Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

A review on the structure-function relationship aspect of polysaccharides from tea materials

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Accepted author version posted online: 11 Oct 2013.Published online: 11 Oct 2013.

To cite this article: Critical Reviews in Food Science and Nutrition (2013): A review on the structure-function relationship aspect of polysaccharides from tea materials, Critical Reviews in Food Science and Nutrition

To link to this article: http://dx.doi.org/10.1080/10408398.2012.678423

A review on the structure-function relationship aspect of polysaccharides from tea materials

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Abstract

Tea (*Camellia sinensis*) has a long history of medicinal use in the world. The chemical components of tea mainly consist of polyphenols, proteins, polysaccharides, chlorophyll, alkaloids, and so on. Great advances have been made in chemical and bioactive studies of catechins and polyphenols from tea in recent decades. However, the polysaccharides from tea materials have received much less consideration than that of polyphenols. The number of relevant publications on the polysaccharides from tea leaves and flowers has increased rapidly in recent years. This mini-review summarizes the structure-function relationship of polysaccharides from tea leaves and flowers. The application of purified polysaccharides from tea material as functional or nutritional foods was still little. It will help to develop the function foods with tea polysaccharides and better understand the structure-bioactivity relationship of tea polysaccharides.

Keywords *Camellia sinensis*; polysaccharides; structure**;** nutrition; function

Tea (*Camellia sinensis*) has a long history of medicinal use in Asian countries such as China, Japan, India and Thailand as ancient as 50,000 years ago (Chopade et al., 2008). Tea is the most popular and lowest cost beverages in the world, next only to water (Hicks, 2009). Tea leaves are popularly consumed with unfermented (green tea), semi-fermented (oolong tea), fermented (black tea), Pu'erh tea, and red tea forms (Chen et al., 2009). The chemical composition of tea mainly consist of polyphenols (TPP), polysaccharides (TPS), chlorophyll, proteins, and alkaloids (Forester and Lambert, 2011; Lee et al., 2009; Stalmach et al., 2010; Müller et al., 2010; Yang and Wang, 2011; Yuan, 2011). Great advances have been made in chemical and bioactive studies of catechins and polyphenols from tea in recent decades. However, TPS has received much less consideration than TPP. Recently, the number of relevant publications on TPS has increased rapidly. This mini-review summarizes the structure-function relationship of TPS.

Content of polysaccharides in leaves and flowers of tea

Tea leaves are graded according to their properties (color, aroma, taste and shape) to 1^{st}-6^{th} level. The leaves of 1^{st} class are much tender than that of 6^{th} class, because the 1^{st} class leaves consisted mainly of one leaf and one bud, whereas the 6^{th} class tea leaves consisted mainly of banjhi leaves, that is, coarse tea (Wang et al., 2001). People like the tender leaves, so the high-grade green tea in market is usually made from the top two leaves of *C. sinensis.* Moreover, many low-grade coarse green tea leaves were discarded because of their worse taste and color. TPS is another main bioactive component of tea leaves other than TPP, especially in the low-grade tea leaves. Wang et al found that the contents of TPP, catechin, and caffeine in the s 6^{th} class tea leaves were less than those in the 1^{st} class tea leaves by 20%, 40%, and 25%, respectively;

however, the content of TPS in the 6th class leaves was twice as high as that of the 1st class leaves (Wang et al., 2001). The contents of TPS were ranged from 0.8% to 1.5% in the low-grade leaves and from 0.4% to 0.9% in the high-grade leaves (Wang et al., 1995).

Compared with tea leaves, tea flowers have similar chemical compositions and contain less caffeine but comparable amounts of total catechins. Tea flowers contain many nutritional compounds, such as proteins, vitamins, amino acids, tea polyphenols and caffeine. From this point, tea flowers are also of important value as leaves. However, there are few reports about the polysaccharides from tea flower (TFPS). TFPS is the main effective components in tea flowers, accounting for a comparative large proportion. We found that the content of total saccharides in tea flowers (62.84%) were much higher than that of leaves (22.80%) (Wei et al., 2010). From this point, tea flowers are also of important application value as leaves.

Fractionation of crude TPS

According to the literatures, the scheme of separation and fractionation of crude polysaccharides from tea materials was summarized in **Figure 1**. The tea materials (leaves and flowers) were pre-extracted with alcohol to remove small molecules. The dry residue was extracted polysaccharides with hot water. Then, the extract was filtered and evaporated under vacuum. Subsequently, the concentrated liquid were precipitated with alcohol and centrifuged. The final precipitate was re-dissolved in double-distilled water. After removed the proteins, the water phase was dialyzed. The dialysate was concentrated and freeze-dried to obtain crude TPS. The gel filtration column chromatography was further used to fractionate crude TPS. Crude TPS was dissolved with distilled water, filtered by 0.4 μm membrane and then injected into the column. TPS was first

eluted with buffer followed by the sequential elution with NaCl (0.1, 0.2, and 0.3 M) (**Figure 2**). Solution collected was determined by phenol-sulfuric acid method and UV spectroscopy. Similar solutions were combined, dialyzed, concentrated and lyophilized to obtain TPS fractions (**Figure 2**).

Structure of TPS

TPS were found to be mostly glycoconjugates in which protein carries and one or more carbohydrate chains were covalently attached to the polypeptide backbone via N- or O-linkages (Nie et al., 2011). **Table 1** summarizes the molecular weight, monosaccharide composition, and protein content of TPS. As shown in **Table 1**, thirty-two TPS were reported. **Figure 3** showed that 29 of 32 (90.62%) TPS contain arabinose (Ara) and 30 of 32 (93.75%) contain Gal. However, only 5 of 32 (15.62%) TPS contain glucuronic acid (GluA) and galacturonic acid (GalA).

