Biochemical Changes in Chaenomeles Fruits and Fruit Juice during Ripening and Storage

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SUMMARY

In this paper, studies on the changes in characteristics and chemical composition of Japanese quince (*Chaenomeles japonica*) fruits and fruit juice during fruit ripening and storage are reported. Juice was extracted from unripe, mature and stored fruits, respectively, and pH, density, viscosity, turbidity, content of soluble and insoluble solids, titratable acidity and content of vitamin C and phenols were analysed. Fruit biochemistry was clearly influenced by genotype and site of cultivation of plants. Ripening was associated with an increase in density, soluble solids, titratable acidity and content of vitamin C in the juice. During storage at 1 °C and 5 °C, respectively, the general tendencies for changes in fruit biochemistry were similar for all genotypes analysed. The characteristics of the fresh fruit were well preserved at 1 °C, even during two months of storage. However, after three months a significant loss in juice yield was observed. This was accompanied by browning of the fruit skin, an increase in turbidity of the juice, changes in the content of vitamin C and a modification of aroma. The fruits kept at 1 °C and 85% relative humidity could be stored for a maximum of nine weeks without significant loss in quality. At 5 °C and 80% relative humidity, the corresponding storage period decreased to four weeks.

INTRODUCTION

Japanese quince (*Chaenomeles japonica*) is an East Asian dwarf shrub, with interesting potential as a fruit crop. The yellow fruits are aromatic (Lesinska *et al.* 1988, Rumpunen 1995) and rich in juice and fibre (Lesinska 1987, Golubev *et al.* 1991, Thomas *et al.* 2000, Thomas & Thibault, 2002). They are very firm and too acid to be consumed fresh, but useful after processing (Rumpunen *et al.* 2000). Japanese quince is presently cultivated on a small scale in Northern Europe in countries around the Baltic Sea (Rumpunen *et al.* 1998). Plant breeding programmes aimed at improving the plant as a fruit crop are now making progress (Rumpunen 2002).

Chaenomeles juice may be a useful ingredient in natural products due to its high acidity and clear nature. Another property of great interest is its antioxidant activity, due to the high content of vitamin C and phenolic compounds. Thus an application as an acidifying agent with antioxidant properties seems promising. However, due to its novelty, no information is available on changes in fruit biochemistry during development and storage. Refrigeration may preserve the sensory and nutritive properties of the

fruit and its juice, thus prolonging shelf life and availability of fruits for processing (Holdsworth 1988). Both ripening and storage conditions influence fruit characteristics, and should be studied to enable prediction of optimum time for harvesting and maximum period of storage without loss of internal fruit quality (Burda *et al.* 1990, Ackermann *et al.* 1992, Hernández & Alique 1995, Pérez-Ilzarbe *et al.* 1995, Al-Maiman & Ahmad 2002). Therefore characteristics and biochemistry of fruits and fruit juice were investigated during late stages of fruit development and postharvest storage for samples of Japanese quince (*C. japonica*).

MATERIALS AND METHODS

Origin of samples

Fruits were picked from 19 genotypes of *C. japonica*, and from one genotype of a hybrid taxon, *C. japonica* x *speciosa*, grown in Finland and Sweden. Background information on origin of the plant material, purpose of the study and time of sampling are given in Table 1. Juice was prepared from fresh fruits sampled during late stages of fruit development, as well as from stored fruits (Barceló *et al.* 2000, Vila *et al.* 2002).

Fruit ripening and site of cultivation

Changes due to fruit ripening and site of cultivation were investigated for 4 genotypes. All genotypes were grown in two different sites in Finland, a northerly site, Hammaaslahti (H), and a southerly site, Kärkölä (K), separated by 400 km in a north-south direction (Table 1). Fruits were sampled 3 times: 4 weeks before estimated maturity, 2 weeks before estimated maturity and finally at maturity. The unitary weight was then determined and the samples were put in plastic bags, frozen under liquid nitrogen, and sent by plane to Spain where the samples were kept at -18 °C until analysis.

