In Vitro Antioxidant Activity of Feruloyl Arabinose from Maize Bran

LIN Qi-ling[1], WEN Qi-biao[1], OU Shi-yi[2], LAI Fu-rao[1]

(1.College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China)

(2.Department of Food Science and Technology, Jinan University, Guangzhou 510632, China)

Abstract: In this study feruloylated oligosaccharides (FOs) was released from maize bran by hydrochloric acid hydrolysis, and feruloyl arabinose (F-Ara) was obtained by D301 macroporous resin chromatography followed by polyamide resin purification from FOs. After structural identification, the antioxidant activity of F-Ara was evaluated in vitro by DPPH and superoxide radical scavenging activity assay and reducing power assay. F-Ara exhibited a good radical-scavenging activity in vitro when compared to standard antioxidants such as ferulic acid (FA), BHA and L-ascorbic acid (VC). The radical-scavenging activity depended on the concentration and increases with increasing dose of sample. The highest reducing value of F-Ara compared with FA and VC at a dosage of 0.6 mmol/L indicated that F-Ara had a considerable reducing power. The present study suggested that F-Ara can be a natural and efficient antioxidant used in food, medicine and cosmetic.

Key words: antioxidant activity; feruloyl arabinose; free radical; maize bran

Article No.: 1673-9078(2012)1-33-38

玉米皮阿拉伯糖阿魏酸酯体外抗氧化活性的研究

林奇龄[1]，温其标[1]，欧仕益[2]，赖富饶[1]

（1.华南理工大学轻工与食品学院，广东广州 510641）（2.暨南大学食品科学与工程系，广东广州 510632）

摘要：本文采用稀盐酸水解玉米麸皮得到低聚糖阿魏酸酯(FOs)，经 D301 大孔树脂的除杂和聚酰胺柱层析的分离获得 FOs 中的 F-Ara 组分，在分析结构后，对该组分进行了体外清除 DPPH 和超氧阴离子自由基的能力测定和还原力测定。结果表明，与标准物阿魏酸(FA)、BHA 和维生素 C 相比，F-Ara 均表现出良好的体外清除自由基活性，其活性随浓度的增加而增大；在试验浓度为 0.6 mmol/L 时，与标准物阿魏酸和维生素 C 相比，F-Ara 显示出最高的还原值，说明 F-Ara 具有较强的还原力。以上结果揭示 F-Ara 可成为一种能应用到食品、药品和化妆品的天然高效的抗氧化剂。

关键词：抗氧化活性；阿拉伯糖阿魏酸酯；自由基；玉米皮

Maize is one of the major crops in China. The maize industry produces a large number of maize bran every year, which is generally used for animal feed or discarded as agricultural waste. However, it still contains useful substances such as phenolic compounds (e.g., ferulic acid (FA) and *p*-coumaric acid) [1]. FA is the most predominant phenolic compound in maize bran and represents up to 3.1% of the dry weight of maize bran [2,3]. It is well known that FA has many physiological functions, including anti-oxidant, anti-microbial, anti-inflammatory, anti-thrombosis, and anti-cancer activities [4]. Therefore, maize bran is promising for producing FA and its derivatives as functional substances.

In maize bran, FA is ester-linked at the C-5 position to α-L-arabinosyl residues which are substituents of the xylan backbone [5]. Following mild acid hydrolysis, relatively weak glycosidic linkages are split and the ester linkages involving FA remained [6]. The major products of hydrolysis are referred to as feruloylated oligosaccharides (FOs). Because of the presence of FA residues, interest in these oligosaccharides is motivated by their biological activities. One of the most active domains of the research is on their antioxidant activity. In normal rat erythrocytes, FOs show in vitro antioxidant activity against hemolysis induced by free radicals [7]. In diabetic rats, FOs are suitable in vivo antioxidants for protection against oxidative damage [8]. In human lymphocytes, FOs exhibited significant protective effect against oxidative DNA damage in cells induced by H_2O_2 under in vitro conditions [9].