The separation methods and tea source significantly affected the structures of TPS. Chen et al analyzed a tea polysaccharide conjugate, which was composed of arabinose, ribose, xylose, glucose, and galactose in mole ratios of 1.0:0.8:2.7:0.9:0.4 (Chen et al., 2007). They purified a polysaccharide conjugate from green tea, which consisted of arabinose, ribose, xylose, glucose, galactose, and glucuronic acid with mole ratios of 1.00: 0.77: 2.65: 0.88: 0.42: 2.13 (Chen et al., 2005). Chen et al further isolated an acidic polysaccharide bound with protein consisting of arabinose, ribose, xylose, glucose and galactose with a molar ratio of 4.9: 2.2: 3.1: 1.8: 1.0 (Zhou et al., 2008). However, Wang separated four polysaccharides fractions from low-grade coarse green tea, namely, NTPS1, ATPS2, ATPS3, and ATPS4 (Wang et al., 2001). NTPS1 mainly

consisted of galactose accounting for 86.5%. ATPS2, ATPS3, and ATPS4 consisted of rhamnose, arabinose, galactose and galacturonic acid. Chen et al studied the antioxidant activity of polysaccharide conjugate from green tea with a molecular weight (MW) of 120 kD (Chen et al., 2005) and isolated three fractions of water-soluble polysaccharide conjugates with MW of 268, 118, and 42 kD (Zhou et al., 2008). These tea polysaccharide conjugates were protein-bounded polysaccharides with large molecular weight (Chen et al., 2007). Wang et al determined the MW of polysaccharides from coarse tea by HPLC and Sephadex G-200 gel as 107 kD (Wang et al., 2001). Lee et al obtained a acidic polysaccharide from green tea with a MW of 80 kD (Lee et al., 2006). The MW of tea polysaccharides obtained by Wang (Wang et al., 2001) was within the range of 6.2-24.8 kD, which was much smaller than those obtained by above data (Chen et al., 2009; Chen et al., 2007; Zhou et al., 2008). These data confirmed that NTPS1, ATPS2, ATPS3, and ATPS4 were new tea polysaccharides, which might be related to the tea raw material source and extraction methods. Due to their inherent flexibility, the exact structures (high order structure) of most TPS in solution are still not clear (Nie et al., 2011). The common physiochemical and structural features of TPS are concluded as: 1) TPS is a soluble glycoconjugate in which a protein carries one or more carbohydrate chains covalently attached to a polypeptide backbone, usually via N- or O- linkages; 2) TPS mainly consisted of rhamnose, arabinose, glucose and galactose with little xylose and mannose. Among these monosaccharides, glucose and galactose accounts for higher ratios; 3) The molecular weight of TPS from unfermented (green tea) is higher than those from semifermented (oolong tea) and fermented (black tea); 4) The extraction methods and materials of the coarse tea significantly affected the

physiochemical and structural features of TPS; 5) TPS usually combine with uronic acid, proteins, inorganic elements to form complexes (Nie et al., 2011).

However, there are few reports about polysaccharide conjugate from the tea flowers. Recently, Wang et al (2010) isolated a polysaccharide conjugate from tea flowers. The components of TFPS by water extraction mainly consist of two kinds of polysaccharides (Wei et al., 2010). Crude TFPS was separated on a DEAE Sepharose FF gel filtration column to obtain TFPS1 with a yield of 18%. TFPS1 was a protein glycoconjugate with an average MW of 450-500 kD and consisted of rhamnose, arabinose, mannose, glucose, and galactose with a molar ratio of 1.0:2.9:0.5:1.3:3.3. The backbone of TFPS1 was composed of Glu and Gal. Ara and Gal were main components of side chain or edge backbone of TFPS1. The main glycosyl residues of TFPS1 were α- and β-configuration, including signal of β-D-galactose, α-L-arabinose, α-D-mannose, α-rhamnose, and α-D-glucose.

Wang et al (2010) further compared the compositions of TPS and TFPS obtained by different extraction methods. TFPS obtained by different processes consisted of rhamnose, arabinose, glucose, galactose, xylose, mannose, and galacturonic acid. And glucuronic acid was not found in the TFPS. However, only TPS obtained by hot water extraction was composed of these six monosaccharides. The components of TPS by hot water extraction mainly consisted of three kinds of polysaccharides with the MW of 413 kD, 104 kD, and 1165 D, respectively. The components of TFPS by hot water extraction were made up mainly of four polysaccharides with the molecular weight of 483 kD, 168 kD, 120 kD, and 1059 D, respectively. The MW of TFPS was higher than that of TPS.

Han et al isolated the water-soluble crude polysaccharide tea flower polysaccharide (TFP) by boiling-water extraction and ethanol precipitation and two polysaccharide fractions termed TFP-1 and TFP-2 were obtained (Han et al., 2011). The structural features of TFP-1 and TFP-2 were investigated by high-performance liquid chromatography (HPLC), gel-permeation chromatography (GPC), rheometer, infrared (IR) spectra, nuclear magnetic resonance (NMR) spectroscopy, atomic force microscope (AFM), and scanning electron microscope (SEM). Results indicated that TFP-1 was composed of glucose: xylose: rhamnose: galactose = 1.0:1.2:0.81:0.98 with a MW of 167.5 KDa, while TFP-2 comprised glucose: xylose: rhamnose: arabinose = 1.0:0.76:2.3:2.3 with a MW of 10.1 KDa.

Wei et al (2011) reported the composition and biological activities of polysaccharides from tea seed (TSPS) obtained by water extraction were investigated. The properties and chemical compositions of TSPS were analyzed with HPGPC, IC, and IR methods. The results showed that TSPS consisted of three kinds of polysaccharides with the molecular weight of 500 kDa, 130 kDa, and 5 kDa. TSPS consisted of rhamnose, xylose, arabinose, glucose and galactose, GalA, GulA, with a molar ratio of 4.9:1.7:11.1:27.2:14.0:3.4:1, sugar backbone of TSPS might consist of glucose, but branched chain may consist of rhamnose, xylose, arabinose, and galactose.

Ele-Ekouna (2011) isolated and identified two distinct pectin fractions (P1 and P2) from green tea leaves based on their solubility in water. Polyphenols were detected only in the easily water soluble fraction (P1). The estimated uronic acids/neutral sugars ratio was 1.7 in the easily water soluble pectin fraction (P1), and 1.0 in the less water soluble fraction (P2). Homogalacturonan sequences (HGAs) corresponded to about 62% of the P1 pectin fraction but only 47% of the P2 fraction. After degradation of P1 and P2 with pectin lyase, chemical studies revealed

rhamnogalacturonan RG I and RG II regions present in P1, whereas only RG I sequences were detected in P2 (**Figure 4**) (Ele-Ekouna et al., 2011). The degree of substitution was lower for HGAs of P1 than P2.

Immunostimulating activity

A number of medicinal and clinical researches *in vivo* and *in vitro* suggested that the TPS showed an important role for human health. The bioactivities of TPS, such as hypoglycemic, hypolipidemic antiatherogenic, anticoagulant and antithrombotic effects, immunomodulatory, anti-cancer and anti-oxidant activities have been reviewed by several research groups (Nie et al., 2011). Here, the structure-function relationship aspects of TPS and TFPS were reviewed.

