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Changes in dietary fibre, polygalacturonase, cellulase of navel orange (*Citrus sinensis* (L.) Osbeck 'Cara Cara') fruits under different storage conditions

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ABSTRACT

The consumption of dietary fibre plays an important role in the prevention of diseases, such as constipation, haemorrhoids. Recently, chemical and physical properties of citrus fibres have been widely studied. In this paper, the polygalacturonase (PG) and cellulase (Cx) gene expression of Cara Cara (*Citrus sinensis* (L.) Osbeck) navel orange fruit stored on tree (ST) was compared with fruit stored in room (SR). The results showed that the mRNA expression levels of PG increased significantly in the fruits of ST, in contrast, the expression levels of Cx increased slightly only in peel of ST. Total pectin (TP) and protopectin of ST fruits pulp were higher than those of SR at every time point. The contents of insoluble dietary fibre (IDF), hemicellulose (HC), cellulose (CEL) and lignin of ST fruits were less than that in SR. However, in fruits from ST, a significant increase of soluble dietary fibre (SDF) and water soluble pectin (WSP) occurred, compared with fruits of SR. Our studies indicated that fruit stored on tree is quite useful for regulating the gene expression and controlling contents of dietary fibre on Cara Cara navel orange.

1. Introduction

Dietary fibre is often classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) (Hadfield and Bennett, 1998). It consists of a variety of non-starch polysaccharides which include cellulose (CEL), hemicellulose (HC), pectin, β-glucans, gums and lignin (Lamghari et al., 2000). Dietary fibre is composed mainly of remnants of edible plant cells; parenchymatous tissues are known to be the most important source of vegetable fibre (DeVries and Faubion, 1999). Cell walls of fruits, vegetables and cereals make up most of the dietary fibre intake (Jiménez et al., 2000). Recently, increasing attention has been given to their beneficial physiological effects of dietary fibre on humans and animals (Anderson et al., 1994). High dietary fibre diets are associated with the prevention, reduction and treatment of some diseases, such as diverticular and coronary heart diseases (Hadfield and Bennett, 1998). It is widely known that dietary fibre obtained by different methods and from different sources, behave differently during their transit through the gastrointestinal tract, depending on their chemical composition and physicochemical characteristics and on the processing that food undergo (Chau and Huang, 2003).

Storing fruit on tree (ST) is one of the most effective ways of extending the harvesting periods, as it is cost saving and can avoid food safety problem since no chemical is applied. Storing fruit on tree not only sustains the quality of navel orange, but also enhances the accumulation of nutrients, improves its flavor appearance. Storing fruits in room (SR) needs space and chemical treatment, which is time-consuming and labor-intensive, leading to loss of fruit lustre and machinery harm (Drake et al., 2006). Therefore, the technology of ST has been used extensively in some countries like USA and Brazil.

In general, fruits are harvested once they are physiologically mature when postharvest ripening begins, and fruits acquire the organoleptic characteristics to be consumed. Fruit texture not only influences consumer choice, but also affects several commercial traits such as shelf-life, transport capability and disease resistance. Softening, an extremely important postharvest event is the result of texture modifications that take place during postharvest ripening (Abu-goukh and Bashir, 2003). During softening, physical injury always occurs and fruits are more susceptible to diseases. It is known that fruit softening is associated with cell wall disassembly (Srisuma et al., 1991; Seymour and Gross, 1996; Majumder and Mazumdar, 2002) and modifications to pectin (Marin-rodriguez et al., 2002), in which polygalacturonase (PG) and cellulase (Cx) are involved (White, 2002). Increases in PG activity and mRNA levels have been observed during ripening in several fruits (Fischer and Bennett, 1991; Hadfield and Bennett, 1998). It has been suggested that other hydrolase enzymes such as

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Cx may also be involved in the metabolism of HC, CEL that accompanies fruit softening. Xyloglucans, the predominant HCs in dicotyledonous plants, are also thought to play an important role in cell wall architecture. In many fruits, including melon (Rose et al., 1998) and avocado (Sakurai and Nevins, 1997), HC undergoes substantial depolymerization. Enzymes such as xyloglucan endo-transglycosylases and Cx have been proposed as allies that cooperate in the modification of the HC network during fruit ripening (Rose and Bennett, 1999). The fruit of transgenic tomato plants with altered PG or Cx gene expression levels showed essentially normal softening, suggesting that altering the expression of a single cell wall-related gene is insufficient to affect the softening process (Giovannoni et al., 1989; Brummell et al., 1999). Thus, it is important to investigate a range of enzymes in order to clarify the mechanisms of tissue softening or dietary fibre changes in fruit.

