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Physico-chemical characterization of starch ferulates of different degrees of substitution

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Abstract

Starch ferulates were prepared by reacting potato starch with ferulic acid chloride, using pyridine as a catalyst in dimethyl sulfoxide. Starch ferulates of different degrees of substitution (DS) were prepared and their formation was confirmed by the presence of the carbonyl signal around 1726 cm^{-1} in the FT-IR spectra. The thermal characteristics of the native starch and starch ferulates of different degrees of substitution were studied using TGA, DTG and DSC and the studies revealed the starch ferulates to be thermally more stable than the native starch. The starch esters exhibited 50% weight loss at temperatures from 332 to 375 °C while the native starch underwent 50% weight loss at 321 °C. The ^{1}H and ^{13}C NMR studies confirmed the structure of the modified starch. X-ray diffraction studies revealed the loss of the ordered B-type crystalline structure, characteristic of potato starch. The microstructural imaging of the starch esters exhibited a networking which was enhanced with increasing degree of substitution. The starch ferulates also exhibited DPPH radical and ABTS radical cation-scavenging activity.

Keywords: Starch; Esterification; Ferulic acid; Physico-chemical characterization

1. Introduction

Starch is an attractive raw material due to its abundant supply, low cost, biodegradability, biocompatibility and ease of chemical modification. Indeed, modified starches have been approved for food use, in which they act as thickeners, gelling agents, as sizing agents in textiles and as adhesives for paper and paper products. In recent years, several authors (Aburto, Alric, & Borredon, 1999; Fang, Fowler, Tomkinson, & Hill, 2002) have reported the preparation of modified starches with high degrees of substitution, using organic solvents. Reactions with starches, to prepare highly substituted derivatives, are not easy, mainly because of the almost impossible proposition of dissolving the granular starch in a suitable medium without significant degradation (Sagar & Merill, 1995). The introduction of an ester group into polysaccharides constitutes an important synthetic task, as it modifies their original hydrophilic nature and yields enhanced or new thermal and mechanical properties (Aburto et al., 2000).

Functional foods, enriched in biologically active compounds are becoming increasingly available in many countries and the potential markets are enormous. Ferulic acid is one of the most abundant hydroxy cinnamic acids, present at relatively high concentrations in the cell walls of several plants (Hartley & Ford, 1989; Hartley & Harris, 1981). The antioxidant activity of ferulic acid is well recognized (Rice Evans, Miller, & Paganga, 1996) and it is also reported as antihepatotoxic (Kiso, Suzuki, Watanabe, Oshima, & Hikino, 1983) and offers various benefits for the cardiovascular system (Rukmini & Raghuram, 1991). Apart from its anti-inflammatory action (Chawla, Singh, Murthy, Gupta, & Singh, 1987), ferulic acid as a constituent of synthetic lignins, may contribute to the defence against viral infections, including AIDS (Lai et al., 1992). Free ferulic acid does not enter the enterohepatic circulation

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(Chang, Xu, Cheng, & Feng, 1993) and therefore cannot easily reach the colon through oral or intravenous administration. Moreover, the dietary fibre-bound ferulic acid is only partly released by the microorganisms in the colon (Ou, Gao, & Li, 1999). Enzyme-resistant starches, e.g. potato and banana starches (Sugimoto, Fujita, Takaya, & Fuwa, 1980), are almost completely fermented in the colon (Baghurst, Baghurst, & Record, 1996) and may serve as a satisfactory carrier of ferulic acid and as a potential prebiotic (Fiedorowicz, Chaczatrian, Kapusniak, Tomasik, & Tomasik, 2003). Ferulic acid has been considered to be a new candidate chemopreventive agent for colon carcinogenesis because of its strong suppressive effects on colon tumor cell proliferation. The release of ferulic acid from starch ferulate by colon microorganisms has been found to be much higher than that from dietary fibres from wheat bran (Ou, Li, & Yang, 2001).

Ou et al. (2001) succeeded in synthesizing starch ferulate of low degree of substitution (DS) using starch derived from maize. The present study has attempted to synthesize starch ferulates of higher degrees of substitution from enzyme-resistant potato starch and to characterize their physicochemical properties.

2. Materials and methods

2.1. Materials

Potato starch was isolated and purified from the fresh tubers of potato (*Solanum tuberosum*), using the modified procedure of Willinger (1964). The amylose and amylopectin contents of starch were determined simultaneously (Landers, Gbur, & Sharp, 1991). The ash, protein and fat contents of the starch were determined according to the standard AOAC methods (AOAC, 1995). Ferulic acid was procured from Sigma (St. Louis, USA). Thionyl chloride, DMSO and pyridine were obtained from Merck, India, Sisco Research Laboratories and CDH, India, respectively. All the reagents used were of analytical grade.

2.2. Esterification

The esterification of starch was carried out in two steps. The first step involved the synthesis of ferulic acid chloride by refluxing ferulic acid with thionyl chloride at 80 °C for 3 h. Upon completion of the reaction, the thionyl chloride was removed by repeated distillation, using chloroform, and the acid chloride formed was dried under nitrogen. Stoichiometric quantities of the acid chloride were added to potato starch dissolved in DMSO, followed by pyridine, added in a 2:1 ratio with respect to the acid chloride and refluxed at 110 °C for 1 h under nitrogen and continuous stirring. The product was precipitated using 95% alcohol (300 ml × 3). The precipitate was then centrifuged, washed with 70% alcohol, vacuum-dried at 50 °C and used to determine the degree of substitution (Table 1).

2.3. Determination of the degree of substitution (DS)

Starch ferulate (0.25 g) was extracted in 25 ml of a solution of 1:1 mixture of 1% (w/v) of $NaBH_4$ and 8% (w/v) NaOH so that the final concentrations were 0.5% and 4%, respectively. The extracts were then acidified with HCl to pH 2.5 and extracted with two volumes of ethyl acetate (×3). The content of free ferulic acid was then determined spectrophotometrically at 320 nm (Rybka, Sitarski, & Raczynska-Bojaowska, 1993) in a UV–visible spectrophotometer (Shimadzu, UV 2100), using ethyl acetate as blank.

