APPLY A NEW VALVE FOR FLOW INJECTION TECHNIQE TO DETERMINE THE TOTAL PHENOLS AND ANTIOXIDANT ACTIVITY IN WHEAT (*Triticum aestiruml*)

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Abstract

In this study, a new design valve was used to estimate the total phenols and antioxidant activity based on continuous flow injection analysis was developed for the assessment with Folin-Ciocalteu reagent (FCR) reducing capacity in several types of food products using gallic acid as the standards. Solution for mixing of sample and reagent were tested (continuous flow of FCR, merging zones, and intercalated zones approaches); lower reagent consumption and higher determination throughput were attained for the merging zones approach. The application of the proposed method to pure compounds and wheat extract provided results with detection limit was 0.05 mg L^{-1} for both standards, and the determination frequency was about 55 h^{-1}, good repeatability was attained (RSD < 2.48%, n = 10) for gallic acid, whereas 120 h^{-1}, (RSD 0.65, n = 10) for ascorbic acid. Highest total phenols in wheat extract was (2.50 mg/g GAE, 2.0 mg/g GAE) for using FIA-FCR, FIA-UV detection respectively. Development of FIA-DPPH, FIA-FRAP system were applied for determination of radical scavenging activity RSA%, contents of antioxidant compounds as well as, the reducing power activity RPA% of obtained wheat extract has been investigated. Radical scavenging activity was found to exhibit IC_{50} value for extract concentration of 7.6 µg/mL DPPH, $1/IC_{50}$ 0.329 µg/mg TP GAE. The reducing power RP was 99.9 mg RP / g wheat. All investigated methanolic wheat extract possess reductive capabilities.

تطبيق صمام جديد في تقنية الحقن الجرياني يستعمل لتقدير الفينولات الكلية وفعالية مضادات الاكسدة في الحنطة

الملخص

تم في هذه الدراسة تصميم صمام جديد يستعمل في تقدير الفينولات الكلية ومضادات الاكسدة مبنيا على تقنية الحقن الجرياني، طور هذا الصمام ليكون متلائم مع كاشف فولن (FCR) لتقدير القدرة الاختزالية لانواع متعددة من الغذاء باستعمال حامض الكاليك كمحلول قياسي. اختبرت تقنية الحقن الجرياني المستمر المناطق المتداخلة مع كاشف فولن وقد تميزت بالاستهلاك القليل للكاشف مع سرعة نمذجة عالية. التطبيق لهذه التقنية كان للمركبات النقية اضافة الى مستخلص الحنطة اعطت نتائج بحد كشف (0.05 mg L^{-1}) لكلا المحلولين القياسيين مع سرعة نمذجة 55 نموذج في الساعة، التكرارية كانت جيدة بمعامل الانحراف النسبي (RSD < 2.48%, n = 10) لحامض الكاليك، بينما كان 120 نموذج في الساعة باستعمال تقنية FIA-UV بمعامل انحراف نسبي (RSD 0.65, n = 10) لحامض الاسكوربيك. اعلى محتوى للفينولات وجدت عند (2.50 mg/g GAE, 2.0 mg/g GAE) باستعمال تقنيتي FIA-FCR, FIA-UV على التوالي. ثم طورت هذه التقنيات

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FIA-DPPH, FIA-FRAP لتتناسب مع تقدير فعالية مضادات الاكسدة RSA% مع القدرة الاختزالية RPA% للمركبات الفينولية في مستخلص الحنطة حيث كانت IC_{50} = 7.6 µg/mL DPPH وال IC_{50} = 0.329 µg/mg TP GAE . اما $1/IC_{50}$ القوة الاختزالية فقد كانت RP = 99.9 mg RP / g حنطة، وقد بينت كل الفحوصات التي اجريت على مستخلص الحنطة لها قدرات اختزالية.

Introduction

Wheat or Triticum aestiruml is one of the world's three most important cereals. Most wheat grain is used as wheat flour and its products are recognized as an important source of essential nutrients. These nutrients are located in various parts of the grain (Yuzhong *et al*, 2011). Wheat grain is an excellent raw material for healthy and tasty foods. Like other cereals, it provides carbohydrate and protein as well as a variety of micronutrients, in particular certain B vitamins, vitamin E, and minerals. The bran fraction contains the highest amount of phenolic compounds within the grain that are attached to the indigestible cell wall polysaccharides of the fiber. One hypothesis is that these phenolic compounds, acting as antioxidants, play an important role in the protective effect of whole-grain consumption. Rye breads provided higher antioxidant capacity (TEAC, PRSC, DPPH RSA, and FCR) than wheat bread (C. Martinez-Villaluenga *et al*, 2009; Nuria, 2010). Currently, the worldwide cereal-based food production is based mainly on wheat grain processing and, to a smaller extent, on rye-based products. An alternative to those commonly cultivated and consumed wheat and rye-based food could be processed spelt grain (Henryk *et al*, 2008). Recently, epidemiological studies have shown that the consumption of whole grains and grain-based products is associated with the reduced risk of oxidative-stress related chronic diseases and age-related disorders, such as cardiovascular diseases, carcinogenesis, and the mammalian cells possess intracellular defenses such as superoxide dismutase, catalase or glutathione peroxidase, in order to protect the cells against excessive levels of free radicals (Isabel *et al*. 2007). Parts of the health benefits of whole grain flours are attributed to the presence of antioxidants. In addition to the most common antioxidants, such as vitamin C, vitamin E (tocopherols and tocotrienols) and carotenoids, grains also contain some phyto-antioxidants, including phenolic acids and flavonoids. Polyphenols represent group of compounds that have more than one phenolic hydroxyl group attached to one or more benzene ring (Jiri *et al*. 2013). Several methods using simple SIA with CL detection for the measurements of antioxidant activity against hypochlorite ion and have successfully applied the method to the evaluation of several antioxidants (Aoi *et al*, 2006). Therefore, the main objective of the present work was the development of high-throughput and automatic methods for the assessment of TAC in biological samples using (FIA) mode with FCR, DPPH, FRAP assays, after the study of reaction conditions and application to compounds with known antioxidant activity. Phenol itself does not act as an antioxidant, but substitution of bulky alkyl groups into 2-, 4- and 6-positions increase the electron density on the hydroxyl group by an inductive effect and thus increase hydrogen donation ability,