Despite the importance of citrus fruit as a source of dietary fibre, little is known about its biosynthetic regulation during fruit maturation and storage. A mutant of the "Washington navel" orange from Venezuela, named Cara Cara (*Citrus sinensis* (L.) Osbeck) which produces fruits of a distinctive red color, is the only popular red-fleshed navel orange in China. However, the effect of storage on the dietary fibre of the Cara Cara navel orange has not been reported.

The aim of this research is to study the effect of storage methods on dietary fibre, PG and Cx enzymes of orange grown on sandy-loam soil, to identify the relationship between gene expression of PG, Cx with dietary fibre changes.

2. Materials and methods

2.1. Fruit sample preparation

Healthy and uniform fruits of Cara Cara navel orange were harvested from trees grafted on trifoliate orange (*Poncirus trifoliata* (L.) Raf) at Citrus Experimental Orchard in Zigui country, Hubei Province (China). 30 orange trees grown under identical condition, were randomly divided into three groups as three replications. And in every group, five trees for ST and others for SR were established in a randomized block design.

One hundred and fifty fruits of SR were harvested on 20 December 2005, and stored at 6 °C at 75–80% relative humidity. One hundred and fifty fruits of ST were harvested on 5 January (15 days after maturity), 20 January (30 days after maturity), 4 February (45 days after maturity), 19 February (60 days after maturity), 6 March (75 days after maturity) and 21 March (90 days after maturity) 2006. The fruits were washed, peeled, cored and diced. Some pulp and peel were frozen in liquid nitrogen, ground to fine powder and stored at −70 °C until analysis, the others were pressed and the mash was dried to a constant weight. Obtained dried orange pulp and peel were crushed and pulverized, and used as a raw material for further isolation of dietary fibre. The samples were assayed and analyzed at 15-day intervals during all the storage. In this study, flesh firmness, TDF (total dietary fibre), IDF, SDF, TP (total pectin), WSP (water soluble pectin), protopectin, HC, CEL, lignin, activities of PG, Cx enzymes and gene expression in the pulp and peel of the Cara Cara fruit from ST and SR at different storage periods were determined.

2.2. Measurement of flesh firmness

Flesh firmness was measured on three locations around the equatorial plane of peeled fruit using GY-1 (Stable Micro Systems Ltd. of Mudanjiang, China) rheometer with a 10 mm plunger.

2.3. Measurement of TDF, IDF, SDF, WSP, protopectin, TP, CEL, HC and lignin

TDF, IDF and SDF were extracted according to the Association of Official Analytical Chemists (AOAC) method (Thomas et al., 2000). Duplicates (2× 1 g) in a phosphate buffer (0.08 mol/L, pH 6) were analyzed for soluble (SDF) and insoluble (IDF) dietary fibre. Samples were treated with a thermo-stable α-amylase (Termamyl 120 L, Novo Nordisk A/S, Denmark) and then digested with a protease (P 5380, Sigma–Aldrich, France) and an amyloglucosidase (E.C. 3213, A-3042, Sigma) to remove protein and starch, respectively. SDF and IDF were separated by filtration (G4 funnel). The retentate (IDF) was first dried by solvent exchange, then under vacuum overnight at 40 °C. The filtrate, containing SDF, was recovered after alcoholic precipitation with 4 volumes of 96% ethanol, then dried by solvent exchange and under vacuum overnight at 40 °C. A control was performed following the same procedure. An estimated content of TDF was then obtained as the combined values of SDF and IDF after corrections for proteins and control were made.

WSP and protopectin determined by colorimetry of Carbazole-Vitriol (Marin-rodriguez et al., 2002). TP is the sum of WSP and protopectin.

A 0.5 g sample was extracted with 40 mm neutral wash buffer (3% dodecyl sodium sulfate) in a 50-ml reaction tube. The test tube was immediately transferred to a water bath maintained at 100 °C for 1 h, and centrifuged at 3500 rpm at 4 °C for 20 min, washed the residue to pH 6.5–7.0. Contents of CEL, HC and lignin were determined by the quantitative analysis method of Wang and Xu (1987).