However, research has mainly focused on the mixture of FOs and limited information is available on

Received data: 2011-10-12

the extract active fraction in FOs responsible for the observed antioxidant activity. In this article, we assessed the antioxidant activity of 5-*O*-(*trans*-feruloyl)-L-Ara*f* (feruloyl arabinose, F-Ara) isolated from FOs from maize bran using different in vitro test systems. The antioxidant activity was accessed with respect to scavenging of DPPH and superoxide radical and reducing power.

1 Experimental

1.1 Materials and Chemicals

Maize bran was obtained from an animal feed company in Shijiazhuang, Hebei, China. The bran was milled and passed through a 0.5 mm sieve. Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1 from *Bacillus licheniformis*, 120 KNU/g) was purchased from Novo Nordisk, Bagsvaerd, Denmark. Protease papain (EC 3.4.22.2 from papaya, 600 KNU/g) was purchased from Yuantian, Guangzhou, China. D301 (weak alkali styrene-type anion exchange resin) was obtained from Zhengguang Industrial Co., Ltd, Zhejiang, China. Polyamide resin was obtained from Sijiashenhua, Zhejiang, China. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Chemicals Co., St. Louis, MO,USA. All other reagents and chemicals used in the experiment were of analytical grade.

1.2 Preparation of maize bran insoluble fiber

Maize bran (100 g) was dried in an oven for 4 h at 105 ℃ and subsequently ground to pass a 60-mesh sieve. After defatted by n-hexane, maize bran was suspended in water (1000 mL) and the heat-stable α-amylase (7.5 mL) was added. Beakers were heated in a boiling water bath for 1 h and shaken gently every 5 min. The pH was adjusted to 7.5, and samples were incubated with protease (1 g) at 60 ℃ for 30 min with continuous agitation. After cooling the samples to room temperature, the suspension was centrifuged at 3000 *g* for 10 min, and the residue was washed with hot water (70 ℃) until no cloudiness was evident, and was finally dried at 40 ℃ overnight in an oven to get maize bran insoluble fiber [2].

1.3 Isolation of feruloylated oligosaccharides

Mild acid hydrolysis of maize bran insoluble fiber was carried out as described by Allerdings et al [10], but with minor modifications. Insoluble fiber (100 g) was treated with 50 mmol/L HCl (1.5 L) under reflux for 3 h at 100 ℃. After centrifugation (3000 *g*, 10min), the supernatant was filtered (<10 μm) on a sintered glass funnel to get maize bran hydrolysate.

One hundred and fifty milliliter of the hydrolysate was applied to a column (30×4.5 cm) of D301 resin (previously washed with 5%NaOH and then 5% HCl). Elution was carried out with 8 column volumes of H_2O, 5 column volumes of 60% (*V/V*) EtOH/H_2O, and 8 column volumes of EtOH. The fraction eluted with 60% EtOH/H_2O was evaporated at 40 ℃ under vacuum to dryness, then dissolved in water (10 mL) and applied to a column (30×4.5 cm) of polyamide resin. Elution was carried out at room temperature and a flow rate of 5 mL/min was maintained. A gradient was used, successively, comprising 10, 20, 30, 40, 50, and 60 % (*V/V*) EtOH/H_2O in 250 mL, respectively. The absorbance of the eluent was monitored continuously at 320 nm with an UV detector and fractions were collected in each tube every minute. Fractions corresponding to separate peaks of the chromatogram were pooled, concentrated (40 ℃ in vacuum) for further analysis.

1.4 Identification of feruloyl arabinose

Molecular weights of F-Ara were determined using electro-spray ionization mass spectrometer (ESI-MS) (mass spectrometer API4000 Q TRAP (ion-source: gas auxiliary electro-spray ionization), Applied Biosystems Co., USA).

Esterified FA from F-Ara was determined according to the method of Yuan et al [11]. Sample was analyzed for FA using high-performance liquid chromatography (HPLC) using a ZORBAX SB C18 column (150 mm ×4.6 mm, 5 μm). FA was identified by comparison of its relative retention time with standard compound (FA).The isolated FOs were also analyzed by HPLC in the same chromatographic condition.

