ANALYSIS OF TOTAL INDIVIDUAL POLYPHENOLS (TIP) FOR LABELLING FUNCTIONAL CONTENT OF COMMERCIAL ANTIOXIDANT FRUIT JUICES WITH HEALTH CLAIMS

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9th International Food Data Conference

Introduction

Nowadays there is a growing interest in the commercialization of antioxidant fruit juices rich in polyphenols, which may have beneficial effects on consumers' health. Analyses of Total Polyphenols content (TP) are usually accomplished by simple colorimetric methods (e.g. Folin-Ciocalteu), but they suffer from low specificity and offer a limited information. Thus, polyphenols compositional characterizations of foods were done by a HPLC method with a high-resolution column, and simultaneous UV-Visible and fluorescence detection for identification and quantification of polyphenols.

Objectives

A simple, improved, fast and reliable HPLC method able to measure the Total Individual Polyphenols content (TIP) present in juices without sample hydrolysis. Five red fruit juices were analyzed for method characterization.

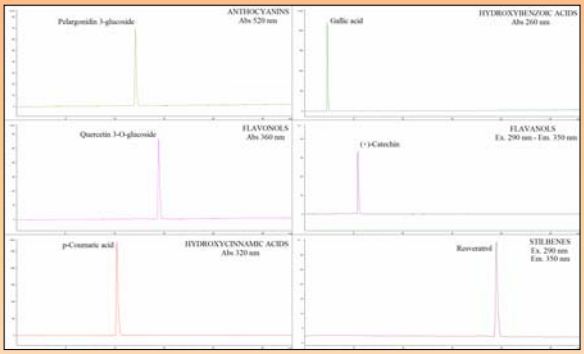
Materials and methods

Fruit juice samples		(J. García Carrión S.A., Spain)
Sour cherry juice concentrate (*Prunus cerasus L.*) Cranberry juice concentrate (*Vaccinium oxycoccus L.*) Red grape juice concentrate (*Vitis vinifera L.*) Blackcurrant juice concentrate (*Ribes nigrum L.*) Blueberry juice concentrate (*Vaccinium myrtillus L.*)	Fruit juices concentrated were five times diluted in water and were filtered at 0.45µm	
Colorimetric method	(Skerget *et al.* 2005)	Folin-Ciocalteu method
HPLC		Agilent Technologies (USA)
Modular liquid chromatographic system serie 1200 Binary pump serie 1200 Photodiode array detector (PDA) A) TFA/water (0.5/99.5 v/v) B) TFA/AcN/water (0.5/50/49.5 v/v) Fluorescence detector Thermostatized autosampler Zorbax SB-C18, 1.8µm, 15cm x 4.6mm i.d. column Gradient elution (flow rate 1mL/min):	Temperature: 25ºC Sample injection: 2µL	

Time (minutes)	0	1.2	14	28	34	38.8	39.2	40
% B	8	18	32	60	100	100	8	8

Results

The HPLC analysis method obtained TIP (total polyphenol individual content) as the sum of the individual polyphenol contents presents in fruit juice. The different phenolic compounds were recovered in chromatograms and identified according to retention times of standard pure compounds, the characteristics of UV-Vis or fluorescence spectra, their order of elution, and the comparison with bibliography data.



A standard compound of every polyphenol group was used for quantification and its detection conditions were: pelargonidin 3-glucoside (anthocyanins, 520 nm), quercetin 3-glucoside (flavonols, 360 nm), p-coumaric acid (hydroxycinnamic acids, 320 nm), gallic acid (hydroxybenzoic acids, 260 nm), catechin (flavanols, λex 290nm - λem 350 nm) and resveratrol (stilbenes, λex 290nm - λem 350 nm). No peaks overlapping of main compounds were observed during fruit juice analysis.

As an example of analyzed fruits, analysis by HPLC of **sour cherry juice** is shown in Table 1. A TIP value of 386 mg polyphenols equivalents/100 mL juice, and a total polyphenol content (TP) of 185 mg gallic acid equivalents/100 mL juice were obtained.