2.4. Analysis of activities of PG and Cx

The procedure of enzymes extraction at 4 °C was adapted from Lohani et al. (2004). PG activity was determined by measuring the reducing groups released from polygalacturonic acid (Orange, Fluka Chem. Co.). Reducing groups were measured according to the technique described by Pathak and Sanwal (1998) and Lohani et al. (2004) with slight modification. The reaction mixture contained 0.5 ml sodium acetate (0.2 M, pH 4.0), 0.4 ml of 1% (w/v) solution of citrus pectin (Sigma), containing 0.6% (w/v) sodium chloride (pH 4.5) and 0.1 ml crude enzyme in a total volume of 1.0 ml. The mixture was incubated at 37 °C for 30 min followed by addition of DNS (3,5-dinitrosalicylate). The reaction was stopped by heating in a boiling water bath for 5 min. A blank was prepared for each sample by boiling the reaction mixture before addition of substrate. The concentration of the reducing groups was determined with D-galacturonic acid as a standard after measuring the absorbance at 540 nm with SHIMADZU UV-2450 spectrophotometer (Tokyo, Japan). One unit (U) of enzyme is the amount which catalyses the formation of 1 μg of reducing groups per minute per gram of original fresh weight of fruits.

Cellulase activity was determined by measuring the reducing groups released from carboxymethyl cellulose (Lohani et al., 2004). The concentration of the reducing groups was determined with D-glucose as a standard, as in the PG assay. The reaction mixture contained 0.1 ml of crude enzyme, 0.4 ml of 1% (w/v) carboxymethyl cellulose and 0.5 ml 0.1 M sodium acetate buffer (pH 5.0). Incubation was carried out at 50 °C for 30 min followed by addition of DNS. In control tubes the substrate was added after the heat treatment. After heating in a boiling water bath for 5 min, the absorbance was measured at 540 nm. Cellulase activity was determined as units, one unit being defined as the amount of the enzyme that catalysed the formation of 1 μg reducing groups per minute per gram of original fresh weight of fruits.

2.5. Semi-quantitative reverse transcriptase polymerase chain reaction (semi-quantitative RT-PCR)

Total RNA was isolated from fruit pulp tissue with the RNArose Reagent Kit (Biological Industries Co., Shanghai, China).

After RQ1 DNase (Promega) treatment, the RNA was used to synthesize first strand cDNA by RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). cDNA was used as a template for amplifying a partial sequence of the Cara Cara PG, Cx gene. The primers of PG and Cx gene were PG-F1 (5′-CACCATGCGAAGGCTTATTT-3′) and PG-R1 (5′-AGCAAGGAGGCGGTTCTACT-3′); Cx-F1 (5′-TTTTCAGGGCAACGAATGAC-3′) and Cx-R1 (5′-TAATAGAACGTGGGCAC-CAG-3′), generating two segments of 123 bp of PG and 116 bp of Cx. β-Actin was used as internal control to verify the RT-PCR reaction. The primers of internal control gene were ACT-F1 (5′-ATCTGCTGGAAGGTGCTGAG-3′) and ACT-R1 (5′-CCAAGCAGCATGAAGATCAA-3′), producing a fragment of 100 bp. The designs of the degenerate PCR primers by Primer 5, genes were based on conserved amino acid sequences in the genebank (EF185420, AF000136). The PCR reaction mixture (20 μl) contained 2 μl 10× PCR buffer, 2 μl dNTPs (2 mM), 1.2 μl $MgCl_2$ (25 mM), 1 μl (10 mM) of each primer and 0.2 μl *Taq* (5 U, Fermentas). The cycling conditions were 94 °C, 2 min for initial denaturation; 94 °C, 30 s (denaturation), 60 °C, 30 s (annealing), 72 °C, 1 min (extension) for 40 cycles; and a final extension at 72 °C for 10 min. The RT-PCR products were separated on 1.5% (w/v) agarose gels, stained with ethidium bromide. The expression level of each gene was calculated after normalization to the level of the actin gene PCR product from the same sample.

2.6. Real time PCR

Relative quantification PCR studied with Applied Biosystems 7500 RealTime PCR System and Power SYBR® Green PCR Master Mix (ABI). The primers of target gene and internal control gene were accord with Semi-quantitative RT-PCR. The cycling conditions were: 50 °C, 2 min; 95 °C, 10 min; 95 °C, 15 s, 60 °C, 1 min for 40 cycles.