Postharvest storage

During postharvest storage, changes in fruit and juice characteristics and biochemistry were studied for 20 genotypes. The conditions for storage were 1 °C and 85% relative humidity (RH) for the 4 previously studied Finnish genotypes, and 5 °C and 80% RH for the other 16 genotypes. Thus, different genotypes were studied at 1 and 5 °C respectively. Therefore, only the general tendencies in the results should be highlighted and absolute values should not be directly compared among treatments. The period of study was 17 weeks for fruits kept at 1 °C, (sampled at 0, 4, 9, 13, and 17 weeks of storage), and 9 weeks for fruits kept at 5 °C (sampled at 0, 3, 6 and 9 weeks of storage). On each sampling occasion, the unitary weight was determined and the samples were placed in plastic bags, frozen under liquid nitrogen and sent by plane to Spain where the samples were kept at -18 °C until analysis.

Characteristics and fractionation of fruits

After sampling, the fresh fruits were weighed and fractionated into juice, seeds and residual pulp. Juice was extracted by halving and squeezing the fruits, using a Frutelia AV5 juice extractor from Moulinex (France). Juice was then analysed fresh.

Physico-chemical analysis of fruit juice

The characteristics studied in the juice were pH, density, content of soluble and insoluble solids, viscosity, turbidity, titratable acidity, content of vitamin C and phenolic compounds. These parameters were determined following the methods of the International Federation of Fruit Juice Producers, the Association of Official Analytical Chemists and others (Singleton & Rossi 1965, Antolovich *et al.* 2000).

Statistics

Samples were analysed in triplicate and an average estimate for each characteristic was calculated for the

Taxon	Genotypes (n) and origin ¹	Study	Week for sampling (0 = maturity)
C. japonica	4, Hammaaslahti and Kärkölä, Finland	Fruit ripening and site	-4, -2 and 0
C. japonica	4, Hammaaslahti and Kärkölä, Finland	Fruit storage (1 °C)	0, 4, 9, 13, and 17
C. japonica	12, Kristianstad, Sweden	Fruit storage (5 °C)	0, 3, 6, and 9
C. japonica	3, Kärkölä, Finland	Fruit storage (5 °C)	0, 3, 6, and 9
C. japonica x speciosa	1, Kristianstad, Sweden	Fruit storage (5 °C)	0, 3, 6, and 9

Table 1. Origin of plant material, purpose of study and period of sampling.

¹Hammaaslahti (H) 62°25'N, 29°50'E, Kärkölä (K) 60°54'N, 25°14'E, Kristianstad 56°07'N, 14°10'E

different sites and treatments, respectively. Results were then presented in graphs to illustrate general tendencies for fruit biochemistry changes during fruit development and postharvest storage. Detailed results on genotypic differences will be published elsewhere.

RESULTS AND DISCUSSION

Changes in characteristics due to fruit ripening and site of cultivation

During late stages of fruit development, fruit weight increased by 20% for fruits picked in Kärkölä at the southerly site, but no obvious trend was noticed for fruits picked in Hammaaslahti at the northerly site (Figure 1A). The unitary fruit weight was always higher for fruits picked at the southerly site compared to samples picked at the northerly site. From this information, it may be concluded that the milder climate in south Finland (Kärkölä) promoted fruit development.

The residual pulp fraction increased by about 10% for all samples, irrespective of origin (Figure 1B). Major changes took place during the last two weeks before final harvest. The residual pulp fraction became in general higher from fruits picked at the southerly site than from fruits picked at the northerly site.

The juice fraction showed a converse relationship compared to the pulp fraction (Figure 1C). Again, the changes occurred during the last two weeks before final harvest.

The percentage of seeds was initially higher in fruits picked at the northerly site than in those from the southerly site with a range between 11 and 14% (Figure 1D). At maturity no difference was found among sites of cultivation. The percentage of seeds decreased in the period 2–4 weeks before maturity and then remained practically constant.

