were HPLC grade and had been degassed with helium at room temperature. Detection was performed by scanning from 210 to 360 nm with an acquisition speed of 1.2 s.

Identification and quantification of components. Identification was carried out by comparing retention times and spectral data with those of standards [15] from Sigma and Aldrich (Steinheim/Albuch, Germany). Components for which no standards were available had been identified previously, as follows: *p*-coumaroylquinic acid and feruloylglucose by hydrolysis [3]; procyanidins, B_2 (dimer), C_1 (trimer) and T_4 (tetramer), by acid hydrolysis and formation of derivatives with phloroglucinol [16]. A sample of previously identified phloretin-2-xyloglucose was kindly provided by Dr. F.A. Tomás-Barberán [17].

Quantitative determinations were carried out by heating measurements, using calibration curves of the standards. The esters *p*-coumaroylquinic acid and feruloylglucose were quantified using the calibration curves of the respective cinnamic acids, *p*-coumaric and ferulic acids. Procyanidins were quantified as (−)-epicatechin. Phloretin-2-xyloglucose was quantified as phloridzin.

Table 1 Mobile phase gradient composition and flow rate (gradient curve no. 5, concave). [*A* Water/acetic acid (98:2, v/v), *B* water/acetonitrile/acetic acid (78:20:2, v/v/v)]

Time (min)	Flow rate (ml/min)	A (%)	B (%)
0	1.0	100	0
55	1.0	20	80
57	1.2	10	90
70	1.2	10	90

Results and discussion

The phenolic compounds identified in the samples of peach and apple juices (Fig. 1) fell into two general classes, cinnamic compounds and flavonoids. The former includes caffeic, ferulic, and *p*-coumaric acids and various of their derivatives (chlorogenic and *p*-coumaroylquinic acids and feruloylglucose). The latter includes flavonols (quercetin glycosides), dihydrochalcones (phloretin glycosides), flavan-3-ols [(+)-catechin and (−)-epicatechin], and procyanidins (dimer B_2, trimer C_1 and tetramer T_4). The sample preparation and chromatographic conditions applied in the

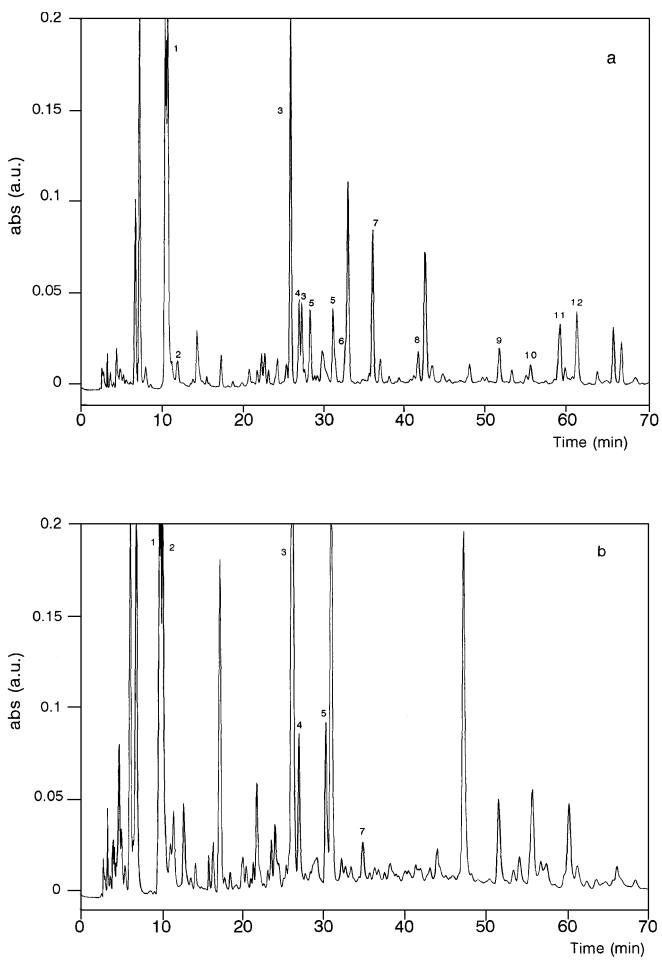

Fig. 1 Chromatograms at 280 nm of **a** apple juice with thermal and enzymatic treatments (Z_3) and **b** peach juice with thermal treatment (Z_1). [*1* 5-Hydroxymethyl-furfural (HMF), *2* 2-furanoic acid, *3* chlorogenic acid, *4* caffeic acid, *5* *p*-coumaroylquinic acid, *6* feruloylglucose, *7* *p*-coumaric acid, *8* ferulic acid, *9* quercetin-3-galactose, *10* phloretin-2-xyloglucose, *11* quercetin-3-xylose, *12* phloretin-2-glucose]

