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The Studying on Dietary Fiber Change of Navel Orange (*Citrus sinesis* (L.) Osb.) During Fruit Development and Maturation

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Abstract: This study investigated the dietary fiber content change, including WSP, HP, TP, SDF, IDF, TDF, NDF, ADF, CEL, HC and Lignin, in Robertson Navel orange fruit. The changes of all above were measured in fruit from Mingshan and Ya'an in Sichuan Province, P.R. China. The results showed that the content of WSP increased with the development and maturation of the orange fruit; the HP, TP and HC contents were reached the highest level during the period of greatest increase in bulk, dropped during the yellow color stage and then increased during the maturation stage. SDF, IDF, TDF, NDF and ADF contents increased until the 100 days after flowering and then decreased. There fore, CEL and Lignin content trends were similar to the pectin content trend but lower in quantity. Navel oranges can be an important source of dietary fiber.

Key words: Naval orange, fruit development, maturation, ditetary fiber, WSP

INTRODUCTION

The composition of and change in fruit texture are key problems for horticulture and plant physiology (Shengming *et al*., 2001). The Water Soluble Pectin (WSP) content increases during the maturation of the fruit and the cellulose breaks down into smaller units (Knee and Bartly, 1981). Much research has been done on cell wall composition and change, focusing on fruit ripening and post-harvest physiology (Andrews and Li, 1995). Such, research usually focuses on hydrolyzing enzymes or cell wall composition changes. There are only a few reports on dietary fiber content, which is also an important component of fruit texture (Andrews and Li, 1995; Jianfeng *et al*., 2004; Bingye and Shu, 2004). Navel oranges are an important edible fruit for most people around the world, but their peels are discarded as useless. Changes in the texture of the fruit's flesh affect taste. This study describes dietary fiber analysis of navel oranges with respect to change during fruit development and maturation and provides reference points for further research on navel orange texture change and on dietary fiber.

MATERIALS AND METHODS

Plant material: Robertson navel orange trees (*Citrus sinesis(L.)* Osbeck cv. Robertson Navel Orange) were on tangerine rootstock, planted in 1992 and 1995, were used for experiments in 2002 and 2004. The trees, which were in the full fruiting period, were located in Ya'an and Mingshan in the west of Sichuan province. Soil and water conditions, fertilizer and growth management routine were controlled. Trees in similar states of health, moderately vigorous; with similar size of trees and with similar fruit density were selected. Five individual trees were marked with lab cards. Samples were collected from the trees starting from 85 Days after Anthesis (DAA) to the ripening day, depending on the location, Ya'an or Mingshan. Fruits were collected from the exterior of each tree, from the east side, the south side, the west side, the north side and the top. Ten fruit samples in total were collected each time by random sample and stored with ice in a Styrofoam container for transport to the lab. In the laboratory the samples were stored at -20°C.

Sample pretreatment: Three fruits were selected, thawed and cut into thin slices. The slices were dried at 115°C for 10-15 Min. in an oven. They were then dried at 65°C to constant weight. The samples were milled to 1mm, placed in airproof bags and stored at -20°C.

Method of analysis: Soluble Dietary Fiber (Dovel and Harris, 1982; Yingming *et al*., 2002) (SDF), Insoluble Dietary Fiber (Mertens, 2003; Hall, 2003) (IDF) and Total Dietary Fiber (Stombaugh *et al*., 2004) (TDF) were determined by different methods, with the following modifications: Samples were filtered using a 3500r frozen

