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Preparation, Characteristics And Bioactivity Of Polysaccharide Iron Complex

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ABSTRACT

A new onion polysaccharide-iron (III) complex (OPS-iron) was preparated and characterized. The preparation conditions of OPS-iron (III) were optimized and the physicochemical properties were characterized by physicochemical methods, scanning electron microscopy (SEM), electron paramagnetic resonance (EPR) spectroscopy, Ultraviolet-visible spectroscopy (UV-Vis), and Fourier transform infrared (FTIR) spectroscopy, respectively. In vitro antioxidant activity of polysaccharides and OPS-Fe were evaluated by determining the radical (DPPH and hydroxyl radical) scavenging abilities. The highest iron content of OPS-iron (III) complex (20.18%) was obtained at the conditions: the ratio of OPS and $FeCl_3 6H_2O$ was 3:5 (w/w), the pH value of alkali solution was 10, the reaction temperature was 30 ◦C and the reaction time was 6 h. The iron (III) was shown to be bound through the binding sites of the polysaccharide OPS and it could form spatially separated iron centers on the polysaccharide backbone. OPS-iron (III) complex was found to have good digestive availability and antioxidant activities in the in vitro assays, which suggested the OPS-iron (III) complex might be used as a new iron supplement candidate. In vitro free radical scavenging activity of OPS-Fe was significantly better than OPS. The IC_{50} of OPS-Fe against DPPH and hydroxyl radical were 3.785 mg/mL and 1.460 mg/mL, respectively

Key words: polysaccharide-iron (III) complex , FTIR, Antioxidant properties

INTRODUCTION

About 80% of onion bulb (Allium cepa) dry matter consists of nonstructural carbohydrates **[1].** The predominant of these nonstructural carbohydrates consist of glucose, fructose, sucrose, and low-molecular-weight fructooligosaccharides [2] Fructans, fructooligosaccharides (FOSs) and polyfructosylsucroses of varying molecular size, are the main carbohydrate reserve in onion, as well as in other vegetative organs and plants including alliaceous organs (bulbs). Fructans accumulate during bulbing, and are then catabolized during the regrowth and the sprout development of the bulbs **[3]**.

Iron is an essential mineral that is involved in many physiological functions, and it plays a significant role in numerous metabolic processes and immune functions [4]. Iron deficiency in the body can lead to iron deficiency anaemia (IDA) or dysfunction. Iron deficiency is highly prevalent in all age groups [5]. Some studies reported that 46% of children aged 5–14 years old and 48% of pregnant women in the world are anaemic [6]. The majority of IDA cases were due to iron deficiency in the diet, poor iron

absorptionand excessive loss of iron [7]. Fortifiers of iron are often used to control IDA, such as oral supplements of ferrous salts (ferrous sulphate, ferrous succinate and ferrous gluconate) [8]. However, ferrous salts are chemically unstable due to the presence of free iron ions, which easily produce free radicals, leading to lipid peroxidation and gastrointestinal side effects [9,10,11]. Although low-cost oral iron supplements have been improved, IDA is still considered to be the most common nutrition deficiency worldwide [12]. There were two major problems associated with the use of iron in biological systems, which were its gastrointestinal side effects and the iron-induced oxidative stress.

Recent research indicated that polysaccharide-iron complex could act as iron supplement to treat Iron Deficiency Anemia (IDA) for stability, water-solubility, and fewer side effects, especially non-toxicity under high concentrations, and when the iron was detached from the ligand polysaccharides, the various biological activities of the polysaccharides could also function in the body.

Polysaccharide-iron complexes, synthesized by the reaction of ferric chloride and mono-, di- and polysaccharides such as glucose, fructose, maltose, cellulose, starch, inulin, and tea polysaccharides were also studied [13]. It was reported that most if iron preparations were made of iron-carbohydrate complexes consisting of a polynuclear iron-oxyhydroxide/oxide core surrounded by a carbohydrate ligand, which could stabilize the complex and protect it against further polynuclearization [14]. While there were still some questions about whether coordination bonds between iron and the alcoholic hydroxyl group of polysaccharides were formed, or ironoxide particles were just ‘packed’ in polysaccharides [15] In recent years, some studies had focused on the structure of the polysaccharide-iron(III) complexes, and two main mechanisms were illustrated. One hypothesis was the site-binding model, which described as the iron was bound through the binding sites of the saccharide and formed spatially separated iron centers along the polymeric backbone [16]. The second hypothesis assumed that the high solubility of the metal hydroxide might be due to the nonspecific interactions between the FeOOH precipitate and the polysaccharide (colloidal model) [17] In the combination of these two mechanisms, the donor group of the polysaccharide acted as nucleation sites for the iron ions, which then bound further to the iron(III) ion through the formation of hydroxide bridges [18].

Iron deficiency and iron deficiency anemia are the most common nutritional disorders in the world, affecting more people than any other condition. one the less, oral administration of iron supplements is typically associated with various gastrointestinal side effects resulting from the release of free iron ions [19]. Hence, the development of new effective iron supplements exhibiting no or fewer side effects is highly desirable.

A polysaccharide-Fe (III) complex was reported to be an effective oral iron supplement, exhibiting good chemical stability, water solubility, and few side effects [20]. Furthermore, the complex showed no toxicity under high concentrations [21]. However, there are several problems associated with the use of polysaccharide-Fe (III) complexes. For instance, during the iron digestion process, the structure of some polysaccharide- Fe (III) complexes can be destroyed in the condition of the acidic pH of gastric juice. At a low pH, Fe ions are released and dissolved [22]. Moreover, some insoluble ferric compounds that cannot be absorbed by the small intestine may form in the intestinal juice [23]. The major drawback of such polysaccharide based carriers is their failure to retain Fe ions in gastric juice. Thus, the most significant challenge for the polysaccharide- Fe(III) complex is steadily pass through the gastrointestinal tract. The

excellent gelling capacity of pectin in gastric acid conditions has been increasingly recognized []. Not ably, pectin does not degrade by the action of digestive enzymes in the small intestine [25]. Therefore, the use of pectin as a carrier for Fe ions appears reasonable.

In this study, we have prepared and characterized the physicochemical properties of a novel polysaccharide iron(III) complex in OPS-iron(III)) using scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). The bioavailability and antioxidant activity of the OPS-iron(III) complex were studied by various in vitro assays.

2. Materials and methods

Materials and chemicals

Samples Collection the Allium cepa (Onions) was purchased from a local market at Zagazig, Egypt, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The dextran standards were obtained from the American Polymer Standards Corporation. All other chemical reagents used in the experiments were of analytical grade.

Extraction and Purification of OP.

Dried onion powder was washed with distilled water (solid to liquid ratio of 1:15) for 15 min under constant mechanical stirring at 600 rpm and then centrifuged to remove the supernatant. polysaccharides was extracted using deionized water (solid to liquid ratio of 1:20). The pH value was adjusted to 1.5-2.0 using 2 M HCl, and the mixture was then heated to 90-95 °C in a water bath and maintained for 60 min under constant mechanical stirring at 600 rpm. After extraction, the soluble portion was recovered via filtration. The filtrate was collected for alcohol precipitation and cooled to room temperature. Subsequently, 1.5 times the volume of 95% ethanol was added to the filtrate. After 4 h, the precipitate was washed three times with 5% (v/v) HCl in 95% ethanol and anhydrous ethanol. Thereafter, the precipitate was freeze-dried. The degree of esterification was determined at 61.74% using a titrimetric method.