such as BHA. The effective antioxidant activity of BHA is due to the strong electron donating potency of its methoxy substituent. However, methylation of the hydroxyl groups eliminated the antioxidant activity effects, indicating that the antioxidantive effect is correlated to the hydroxyl groups (Yong, 2007). A schematic diagram of the flow system with single line is shown in Fig.1.

Materials

All chemicals and reagents were of analytical grade and used without further purification. Folin Ciocalteu (EC)-No 127212008), DPPH free radical (2,2-Di(4-*tert*-octylphenyl)-1-picrylhydrazyl) (CAS-No 84077-81-6), gallic acids (CAS-No 149-91-7), ascorbic acid (CAS-No 50-81-7), Sodium carbonate (CAS-No 497-19-8), potassium ferricyanide (CAS-No 13746-66-2), trichloro acetic acid (TCA) (CAS-No 76-03-9), ferric chloride (CAS-No 7705-08-0), disodium hydrogen phosphate (CAS-No 7558-79-4) and sodium dihydrogen phosphate (CAS-No 7558-80-7) were purchased from Sigma–Aldrich (Germany). The polyphenol stock solutions were prepared by dissolving an appropriate amount of the compound in 80% methanol (Environmental grade, Alfa Aesar, Barcelona, Spain). All the stock solutions are keep away from the light and stored for not more than 48 hours. Diluted solutions were prepared daily from these stock solutions. Wheat sample was purchased from local market.

Apparatus

Flow injection analysis set up consisted of; a peristaltic pump (model ISM 834; Ismatec, Germany) connected to low-pressure homemade valve which contains ten ports with three loops. Centrifuge (EBA 20 Hettich, 5000 rpm, Germany), shaker water path (Memmert, Germany), balance (Denver instrument, Germany), Heater (ardeas 51, Germany). Measurements were carried out using a double-beam spectrophotometer APEL PD-303UV detector with flow cell 394 µL (Japan). Recorder (Siemens C 1032, Germany). UV-VIS spectrophotometer (Shimadzo 1800, Japan). Valves were made in the laboratory, while the reaction coil was made in a glass workshop.

Design of valve

A simple innovated homemade valve of FIA system (Fig. 2.) was used for all experiments.

Select one loop

In case of using a single loop (L), we can calculate the volume carefully through the injection L_3 individually, and close of the sub-valves SV2 and SV7 of the point (b) and opened towards (a) & (c), either the rest of the sub-valves must be closed off (c) and opened toward the (a) & (b). The process injection upload component (ascorbic acid and kiwifruit extract) on L_3 as shown in Fig. 1(A). After that the washing process take place, through the close of all sub-valves towards c, and open towards (a) & (b) as shown in Fig. 4. Injection process component to flow cell conducts through close SV2 & SV7 towards (b) and open towards (a) & (c) and the sub-valves SV3 and SV8, it shuts off (a) and open towards (c) & (b) as shown in Fig. 1(D).

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Selection of two loops

The antioxidant activities of standard and sample solutions were estimated according to FIA-DPPH method. For loading of two components (gallic acid and DPPH assay) into two loops, choosing loops (L_3, L_2) because they are close to each other and they have the same distance from the mixing point, which makes the process more efficient. The process of loading DPPH solution on L_3 was carried out to close the sub-valves SV2 & SV7 towards b and open towards (c-a), other sub-valves were opened towards (a-b) and closed towards the (c) Fig. 1(A), while the gallic acid was loading on L_2 with the same processes of loading L_3 Fig. 1(B). The washing process of the remains of the components inside the cavity of main valve; all sub-valves closed towards (c) and opened towards (a-b), except SV5 was closed towards (a) and opened towards (c-b), and SV3 was opened at all directions (a-b-c) as a Fig.4. Injection process takes place after the close of SV2, SV7 towards (b), and open towards (a-c), and SV4, SV9 were closed toward (a) and opened toward (b-c) and SV3, SV8 opens at all directions (a-b-c). Then allow to the carrier stream to pass through the valve to introduce the components to flow, these processes were shown in Fig 1(C).