There are essentially three main types of Camellia tea, which are Green, 'Oolong' and Black. The difference lies in the 'fermentation', which actually refers to oxidative and enzymatic changes within the tea leaves, during processing. Green tea is essentially unfermented, Oolong tea is partially fermented and Black tea is fully fermented. The fermentation significantly affected the bioactivities of TPS. The bioactivities of TPS and their conjugates also can be affected by many factors including chemical components, MW, structure, and conformation (Nie et al., 2008). The MW of polysaccharides played an important role on their bioactivities. TPS from black tea (BTPS) consisted of a high proportion of low MW fractions (3.8-32.7 kD), which was associated with higher bioactivities than those of TPS from green tea (GTPS, 9.2-251.3 kD) and TPS from oolong tea (OTPS, 5.3-100.9 kD) (Chen et al., 2009). BTPS showed a dose-dependent effect on α-glucosidase inhibitory activity. But GTPS and OTPS hardly inhibited α-glucosidase activity in the same condition (Chen et al., 2009). TPS with high-MW was the main

substance with an immunostimulating activity and the polyphenol-polysaccharide complex is a potential immunostimulator.

Monobe et al (2010) found that the immuno-stimulating activity of TPS depended on the content of total catechin in the leaf extract and the activity of TPS from immature tea leaves was higher than that of TPS from mature tea leaves. Nakamura et al (1997) reported that a complex mixture of tannins with polyphenols and polysaccharides inhibited tumor promotion and carcinogenesis in mice and mice cell lines. In the treatment of diabetes in non-obese diabetic mice, water-soluble tea polysaccharide conjugates showed dose response and better efficacy than alkali-soluble polysaccharide conjugates (Wang et al., 2001).

Han et al. isolated a TFP from tea flowers consisting of two kinds of polysaccharides with the peak MW of 31 kD and 4400 D (Han et al., 2010). Effects of TFP on plasma IL-2 and IFN-γ levels were determined by ELISA. The plasma IL-2 significantly increased in TFP groups at M-TFP and H-TFP ($P< 0.01$) compared with that in control group and the similar observation also indicated in the plasma IFN-γ ($P< 0.05$ and $P< 0.01$), compared with the normal group. TFP markedly augmented IL-2 and IFN-γ in plasma of S180 bearing mice in dose dependent manner. While the proportion of the $CD8^+$ T cell was not affected, the percentage of $CD4^+$ T cells significantly improved in M-TFP and H-TFP groups compared with control group ($P < 0.01$). Furthermore, there was a definite trend toward an increased ratio of $CD4^+$ T cell and $CD8^+$ T cells in the TFP administered groups in dose-dependent manner except the high dose group (Han et al., 2010).

Antidiabetic activities

Ni et al (2004) investigated the influence of polysaccharide extracted from Oolong tea (OTPS) on hepatic-nephritic anti oxidation and histomorphology in mice with streptozotocin (STZ)-induced diabetes (MD). The results showed that SOD and GSH-PX activity was increased and MDA content decreased in liver and kidney of MD mice treated with OTPS for 4 weeks. OTPS had the function to improve the anti-oxidative potential and protect the liver and kidney of MD mice (Ni et al., 2004).

The inhibitory effect of TPS on α-glucosidase increased with increasing neutral polysaccharides content in TPS (Wei et al., 2010). TFPS obtained by traditional water extraction (TFPS-TWE) mainly consisted of two kinds of polysaccharides with the molecular weight of 31kD and 4400D (Wang et al., 2010). The molecular weight of TFPS obtained by microwave-assisted water extraction (TFPS-MAW) is higher that of TFPS obtained by traditional water extraction. TFPS-TWE showed much stronger inhibitory effect on α-glucosidase than does TFPS-MAW. The TFPS-TWE (2 mg/mL) exhibited a strongly inhibitory effect on α-glucosidase with the inhibitory rate of 83.3 %. However, TFPS-MAE had very low inhibitory effects on α-glucosidase.

Wang et al (2010) compared the α-glucosidase inhibitory and α-amylase inhibitory activities of polysaccharides from leaves and flowers of green tea obtained by hot water extraction (HWE), boiled water extraction (BWE) and enzymatic extraction (EE). TFPS obtained by BWE generally had very low inhibitory effects on α-amylase as well as HWE and EE. The inhibitory percentages of α-amylase at the concentration of 1.0mg/mL for TLPS and TFPS were less than 10.0% while arcabose were tested in around 75% inhibition at the concentration of 0.1 mg/mL. The inhibitory percentages of TFPS against α-amylase were all lower than α-glucosidase for different extractions (Wang et al., 2010).

Chen et al (2010) extracted water-soluble TPS (TPC-W) and alkali-soluble TPS (TPC-A) from green tea by hot and alkali water, respectively. The daily oral administration of 150 mg/kg TPC-W can significantly decrease the level of blood glucose in non-obese diabetic mice. The anti-glutamic acid decarboxylase (GAD) antibody levels in NOD mice treated with 150mg/kg of TPC-W decreased 27% (Chen et al., 2010). The results demonstrated that both TPC-W and TPC-A can suppress spontaneous diabetes mellitus in NOD mice (Chen et al., 2010).

Han et al (2011) isolated two water-soluble polysaccharide fractions (TFP-1 and TFP-2) from tea flower. The weight-average molar mass of TFP-1 and TFP-2 were determined as 15.9×10^4 and 1.12×10^4 g/mol. Moreover, TFP-1 and TFP-2 can inhibit α-glucosidase and α-amylase. Moreover, continuous administration of TFP-2 with 75, 150 and 300 mg/kg/b.wt for 3 weeks caused a significant decrease in blood glucose levels in alloxan-induced diabetic mice (Han et al., 2011).

Chen et al (2011) obtained three unexplored polysaccharide-conjugate fractions termed gTPC1, gTPC2 and gTPC3 from hot-water extracts of low-grade green tea on a DEAE-cellulose DE-52 column chromatography. As compared with cell injury group, gTPC1–3 at all of three dose were found to remarkably protective on HUVE cells against impairments induced by high glucose in a dose-dependent manner (Chen et al., 2011). To contribute toward our understanding of the cell-based protection mechanism of gTPC1–3 scavenged the self-oxidation of 1,2,3-phentriol assay by 55.1%, 47.6% and 47.9% at the concentration of 300 μg/mL, respectively (Chen et al., 2011). gTPC1–3 could be developed as non-cytotoxic candidates of therapeutic agent for diabetic vascular complications.