2.7. Statistical analysis

All data presented were means of three replicates along with standard errors of means. Data were further subjected to analysis of variance, and means were compared using least significance difference (LSD) test (SAS Institute, Cary, USA). The correlation was established by the CORR of SAS 8.1 statistical data analytical software. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Ratio of dry weight with fresh weight and flesh firmness

The ratios of dry weight with fresh weight (DW/FW) of Cara Cara fruits pulp were similar at different storage stages (Fig. 1). However, they were inconsistent in the peels, which increased to the maximal value until 30 days after maturity and then decreased for ST, higher than those of fruits from SR. Fruits stored in different environments had nearly identical softening rate. The firmness of flesh decreased significantly in all treatments over time. However, fruits from SR retained greater firmness than those from ST, which decreased quickly after storage.

3.2. Contents of TDF, IDF and SDF

TDF contents in pulp of ST fruits were slightly higher than those of SR during the first 30 days (Fig. 2A). In pulp of fruits by ST

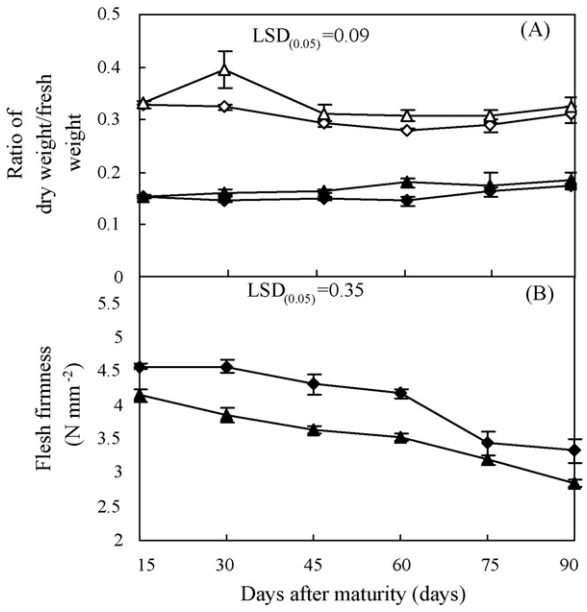

Fig. 1. Changes of ratio of dry weight with fresh weight and flesh firmness in Cara Cara fruits during different storage periods. (A) Ratio of dry weight/fresh weight; (B) flesh firmness (N mm^{-2}). The data are displayed with mean ± S.D. (bars) of three replications. (◆) Pulp of SR fruit; (◇) peel of SR fruit; (▲) pulp of ST fruit; (△) peel of ST fruit.

storage, it gradually decreased slightly, while in peel, it first decreased until 30 days after maturity and then increased gradually and subsequently maintained constant. Nevertheless, TDF contents of SR showed minor fluctuation during the course. SDF contents of fruits from ST were significantly higher than those from SR (Fig. 2B). In pulp of fruits in ST storage, they increased gradually up to 60 days after maturity, followed by slight decrease, while in SR they decreased continuously during the whole periods. Contents of IDF in peel increased progressively until 75 days after maturity before a gradual decline. But in the pulp of ST fruits, they decreased during the storage, while for SR a different trend exhibited (Fig. 2C).

3.3. Contents of WSP, protopectin and TP

TP and protopectin of ST fruits pulp were higher than those of SR at every time point (Fig. 2D and E). In pulp, there was a slight increase in TP contents in ST fruits, which stopped increasing after 45 or 60 days and then decreased gradually; at the same time protopectin maintained a constant level and decreased slightly after 75 days during the storage periods. In peel, there were significant differences in TP contents between fruits from ST and SR. In addition, protopectin contents of ST were higher compared with that of SR (Fig. 2D and E). The WSP contents increased sharply in the fruits pulp during the early storage stage and subsequently dropped dramatically in different storage types. In the early storage stage, the WSP contents of fruits from ST were higher than that from SR. In later storage periods, there were no significant differences in WSP contents of pulp between fruits from ST and SR. But there were inconsistent patterns of WSP changes during storage of Cara Cara fruits peel of different storage types, it increased dramatically in the middle of storage, while that did not appear in fruits from SR (Fig. 2F).

