

Antioxidant activity and physicochemical properties of rice starch-chitosan-based films containing green tea extract

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Abstract

Rice starch-chitosan-based films were developed with the addition of green tea extract (GTE) of 10 percent (weight of dried green tea leaves per volume) at different levels (0, 2 and 5 g). The antioxidant activity and the physicochemical properties were investigated along with the mechanical properties to determine their potential uses for food packaging applications. The average film thickness of 0.170±0.006 mm was obtained. The results suggested that incorporation of GTE into the rice starch-chitosan-based films significantly ($p<0.05$) improved the total phenolic content (TPC), the antioxidant activity, the tensile strength (TS) and the water vapor permeability (WVP) with an increase of GTE content. However, the elongation at break (E) and the water solubility (WS) of the films added with GTE were decreased. Furthermore, the film integrated with GTE appeared darker as confirmed by the decreasing in the L* values. The changes in the Fourier Transform Infrared (FTIR) spectra of the film incorporated with GTE indicated that there were some molecular interactions between the components in the films. The results from this study suggested that the rice starch-chitosan-based films containing GTE hold a considerable promise for future food packaging applications.

Keywords: Rice starch film, Chitosan, Green tea extract, Antioxidant activity

1. Introduction

Starch has been widely used to produce the biodegradable film in recent years (Wittaya, 2012) as it is a natural polymer that could be an alternative solution to the environmental problem caused by plastic uses. Starch has shown a promising application as a food packaging due to its plentiful availability, biodegradability, low cost, and flexibility (Parra *et al.*, 2004). Rice starch and its major components, amylose and amylopectin, are biopolymer, which are the attractive raw materials for using as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability. However, a wide application of starch film is limited by its poor mechanical properties. This constraint has led to the improvement of the properties of rice-based films either by modifying its starch properties and/or incorporating other materials (Zhang *et al.*, 2009).

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Heat Moisture Treatment (HMT) is a physical modification that involves low moisture levels, usually in a restricted range of 10–30%, and heating at high temperatures (90–120°C) for a period of time ranging from 15 min to 16 h. HMT allows control of molecular mobility at high temperatures by limiting the amount of water. HMT-induced changes in starch structure and properties have been found to vary with starch source and amylose content. For instance, tuber starches are more sensitive to HMT than legume or cereal starches. According to Gunaratne and Hoover (2002), HMT promotes the interaction of polymer chains by disrupting the crystalline structure and dissociating the double helical structure in the amorphous region, followed by the rearrangement of the disrupted crystals. Crystalline disruption near the granule's surface can facilitate the attack of α -amylase within it. When the crystals are not disrupted by HMT, increased enzymatic susceptibility results due to the disruption of the double helices by treatment in amorphous regions. This disruption facilitates enzymatic access to the sites of interaction between the amylose chains during the rearrangement of the polymer chains (Zavareze and Dias, 2011).

Chitosan is a functional biopolymer, having intrinsic antimicrobial and antioxidant properties and consequently has high potential to be used as a biodegradable active packaging. Chitosan films are biodegradable, biocompatible, flexible, durable, strong, tough and hard to break. The chitosan films have moderate values of water and oxygen permeability. It helps decrease the respiratory rate of food and also inhibit the microbial growth in food. Most of the mechanical properties of chitosan films are comparable to those of commercial polymers of medium strength such as cellulose. The mechanic and permeable properties of chitosan films can be controlled by selecting its molecular weight, a suitable solvent system, and the addition of plasticizer agents, dispersants and compatibility (Martínez-Camacho *et al.*, 2010). Green tea (*Camellia sinensis*), is a non-fermented tea product. It has a strong antioxidant property as it contains about 36% of polyphenolic compounds. The phenolic compounds are able to scavenge reactive oxygen and nitrogen species. Thus green tea has been used to delay the onset of lipid oxidation in various foods (Siripatrawan and Harte, 2010).

As for the improvement of biodegradable film for food packaging application, it was anticipated that adding the green tea extract (GTE) into the rice starch-based film might improve the anti-oxidant property of the film. While adding the chitosan into the rice starch-based film was expected to improve its mechanical property. Therefore, the aims of this study were (i) to develop the environmentally friendly active films from rice starch and chitosan, incorporated with green tea extract at various concentrations and (ii) to characterize the antioxidant activity, the physicochemical properties, the mechanical properties as well as the molecular miscibility of the rice starch-chitosan and GTE.