DS value was determined titrimetrically according to the method of Wurzburg (1964) with slight modifications. Powdered starch ferulate (0.125 g) was weighed accurately, treated with 6.25 ml of 75% ethanol, warmed at 50 °C and held at that temperature for 30 min, followed by the addition of 5 ml of 0.5N NaOH. The flask was stoppered and allowed to stand for 72 h with occasional shaking. The excess NaOH was back-titrated against 0.5N HCl, using phenolphthalein as indicator. A blank was simultaneously titrated with native starch instead of the ester. DS was calculated as:

$$\%\text{feruloyl} = \frac{[\text{ml(blank)} - \text{ml(sample)}] \times \text{normality of acid} \times 0.177 \times 100}{\text{sample weight in grams (dry basis)}}$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \%\text{feruloyl}}{177 \times 1000 - (176 \times \%\text{feruloyl})}$$

where 162 = molecular weight of glucose unit; 177 = molecular weight of feruloyl group; 176 = molecular weight of feruloyl group-1.

Reaction efficiencies were calculated as the ratio of measured DS to theoretical DS (moles of starch ferulate/moles of anhydroglucose residue).

Table 1
Esterification yields and degrees of substitution of starch ferulates

Type	No. of moles of acid chloride	No. of moles of starch ferulate	Theoretical DS of starch	DS obtained by		Reaction efficiency (%)	Yield (%)
				Alkaline saponification	Spectrophotometry		
1	0.0072	0.014	0.514	0.150	0.125	29.2	96.2
2	0.0102	0.014	0.728	0.360	0.344	49.6	94
3	0.014	0.014	1.00	0.50	0.519	50	97
4	0.0224	0.014	1.60	0.776	0.757	48.5	97

2.4. Determination of viscosity

The viscosities of the native and modified starch esters in dimethylsulfoxide (DMSO) were determined using a Synchro-electric Brookfield viscometer (Model RVT, MA, USA) at three different rpm, using spindle number 21, and expressed in Pa s. Both starch and starch ferulate (1%w/v) were dissolved in DMSO at 80 °C under stirring and the viscosity was measured in triplicate at 30 ± 1 °C.

2.5. Thermal analyses

2.5.1. Thermogravimetric analyses

Thermogravimetric analyses were performed in a Simultaneous DTA-TG Apparatus (DTG-60; Shimadzu, Japan). Samples (2–8 mg) were heated at a rate of 20 °C/min from ambient temperature to 800 °C. Nitrogen was used as the purge gas at a flow rate of 20 ml/min. In order to determine the thermal stability of native starch and the starch esters, the following values were determined: $T_{x\%}$ – temperature corresponding to x% mass loss.

2.5.2. Differential scanning calorimetry (DSC) analyses

Thermal properties of the native potato starch and the starch ester were characterized using a Perkin–Elmer Pyris DSC 6 (Perkin–Elmer, Boston, MA). Nitrogen, at the rate of 30 ml/min, was used as purge gas; 5–9 mg of powdered material were sealed in aluminium pans and heated from 20 °C up to 200 °C in the case of starch ester and up to 250 °C in the case of native starch at the rate of 10 °C/min, followed by a cooling cycle back to 20 °C at the same rate.

2.6. Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of the native starch and starch ester were recorded in an IR spectrometer (Nicolet Magna 4R 560, MN, USA), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in the ratio 1:200.

2.7. X-ray diffraction studies

X-ray diffraction patterns of native potato starch, ferulic acid and starch ferulate were analyzed using an X-ray diffractometer (XPERT, Philips, Eindhoven, The Netherlands) with Nickel filtered Cu Kα radiation ($\lambda = 0.154$ nm) at a voltage of 40 kV and current of 30 mA. The diffractometer was equipped with an automatic divergence slit and the scattered radiation was detected in the angular range of 5–40 (2θ), with a scanning speed of 2 ° (2θ)/min and step size of 0.06 (2θ).

2.8. NMR analysis

The NMR spectra were recorded using a Bruker Spectrometer at 500 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR, respectively. The control starch and starch ferulate were dissolved in DMSO-d_6 and the analysis was carried out at 50 °C.

2.9. Microstructure studies by SEM

The morphological features of the native potato starch and starch ferulates of different DS were studied with a JSM-5600 LV scanning electron microscope of JEOL, Tokyo, Japan. The dried samples were mounted on a metal stub and sputtered with gold in order to make the sample conductive, and the images were taken at an accelerating voltage of 15 kV and 2500× magnification.

2.10. Determination of solubility

The solubilities of the starch ferulates were measured at 5% (w/v) concentration in a range of organic solvents, with stirring at room temperature and also under heating.

2.11. Spectrophotometric analyses

The UV–visible spectra of starch, starch ferulates of different DS in DMSO solution (at a concentration of 100 mg/ml) and ferulic acid in ethanol (10 μg/ml) were obtained using a UV–visible spectrophotometer (Shimadzu, Kyoto, Japan).

2.12. Evaluation of antioxidant activity

2.12.1. Rapid screening of radical-scavenging capacity of starch ferulates by dot-blot and DPPH staining

An aliquot (5 μl) of each dilution of the starch ferulate and the standard compound, ferulic acid were carefully loaded onto a 10 × 20 TLC layer (Silica gel 60F_{254}; Merck) and allowed to dry. Drops of each sample were loaded in the order of increasing concentration along the row. The staining of the silica plate was based on the procedure of Solver-Rivas, Carlos Espin, and Wichers (2000).

2.12.2. Determination of ABTS radical cation decolorisation capacity

The experiments were carried out using an improved ABTS decolorisation assay Re et al. (1999) and involved the generation of $ABTS^{+}$ chromophore by the oxidation of ABTS with potassium persulfate. It is applicable for both hydrophilic and lipophilic compounds.