Preparation of OPS

The OPS was extracted and purified following the method of [29]. Briefly, the dried ground I. obliquus was refluxed with 80% (v/v) ethanol at 80 ◦C for 2 h for three times. The dried residue (50.0 g) was extracted thrice with 1 L-distilled water for 2 h each at 100°C. The combined extract was filtered through a Whatman No. 1 filter paper and the filtrate was then concentrated with a rotary evaporator at 50 ◦C under vacuum. The proteins in the extract were removed using the Sevag reagent. After removal of the protein, anhydrous ethanol was added to precipitate the polysaccharides (48h, 4°C). After centrifugation (3000 × g, 15 min), the precipitate was decolorated with acetone and anhydrous ethanol extraction in turn, and then freeze-dried. The polysaccharide was obtained and named as OPS.

Synthesis of OPS-iron(III)

The OPS-iron(III) complex was prepared according to the method of [13] with minor modification. Briefly, 1 mL of 2 M $FeCl_3$-$6H_2O$ was added drop wise under continuous stirring to the aqueous solution containing 2.50% (w/w) of OPS and 0.125% (w/w) of sodium citrate. The pH values were adjusted by the addition of 2 M of HCl or 2 M of NaOH solution. The reaction temperature was controlled by the shaking incubator. After the preset reaction time, the reaction mixture was then centrifuged at 3000 × g for 15 min. The supernatant was concentrated and then dialyzed in distilled water to remove unbound ions (Cl^-, Na^+, and Fe^{3+}). Finally, the dialyzate was concentrated, precipitated by anhydrous ethanol, and then was centrifuged and freeze dried. The polysaccharide-iron(III) complex was obtained and named as OPS-iron (III).

Periodate oxidation and Smith degradation

The sample (30 mg) dissolved in 12.5 mL of distilled water was mixed with 12.5 mL of 30 mmol/L $NaIO_4$. The solution was kept in the dark at room temperature; 0.1 mL aliquots were withdrawn at 24 h intervals, diluted to 25 mL with distilled water and read in a spectrophotometer at 223 nm [26] Periodate consumption was calculated on the basis of the change of the absorbance at 223 nm. The solution of periodate product (2 mL) was used to assess the amount of formic acid by titration with 0.005 mol/L NaOH. Ethylene glycol (2 mL) was added, then the experiment of periodate oxidation was over. The solution of periodate product was extensively dialyzed against tap water 72h and distilled water for 48 h, respectively. The content inside was concentrated and reduced with $NaBH_4$ (100 mg), and the mixture was left for 24 h at room temperature, neutralized to pH 6.0 with acetic acid (50 mL/100 mL), dialyzed as described above, and re-concentrated to 10 mL. One-third of the solution mentioned above was freeze-dried, fully hydrolyzed and analysed by HPLC [27]. Two-thirds of the solution was added to the same volume of 1 mol/L sulfuric acid, kept for 40 h at 25 ?C, neutralized to pH 6.0 with $BaSO_4$, and filtered for analysis by smith degradation. The filtrate was dialyzed (molecular weight cut off 3 kDa), and the content out of the dialysis bag was analyzed by HPLC; whereas the contents inside the dialysis bag were mixed with four volumes of absolute ethanol and centrifuged. The supernatant and precipitate were also analyzed by HPLC [28].

Partial hydrolysis

The OPS (100 mg) was partially hydrolyzed with 0.05 M TFA (3 mL) for 16 h, at 80?C. The hydrolysate was mixed with four volumes of ethanol absolute and kept at 4 ?C overnight. The precipitate was removed by centrifugation at 5000 rpm for 20 min (OPS), and the supernatant was dialyzed against distilled water for 48 h in a dialysis bag (molecular weight 3 kDa cut off). Each of solutions in and out of the dialysis bag was collected for further analysis. Ethanol was added to the solution in the bag after dialysis, and the precipitate and supernatant designated was recovered after centrifugation. The fraction out of dialysis bag (AEP-4) and all other fractions were analyzed by HPLC [30).]

HPLC analysis for monosaccharide composition

The sample (5 mg) was hydrolyzed with 2 mL of 3 M trifluoroacetic acid (TFA) to fill the sample bottle (2 mL) which was then sealed, wrapped in Teflon tape and kept at 110°C for 4 h. After hydrolysis was complete, the residual acid was removed with methanol then dried under vacuum. The dried product

was mixed with 450 µL of 0.5 mol/L PMP-formaldehyde solution to dissolve fully. Then 450 µL of 0.3 mol/L NaOH was added to allow a derivatization reaction at 70°C for 2 h. An equal amount of HCl was used to neutralize the mixture at the end of the reaction then the reaction mixture was extracted with 1 mL chloroform 3 times. The extracts were microfiltered before analysis. High performance liquid chromatography was used to determine the monosaccharide composition. The analysis was performed with a 1260 Infinity II system (Agilent Technologies, Santa Clara, CA, USA) equipped with an InfinityLab Poroshell 120 EC-C18 column (Agilent Technologies, 150 mm × 4.6 mm), and a UV detector with a 250 nm detection wavelength. The eluents used were a mixture of phosphate buffer and acetonitrile (85:15, v/v) injected at a rate of 1 mL/min, at a temperature of 40°C. The sugars were identified by comparison with reference sugars (rhamnose, fructose, arabinose, xylose, mannose, galactose, galacturonic acid, and glucose).

Determination of the Molecular Weight.

The molecular weight of the sample was determined using high performance size-exclusion chromatography on an Empower 3 system equipped with a Waters AQ 450 column (4.6 × 150 mm, 2.5 µm) and a refractive index detector. The sample (4.0 mg/mL) was centrifuged at 6000 rpm for 15 min and then filtered through a 0.22 µm filter. Then, 10 µL of the supernatant was injected during each run. The column and detector temperatures were maintained at 35 °C. A 0.05 M solution of $NaNO_3$ was used as the mobile phase at a flow rate of 0.5 mL/min. The calibration curve was calculated using dextran standards with known molecular weights.

Fourier transform infrared (FTIR) analysis

FTIR spectroscopy was used to investigate the vibrations of molecules and polar bonds between the different atoms. Infrared spectrum analysis was performed using Fourier transform infrared (FT-IR) Spectrometer (TENSOR 27, Bruker, German). Two milligrams of OPS, OPS- iron(III) and FeOOH were mixed with 200 mg potassium bromide (KBr), respectively, compacted into disks for the collection of infrared spectra at 4000–400 cm^{-1} wavelength. The spectra were recorded with 4 cm^{-1} resolution, 16 scans coaddition and were analyzed using Opus software 6.5 (Bruker Optics Limited).

Physicochemical Properties.