2. Materials and Methods

2.1 Materials

Native rice starch was purchased from Cho Heng Rice Co., Ltd., Bangkok, Thailand. Chitosan with the degree of deacetylation of 80–95% was obtained from Seafresh Industry Public Company Limited, Bangkok, Thailand. Glycerol, acetic acid and silica gel were obtained from Lab Valley Limited partnership. Gallic acid, Folin-Ciocalteu (F–C) reagent, Sodium carbonate, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma – Aldrich (St. Louise, MO, USA). Green tea dried leaves were purchased from Thai Tea Suwirun Co., Ltd., Chiang Rai, Thailand. All chemicals and reagents were of analytical grade and were used as received.

2.2 Film preparation

The chitosan solution (1% w/v) was prepared by dispersing chitosan in 1% v/v of acetic acid and heated at 60°C for 30 min with continuous stirring. Native rice starch was adjusted to 25% moisture content and put into autoclave at 105°C for 90 min to obtain the heat-moisture modified rice starch. The modified rice starch was dissolved in distilled water at concentrations of 2 g/100 ml and heated at 90°C for 10 min. A series of starch-chitosan blended film solutions was prepared by mixing the modified rice starch solutions with the chitosan solution in 1:1 ratio. Glycerol was added as 20% (w/w) of the total solid weight in rice starch-chitosan film solution. The green tea extract (GTE) solution was prepared by mixing green tea dried leaves with the distilled water (10% w/v) and heated at 100°C for 7 min. The tea residues were then removed. The GTE was obtained. The GTE was then mixed with starch-chitosan-blended film forming solution at the concentration of 0, 2, and 5 g, respectively. The films forming mixtures were casted into the plastic petri dishes (9-in dia.) and dried at room temperature for at least 72 h. The rice starch-chitosan-GTE-based films (RS-CS-GTE-based films) were peeled and kept in a desiccator prior to experimental uses.

2.3 Film thickness

The film thickness was measured with a digital micrometer at five random locations on the film. The mean thickness values of each sample were calculated according to the method of Siripatrawan and Harte, 2010.

2.4 Water solubility (WS)

The water solubility of the films was defined as the percentage of dissolved specimen dry matter after 24 h of immersion in distilled water. Films, previously equilibrated at 50% RH, were cut to 1 cm × 4 cm strips, and immersed in 50 ml of distilled water. After 24 h, the strips were taken out and dried at 105±1°C for 24 h and the final dry weight (W_f) was measured.

The initial dry weight (W_i) was determined by drying the strips in an oven at 105°C to constant weight. All the tests were conducted in triplicate and the means were reported.

The water solubility was calculated according to the following equation (Wang *et al.*, 2013);

$$WS (\%) = [(W_i - W_f) / W_i] \times 100\%$$

2.5 Water vapor permeability (WVP) and water vapor transmission rate (WVTR)

The test cups were filled with 10 g of silica gel to produce a 0% RH below the film. A sample was placed in between the cup and the ring cover of each cup coated with paraffin wax. The water vapor transmission rates (WVTR) of each film were measured at 50±2% RH and 30±2°C. After taking the initial weight of the test cup, it was placed into a growth chamber. Weight gain measurements were taken by weighing the test cup every 1 h for 8 h. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). The WVTR was expressed in gram units, per square meter, per hour. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. The WVP of the film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film (Bourtoom and Chinnan, 2008).

2.6 Tensile strength (TS) and elongation at break (E)

Tensile strength (TS) was measured with a Universal Testing Machine (UTM, TA-XT2, Texture Technologies Corp.), nine samples, 2 cm × 5 cm, were cut from each films. The crosshead speed was set at 30 mm/min with 100N load cell used. Tensile strength is calculated by dividing the maximum load by the original minimum cross sectional area. The result is expressed in megapascal (MPa).

$$\text{Tensile strength} = [\text{maximum load}] / [(\text{original width}) \times (\text{original thickness})]$$

2.7 Color

A CIE colorimeter was used to determine the film L^* , a^* , and b^* color values [$L^* = 0$ (black) to 100 (white); $a^* = -60$ (green) to +60 (red); and $b^* = -60$ (blue) to +60 (yellow)].

$$\Delta L^* = L^*_{\text{sample}} - L^*_{\text{standard}}; \Delta a^* = a^*_{\text{sample}} - a^*_{\text{standard}}; \Delta b^* = b^*_{\text{sample}} - b^*_{\text{standard}}$$

2.8 Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the films were measured by using a PerkinElmer FTIR Spectrometer with an ATR attachment to investigate the interactions of rice starch-chitosan and green tea extract in the films.

