Determination of Total Polyphenols in Beers by Flow Injection Analysis

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ABSTRACT

Two automatic flow injection methods for the determination of total polyphenols in beers based on classical reactions (the Folin–Ciocalteu reagent method and the International Official method) are proposed. Their usefulness was tested by applying them to different beers. The results obtained agree with those provided by the corresponding batch methods.

INTRODUCTION

The determination of total polyphenols in beers is necessary to control its production and it is also useful as an indicator of the type of fermentation and ageing. There are several techniques, most of them being based on the formation of a coloured compound between a reagent and the polyphenolic fraction of the sample. Among them, the two most important ones are the Folin–Ciocalteu reagent method and the Jerumanis method. The former has a low sensitivity (Ribereau–Gayon, 1976) when the concentration of polyphenols is low (under $100 \, mg \, litre^{-1}$); in these cases, the Jerumanis method (De Clerck & Jerumanis, 1967) and the International Official method (Analytica EBC, 1987) are recommended for beers. Another difficulty related to the different methods is the selection of a suitable standard, due to the heterogeneity of the polyphenolic fraction of natural products.

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Flow injection analysis (FIA) has proved to be a very useful and versatile automation technique (Valcárcel & Luque de Castro, 1987). It has been widely used in food with good results (Lemieux *et al.*, 1989). However, in spite of its advantages, no method has so far been developed to automate the determination of total polyphenols in beers by FIA, though it has been done in wines and grape musts (Peris-Tortajada *et al.*, 1986; Buitrago *et al.*, 1986).

The purpose of the current work is to automate the determination of total polyphenols in beers by FIA by means of the Folin–Ciocalteu reagent method and the International Official method. This includes the comparison between the results and those obtained by the corresponding batch methods and the application to commercial samples chosen in the Spanish market.

MATERIALS AND METHODS

Materials and instrumentation

The following reagents were used:

Aqueous solution of sodium carbonate, 20% w/v.

Folin–Ciocalteu reagent (FCR) for analysis.

Carboxymethyl cellulose/ethylene diamine tetraacetic acid (CMC/ EDTA): 10 g of sodium carboxymethyl cellulose and 2 g of disodium ethylene diamine tetraacetic acid are added to distilled water with stirring and the solution is diluted to 1 litre with distilled water. The solution is freshly prepared each month.

Ferric reagent: 3·5 g of ammonium iron (III) citrate are dissolved in 100 ml of distilled water. The solution is freshly prepared each week.

Ammonia reagent: Concentrated ammonia ($d = 0.92\,\text{g ml}^{-1}$) is diluted 1:3 with distilled water.

All reagents were of analytical grade. Instruments used were, a Bausch and Lomb Spectronic 2000 spectrophotometer, equipped with a Hellma 178.12QS flow cell (inner volume 18 μl) and a Linseis L-600 recorder, a Gilson Minipuls-3 peristaltic pump, a Selecta 512 ultrasonic bath, a Rheodyne 5041 injection valve and a Tecator TM III chemifold.

Sample pre-treatment

Samples were degassed for 10 min in the ultrasonic bath and then suitably diluted with distilled water to fit the concentration into the linear range of the calibration graph (both methods).

RESULTS AND DISCUSSION

On account of their different characteristics, each proposed method is discussed separately. The procedure involved was as follows: selection of the most suitable standard; optimisation of the FIA (injected volume, reactor lengths, flow-rate) and chemical variables (reagent concentrations, pH), carried out by the univariate method; construction of the calibration graph and study of the reproducibility of the method.

Folin–Ciocalteu reagent method (A)

The FIA configuration used is shown in Fig. 1(a). The sample was injected into the Na_2CO_3 stream and later merged with the FCR, thus obtaining a homogeneous pH in the stream circulating through R_2, where the reaction took place, and the product was monitored at 750 nm. Table 1 shows the main features of this method. Precision was evaluated by injecting 11 samples of 50 mg litre^{-1} of coumaric acid in triplicate. The ranges over which the variables were studied and their optimum values are summarised in Table 2.