The ABTS radical cation ($ABTS^{+}$) was produced by reacting a 7 mM stock solution of ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature before use. The $ABTS^{+}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Shimadzu UV–visible Spectrophotometer, Model 2100). Absorbance was measured 7 min after the initial mixing of different concentrations of starch ferulate with 1 ml of $ABTS^{+}$ solution. All determinations were carried out in triplicate and the mean values were taken.

3. Results and discussion

3.1. Starch ferulate synthesis

The solubilisation of the swollen starch in DMSO activates the exposed hydroxyl groups to attack by electrophilic reagents. Pyridine acts, not only as a base, but also as a nucleophilic acylation catalyst. The hydrochloric acid formed as a by-product reacts with the excess pyridine to form the pyridinium salt (Fang et al., 2002). The percentage yield of starch ferulates obtained is given in Table 1. The formation of the starch ferulate (Fig. 1) was confirmed by IR.

3.2. DS of starch ferulate

The DS of a starch derivative is defined as the number of hydroxyl (OH) groups substituted per D-glucopyranosyl structural unit of the starch polymer. Since each glucose unit possesses three reactive hydroxyl groups, the maximum possible DS value is 3. The primary OH group on C-6 is more reactive and is esterified more readily than the secondary ones on C-2 and C-3 due to steric hindrance. The DS varies with the source of starch, amylose and amylopectin fractions, stoichiometric amounts and reaction time. The esterification of starch with organic acids results in thermoplastic and hydrophobic materials when the DS is high enough (Rudnik, Matuschek, Milanov, & Kettrup, 2005).

The DS of the starch ferulates increased with higher ratios of acid chloride to starch. Higher ferulic acid chloride concentration resulted in a higher rate of molecular collision and a greater availability of ferulic acid chloride molecules in the vicinity of starch (Xu, Miladinov, & Hanna, 2004). The reaction efficiency, however, decreased slightly at higher molar concentrations of acid chloride as the esterification reactivities of the hydroxyl goups in the anhydroglucose unit were different. The hydrophobicity of starch increases with the increasing DS, which in turn increases the miscibility of starch with other hydrophobic polymers (Thiebaud et al., 1997).

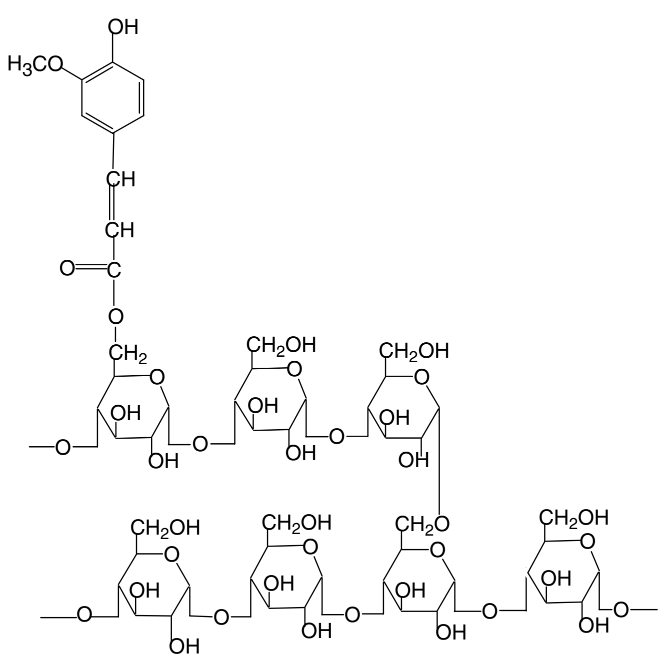

Fig. 1. Structure of starch ferulate with ferulic acid attached to the sixth carbon atom of the glucose unit in starch.

3.3. Viscosity

The viscosity profile of both native potato starch and the synthesized starch ferulates, when spindle number 21 was used, are shown in Table 2. On esterification, the starch exhibited a reduction in viscosity, supporting an enhanced hydrophobicity for the starch ester compared to the native starch. Chemical modifications cause rupturing of some or all of the starch molecules, thereby weakening them and decreasing their capacity to swell. Also, a certain degree of depolymerization, which must have occurred during the esterification reaction, might also have contributed to the reduction in viscosity. The viscosity was found to decrease with increasing rpm, revealing the shear-thinning behaviour and pseudoplastic nature of both native starch and the starch ester.

3.4. Thermogravimetric analyses

The thermogravimetric spectra were used to determine the weight loss of the material on heating. The TGA and DTG (derivative thermogravimetric) curves for the native starch and starch ferulates are shown in Figs. 2 and 3. The starch and starch esters of low DS showed a three stage weight loss, the first minor one corresponding to the loss of water around 60–100 °C and the other two corresponding to its decomposition. Water is the main prod-

Table 2
Viscosity profiles of potato starch and starch ferulates

Sample	Brookfield (rpm)	Brookfield viscosity in Pa s
Native potato starch	10	0.055
	20	0.047
	50	0.034
Starch ferulate 1	10	0.025
	20	0.020
	50	0.010
Starch ferulate 2	10	0.025
	20	0.020
	50	0.009
Starch ferulate 3	10	0.020
	20	0.015
	50	0.007
Starch ferulate 4	10	0.017
	20	0.012
	50	0.005

uct of decomposition at temperatures below 300 °C (i.e. water formed by intermolecular and intra-molecular condensation of the starch hydroxyls). The native starch underwent 50% weight loss at 321 °C while, in the case of starch ferulates, the temperature ranged from 332 to 375 °C for esters with different DS (Table 3). The DTG curve for native starch showed three peaks, the first one corresponding to the loss of water at around 60–100 °C, followed by a two stage decomposition. With increase in DS there was a shifting of peak maximum towards higher temperatures. The starch ferulates were found to be more thermally stable than native starch. Since the main decomposition mechanism of starch is the dehydration reaction between starch hydroxyls, the lower the number of hydroxyl groups remaining, the better is the thermal stability of the starch esters (Rudnik et al., 2005).