The Fe content was determined according to the 1,10-phenanthroline method previously described by Wang et al., 2015 with minor modifications. Briefly, 20 mg of the complex was dissolved in 100 mL of 1 M HCl. Then, 1 mL of sample was mixed with 2 mL of 0.2% ascorbic acid, 2 mL of sodium acetate trihydrate buffer solution (pH = 4.5), and 1 mL of 10% 1,10-phenanthroline solution. After 20 min, the UV-vis absorbance of the complex was recorded at 510 nm (PerkinElmer, U.S.A.). A ferrous sulfate solution was used to obtain a calibration curve ($y = 0.1511x - 0.0123$; $R^2 = 0.9995$), and the iron content of the complex was calculated. The total carbohydrate and galacturonic acid contents were determined using a phenol sulfuric acid ([31] and colorimetric carbazole method[32] respectively.

DPPH Radical Scavenging Assay.

The DPPH radical scavenging activity was evaluated according to a previously reported method[33]. All samples were dissolved in distilled water, and then 1 mL of sample solutions was mixed with 4 mL of 0.1 mmol/L DPPH in methanol. The mixture was shaken for 30 min at room temperature. The absorbance of the mixture was then determined at 517 nm. Ascorbic was used as the positive control. The following formula was used to calculate the DPPH radical scavenging activity:

$$= \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Qualitative identification

Ten milligrams of dried OPS-iron(III) complexes were dispersed in 2 mL of H_2O and 2 mL of 1 M HCl, respectively. The mixtures were stirred at room temperature for 2 h. Potassium thiocyanate (KSCN) and potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$) were then used to detect the two solutions, respectively. The reaction phenomena were recorded.

Quantification of iron content

The quantification of iron content was determined according to the method of [13] with minor modification. Ten milligrams of dried OPS and OPS-iron (III) complexes were dispersed separately n 20 mL of 1 M HCl for 24 h in order to break the complexes and make soluble all released iron. Each sample (1 mL) was mixed with 1 mL of 10% hydroxylamine hydrochloride, 2.5 mL of 10% 1, 10-phenanthroline and 5 mL of sodium acetate trihydrate buffer solution (pH 4.5). After 30 min, UV–Vis (UV-2450, Shimadzu, Japan) detection was performed at = 510 nm. The calibration curve was obtained by using standard solutions of ferrous ammonium sulfate in distilled water ($y = 0.0443x + 0.0036$; $R^2 = 0.9995$). The iron content in OPS-iron (III) complex was calculated.

Intrinsic viscosity of the OPS and OPS-iron(III)

The intrinsic viscosity was measured according to Chen et al., 2013. Different concentrations of OPS and OPS-iron (III) solutions (1.0–5.0 mg/mL in 0.2 M phosphate buffer) were centrifuged and filtered through 0.45 m filter membrane, respectively. An Ubbelohde glass capillary viscometer equipped with viscometer bath was used to determine the passage time of every sample solution flowing through the capillary. The temperature was maintained at 25 ± 0.5 □C. The different passage time between two times was controlled under ±0.1 s.

Morphological analysis

Scanning electron micrographs were obtained with an environmental scanning electron microscope (S-4800, Hitachi, Japan). The OPS and OPS-iron (III) powders were placed on a specimen holder with the help of double-sided adhesive tapes and coated with gold powder. Each sample was observed with 500 and 20.0k fold magnification at 3.0 kV accelerated voltage during micrography.

Electron paramagnetic resonance (EPR) analysis

EPR spectra were recorded with a Bruker A300 spectrometer, operating at X-band frequency (9.6 GHz). EPR spectra of OPS and OPS-iron (III) in solid state were recorded at room temperature in quartz tubes (3 mm i.d.). $CuSO_4$ was taken as the standard for the spin abundance (2.4 × 1021 spins/g) and DPPH was the standard for the g-factor. Measurement conditions used were modulation amplitude of 1.02 mT, modulation frequency of 100 kHz, microwave frequency of 9.76 GHz and nominal microwave power of 57.29 MW.

In Vitro Fe Release of OPS-Fe(III) and $FeSO_4$.

The release of Fe from the OPS –Fe (III) complex was measured according to the 1,10-phenanthroline method. The OPS –Fe (III) complex and $FeSO_4$ were dissolved in distilled water to get a final concentration of 2 mg/mL. A simulated gastric fluid containing 2 g of NaCl, 3.2 g of pepsin (15,000 units), and 7 mL of HCl at a pH of 1.2 was used. Then, 5 mL of 2 mg/mL solution of the OPS –Fe (III) complex or a 2 mg/mL solution of $FeSO_4$ was mixed with 5 mL of simulated gastric fluid and incubated for 2 h. The solution was then added to the simulated intestinal fluid (phosphate buffer media, pH = 6.8) and incubated for an additional 4 h. During the entire process, the fluids were maintained at 37 °C and 100 rpm. Next, 5 mL of the samples was with drawn for the analysis of Fe release. The withdrawn volume was immediately replaced with an equivalent volume of fresh fluids. $K_3[Fe(CN)_6]$-$6H_2O$ and KSCN were used to identify free Fe^{2+} and Fe^{3+} in the simulated gastric fluid of the OPS -Fe(III) complex. The release of Fe ions from the OPS –Fe (III) complex was measured at 510 nm using the 1,10-phenanthroline method. Polarized microscopy (NOVEL, Nanjing, China) was used to observe the freshly prepared gel in the simulated gastric fluid at 50× magnification. The OPS –Fe (III) complex in the simulated gastric fluid was placed on a glass slide without a cover glass at room temperature. Viscosity was measured using a rotational rheometer (MCR 102, Anton Paar GmbH, Austria). Parallel plates with a diameter of 40 mm and a gap size of 1 mm were used. A temperature of 37 °C was maintained, and the shear rate was 0.1-100 s^{-1}. The viscosity curve was recorded.

Iron release from OPS-iron(III) complex in vitro assay

The iron release from OPS-iron (III) complex was measured according to the method of [34] with minor modification. Aliquots (50 mg) of OPS-iron (III) complexes were placed in a round-bottomed volumetric flask. Two types of dissolution media were used during the two successive stages. Firstly, 0.1 M HCl media (pH 1) was used for 2 h, then phosphate buffer media (pH 6.8) was used for 4 h. The dissolution media (500 mL) were maintained at 37 ◦C in the whole process. The paddle was rotated at 100 rpm and 5 mL of samples were withdrawn at the predetermined time intervals (every 20 min) for the analysis of the released iron ions. The withdrawn volume was immediately replaced with an equivalent volume of the fresh medium maintained at the same temperature. The obtained solution was filtered through 0.45 m filter membrane and 100 L of each release solution was adjusted to 1 mL with distilled water. The released iron amount was determined by the 1,10-phenanthroline method with ferrous ammonium sulfate as the standard ($y = 0.0443x + 0.0036$; $R^2 = 0.9995$).