The measuring probe directly touched the surface of the films. A spectral resolution of 4 cm^{-1} was employed and 64 scans were acquired for each spectrum in the range of 4000 to 1000 cm^{-1} (Wang *et al.*, 2013).

2.9 Total phenolic content (TPC)

The total phenolic content (TPC) was measured by dissolving a 25 mg of each film sample in 3 ml of distilled water. Total phenolic content of the film samples was determined according to the Folin-Ciocalteu method as described by Siripatrawan and Harte, 2010. Briefly, 1 ml of film extract solution was mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml of sodium carbonate solution. The mixture was stored at room temperature for 1 h. The absorbance of the mixture was then measured at 765 nm using a spectrophotometer.

2.10 Antioxidant activity

The antioxidant activity of the film samples was evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay according to the method of Siripatrawan and Harte, 2010. Briefly, the 25 mg of each film sample was dissolved in 3 ml of distilled water for using as the film extract solution. The 50 μ l of the film extract solution were then mixed with 1950 μ l of DPPH solution. The mixture was vortexed and incubated in the dark at ambient temperature for 30 min. The absorbance was then measured at 517 nm.

2.11 Statistical analysis

Data were expressed as means \pm standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS 16.0 for Windows. The significance level of $P < 0.05$ was considered significantly different.

3. Results and Discussion

3.1 Film thickness

The thicknesses of various RS-CS-GTE-incorporated films were measured. The results obtained are shown in Table 1. The film thicknesses of all conditions were not significantly different ($p > 0.05$). This could be due to the thickness of the film was controlled by the define volume of film solution and the constant diameter of the casting mold.

Table 1 Thickness of the rice starch-chitosan blended films incorporated with green tea extract at various concentrations.

Films	Thickness (mm)
0 g GTE	0.177 \pm 0.025*a
2 g GTE	0.167 \pm 0.006a
5 g GTE	0.167 \pm 0.012a

Note: * Values are given as mean \pm standard deviation.

Different letters in the same column indicate significant difference ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.2 Water solubility (WS)

The WS values of various films are shown in Table 2. The results demonstrated that the water solubility of the films decreased with the addition of green tea extract. Generally, higher solubility would indicate lower water resistance. So, the GTE composite films have a higher water resistance. This might be due to OH- groups of the phenolic compound in GTE formed the H-bonding with the NH₃⁺ groups of the chitosan backbone (Bourtoom and Chinnan, 2008; Siripatrawan and Harte, 2010). The stronger intermolecular H-bonding, among the OH- groups of rice starch and that of GTE and the NH₃⁺ groups of the chitosan, decreased the affinity of the composite film toward water and resulted in a decrease in the water solubility.

Table 2 Water solubility of the rice starch-chitosan blended film incorporated with green tea extract at various concentrations.

Films	Water solubility (%)
10% (GTE 0 g)	50.21 ± 7.79 ^{*b}
10% (GTE 2 g)	48.37 ± 0.95 ^b
10% (GTE 5 g)	36.01 ± 3.91 ^a

Note: * Values are given as mean ± standard deviation.

Different letters in the same column indicate significant difference ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.3 Water vapor permeability (WVP) and Water vapor transmission rate (WVTR)

The WVP of GTE infused films are shown in Table 3. It was found that the WVP values increased when the GTE content in the film were increased. These results could cause by the hydrogen or covalent bondings of polyphenol compounds in GTE with reactive groups of chitosan (Siripatrawan and Harte, 2010). These resulted in the increasing in the film polarity and the increasing in the interaction of permeating water molecules with polar groups in the film's structure (Bourtoom and Chinnan, 2008).

Table 3 Water vapor permeability (WVP) and water vapor transmission rate (WVPR) of the rice starch-chitosan blended film incorporated with green tea extract at various concentrations.