Monosaccharide composition was analyzed by high-performance anion-exchange chromatography using a pulse amperometric detector (HPAEC-PAD) as described by Yuan et al [7]. The sample was analyzed by HPAEC-PAD on a Dionex BioLC system using a Amino Pac PA10 column (2 mm×250 mm). Monosaccharide composition was identified by comparison to relative retention times of authentic standards (arabinose and xylose).

1.5 Determination of antioxidant activity of feruloyl arabinose

1.5.1 DPPH radical scavenging assay

The scavenging activity of F-Ara on DPPH radicals was measured according to the method of Yuan et al [7] with minor modifications. FA and butylated hydroxyanisole (BHA) were used as reference materials. An aliquot of 0.5 mL of sample solution at different concentrations (0.05~2 mmol/L) was mixed with 2 mL of Tris–HCl buffer (100 mmol/L, pH 7.4) and 2.5 mL of 0.2 mmol/L ethanolic solution of DPPH. The reaction mixture was shaken well and incubated for 30 min in the dark at room temperature, and the absorbance of the resulting solution was measured at 517 nm against 50% (v/v) aqueous ethanol using a UV-9600 UV/VIS Recording Spectrophotometer (Rayleigh Analytical Instruments, Beijing, China). The radical-scavenging activity of the tested samples was measured as a decrease in the absorbance of DPPH and was calculated by the following equation:

DPPH scavenging activity(%)={1−(*B*−*C*)/*A*}×100

where *A*, *B* and *C* are the initial absorbance of the blank, the absorbance of test sample and DPPH solution, and the absorbance of test sample without the DPPH solution, respectively.

1.5.2 Superoxide radical scavenging assay

The superoxide radical-scavenging activity was estimated using the spectrophotometric monitoring of the inhibition of pyrogallol autoxidation as described by Li et al [12] with some modifications. Pyrogallol solution (0.2 mL and 45 mmol/L) was added into a tube containing F-Ara (0.3 mL and 0.25~8 mmol/L) previously dissolved in phosphate buffer (2.7 mL and 0.05 mmol/L, pH 8.2) at 25 ℃. The mixture was incubated at 25 ℃ for 3 min and the optical density (OD) was measured at 420 nm using a spectrophotometer. The antioxidant activity was determined as the percentage of inhibiting pyrogallol autoxidation, which was calculated from OD in the presence or absence of pyrogallol and F-Ara. FA and L-ascorbic acid (Vitamin C, VC) were used as controls.

1.5.3 Reducing power assay

The reducing power of F-Ara was determined according to the method of Gulcin et al [13] with some modification. The sample (0.75 mL) at different concentrations (0.1~1 mmol/L) was mixed with 0.75 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 0.75 mL of 1% potassium ferricynide and the mixture was incubated at 50 ℃ for 20 min. Then, 0.75 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 *g* for 10 min. The upper layer (1.5 mL) was mixed with 1.5 mL of deionized water and 1.5 mL of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank (containing all reagents except the test sample). FA and VC were used as controls. The reducing power of the tested sample increased with the absorbance value.

1.6 Statistical analysis

All the tests were done in triplicate and data were reported as the mean values and standard deviation. Data were analyzed by an analysis of variance and significant differences between means were determined by Duncan's multiple range tests. Differences in the statistical tests were considered significant when $P<0.05$.

2 Results and discussion

2.1 Isolation and identification of feruloyl arabinose

The isolation procedure of FOs involved mild acidic hydrolysis of maize bran fiber, pre-separation of the hydrolyzate using a D301 resin column and further separation by polyamide resin chromatography. Owing to the complex structure of maize bran arabinoxylan which decreases the access of carbohydrolases to the xylan backbone [14], the method of mild acidic hydrolysis was chosen for preparing FOs. A treatment with 50 mmol/L HCl at 100 ℃ for 3 h was applied since it gave appreciable amounts of FOs with less release of free FA. The hydrolysate was loaded onto a D301 resin column which was eluted with water, 60% EtOH/H_2O, and EtOH. The fraction obtained by eluting with 60% EtOH/H_2O contained approximately 80% of esterified FA of the hydrolysate and was, therefore, selected to isolate FOs using polyamide resin chromatography.