The level of blood glucose in ICR mice administrated TPS previously didn't increase obviously

after injected alloxan, with the activity of SOD and GSH·Px increasing and the content of MDA decreasing (Wu et al., 2003). Moreover, the activity of liver glucokinase was higher than those without TPS.

Wang et al (2005) isolated and purified an acidic polysaccharide (ATPS) from course green tea. ATPS was further purified by DEAE52 to give ATPS-I. The average molecular weight distribution between ATPS and ATPS-1 was almost the same. ATPS showed hypoglycemie effects on alloxan induced diabetic mice (Wang et al., 2005).

Cai et al (2011) reported that TFPS can decrease the blood glucose level, increase the weight growth rate efectively, and decrease SD rat blood glucose level after the second time alloxan injected. Crude tea flower polysaccharide had significant hypoglycemic action in diabetic rat induced by alloxance, which has a prevention effect on hyperglycemia (Cai et al., 2011).

Zhou et al (2009) separated and purified pu'er TPS complex and studied the hypoglycemic effect on alloxan-induced mice. The pu'er TPS could effectively lower the blood glucose levels in diabetic mice. The high doses of TPS (160 mg/kg) showed higher hypoglycemic effect than that of low doses of TPS (80 mg/kg) (Zhou et al., 2009).

Wang et al (2006) investigated the preventive effect of TPS on type I diabetes in nonobese diabetic mice. TPS can lower incidence of diabetes, improve the serum C-peptide level, and decrease the lymphocytic infiltration in pancreatic islets. TPS also showed stronger proliferation of CD8 T subsets and lower ratio of CD4/CD8 subgroup in splenocytes as compared with normal saline group (Wang et al., 2006).

Antioxidant activity

Many reports found the crude TPS showed good antioxidant activities. In our recent report, the antioxidant activities of crude TPS from tea leaves of Xihu Longjing (XTPS), Anxi Tieguanyin (TTPS), Chawentianxia (CTPS) and Huizhoulvcha (HTPS) exhibited lower DPPH scavenging activities than Vc within 25-200 μg/mL (Xiao et al., 2011). TPS-protein conjugate was extracted and purified from old tea leaves from Wuyuan county of Jiangxi province in China (Nie et al., 2005). The TPS showed distinctive anti-oxidative activity.

The antioxidant abilities of TPS-protein conjugates depend on the protein content (Nie et al., 2008). With increasing of the protein content, the antioxidant activities of TPS-protein conjugates enhanced (Nie et al., 2008). The protein may affect the physico-chemical properties of TPS and hence their bioactivities. The in vivo and in vitro antioxidant activities of crude tea polysaccharides were found to be superior to tea polysaccharide fraction (Zhou et al., 2007). This can be explained by the higher proportion of tea polyphenols, tea pigments, vitamins, and other antioxidant substances in the cruder fractions. Compared with TPC-1 (26.8 kD) and TPC-2 (11.8 kD), TPC-3 (4.2 kD) exhibited the highest antioxidant activities, according to the deoxyribose assay, the photoreduction of NBT assay and the lipid peroxidation inhibition assay (Nie et al., 2008). There was a distinguished difference among OTPS, GTPS, and BTPS on the antioxidant activities (Chen et al., 2009). Scavenging rates of GTPS, OTPS and BTPS on DPPH radicals were 47.9%, 23%, and 61.7%, respectively (Chen et al., 2009).

Wang et al (2011) isolated crude tea polysaccharides from coarse green tea leaves by precipitation and ultrafiltration. The dry tea leaves were extracted with 10-times distilled water at 90 °C for 2 h and repeated twice. The extracts were concentrated and precipitated with 95 % ethanol, and then freeze-dried to yield crude tea polysaccharides (TPS1). TPS1 was fractionated

by means of two ultrafiltration membranes (MW_{cutoff} =100 kD and 10 kD), precipitation and the dialyzed fractions were freeze-dried to obtain TPS2, TPS3, and TPS4 (Wang et al., 2011). The contents of neutral sugar in TPS, TPS1, TPS2 and TPS3 were determined as 48.90%, 55.70%, 71.60 %, and 59.60%, respectively. TPS2 showed highest neutral sugar content. The contents of uronic acid in TPS, TPS1, TPS2 and TPS3 were 28.52%, 34.82%, 10.86% and 3.95%, respectively. The MW of TPS1, TPS2, and TPS3 were about 240, 21.4 and 2.46 kDa, respectively. The Mw/Mn value of TPS3 (1.03) was close to 1; however, Mw/Mn values of TPS1 and TPS2 were 3.57 and 1.57, which indicated that TPS3 was more homogeneous. The contents of protein and polyphenol were relatively low in TPS1 (2.41 % and 4.58 %) and TPS3 (1.51 % and 4.64 %). However, the contents of protein and TPP were high in TPS (4.00 % and 8.86 %) and TPS2 (3.75 % and 11.83 %) (Wang et al., 2011).

Then, the DPPH radical scavenging, total antioxidant, non-enzymatic lipid per-oxidation induced by Fe^{2+}/ascorbate, hydroxyl radical scavenging, superoxide anion scavenging activities, metal chelating, reducing power, and self-oxidation of 1,2,3-phentriol assay of TPS1-4 were investigated.

The DPPH scavenging activity of these fractions were determined as TPS1>TPS3>TPS2>TPS (Wang et al., 2011). The content of polyphenol was relatively low in TPS1 (4.58 %) and TPS3 (4.64 %). However, TPS1 and TPS3 showed higher DPPH radical scavenging activity than the fractions (TPS and TPS2) with higher level of TPP. The EC_{50} values of TPS1, TPS3, TPS2 and TPS were determined as 36, 61, 96 and 119 μg/ml, respectively (Wang et al., 2011). These results illustrated that the TPP are the major anti-oxidant in the crude extract TPS and polysaccharides are the major anti-oxidant in TPS1 and TPS3. TPS with lower MW showed

higher radical scavenging activity. The crude TPS from coarse green tea leaves can be used as a new antioxidant and functional food.

Wu et al (2011) studied the characterization and antioxidant activity of the TPP-β-glucan complex. The complexation and blending of TPP and beta-glucan exhibited different impacts on the index of antioxidant capacities. In the concentration range of 0.5-2.5 mg/mL, the complex showed highest $O_2\cdot$ scavenging activity, whereas the highest OH· scavenging activity was found with the physical mixture. For antioxidant testing *in vivo*, there was no significant difference between the complex and the physical mixture in terms of glutathione peroxidase activity and levels of malondialdehyde and total antioxidant capacity in serums (Wu et al., 2011). However, the complex exhibited much higher activities of superoxide dismutase and glutathione peroxidase in livers than the physical mixture (Wu et al., 2011). It provided a deeper understanding of the influence of molecular interaction between TPP and polysaccharides on their antioxidant activities.