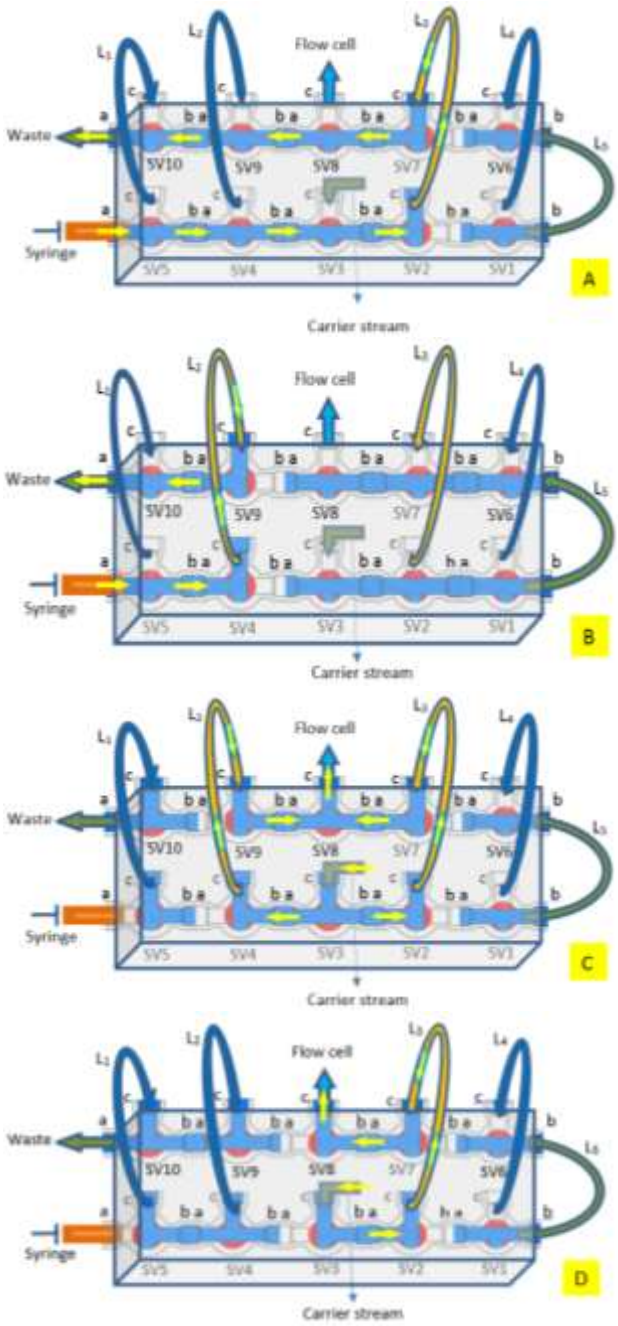

Fig. 1. The process of selection one and two loops for inject the sample and reagent, A loading reagent on L_3, B loading sample on L_2 and C, D were shown passing the carrier stream through the valve to introduce the components into the flow cell.

Selection three loops

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The processes for injection of three components to flow cell, were done to load three loops (L_4,L_3,L_2) with three components (gallic acid on L_2, FCR on L_3, buffer solution on L_3 when used FIA-FCR and $FeCl_3$ on L_2, ascorbic acid on L_3, P.B.T mixture on L_4 when used FIA-FRAP). To load a third component on L4, the sub-valve SV1, SV6 are closed towards (b) and open them towards (a-c). All the others sub-valves are closed towards (c) and open them towards (a-b). In the same way the loading of L_3 with the second component by closing of SV2, SV7 towards (b) and open them towards (a-c), and loading L_2 with the first component by the close of SV4, SV9 towards (a) and opening them towards (b-c). The rest of the valves must be keep closed towards (c) using syringe to inject the components on loops, these processes as shown in Fig. 2 (D,E). The washing process for the remaining of the components inside the cavity of the main valve. This process was conducted after loading of all components. All sub-valves must be close towards (c) and open towards (a-b), except SV5 must be closed towards (a) and open towards (c-b), and keep SV3 open at three directions (a-b-c) as shown in Fig. 4. The injection process takes place after the closure of SV1, SV6 towards (b), and open it towards (a-c). SV4, SV9 must be close towards (a) and open towards (b-c), SV3, SV8 are open to three directions (a-b-c), and then the carrier stream is allowed to pass through the main valve to introduce the components to flow cell, as shown in Fig. 2 E.

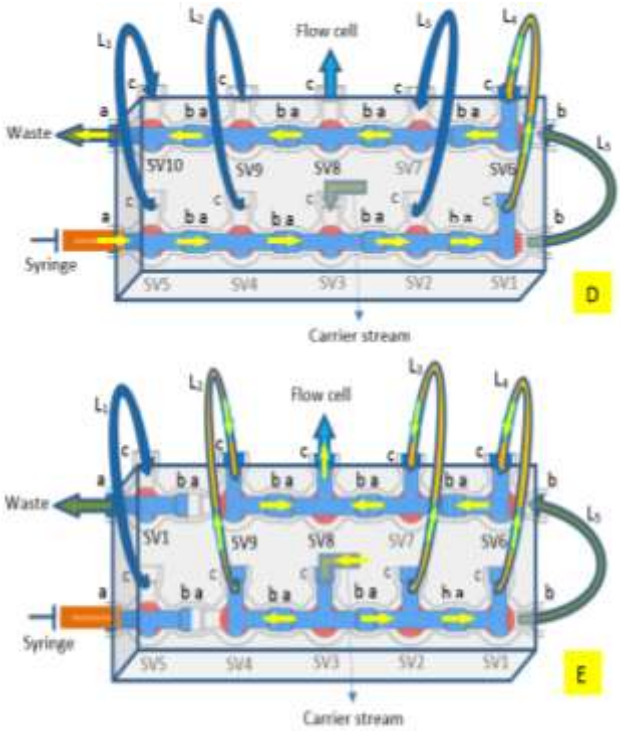

Fig. 2. Selection of three loops to inject the sample with two components, this process is done by loading L_4,L_3,L_2, as shown in Fig. 1 (A,B) and Fig. 2 (D). The carrier stream is allowed to pass through the valve to introduce the components into the flow cell as shown in (E)