FIA variables
The optimisation was carried out using coumaric acid solutions of 150 ppm as samples, due to reasons that are explained later. Different sample volumes were tested and all of them produced acceptable analytical signals; the
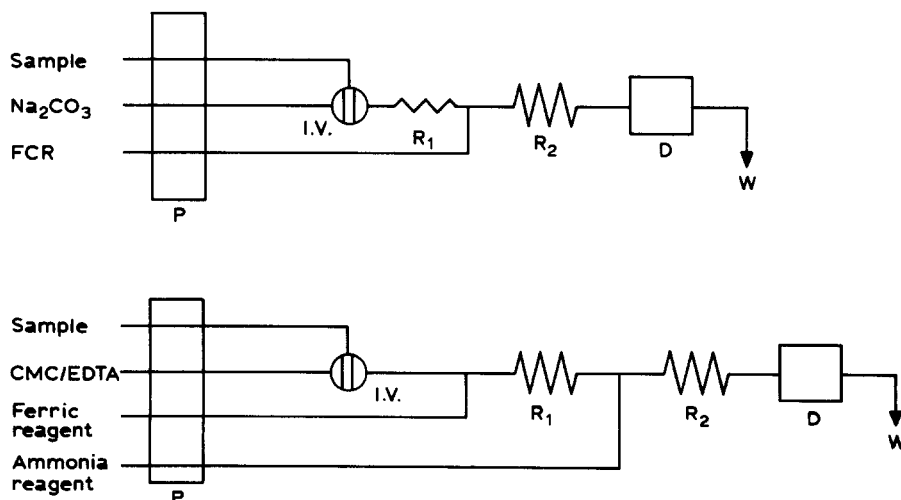
Fig. 1. (a) FIA configuration for the Folin–Ciocalteu reagent method; (b) FIA configuration for the International Official method. P, peristaltic pump; IV, injection valve; R_1 and R_2, reactors; D, UV/V detector; W, waste.

TABLE 1
Features of the Proposed Methods

Method A
  Linearity range: 0–100 mg litre^{-1} of coumaric acid
  Correlation coefficient: $r = 0.9922$
  Precision: RSD = 1.58 per cent
  Sampling frequency: 42 h^{-1}

Method B
  Linearity range: 0–100 mg litre^{-1} of coumaric acid
  Correlation coefficient: $r = 0.9987$
  Precision: RSD = 1.89 per cent
  Sampling frequency: 38 h^{-1}

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minimum volume was then chosen as optimum (19·7 μl) so as to avoid high sample consumption and to achieve good sampling frequency. Reactor lengths over the range between 30 and 300 cm were assayed, the most suitable one being 30 cm for R_1 and 200 cm for R_2. As regards flow-rates, the range studied was between 0·3 and 1·6 ml min^{-1}, 0·8 being chosen as a compromise between sensitivity (low flow-rates) and sampling frequency (high flow-rates).

Chemical variables
Two difficulties arose at this point, both of them related to the pH value of the medium. First, if the pH was high (over 10·5) (Na_2CO_3 and FCR concentrations given by the batch method), a precipitate appeared hindering measurements and obstructing the tubes. Obviously, a lower Na_2CO_3 concentration was necessary to avoid this. Besides, the FCR concentration was also found to be determinative concerning the appearance of a precipitate. On the other hand, if the pH of the reagent mixture was lower than 4, the presence of CO_2 was unavoidable, as a consequence of the reaction between the carbonate ions and the acid of the FCR. The compromise solution consisted of working at lower Na_2CO_3 levels (15, 10, 5 and 1% w/v). With a 1:100 dilution of the FCR the best results were obtained (no precipitate and a good analytical signal), the final dilution of the original Na_2CO_3 solution being 1:20 (concentration = 0·94 M).

    Other alkalising reagents such as NaOH and NH_4OH were also tested in an effort to avoid the difficulties concerning the use of Na_2CO_3. No better results were obtained.

Kinetic behaviour of standards and samples
According to the bibliography, no universal standard is available for any problem, owing to the fact that the composition and proportion of

TABLE 2
Ranges Studied and Optimum Values of the Variables

Method	Variable	Range studied	Optimum value
A	Injection volume/μl	19·7–170	19·7
	Flow-rate/ml min^{-1}	0·5–1·3	0·8
	Length R1/cm	20–100	30
	Length R2/cm	60–300	200
	[Na$_2$CO$_3$], per cent m/V	0·5–10·0	1·0
	[FCR]	C/50–C/200	C/100
B	Injection volume/μl	19·7–150	19·7
	Flow-rate/ml min^{-1}	0·40–1·50	0·75
	Length R1/cm	30–150	60
	Length R2/cm	60–300	200
	[CMC-EDTA]	C/10–C	C
	[Ferric rgt.]	C/20–C	C/20
	[NH$_4$OH], per cent v/v	0·5–5·0	1·0

polyphenols in samples is variable. Gallic acid is, however, the most widely used in the official methods.

In FIA we have noticed that gallic acid for beers gives results which differ considerably from those obtained with the batch method. Different measuring times are undoubtedly responsible for these discrepancies. To find out the most suitable one, different polyphenol standards were selected: gallic acid, coumaric acid, ferulic acid, catechin, quercetin and 2-hydroxybenzoic acid. Ferulic acid and quercetin were discarded because of their low solubility in the working medium and nearly null analytical signal in the FIA conditions. In all the other cases 100 mg of each standard were dissolved and diluted to 1 litre with distilled water.

The procedure involved in the kinetic study was as follows: the flow was stopped when the highest FIA signal was reached, obtaining then a graphic record which corresponds to the absorbance increase until the signal became stable. With reference to the beer, the most similar kinetic behaviour turned out to be that of the coumaric acid. Therefore, coumaric acid was selected as standard for the Folin–Ciocalteu reagent method.