3.4. Contents of CEL, HC and lignin

There were no significant differences in CEL contents in the pulp between fruits from ST and SR. That content maintained a constant

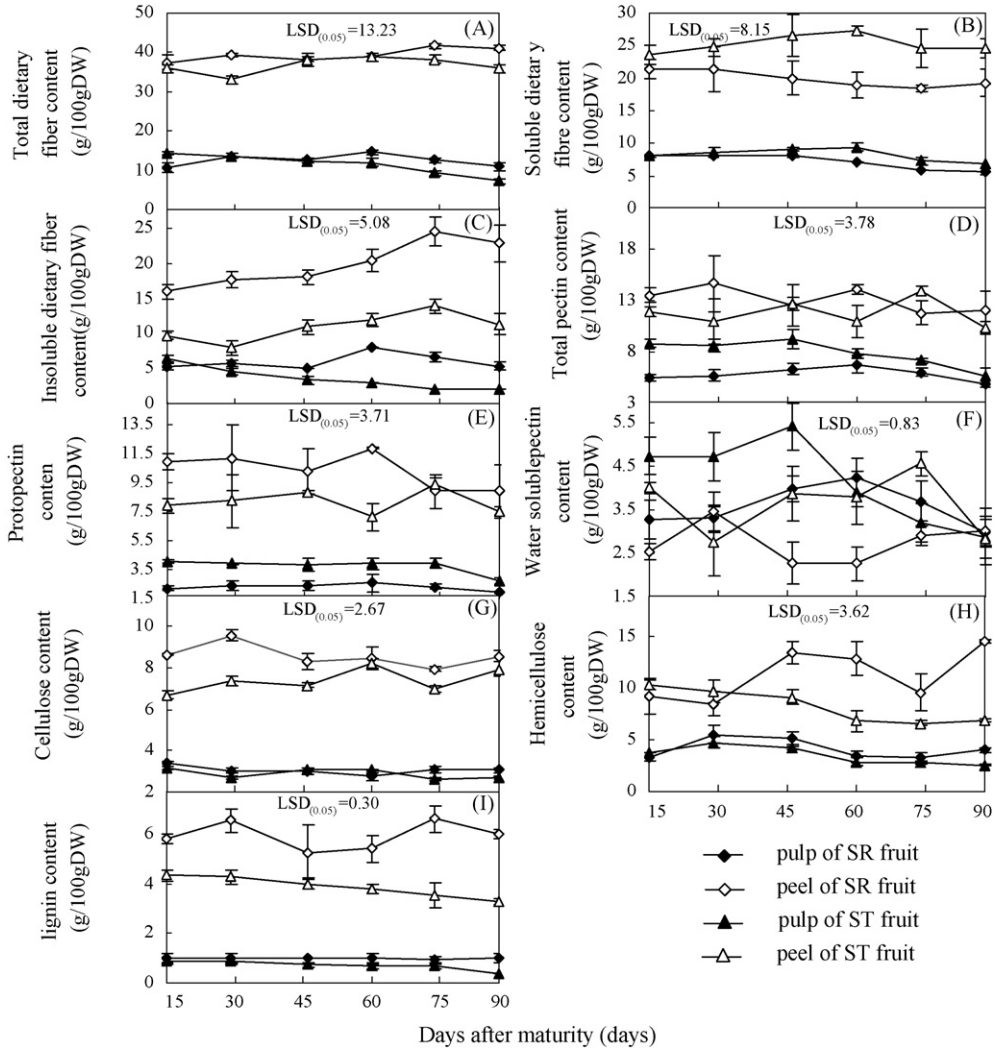

Fig. 2. Changes of dietary fibre contents in Cara Cara fruits during different storage periods. The data are displayed with mean ± S.D. (bars) of three replications. (A) TDF (total dietary fibre); (B) SDF (soluble dietary fibre); (C) IDF (insoluble dietary fibre); (D) TP (total pectin); (E) WSP (water soluble pectin); (F) protopretin; (G) CEL (cellulose); (H) HC (hemicellulose); (I) lignin.

level over time (Fig. 2G). In peel, CEL contents of fruits from SR were higher than that from ST which agreed with the trends of lignin contents. The lignin contents of ST fruits decreased gradually during all storage periods, but there were no significant differences in peel of fruits from SR between early storage periods and later one (Fig. 2I). The HC contents in pulp of fruits from ST were lower than that from SR. In peel of ST fruits, it decreased gradually while that of SR slightly increased in general (Fig. 2H).

