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Characterisation and preliminary lipid-lowering evaluation of starch from Chinese yam

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Abstract

The physicochemical properties and preliminary lipid-lowering activities of starch separated from the tuber of Chinese yam (*Rhizoma dioscoreae* cv. Anguo) were investigated and compared with potato starch, to determine the possibility of using it for human consumption. The two starches showed differences in physicochemical properties, such as swelling power, turbidity, gelatinisation and crystalline properties. Chinese yam starch significantly decreased the serum total cholesterol, triglyceride and LDL-cholesterol levels in hyperlipidaemic rats. The serum total cholesterol, triglyceride and LDL-cholesterol decreased by approximately 33.8%, 46.2% and 27.5%, respectively. The HDL-cholesterol level was not modified significantly, potato starch also reduced total cholesterol, triglyceride and LDL-cholesterol but the results were not significant. The results showed that starch from Chinese yam will lower blood lipid levels.

Keywords: Chinese yam; Starch; Physicochemical properties; Blood lipid

1. Introduction

There are more than 600 species of yam (*Dioscorea* spp.) in the world, 93 of which are found in China (Fu, Chen, Huang, & Li, 2005). Chinese yam (*Rhizoma dioscoreae, Dioscorea opposita)* has been used in traditional Chinese medicine for many years, to strengthen stomach function, alleviate anorexia, and cure diarrhoea (Lee et al., 2002). It has also been used as a delicious food in Chinese diets. Chinese yam is produced in Hebei, Shanxi and Shandong, with the best from Xinxiang county of Henan province. It is harvested in winter and processed in a procedure of washing, peeling, steaming with sulphur, drying, softening and slicing.

As reported in the literature, there are many chemical components contained in the Chinese yam such as mannan, allantoin, dopamine, batatasine, phytic acid, abscisinII, amino acids, glucoprotein, choline, ergosterol, campesterol, saponins, starch, non-starch polysaccharides and so on (Ma et al., 2005; Mishra & Gaikar, 2004; Yang, Lu, & Hwang, 2003). Furthermore, the tuber of Chinese yam contains, on average, 2.26% potassium, 0.2% phosphorus, 0.2% calcium, 0.14% magnesium, 5.51% ash, 53.6 mg/kg iron, 29.2 mg/kg zinc, 10.6 mg/kg copper and 5.38 mg/kg manganese (Zhou, Wu, Zhang, & Yan, 2004). Most of the chemical components have been extensively studied in the past decades. However, there are few investigations on the physicochemical and physiological properties of starch present in Chinese yam. Research on the properties of Chinese yam starch is very important due to its ready availability and its potentially extensive utilisation in the food and non-food industries.

Extensive research has been conducted on the structure and functional properties of the commercial starches obtained from seeds (corn, waxy corn, high amylose corn,

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wheat and rice) and from tubers and roots, particularly potato, sweet potato and cassava, due to their ready availability and comprehensive usage in food and pharmaceutical fields (Singh, Singh, Kaur, Sodhi, & Gill, 2003). In our previous reports, the physicochemical properties of starches from several *R. dioscorea* cultivars were studied (Pang et al., 2007; Wang, Gao, Liu, et al., 2006; Wang, Gao, Yu, et al., 2006; Wang, Liu, et al., 2006; Wang, Yu, et al., 2006; Wang, Yu, Yu, et al., 2007; Wang, Yu, Gao, et al., 2007).

Hyperlipidaemia (HL) is a major cause of mortality throughout the world (Ginsberg, 2002). From epidemiologic and experimental investigations, it is well known that diet plays an important role in the regulation of cholesterol homeostasis. Modern research shows that *R. dioscorea* has the ability to lower blood lipids. A water decoction of Chinese yam decreased the serum total cholesterol and triglyceride levels in hyperlipidaemic mice (Hang, 1994). Chen, Wang, Chang, and Wang (2003) reported the effects of Taiwanese yam powder on the lipid metabolism in adult mice. The results showed that a 25% or 50% yam diet decreased the serum total cholesterol, triglyceride and LDL-cholesterol levels in normal mice. It has also been reported (Chen et al., 2003) that viscous mucilage composed of soluble glycoprotein, dietary fibre, and plant saponins (diosgenin) might modulate lipid metabolism. Soluble polysaccharides, especially viscous fibre, have consistently been shown to reduce serum total cholesterol and LDL-cholesterol levels in hyperlipidaemic rats/mice (Jenkins, Vuksan, & Jenkins, 2001; Thewles, Parslow, & Coleman, 1993; Uchida et al., 1984). However, the effect of starch from Chinese yam on lipid metabolism in hyperlipidaemic rats remains to be investigated.

