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Full Length Research Paper

Effect of green tea polyphenols on the gelatinization and retrogradation of rice starches with different amylose contents

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The effect of green tea polyphenols (GTP) on the property of rice starch was investigated. 0, 5, 10 and 15% of GTP (based on rice starch weight) was added to rice starches with high (HAC), intermediate (IAC) and low (LAC) amylose contents, respectively. The gelatinization and retrogradation properties of rice starches with different amylose content containing different levels of GTP were analyzed by differential scanning calorimetry (DSC). The results exhibited that GTP could retard retrogradation of the three rice starches, which was further proved by X-ray diffraction investigation. In the DSC analysis, the temperature and enthalpy of starches gelatinization decreased with the concentration of GTP increasing. After storage at 4°C for 20 days, retrogradation enthalpy (ΔH_{ret}) for all rice starches with different levels GTP began to appear, and the ΔH_{ret} and degree of retrogradation (DR%) of the three rice starches significantly decreased with the increased level of GTP. The X-ray diffractogram indicate that all the three gelatinized rice starch gels showed B-type X-ray diffraction patterns, and the B-type X-ray diffraction patterns gradually disappeared with the concentration of GTP increasing.

Key words: Green tea polyphenols, rice starch, gelatinization, retrogradation, differential scanning calorimetry, X-ray diffraction.

INTRODUCTION

Starches are widely used in many food products, at present, maize starch and potato starch are mostly utilized. Compared to maize and potato starch, the use of rice starch is limited due to its high starch retrogradation rate during storage that shortens their shelf lives. Retrogradation is used to describe the changes that occur upon cooling and storage of gelatinized starch (Fredriksson et al., 1998). Björck (1996) indicated that retrogradation means fully reversible recrystallization in the case of amylopectin and partial irreversible recrystallization in the case of amylose. The firmness or rigidity of starch gel increases markedly with retrogradation (Collison, 1968).

Industrially, starch is treated with various chemical or physical modifications to prevent starch retrogradation. Retrogradation of starch has been reported to be suppressed by cross-linking using Sodium trimetaphosphate and sodium tripolyphosphate mixture, as indicated by their lower enthalpy of retrogradation after storage which may be because of the restricted mobility of cross-linked amylopectin branches due to the presence of phosphate groups (Chung et al., 2004). Both normal and

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Abbreviations: DR%, Degree of retrogradation; **GTP,** green tea polyphenols; **TP,** tea polyphenols; **HAC,** rice starches with high amylose content; **IAC,** rice starches with intermediate amylose content; **LAC,** rice starches with low amylose content; **ΔH_{ret},** retrogradation enthalpy; **ΔH_{gel},** enthalpy of gelatinization; **Native HAC,** native rice starches with high amylose content; **Native IAC,** native rice starches with intermediate amylose content; **Native LAC,** native rice starches with low amylose content.

waxy oxidized starches showed lower values of retrogradation compared to their native counterparts due to the restricted retrogradation (Kuakpetoon and Wang, 2001). However, many chemical modification used in food products is not safe enough for health due to questionable chemicals. In addition, physical modification can prevent the retrogradation of rice flour but not rice starch (Wu et al., 2010).

Green tea has attracted significant attention recently, both in the scientific and in consumer communities for its health benefits for a variety of disorders, ranging from cancer to weight loss. Green tea extract have drawn increased attention in recent years, not only as flavouring and food preservatives due to its antioxidant activity and antimicrobial effect but also for preventing firmness. Zhou et al. (2008) reported that polysaccharides of tea extract could retard the retrogradation of wheat starch. It is well known that polyphenolic compounds, particularly the catechins are the main compounds of green tea, which make up 30% of the dry weight of green tea leaves. These catechins are present in higher quantities in green tea than in black or oolong tea (Zaveri, 2006). Previous studies have mostly focused on antibacterial, antioxidative and acti-tumor activities of tea polyphenols (TP) (Zaveri, 2006). There are few studies on the relationship between starch and TP. Zhu et al. (2009) studies effect of extract of phytochemicals from green tea on the pasting, thermal, and gelling properties of wheat starch. Our previous study investigated the effect of tea polyphenols on the retrogradation of a high amylose rice starch (Wu et al., 2009). The interaction and association between TP and gelatinization and retrogradation of rice starch are not well understood.

In this study, the effect of green tea polyphenols (GTP) on gelatinization and retrogradation of rice starches with different amylose contents were investigated by using differential scanning calorimetry (DSC) and X-ray diffraction. Our study suggests that the temperature and enthalpy of starches gelatinization decreased with the concentration of GTP increasing, and GTP retarded retrogradation of all the tested rice starches.