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centrifugal filter for 15 min. instead of an air pump sieve. The step of hydrolyzing á-amylase was omitted because no soluble amylum was detected in the samples. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content were determined by Van Soest's method (Van Soest and Wine, 1967), using an automatic fiber analyzer from the VELP corporation in Italy. Acid Cellulose (CEL), Hemicellulose (HC) and acid detergent lignin (Lignin) analysis were done with a procedure designed by Yuwan (1987) modified as follows: A milled sample weighing precisely 0.5 g was hydrolyzed for 1 h at 100°C by neutral detergent; the crude extract was centrifuged at 3500r for 15 min. at 2 °C, the residue was washed by double distilled water and acetone 3 times, respectively. Then the residue was incubated with 50 mL 2 mol L^{-1} HCL, the reaction vessel was immersed in boiling water for 50 min, then centrifuged at 3500r for 15 min at 2 °C again. The residue was washed with double distilled water until the filtrate reached pH6.5-7.0 and water was added to make a total volume of 100 mL. The hemicellulose content was determined by 3, 5-dihydroxytoluene method, using xylose as the standard. The remaining residue was hydrolyzed in 5 mL of 72% H_2SO_4 at 35°C for 1 h, diluted with 25mL double distilled water and immersed again in boiling water for 1 h. Cellulose was determined from the filtrate by the anthrone reagent method. During distillation for CEL and HC, water vapor must be recirculated in order to keep the volume and temperature constant. The last step was lignin content analysis, which used the residue from the process so far, dried to constant weight at 80°C. The pectin (both WSP and HP) content was analyzed by the carbazole spectrophotomeoric method (Daliang Light Industry College, 1998), using D-galacturonic acid (Sigma Chemical Co.) as a standard. All of the above protocols were repeated three times.

RESULTS

The results showed that in the two habitats of Ya'an and Mingshan the pectin content of HP and TP in navel oranges increased from the young fruit stage to expansion stage (Fig. 1). When the fruit color turned to yellow (155d–190d) the pectin maintained a more or less constant level, then increased during the ripening stage (190d–220d). The WSP content showed little difference and had an upward trend: There was a small peak about 130 days after anthesis during the expansion stage, then a slight decrease when the fruit turned to yellow color; then a large increased. Before ripening, WSP content rose sharply, with the highest point 220 days after anthesis.

The result content on dietary fiber in navel orange fruit (Fig. 2) shown that SDF was lower than IDF and TDF.