Films	WVP (g.mm.m ⁻² .h ⁻¹ .mmHg)	WVPR (g m ⁻² h ⁻¹)
10% (GTE 0 g)	0.0003 ± 0.0000 ^a	14.3616 ± 0.3002 ^a
10% (GTE 2 g)	0.0004 ± 0.0000 ^a	15.5288 ± 0.4002 ^a
10% (GTE 5 g)	0.0004 ± 0.0000 ^a	14.2731 ± 2.0761 ^a

Note: * Values are given as mean ± standard deviation.

Different letters in the same column indicate significant difference ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.4 Tensile strength (TS) and Elongation at break (E)

The TS of the films increased with the increasing of GTE concentration. However, the elongation at break behaved inversely to the TS as shown in Table 4. The increasing in TS values as the GTE content increased could cause by the attribution of the hydrogen bonds formation between the OH- groups of polyphenolic molecules and that of the rice starch molecules and the NH₃⁺ reactive group of the chitosan backbone (Xu *et al.*, 2005).

This contributed to the stronger films. While the decrease of E values as the GTE added, indicated that the film became less flexible. The decrease in film stretch ability (extensibility) might be the results of the limited chain movement caused by the interactions of chitosan and the rice starch and GTE (Detduangchan *et al.*, 2014).

Table 4 Tensile strength (TS) and elongation (E) at break of the rice starch-chitosan blended films incorporated with green tea extract at various concentrations.

Films	TS (MPa)	Elongation (%E)
10% (GTE 0 g)	0.0978 ± 0.0744a	11.9333 ± 1.1140b
10% (GTE 2 g)	0.1969 ± 0.0538a,b	7.7667 ± 2.1795a,b
10% (GTE 5 g)	0.3237 ± 0.1263b	8.6333 ± 2.2590a

Note: Values are given as mean ± standard deviation.

Different letters in the same column indicate significant difference ($p > 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.5 Color

The color values of the films are exhibited in Table 5. Since L* = 0 is black and L* = 100 is white, thus the reducing of L* values could imply that the films became darker when adding GTE. The incorporation of GTE on the L* values in the range of 2 to 5 g was significantly different ($p > 0.05$). In addition, the values of a* and b* were found increase significantly ($p > 0.05$), which are the indication of the tendency toward redness and yellowness.

Table 5 Color values of the rice starch-chitosan blended film incorporated with green tea extract at various concentrations.

Films	L*	a*	b*
10% (GTE 0 g)	87.70 ± 1.24c	0.27 ± 0.15a	5.27 ± 0.74a
10% (GTE 2 g)	40.42 ± 4.20b	0.60 ± 0.06a	10.48 ± 0.35b
10% (GTE 5 g)	35.05 ± 0.19a	4.23 ± 0.28b	19.06 ± 2.28c

Note: Values are given as mean ± standard deviation.

Different letters in the same column indicate significant difference ($p > 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.6 Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis

FTIR spectroscopy was used to examine the interaction among the molecules of rice starch, chitosan and polyphenolic molecules in the films. The FTIR spectra of various film conditions are present in Figure 1. The broad band of all films conditions at 3279.05 cm^{-1} , 3282.93 cm^{-1} and 3271.76 cm^{-1} were the results of the O-H stretching. The peak at 2934.99 cm^{-1} , 2932.68 cm^{-1} and 2936.57 cm^{-1} corresponded to the C-H stretching. The peak at 1646.55 cm^{-1} , 1646.93 cm^{-1} and 1647.29 cm^{-1} could be due to the O-H bendings of water in rice starch. While at 1415.92 cm^{-1} , 1416.68 cm^{-1} and 1416.40 cm^{-1} peak could indicate the bending of CH₂ in rice starch (Bourtoom and Chinnan, 2008). New peak at 1744.6 cm^{-1} suggested the presence of carbonyl group in the film (Xu et al., 2005). When adding GTE into the rice starch-chitosan blended films, the slight shifts in the FTIR peaks occurred at 1453.93 cm^{-1} . This indicated the CH₂ bending of rice starch was lessening as the polyphenols molecules limited its movements.