Changes in chemical composition of juice due to fruit ripening and site of cultivation

The content of insoluble solids in the juice slightly increased during fruit ripening (Figure 1E). Major changes took place during the last two weeks before final harvest. Fruits picked at the northerly site had the highest final content of insoluble solids (4.2% compared to an initial content of 2.8%).

pH was very low and only slightly increased during the two last weeks of fruit ripening (Figure 1F). Fruits picked at the northerly and southerly site reacted in the same way. The small increase is probably due to a high buffering capacity of the organic acids in the fruits.

There was a general tendency for increased juice density during fruit ripening. Fruits picked at the southerly site obtained a higher density than fruits picked at the northerly site (Figure 1G).

The viscosity of juice obtained from fruits picked at the southerly site behaved differently to the viscosity of juice obtained from fruits picked at the northerly site (Figure 1H). In samples from the southerly site the viscosity slightly increased, whereas in samples from the northerly site the viscosity first increased, and then decreased.



Figure 1. Changes in unitary fruit weight (A), content of pulp (B), content of juice (C), content of seeds (D), insoluble solids in juice (E), pH of juice (F), density of juice (G), viscosity of juice (H), turbidity of juice (I), soluble solids in juice (J), titratable acidity in juice (K), vitamin C in juice (L) and phenols in juice (M) during ripening of Japanese quince (*C. japonica*) fruits, sampled at a northerly site, Hammaaslahti (H), and a southerly site, Kärkölä (K), in Finland.

400

375

350 – -4

-2

Weeks before harvest

0

During fruit ripening there was first an increase and then a decrease in turbidity of the juice (Figure 1I). The turbidity was lower when the sample was extracted from fruits picked at the northerly site than from fruits picked at the southerly site.

The content of soluble solids increased during fruit ripening (Figure 1J). The content of soluble solids was higher in juice from fruits picked at the southerly site compared to fruits picked at the northerly site. The average final values were 9.8 and 8.5 °Brix, respectively. In apples too, the content of sugars increases at different rates till harvest, and then the levels remain constant after an initial drop (Ackermann *et al.* 1992).

Titratable acidity increased during fruit ripening (Figure 1K). Chaenomeles juice from fruits picked at the southerly site was slightly more acidic than juice from fruits picked at the northerly site.

Vitamin C and phenolic compounds are valuable fruit internal quality parameters (Gardner *et al.* 2000). Recommendations for healthy eating include the consumption of fruit juices (Williams 1995) whose beneficial health effects are ascribed, in part, to the antioxidant potential of vitamin C and phenolic compounds (Hertog *et al.* 1993). Vitamin C (ascorbic + dehydroascorbic acid) strongly increases in the chaenomeles fruits during late stages of development (Figure 1L). The increase was from 18 to 50 mg of ascorbic acid/100 ml of juice obtained from fruits picked at the southerly site. By contrast, it decreases in *e.g.* apple (Ackermann *et al.* 1992). Juice extracted from fruits picked at the southerly site had a higher final content of vitamin C compared to juice obtained from fruits picked at the northerly site. The milder climate in southern Finland thus seems to promote production and accumulation of vitamin C in chaenomeles fruits.

Chaenomeles fruits from the southerly site had a higher total content of phenolic compounds (Figure 1M) than fruits from the northerly site. There was a slight tendency for the content of phenolic compounds to decrease during fruit ripening. This pattern is consistent with the decrease in phenolic compounds observed in e.g. cherimoya (Hernández & Alique 1995), in some varieties of apples (Dever *et al.* 1991) and in pomegranate (Al-Maiman & Ahmad 2002) during fruit ripening. The concentration of individual phenolics may, however, develop in a different way (Spanos & Wrolstad 1992).