Table 2 Concentration (mg/ml) of phenolic compounds in peach juice. [*Z* Control sample, Z_1 thermal treatment (120 °C, 20 min), Z_2 thermal treatment (105 °C, 45 s), Z_3 enzymatic treatment + thermal treatment (120 °C, 20 min), Z_4 enzymatic treatment + thermal treatment (105 °C, 45 s), *nd* not detected, *t* traces]

Compound	Z	Z_1	Z_2	Z_3	Z_4
HMF	nd	23.10 ± 1.03	0.74 ± 0.09	33.70 ± 1.12	2.18 ± 0.19
2-Furanoic acid	nd	0.33 ± 0.05	nd	0.65 ± 0.05	0.16 ± 0.04
Catechin	1.55 ± 0.11	nd	nd	nd	nd
Chlorogenic acid	16.80 ± 1.20	13.60 ± 0.77	3.01 ± 0.50	11.70 ± 0.75	8.60 ± 0.66
Caffeic acid	0.03 ± 0.02	0.67 ± 0.11	t	0.63 ± 0.09	0.11 ± 0.05
p-Coumaroylquinic acid	0.49 ± 0.09	0.41 ± 0.07	0.17 ± 0.05	0.26 ± 0.05	0.44 ± 0.08
p-Coumaric acid	0.10 ± 0.03	0.12 ± 0.03	nd	0.25 ± 0.04	0.31 ± 0.05
Tetramer T_4	0.26 ± 0.04	nd	nd	nd	t

Table 3 Concentration (mg/ml) of phenolic compounds in apple juice. [*Z* Control sample, Z_1 thermal treatment (120 °C, 20 min), Z_2 thermal treatment (105 °C, 45 s), Z_3 enzymatic treatment + thermal treatment (120 °C, 20 min), Z_4 enzymatic treatment + thermal treatment (105 °C, 45 s), *nd* not detected, *t* traces]

Compound	Z	Z_1	Z_2	Z_3	Z_4
HMF	nd	10.40 ± 1.02	0.14 ± 0.03	12.90 ± 0.94	0.20 ± 0.04
2-Furanoic acid	nd	0.13 ± 0.05	nd	0.18 ± 0.06	nd
(+)-Catechin	0.48 ± 0.08	nd	nd	nd	nd
Chlorogenic acid	30.60 ± 1.42	16.25 ± 1.02	9.61 ± 0.95	4.08 ± 0.83	2.99 ± 0.81
Caffeic acid	nd	0.23 ± 0.05	t	0.32 ± 0.06	0.42 ± 0.06
Procyanidin B_2	1.72 ± 0.09	nd	0.45 ± 0.07	nd	nd
p-Coumaroylquinic acid	0.56 ± 0.08	0.61 ± 0.07	0.62 ± 0.09	0.34 ± 0.07	0.25 ± 0.07
(−)-Epicatechin	0.26 ± 0.08	nd	nd	nd	nd
Feruloylglucose	0.20 ± 0.06	0.16 ± 0.05	0.19 ± 0.06	0.02 ± 0.01	0.04 ± 0.03
p-Coumaric acid	0.09 ± 0.03	0.12 ± 0.06	nd	0.37 ± 0.09	0.49 ± 0.11
Trimer C_1	0.59 ± 0.07	nd	nd	nd	nd
Ferulic acid	nd	0.01 ± 0.01	nd	0.02 ± 0.01	0.03 ± 0.01
Quercetin-3-galactose	2.16 ± 0.36	0.99 ± 0.16	1.00 ± 0.07	1.01 ± 0.07	1.13 ± 0.09
Phloretin-2-xyloglucose	3.97 ± 0.28	1.13 ± 0.08	0.98 ± 0.08	0.29 ± 0.05	0.15 ± 0.03
Quercetin-3-xylose	2.81 ± 0.26	1.23 ± 0.14	1.10 ± 0.09	0.96 ± 0.08	1.07 ± 0.08
Phloretin-2-glucose	4.41 ± 0.27	2.48 ± 0.16	1.64 ± 0.09	0.46 ± 0.06	0.49 ± 0.06

analysis of phenolic compounds also allowed us to study furfural derivatives, i.e. 5-hydroxymethyl-furfuraldehyde (HMF) and 2-furanoic acid (Fig. 1).

