Original paper

Automated determination of total polyphenols in apple juice

Juan Mangas¹, Belén Suárez¹, Domingo Blanco²

¹ Instituto de Experimentación y Promoción Agraria, E-33300 Villaviciosa, Spain

² Departamento de Química Física y Analítica, Universidad de Oviedo, E-33006 Oviedo, Spain

Received March 1, 1993

Automatische Bestimmung aller Polyphenole in Apfelsaft

Zusammenfassung. Es wurde eine automatische Methode zur Erfassung der gesamten Polyphenole in Apfelsaft mittels Folin-Ciocalteu-Reagens angewandt. Chlorogensäure wurde als Standard gewählt, da mit ihr ein ähnliches kinetisches Verhalten in der Standardprobe beobachtet wurde. Es wird eine unkomplizierte Methode mit guter Reproduzierbarkeit (Probenfrequenz C.V. < 1%), Probenfrequenz (48 pro h) und gutem Verhältnis zur traditionellen schrittweisen Methode vorgeschlagen.

Abstract. An automated method of total polyphenols analysis in apple juice using the Folin-Ciocalteu reagent is presented. Chlorogenic acid was selected as the standard since a similar kinetic behaviour, standard-sample, was observed. A straightforward method with good reproducibility (CV < 1%), sampling frequency (48 h⁻¹) and correlation with the traditional batch method ($r = 0.9924$) is proposed.

Introduction

The determination of total polyphenols in apple is necessary to control the processing of apple products, especially cider and apple juice. The phenolic components participate in certain apple fruit characteristics, such as colour, bitterness, and astringency, which are responsible for organoleptic properties. However, the phenolic composition of the fruit can be greatly changed by its processing, which involves new chemical and sensory characteristics.

The current analytical control of polyphenol compounds is based on the formation of a coloured component between a reagent and the polyphenols. In particular, the Folin-Ciocalteu reagent method is used in the determination of total polyphenols in foods [1–3]. However, nitrogenous bases, amino acids and proteins interfere with this method [4] and, moreover, it has less sensitivity than the Jerumanis method.

Flow injection analysis (FIA) allows us to carry out food analysis with good reproducibility, sampling frequency, selectivity and sensitivity. In addition, further advantages of FIA include a simple and inexpensive set-up [5]. The aim of this paper was the automation of total polyphenols analysis in apple juice and to compare the FIA results with those obtained by a batch method.

Materials and methods

Apple juice. The apple juice was obtained by means of a grating mill and pilot hydraulic press. Prior to analysis, the apple juice was suitably diluted with milli-RO water to fit the concentration in the linear range of the calibration graph.

Reagents. All reagents used were of analytical grade and were employed as supplied, without further purification. The Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany) and (-)-epicatechin, and chlorogenic, *p*-coumaric and tannic acids were purchased from Sigma (St. Louis, Mo., USA).

Instrumentation. The FIA apparatus used to analyse total polyphenols consisted of a Tecator FIAstar 5010 Analyzer, a 5023 spectrophotometer, a 5032 controller, a 5017 Sampler, a rheodyne injection valve and a TM II Chemifold. A diagram of the FIA apparatus employed is shown in Fig. 1.

Folin-Ciocalteu index. The Folin-Ciocalteu batch method is described as follows. The sample and Folin-Ciocalteu reagent were allowed to stand at 25° C for 3 min. Sodium carbonate (7.5%) was then added and the resulting mixture was allowed to stand at 70° C for 10 min. Finally, the coloured compounds were detected at 673 nm.

Fig. 1. Diagram of basic flow injection analysis (FIA) apparatus: *P* pumps; *A* autosampler; *C* carrier; *R* reagent; *V* injection valve; *C*₁, *C*₂ coils; *D* detector

Fig. 2. FIA configuration used for total polyphenol analysis. Carrier: Folin-Ciocalteu Reagent (1/100); Na_2CO_3 : 1%

Results and discussion

The chemical FIA variables (pH and reagent concentrations) were fixed to avoid the presence of CO_2 and the precipitation of tungsten and molybdenum salts, as reported by Peris et al. [6, 7]. The FIA configuration used is shown in Fig. 2. In order to find out the most suitable standard, the kinetic behaviour of standards and sample was studied. Chlorogenic and coumaric acids and (-)epicatechin are typical apple polyphenols, and tannic acid is most widely used in the batch methods. Based on our observations, chlorogenic acid was selected (similar kinetic behaviour to the standard and sample) to carry out the analytical study. The FIA variables, namely flow, channel length, sample volume and temperature were optimized. The ranges of the FIA variables studied are described in Table 1.