Five fractions (F1~F5) were eluted by a stepwise gradient using ethanol-water from a polyamide resin column (Fig.1) and analyzed by HPLC. Fractions F1, F4 and F5 yielded several peaks of absorbance at 320 nm in chromatogram of HPLC, which indicate a poor quality in purity. Fraction F3 gave the only one peak of absorbance at 320 nm indicating the purity of F3 reaching HPLC-purity level. Using the described isolation procedure, about 600 mg of F3 were isolated out of 100 g insoluble maize fiber. Fractions corresponding to F3 were

pooled, identified for structure, and evaluated for antioxidant activity.

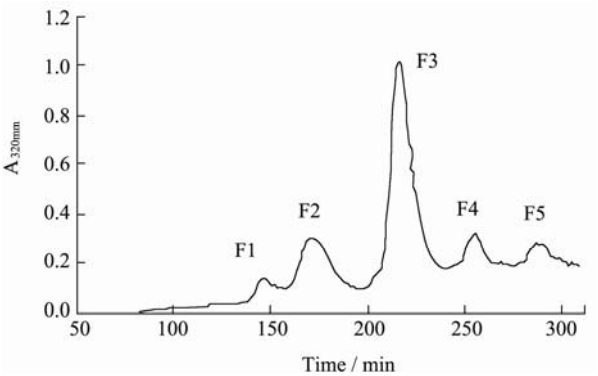

Fig.1 Poluamide resin chromatogram of feruloyated oligosaccharides obtained by D301 macroporous resin chromatochraphy

Analysis of F3 by ESI-MS gave a sodium adduct ion with *m/z* 349.3 $[M+Na]^+$ in positive ion mode and a deprotonated ion with *m/z* 325.5 $[M–H]^-$ in negative ion mode, indicating a molecular mass of 326, corresponding to one FA and one arabinose.

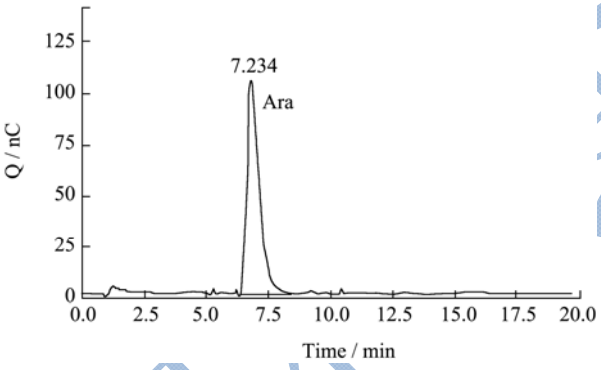

Fig.2 Ion chromatogram of monosaccharide composition of F3

Thus, compared with the published literature [5,10,15], the structure of F3 was determined to be 5-*O*-(*trans*-feruloyl)-L-Ara*f* (F-Ara) (Fig.3).

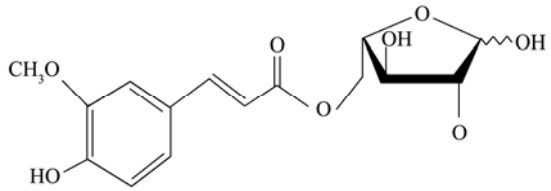

Fig.3 Structure of F-Ara

The hydrolyzed products of F3 by treatment with mild alkaline were analyzed by HPLC. Comparison of its relative retention time with standard compound (FA) confirmed the presence of FA. The glycosyl residue composition of the de-esterifiedproducts of F3 was analyzed by HPAEC-PAD. The results showed that only arabinose was discovered in the monosaccharide composition of F3 (Fig.2).