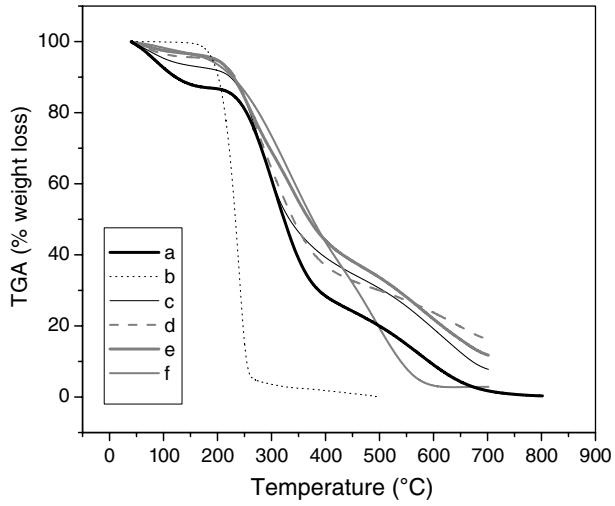

Fig. 2. TGA thermogram of (a) native starch, (b) ferulic acid and starch ferulates of different DS (c) starch ferulate 1, (d) starch ferulate 2, (e) starch ferulate 3 and (f) starch ferulate 4.

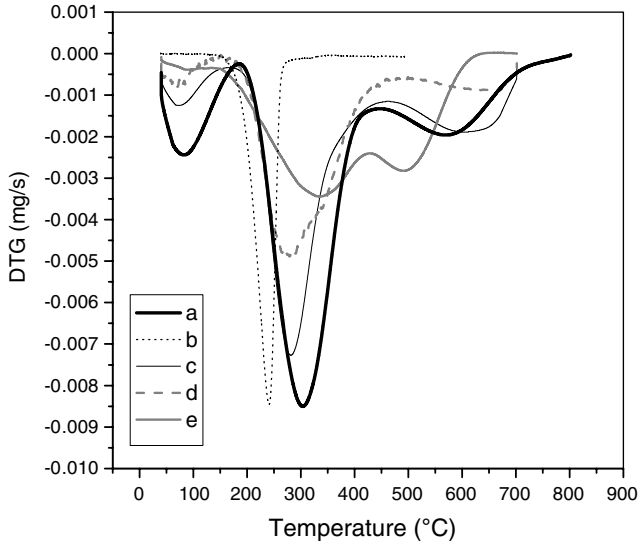

Fig. 3. Derivative thermogravimetric curves of (a) native starch, (b) ferulic acid and starch ferulates of different DS (c) starch ferulate 1, (d) starch ferulate 2 and (e) starch ferulate 4.

Table 3
Thermal characteristics of starch and starch ferulates (% weight loss at different temperatures)

Sample	$T_{5\%}$ (°C)	$T_{25\%}$ (°C)	$T_{50\%}$ (°C)
Starch	81.5	267.4	321.0
Ferulic acid	190.4	216.4	234.3
Starch ferulate 1	105.3	271.9	332.5
Starch ferulate 2	191.1	271.9	340.1
Starch ferulate 3	196.7	279.5	370.3
Starch ferulate 4	198.5	292.2	375.4

3.5. DSC

DSC was used to measure the occurrence of exothermal or endothermal changes with increase in temperature (Fig. 4) however, the DSC profile of native starch and starch ferulate did not reveal much significant data except for the broad endothermal peak around 60–100 °C, corresponding to the evaporation of water, as supported by the TGA and DTG data. On gelatinization, the hydrogen bonding between the adjacent glucose units in the swollen granule of starch is disrupted and the crystallinity is destroyed (St. Pierre, Favis, Ramsay, Ramsay, & Verhoogt, 1997).

3.6. FT-IR

In potato starch, the finger print region of the spectrum consists of three characteristic peaks between 923 and 1162 cm^{-1}, attributed to the C–O bond stretching (Fig. 5) (Goheen & Wool, 1991). The bands at 1659 cm^{-1} and 1467 cm^{-1} were assigned to the δ (O–H) bendings of water and CH_2 respectively (Mano, Koniarova, & Reis, 2003). The sharp band at 2926 cm^{-1} is characteristic of C–H stretches associated with the ring methine hydrogen atoms.

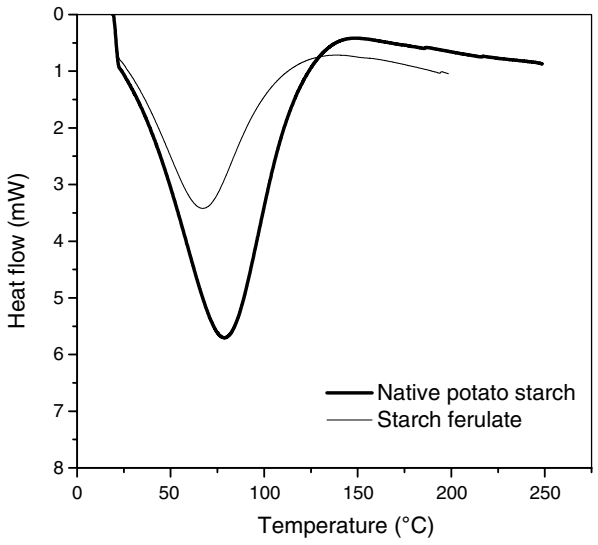

Fig. 4. DSC thermogram of native starch and starch ferulate.