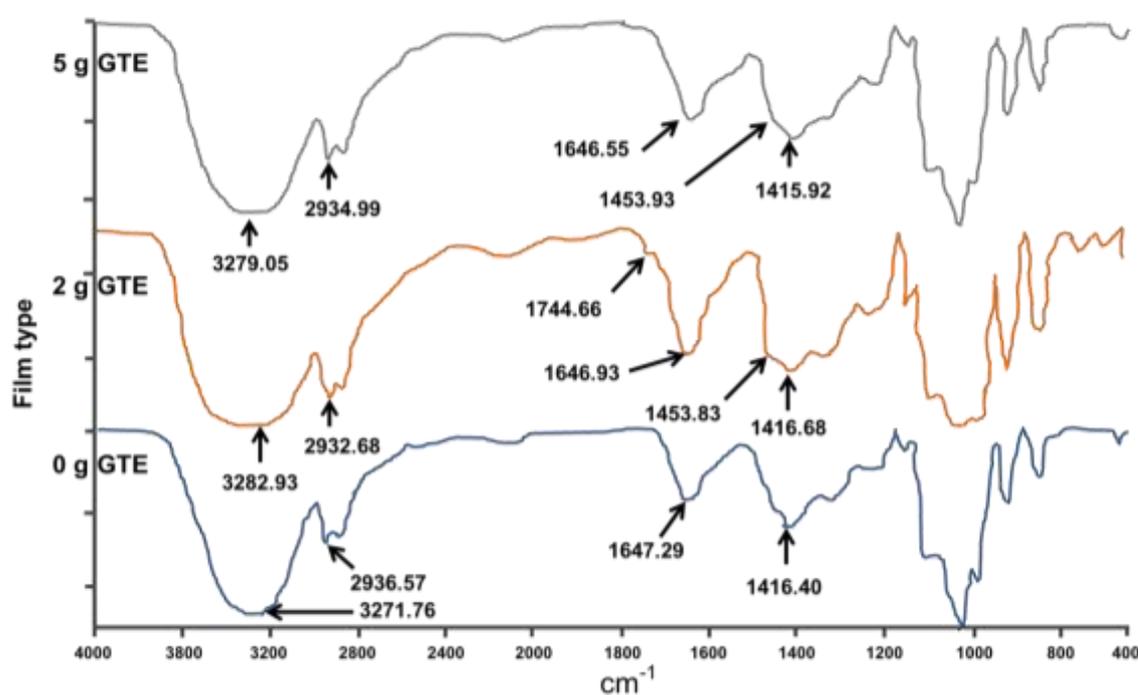


Figure 1 ATR-FTIR spectrum of the rice starch-chitosan blended film incorporated with green tea extract at different concentrations. Values are given as mean \pm standard deviation. Different letters in the same column indicate significantly different ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.7 Total phenolic content (TPC)

As mentioned earlier, green tea contains high content of phenolic compounds. Thus, it was expected that the TPC of the GTE added films would be higher than that of the typical film. The results suggested that when incorporating GTE into the starch-chitosan blended films, the TPC of the films were much higher than that of the films without GTE addition and the TPC increased significantly ($p < 0.05$) with an increasing of GTE concentration as shown in Figure 2.

