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Pectin – accumulation during winter squash growth and differences among varieties

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ABSTRACT

Nineteen winter squash varieties were investigated to improve the understanding of pectic substance accumulation during fruit growth and to explore differences among cultivars. The results showed that there were significant differences in pectin content among varieties ($P < 0.05$). Some *Cucurbita maxima* varieties were better sources of pectin, in which total pectin (TP) reached 2.47% of fresh wt. Acid soluble pectin (ASP) was the main fraction of pectin in winter squash, and water soluble pectin (WSP) and oxalate soluble pectin (OSP) were minor components of TP. WSP and OSP increased during fruit growth while ASP and TP first increased and then decreased during fruit growth, peaking at different times for early and mid to late maturity varieties. TP differed between species and maturity classes; TP in *C. maxima* was about 1.5 times that in *C. moschata* ($P < 0.05$). Likewise, TP was 2.3 times higher in early maturity varieties than that in mid to late maturity varieties ($P < 0.05$).

1. Introduction

Winter squash (*Cucurbita spp.*) is commonly consumed in China and other countries. Annually on a worldwide basis there are 1.5 million ha grown and in 2007 yield exceeded 20 million MT, and China accounts for about 22% of the production area and 31% of worldwide production (Liu et al., 2008). Extensive research has been aimed at improving disease-resistance, yield and nutrition of winter squash by breeding in China (Luo, 2000; Sun et al., 2004; Liu et al., 2008) and also on how to process winter squash after harvest (Teotia, 1992; Wang et al., 2012).

It is well known that pectin is widely used in the food industry as a gelling, thickening and stabilizing agent (King, 1993). The consumption of pectin has also been associated with improved human health by reducing serum cholesterol or by reducing the incidence of diabetes (Mermelstein, 1979; Sheldon, 1987; Jia, 1998; Tang, 2001). Although pectins are traditionally obtained from citrus and apples, other sources exist and it is important to analyze their potential (de Escalada Pla et al., 2007).

The potential utilization of pectin from winter squash has also received some attention and findings have shown that squash pectins are highly esterified, ranging from 74% to 87% in the three varieties analyzed (Tinay, 1982). The jelly grade of pectin from winter squash reached 120 (Zhang, 1990), and pectin products isolated from 'Butternut' squash demonstrated an interesting range of thickening properties (Fissore et al., 2009a). All of the above observations indicate that pectin from winter squash may have value in the food industry.

Pectins are structural polysaccharides existing in cell walls of higher plants, generally present in three states including acid-, water- and oxalate- soluble pectin (ASP, WSP and OSP, respectively). ASP can be converted to WSP by pectic enzymes, while WSP can degrade further to OSP (i.e., pectic acid) by loss of methyl esters (Ranganna, 1977). The extraction of pectins from winter squash has been examined (Alexander et al., 1995; Qiang, 2002; Liang, 2003; O'Donoghue and Somerfield, 2008; Ptichkina et al., 2008; Fissore et al., 2007, 2009b, 2010), but, there is little information about how pectins may change during fruit development and may differ among varieties and maturity classes of winter squash especially for the species *C. maxima* and *C. moschata*. A better understanding of the effect of species and maturity on pectin content in winter squash may reduce this information gap leading to greater utilization of winter squash as a source of pectin in the food industry and could also lead to breeding new winter squash varieties with higher pectin contents.

Consequently, the objectives of this research were: (1) to determine content of pectic fractions including ASP, WSP and OSP and total pectin (TP) during the growth of four winter squash varieties representing two species (*C. maxima* and *C. moschata*) and two maturity classes, early and mid to late maturity, and (2) to examine differences of TP and pectic fractions among nineteen local and market varieties of winter squash in China and understand the

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Table 1
Species, origin and maturity of nineteen winter squash varieties used for pectin evaluation.

Variety	Species	Maturity[a]	Origin
'Jixiang' No. 1	*C. maxima*	Early	Beijing, China
'Wuyuezao'	*C. maxima*	Early	Hubei, China
'Chestnut'	*C. maxima*	Early	Japan
'Green Chestnut'	*C. maxima*	Early	Japan
'Red Chestnut'	*C. maxima*	Early	Japan
'Daji'	*C. maxima*	Early	Taiwan, China
'Kangle'	*C. maxima*	Early	Taiwan, China
'Xiangrui'	*C. maxima*	Early	Taiwan, China
'Jarrahdale Large'	*C. maxima*	Mid to late	Australia
'Queensland Blue'	*C. maxima*	Mid to late	Australia
'Ainan' No. 1	*C. moschata*	Early	Shanxi, China
'Ainan' No. 4	*C. moschata*	Early	Shanxi, China
'Zhangping'	*C. moschata*	Mid to late	Fujian, China
'Miyun' No. 1	*C. moschata*	Mid to late	Beijing, China
'Miyun' No. 2	*C. moschata*	Mid to late	Beijing, China
'Feixian'	*C. moschata*	Mid to late	Shandong, China
'Luotuobo'	*C. moschata*	Mid to late	Hebei, China
'Hunan'	*C. moschata*	Mid to late	Hunan, China
'Shiqian'	*C. moschata*	Mid to late	Guizhou, China

[a] Early means the winter squash variety was ready to harvest in about 90–100 days and the first female flower was located on node 8 to 12 of the main stem; Mid to late maturity means the variety was ready to harvest in 120–150 days and the first female flower was located above node 16 of the main stem.

effect of species and maturity classes on pectin. The findings will be useful for winter squash breeders, farmers, food manufactures and consumers.