Tables 2 and 3 give the quantities of phenolic compounds and furfural derivatives in treated and untreated fruit juices. Because of the diversity of the phenolic components present, not all of the phenolic compounds analysed followed the same trend in each of the processes tested.

Chlorogenic acid was the main cinnamic acid derivative identified in both peach and apple juices. The chlorogenic acid content decreased with all the processes, although the extent of these variations depended on the nature of the fruit. For peach juice (Table 2), the weakest thermal treatment (Z_2) reduced the chlorogenic acid content more compared with the strongest treatment (Z_1). However, no corresponding increase in the amount of the free cinnamic acid, caffeic acid, was observed in Z_2. When enzymatic treatment was applied before the weakest thermal treatment, i.e. Z_4, the decrease of chlorogenic acid was less dramatic than that occurring in treatment Z_2, and some release of caffeic acid was observed. Addition of pectinase before the strongest treatment, i.e. Z_3, resulted in a slight decrease in the chlorogenic acid content compared to Z_1, while no significant difference was observed in the caffeic acid content. The compounds *p*-coumaroylquinic acid and *p*-coumaric acid, also identified in peach juice, exhibited the same trend as chlorogenic acid and caffeic acid with the different processes.

These results can be explained by the presence of endogeneous enzymes acting on cinnamic acid derivatives that are not only denatured by process Z_2 (thermal treatment at 105 °C for 45 s), but are even activated by those conditions. The low amounts of free cinnamic acids detected in Z_2 indicate that the degradation of cinnamic acid derivatives by endogenous enzymes does not lead to the production of free cinnamic acids, or

that there are other kinds of endogenous enzymes that degrade the cinnamic acids once they are produced. It also seems that these endogeneous enzymes show higher specificity for caffeic acid derivatives rather than for *p*-coumaric and ferulic acid derivatives. In the presence of a large amount of pectinase (Z_4), the degradation of cinnamic acid esters leads to a greater release of free cinnamic acid, thanks to the competitive action of the pectinase. Thermal treatment in Z_1 and Z_3 (120 °C, 20 min) may denature endogenous enzymes or, at least, not activate them.

The weakest thermal treatment (Z_2) applied to apple juice also reduced the content of chlorogenic acid more compared with the strongest one (Z_1), again with no corresponding increase in the caffeic acid content in Z_2 (Table 3). When enzymatic treatment was applied before both thermal treatments (Z_4 and Z_3), a notable decrease in the amount of chlorogenic acid was observed, with a corresponding increase in the amount of the free acid, caffeic acid. No significant variations were detected in the amounts of feruloylglucose and *p*-coumaroylquinic acid with processes Z_2 and Z_1. However, addition of pectinase (Z_4 or Z_3) resulted in a substantial decrease in the concentration of both derivatives, with corresponding increases in the amounts of the free acids, ferulic and *p*-coumaric acids.

The variations in the amounts of chlorogenic acid in apple juice with the different processes can again been partly attributed to the action of endogenous enzymes, the occurrence of which seems to be less important in apple than in peach juices. These endogenous enzymes seem also to be more specific for caffeic than for *p*-coumaric and ferulic acid derivatives.

The results indicate, therefore, that for processes Z_2 and Z_4 (105 °C, 45 s), the heating time was too short to inactivate the enzymes naturally present in the fruit (e.g. peroxidases and polyphenol oxidases), which agrees with previous studies by other authors (e.g. [18]).

Flavan-3-ol monomers [(+)-catechin and (−)-epicatechin] in both peach and apple juices were undetectable following all processes applied (Tables 2 and 3). Procyanidins (tetramer T_4 in peach, and trimer C_1 and dimer B_2 in apple) followed the same pattern, with the exception of B_2, which was detected in Z_2 in apple juice. In view of the marked stability of such compounds, according to other reports in the literature [19], the latter results may be attributable to an increase in polymer size, resulting in the formation of substances that were undetectable in the analytical conditions employed in the present study.

Quercetin derivatives (quercetin-3-galactose and quercetin-3-xylose) and phloretin derivatives (phloretin-2-glucose and phloretin-2-xyloglucose) present in the apple juice (Table 3) underwent a similar decreasing trend with both thermal treatments (Z_2 and Z_1). However, the nature of the aglycon appeared to affect the results in the processes involving use of pectinase (Z_4 and Z_3). The decrease in quercetin glycosides was similar for both the untreated and enzyme-treated processes, but the phloretin derivatives were more affected by processes involving enzymatic treatment. This indicates that the pectinase exhibited higher specificity for phloretin derivatives.