MATERIALS AND METHODS

Rice starches with different amylose content were purchased from Puer Biological Technology Co.Ltd (Yun Nan, China). The amylose contents in rice starches were 28.3% (high amylose content), 13.7% (intermediate amylose content) and 1.15% (low amylose content), respectively. The amylose contents were determined using the colorimetric method described by McGrance et al. (1998). The moisture content of rice starch with high amylose content (HAC) was 11.2%, the moisture content of rice starch with intermediate amylose content (IAC) was 12.6%, and the moisture content of rice starch with low amylose content (LAC) was 13.5%, determined by heating at 105°C for 5 h. All rice starches contained crude protein less than 0.45% and ash less than 0.15% (data from Puer Co.). GTP was obtained from Novanat Bioresources Co. Ltd. (Shanghai, China) with total polyphenols of 99.97%, contain of 46.8% (–) epigallocatechin-3-gallate (EGCG), 24% (–) epicatechin-3-gallate (ECG), 12.8% (–) epigallocatechin (EGC), 8% (–) epicatechin (EC), analyzed by high-pressure liquid chromatography.

Differential scanning calorimetry (DSC)

Gelatinization and retrogradation thermal properties of the samples were measured using 61 DSC-Pyris Diamond (Perkin-Elmer Corp., Norwalk, CT, USA). 0, 5, 10 and 15% of GTP (based on rice starch weight) were added to HAC, IAC and LAC, respectively. The calorimeter was calibrated with an indium standard. Samples of the mixtures of rice starch and GTP (about 2 mg) were accurately weighed into aluminum DSC pans, and deionized water was added by micropipette at a water-sample ratio of 2:1. The sample pans were sealed and equilibrated at room temperature for 24 h before analysis. For all DSC runs, a sealed empty aluminum pan was used as reference. The samples were heated. The scanning temperature range and the heating rate were 25 to 100°C and 10°C/min, respectively. The onset temperature T_o, peak temperature T_p and conclusion temperature T_c were determined from the first run heating DSC curves, the range of gelatinization (Tc- To) was calculated. Enthalpy of gelatinization (ΔH_{gel}) was evaluated based on the area of the main endothermic peak. Then the gelatinized samples were stored at 4°C for 5, 10, and 20 days and rescanned under the same conditions to determine the temperature and enthalpy changes due to retrogradation. The retrogradation temperature (T_o, T_p and T_c) and enthalpy (ΔH_{ret}) were determined from the second run heating. In addition, the degree of retrogradation (DR%) was calculated as the ratio of the retrogradation enthalpy to the gelatinization enthalpy in run heating (Rodríguez-Sandovala et al., 2008). Analyses were performed in triplicate.

X-ray diffraction

The samples with rice starches containing 0, 5, 10 and 15% GTP (based on rice starch weight) respectively were added double deionized water and gelatinized by steam heating for 20 min in the closed thermostat water bath. These samples were cooled to room temperature then stored at 4°C condition for 10 days. The freeze-dried samples were ground and then powder samples passed through a 100 mesh sieve before testing. The recrystallization analysis of samples was carried out using a Rigaku D-Max-2500 X-ray diffractometer (Rigaku, Tokyo, Japan) equipped with a copper tube operating at 40 kV and 250 mA. Diffractograms were obtained, the scanning region of the diffraction angle (2 θ) was from 4 to 50° at a rate of 4° /min, a step size of 0.02°, 1°/1° divergence slit/scattering slit, 0.30 mm receiving slit. MDI Jade 5.0 was used to analyze the diffractograms.

Statistical analysis

The data reported in the tables were average of triplicate observations. Data obtained windows version 13.0 following the procedure described by Miller and Miller (1993). Confidence interval of sample means was reported at the 95% confidence probability. Comparisons of means were made using least significant difference (LSD) and shortest significant ranges (SSR) at 5% significance level (P<0.05).

RESULTS AND DISCUSSION

Thermal properties

The gelatinization transition temperatures and

Table 1. Gelatinization temperatures and enthalpy of the mixtures of rice starch with different amylose contents and green tea polyphenols at various ratios.

