Food Chemistry **47** (1993) 201–204
Analytical Methods Section

A new flow-injection spectrophotometric method for the determination of tannins in tea and beer using iron(III) and 1,10-phenanthroline

Consuelo Tomàs, Mercedes Celeste, Andreu Cladera, Enrique Gómez, José Manuel Estela & Víctor Cerdà

Departament de Química, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain

(Received 6 June 1992; revised version received 8 November 1992; accepted 10 November 1992)

A new flow-injection spectrophotometric method for the determination of tannins in tea and beer based on the reduction of Fe(III) to Fe(II) by tannins and the subsequent formation of the coloured complex Fe(II)-1,10-phenanthroline is proposed. The FIA set-up used is controlled by a personal computer running appropriate software for automatic sample injection and data acquisition and processing. The calibration curve obtained is linear between 0–30 mg litre^{-1} gallic acid and the relative standard deviation for a standard containing 10 mg litre^{-1} gallic acid is 0·7% (n = 10). The sample throughput achieved is 60 sample h^{-1}. Unlike the batch method, on which it is based, the proposed method requires no sample pretreatment in order to avoid interferences; it only involves appropriate dilution of the samples, and so provides more reliable results in a substantially shorter time.

INTRODUCTION

The determination of the total polyphenol content of beverages is a common practice in agricultural food industries. Some products, such as beer and tea, contain a large variety of polyphenolic compounds with similar chemical features, which hinders their individual determination and compels analysts to quantify them jointly as the so-called 'total polyphenol number' by comparing the analytical response of the sample to a calibration curve constructed from a standard polyphenol (usually tannic or gallic acid). Hence the result obtained is an average of the different analytical responses of the phenol compounds in the unknown sample, and the total polyphenol number is an arbitrary parameter representative of the tannin content and of great significance for characterization purposes. Nevertheless, the meaning of 'tannin' depends upon the analytical method being used because there is no universally accepted definition of the word.

The total polyphenol number may be determined by a number of methods, which can be classified according to the type of reaction involved (Deshpande *et al.*, 1986), namely: (a) redox reactions (e.g. the Folin—Ciocalteau, Jerumanis and Lowenthal methods); (b) precipitation reactions with organic (polyamides, gelatin, polyvinylpyrrolidone) and inorganic (aluminium, zinc, lead or copper salts) compounds; (c) condensation reactions (e.g. the vanillin reaction); and (d) enzymatic reaction.

In this paper a redox reaction has been selected to measure the total polyphenol number. Tannins reduce Fe(III) to Fe(II), which subsequently reacts with 1,10-phenanthroline to form a coloured complex with maximum absorbance at *c.* 510 nm. This reaction is the basis for a batch method (Lau *et al.*, 1989) in which the sample is subjected to an additional treatment in order to assess the effect of potential interferents on the analytical signal. Thus the tannins in the sample are precipitated with gelatin (Onishi & Hara, 1964) and the effect of other reductants remaining in the sample is measured.

In this work, a fully automated flow-injection method has been developed which avoids the effect of interferents, and thus requires no sample pretreatment, thereby avoiding potential sources of error and reducing the analysis time.

EXPERIMENTAL

Reagents

The solutions used in this work included the following:

—250 mg litre^{-1} gallic acid (GA), prepared from the monohydrate product (Fluka, Buchs, Switzerland, HPLC grade).

Food Chemistry 0308-8146/93/$06.00

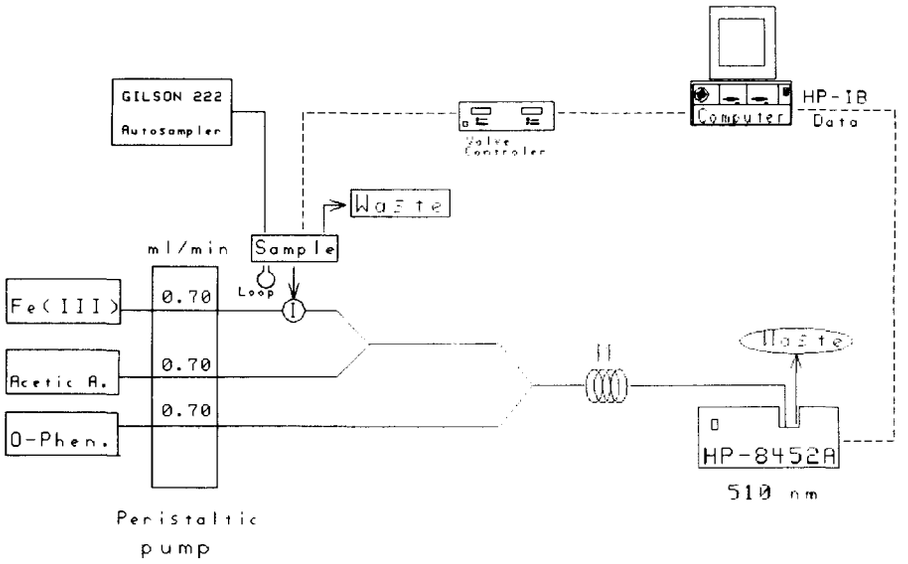

Fig. 1. Scheme of the FIA manifold used. I = injection valve (105 μl), M = mixing loop (0·5 m) HP-IB = Hewlett-Packard-Interface Bus.