Sun et al (2011) investigated the antioxidant effects of crude TPS in exhausting training mice. Three groups of mice were administrated with TPS (100, 200 and 300 mg/kg, respectively). The results showed that MDA levels in plasma, lever and heart were reduced after 30 days of treatment (Sun et al., 2011). Compared with control mice, blood, liver and heart superoxide dismutase, catalase, GPx activities were significantly increased after 30 days of TPS treatment. Overall, both TPS extracts possessed good antioxidant properties and can be developed as anti-fatigue medicines (Sun et al., 2011).

However, whether or not the antioxidant abilities of crude TPS depend on TPP in the crude TPS is not understand. We isolated TPS fractions (NTPS, ATPS1, ATPS2, and ATPS3) from crude

TPS and their antioxidant activities were compared. The crude TPS showed stronger antioxidant activities than that of TPS fractions. However, very low concentration of EGCG (6.15 μg/mL) significantly enhanced DPPH radical scavenging potential of TPS fractions for each dose (**Figure 5**). Moreover, very low concentration of EGCG (8.0 μg/mL) significantly enhanced the reducing power of TPS fractions (**Figure 6**). EGCG causes a synergistic increase in the antioxidant activities of TPS fractions. These results illustrated that TPS fractions hardly exhibited antioxidant activities and TPP are the major antioxidants in the crude TPS. The antioxidant activities of dextrans in the absence and presence of EGCG were investigated to illustrate that the antioxidant activities of TPS is similar to dextrans.

Wang and Xia (2010) elucidated the mechanism of TPS reducing blood glucose. The scavenging effects of TPS1 (89% tea polysaccharides) and TPS2 (51% tea polysaccharides) on hydroxyl radical, superoxide radical key carbohydrate metabolic enzymes activities, and the glucose transportation to 3 T3-L1 were careful investigated *in vitro*. TPS2 showed higher radical scavenging abilities than that of TPS1. It illustrated that TPS with higher purity exhibited lowered antioxidant activity. The effect on α-glucosidase activities and transportation of glucose to 3T3-L1 cell were also no obvious. However, TPS significantly enhanced the activities of hexokinase (HK) and glucokinase (GK).

Antitumor activity

The inhibition effect of TFPS from tea flowers on sarcoma 180 tumor (S180)-bearing mice was observed at dosages of 75, 150 and 300 mg/kg by systematically to measure the S180 tumor inhibition rate, mice survival rate and cellular immunity (Han et al., 2010). The result showed

that continuous administration of TFPS for 10 day continuously was found to inhibit the growth of transplanted S180 and prolong the mice survival days.

Wei et al (2011) reported the antitumor activity of TPS from tea seed (TSPS) obtained by water extraction. TSPS significantly inhibited the growth of K562 cells. TSPS with higher concentrations (100, 200 and 400 μg/ml) showed higher proliferation effects on lymphocyte. It demonstrated that the polysaccharide had a potential application as natural antitumor drugs (Wei et al., 2011).

Fan et al (2011) obtained glycan from green tea extracted with boiling water. Tea glycan, with an average MW of 8.3×10^5 Da, consisted of Rha, Ara, Xyl, Man, Gal, and Glc) with a molar ratio of 1.06:2.31:5.17:0.91:3.06:4.24. Cytotoxic effects of ethanol extracts and tea glycan against SKOV-3 cells were compared with MTT assay. However, tea glycan showed very weak antitumor activity against the SKOV-3 cells. Ethanol extract of tea showed significantly higher antitumor activity against the SKOV-3 cells than tea glycan. These results indicate that the higher antitumor activity of ethanol extracts from tea may be related to their polyphenol contents (Fan et al., 2011).

Liu et al (2006) reported the the inhibitory effect of the honeybee pollen polysaccharide (HPP) from tea flower on tumor growth in S-180 sarcoma bearing mice. Results in comparison with the tumor control group, the tumor growth was significantly inhibited and the tumor weight was reduced by 43.6% in the HPP (800 mg/kg/d ×10d) experimental group (Liu et al., 2006).

Protecting skin

The protective effects of TPS1 (92% TPS), crude TPS2 (20% TPS) and TPP (98%) on skin were investigated by Wei et al (Wei et al., 2009). The abilities of TPS and TPP to protect the skin were assessed in four aspects: moisture absorption and retention, sunscreen, promoting the proliferation of fibroblast cells and tyrosinase inhibitory effect. The TPS and TPP absorbed and reserved moisture perfectly. TPS with higher purity had better moisture absorption and retention abilities. TPS1 hardly prevented the skin from the sun ultraviolet and had little promoting effect on fibroblasts proliferation. The results indicated that TPP and TPS had complementary advantages and they should be appropriately combined to achieve higher performance when applied as active components of cosmetics (Wei et al., 2009).

In MTT assay, it was shown that the effect of TPS1 on skin fibroblasts proliferation was not obvious ($P>0.05$) (Figure 3a). Cell viability was significantly reduced in a dose-dependent manner after exposure of fibroblasts to TPS2. The low concentrations (20-200 μg/mL) of TPS2 showed a stimulation of cell proliferation, but with the increasing concentration, effect of TPS1 on skin fibroblasts proliferation decreased rapidly. The highest cell viability was 163.75% with the TPS2 concentration of 20 mg/L. The high concentration (500 μg/mL) of TPS2 was cytotoxic and inhibited the cell proliferation (Wei et al., 2009).

TPS as nutritional food for diabetes

Diabetes mellitus, particularly Type 2 or late onset diabetes, is a major and rapidly increasing health problem all over the world, because of its chronic nature and disabling complications. The hypoglycaemic (lowering blood glucose levels) effect of tea has been known for centuries in China and Japan, where tea is a remedy for diabetes. TPS are important in the prevention of

degenerative type diseases. These include cardiovascular disease and diabetes type 2. Polysaccharides can also act as an anticoagulant. It reduces the stickiness of platelets making it harder for them to build up in artery walls. It (they) have anti-thrombotic effects and blood lipids are reduced. HDL cholesterol may be raised while LDL levels are decreased. The hypoglycaemic effect of TPS has been studied scientifically in the past five years. Recently, TPS were found to inhibit glycosidase (inhibiting the uptake of glucose) and from food recently eaten (Chen et al., 2009; Wang et al., 2010). The glucosidase inhibitors are currently interesting to researchers owing to their promising therapeutic potential in the treatment of diabetes, HIV infection, metastatic cancer, and lysosomal storage diseases (Chen et al., 2009). Many reports have focused on the effective α-glucosidase inhibitors from natural materials to find a functional food use as anti-diabetes drugs (Chen et al., 2009).