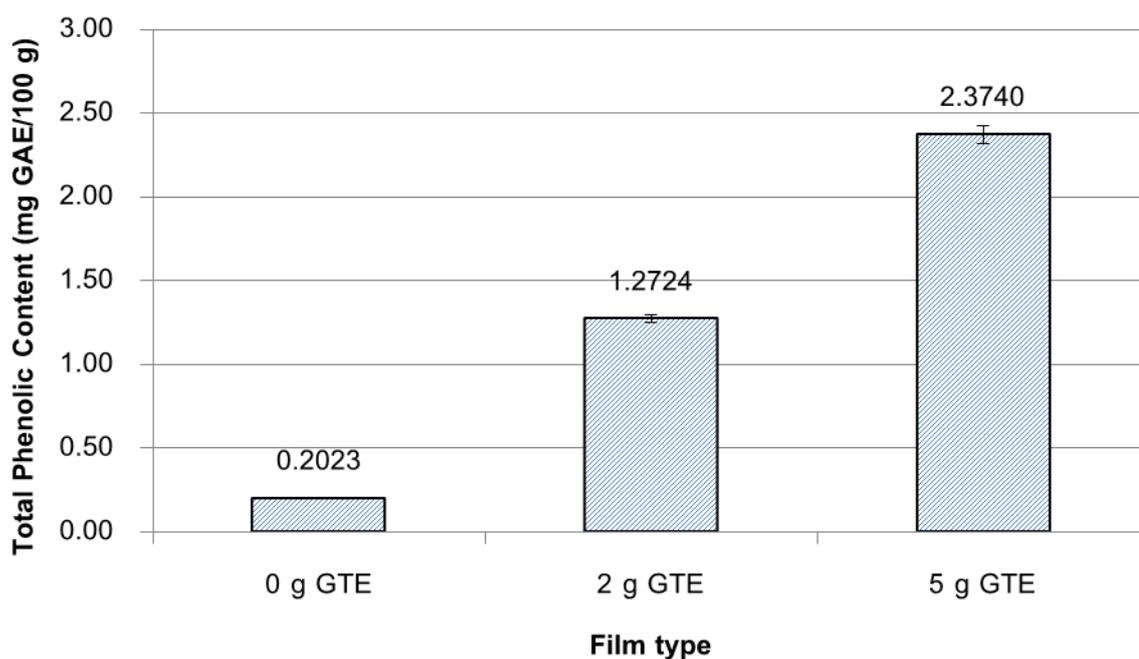


Figure 2 Total polyphenolic content of the rice starch-chitosan blended film incorporated with green tea extract at different concentrations. Values are given as mean \pm standard deviation. Different letters in the same column indicate significantly different ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.8 DPPH free radical scavenging activity

Phenolic and polyphenolic compounds found in tea are the main contributor to the antioxidant activity, which are the highly effective free radical scavengers and antioxidants (Siripatrawan and Harte, 2010). Thus, the correlation between TPC values and the antioxidant activities of the RS-CS-GTE incorporated films were expected to be closely related. When incorporating GTE into the rice starch-chitosan blended film, the DPPH free radical scavenging activity of the films was much higher than that of the films without GTE and increased significantly ($p \leq 0.05$) with increasing of GTE concentration as shown in Figure 3. The phenolic

compounds present in GTE are responsible for the antioxidant activity because polyphenols can improve scavenging activity and antioxidant activity (Wang *et al.*, 2013).

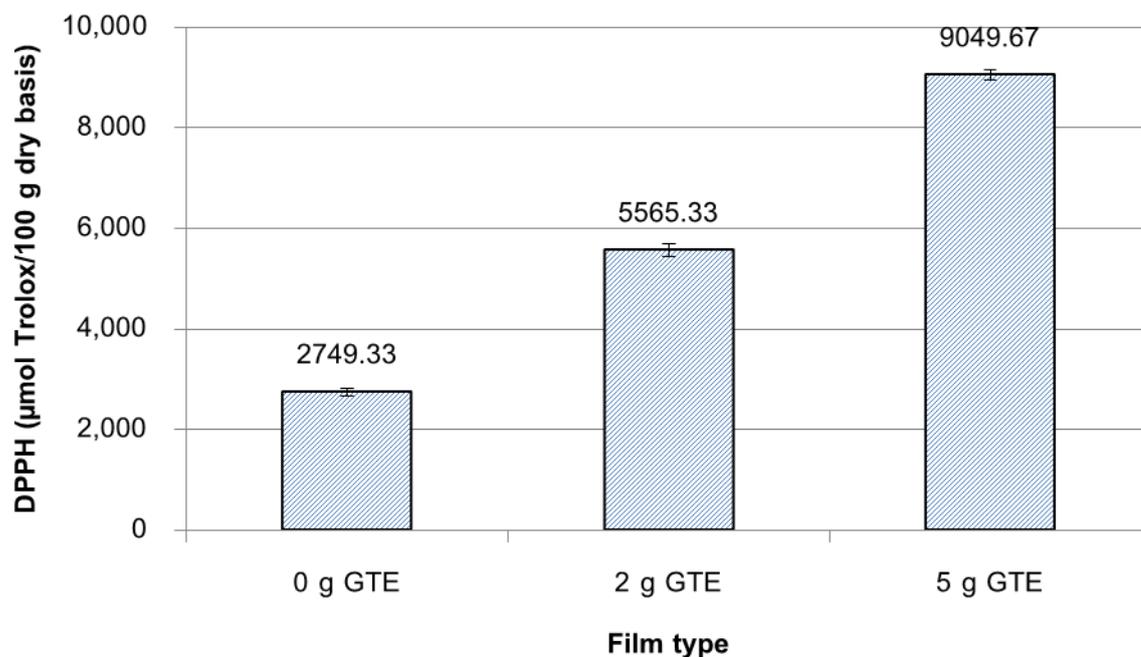


Figure 3 DPPH free radical scavenging activity of the rice starch-chitosan blended film incorporated with green tea extract at different concentrations. Values are given as mean \pm standard deviation.