The objective of this study is to characterise the starch from Chinese yam and to evaluate its preliminary effect of lowering blood lipid.

2. Materials and methods

2.1. Materials

Dioscorea opposita cv. Anguo (D. AG) was provided by Henan Agricultural Academy of Science and was identified by Researcher Liu Hongyan, Henan Academy of Agricultural Sciences, Zhengzhou, Henan Province, China. Native potato starch (16% moisture) containing 20% amylose and 80% amylopectin by weight, was obtained from Xuanwei Runkai Starch Company (Xuanwei, Yunnan Province, China).

2.2. Starch isolation

The dried tubers of D. AG were washed, cut into small pieces and milled to pass through a 120 mesh sieve. The D. AG powders were immediately steeped in water containing 2%$NaHSO_3$ to remove impurities, such as protein. After precipitation, the supernatant was removed by suction and the settled starch layer was resuspended in distilled water. After precipitating and resuspending seven or eight times, the slurry containing starch was centrifuged in wide-mouthed cups at 3000 rpm for 10 min. The supernatant and upper non-white layer, which included cellulose, were removed. The white layer (starch layer) was resuspended in distilled water and recentrifuged 3–5 times. The starch was then collected and dried at room temperature.

2.3. Physicochemical properties of starch

Apparent amylose content of the isolated starch was determined in triplicate by using the method of Williams, Kuzina, and Hiynka (1970). Swelling power was determined in triplicate, according to the method of Leach, McCowen, and Schoch (1959). Turbidity was measured as described by Perera and Hoover (1999).

Scanning electron micrographs (SEM) were obtained with an environmental scanning electron microscope (XL-3; Philips Eindhoven, The Netherlands). Starch sample was suspended in acetone, to obtain a 1% suspension. One drop of the starch–acetone suspension was applied on an aluminum stub using double-sided adhesive tape and the starch was coated with gold powder to avoid charging under the electron beam after the acetone volatilised. An accelerating potential of 20 kV was used during micrography.

Particle size analysis of starch was done using a laser light scattering particle size analyser (Mastersizer S, Version 2.15, Malvern Instruments Ltd., Malvern, UK). The focal length was 100 mm. Thermal characteristics were tested according to the method of Wang et al. (2006). Onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH_{gel}) were calculated automatically. The gelatinisation temperature range (R) was computed as ($T_c - T_o$), as described by Vasanthan and Bhatty (1996). Enthalpies were calculated on a starch dry weight basis. The peak height index (PHI) was calculated by the ratio $\Delta H/(T_p - T_o)$, as described by Krueger, Knutson, Inglett, and Walker (1987). X-ray powder diffraction (XRD) measurements were done according to the method of Wang et al. (2006).

2.4. Animals and treatment

Kunming male Wistar rats (7-week-old rats, weighing 210 ± 20 g) were purchased from the Department of Laboratory Animal Science, Department of medicine, Beijing University. The rats were housed individually in cages under normal laboratory conditions, with free access to food and water. The animal facility was maintained on a 12 h light/dark cycle at a temperature of 23 ± 1 °C and relative humidity of 60 ± 5%. The blank control rats were fed a basal diet (BD) at 750 g/kg of body weight per day. HL was induced by a high-fat diet (HFD), fed at 750 g/kg of body weight per day for 4 weeks. The approximate compo-

sition of high-fat feed was as follows: 2% cholesterol, 10% lard, 5% vitellin, 0.2% methylthiouracil plus 82.8% normal feed. HL in the rats was confirmed by measurement of the plasma blood lipid level. Rats with HL were randomly divided into three groups: HFD feeding only, HFD with potato starch (HFD & PS) and HFD with D. AG starch (HFD & DS), both at 6.5 g/kg of body weight per day (HFD + PS and HFD + DS, respectively). The HFD + PS and HFD + DS diets were prepared by adding the two starches into the HFD and blending the mixture uniformly. The rats were fed diets by stomach perfusion every day until the 8th week.