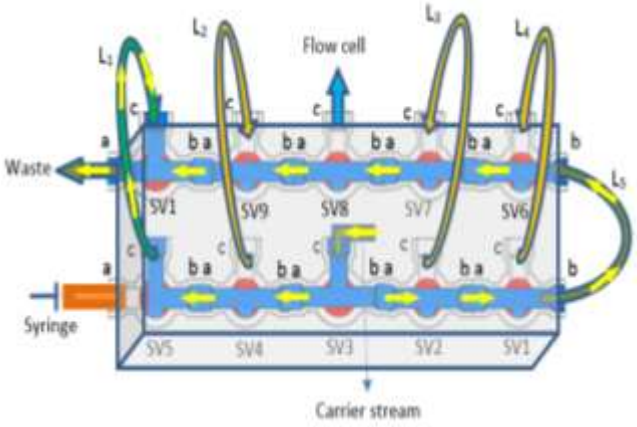

Fig. 3. Washing process was done after loading each component, the orange loops represents, which are loaded by components, the green loops represent the passage of the carrier stream through the valve to remove residual components inside the cavity of valve.

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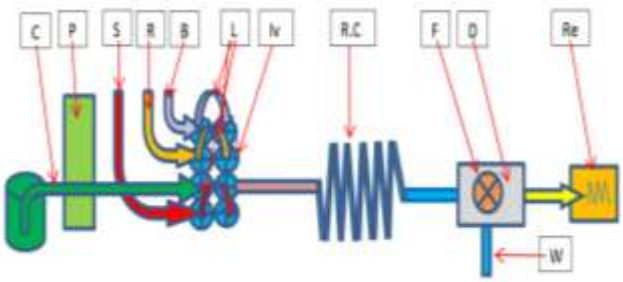

Fig. 4. Schematic of FIA system, P, peristaltic pump; S, sample solution; C, carrier; D, detector; W, waste; Iv, injection valve; R, reagent; B, buffer solution; L, loops; R.C, reaction coil; F, flow cell; Re, recorder; W, waste.

Experiments

The total phenols of standard and sample solutions were estimated by the FIA-FCR method was optimized with the physical and chemicals conditions for the FIA technique as follow; Gallic acid is loaded in L_2 (117 µL), buffer solution (7.5% Na_2CO_3) is loaded in L_4 (235 µL), the reagent (Folin-Ciocalteu) loaded in L_3 (157 µL)**,** length of reaction coil was 100 cm, the carrier stream was distilled water. Flow rate 2 mL.min^{-1} at the temperature of 30 ºC, the response measured at 760 nm. Determination frequency was 55 per hour, these data are shown in Table 1.

Table 1. Analytical values of statistical treatment with optimum working conditions for the GA determination with FCR system.

Param-eters	Length of R.C cm	Flow rate	Loading site	volume of loops µL	Temp ºC	Buffer soln.	FCR conc. M	Replication	LOD & LOQ mgL^{-1}
Optimum value	100	2.0 mL. min^{-1}	GA L_2 FCR L_3 Buf. L_4	L_4=235 L_3=157 L_2=117	30	7.5%w/v	0.2 λmax 760 nm	$\bar{d}$ = 5.02, SD = 0.124, RSD% =2.48	Math.= 1.41 Prac.= 0.05 LOQ = 4.28

Calibration graph for FIA-FCR method

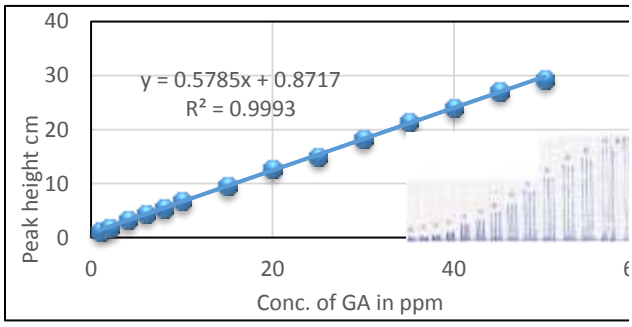

Fig. 5. Calibration curve of different concentrations of gallic acid responses expressed as an average peak height cm, which reacts with 0.2 M FCR.

FIA-UV determine of total phenols.

Under standard conditions that have been studied and shown in Table 2, prepare variation concentrations of ascorbic acid (AA) and measure the response at λmax 280 nm. Calibration curve was constricted by plotting a linear relationship between the concentration of the acid and the response as a peak height.

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Table 2. Analytical values of statistical treatment with optimum working conditions for determination of ascorbic acid concentration by UV detection.