International official method (B)

The FIA configuration used is shown in Fig. 1(b). The sample was injected into the CMC-EDTA stream and later mixed with the ferric reagent. After this merging point, there is another confluence point for the ammonia

reagent. The compound formed along the last reactor shows maximum absorption at 600 nm.

Table 1 shows the main features of this method. The precision of the method was studied on eleven different samples of 50 mg litre^{-1} of coumaric acid injected in triplicate. As in the other method, the optimisation was carried out with aqueous solutions of coumaric acid. The studied ranges and optimum values of the variables are shown in Table 2.

FIA variables
Because of the slight influence of the injection volume on the analytical signal, a small volume (that of the injection valve without loop) was considered optimum. The length of R_1 chosen was the minimum required to ensure a suitable connection. A length of R_2 of 200 cm yielded the maximum signal; shorter lengths resulted in insufficient reaction development, while greater lengths gave rise to decreased signals as a result of the effect of dispersion exceeding the increase due to the evolution of the reaction. Finally, high flow-rates gave rise to decreased signals resulting from higher viscosities due to the CMC-EDTA. To avoid long residence times of the reacting plug in the reactor, a compromise value of 0·75 ml min^{-1} was chosen.

Chemical variables
High concentrations of the ferric and ammonia reagents gave rise to bubbles and precipitate, respectively; thus in both cases dilution was necessary. Optimum values (Table 2) are referred to those stated under 'Materials and Methods' which are denoted by C in this table.

Kinetic behaviour of standards and samples
The procedure involved for the selection of the standard was the same as the one described above (see the other method). Coumaric acid was also the compound whose kinetics were the most similar to those of the analysed beer, it being chosen as standard for this method.

Application of the methods to commercial samples

The proposed methods were applied to different beer samples (Table 3) whose content of polyphenols had previously been determined by the corresponding batch methods. Representative Spanish and foreign brands were selected and each sample was analysed in triplicate. The results obtained—average values of three cans of each beer—are shown in Table 3.

It can be seen that the FCR method gives higher values than the official

TABLE 3
Application of the Methods to Commercial Samples of Beer

Beer	*Alcoholic grade*	*Per cent dry extract*	*Method*[a]			
			A (FIA)	*A (Batch)*	*B (FIA)*	*B (Batch)*
Adlerbrau	5·5	13	42	41	33	39
El Aguila	4·5	11	36	38	27	28
Cruz Campo	5·4	13	42	41	28	35
Golden Turia	5·4	13	42	45	28	33
Mahou	5·5	13	40	39	32	37
San Miguel	5·4	13	40	47	29	34
Skol	5·5	13	41	45	26	35
Voll-Damm	7·2	17	51	49	42	45
Carlsberg	5·5	13	37	42	27	25
Hannen Alt	5·0	12	47	50	36	46
Heineken	5·0	12	36	38	26	30
Guinness	5·0	12	72	82	63	66
Kronenbourg	4·6	11	43	44	28	29
Eichbaum	5·0	12	44	47	32	31

[a] Concentration, mg litre^{-1} (coumaric acid).

method in FIA as well as in the batch procedure. Batch and FIA results are quite similar in the FCR method whereas in the other method batch values generally exceed those obtained by FIA.

CONCLUSIONS

Satisfactory results in the determination of the content of total polyphenols in commercial samples of beer were obtained by the two proposed methods, both of them having a high reproducibility.

These two FIA methods are straightforward with good sampling frequencies, low sample and reagent consumption and time saving (always referred to the corresponding batch methods). They do not require expensive instrumentation and their features make them suitable for adaptation to analysis and quality control laboratories.

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REFERENCES

Analytica (1987). *European Brewery Convention* (4th edn) Method 9.1.1, London.

Buitrago, J., Cela, R. & Pérez-Bustamante, J. A. (1986). Determinación automatizada del índice de polifenoles totales en vinos blancos mediante análisis por inyección en flujo. *Afinidad*, **XLII, 406**, 530–6.

De Clerck, J. & Jerumanis, J. (1967). *Bull. Anc. Etud. Brass. Louvain*, **63**, 137.

Lemieux, L., Puchades, R. & Simard, R. E. (1989). Application of FIA-techniques to food analysis. Lebensm.-Wiss. u. Technol., **22**, 254–63.

Peris-Tortajada, M., Pérez, M. & Maquieira, A. (1989). Automated determination of total polyphenols by means of the Folin–Ciocalteu reagent. *Química Analítica*, **8**, 211–22.

Ribereau-Gayon, P., Peynaud, E. & Sudrau, P. (1976). *Traité D'enologye. Sciences et Technique du Vin. Vol. 1. Analyse et Controle des Vins*, Bordas, Paris, pp. 471–513.

Valcárcel, M. & Luque de Castro, M. D. (1987). *Flow Injection Analysis: Principles and Applications*. Ellis Horwood, Chichester, UK.