Samples	T_o (℃)	T_p (℃)	T_c (℃)	ΔH_{gel} (J/g)	R (℃)
HAC+0% GTP	66.39 ± 0.41^a	71.40 ± 0.66^a	76.12 ± 0.37^a	9.68 ± 0.13^c	9.73^d
HAC+5% GTP	63.56 ± 0.32^d	69.78 ± 0.23^b	75.46 ± 0.53^b	9.66 ± 0.23^c	11.90^b
HAC+10% GTP	61.33 ± 0.58^f	67.39 ± 0.47^d	73.31 ± 0.31^c	5.86 ± 0.10^f	11.98^b
HAC+15% GTP	59.26 ± 0.12^h	66.04 ± 0.26^e	71.73 ± 0.10^e	3.25 ± 0.07^g	12.47^a
IAC+0% GTP	64.33 ± 0.35^c	67.70 ± 0.43^d	71.64 ± 0.06^e	10.45 ± 0.21^b	7.31^g
IAC+5% GTP	61.50 ± 0.41^f	65.17 ± 0.54^f	69.48 ± 0.11^g	10.24 ± 0.22^b	7.98^g
IAC+10% GTP	59.07 ± 0.09^h	63.12 ± 0.17^h	67.59 ± 0.29^i	6.35 ± 0.05^e	8.52^f
IAC+15% GTP	56.95 ± 0.14^j	61.08 ± 0.28^i	66.24 ± 0.18^j	4.96 ± 0.13^f	9.29^e
LAC+0% GTP	65.03 ± 0.62^b	68.14 ± 0.53^c	72.51 ± 0.07^d	11.86 ± 0.38^a	7.48^g
LAC+5% GTP	62.92 ± 0.19^e	66.37 ± 0.25^e	71.55 ± 0.38^e	11.51 ± 0.27^a	8.63^f
LAC+10% GTP	60.62 ± 0.24^g	64.32 ± 0.18^g	70.00 ± 0.63^f	7.05 ± 0.31^d	9.38^e
LAC+15% GTP	58.13 ± 0.08^i	63.48 ± 0.09^h	68.45 ± 0.54^h	5.12 ± 0.16^e	10.32^c

The values are average ± standard deviation, n = 3. Values followed by the same letter in the same column are not significantly different ($p < 0.05$). R, the range of gelatinization.

gelatinization enthalpy of samples added GTP at various ratios are shown in Table 1. Adding GTP significantly affected the gelatinization properties of rice starch with different amylose content. Among native rice starches, HAC starch had the highest gelatinization temperature and IAC had the lowest. However, the ranges of gelatinization temperature were 9.73, 7.31 and 7.48℃ for HAC, IAC and LAC, respectively. This difference in range of gelatinization (R) suggests that the degrees of heterogeneity of crystallites within granules of the studied starches are different. Furthermore, high amylose starches with longer average chain were reported to exhibit higher transition temperatures (Jane et al., 1999). The enthalpy value of gelatinization (ΔH_{gel}) in LAC was the highest and the lowest in HAC. LAC starch had the highest heat of gelatinization due to being composed of most amylopectin, its starch granule's crystallinity is considered higher compared with those of HAC and IAC. Kreuger et al. (1987) reported that more thermal energy is needed to initiate melting in the absence of amylose-rich amorphous regions. After adding GTP to rice starches with different amylose content, the effect of varying concentration of GTP on the gelatinization of rice starches with different amylose content is shown in Table 1. The gelatinization temperatures (T_o, T_p and T_c) significantly decreased with the content of the GTP increasing GTP. When the 10% GTP was added to rice starches, the enthalpy value of gelatinization (ΔH_{gel}) began to decrease significantly. The increase in gelatinization temperature range (R) implied that the crystalline of rice starches became heterogeneous due to the adding of GTP. The effect of GTP on the gelatinization enthalpy was to disrupt the crystallites. It may be that the hydrophilic character of GTP OH groups might interact with side chains of amylopectin and bind to the amorphous region of starch granules to various degrees and thus change the coupling forces between the crystallites and the amorphous matrix (Wu et al., 2009). The result facilitates the easy hydration of starch granules, requiring less thermal energy for gelatinization (Zhu et al., 2009).

When a stored starch gel is reheated in a DSC, an endothermic transition occurs that is not present in the DSC scan of the freshly gelatinized sample. Such a transition is generally attributed to the melting of recrystallized amylopectin (Jaspreet et al., 2007). The retrogradation starches showed lower transition temperature and enthalpy in the second run heating DSC than native starches in the first run heating DSC, because starch crystallinity had been weakened (Sasaki et al., 2000). The retrogradation transition temperature was among 41 to 59℃, the retrogradation enthalpy and retrogradation ratio of gelatinized starch added GTP at various ratios are showed in Table 2. LAC had the highest ΔH_{ret}, and IAC had the lowest. The retrograded amylose was prepared for the crystal nuclei, which increased the nuclei crystal growth; so, the higher the amylose content, the faster the starch retrograded and the higher the ΔH_{ret} value. For waxy rice and very low amylose content rice, the amylopectin content is very high, so amylopectin recrystallization is very easy, and showed high retrogradation enthalpy (Shifeng et al., 2009). In native rice starch with different amylose content, ΔH_{ret} and DR% of gelatinized rice starch significantly increased with storage time increasing. However, when GTP was added to rice starch, ΔH_{ret} of all samples with GTP could not be detected after 5 days of storage. ΔH_{ret} of HAC, IAC and LAC with 5% GTP was detected after 10 days of storage and was 2.33, 1.08 and 3.19 J/g, respectively. Only after 20 days of storage, the HAC, IAC and LAC with 10 and 15% GTP began to show retrogradation endotherm on the DSC. Twenty days after