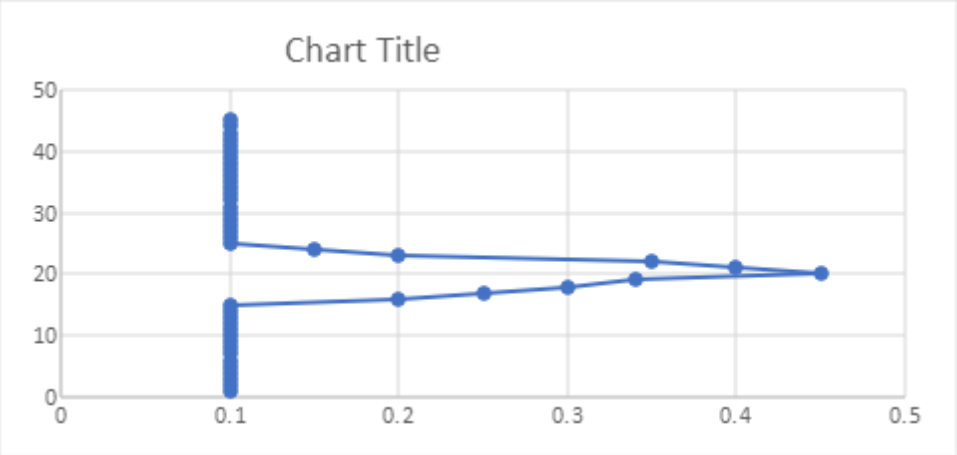
RESULTS AND DISCUSSION

Separation and purification of polysaccharides

The crude polysaccharides were extracted from onion. then purified by DEAE-25 ion exchange column chromatography. The OPS-1 and OPS-2 fractions were obtained in the tubes (Figure 1(a)). The main fraction, OPS-1, was collected and further purified on Sephadex gel chromatography for subsequent experiments. The curve of OPS-1 after passing the Sephadex G-50 gel chromatography showed a single, symmetrical and sharp elution peak (Figure 1(b)), which indicated that it was homogeneous polysaccharide (32). The lyophilized sample of OPS-1 was white flocculent powder with no proteins that showed a negative response in Bradford's method.

Molecular weight and homogeneity detection

The logarithm of the average molecular weight (lgMw) is related to the average partition coefficient in chromatographic columns [35]. The molecular weight of OPS-1 was determined by HPGPC (Figure 2). The curve showed a single and symmetrically sharp peak revealing that OPS-1 had a good homogeneity [41]. According to the retention time, the average molecular weight of OPS-1 was estimated to be 34.0 kDa. The result showed that the purity of the component improved after separation and purification. Analysis of monosaccharide composition. The monosaccharide composition of OPS-1 was quantitatively analyzed by HPLC. As shown in Figure 3(b), the type of monosaccharide of OPS-1 was identified based on the retention times of the derivatization of monosaccharide standards within 30 min. OPS-1 consisted of Fru, Rha, GalA, Glc, Gal, Xyl and Ara at a molar ratio of 2.63:2.40:1.64:6.28:1.95:2.48:5.01. The biological activity of polysaccharides is usually related to their monosaccharide compositions



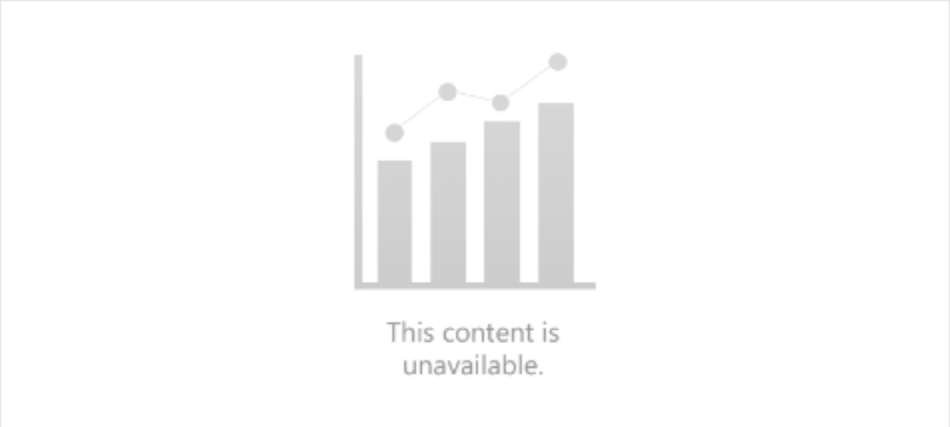
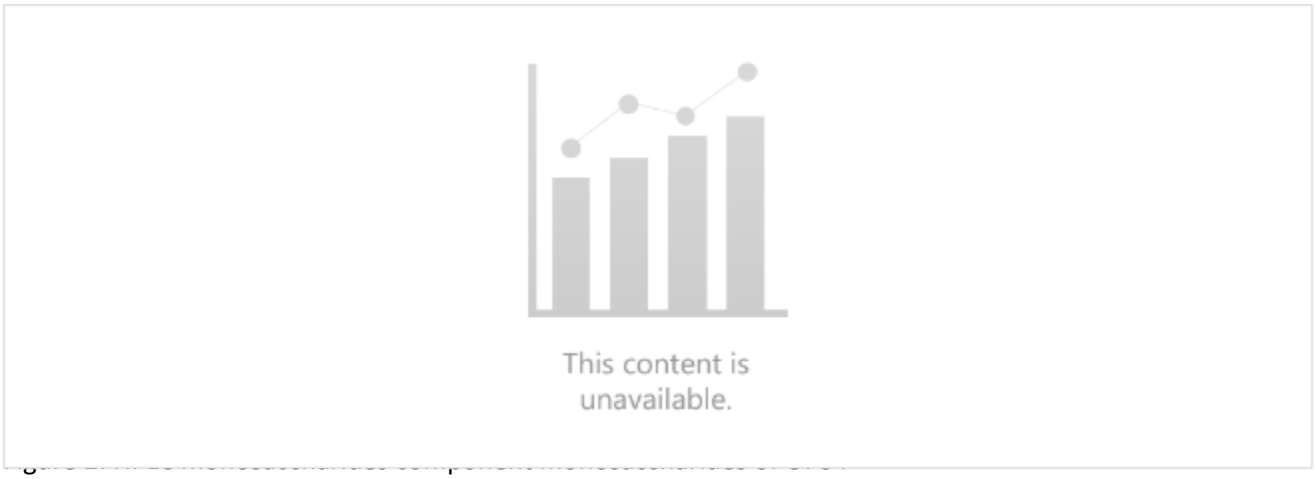


Figure 1. Chromatographies of the polysaccharides from onion. The crude polysaccharide separated by DEAE-52 ion exchange column (a) and eluted with a step-wise gradient of 0, 0.1, 0.2, 0.3 and 0.4 M NaCl-Tris-HCl buffer solution at a flow rate of 1.0 mL/min and 3 mL was collected consecutively in each tube. The fraction eluted with 0.1 M NaCl was pooled, and named as OPS-1. The elution curve of a homogeneous polysaccharide (OPS-1) isolated from OPS-1 (b) by Sephadex gel chromatography. Tris-HCl buffer solution was used as the eluent at a flow rate of 2.5 mL/min with 2 mL collected in each tube.

IR analysis of OPS

The infrared spectra of OPS was shown in Figure 4(a). The broad peak at 3,500–3,000 cm^{-1} was the absorption peak of the O-H stretching vibration in the polysaccharide molecules [36]. The medium- and high-intensity peaks near 2,942 cm^{-1} was caused by the C-H stretching vibration of the alkyl groups [37]). The peaks at 1,630 and 1,220 cm^{-1} indicated the presence of COOH groups in the polysaccharide chain of OPS [38]. The absorption bands at 1550 cm^{-1} was attributed to the stretching vibration of the C–O bond of carboxyl group, and the same absorption peak was also presented at yellow pear residue polysaccharides [39] and the flesh of polysaccharides [40]. The absorption peak at 1,460 cm^{-1},1400 cm^{-1} were caused by the C-H in plane bending vibration [36]33) .