3.5. Activities of PG and Cx

There were significant differences in PG activity between fruits from ST and SR. PG activity of SR fruits decreased gradually in all storage periods. Whereas, there was a same trend between PG activity of ST pulp and peel which reached the maximum until 45 days after maturity, and then declined gradually. PG activity of pulp was higher than that of peel in different storage methods. In general, Cx activity increased in two storage methods (Fig. 3). Additionally, Cx activity in pulp from ST fruits increased dramatically than that from SR. Cx activity in peel of ST fruits maintained at a basal level even after 30 days of maturity and increased gradually after 60 days, while the levels increased in first 30 days and undulated after that in fruits from SR. However, there appeared to have negative relationship between levels of PG activity and content of protopectin ($r = -0.78535$, fruits pulp of SR; $r = -0.53782$, fruits peel of SR; $r = -0.07529$, fruits pulp of ST; $r = -0.04387$, fruits peel of ST) and between Cx activity and content of lignin of ST fruits ($r = -0.62004$, in pulp; $r = -0.31841$, in peel).

3.6. Effects on gene expression of PG, Cx in fruit of Cara Cara

PG and Cx gene expression were analyzed in fruit of Cara Cara navel orange during the storage periods. The storage approaches significantly affected the PG gene expression, but these treatments have slight effects on the expression of Cx (Figs. 4 and 5). The result of semi-quantitative RT-PCR and real time PCR showed that PG mRNA levels in ST fruits were higher than that of SR fruits. And the expression of PG gene in SR fruits pulp remained very low levels during all storage periods. The transcript levels of PG increased in the ST fruit peel and reached a maximum after 45 days of maturity. The expression pattern of Cx in ST fruit peel was similar to PG, which increased and reached a maximum on day 60. However, PG and Cx in the SR peel there appeared to have another expression pattern, which did not have the stage of up regulation. The Cx mRNA levels in the stored fruit remained very low levels compared with PG during storage period. There were no significant

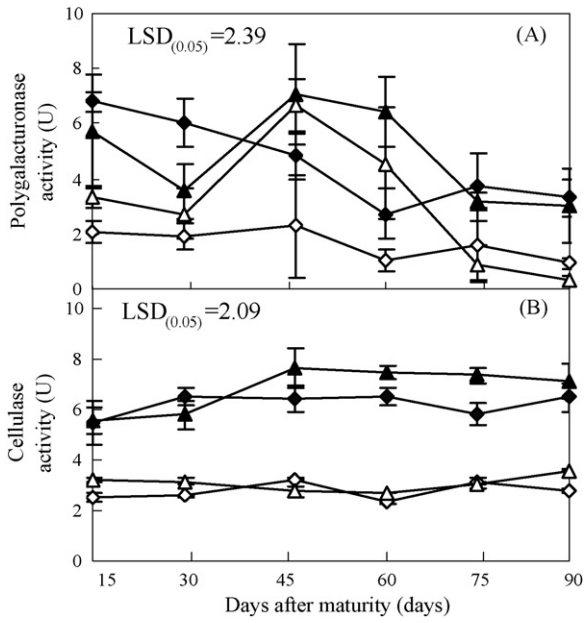

Fig. 3. Changes of polygalacturonase and cellulose activities in Cara Cara fruits during different storage periods. The data are displayed with mean ± S.D. (bars) of three replications. (A) polygalacturonase (PG); (B) cellulase (Cx). (◆) pulp of SR fruit; (◇) peel of SR fruit; (▲) pulp of ST fruit; (△) peel of ST fruit.