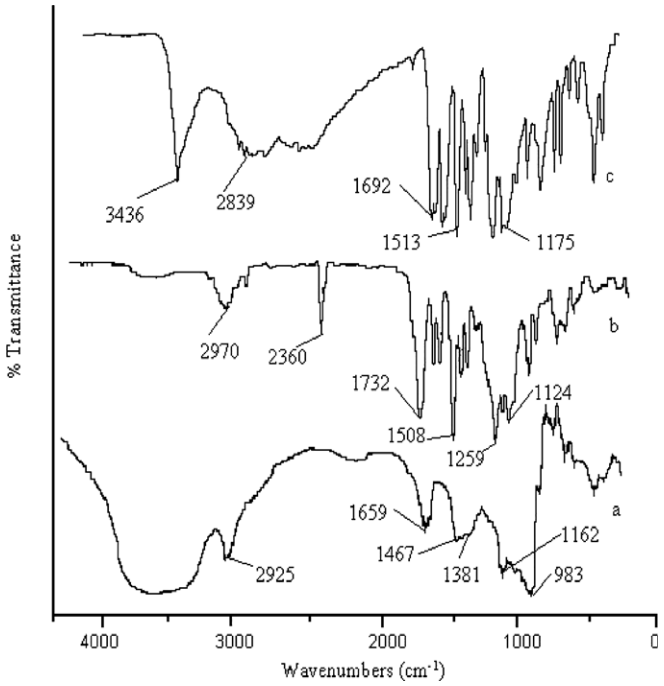

Fig. 5. IR Spectra of (a) native potato starch, (b) ferulic acid chloride and (c) ferulic acid.

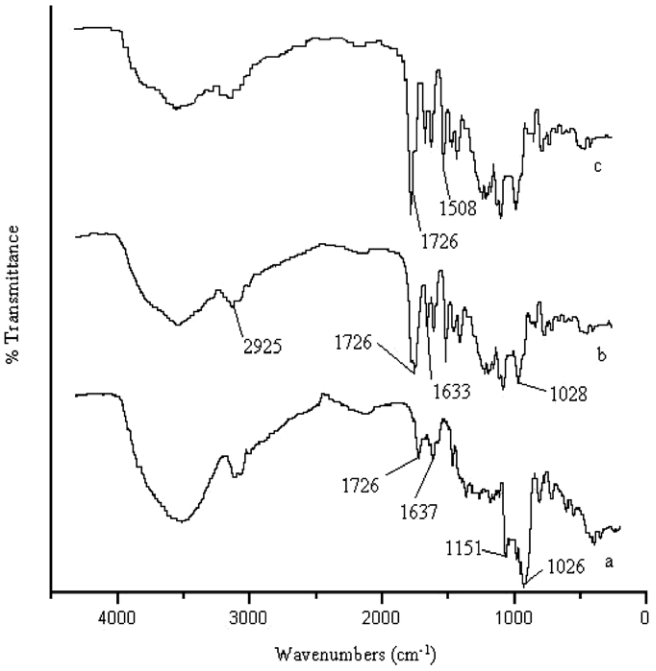

Fig. 6. IR Spectra of starch ferulates of different degree of substitution: (a) starch ferulate 2, (b) starch ferulate 3 and (c) starch ferulate 4.

An extremely broad band occurs at 3400 cm^{-1}, due to the hydrogen-bonded hydroxyl groups that contribute to the complex vibrational stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of starch (Fang et al., 2002).

The spectrum of ferulic acid consisted of a sharp peak at 3436 cm^{-1} corresponding to the hydroxyl group. The sharp bands at 1619, 1593, 1513, 1434 cm^{-1} are due to C–C skeletal vibrations and are characteristic of the aromatic ring. The sharp bands at 804 and 850 cm^{-1} are due to the two adjacent hydrogen atoms. The formation of the acid chloride was confirmed by the presence of carbonyl absorption in the FT-IR spectrum at 1732 cm^{-1} and absence of the broad hydroxyl band of the acid in the region of 3500–3000 cm^{-1}. Esterification resulted in the presence of a carbonyl signal in the FT-IR spectrum around 1726 cm^{-1} (Fig. 6), distinct from the carbonyl signals of both unreacted acyl chloride around 1732 cm^{-1} or the hydrolysis products, carboxylic acids, around 1700 cm^{-1} and their salts around 1640 cm^{-1}. The occurence of two peaks of strong intensities at 2926 and 2860 cm^{-1} in the spectra is attributed to the methyl and methylene C–H stretching associated with the feruloyl substituents. In the starch ferulate, the broad peak around 3400 cm^{-1} was reduced in intensity owing to the reduction in the number of hydroxyl groups. The intensity of the carbonyl group peak at 1726 cm^{-1} also increased with increase in DS (Fig. 6).

3.7. XRD

The native potato starch powder had a typical B-type crystalline structure [31], as shown in Fig. 7 with a strong

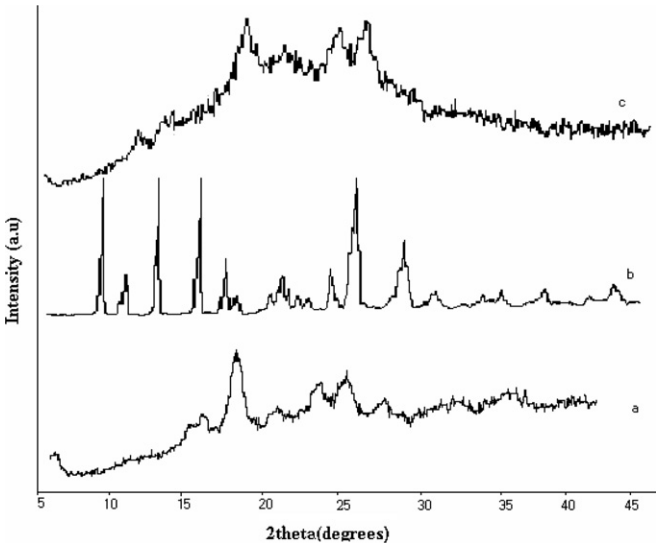

Fig. 7. XRD profile of (a) native potato starch, (b) ferulic acid and (c) starch ferulate.

diffraction peak at around 17° (2θ) and a few small peaks at around 2θ of 15°, 20°, 22° and 24°. Linear amylase, composed of α-1,4 glucopyranose units, contributed to amorphous regions, while the branched amylopectin, composed of α-1,4 and α-1,6 glucopyranose units contributed to the crystalline region. The intra and intermolecular hydrogen bonds were responsible for the highly ordered crystalline structure (Xu et al., 2004). Ferulic acid has strong crystallinity peaks at 2θ of 9°, 10.4°, 12.7°, 15.6°, 17.3°, 26.3° and 29.4°, and several minor peaks at 21°, 24.5°, 31.5°, 34.6°, 36° and 39°. On esterification, the

feruloyl groups replaced some of the hydroxyl groups on starch, reducing the formation of intermolecular hydrogen bonds and thereby the destruction of the ordered crystalline structure. The starch ferulate ester had broad peaks at 17.7°, 20.2°, 21.8°, 23.8° and 25.47°.