TPS can decrease the blood pressure and improve coronary artery capacity. The glucose levels in blood are reduced which is a benefit in treating diabetics. β cell function in the pancreas was improved, as well as do anti-diabetic properties. Anti-radiation effects may be noted and free radicals can be all but eliminated. There is anti-viral activity, and it improves blood reproduction and maintenance. Oxidative stress is another contributor to chronic illnesses such as diabetes and cancer. It's well known that green tea contains a number of antioxidants that scavenge the cell-damaging oxygen-derived free radicals (Wang et al., 1995). It was found that TPS in black tea has stronger scavenging free radicals than those in green tea (Chen et al., 2009).

However, it's not clear whether simply drinking tea would help to treat diabetes. Chen's team used chemical extraction methods -- not simple brewing -- to derive TPS they purchased at local markets (Chen et al., 2009). The application of purified TPS as functional or nutritional foods

was still little. TPS has been applied as the supplementary material to the production of the beer (You et al., 2008). Processing technology of the beer had been decided without changing the flavor and nutrition of the traditional beer (Han et al., 2001). TPS was used an anti-bloom for chocolate (Gao et al., 2001). The future challenge is to develop the function food with TPS and better understand the structure-bioactivity relationship of TPS. This presents a good opportunity for scientists to elucidate the nutritional roles of TPS and design high potential antidiabetes foods.

Acknowledgements

The authors are grateful for financial sponsored by Shanghai Rising-Star Program (11QA1404700), Natural Science Foundation of Shanghai (10ZR1421700), "Chen Guang" project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (09CG46), Program of Shanghai Normal University (SK201006), Leading Academic Discipline Project of Shanghai Municipal Education Commission (J50401), Innovation Program of Shanghai Municipal Education Commission (10YZ68).

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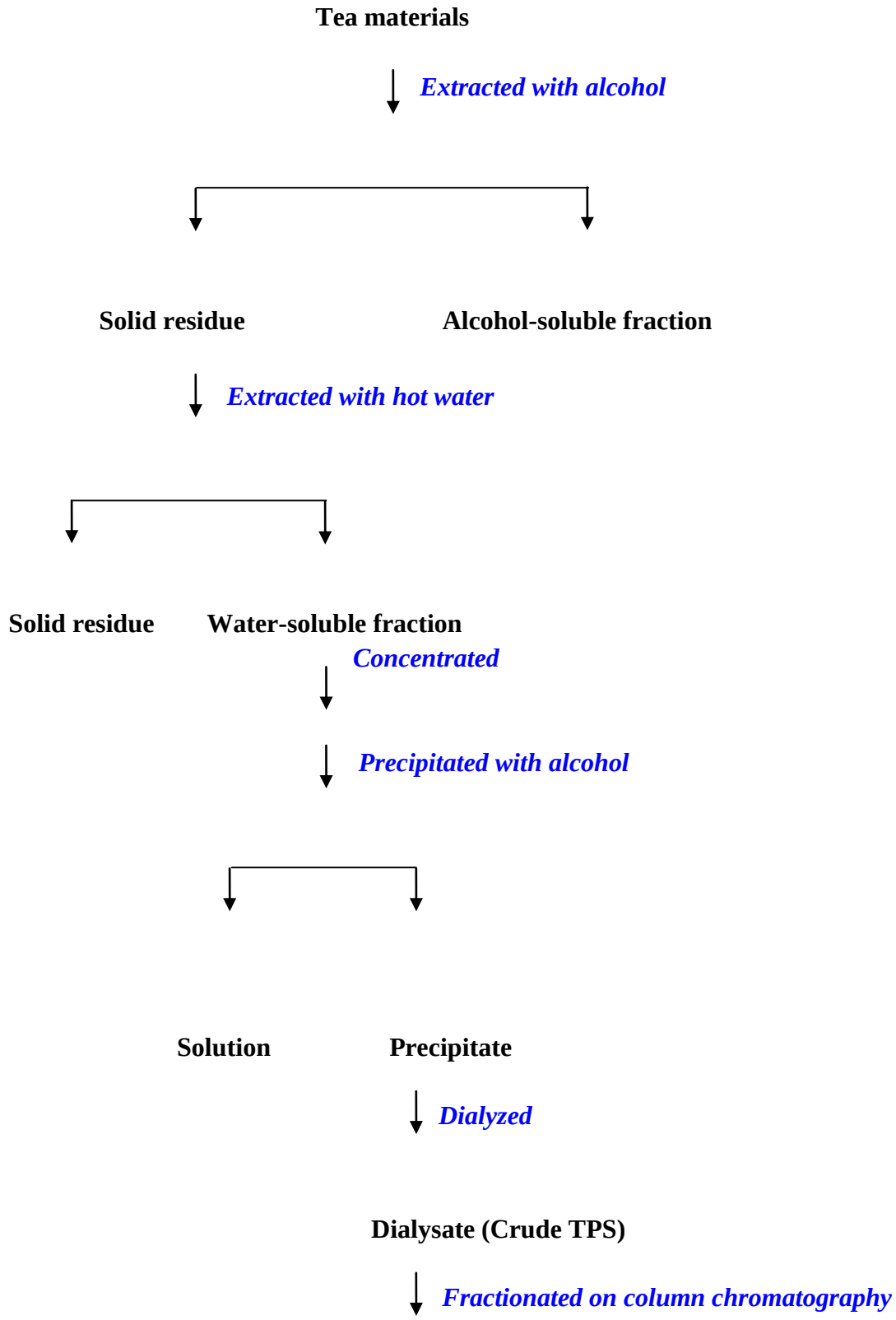
Table 1 The molecular weight, monosaccharide composition and protein in TPS and TFPS.