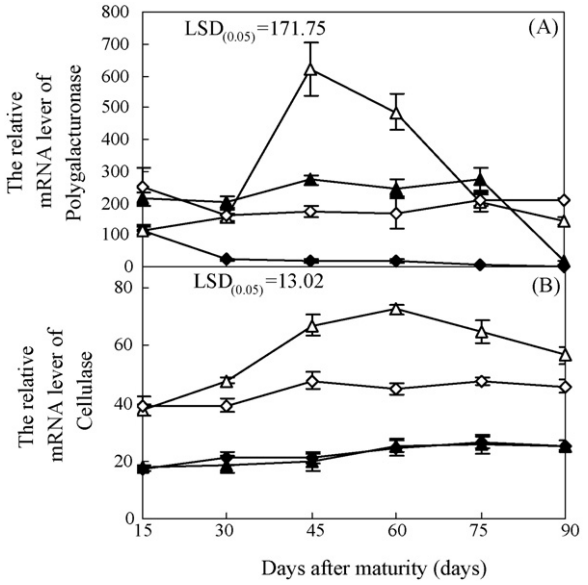

Fig. 4. Dacturonase (PG) and cellulase (Cx) transcripts by SYBR green real time PCR in different storage period of Cara Cara navel orange. The data are displayed with mean ± S.D. (bars) of three replications. (A) polygalacturonase (PG); (B) cellulase (Cx). (◆) pulp of SR fruit; (◇) peel of SR fruit; (▲) pulp of ST fruit; (△) peel of ST fruit.

differences in Cx mRNA levels between fruit pulp from ST and SR. The transcript levels of PG and Cx in peel were higher than that in pulp in all stored fruits.

4. Discussion

The change of dietary fibre is largely associated with breakdown of fruit cell wall. The primary cell wall is composed of several polymers, including CEL, glycans and pectins that are modified during fruit ripening (Brummell and Harpster, 2001; Giovannoni, 2001). The effects of several cell wall modifying enzymes on fruit ripening and softening have been studied in many plant species;

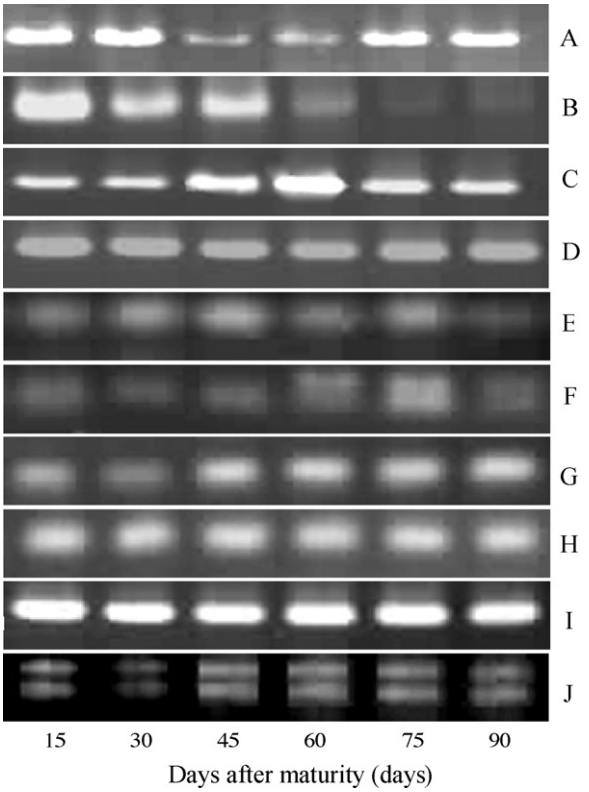

Fig. 5. Detection of polygalacturonase (PG) and cellulase (Cx) transcripts by semi-quantitative RT-PCR in different storage period of Cara Cara navel orange. PCR annealing temperature is 60 °C, β-actin was used as loading control. (A) PG expression of pulp on tree; (B) PG expression of pulp in room; (C) PG expression of peel on tree; (D) PG expression of peel in room; (E) Cx expression of pulp on tree; (F) Cx expression of pulp in room; (G) Cx expression of peel on tree; (H) Cx expression of peel in room; (I) β-actin; (J) RNA.

the most intensively studied enzyme is PG. The result of semi-quantitative RT-PCR and real time PCR showed that PG mRNA levels in ST Cara Cara navel orange fruit were upregulated, as was with enzyme activity. The content of WSP increased sharply in the fruit of ST. These studies indicated that PG of orange fruit could be promoted by storage on tree, and fruit ripening and softening were going on. In some sense, fruit stored on tree is one of the stages of fruit growing. Whereas, PG activity was decreasing during all the SR storage period, there was no stage of up regulation. These results suggested that the ripening and softening of Cara Cara navel orange was slowed down after storage in room.