Table 2. The enthalpy and degree of retrogradation of mixtures of rice starch with different amylose contents and green tea polyphenols (GTP) at various ratios after 4°C storage.

Samples	5 days		10 days		20 days	
	ΔH_{ret} (J/g)	DR%	ΔH_{ret} (J/g)	DR%	ΔH_{ret} (J/g)	DR%
HAC+0% GTP	2.18 ± 0.18^{b}	22.5^{b}	4.98 ± 0.41^{b}	51.4^{b}	7.65 ± 0.56^{b}	79.0^{b}
HAC+5% GTP	ND	-	2.33 ± 0.22^{d}	24.1^{e}	4.43 ± 0.39^{e}	45.9^{e}
HAC+10% GTP	ND	-	ND	-	1.77 ± 0.24^{h}	30.2^{g}
HAC+15% GTP	ND	-	ND	-	0.38 ± 0.21^{i}	11.7^{i}
IAC+0% GTP	2.09 ± 0.15^{b}	20.0^{c}	3.43 ± 0.44^{c}	32.8^{c}	6.86 ± 0.31^{c}	65.6^{c}
IAC+5% GTP	ND	-	1.08 ± 0.32^{e}	10.5^{f}	3.21 ± 0.33^{f}	31.3^{g}
IAC+10% GTP	ND	-	ND	-	1.62 ± 0.14^{h}	25.5^{h}
IAC+15% GTP	ND	-	ND	-	0.31 ± 0.11^{i}	6.3^{j}
LAC+0% GTP	3.14 ± 0.34^{a}	26.5^{a}	6.43 ± 0.55^{a}	54.2^{a}	9.86 ± 0.62^{a}	83.1^{a}
LAC+5% GTP	ND	-	3.19 ± 0.38^{c}	27.7^{d}	5.92 ± 0.47^{d}	51.4^{d}
LAC+10% GTP	ND	-	ND	-	2.40 ± 0.40^{g}	34.0^{f}
LAC+15% GTP	ND	-	ND	-	0.64 ± 0.23^{i}	12.5^{i}

The values are average ± standard deviation, n = 3. Values followed by the same letter in the same column are not significantly different ($p < 0.05$). ND: not detectable.

the DSC test, it was also clearly shown that ΔH_{ret} and DR% significantly decreased with concentration of GTP increasing. The incorporation of green tea extract both increased the shelf life and improved the flavor of food, a successful example has been their application in mooncake, a traditional cake eaten during the Chinese Middle Autumn Festival. A more recent example is the addition of green tea extract to bread by Wang and Zhou (2004). In other words, during gelatinized wheat starch stored, the gel hardness increased due to the retrogradation, however, green tea extract (mainly phenolic compounds) considerable reduced the gel hardness to increase the shelf life of bread. In this study, the retrogradation trend of rice starch, with high amylose content or low, is significantly inhibited by the addition of GTP.

Recrystallization analysis

Information on the starch granule crystallinity was obtained using X-ray diffraction. The X-ray diffraction patterns and corresponding crystallinity of native rice starch and gelatinized rice starch gels containing different concentration of GTP are shown in Figure 1. Based on the X-ray diffraction pattern, the crystalline region of starches granules added to GTP was significantly affected. The scattering angle, at which the diffraction intensities observed was 2 θ. Native rice starches with different amylose contents (HAC, IAC and LAC) all showed typical A-type X-ray diffraction pattern with strong peaks at 2 θ close to 15.4, 17.3, 18.5, and 23.5, in comparison with native starch granules, gelatinized LAC with 0 and 5% GTP showed diffractogram characteristics of B-type starches. However, gelatinized HAC and IAC containing 0 and 5% GTP showed both B-type and V-type X-ray diffraction patterns. Gelatinized HAC and IAC containing 10 and 15% GTP showed only V-type X-ray diffraction patterns. However, gelatinized LAC containing 10 and 15% GTP did not showed any X-ray diffraction pattern. B-type crystallinity is characterized by a well-defined peak at 16.9 (2 θ). The B-ray diffraction pattern of all samples with 10 and 15% GTP disappeared in X-ray diffractogram after 10 days of storage. Retrograded starch gives a B-type X-ray diffraction pattern due to the partial loss of granule crystallinity during heating treatment (Kugimiya et al., 1980), which resulted in lower intensities of X-ray diffraction peaks of samples. Amylose-lipid complexes are shown to possess V-type X-ray diffraction patterns with peak at 2 θ close to 20 (Eerlingen et al., 1994). All the diffractograms of rice starches with high and intermediate amylose content show an obvious peak at approximately 20 (2 θ), however all the diffractograms of rice starch with low amylose content do not nearly show the characterized peak, which obviously be indicative of the amylose-lipid complex formation.