Furfural derivatives were only detected in the treated juices (Tables 2 and 3). Concentrations of 5-hydroxymethyl-furfuraldehyde and 2-furanoic acid rise as heating time and temperature increase (Z_1 and Z_3). Addition of pectinase (Z_3 and Z_4) slightly increased the formation of furfural derivatives. Higher quantities in the peach juice than in the apple juice were recorded in all cases, probably because of the higher initial amount of pectins in the peach juice.

In conclusion, the two thermal treatments applied to peach and apple juices affected their phenolic composition. Changes in the amounts of cinnamic acids and their derivatives could be associated with the effect of heating on the activities of endogeneous enzymes, especially in peach juices. Flavan-3-ol monomers were undetectable with the thermal treatments tested. The amounts of quercetin and phloretin derivatives in apple juice decreased with both thermal treatments, but no influence of heating conditions was observed. Furfural derivatives appeared with thermal treatments, their quantities increasing with heating time and temperature. Addition of pectinase before thermal treatments gave rise to specific changes in phenolic composition. Caffeic, *p*-coumaric and ferulic esters were hydrolysed to give the corresponding cinnamic acids, though the final effect depended on the nature of the fruit juice. Regarding flavonoids, while no significant variations were observed in the quantity of quercetin derivatives with the addition of the enzyme, phloretin derivatives were intensely degraded by the pectinase. A very slight increase in the quantity of furfural derivatives was observed when juices were treated with pectinase before thermal treatments. Determination of phenolic compounds as well as furfural derivatives in industrial and commercial fruit juices may be very useful in the identification of the type of processing employed. The absence of flavan-3-ols could indicate thermal treatment, whereas the levels of furfural derivatives may be used as an indicator of heating conditions. Pectinase treatment could be detected by looking at the amount of phloretin derivatives or at the relationship between contents of quercetin and phloretin derivatives.

Acknowledgements The authors are grateful to the financial support from the Spanish Comisión Interministerial de Ciencia y Tecnología (Project ALI92-0928) and the technical assistance provided by Luis Piñal.

References

1. Macheix JJ, Fleuriet A, Billot J (1990) Fruit phenolics. CRC, Boca Raton, Fla

2. Higuchi T (1990) Wood Sci Technol 24: 23–63
3. Peréz-Ilzarbe FJ, Hernández T, Estrella I (1991) Z Lebensm Unters Forsch 192: 551–554
4. Joshi VK, Chauhan SK, Lal BB (1991) J Food Sci Technol 28: 64–65
5. Spanos GA, Wrolstad RE (1992) J Agric Food Chem 40: 1478–1487
6. Schols HA, Veld PH, Van Deelen W, Vorgen A (1991) Z Lebensm Unters Forsch 192: 142–148
7. Verspuy A, Piilnk W, Vos L (1970) Fluessiges Obst 37: 518–519
8. Dixon JM (1985) Food Eng Int 1: 24–28
9. Mitek M, Drzazga B (1989) Acta Aliment Pol XV 1: 3–12
10. Van Buren J, de Vos L, Pilnik W (1976) J Agric Food Chem 63: 448–451
11. Mattick LR (1988) In: Nagy S, Attaway JA, Rhodes ME (eds) Adulteration of fruit juice beverages. Dekker, New York, pp 175–193
12. Aleixandre JL, Serrano P (1989) Aliment Equip Tecnol 5: 83–86
13. Meydav S, Berk Z (1978) J Agric Food Chem 26: 282–286
14. Dinsmore HL, Nagy S (1972) J Food Sci 37: 768–771
15. Bartolomé B, Bengoechea ML, Gálvez MC, Peréz-Ilzarbe FJ, Hernández T, Estrella I, Gómez-Cordovés C (1993) J Chromatogr A 655: 119–125
16. Pérez-Ilzarbe FJ, Martínez V, Hernández T, Estrella I (1992) J Liq Chromatogr 15: 637–646
17. Tomás-Barberán FA, García-Viguera C, Nieto JL, Ferreres F, Tomás-Lorente F (1993) Food Chem 46: 33–36
18. Avisse C, Varorvaux P, Dupuy P (1974) C R Seances Acad Agric Fr 60: 741–748
19. Waterman PG (1988) In: Cody V, Middleton E, Harborne JB, Berets A (eds) Plant flavonoids in biology and medicine II: biochemical, cellular, and medicinal properties. Liss, New York, pp 77–92