2.2 Effect of scavenging on DPPH

DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated. The assay is based on the measurement of the scavenging capacity of antioxidants towards DPPH. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine [16]. The profile of scavenging activities of F-Ara and reference materials on DPPH is shown in Fig.4. All of test samples were capable of scavenging DPPH radicals in a concentration-dependent manner. The scavenging effect of F-Ara, FA and BHA on DPPH radicals significantly (P <0.05) increased from 0.05 to 1 mmol/L and subsequently followed by a much slower increase from 1 to 2 mmol/L. At a concentration of 2 mmol/L, the DPPH radical-scavenging activity of F-Ara, FA and BHA reached 77.3±2.6%, 82.0±3.1% and 82.1±2.8%, respectively, indicating that F-Ara, FA and BHA showed similar DPPH radical scavenging activity (P>0.05).

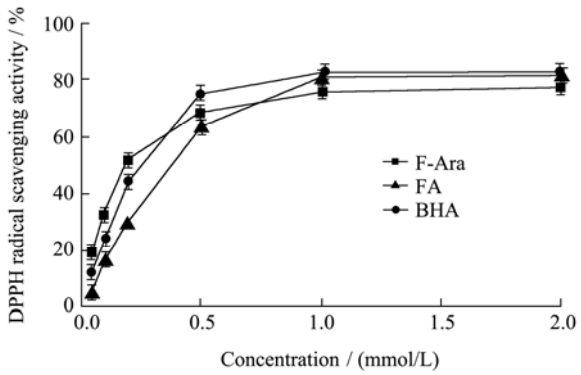

Fig.4 DPPH radical scabenging of F-Ara from maize bran (n=3)

The effective concentration for 50% scavenging (EC_{50}) determined using the regression equation indicated that for 50% scavenging of DPPH radical, a sample concentration of 0.19 mmol/L of F-Ara is

required. For FA and BHA, EC_{50} values were 0.37 and 0.23 mmol/L respectively. A higher DPPH radical-scavenging activity is associated with a lower EC_{50} value [17]. It was evident that F-Ara did show hydrogen-donating ability to act as antioxidants which could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

2.3 Effect of scavenging on superoxide radical

Numerous biological reactions generate superoxide radical which is a highly toxic species. Although it cannot directly initiate lipid oxidation, superoxide radical is a potential precursor of damaging oxygen species, such as hydroxyl radical, and thus the study of the scavenging of this radical is important [18]. The scavenging activities of F-Ara and the controls on superoxide radicals are shown in Fig.5. It was found that F-Ara, FA and VC revealed considerable superoxide scavenging activities at all the concentrations，which increased with the increase of their concentrations. F-Ara and FA at 4 mmol/L exhibited 86.2±1.6% and 79.9±1.1% superoxide radical-scavenging activity, respectively. These values were significantly (P<0.05) lower than that of the same dose of VC (93.9±2.3%). The data indicate that superoxide radical-scavenging activity of those samples followed the order: VC>F-Ara>FA.

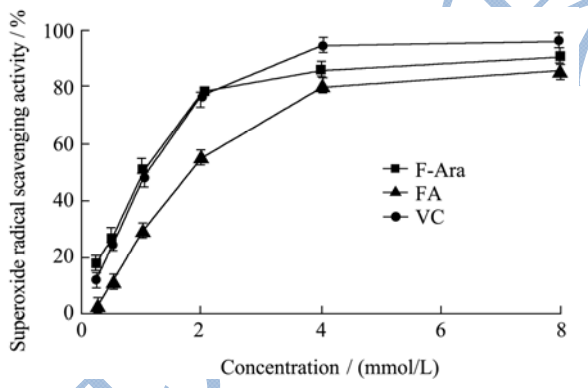

Fig.4 Superoxide radical scavenging of F-Ara from maize bran (n=3)

EC_{50} values, in scavenging abilities on superoxide radical, were comparable for F-Ara (1.05 mmol/L) and VC (1.08 mmol/L), and more effective (P<0.05) than that of FA (1.77 mmol/L).

This assay is dependent on the reducing activity of test compound by a superoxide radical-dependent reaction, which releases chromophoric products [12]. The results imply that F-Ara is a good superoxide scavenger and its capacity to scavenge superoxide may contribute to its antioxidant activity.