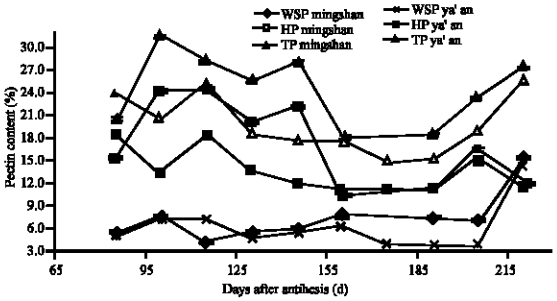

Fig. 1: Changes of pectin content in robertson navel orange fruit

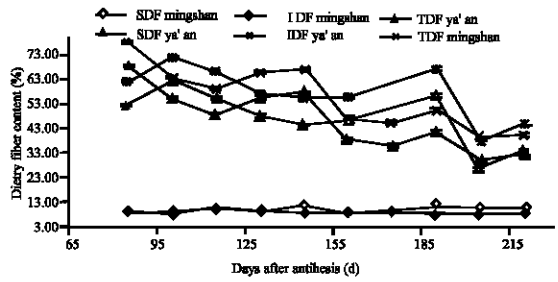

Fig. 2: Changes of dietary fiber content in Robertson Navel orange fruit

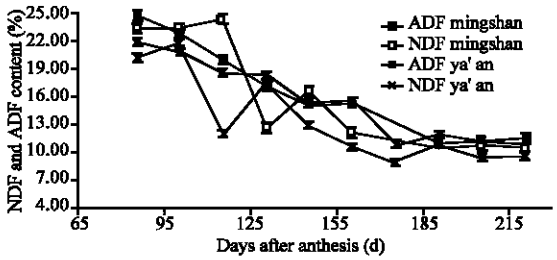

Fig. 3: Changes of ADF and NDF content in robertson navel orange fruit

Its content was about 10% during all of the fruit development stages and showed no significant difference between Mingshan and Ya'an. IDF is the basic component dietary fiber of oranges. IDF content decreased from 70-30% from the young stage to the maturation stage. The trend was nearly linear conforming to the following equations: $IDF_{Mingshan} = -0.1711t+72.849$, $R^2 = 0.535$; $IDF_{Ya'an} = -0.2488t+84.264$, $R^2 = 0.7934$. TDF content was similior to IDF content during fruit development and maturation, conforming to the following equations: $TDF_{Mingshan} = -0.1642t + 82.014$, $R^2=0.5097$ and $TDF_{Ya'an} = -0.252t + 94.009$, $R^2 = 0.8049$. IDF and TDF content had a significant difference between Ya'an and Mingshan.

The changes in NDF and ADF in Mingshan and Ya'an are shown in Fig. 3. Both NDF and ADF decreased

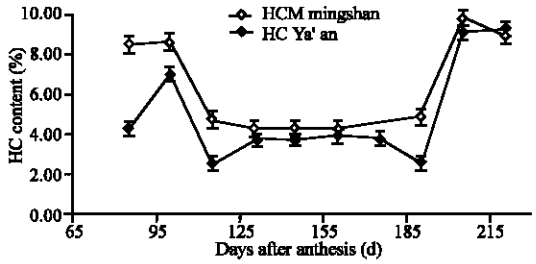

Fig. 4: Changes of hemicellulose content in Robertson Navel orange fruit

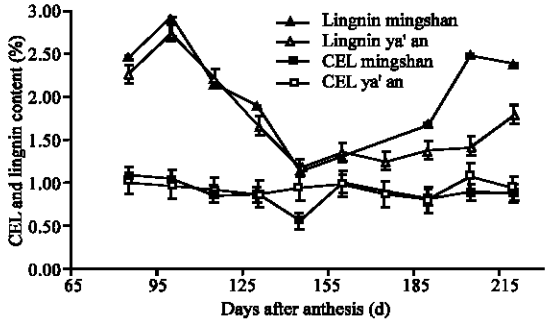

Fig. 5: Changes of cellulose and lignin content in Robertson Navel orange fruit

with the development of the fruit, but their ranges were lower than those of IDF and TDF. NDF contents increased from 100 days after anthesis, then declined slowly. Starting around day 160 after anthesis, when the fruits entered into the maturation stage, NDF and ADF contents change little, remaining at about 10% until harvest occurred on about day 220. ADF and NDF in fruits during the development and the maturation had no significant difference between Ya'an and Mingshan.

The changes in CEL, HC and Lignin content in the navel orange fruit are shown in Fig. 4-5. The contents of CEL and Lignin were lower than HC during development and maturation. CEL was about 1%, Lignin about 3% and HC about 2-10%. The CEL and Lignin contents showed a slight drop from day 85 to day 120 after anthesis. From day 120 to day 190 it continued to drop slowly, then rose slightly to day 220. There was a great change in HC content. From day 85 to day 120 it increased and then declined. At about day 100 there was a large increase to 10%. From day 120 to day 190 the HC contents were about 4%, changing little. At the maturation stage HC climbed suddenly to about 10%. At day 220 it was about 9%.

DISCUSSION

Studies have shown that sweet orange fruits accumulate pectin and nonstarch polysaccharides starting in the young fruit period. Lignin is accumulated as part of the secondary cell wall during cell wall growth. In oranges, pectin is located mainly in the peel. Most of the HP is located in the endocarp and epicarp, WSP mostly in the sarcocarp (Tianfu, 1999). This experiment proved that HP, HC, NDF, ADF and IDF content was higher in the early development stage. The upward trend at day 100 after anthesis, the period of maximum fruit growth, indicates that pectin, CEL and HC are the foundation of fruit development (Joseph and Liang, 1989; Daniel, 2000). Reasons for the decline in the post-expansion and maturation periods may be the rapid sugar accumulation and the hydrolyzation of the cell wall by pectinmethylesterase, Polygalacturonase (PG) and β-glycosidase during maturation. The CEL and HC content are related to water conditions in the environment.