—0·015 mol litre^{-1} 1,10-phenanthroline, prepared from its hydrochloride (Merck, Darmstadt, Germany).
—0·01 mol litre^{-1} Fe(III), made by dissolving the required amount of $FeCl_3 \cdot 6H_2O$ in 0·4 mol litre^{-1} HCl.
—0·5 mol litre^{-1} acetic acid, pH 3·5 to which was added 0·01% polyvinyl acid as surfactant in order to minimize the memory effects arising from adsorption on tube and reactor walls.

All the above solutions were made in distilled water from a.r. grade chemicals unless otherwise stated.

Apparatus and software†

Figure 1 depicts the instrumental set-up used. The FIA manifold consisted of a Gilson (Villiers-le-Bel, France) Minipuls-3 eight-channel pump, a Rheodyne (Cotati, California, USA) 50 injection valve that was controlled by the computer via a customized mechanical actuator, and PTFE tubing of 0·5 mm i.d. for the injection loop and the reactors. Signals were measured by a Hewlett-Packard (Waldbronn, Germany) HP 8452A diode array spectrophotometer furnished with a flow-cell of 18 μl volume and 1 cm pathlength, and interfaced to a PC computer. Samples were injected via a Gilson Sample Changer 222 sampler. Data aquisition and treatment and instrumental control were commanded by the programme DARRAY (Cladera *et al.*, 1991), which was previously developed at our laboratory

Reference method

We used the method reported by Lau *et al.*, (1989) which has been contrasted with the reference method based on the well-known Folin—Denis reaction (Bajaj & Devsharma, 1977; Horwitz, 1980). This latter method requires the background signal to be corrected by precipitating tannins with gelatin in order to obtain reliable results.

† The software used can be obtained on request from SCI-WARE. Banco de Programas, Departament de Química, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain.

Procedure

Preparation of samples

Beer samples were degassed prior to analysis. An amount of 0·500 g of tea was extracted into 100 ml of boiling distilled water for 1 h, after which the mixture was allowed to cool and filtered, and the filtrate was made to 250 ml with further distilled water.

Determination

The sample to be analysed was injected into a stream of 0·01 mol litre^{-1} Fe(III) via a 105-μl loop (Fig. 1). and was subsequently merged with a stream of 0·5 mol litre^{-1} HAcO/NaAcO at pH 3·5 and then with another containing 0·015 mol litre^{-1} 1,10-phenanthroline. The reaction mixture was then passed through a mixing

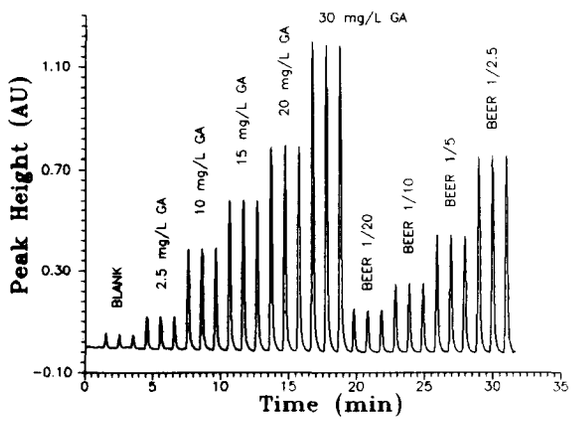

Fig. 2. FIA recording corresponding to a calibration graph run from gallic acid (Fluka) and different dilutions of a beer sample obtained by the proposed method.

loop. The flow-rate was set to 0·7 ml min^{-1} for all channels. Signals were acquired by monitoring the reaction spectrophotometrically; each point was taken as the difference between the readings at the wavelength of maximal absorption (510 nm) and those at the wavelength of zero absorption (680 nm). The data thus acquired were processed by the software in order to calculate the corresponding analyte concentrations.

Once the system had stabilized, a calibration graph was constructed from standards containing 0–30 mg $litre^{-1}$ gallic acid. Then, tea and beer samples were analysed after appropriate dilution (1:10 and 1:20) with distilled water. The sampling rate thus achieved was 60 samples h^{-1}. A typical FIA recording is shown in Fig. 2.