NO. of replicate (N)	1	2	3	4	5	6	7	8	9	10	11	12
Peak height cm (Replication)	8.6	8.6	8.6	8.6	8.6	8.6	8.7	8.6	8.6	8.7	8.5	8.5
Mean $\bar{d}$	8.6											
SD	0.05											
RSD%	0.65											
Flow rate	3 $ml.min^{-1}$											
Length of L_3	40 cm equivalent 314µL											
LOD	0.05 mgL^{-1} practically, 0.64 mgL^{-1} mathematically											
LOQ	2.14 mgL^{-1} mathematically											
Dispersion	1.36 for 10ppm, 1.5 for 5ppm											
Determination frequency	120 per hour											

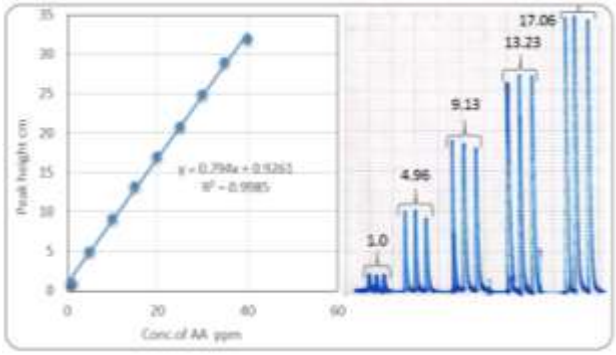

Fig. 6. Calibration graph of responses standard AA expressed as an average peak height cm, for determination of TP content by UV detection.

FIA-DPPH system determination of Antioxidants.

New design valve of the FIA-DPPH method way used for the determination of antioxidant activity. The change in colour caused by the reaction between free radical and sample was measured as a peak height. The sensitivity of the measurement depends on the choice of free radicals, reaction time, length and shape of reactor as well as flow rate (Donatha, 2012). Choice of test substances and reagents for the FIA, GA and DPPH were used as test substances to measure the antioxidant activity of TP in the extracts, using FIA system optimization. The antioxidant activity can be measured from the decrease of the absorption of a free radical (R•) after reacting with an antioxidant (AH) (Sumathy & Ajesh, 2013)

$$R\bullet + AH \longrightarrow R\text{-}H + A\bullet$$

The Injection process of DPPH.

This process was determined by modification Blois method. Suitable volumes of 0.2 mM 80% methanolic solution of DPPH free radical was loaded on L3, and suitable volumes of standard gallic acid solution or crude extract methanol solution of TP was loaded on L_2 (containing 50 – 400 µg of dried sample). Carrier stream (distilled water) was allowed to transfer the components to the flow cell passing through the reaction coil. The response was

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measured at 517 nm and the activity is given as % DPPH radical scavenging calculated according to the following equation: % DPPH Radical Scavenging Activity (%RSA) = [(control response (Ctrl) – extract response)/control response (Ctrl)] x100 (Sathyaprabha & Kumaravel, 2011).

Effect of gallic acid concentration.

After establishing the physical conditions of the system, the concentration of DPPH was fixed at 0.2 mM. Gallic acid concentration was studied in a range from (1 to 25) ppm. At concentrations higher than 25 ppm there was no significant increase in the response as (RSA%), and this is illustrated in Table 4 & Fig. 7. Analytical values of optimum working conditions were illustrated in Table 3.

Table 3. Analytical values of statistical treatment with optimum working conditions for determination of RSA% (DPPH) with GA concentration.

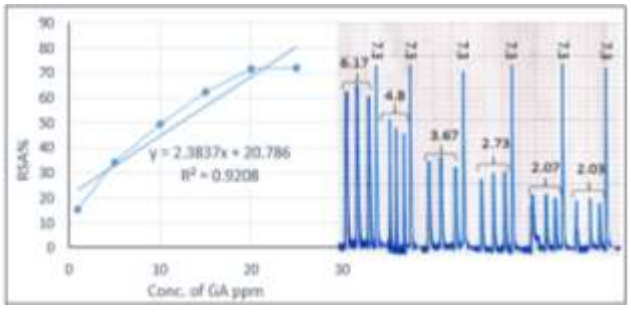
Param-eters	Length of RC	Flow rate	Loading site	Length of loops cm	Df *	DPPH conc.	IC_{50}	LOD
Optim. value	150 cm	2 ml. min^{-1}	GA L_2 DPPH L_3	L_3=30 L_2=30	65 h^{-1}	0.2 mM λmax 517nm	10 µg/mL DPPH	Practically 0.05 mg/L(GA) 0.1 mM (DPPH)

* Determination frequency

Table 4. Measuring response by changing concentration of GA and fixing conc. of DPPH at 0.2 mM

Conc. of GA ppm	Peak height cm NO. of replicate (N) 1	2	3	Mean $\bar{d}$	Ctrl	%RSA	SD	RSD %	$\bar{d} \pm t \frac{SD}{\sqrt{n}}$
1	6.2	6.3	6.0	6.17	7.3	15.5	0.12	2.02	6.17 ± 0.37
5	5.0	4.8	4.6	4.8	7.3	34.24	0.16	3.40	4.8 ± 0.49
10	3.5	3.8	3.7	3.67	7.3	49.77	0.12	3.40	3.67 ± 0.37
15	2.6	2.8	2.8	2.73	7.3	62.55	0.09	3.44	2.73 ± 0.28
20	2.1	2.1	2.0	2.07	7.3	71.68	0.04	2.28	2.07 ± 0.14
25	2.0	2.1	2.0	2.03	7.3	72.14	0.04	2.31	2.03 ± 0.14

Fig. 7. The relationship between the RSA% values versus different conc. of GA, using constant conc. of DPPH at 0.2 mM.