2. Materials and methods

2.1. Field trials, sampling and preparation

Field trials were carried out at the Nankou Experimental Station, a substation of the Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences, located some fifty kilometers northwest of Beijing. The experiment was laid out as a randomized complete block with three blocks. Nineteen winter squash varieties from China, Japan and Australia were seeded at the beginning of April and then transplanted at the end of April (Table 1). When plants flowered, they were hand pollinated in the morning and tagged with the date of pollination.

Four varieties, representing both winter squash species and both maturity classes were chosen as subjects for determining pectin differences during development. Varieties selected for sampling included the *C. maxima* varieties 'Chestnut' (early) and 'Jarrahdale Large' (mid to late) and the *C. moschata* varieties, 'Ainan' No. 1 (early) and 'Miyun' No. 1 (mid to late). Selection of these varieties was based on experience and known characteristics. For early maturity varieties belonging to *C. maxima*, most were bred with Japan parents, so 'Chestnut' was chosen as a representative variety. For early varieties belonging to *C. moschata*, 'Ainan' No.1 and No.4, bred by Shanxi Agricultural Academy, had similar shape and size but different colors. The *C. maxima* 'Jarrahdale Large' is a well known variety from Australia and 'Miyun' No.1 from Beijing had the largest yields, over 45 t/hectare, among all the varieties. Five fruits, one from each of five plants in a block of each variety were harvested every ten days from pollination to maturity 50 days after pollination, except for 'Ainan' No. 1 which ripened on the 40th day.

To compare pectin composition among the other winter squash varieties, five fruits from each block of each variety were also sampled 50 days after pollination. To reduce variability, all the samples for this experiment were obtained from fruit arising from pollinations conducted within a three-day period.

To prepare samples for analysis, each winter squash was separated into edible flesh and other parts by removing peel, core and seeds. Twenty gram of edible flesh was obtained from each of the five winter squashes. After slicing, samples from each winter squash were mixed to provide a 100 g sample, and frozen under liquid nitrogen. Frozen samples were then packed in aluminum foil containers, sealed and stored at −35 °C until used for analysis. When analyzed, the 100 g sample was homogenized with 100 ml distilled water using a homogenizer (Philips HR2864, China). Ten gram samples of the homogenate were taken for pectin analysis.

2.2. Pectin analysis

Interfering substances such as free sugars were removed from the homogenate by placing the 10 g sample in a 100 mL beaker, and adding 50 ml of 95% ethyl alcohol (AR, Beijing Chemical Co.). This solution was stirred for about 30 min, and then filtered with filter paper. The washing operation was repeated until sugar was not detected by α-naphthol–sulfuric acid.

Pectin fractions including WSP, OSP and ASP were prepared from the washed residue using the methods of Ranganna (1977). Briefly, this method involves sequential extraction with water (WSP), and then ammonium oxalate (OSP) at room temperature, followed by extraction with boiling hydrochloric acid (ASP). To quantitate each fraction, 10 mL was diluted to 100-mL in a volumetric flask with distilled water. One mL of each extract solutions was pipetted into a test tube. Subsequently, 0.5 mL of 0.15% carbazole reagent (AR, Beijing Chemical Co.) was added, then 6.0 mL of conc. H_2SO_4 (AR, Beijing Chemical Co.) were added while providing constant agitation. The tube was then closed with a rubber stopper and allowed stand for approximately 10 min in a tap water bath (25 °C) for the color to develop. Exactly 15 min after adding acid, the transmittance of the sample was measured at 525 nm by spectrophotometer (TU-1800, Beijing Purkinje General Instrument Co., Ltd.) after setting the instrument to 100% transmittance with a reagent blank. Concentration of pectic substances was calculated based on a standard curve using anhydrous galacturonic acid (Sigma Co.). Total pectin (TP) % was calculated as WSP % + OSP % + ASP %.

2.3. Data analysis

Statistical analyses were conducted using SAS 8.1 (SAS Institute Inc., Cary, NC, USA). Triplicates were done for each variety, the data were analyzed using analysis of variance (ANOVA), and the differences between the means were tested using Duncan's multiple range test ($P < 0.05$). Standard errors were calculated using Excel (Microsoft Office, 2003)

3. Results

3.1. Pectins in winter squash during fruit development

Concentrations of pectic substances in four winter squash varieties sampled during fruit development are presented in Fig. 1(a)–(d). Regardless of species or maturity class, WSP tended to increase during development (Fig. 1a), but there were differences among varieties. For example, WSP content of 'Chestnut' (early maturity, *C. maxima*) increased from 27 mg/100 g to 71 mg/100 g, while 'Jarrahdale large' (mid to late maturity, *C. maxima*) increased only slightly 7 mg/100 g from 23 to 30 mg/100 g. For OSP (Fig. 1b), concentrations tended to increase during development for three of the varieties, 'Jarrahdale Large', 'Chestnut' and 'Ainan' No. 1, especially when comparing values for fruits obtained 10 d after pollination to those for ripe fruits; for 'Miyun' No. 1, there was little difference in OSP between very young and mature fruit (Fig. 1b). ASP contents (Fig. 1c) for two early maturity varieties, 'Chestnut' and 'Ainan' No. 1, increased until 30 days after pollination, and then