2.5. Blood lipid level measurement

After 8 weeks of D. AG starch feeding, the blood was sampled from the orbital vein of the rats, after fasting for 12 h. Plasma samples were obtained by centrifuging the heparinised blood at 3000 g for 10 min. The total cholesterol (TC), triglyceride (TG), and high density lipoprotein cholesterol (HDL-C) concentrations in the serum were determined enzymatically, using commercially available reagents kits (Biosino Biotechnology Company Ltd, Chinese Academy of Sciences, Beijing, China). Low density lipoprotein (LDL) fraction was separated from the HDL fraction by precipitation with dextran and $MgCl_2$. The LDL cholesterol (LDL-C) level was calculated according to the Friedewald equation: LDL-C = TC – (TG/2.2 + HDL-C).

2.6. Statistics

All the data were expressed as means ± standard deviation of three replications, and Student's test was used for the statistical analysis. The values were considered to be significantly different when the p value was less than 0.05.

3. Results

3.1. Physicochemical properties of starch from D. AG

The amylose content observed for D. AG starch was the same as that of potato starch (Table 1). Swelling power of the two starches differed significantly. Potato starch had the higher swelling power of 56.8 g/g, while it was only 10.5 g/g for D. AG starch. D. AG starch showed the higher turbidity (1.15%), compared to potato starch (0.89%). The two starches have a similar mean diameter (□30.0 μm).

Table 1
Amylose content, swelling power, turbidity and mean diameter of two different starches

Samples	Amylose (%)	Swelling power (g/g)	Turbidity (%)	Mean diameter (μm)
D. AG starch	19.4	10.5b	1.15a	30.5
potato starch	20.0	56.8a	0.89b	30.0

Means with different letters are significantly different ($p < 0.05$).

3.2. Morphology of D. AG starch

Scanning electron micrographs of the two starch granules are illustrated in Fig. 1. The granule size of native D. AG starch is variable and ranges from 5 to 50 μm. Two distinct populations in the size distribution of starch granules (large granules with diameters ranging from 25 to 50 μm and small granules with diameters ranging from 5 to 15 μm) could be found. The granule surface appeared smooth, with no evidence of any fissures or pores, and was disk-like or oval in shape. D. AG starch showed the presence of a fairly large number of large-sized, disk-like granules. The SEM of potato starch showed the presence of starch granules from small to large and round to oval with diameter ranges between 5–20 μm and 20–50 μm, for small and large granules, respectively. The surface of the granules was also very smooth without any fissures or pores.

3.3. Gelatinisation of D. AG starch

Differential scanning calorimetry (DSC) parameters of the two starches are summarised in Table 2. The transition temperatures (T_o, T_p and T_c), range ($T_c - T_o$), enthalpies of gelatinisation (ΔH_{gel}) and peak height indices (PHI) of the two starches differed significantly. D. AG starch showed the higher T_o, T_p and T_c values of 70.2, 80.7 and 86.9 °C, compared to potato starch (58.0, 61.6 and 67.9 °C). D. AG starch had the higher ΔH_{gel} value of 12.5 J/g, while it was 11.8 J/g for potato starch. Potato starch shows the higher PHI value and lower R value.

3.4. Crystal properties of D. AG starch

The X-ray powder diffraction patterns of D. AG and potato starches are presented in Fig. 2. Potato starch showed a typical B-type X-ray diffraction pattern. It gives the strongest diffraction peaks at an angle 2θ of 20° and a few small peaks at around 2θ values of 17°, 23°, 26°, 28° and 30° (Fig. 2). A representative peak appears at about 6.3° 2θ which is characteristic of B-type starch. As for D. AG starch, the peak at around 2θ value of 6.3° is characteristic of B-type starch, while peaks at 19.7°, 20.8° and 26.6° 2θ are indicative of the A-type starch, showing that native D. AG starch is a typical C-type starch, i.e., a mixture of A-type and B-type starches.