IC_{50} = 10 µg/mL DPPH

2. *FIA-FRAP determine (RPA%)*

FRAP as standard control reducing power was prepared according to the standard procedure (Pavel Stratil., 2008) and the absorption spectra was obtained by a spectrophotometer. The maximum absorption wavelength of the yellow color product was 700 nm. This was determined as described previously. Briefly, the 5 ml of 10 mgL^{-1} AA was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 1%). Then the mixture was incubated at 50C^o for 30 min. Afterwards 2.5 ml of trichloroacetic acid (TCA 10 %) was added to the mixture, which was then centrifuged at 5000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Increasing absorbance of the reaction mixture indicates increasing reducing power of the sample. Modification of above procedure to proportional FIA system achieved by controlling the conditions parameters of a volume and length of loos, length of RC, effect of tem., effect of reagent components percentage mixture, effect of $FeCl_3$ conc. and effect of flow rates. This procedure divided into three parts, part one consists from proportion volumes mixture (P.B.T) of potassium ferricyanide ($K_3Fe(CN)_6$; 1%) and phosphate buffer (0.2 M, pH 6.6). Then the mixture was incubated at 50 ^oC for 30 min. Afterwards trichloroacetic acid (TCA 10 %) was added to the mixture, which was then centrifuged at 5000 rpm for 10 min. Finally, the supernatant solution was mixed with 2.5 ml of distilled water. This mixture was prepared freshly every day. Ascorbic acid was used as a standard control. The RPA% of the sample extract was determined against a standard curve of the FRAP value was expressed as mgL^{-1} of AA equivalents per 100 mL of juice or mg of AA equivalents per g of crude (mg/g EPA). The relative percentage reducing power of the sample as compared to the maximum absorbance tested which appeared in Ascorbic acid was calculated using the following equation (Sadananda, *et al*, 2014; Mohammed, *et al*, 2010):

FRAP value = Absorbance (sample +FRAP reagent) - Absorbance of FRAP reagent (Ctrl); RPA% = (A-Ctrl) / Amax ×100

Here, Amax = maximum absorbance tested at the highest concentration, Ctrl= absorbance of (P.B.T) & $FeCl_3$ concentrations without sample, and A= absorbance of FRAP reagent with different concentrations of the sample. Choose the valve shown as in Fig. 2(E) and the paragraph (3.3) to curry out this experiment.

Optimization Conditions

The results that were obtained from experimental optimization conditions are shown in the Table 5. A calibration curve of the AA concentration versus reducing power activity RPA% over the range of (1–25) mgL^{-1} was plotting with three times for each reading. Results obtained shown in the Fig. 8.

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Table 5. Analytical values of statistical treatment with optimum working conditions for RPA% determination of AA with FRAP reagent.

Param-eters	Length of RC	Flow rate	Loading site	Length of loops	Df*	Vol. ratio of mixture	Conc. of Components	LOD & LOQ $mg.L^{-1}$
Optimum value	150 cm	2 ml. min^{-1}	$FeCl_3$ L_2 AA L_3 P.B.T L_4	L4=30cm V=235.5 L3=25cm V= 196.2 L2=15cm V= 117.8	45 h^{-1}	3/2/2 v/v/v B/P/T T(TCA)	(0.1% $FeCl_3$, 1% P, 10% T) w/v 0.2 M B, pH 6.6 λmax 700nm	LOD Mathematicall y 0.82 Practically 0.2 LOQ 2.75

***** Determination frequency

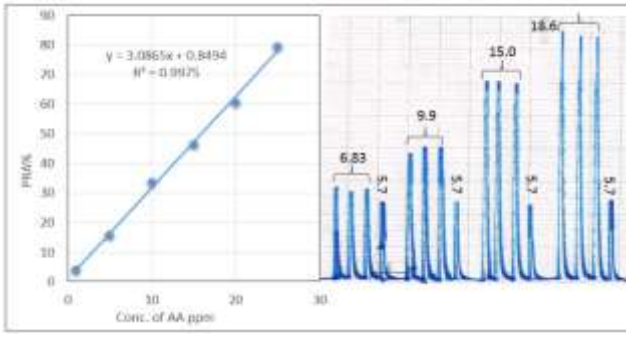

Fig. 8. Calibration curve of responses for standard AA, expressed as an average peak height cm, for the determination of RPA%.

Extraction of Polyphenols

Method that was described by the International Organization for Standardization (ISO) 14502-1 was used in this study. Fruits and vegetables were bought from local markets. For examination only healthy looking of them were chosen, and were washed to remove any amount edible portions. The peels were air dried for about a period of one week and ground to a fine powder and passed through a mesh sieve. These powdered samples were later extracted with 80% methanol at room temperature. Briefly, 0.200 ± 0.002 g of grinded wheat flour was weight in an extraction tube, and 10 mL of 80% methanol at 50 °C was added. The extract was mixed by using a vortex machine and shaken in a water-bath at 50 °C for 2 hr. After cooling at room temperature, the extract was centrifuged at 5000 rpm for 10 minutes, and the supernatant liquid was decanted in a graduated tube. The residue was re-extracted twice under the same conditions to ensure complete extraction (Claudia, *et al*, 2008). Both crud extracts were pooled and treated with trichloroacetic acid (TCA) 10% to

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separate the phenol extraction from proteins, deproteinize was used acetone precipitation method (Xiaocui, *et al*., 2013). The volume adjusted to 20 mL with cold 80% methanol. (1, 2, 3, 4, 5) milliliter of the extract was diluted with 80% methanol to 20 mL.

Determination of total phenols and antioxidant activity in wheat.

FIA-FCR determination

Table 6. Summary results for determination of total phenols in wheat using 0.2 M FCR.