The high-intensity absorption peaks at 1034 cm^{-1} were mainly caused by the deformation and vibration of the single C-O-C and C-O bonds in pyran rings [13]. The weak peak at 909 and 868 cm^{-1} showed the characteristic absorption of the β- and α-glycosidic bonds, respectively, indicating their presence in OPS

Periodate oxidation–Smith degradation

For OPS, a total of 1.18 mol $NaIO_4$ was consumed per mole of sugar residues, and 0.15 mol formic acid was produced. Thus, it was inferred that the (1 ?6)-linked glycosyl bonds or non-reducing terminal residues amounted to 15 % approximately. The oxidized products was analyzed by HPLC after Smith degradation and showed the existence of arabinose, glucose, galactose, fructose and glycerol in the ratio of 1.5:4:1:1.2:7.6, respectively.

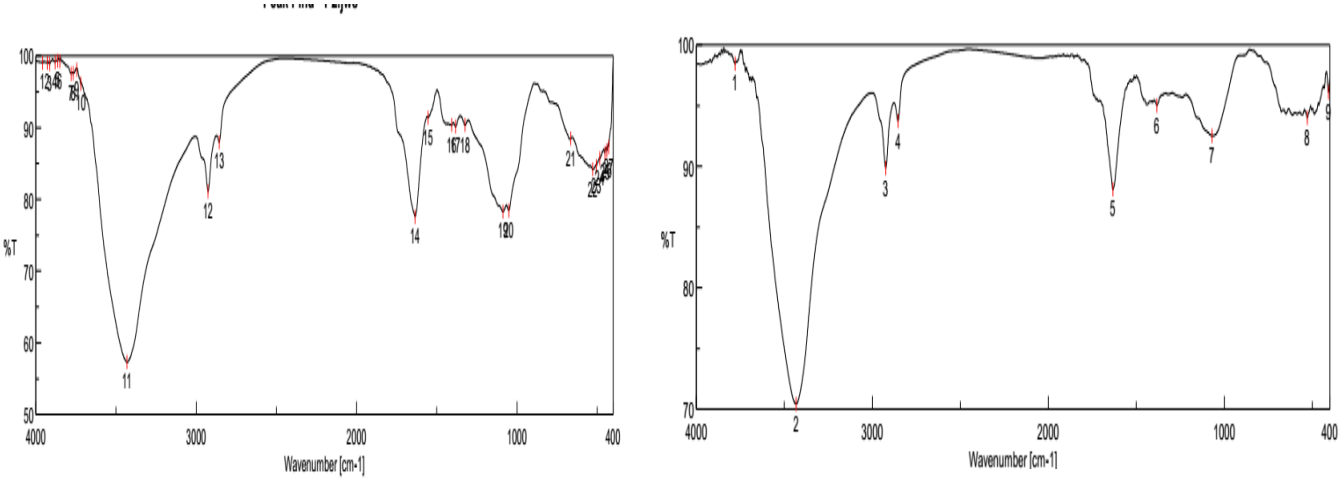


Fig. 3. Spectroscopic characterization of the OPS and OPS-iron(III). FTIR spectra. **Physicochemical characterization of OPS-iron(III) complex**

Synthesis of OPS-iron (III) complex

Based on the previous studies on the synthesis of polysaccharide and iron (III) ion, both sodium citrate and OPS were the ligands for iron (III) [13] Sodium citrate acted as a bridge to associate OPS and iron(III), and then dissociated from OPS-iron(III). The sodium citrate was available for further reaction to produce more OPS iron(III).

The OPS-iron (III) complexes with high iron content were acquired when the solution pH value was set at 10. This might be due to that the OPS contained alcoholic oxygen donor atoms could coordinate to the iron (III) ion after deprotonation of alcoholic hydroxyl groups in the alkaline solution (pH 10) [42]. Or it might be the reason that the carboxylic group of polysaccharides were negatively charged in alkaline media and hence had greater affinity to cations [18]. OPS iron(III) complexes with different colors were obtained depending on the different pH values and the color varied from yellow to reddish brown with pH values changed from 4 to 10. Water solubility of the synthesized OPS-iron (III) complex was also different based on the different synthesis conditions. The optimum conditions for preparing OPS-iron(III) were the temperature of 30□C, pH value of 10 and reaction time of 6 h.

Qualitative identification of OPS-iron(III) complex

The OPS-iron(III) complex obtained at the optimum conditions was a reddish brown powder and had good solubility in water. When potassium thiocyanate (KSCN) was separately added into the aqueous solution and HCl solution of the complex, there was no change in the former but blood red substance in the later. And similarly, there was no dark blue precipitate occurred when potassium ferrocyanide ($K_4[Fe(CN)_6]$-$3H_2O$) was added into aqueous solution while dark blue precipitate appeared in the HCl solution of the OPS iron(III) complex. The results verified that there was no free iron (III) ions existed in

the OPS-iron(III) aqueous solution, the iron(III) ions were coordinated to the OPS instead of simply physical mixture, and the HCl could break the complexes and make iron (III) ions released

Intrinsic viscosity and particle size distribution.

The plot of OPS concentration vs. specc-1 was presented in Fig. 1. The intrinsic viscosity [⍰] of OPS was found to be 0.076 mL/mg while the [⍰] of OPS-iron(III) was decreased to 0.050 mL/mg. The results were in accordance with the findings of [18], in which the intrinsic viscosity [⍰] of alginate was found to be decreased from 0.54 to 0.49 mL/mg with iron loading. The average particle size of the OPS and OPS-iron(III) were determined to be 20.1 and 104.8 nm, respectively. The viscosity of OPS and OPS-Fe are concentration dependent, when the concentration was 0.5 mg/mL and 1 mg/mL, the viscosity of OPS was from 2.40 cps to 2.58 cps while that was 2.82 cps to 4.00 cps in OPS-Fe. In addition, the addition of metal ions may also cause a change in the pH of the solution, which was one of the reasons for the viscosity changed of the polysaccharides solution [34].

The g as coordin a conforr PS was inc ne d.