RESULTS AND DISCUSSION

In order to assure the best possible performance from the proposed method, chemical and FIA variables were optimized by the Univariate method.

Chemical variables

The influence of the Fe(III) concentration was investigated by using solutions of the same acidity (0·4 mol $litre^{-1}$ HCl) containing between 0·001 and 0·075 mol $litre^{-1}$ Fe(III). Both the peak height and the blank signal were found to increase with increase in the Fe(III) concentration. An intermediate iron concentration (0·01 mol $litre^{-1}$) was chosen as a compromise.

The 1,10-phenanthroline concentrations tested ranged between 0·001 and 0·1 mol $litre^{-1}$. The signal increased with increasing concentration of this reagent, but saturated above 0·01 mol $litre^{-1}$. On the other hand, increased concentrations also resulted in peak tails and irreproducible signals. A 0·015 mol $litre^{-1}$ concentration of 1,10-phenanthroline was chosen as optimal.

One of the channels was originally used to circulate an acetic acid/acetate buffer of pH 3·5–5. Increasing pH values in this range resulted in peaks of increasing width and constant height. A pH of 5 gave rise to double peaks. On the other hand, increasing concentrations of the buffer resulted in increasing peak width and decreasing height. Also, using water or acetic acid alone provided narrow, lower peaks. A stream of 0·5 M HAcO/NaAcO at pH 3·5 was therefore used.

FIA variables

Several injected samples volumes (50, 75, 105, 130 and 190 μl) were tested. Both the signal and the peak width were found to increase with increase in the sample volume, so 105 μl was chosen as optimal in order to accomplish a high sampling frequency and an acceptable analytical signal.

The best reactor arrangement was found to consist of a single one placed immediately before the spectrophotometer cuvette. Also, of the different reactor lengths tested (0–2 m) 0·5 m was selected, as longer lengths resulted in major peak broadening.

The temperature was found to have little influence at the chosen reactor length, so the reaction was conducted at room temperature.

Interferences

Sulphur dioxide and ascorbic acid were found to interfere with the determination of polyphenols by the proposed method on account of their reducing character. The tolerated concentrations of these two species in the determination of 10 mg $litre^{-1}$ gallic acid (error < 10%) were 2 mg $litre^{-1}$ SO_2 and 3 mg $litre^{-1}$ ascorbic acid, respectively. Therefore, the proposed method is unsuitable for samples containing these species (e.g. wine). On the other hand, it is indeed perfectly applicable to such samples as tea and beer, which normally contain very low or nil concentrations of these species. As far as reducing sugars are concerned, glucose was tolerated at least up to 1000 mg $litre^{-1}$ for 10mg $litre^{-1}$ gallic acid.

Application to real samples

The calibration curve obtained under the above-described conditions conformed to the following equation:

Peak height (AU) = $A + B$ [Standard] (mg $litre^{-1}$) (r = 0·9996) (n = 5) where AU = absorbance units, A = 0·047, B = 0·031 and [Standard] = 0–30 mg $litre^{-1}$. The relative standard deviation for a standard con-taining 10 mg $litre^{-1}$ gallic acid was 0·7% (n = 10). The equation of the calibration curve for the batch method is defined by

$$\text{Absorbance (AU)} = A + B\,[\text{Standard}]\ (\text{mg litre}^{-1}) \quad (r = 0.9997)\ (n = 5)$$

where A = 0·032 and B = 0·470 with an applicability range of 0–2 mg $litre^{-1}$ for the same standard.

The proposed method was applied to real samples for comparison with its batch counterpart. Thus, five samples of tea and another five of beer were analysed as described under Procedure and subsequently diluted with distilled water in the following proportions: 1:2·5, 1:5, 1:10 and 1:20 (beers), and 1:5, 1:10 and 1:20 (teas). The results were found to depend upon the dilution used: the higher this was, the greater was the polyphenol number obtained. The beers behaved similarly in this respect, whereas the teas were less markedly affected. These results materialized as a

Table 1. Recoveries obtained at different dilutions of a beer sample (Carlsberg)

Dilution	GA added (mg $litre^{-1}$)	GA found (mg $litre^{-1}$)	Recovery (% $litre^{-1}$)
1 : 20	0	3·00	—
1 : 20	5	7·72	96·5
1 : 20	10	12·30	94·6
1 : 10	0	5·37	—
1 : 10	10	15·00	97·6
1 : 10	20	24·20	95·4
1 : 5	0	8·95	—
1 : 5	10	17·33	91·5
1 : 5	20	25·53	88·2

Table 2. Recoveries obtained at different dilutions of a tea sample (Compañía de la Indias)