Volume of Wh. ex.	Conc. of TP in ppm	Peak height cm NO. of replicate (N) 1	2	3	Mean $\bar{d}$	SD	RSD %	TP mg/g GAE	$\bar{d} \pm t \frac{SD}{\sqrt{n}}$
1/20	1.37	1.6	1.5	1.6	1.56	0.04	3.00	2.7	1.56 ± 0.14
2/20	2.41	2.0	2.0	1.8	1.93	0.09	4.87	2.5	1.93 ± 0.28
3/20	4.00	2.5	2.3	2.4	2.40	0.08	3.40	2.6	2.4 ± 0.24
4/20	4.85	2.7	2.6	2.7	2.67	0.04	1.76	2.4	2.67 ± 0.14
5/20	5.80	2.9	2.9	2.9	2.9	0.00	0.00	2.3	2.9 ± 0.00

The average of total phenols in wheat (Wh) = 2.5 mg/g GAE

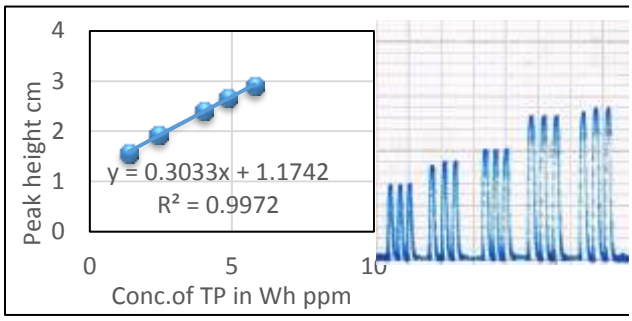

Fig. 9. The relationship between the responses expressed as an average peak height cm versus deferent concentrations of total phenols in wheat using 0.2M FCR.

FIA-DPPH determination

IC_{50} was determined from the linear regression equation. Regression equations had correlation coefficients ≥ 0.91. The IC_{50} was expressed as µg solids/mL DPPH and the antioxidant activity of the sample was reported as $1/IC_{50}$ mg/ml DPPH.

IC_{50} of wheat extract = 7.6 µg/mL DPPH, $1/IC_{50}$ = 0.329 µg/mg TP GAE

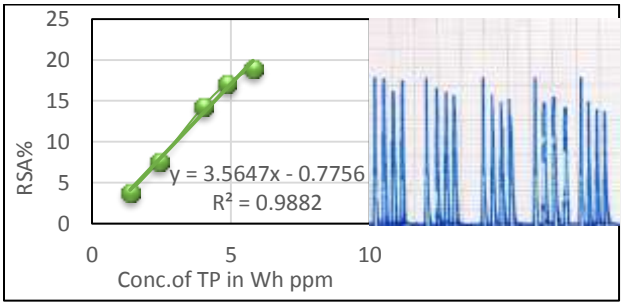

Fig. 10. The relationship between the RSA% expressed as an average peak height cm versus deferent concentrations of total phenols in wheat using 0.2 mM DPPH.

FIA-UV determination

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Table 7. Results for determination of total phenols in wheat using UV detection.

Volume of Wh. ex.	Conc. in ppm	Peak height cm NO. of replicate (N)			Mean	SD	RSD	TP mg/g	$\bar{d} \pm t \frac{SD}{\sqrt{n}}$
		1	2	3	$\bar{d}$		%	AAE	
1/20	1.0	1.0	1.0	1.0	1.0	0.0	0.0	2.0	0.0
2/20	2.12	2.1	2.1	2.1	2.1	0.0	0.0	2.12	0.0
3/20	2.92	2.9	2.9	2.9	2.9	0.0	0.0	1.95	0.0
4/20	4.03	4.0	4.0	4.0	4.0	0.0	0.0	2.0	0.0
5/20	4.84	4.8	4.8	4.8	4.8	0.0	0.0	1.94	0.0

The average of total phenols in wheat = 2.0 mg/g GAE

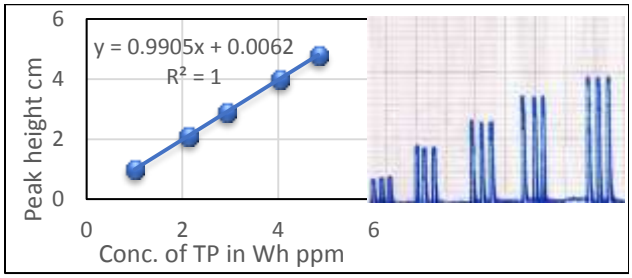

Fig. 11. The relationship between the responses expressed as an average peak height cm versus deferent concentrations of total phenols in wheat using UV detection.

FIA-FRAP determination

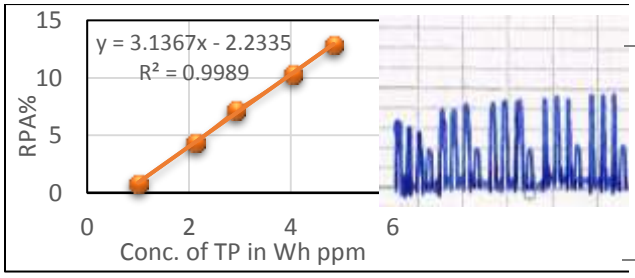

Fig. 12. The relationship between the RPA% expressed as an average peak height cm versus deferent concentrations of total phenols in wheat using FRAP method.

Average of reducing power (RP) of Fe^{2+}/Fe^{3+} = 99.9 mg RP / g Wheat

Statistical Analysis

Plots and fittings were carried out by using Genstat statistics program. Data were analyzed by Analysis of variance, considering P < 0.05 as least significant differences of means followed by regression statistics and variance (ANOVA) as the following table.