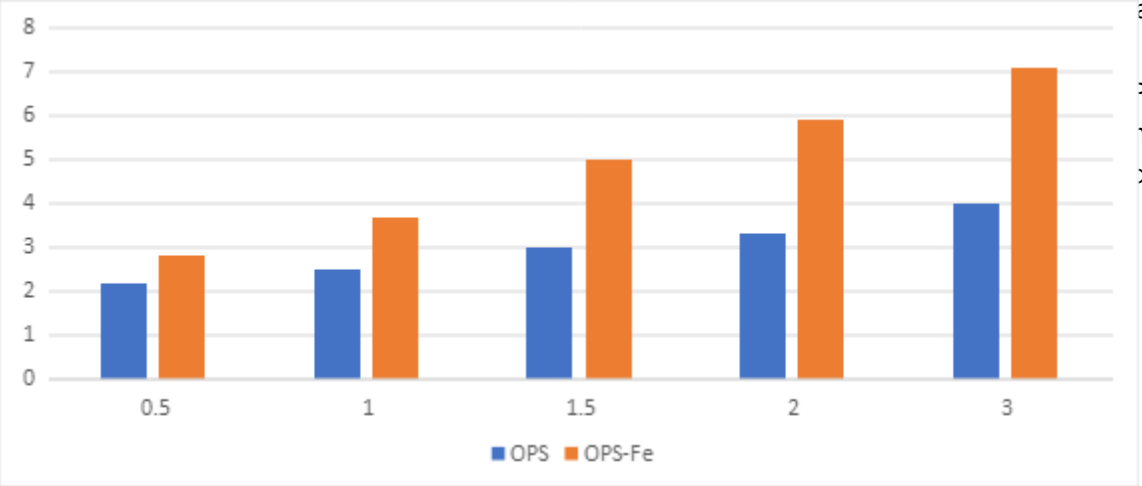
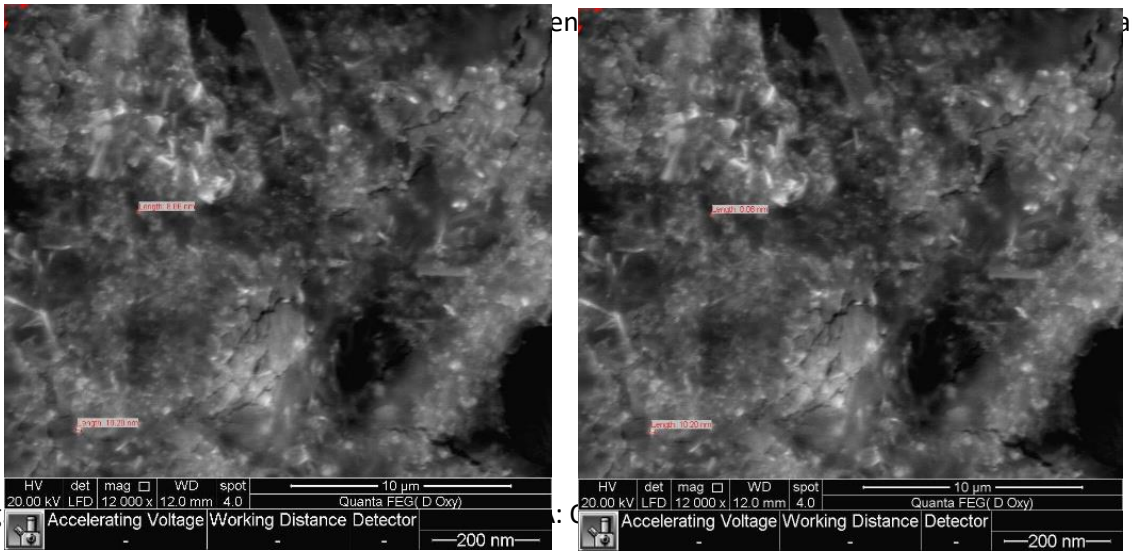


Fig. 4. Intrinsic viscosity [⍰] of OPS and OPS-iron(III).

Morphological analysis

The microphotographs showed the morphology of fragmented OPS and OPS-iron (III) granules (Fig. 2). The surfaces of the polysaccharides and complex showed obvious variations in size and shape. The OPS particle was in the shape of irregular lumps under the 500 fold exaggeration condition while the OPS-iron (III) complex appeared as flake-like morphology. Seen from the image at 20.0k fold augmentation, OPS exhibited relatively regular round-like shape. In contrast, the surface of OPS-iron (III) was flat and smooth, the lateral thickness was 1–1.2 nm. The mechanism might be due to that there was increased

intermolecular OPS-iron (III) cross-linking when iron was coordinated to the OPS and the conformational



Fig

Effect of pH on the stability of OPS-Fe

OPS-Fe solution (2 mg/mL) was prepared, and the pH was adjusted with the NaOH and HCl solution. The NaOH was added dropwise to the OPS -Fe solution. When the pH reached 14, no turbidity occurred, and NaOH was continuously added dropwise, and no brown-red precipitate formed. It proved that OPS -Fe was stable in neutral and alkaline media. When the pH dropped to 3, added the HCl to the OPS -Fe solution, no turbidity occured. Therefore, OPS -Fe was stable at pH 3 ~ 14. When $FeCl_3$ started to be cloudy, the pH was 2.65, when a lot of cloudiness appeared, the pH was 6.72; when $FeSO_4$ started to be cloudy, the pH was 3.35, and when a lot of cloudiness appeared, the pH was 6.79 (Table 3). It showed that OPS -Fe was not possessed iron ions, but it was connected to the polysaccharides by a chemical bond []. One of the main indicators for evaluating the bioavailability of iron supplements was whether the iron supplement was soluble in the duodenum (physiological pH 6 ~ 7), the main absorption site of iron in the body. OPS -Fe was soluble and stable at pH 3 ~ 14 and the structure of the complex had not been destroyed, which indicated that it had high bioavailability [].

Table 3. Effects of pH on the stability of OPS -Fe

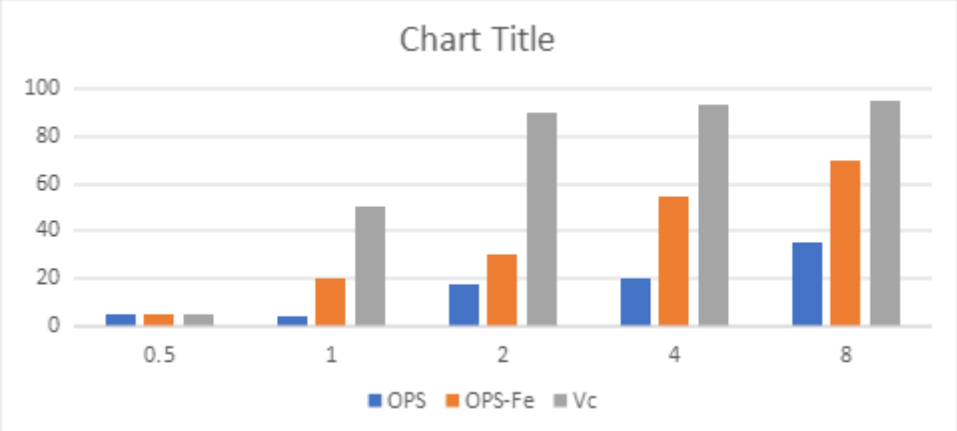
	$FeCl_3$	$FeSO_4$	OPS-Fe
pH at the beginning of turbidity	2.65	3.35	□
pH at lot of turbidity	6.72	6.79	□

Antioxidant activity analysis of OPS and OPS -Fe

In Figure 7a, it showed the scavenging activity of OPS, OPS-Fe, and Vc against DPPH free radical. The result indicated that DPPH free-radical scavenging activities of OPS and OPS -Fe were concentration-dependent. The IC_{50} was defined as the effective concentration of samples, which scavenged 50% radicals. IC_{50} values of OPS, OPS-Fe, and Vc were 9.06, 3.79 and 0.005 mg/mL, respectively. According to the results of DPPH free-radical scavenging activities, the OPS-Fe had stronger anti-oxidative activity. Hydroxyl radical scavenging activity of OPS and OPS-Fe are signifcantly lower than that of Vc (Figure 7b).