Table 1. Studied ranges of flow injection analysis (FIA) variables

Parameters	Range of values
Flow	0.6–1.0 ml/min
Channel length	300–1000 mm
Temperature	25–60° C
Injection volume	40–200 µl

Table 2. Features of the FIA method

Parameters	Values
Linearity range	0–105 ng/L chlorogenic acid
Correlation coefficient	0.9982
Reproducibility	<1%
Sampling frequency	48 h ⁻¹

Table 3. Analysis of the total polyphenols in 36 apple cultivars

Cultivar	FIA method (mg/L)	Batch method (mg/L)	ε
1	1706.41	1793.34	4.84
2	1385.89	1370.56	1.11
3	2267.30	2299.75	1.41
4	1722.44	1700.42	1.29
5	1209.61	1189.37	1.70
6	1097.44	1161.49	5.51
7	2571.79	2439.13	5.43
8	1321.79	1412.37	6.41
9	1738.46	1793.34	3.06
10	905.12	947.77	4.50
11	1690.38	1742.24	2.97
12	2010.89	2109.27	4.66
13	825.00	751.09	9.84
14	1417.95	1551.75	8.62
15	1546.15	1561.04	0.95
16	712.85	669.02	6.55
17	1754.48	1728.30	1.51
18	1097.44	1077.86	1.81
19	1783.53	1830.51	2.56
20	2363.46	2425.20	2.54
21	2075.00	2053.52	1.04
22	3389.10	3233.60	4.80
23	2683.97	2592.45	3.53
24	1321.79	1356.62	2.56
25	2587.82	2480.95	4.30
26	1594.23	1505.29	5.90
27	2828.20	2871.21	1.49
28	1658.33	1523.88	8.82
29	1946.79	1904.85	2.20
30	1417.95	1393.79	1.73
31	1466.02	1403.08	4.48
32	2427.56	2295.11	5.77
33	2139.10	2053.53	4.16
34	2155.12	2044.22	5.42
35	1754.48	1756.18	0.09
36	1482.05	1514.58	2.15

ε, relative error; FIA, flow injection analysis

When flow was reduced, a lower sampling frequency and a higher sensitivity was detected. This is in agreement with the well-known conclusion of the “tank-in-series” model, i.e. high flow produces a greater height equivalent to a theoretical plate and dispersion [8]. Likewise, an increase in the sensitivity can be produced with longer reaction time.

As regards channel length, no maximum was detected in the detector signal when the length was changed. This seemingly indicates that the kinetic process is very quick and it would be necessary to use shorter channel length to optimize the analytical signal. Therefore, dispersion plays a major role in the sensitivity observed when the length is increased.

As predicted, an increase in temperature gave rise to a higher analytical signal. However, over 40° C a strong degasification was observed, which appears to arise from the use of sodium carbonate. Finally, the injection volume was fixed at 40 µl, which allowed the development of an adequate sensitivity, good sampling frequency and lower sample consumption.

The features of the analytical method proposed are described in Table 2. Likewise, a good correlation between traditional batch and FIA methods was obtained ($r = 0.9924$) (Table 3), and an average error below 4%, between both methods, was determined.

The FIA method for the analysis of polyphenols in apple juice presented has good sensitivity, reproducibility, sampling frequency and correlation with the traditional batch method. Therefore, the automated method proposed could be suitable for controlling the polyphenols of the fruits employed in cider and apple-juice making.

Acknowledgement. This work was supported by the Comisión Interministerial de Ciencia y Tecnología (Project, ALI 89-0101-CO2-O1).

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