3.5. Effect of D. AG starch on the blood lipid and body weight of the experimental hyperlipidaemic rats

Table 3 shows the serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride concentrations and the body weight changes in rats fed different feeds. Rats were in good health throughout the study. Body weight gains in the HFD, HFD + PS and HFD + DS groups were higher than

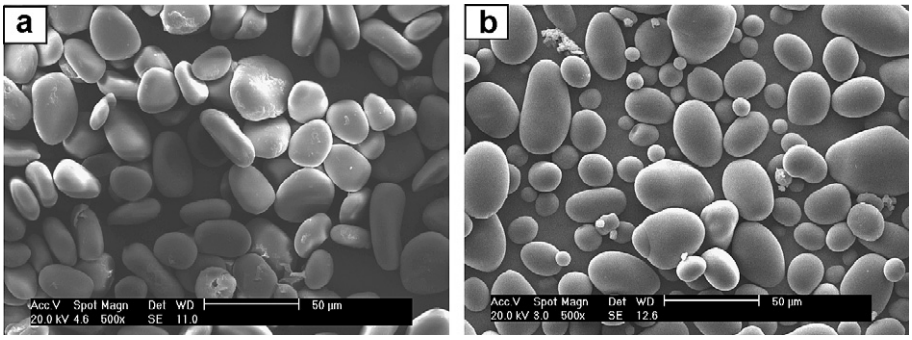

Fig. 1. Scanning electron micrographs of Chinese yam and potato starches a: Chinese yam starch; b: Potato starch.

Table 2
Gelatinisation properties of two different starches

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	PHI	R
D. AG starch	70.2a	80.7a	86.9a	12.5a	1.19b	16.7a
Potato starch	58.0b	61.6b	67.9b	11.8b	3.29a	9.1b

Means with different letters are significantly different ($p < 0.05$).

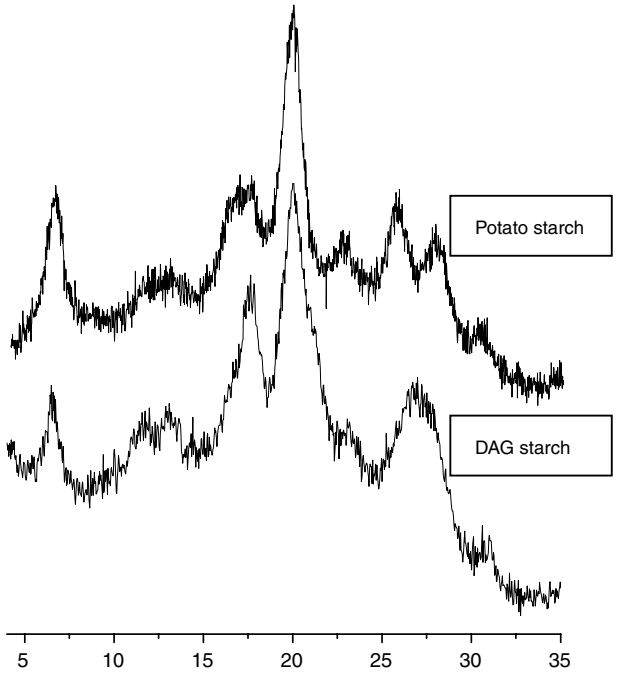

Fig. 2. X-ray diffraction pattern of Chinese yam (D. AG) starch.

those in the BD group, of which the HFD + DS group showed the lowest body weight gain. However, the increases were not statistically significant.

The serum total cholesterol and triglyceride levels in the HFD group were significantly higher than those in the BD group, which indicates that the experimental hyperlipidaemia model of rats is successful. The serum total cholesterol, triglyceride and LDL-cholesterol decreased by approximately 5.25%, 15.97% and 13.6%, respectively, for the HFD + PS group, as compared with the HFD group. However, the decreases were not statistically significant. The HFD + DS group was significantly lower in serum

Table 3
Plasma cholesterol, triglyceride and body weight of rats fed experimental diets for 8 weeks*