2.4 Reducing power

Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The reducing properties are generally associated with the presence of reductones, the antioxidant action of which is based on the breaking of the free radical chain by donating a hydrogen atom. Fig.6 shows the dose–response curves for the reducing powers of F-Ara from maize bran and reference materials. High absorbance indicates high reducing power. The reducing power of F-Ara increased from 0.169±0.005 at 0.1 mmol/L to 1.873±0.063 at 1 mmol/L. The reducing power of FA and VC increased from 0.112±0.003 and 0.153±0.005 at 0.1 mmol/L to 1.332±0.045 and 1.705±0.058 at 1 mmol/L, respectively. At a dosage of 0.6 mmol/L, F-Ara showed highest reducing values of 1.162±0.031 compared with FA (0.855±0.024) and VC (1.054±0.037), suggesting that F-Ara had a noticeable effect on reducing Fe^{3+}. The results were found statistically significant (P<0.05).

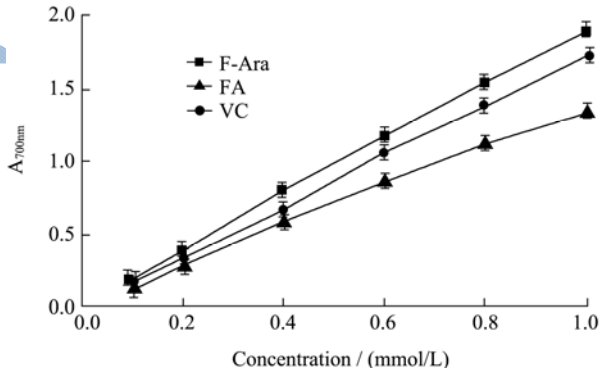

Fig.6 Reducing power of F-Ara from maize bran (n=3)

A high correlation was observed between reducing power and antioxidant activity determined by scavenging of DPPH (r^2=0.9669, 0.9452 for F-Ara and FA, respectively), and scavenging of superoxide radical (r^2= 0.9858, 0.9948 for F-Ara and FA, respectively). The results were in accordance with other investigators who have also reported that antioxidant properties are concomitant with the development of reducing power [17].

In this study, the reducing power of the samples assessed implies that F-Ara was able to donate electron,

hence it should be able to donate electrons to free radicals in actual biological or food systems, making the radicals stable and unreactive.

Natural antioxidants are closely related in their medicinal and beneficial properties. Thus antioxidant capacity is a widely used parameter for assessing the bioavailability of materials. The antioxidant properties of materials should be evaluated in a variety of model systems using several indices to ensure the effectiveness [17]. In this study 3 antioxidant activity methods were employed to evaluate the antioxidant property of F-Ara. Interestingly, F-Ara exhibits a better ability to scavenge free radicals and metal ion as compared to FA in vitro. The result is in agreement with the previous study [15]. The effectiveness of F-Ara could come primarily from the hydrogen-donating ability of its hydrophobic ferulic acid moiety. The presence of electron donating groups on the benzene ring (3-methoxy and more importantly 4-hydroxyl) of ferulic acid moiety gives the additional resonance structures of the resulting phenoxyl radical, contributing to the stability of this intermediate or even terminating free radical chain reactions, and the carboxylic acid group in ferulic acid moiety with adjacent unsaturated C=C double bond can provide additional attack sites for free radicals [9]. In addition, F-Ara also contain hydrophilic arabinose moiety, which might further intensify the stability of resonance structures of the resulting phenoxyl radical. Thereby the rate-limiting hydrogen abstraction reaction of F-Ara is promoted and the termination of free radical chain reactions achieve more rapidly.

Ohta et al [24] reported that orally administered F-Ara existed as the conjugated form of F-Ara (25%) and FA (75%) in the circulation system. The result demonstrates that F-Ara can be absorbed intact from digestive tract to circulation system and metabolized into conjugated F-Ara. The formation of FA conjugates could be due to the hydrolysis of F-Ara by intestinal esterases [3]. F-Ara from FOs is a nonionic chemical species that may pass through cell membranes with a high density of inner negative charges more easily than the negative free phenolic compounds [8]. It is suggested that F-Ara in the circulation system possesses the potential to enter the cells and act as an antioxidant in cells. The antioxidant activity of F-Ara in cells is needed to further investigate.