Vc had the strongest scavenging activity (IC_{50}, 0.072 mg/mL). IC_{50} of OPS and OPS- Fe were 4.08 mg/mL and 1.46 mg/mL, respectively.

The hydroxyl radical scavenging activity of the OPS-Fe was significantly higher than that of OPS. The polysaccharides of onion contained hydroxyl groups, and as an electron donor, it could interact with free radicals to achieve the effect of scavenging-free radicals. However, when the polysaccharides were coordinated with iron, the electron-donating effect of hydroxyl groups was weakened, but the ability to scavenge free radicals were increased, which may be caused by the combination of iron ions. After the polysaccharides of onion and iron were combined, the space structure of polysaccharides from onion was changed, which made the coordination part cooperated with the exposed active groups, and promoted the interaction with free radicals, thus enhanced the ability of scavenging free radicals [35]



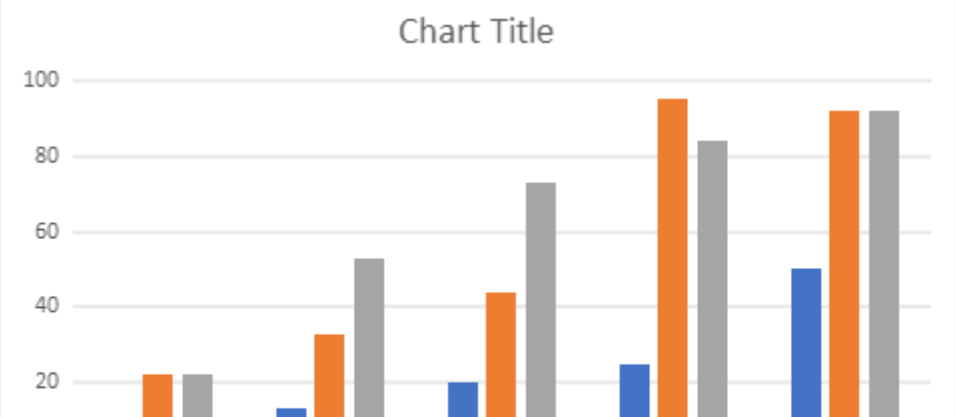


Fig. 6. Antioxidant activities of the OPS-Fe. A: scavenging activity against DPPH free radical; B: lipid peroxidation inhibition activity

Conclusions

The polysaccharide OPS was isolated from the onion. by means of enzymatic and ultrasound co-extraction and a series of purification, including chromatography with DEAE-52 and Sephadex G-50 gel column. Structural analysis revealed that OPS was heteropolysaccharides with different molar ratios of Frc, Rha, GalA, Glc, Xyl, Gal and Ara. In this research, OPS-Fe was obtained by ferric chloride co-heating method, and the optimal conditions were determined by response surface method: reaction temperature was 38.0°C, reaction time was 3.0 h, $FeCl_3$ addition was 2.90 mL and pH was 9, the iron content of OPS- Fe was 15.61%±0.04. UV-vis, FT-IR, XRD, and TEM confrmed that OPS-Fe was a complex with β-FeOOH as the core and polysaccharides on its surface. TGA-DSC and viscosity analysis showed that the stability and viscosity of the modifed polysaccharides increased, and the monosaccharide composition showed that the Gal ratio in OPS-Fe increased, which may be one of the reasons for the enhancement of the antioxidant activity of OPS-Fe. In vitro simulation of human gastrointestinal digestion environment, OPS-Fe was stable at pH [314], it could be reduced to Fe^{2+} at 0.5 h and had a good dissolution. These properties made OPS- Fe slow the gastrointestinal irritation and oxidative toxicity caused by excessive iron intake.**REFERENCES**

[1] Darbyshire B. and R. J. Henry, (1981) “Differences in fructan content and synthesis in some Allium species,” New Phytologist, vol. 87, no. 2, pp. 249–256,.

[2]Benkeblia N., P. Varoquaux, N. Shiomi, and H. Sakai, (2002) “Storage technology of onion bulbs c.v. Rouge Amposta: effects of irradiation, maleic hydrazide and carbamate isopropyl, N-phenyl (CIP) on respiration rate and carbohydrates,” Inter J of Food Science & Technology, vol. 37, no. 2, pp. 169–175,.

[3] Benkeblia N., S. Onodera, and N. Shiomi, (2004) “Effect of gamma irradiation and temperature on fructans (fructo-oligosaccharides) of stored onion bulbs Allium cepa L,” Food Chemistry, vol. 87, no. 3, pp. 377–382,.

[4] Corce, V., Gouin, S.G., Renaud, S., Gaboriau, F. & Deniaud, D. (2016). Recent advances in cancer treatment by iron chelators. Bioorganic & Medicinal Chemistry Letters, 26, 251–256.

[5] Pasricha, S.R. & Drakesmith, H. (2016). Iron deficiency anemia: problems in diagnosis and prevention at the population level. Hematology/oncology Clinics of North America, 30, 309–325.

[6] Cottin S. C., L. Gambling, H. E. Hayes, V. J. Stevens, and H. J. McArdle,(2016) "Pregnancy and maternal iron defciency stimulate hepatic CRBPII expression in rats," e Journal of Nutritional Biochemistry, vol. 32, pp. 55–63,.

[7] Denic S. and M. M. Agarwal, (2007) "Nutritional iron defciency: an evolutionary perspective, "Nutrition Journal , vol. 23, no.7-8, pp. 603–614,.

[8] Lebedev, V.V., Demikhov, V.G., Dmitriev, A.V., Aiari, M. & Fomina, M.A. (2016). A comparative efficacy and safety of using ferrous and ferric iron preparations for management of irondeficiency anaemia. Pediatric Hematology/Oncology & Immunopathology, 15, 5–12.

[9] Mimura, E.C., Bregano, J.W., Dichi, J.B., Greg orio, E.P. & Dichi, I. (2008). Comparison of ferrous sulfate and ferrous glycinate chelate for the treatment of iron deficiency anemia in gastrectomized patients. Nutrition, 24, 663–668.

[10] Cancelo-Hidalgo M.J., C. Castelo-Branco, S. Palacios, J. Haya-Palazuelos, M. Ciria Recasens, J. Manasanch, L. Pérez-Edo, (2013) Curr. Med. Res. Opin. 29 291–303.

[11] Hernandez, Y.G., Calderon, A.M. & Llamo, V.B. (2015). Oxidative effect of severe iron deficiency anemia in male justweaned wistar rats. Revista Cubana De Investigaciones Biomedicas, 34, 44–53.

[12] Sobhani, S.A., Etaati, Z., Mirani, S., et al. (2011). The association between iron status and some immunological factors in the pregnancy. Iranian Journal of Reproductive Medicine, 9, 251–252.

[13] Tang, M. M.; Wang, D. F.; Hou, Y. F.; Buchili, P.; Sun, L. P. (2013) Preparation, Characterization, Bioavailability in Vitro and in Vivo of Tea Polysaccharides-iron Complex. Eur. Food Res. Technol., 236, 341–350.