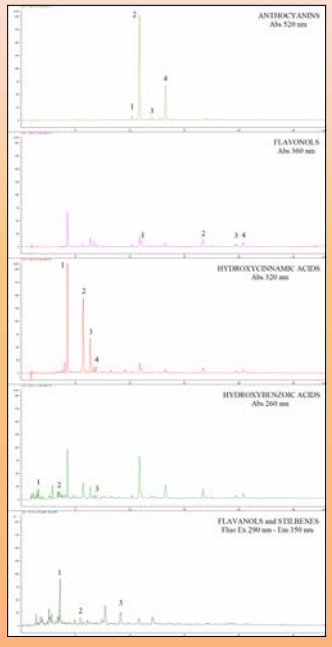


Table 1: Sour cherry polyphenols content (mg/100mL fruit juice)

N.	Compound	Polyphenols content
	Anthocianyns	**106**
1	Cyanidin 3-sophoroside	2.87
2	Cyanidin 3-glucosyl-rutinoside	73.67
3	Cyanidin 3-glucoside	1.59
4	Cyanidin 3-rutinoside	28.05
	Flavonols	**9**
1	Quercetin-3-glucosil-rutinoside	2.14
2	Quercetin-3-O-rutinoside	3.82
3	Kaempferol-3-O-glucoside	0.91
4	Myricetin-3-glucoside	1.80
	Hydroxycinnamic acids	**225**
1	Neochlorogenic acid (3-caffeoylquinic)	101.18
2	p-Coumaric glucoside	79.81
3	Chlorogenic acid (5-caffeoylquinic)	35.86
4	Caffeic acid	7.76
	Hydroxybenzoic acids	**25**
1	Gallic acid	6.45
2	3,4-dihydroxybenzoic acid	6.49
3	Vanillic acid	12.18
	Flavanols and Stilbenes	**14**
1	Epigallocatechin	7.27
2	(+)-Catechin	2.10
3	(-)-Epicatechin	4.76
	TIP (total polyphenol individual content)	***386***
	TP (total polyphenol content)	***185***

Table 2 shows the TIP analysis of five fruit juices analyzed.

Table 2: Polyphenols content (mg/100mL fruit juice)

Compounds	Sour cherry	Cranberry	Red grape	Blackcurrant	Blueberry
Anthocyanins	106.2	13.8	22.4	163.0	284.3
Flavonols	8.7	13.6	3.9	9.7	6.7
Hydroxycinnamic acids	224.6	71.2	21.0	71.0	113.5
Hydroxybenzoic acids	25.1	80.4	0.0	16.8	26.5
Flavanols and Stilbenes	14.1	16.2	8.8	26.2	31.5
TIP (HPLC):	**386**	**238**	**65**	**326**	**503**
TP (Colorimetric method):	**185**	**180**	**215**	**465**	**352**

Conclusions

- Blueberry juice has the highest TIP value, while red grape has the smallest value.
- Blackcurrant juice has the highest TP value, while cranberry has the smallest value.
- Blueberry is the red fruit with the biggest quantity in anthocyanins, stilbenes and flavanols.
- Cranberry is the red fruit with the biggest quantity in flavonols and hydroxybenzoic acids.
- The HPLC method proposed is of special interest for juice industry. It allows to verify the authenticity of fruit juices and to quantify their composition in polyphenols. These data could complete the labelling information of commercial antioxidant fruit juices for beneficial effects on consumers' health.

References

- Obón *et al.* (2011) Red fruit juice quality and authenticity control by HPLC. Journal Food Composition Analysis, 24, 760-771.
- Skerget *et al.* (2005) Folin-Ciocalteu method.

Acknowledge

The present work has been supported by projects and a grant from "Ministerio de Ciencia y Tecnología" (AGL 2007-60455) and the "Fundación Séneca" (16227/BPS/10 and 08702-PI-08). The authors thank J. García Carrión, S.A. (Jumilla, Spain) for supplying the red fruit juices.