Table 9. Analytical values of statistical treatment with optimum working conditions for two methods of FCR, UV to determination of GA.

Source of variation	***d.f.***	***s.s.***	***m.s.***	***F t.***	***F pr.***
Methods	*1*	*254.58*	*254.5*	*3.11*	*0.044*
Residual	*52*	*4263.0*	*8*		
Total	*53*	*9*	*81.98*		
		4517.6			
		7			

Grand mean 14.7

Methods 1 2

12.6 16.9

Least significant differences of means (5% level) l.s.d. 4.04

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Table 8. Summary of results obtained of gallic acid (GA), ascorbic acid (AA) concentrations by using FIA-FCR and FIA-UV methods.

GA, AA Concs. ppm	**No.**	**1**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
FIA-FCR 760 nm	1	1.15	3.9	6.7	10.0	13.0	15.2	18.1	21.6	24.2
	2	1.15	4.0	6.9	9.4	12.6	14.9	18.3	21.4	23.9
	3	1.15	3.8	6.8	9.5	12.9	14.9	18.5	21.5	23.8
FIA-UV 280 nm	1	1.0	5.1	9.3	12.7	17.10	21.4	24.6	28.5	33.0
	2	1.0	5.1	9.2	13.5	17.10	20.5	24.5	28.7	31.0
	3	1.0	4.7	8.9	13.5	17.0	20.5	25.5	30.0	32.0

Results & Discussion

The FIA-FCR method for determination of total phenols, expressed as the ratio between the slopes of the calibration curves determined for gallic acid concentration as a pure compounds and for total phenols in wheat extract. On the other hand, the optimized experimental conditions, in an aqueous solution was determined of gallic acid and the analytical parameters were a limit of detection (LOD) of 0.05 mg L^{-1}, a calibration range of 0.2–50.0 mgL^{-1} (r^2=0.9993) as shown in Fig. 5. Similar results were observed when using ascorbic acid as a standard solution that was determined by FIA-UV detector. The limit of detection (LOD) of 0.05 mg L^{-1}, a calibration range of 0.2–40.0 mg L^{1} (r^2=0.9985). The proposed methods were validated in terms of linearity, repeatability, detection limit, dispersion and accuracy. The repeatability of the developed two methods were assessed by calculating the standard deviation SD and the relative standard deviation RSD% from 10 consecutive determinations of two gallic acid and two ascorbic acid standard solutions (5.0, 10.0) ppm providing values of 0.124 SD, 2.48 RSD% for gallic acid and 0.05 SD, 0.65 RSD% for ascorbic acid. Total phenols content in wheat extract was 2.5 mg/g GAE, 2.0 mg/g AAE for using FIA-FCR, FIA-UV respectively as shown in Figs. 9, 11. Therefore, the FIA-UV method is also suitable for determination of total phenolic content. Moreover, the application of FIA-DPPH method for determination of antioxidant capacity and FIA-FRAP method for determination reducing power activity in food samples is proposed for evaluation of the contribution from phenolic and other reducing substances (as ascorbic acid, for instance). The contribution from other antioxidant compounds with different application to Food Samples. The assessment of DPPH antioxidant activity of wheat extract was performed using the proposed FIA-DPPH system. The peak height obtained for samples was interpolated in the following range of total phenols concentration in wheat extract: A (1, 5, 10, 15, 20, 25) ppm, and B (1.37, 2.41, 4.0, 4.85, 5.80) ppm, where A is the concentration of gallic acid and B is the concentration of total phenols in wheat extract as gallic acid equivalents GAE. Thus, the DPPH free radicals scavenging activity percentage (RSA%) was expressed

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as gallic acid equivalents (mg L^{-1}), and the IC_{50} was 7.6 µg/mL DPPH, $1/IC_{50}$ was 0.329 µg/mg TP GAE as shown in Fig. 10. The results obtained by the proposed methodology (FIA-FRAP) For determination of reducing power RP of wheat extract was 99.9 mg RP / g wheat as shown in Fig. 12.

Conclusion

The FIA-FCR, FIA-UV, FIA-DPPH and FIA-FRAP and the methods presented in this work allowed the in vitro assessment of total phenols and antioxidant capacity of wheat extract samples. Through the appropriate selection of standard compound it was possible to decrease by 7.5 times the analysis time, from the original 30 to 4 min, providing high-throughput results under the optimize conditions. The automation FIA method allowed for reduced intervention by an operator, which is a special requirement in the research/clinical biochemistry field. Moreover, the FIA manifold described here is extremely simple to implement in routine laboratories, easily operated and is also cost-effective, making it an attractive and suitable tool for two possible application areas. First, it can be used for screening biological activity in food products, plant extracts or pharmaceutical compounds and, secondly, it can be used in clinical trials and epidemiological studies as a routine test for biological fluids in order to evaluate and follow up the level of oxidative status. In conclusion, the present automatic methodology for the determination of FCR and other FIA methods represents a suitable tool for routine determinations. It was successfully applied to food samples of wheat extract, providing results that were in agreement with those obtained by the time-consuming batch method proposed for standardization. Moreover, the strict control of reaction conditions (mixing of reagent/sample, reaction time) and the reduced intervention of operator contributed to achieving reliable results, with good repeatability.

Nomenclature

FIA = flow injection analysis, FCR = Folin Ciocalteu reagent, UV = ultra violet, FRAP = ferric ion reducing antioxidant power, TP = total phenols, GAE = gallic acid equivalent, GA = gallic acid, AA = ascorbic acid,

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