Figure 1 shows that intensities of X-ray diffraction peaks of all samples decreased with the concentration of GTP increasing. In general, intensities of X-ray diffraction peaks also indicate the extent of retrogradation of gelatinized starch (Kugimiya et al., 1980; Eerlingen et al., 1994). The investigation done using X-ray diffraction further proves the inhibiting effect of GTP on the retrogradation, and was found to be in agreement with that done using DSC.

Conclusions

Concerns and potential risks regarding the use of synthetic chemical in food production have renewed the

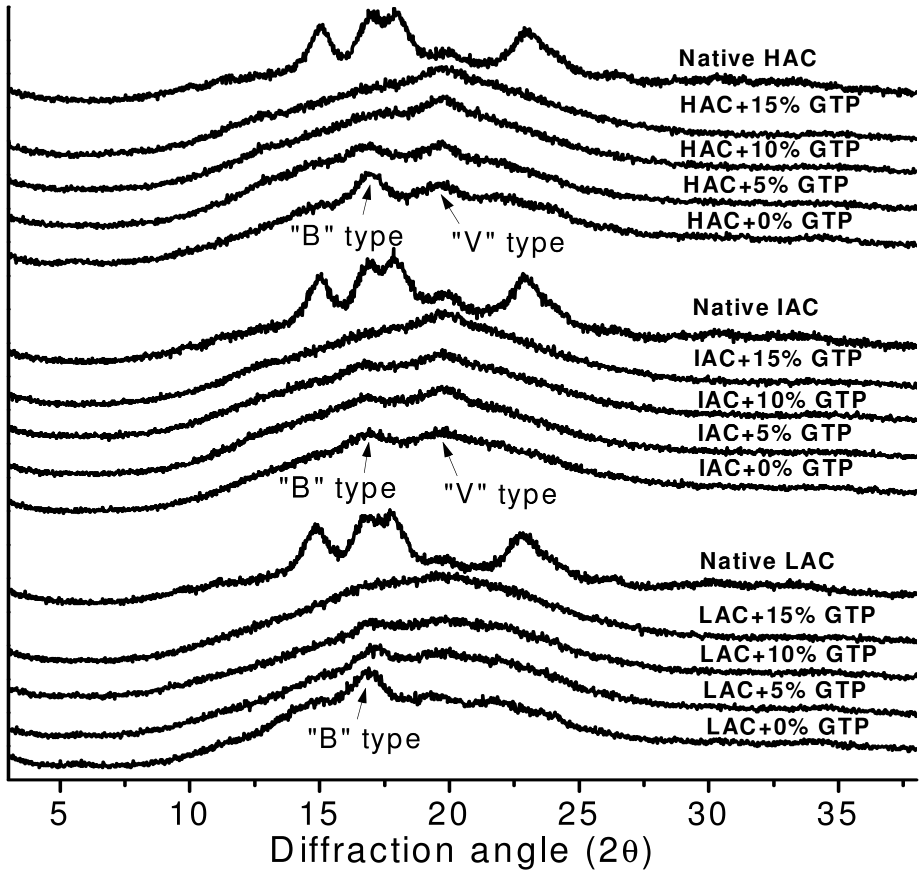

Figure 1. X-ray diffraction of native starch and gelatinized starch gels containing different concentrations of GTP after 10 days storage at 4°C.

interests of consumers using natural and safe alternatives. To address the need of natural and safety alternatives, green tea polyphenols (GTP) are being used in the food industry. The results observed in this work demonstrate that the temperature and enthalpy of starches gelatinization decreased with the concentration of GTP increasing, and GTP retarded retrogradation of all the tested rice starches. Considering GTP is ubiquity, abundance and low cost, it will be attractive to use it as an additive to add to rice products to simultaneously enhance quality and nutrition.

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