Groups	BD	HFD	HFD + PS	HFD + DS
Plasma (mM/L)				
Cholesterol				
TC	2.11 ± 0.24	14.48 ± 3.83[a]	13.72 ± 2.05	9.58 ± 3.58[b]
LDL-C	–	12.99 ± 3.56	11.22 ± 3.14	9.42 ± 3.94[b]
HDL-C	–	0.96 ± 0.24	0.90 ± 0.38	0.82 ± 0.31
Triglyceride	0.52 ± 0.19	1.19 ± 0.60[a]	1.00 ± 0.37	0.64 ± 0.24[b]
Body weight				
Initial (g)	199 ± 13	200 ± 22	198 ± 33	202 ± 24
Final (g)	281 ± 20	290 ± 17	284 ± 18	286 ± 15
Gain (g/8 weeks)	82 ± 3	90 ± 7	86 ± 5	84 ± 6

Data are expressed as means ± standard deviation. $n = 10$.
[a] Represents the comparison between BD and HFD groups in the same row, $P < 0.01$.
[b] Represents comparison between HFD and HFD+PS/HFD+DS groups in the same row, $P < 0.05$.

total cholesterol, triglyceride and LDL-cholesterol levels, compared with the HFD group. The serum total cholesterol, triglyceride and LDL-cholesterol decreased by approximately 33.83%, 46.21% and 27.48%, respectively, for the HFD + DS group. The HDL-cholesterol decreases by about 6.25% and 14.6% for HFD + PS and HFD + DS, respectively, but there was no significant difference between HFD and HFD + PS or HFD and HFD + DS.

4. Discussion

In recent years, the bioactive constituents from Chinese yam have been paid more and more attention by researchers. However, there are few investigations on the properties of starch present in Chinese yam. In the present study, the starch from D. AG was isolated and characterised by SEM, XRD and DSC, and the effect of the D. AG starch on the blood lipid of hyperlipidaemic rats was also evaluated. D. AG starch showed distinct properties, compared with those of potato starch and other cereal starches.

Resistant starch (RS) has been defined as the fraction of starch, which escapes digestion in the small intestine, and may be digested in the large intestine. RS is subdivided into four fractions: RS_1, RS_2, RS_3 and RS_4. They are also named as Type I, II, III and IV starches (Sajilata, Singhal,

& Kulkarni, 2006). D. AG starch and potato starch could be classified as RS_2, which is in a certain granular form and is resistant to enzyme digestion. In general, RS_2 shows typical B- or C-type X-ray diffraction patterns, as were seen for D. AG starch and potato starch. RS shows many physiological effects, such as lowering serum cholesterol and triglyceride level, increasing faecal bulk and lowering colonic pH, increasing short-chain fatty acids, such as acetate, propionate, and butyrate, and so on (Haralampu, 2000). D. AG starch obviously decreased serum total cholesterol, triglyceride and LDL-cholesterol levels in rats, as compared to the HFD group and the HFD + PS group. Most of the serum total cholesterol in animals fed cholesterol and/or high fat diets is associated with LDL-cholesterol (Fukushima & Nakano, 1995). Therefore, lowering the LDL-cholesterol may be an important factor in lowering the serum total cholesterol level in rats fed a high cholesterol diet. D. AG starch had obvious activity in decreasing blood lipid levels in experimental hyperlipidaemic rats. The mechanism could be ascribed to the resistance of D. AG starch. Further investigations are required to clarify the mechanism from enzyme level and gene expression. Potato starch also decreased serum total cholesterol, triglyceride and LDL-cholesterol concentration, but the decreases were not statistically significant. The differences in blood lipid lowering effect between D. AG and potato starches may be due to differences in RS content. The relevant experiments are currently being carried out to resolve these issues.

To our knowledge, Chinese yam can lower blood lipids. The active components for the beneficial lipid-lowering effects may be diosgenin, dietary fibre, mucilage, plant sterols, or a synergism of these active components. Our preliminary results showed that starch could also be responsible for the blood lipid lowering activity of Chinese yam. Therefore, uncooked Chinese yam should be better for modulating the levels of blood lipid than cooked yam.

In conclusion, the starch from Chinese yam showed distinct physicochemical properties and good blood lipid lowering effects. It might be developed as a functional food in the future.

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