3 Conclusion

In conclusion, this study indicated that F-Ara could be obtained using D301 macroporous resin chromatography followed by polyamide resin purification from FOs, which was released from maize bran by hydrochloric acid hydrolysis, and was capable of donating hydrogen to convert DPPH to stable product, directly quenching superoxide radical to terminate the radical chain reaction and acting as a reducing agent on reducing Fe^{3+} significantly. Therefore, this study provided evidence on the potential health benefits of F-Ara.

References

[1] Saulnier L, Marot C, Elgorriaga M, et al. Thermal and enzymatic treatments for the release of free ferulic acid from maize bran [J]. Carbohydrate Polymers, 2001, 45: 269-275

[2] Bunzel M, Ralph J, Marita J M, et al. Diferulates as structural components in soluble and insoluble cereal dietary fibre [J]. J Agric Food Chem, 2001, 81: 653-660

[3] Zhao Z, Moghadasian M H. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review [J]. Food Chemistry, 2008, 109: 691-702

[4] Ou SY, Kwok K C. Ferulic acid: pharmaceutical functions, preparation and applications in foods [J]. J Sci Food Agric, 2004, 84(11): 1261-1270

[5] Saulnier L, Vigouroux J, Thibault J-F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran [J]. Carbohydrate Research, 1995, 272: 241-253

[6] Saulnier L, Crepeau M J, Lahaye M, et al. Isolation and structural determination of two 5,5'-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates [J]. Carbohydrate Research, 1999, 320: 82-92

[7] Yuan X, Wang J, Yao H, et al. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber [J]. LWT, 2005, 38: 877-883

[8] Ou S Y, Jackson M, Jiao X, et al. Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides [J]. J Agric Food Chem, 2007, 55: 3191-3195

[9] Wang J, Sun B, Cao Y, et al. Inhibitory effect of wheat bran feruloyl oligosaccharides on oxidative DNA damage in human lymphocytes [J]. Food Chemistry, 2008, 109: 129-136

[10] Allerdings E, Ralph J, Schatz P F, et al. Isolation and structural identification of diarabinosyl 8-O-4-dehydrodiferulate from maize bran insoluble fibre [J]. Phytochemistry, 2005, 66: 113-124

[11] Yuan X, Wang J, Yao, H. Feruloyl oligosaccharides stimulate the growth of *Bifidobacterium bifidum* [J]. Anaerobe, 2005, 11: 225-229

[12] Li Y, Jiang B, Zhang T, et al. Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH) [J]. Food Chemistry, 2008, 106: 444-450

[13] Gulcin Y, Buyukokuroglu M E, Oktay M, et al. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn subsp. *pallsiana* (Lamb.) Holmboe [J]. Journal of Ethnopharmacology, 2003, 86: 51-58

[14] Allerdings E, Ralph J, Steinhart H, et al. Isolation and structural identification of complex feruloylated heteroxylan side-chains from maize bran [J]. Phytochemitry, 2006, 67: 1276-1286

[15] Ishii T. Structure and functions of feruloylated polysaccharides [J]. Plant Science, 1997, 127: 111-127

[16] Kedare S B, Singh R P. Genesis and development of DPPH method of antioxidant assay [J]. J Food Sci Technol, 2011, 48: 412-422

[17] Ozsoy N, Can A, Yanardag R, et al. Antioxidant activity of *Smilax excelsa* L. leaf extracts [J]. Food Chemistry, 2008, 110: 571-578

[18] Jayasri M A, Mathew L, Radha A. A report on the antioxidant activity of leaves and rhizomes of Costus pictus D. Don [J]. Int J Integr Biol, 2009, 5: 20-26

[19] Ohta T, Sembokum N, Kuchii A, et al. Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system [J]. J Agric Food Chem, 1997, 45: 1644-1648