3.8. Analysis of the NMR spectra

The chemical shifts are reported in parts per million downfield from 0.00 ppm. The ^{1}H NMR spectral signals between 3.2 and 5.2 ppm (Fig. 8a) corresponded to the protons of the constituent repeating α-D-glucopyranosyl units, while the starch ferulate exhibited the characteristic peaks of ferulic acid protons in the region from 6.5 to 8 ppm, except for the methoxy protons of ferulic acid observed as a singlet at 3.8 ppm (Fig. 8b). The presence of a pair of downfield doublets at 6.7 and 6.8 ppm and 7.6 and 7.8 ppm confirmed the presence of vinylic protons in ferulic acid and the aromatic protons were observed in the 6.9–7.4 ppm range.

In the ^{13}C spectra of starch (Fig. 9a), the signal at 100.0 ppm corresponded to the anomeric methine carbon atom (C-1), while the peak at 60.4 ppm was due to the primary carbon atom in starch (C-6). The C-4 carbon has chemical shifts at 78.7 ppm while the peaks at 73.2, 71.9 and 71.5 ppm corresponded to C-3, C-2 and C-5 of

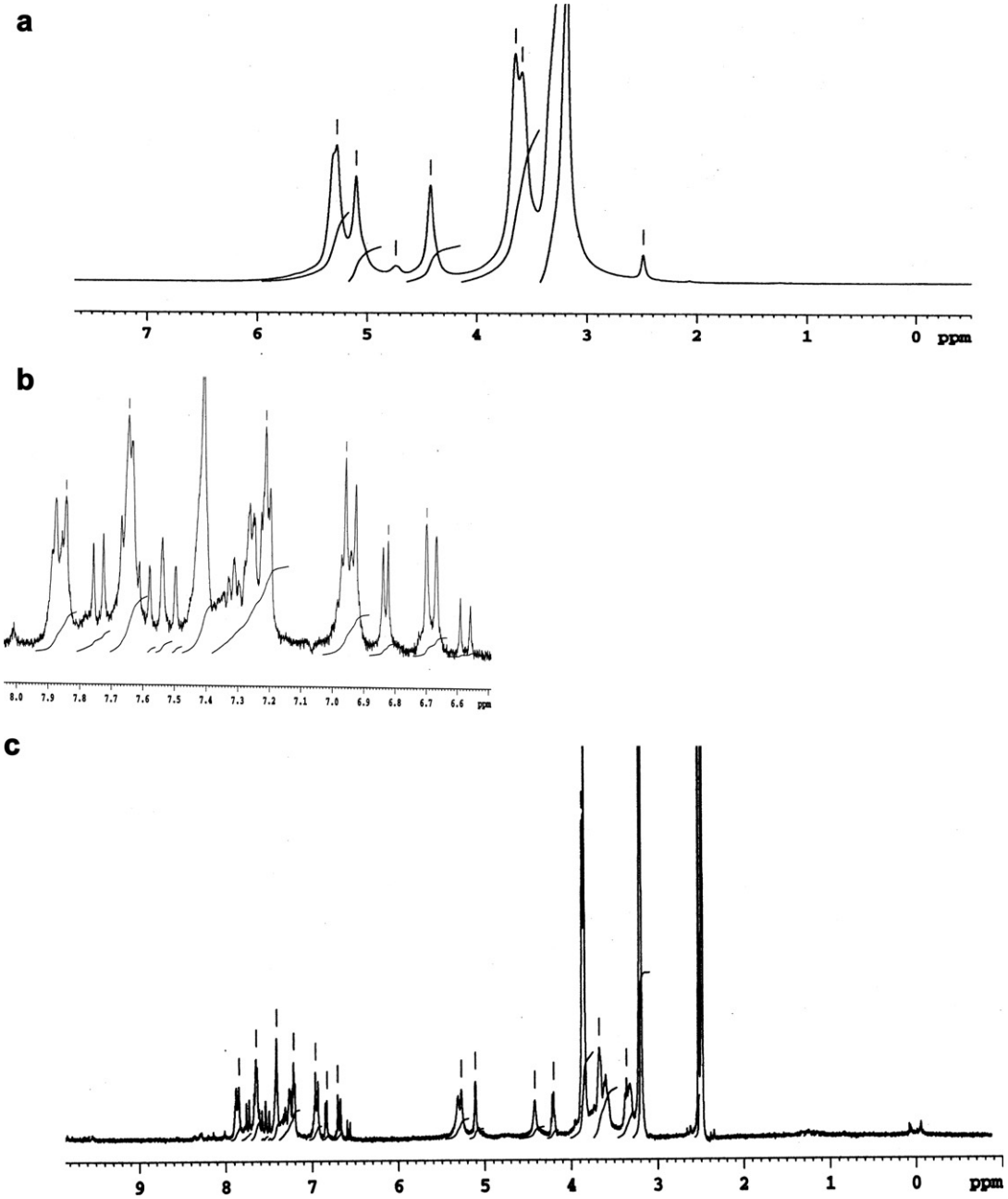

Fig. 8. ^{1}H NMR spectra of (a) starch, (b) starch ferulate and (c) inset (region from 6 to 8 ppm of starch ferulate spectra enlarged).