[14] Koskenkorva-Frank, T.S.; Weiss, G.; Koppenol, W.H.; Burckhardt, S. (2013) The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radical Bio. Med., 65, 1174–1194

[15] Somsook. E., D. Hinsin, P. Buakhrong, R. Teanchai, N. Mophan, M. Pohmakotr, J. Shiowatana, (2005) .Interactions between iron(III) and sucrose, dextran, or starch in complexes. Carbohydr. Res. 61 281–287

[16] Bathia, S.C..N. Ravi, .A (2000) Magnetic Study of an Fe−Chitosan Complex and Its Relevance to Other Biomolecules. Biomacromolecules 1 413–417

[17] F. Jones, H. Colfen, M. Antonietti, (2000) Interaction of κ-Carrageenan with Nickel, Cobalt, and Iron Hydroxides. Biomacromolecules 1 556–563

[18] Sreeram K.J., H. Yamini Shrivastava, B.U. Nair, (2004) Biochim. Biophys. Acta 1670 121–125.

[19] Blanco-Rojo, R.; Vaquero, M. P. (2019) Iron bioavailability from food fortification to precision nutrition. A review. Innovative Food Sci. Emerging Technol., 51, 126-138

[20] Saini, R. K.; Manoj, P.; Shetty, N. P.; Srinivasan, K.; Giridhar, P. (2014) Dietary iron supplements and Moringa oleifera leaves influence the liver hepcidin messenger RNA expression and biochemical indices of iron status in rats. Nutr. Res., 34, 630-638

[21] Torino, A. B.; Gilberti, M. D. F. P.; da Costa, E.; de Lima, G. A.; Grotto, H. Z. W. (2014) Evaluation of red cell and reticulocyte parameters as indicative of iron deficiency in patients with anemia of chronic disease. Rev. Bras. Hematol Hemoter., 36, 424-429

[22] Cheng, C.; Huang, D.-C.; Zhao, L.-Y.; Cao, C.-J.; Chen, G.-T.(2019) Preparation and in vitro absorption studies of a novel polysaccharideiron (III) complex from Flammulina velutipes. Int. J. Biol. Macromol. , 132, 801-810

[23] Wang, H. Chen, Y. Wang, and L. Xing, (2015) "Synthesis and characterization of a new Inonotus obliquus polysaccharide-iron(III) complex," International Journal of Biological Macromolecules, vol. 75, pp. 210–217,

[24] Khramova, D. S.; Vityazev, F. V.; Saveliev, N. Y.; Burkov, A. A.; Beloserov, V. S.; Martinson, E. A.; Litvinets, S. G.; Popov, S. V. (2019) Pectin gelling in acidic gastric condition increases rheological properties of gastric digesta and reduces glycaemic response in mice. Carbohydr. Polym., 205, 456-464

[25] Das, S.; Ng, K. Y.; Ho, P. C. (2010) Formulation and optimization of zinc-pectinate beads for the controlled delivery of resveratrol. AAPS PharmSciTech., 11, 729-742

[26] Linker, A., Evans, L. R., & Impollomeni, G. (2001). The structure of a polysaccharide from infectious strains of Burkholdria cepacia. Carbohydrate Research, 335, 45–54.

[27] El-Sayed, O. H., Ismail, S. A., Ahmed, Y. M., Abd El-Samei, M., & Asker, M. M. S. (2005). Studies on the production of sulfated polysaccharide by locally isolated bacteria. Egyptian Pharmaceutical Journal, 4, 439–452.

[28] Zhang, W. J. (1987). Technology of biochemical research on compound polysaccharide. Hangzhou: Zhejiang University Press (pp. 108–111).

[29] N. Zhang, H.X. Chen, L.S. Ma, Y. Zhang, (2013) Physical modifications of polysaccharide from Inonotus obliquus and the antioxidant properties .Int. J. Biol. Macromol. 54 209–215.

[30] M.M.S. Asker, B.T. Shawky, (2010) Structural characterization and antioxidant activity of an extracellular polysaccharide isolated from Brevibacterium otitidis BTS 44. Food Chem. 123 315–320.

[31] AlKhatib H. S., M. O. Taha, K. M. Aiedeh, Y. Bustanji, and B. Sweileh, (2006) "Synthesis and in vitro behavior of iron-crosslinked Nmethyl and N-benzyl hydroxamated derivatives of alginic acid as controlled release carriers," European Polymer Journal, vol. 42, no. 10, pp. 2464–2474,.

[31] Jain, V. M.; Karibasappa, G. N.; Dodamani, A. S.; Mali, G. V. (2017) Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method. J. Educ. Health Promot. 6, 90-96.

[32] Ma, X.; Jing, J.; Wang, J.; Xu, J.; Hu, Z. (2020) Extraction of low methoxyl pectin from fresh sunflower heads by subcritical water extraction. ACS Omega, 5, 15095-15104.

[32] Niu YG, Yan W, Lv JL, (2013) Characterization of a novel polysaccharide from tetraploid Gynostemma pentaphyllum makino. J Agric Food Chem.;61:4882–4889.

[33] Hosseini, S. S.; Khodaiyan, F.; Kazemi, M.; Najari, Z. (2019) Optimization and characterization of pectin extracted from sour orange peel by ultrasound assisted method. Int. J. Biol. Macromol., 125, 621-629.

[33] Liang XX, Gao YY, Pan Y, (2018). Purification, chemical characterization and antioxidant activities of polysaccharides isolated from mycena dendrobii. Carbohyd Polym.;203:45–51.

[34] He, R. X. (2012) Study on Preparation, Properties and Structure of Soluble Soybean Polysaccharide Iron Complex; Guangzhou China, South China University of Technology,

[35] Bhatia, S. C. and Ravi, N. A (2000) Magnetic Study of an Fe-chitosan Complex and Its Relevance to Other Biomolecules. Biomacromolecules., 1(3), 413–417.

[35] Sun LQ, Wang C, Shi QJ,(2009). Preparation of different molecular weight polysaccharides from Porphyridium cruentum and their antioxidant activities. Int J Biol Macromol.;45:42–47.

[37] Kim GY, Lee JY, Lee JO,(2006). Partial characterization and immunostimulatory effect of a novel polysaccharide–protein complex extracted from phellinus linteus. Biosci Biotechnol Biochem.;70:1218–1226.

[38] Viteri, F. E., E. Casanueva, M. C. Tolentino,(2012) J. D´ıaz-Franc´ es, and A. B. Erazo, "Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women," Reproductive Toxicology, vol.34, no. 1, pp. 125–132,.

[39] Peng, X.-B., Q. Li, L. Ou, L.-F. N,(2010) K. Zeng, "GC-MS, FTIR analysis of black fungus polysaccharides and its inhibition against skin aging in mice," International Journal of Biological Macromolecules, vol. 47, pp. 304–307.

[40] Dubois M, Gilles KA, Hamilton JK,(1956). Colorimetric method for determination of sugars and related substances. Anal Chem.;28:350–35.

[41] Yang JL, Tu JM, Liu H,(2019). Identification of an immunostimulatory polysaccharide in banana. Food Chem.;277:46–53.

[42] Gyurcsik L., B. Nagy, (2000) Metal complexes of carbohydrates and their derivatives: coordination equilibrium and structure, Coord. Chem. Rev. 203 81–149.

[43] Hernandez R., Sacristan J., Nogales A., Fernandez M. and Carmen Mijangos (2010). Structure and viscoelastic properties of hybrid ferrogels with iron oxide nanoparticles synthesized in situ. Soft Matter 6:3910–3917