Our results showed that the contents of TDF, IDF, HC and lignin were decreased, whereas SDF was increased in fruits from ST. As the fruits stored, there was an increase in SDF of fruit pulp in the early storage periods, especially in fruits of ST. In pulp of ST fruits, SDF was the major component, accounting for as high as 43.97% of TDF. By contrast, SDF of SR fruits were relatively low in fully mature fruits, less than 31.51% of TDF, suggesting that ST may promote relative enzyme activity, leading to degradation and depolymerization of pectin substances. The increase in WSP and SDF of fruits was correlated with PG activity. The result of Rao and Paran (2003) indicated that PG was the most important cell wall modifying enzyme on fruit ripening and softening of pepper. In tomato, the high level of endo-PG activity detected in ripe fruits has led to the hypothesis that PG plays an important role in fruit softening (Giovannoni et al., 1991). However, several studies with transgenic tomatoes, in which PG activity was suppressed, indicated that PG activity alone is not sufficient to affect fruit softening and ripening significantly (Giovannoni et al., 1989;

Cooley and Yoder, 1998). Also, in other fruits, such as strawberry and banana, PG activity is very low or absent, despite evidence for pectin solubilization and degradation (Huber, 1984; Smith et al., 1989). However, PG activity in orange pulp showed substantial increase in activity during ripening. These results indicated that PG has a role in changing the texture of the fruit during ripening, ultimately leading to fruit deterioration necessary for seed dispersion (Brummell and Harpster, 2001).

Genes expected to be involved in cell wall degradation such as the gene encoding PG-like proteins were upregulated in ST Cara Cara navel orange. For other important cell wall degrading enzyme, i.e. Cx, only a slightly higher expression was detected in ST fruit of Cara Cara navel orange compared with SR. It is known that Cx degrades both CEL and β-1, 4-glucan backbone of xyloglucan, a hemicellulosic polysaccharide abundant in cell walls of dicotyledons (Sethu et al., 1996). Increase in Cx activity has been reported during ripening of guavas (Abu-goukh and Bashir, 2003), mango (Roe and Bruemmer, 1981) and strawberry (Maurice and Palehett, 1976). In this study, there were no significant differences on Cx activity between fruit stored on tree and in room, suggesting that the changes of dietary fibre was mainly caused by PG. PG might be the key factor in cell wall disassembly and softening of stored Cara Cara navel orange fruit. The role of Cx in fruit softening is uncertain. Therefore, in spite of the high correlation between the increase of Cx activity and the loss of resistance to shearing force, no significant role in tissue softening can be ascribed to Cx at the present time (Abu-goukh and Bashir, 2003).

The flesh firmness of Cara Cara fruits differed markedly under different storage environment. It decreased under all storage conditions, coupled with decrease of HC, CEL and lignin of fruits pulp, which indicated that softening of fruits resulted from an increase in depolymerization and degradation of cell wall polysaccharides (Brummell and Harpster, 2001). Fruits of SR retained greater firmness, concurrent with higher content of cell wall polysaccharides, especially HC. However, the role of HC in the softening process during fruit ripening has not yet been fully elucidated, which is partially due to the apparent irregular behavior of this kind of polymer in various fruit species (Manrique and Lajolo, 2004).

WHO suggested that every one should take in dietary fiber in quantity of 27.00–40.00 g per day. The peel and pulp of citrus fruits are rich in dietary fibre, especially SDF. ST is an effective method for preservation and storage with several advantages such as lower cost, avoiding chemical pollution of fresh-keeping agents and improving quality, etc. Additionally, ST promoted the contents of SDF, which has extraordinary physiological functions such as stimulating intestines peristalsis, regulating metabolism and depressing blood pressure, etc. This study may constitute a system for the study of controlling contents of dietary fibre in citrus and the involvement of ST in fruit maturation and PG, Cx activities.

5. Conclusions

Different parts of the fruits of orange contain large amounts of dietary fibre. However, fruit stored on tree is quite useful for regulating the content of dietary fibre on Cara Cara navel orange. It promoted the contents of SDF, which has extraordinary physiological functions. Cara Cara navel orange fruits stored on tree could enhance the PG and Cx gene expression, increase enzyme activity to improve the content of dietary fibre compared with fruit stored in room. The contents of SDF and WSP in ST fruits were promoted, especially in pulp, while the CEL, HC and lignin contents appeared to be declined compared with the SR fruits. Orange is therefore an interesting potential fibre crop and further studies are needed to determine more precisely the structure and properties of the cell wall polysaccharides, especially pectin.

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