Name	MW/kD	Monosaccharide composition (mol % or mole ratios)									Protein (%)	Source/Ref
		Rha	Fuc	Ara	Xyl	Man	Rib	Gal	Glu	Gal	Glu		
GTPS	9.2-251.5	7.8		41.8	7.1	7.3		18.7	17.0			32.6	Green(Chen et al., 2009)
OTPS	5.3-100.9	16.2		43.7				18.0	21.9			32.7	Oolong(Chen et al., 2009)
BTPS	3.8-32.7	14.4		36.4				19.7	29.4			38.0	Black(Chen et al., 2009)
CS-F2	80	6.28	5.52	6.10	0.28	2.82		3.44		49.25	26.32		Green(Lee et a., 2006)
NTPS-1	21			1.16				1.00	2.04				Green(Wang et al., 2009)
TPS1-4	228-276												Green(Guo et al., 2010)
TGP	126.5	5.88		13.7	1.99	1.00	1.71	33.8	1.84			6.83	Green(Nie et al., 2008)
TPC	120			1.00	2.65		0.77	0.42	0.88		2.13		Green(Chen et al., 2005)
TPC-1	268			3.3	2.7	2.5	2.0	1	4.1			2.8	Green(Chen et al., 2008)
TPC-2	118			1.9		1	1.6		2.7			3.8	Green(Chen et al., 2008)
TPC-3	42			4.5	2.8		2.0	1.0	1.8			4.0	Green(Chen et al., 2008)
TPS	107		6.58	6.49	2.6			41.1	43.3			17.89	Green(Wang et al., 2001)
TPS				1.0	2.7		0.8	0.4	0.9			17.2	Green(Chen et al., 2007)
TPF	120	1.0	1.01	18.9	2.5	5.73		18.6	1.0			3.5	Green(Zhou et al., 2007)
TPS	100-105	1.14		8.11		2.74		5.34	6.97				Green(Zhou et al., 2008)
OTPS2-1	88.77	1.02	1.76	7.05				7.58	2.14			1.1	Oolong(Nie et al., 2004)
REE-TPS	107	4.0		11.6	10.8			35.9	37.7			30.6	Green(Wang et al., 2001)
OTPS	128	1.37		1.89				1.0	1.30			19.59	Oolong(Chen et al., 2009)
ATPS-2	4.43	0.68		0.4				1.0			1.58		Green(Wang et al., 2009)
TPS				4.9	3.1		2.2	1.0	1.8				Green(Chen et al., 2008)
TPS1		4.8		15.4		7.3	1.6	44.9	6.6		19.4		Green(Wei et al., 2009)
TPC-W	6.6-455	8.74	4.69	29.0	0.42	7.11		35.9	14.1			2.29	Green(Pan et al., 2009)
TPC-A	4.13-494	13.8	1.41	36.0	5.24	4.89		32.3	6.28			14.42	Green(Pan et al., 2009)

TPS-II	101	2.98	1.58	4.27	1.0	1.82	6.98	2.25		2.8	Green[(Chen et al., 2010)]
NTPS1	24.8	1.2		3.4		1.2	86.5	7.8			Green[(Wang et al., 2005)]
ATPS2	6.2	18.5		10.9			27.4		43.3		Green[(Wang et al., 2005)]
ATPS3	20.0	17.2		11.4			27.1		44.3		Green[(Wang et al., 2005)]
ATPS4	16.2	29.9		12.7			28.1		29.3		Green[(Wang et al., 2005)]
TFPS1	400-500	1.0		2.9		0.5	3.3	1.3			Flower[(Wang et al., 2010a,b)]
TFP-1	167.5	0.81			1.2		0.98	1.0			Flower[(Han et al., 2011)]
TFP-2	10.1	2.3			0.76		2.3	1.0			Flower[(Han et al., 2011)]
TSPS	5, 130, 500	4.9		11.1	1.7		14.0	27.2	3.4	1	Seed[(Wei et al., 2011)]



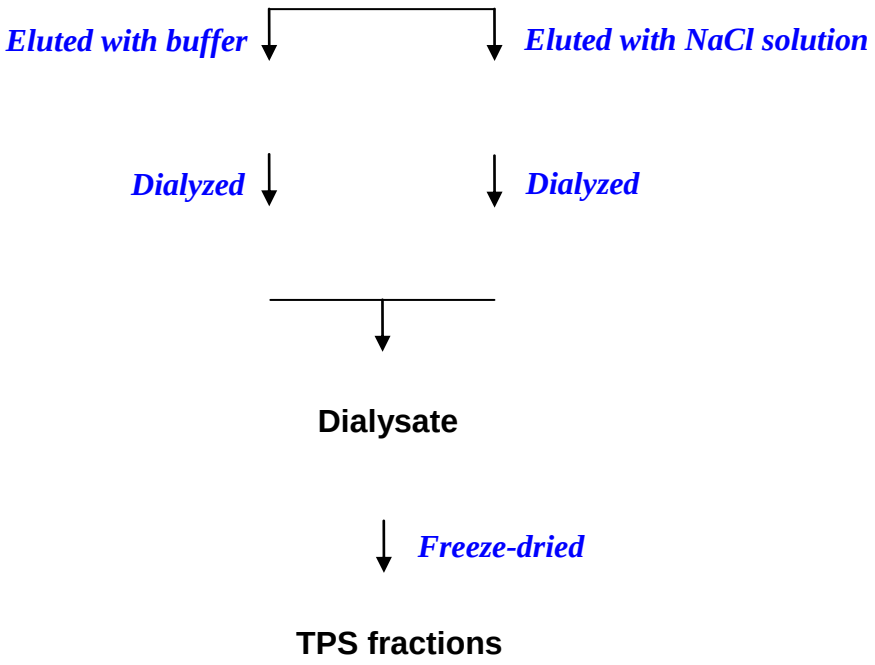


Figure 1. Scheme for separation and fractionation procedures of crude TPS.

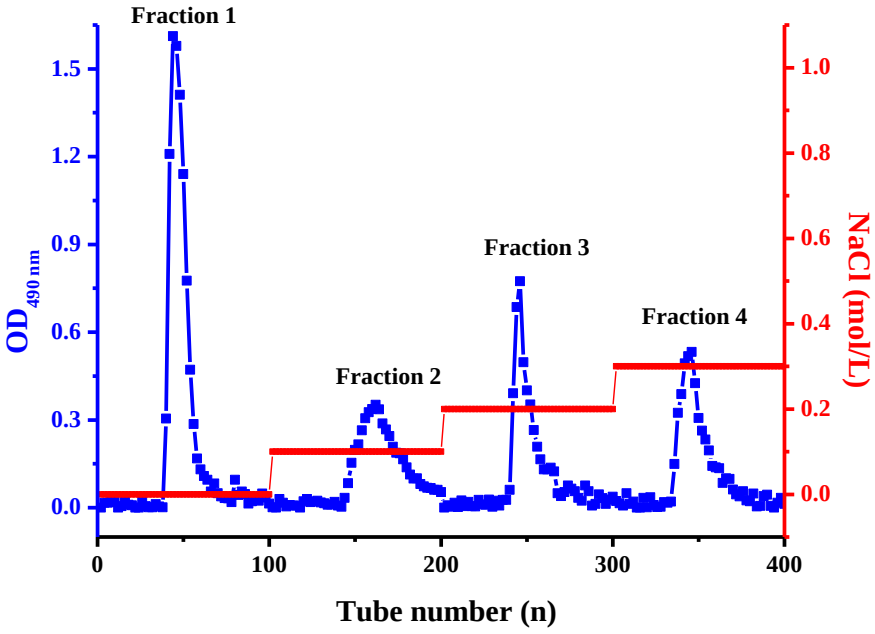


Figure 2. A typical gel filtration profile of crude TPS on a column chromatogramy.