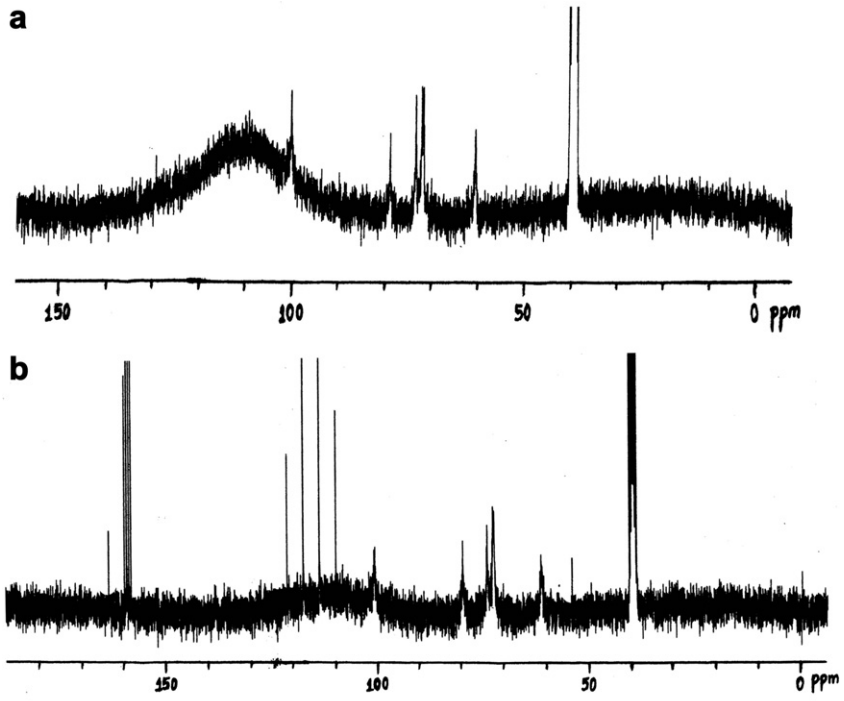

Fig. 9. ^{13}C NMR spectra of (a) starch and (b) starch ferulate.

α-D-glucose. The ^{13}C spectra of starch ferulate (Fig. 9b) showed, in addition to the signals of starch, the carbon signals corresponding to ferulic acid in the region from 109 to 160 ppm. The O–CH_3 signal could be observed as a singlet at 55.9 ppm. The esterification of the free hydroxyl groups of α-D-glucopyranosyl residues caused a resonance downfield shift of the carbon linked to an esterified hydroxyl group, while the resonance of the adjacent carbons shifted upfield. The peak at 62.0 ppm in starch ferulate could be assigned to the C-6 carbon attached to an esterified hydroxyl group. Moreover, the peak at 70.4 ppm may be attributed to a C-5 carbon adjacent to an esterified C-6 carbon. The results of the ^{1}H and ^{13}C NMR spectroscopy confirm the structure of the modified starch.

3.9. SEM observations

Most of the potato starch granules were oval to ellipsoid in shape, with a few spherical ones, and ranged in size from 15 to 60 μm. The starch granules lost their individuality and smoothness on esterification, as a result of gelatinization and substitution of hydroxyl groups, and formed agglomerates. As the DS of the starch ferulates increased, there was an enhancement in the networking nature (Fig. 10), as has been observed earlier by Xu et al. (2004).

3.10. Solubility

The introduction of feruloyl groups into the starch might alter the solubility profile of starch, which depends on the extent of esterification, nature of the group substituted, type of starch, temperature and solvent employed (Fang, Fowler, Sayers, & Williams, 2004).

The starch ferulates were found to be soluble in hot DMSO and in pyridine but insoluble in all the other organic solvents employed, suggesting that the degree of substitution was not high enough to bring about a prominent change in the solubility.

3.11. Spectrophometric analyses

Starch did not show any absorbance in the 200–700 nm wavelength range (Fig. 11). Ferulic acid had an absorbance maximum at 320 nm in the non ionized state, while the starch ferulates had two absorption maxima, one at 300 and another at 330, corresponding to the absorption of ferulic acid ester linked to the starch. The esters of ferulic acid have been found to absorb at longer wavelengths than the free ferulic acid (Fry, 1982). The absorption intensity in the UV range increased with the degree of substitution.

3.12. DPPH radical-scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dinis, Cunha, & Ameida, 1997). The stained silica layer revealed a purple background with yellow spots at the location of the drops, due to the decrease in the local concentration of DPPH, indicating radical-scavenging activity in the case of both ferulic acid and starch ferulate. The intensity of the yellow colour depends on the amount and nature of radical-scavenger present in the sample. Ferulic

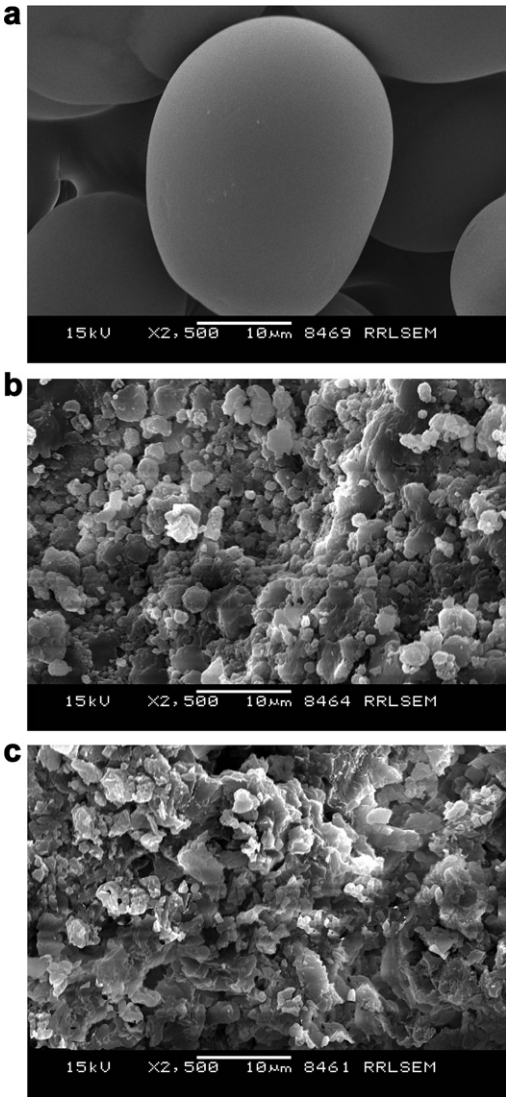

Fig. 10. SEM photographs of (a) native potato starch granules (b and c) starch ferulates of different degree of substitution.