Different letters in the same column indicate significantly different ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test

3.9 Correlations of DPPH value with TPC

The correlation between antioxidant activities (DPPH values) and the TPC values of the rice starch-chitosan blended film incorporated with GTE are shown in Figure 4. The results showed a positive linear correlation between DPPH values and TPC values ($R^2 = 0.9915$). Considering the values of R^2 , it can be interpreted that there are the strong correlations between antioxidant activities (DPPH values) and the TPC values. The results suggested that phenolic compounds could be one of the main components responsible for antioxidant activities of the film (Park, *et al.*, 2012).

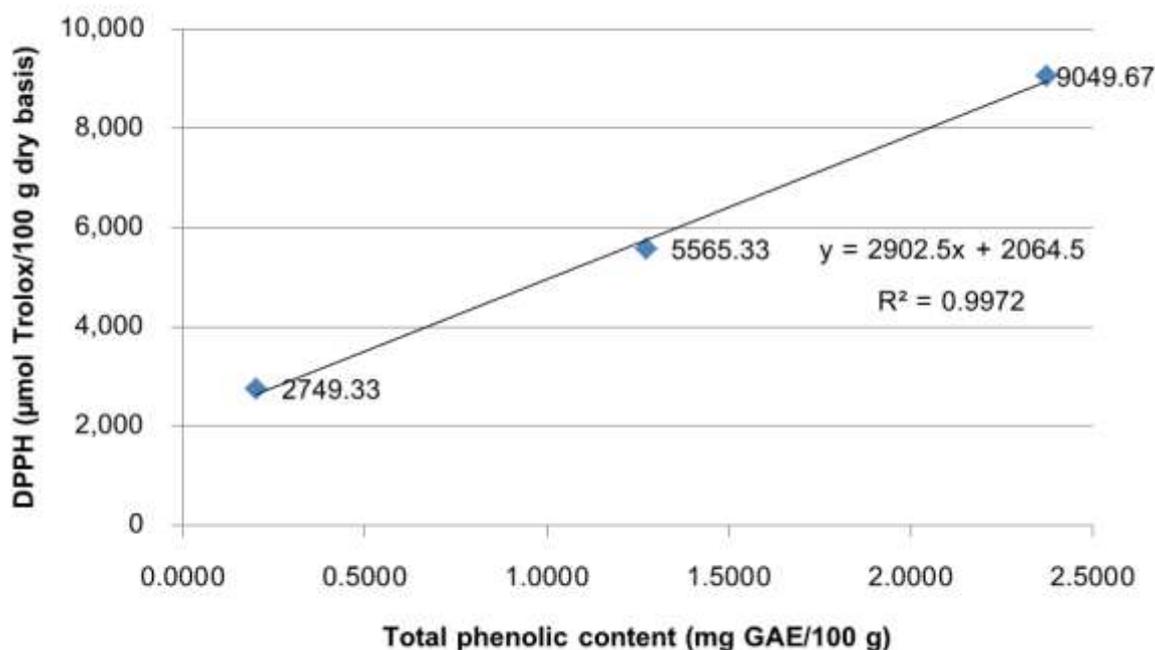


Figure 4 Correlation between total phenolic content and antioxidant capacities measured by the DPPH assay. Values are given as mean \pm standard deviation. Different letters in the same column indicate significantly different ($p \leq 0.05$) when analyzed by Duncan's New Multiple Range Test.

4. Conclusion

The results from this study suggested that incorporation of GTE caused interactions between rice starch-chitosan and GTE. This gave rise to the films darker appearance. Addition of GTE improved water vapor permeability, tensile strength and antioxidant properties but reduced water solubility and elongation at break of the films. The rice starch-chitosan blended film incorporated with GTE shows potential to be used as an environmentally friendly active film.

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