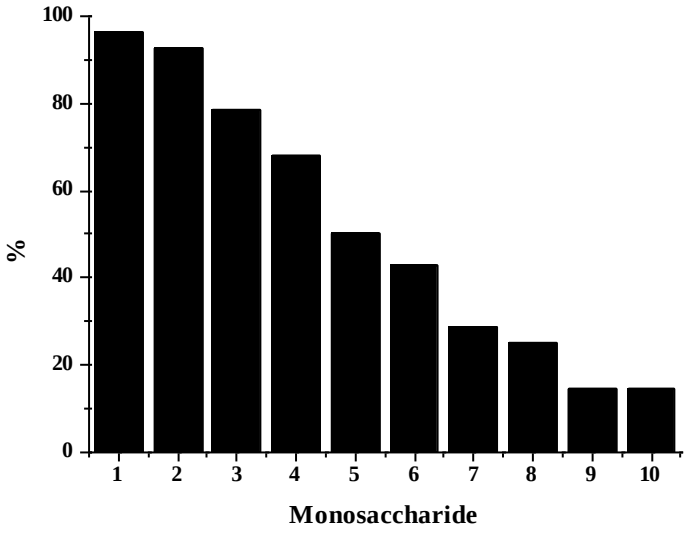


Figure 3 The monosaccharides found in 28 TPS from references. 1. Ara, 2. Gal, 3. Glu, 4. Rha, 5. Xyl, 6. Man, 7. Rib, 8. Fuc, 9. GluA, 10. GalA.

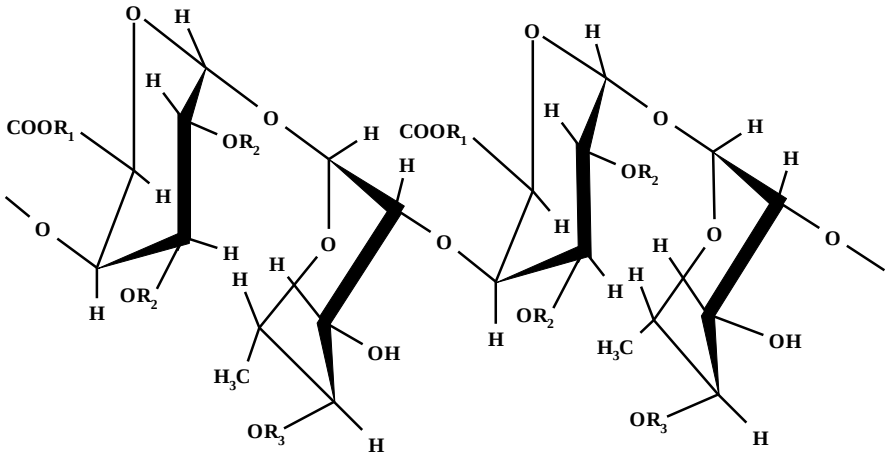


Figure 4. Chemical structures of tea pectin fractions P1A and P2A (Ele-Ekouna et al., 2011).

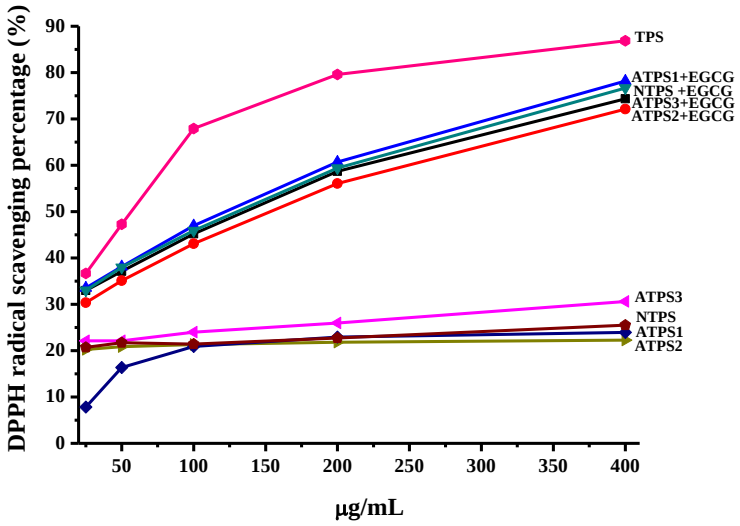


Figure 5. DPPH radical scavenging activities of TPS in the absence and presence of EGCG (6.15 μg/mL).

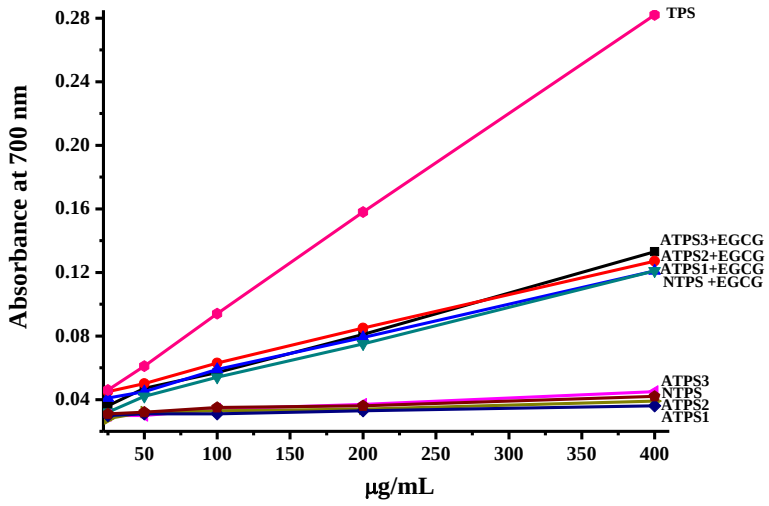


Figure 6. Reducing power of TPS in the absence and presence of EGCG (8 µg/mL).