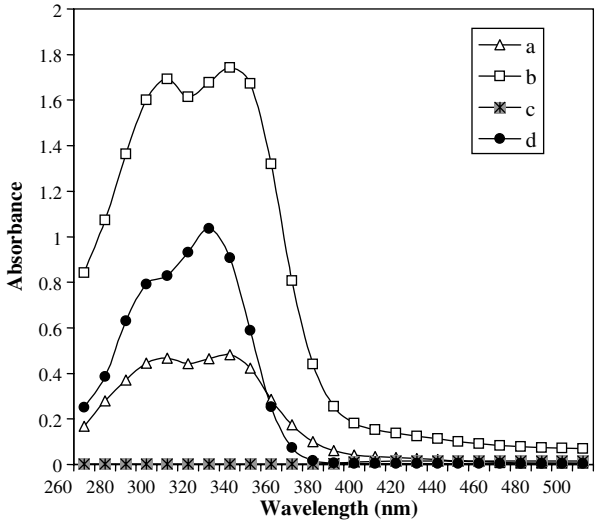

Fig. 11. UV absorption spectra of starch ferulates of different degree of substitution: (a) starch ferulate 1 (△), (b) starch ferulate 2 (□), (c) starch (✻) and (d) ferulic acid (●).

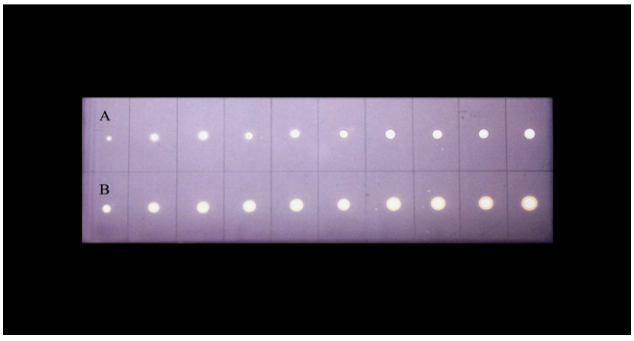

Fig. 12. Dot blot assay on a silica sheet stained with DPPH solution in methanol for rapid detection of DPPH radical-scavenging activity. (A) starch ferulate (100–1000 µg/ml); (B) FA (10–100 µg/ml).

acid exhibited a faster reaction rate than did the starch ferulate (Fig. 12B). Significant radical-scavenging activity was evident at all the tested concentrations and the scavenging activity increased with the increasing concentration of starch ferulate and ferulic acid. The starch ferulates of different DS showed free radical-scavenging capacities at all the concentrations tested.

3.13. ABTS radical cation decolorisation assay

This method measures the ability of starch ferulate to scavenge the radical $ABTS^{+}$ and is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants.

Fig. 13 depicts a steady increase in the ABTS radical-scavenging capacity of starch ferulates up to a concentration of 750 µg/ml. The starch ferulates of higher degree of substitution exhibited a higher degree of scavenging, as

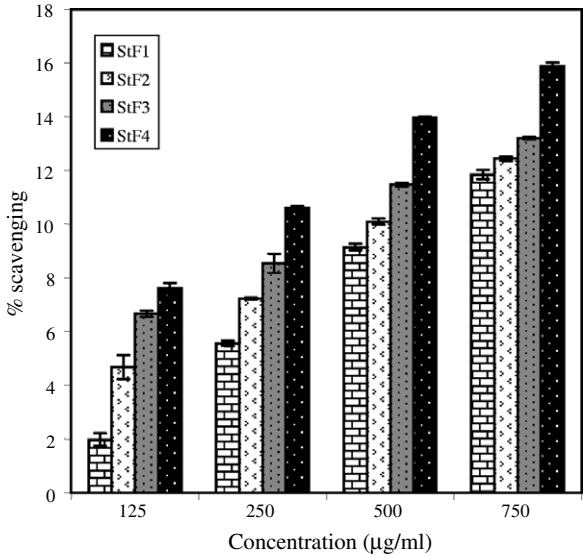

Fig. 13. ABTS radical-scavenging capacity of starch ferulates of different DS.

expected. When the starch ferulates were added to $ABTS^{+}$, a biphasic reaction was observed with a comparatively faster reaction rate in the first 1 min, followed by a relatively slow scavenging. This reaction pattern implies that, for the reaction of $ABTS^{+}$ with starch ferulates, the reduction in absorbance depends on the time point at which absorbance is read.

4. Conclusions

Starch ferulates, with different degrees of substitution, were prepared by reaction of starch with ferulic acid chloride in organic solvent. The DS values of starch ferulate could be controlled by the addition of stoichiometric quantities of the acid chloride. Starch has the disadvantages of hydrophilicity and poor mechanical properties, especially in humid environments and chemical modification is a means to overcome these shortcomings. Structural modification of starch resulted in significant changes of the physicochemical properties of starch and an increased hydrophobicity. Ingestion of ferulic acid, in the form of starch ferulate, facilitates quantitative passage to the colon and thereby a higher bioavailability, at the site of action, compared to free ferulic acid (Curini, Epifano, & Genovese, 2005). The starch ferulates were found to exhibit free radical-scavenging activity which is of significance in the prevention of colon cancer. Non-digestible oligosaccharides, such as feruloyl oligosaccharides, have aroused significant interest in recent years, due to their ability to stimulate the growth of potentially beneficial bacteria, such as *Bifidobacteria,* in the gut. More detailed studies are needed to assess the bifidogenic and dietary fibre properties of starch ferulates.

Development of dietary compounds, as potential cancer chemopreventive agents, is highly desirable, due to their safety, low toxicity and general acceptance as dietary supplements.

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