Dilution	GA added (mg litre^{-1})	GA found (mg litre^{-1})	Recovery (% litre^{-1})
1 : 20	0	4·83	—
1 : 20	5	9·91	100·8
1 : 20	10	15·05	101·5
1 : 10	0	10·10	—
1 : 10	10	20·75	103·2
1 : 10	20	30·50	101·3
1 : 5	0	19·75	—
1 : 5	10	25·15	84·5
1 : 5	20	34·60	87·0

variation in the slope of the standard-addition curves, which were only parallel to the calibration curve at dilutions higher than 1 : 10. Tables 1 and 2 give the analytical recoveries obtained for two additions of standard to variously diluted tea (Compañía de Indias) and beer (Carlsberg)—the other beer and tea brands behaved very similarly in this respect. As can be seen, recoveries were quite satisfactory (*c.* 100%) for dilutions above 1 : 10, which were therefore chosen as optimal for application of the proposed method.

Tables 3 and 4 show the results provided for tea and beer samples by the proposed automatic method and the reference batch method without and with background correction. As can be seen, the proposed FIA method provides results comparable with those yielded by the batch method with background correction. Also, the proposed method is virtually free of the interferences from proteins, sugars, etc. that affect the reference batch method. This avoids the need to precipitate tannins with gelatin in order to measure the background signal, which is time-consuming and error-prone. The fact that the species mentioned above do not interfere with the proposed method may result from their following different kinetics to tannins for Fe(III) reduction; hence, if a short fixed-time kinetic method is used, the extent of reaction of the interferents will be insufficient to exert an appreciable effect on the analytical signal at reading under the set conditions.

Table 3. Results obtained for various tea samples (n = 4), expressed as percent weights of gallic acid (σ)

Sample	FIA method	Batch method	Batch method[a]
Brooke Bond	4·2 (0·2)	5·8 (0·1)	8·1 (0·1)
Hornimans	3·7 (0·1)	4·7 (0·1)	6·9 (0·1)
Lipton	5·4 (0·3)	6·7 (0·1)	9·1 (0·1)
Compañía de las Indias	4·9 (0·1)	5·7 (0·1)	7·9 (0·1)
Twinings	5·2 (0·3)	5·9 (0·1)	8·0 (0·1)

[a] Without background correction using gelatin.

Table 4. Results obtained for various beer samples (n = 3), expressed as mg litre^{-1} of gallic acid (σ)

Sample	FIA method	Batch method	Batch method[a]
Mahou	38·4 (1·6)	40·0 (1·8)	138·5 (0·1)
San Miguel	47·2 (0·5)	36·8 (1·9)	118·8 (1·9)
Carlsberg	49·1 (0·2)	42·2 (1·9)	117·1 (1·9)
Henninger	52·0 (0·4)	40·8 (0·2)	137·9 (0·2)
Heineken	46·7 (0·6)	33·4 (0·2)	122·3 (0·2)

[a] Without background correction using gelatin.

CONCLUSIONS

The proposed FIA method for the determination of the polyphenol number in tea and beer samples provides results comparable to those afforded by the batch method involving background correction by precipitation with gelatin on which it is based and is free of some of the usual interferences present in these types of sample. Tea samples provide more divergent results; however, because of the arbitrary nature of the number, it can equally be valid to compare and classify brands.

The proposed method features the typical advantages of fully automated processes, viz, a high sample throughput and reproducibility, the need for no intermediate manipulations and a low reagent consumption.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the DGICyT (Spanish Council for Research in Science and Technology) for financial support granted for the realization of this work as part of Project PB 90–0359.

REFERENCES

Bajaj, K. L. & Devsharma, A. K. (1977). A Colorimetric method for the determination of tannins in tea. *Mikrochim. Acta*, **11**, 249–53.

Cladera, A., Gómez, E., Estela, J. M. & Cerdà, V. (1991). Determination of iron by flow injection based on the catalytic effect of the iron(III)-ethylenediaminetetraacetic acid complex on the oxidation of hydroxylamine by dissolved oxygen. *Analyst*, **116**, 913–17.

Deshpande, S. S., Cheryan, M. & Salunkhe, D. K. (1986). Tannin analysis of food products. *CRC Rev. Food Sci. Nutr.*, **24**, 401–49.

Horwitz, W. (ed.) (1980). Official Methods of Analysis of the Association of Official Analytical Chemists, 13th edn. Association of Official Analytical Chemists, Arlington, VA, Section 9.100.

Lau, O.-W., Luk, S.-F. & Huang, H.-L. (1989). Spectrophotometric determination of tannins in tea and beer samples with iron(III) and 1,10-phenathroline as reagents. *Analyst*, **114**, 631–3.

Onishi, I. & Hara, T. (1964). Spectrophotometric determination of a small amount of L-ascorbic acid with the iron(III)-1,10-phenanthroline reagent. *Bull. Chem. Soc. Jpn*, **37**, 1314–17.