Experiments by Zhangcheng (1997)showed that ^{14}C is absorbed by the plant even under drought conditions, while at the same time the CEL and HC content increases. This shows that HC changes are due to the development and maturation of the fruit. Another key reason for the change in HC and CEL is climate, especially relative humidity. Mingshan and Ya'an are located in the Middle-northern sub-tropic zone in Sichuan province; both are famous for plentiful rainfall, with more rain in summer than in spring and autumn. Considering the lag-behind affects of HC response to climate factors, the fact that HC content from day 100 to day 190 (summer and early autumn) is lower than during other periods (spring and late autumn) may be related to yearly rainfall changes. But the mechanism of how dietary fiber responds to climate factors is unclearly at this point. Further research is required.

The decline in HC content during maturation was related to cell wall hydrolase as well.

In most of the plant material CEL content is greater than HC content. However, this study shows little difference between the two. The results showed HC content was between 4 and 8%, whereas CEL content was 1% and lignin content was between 2 and 5%. One reason is that the CEL analysis method used was different from other methods. This analysis determined the true cellulose content, whereas the general method of cellulose analysis includes most of the HC. Furthermore, based on physiology theory it is reasonable for the results to show high HC, low CEL and low lignin, because the navel oranges have a crisp taste and leave little residue in mouth when eaten fresh.

The change in dietary fiber indicates that CEL content declines linearly and pectin matter increases and decreases during development and maturation. Such results can probably be explained by cellulose fiber

biosynthesis. Fiber are synthesized during the earlier stage of development (Joseph and Liang, 1989; Cosgrove, 2000) but pectin matter and HC can be synthesized and attached to the middle lamella during all stages of the fruits cell wall growth (Grant Reid, 2000; Robyn *et al*., 1999). HC content is effected by climate factors as well. So, a methodology for regulating and controlling texture quality could be the use of hydrolyzing enzymes during the development and maturation periods. A second method would be to regulate glucosyltransferases, which are the key enzymes for cell wall material synthesis. A third would be bionic nursing, the environmental conditions are managed to create the highest quality texture.

The dietary fibers in fruit are mainly CEL, HC, pectin and hydrophilic colloids, which are indigestible by the enzymes in the human small intestine. The components of dietary fiber include resistant starch, resistant dextrin, resistant oligosaccharides, modified cellulose, gum, mucilages and associated minor substances such as waxes, cutin and suberin. Links between insufficient dietary fiber consumption and constipation, diverticular disease, hiatus hernia, appendicitis, varicose veins, hemorrhoids, diabetes, obesity, coronary heart disease, cancer of the large bowel, gallstones, duodenal ulcers, breast cancer and blood clotting have been hypothesized. Dietary fibers have been named "the seventh nutriment" after sugar, protein, fat, water, minerals and vitamins (Dovell and Harris, 1982; Yingming *et al*., 2002; Mertens, 2003; Hall, 2003; Stombaugh *et al*., 2004; Yun *et al*., 2003). Orange dietary fiber is unique because it is rich in flavones. This study found that the IDF content of Robertson navel orange fruits was 30- 70% and the SDF, the orange pectin, the HC are present in large quantities during all developmental stages, which makes navel oranges a good source of dietary fiber.

Yuan *et al*. (2003) investigated the change in dietary fiber in grapefruit and Satsuma mandarin fruits from the late period of development and maturation, but she did not study navel oranges in detail. In Dovell's investigation (Dovell and Harris, 1982) of dietary fiber in sweet oranges analyzed only ADF and NDF. This study analyzed the dynamic changes of dietary fibers including WSP, HP, TP, SDF, IDF, TDF, NDF, ADF, CEL, HC and Lignin in Robertson Navel orange fruit. Because of the high content of SDF, IDF and pectin in navel orange, how to use navel orange's fruit and peel as a source of dietary fiber efficiently and safely is the next step in further research.

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