Changes in characteristics of fruits during storage

During storage at 1 °C and 85% RH, chaenomeles fruits only slightly decreased in weight (Figure 2A). By contrast, at 5 °C and 80% RH a pronounced weight loss occurred due to evaporation. After nine weeks the fruits had lost 30% of their initial weight.

Following an initial increase, the residual pulp fraction of fruits stored at 1 °C and 85% RH decreased (Figure 2B). A converse pattern was obtained for the juice fraction (Figure 2C). Percentage of seeds slowly decreased (Figure 2D) during the same period, from 12 to 10%.

During storage at 5 °C and 80% RH, the residual pulp fraction increased (Figure 2B). A converse pattern was obtained for the juice fraction (Figure 2C). Percentage of seeds consistently increased (Figure 2D), from 6 to 10%.

At both storage temperatures the appearance of the fruits became unattractive. The main factors in the loss in quality were a slow browning of the fruit skin, growth of fungi (Barceló *et al.* 2000, Vila *et al.* 2002) and a modification of aroma (Jordán *et al.* 1998). The development of browning may be caused by an increase in polyphenoloxidase activity, a higher content of phenolic compounds and the presence of high levels of cinnamic acids (Hernández & Alique, 1995). The period of storage without significant loss of quality noticed in our study (2 months at 1 °C) was longer than for pear (1 month, Rousseas *et al.* 2000) but shorter than for apple (4 months, Róth *et al.* 2000).

Storage fungi are able to grow on chaenomeles fruits as they do on other fruits (Hellín *et al.* 1998, 2001). No pesticides were used during cultivation of the chaenomeles fruits and fruits were not treated with any chemical during postharvest handling. Treatments aimed at reducing the contamination of fruits would most likely result in a prolonged keeping quality.



Figure 2. Changes in unitary fruit weight (A), content of pulp (B), content of juice (C), content of seeds (D), insoluble solids in juice (E), pH of juice (F), density of juice (G), viscosity of juice (H), turbidity of juice (I), soluble solids in juice (J), titratable acidity in juice (K), vitamin C in juice (L) and phenols in juice (M) during storage of Japanese quince (*C. japonica*) fruits at 1 and 5°C.

Changes in characteristics and chemical composition of juice during fruit storage at 1 $^{\rm o}{\rm C}$ and 85% RH

The content of insoluble solids in the juice was 4–6% during the complete period of storage (Figure 2E). The pH of the juice was almost constant pH 2.8 (Figure 2F), although on the last sampling occasion a notable decrease in pH (to 2.4) was detected. The density of the juice varied slightly during storage (Figure 2G). Initially viscosity decreased, but thereafter it strongly increased (Figure 2H). The nephelometric turbidity of the juice showed a slight tendency to increase (Figure 2I), but the content of soluble solids did not change during storage (Figure 2J). Titratable acidity (Figure 2K) decreased in the beginning and then returned to a stable value for the rest of the storage period. The marked decrease in titratable acidity might be due to an increased respiration rate since acids are known to be quickly metabolised during respiration (Kim *et al.* 1993, Rocha & Morais 1995). The content of vitamin C (Figure 2L) increased from 35 to 48 mg of ascorbic acid/100 ml of juice during storage. Phenolic compounds showed a more complicated pattern (Figure 2M). First the content of phenolic compounds increased, then it became stable and finally it increased again. Vitamin C and phenols thus showed a good keeping quality during storage, as in other fruits (Burda *et al.* 1990, Pérez-Ilzarbe *et al.* 1995, Lee & Coates 1999).

The overall adulteration of the juice became more pronounced after the second month of storage. This suggests that a juice of high quality can be obtained from fruits that have been stored for a maximum of nine weeks at 1 $^{\circ}$ C and 85% RH.

Changes in characteristics and chemical composition of juice during fruit storage at 5 $^{\rm o}{\rm C}$ and 80% RH

The content of insoluble solids of the fruit juice increased from 9% to 18% (Figure 2E) when fruits were stored at 5 °C and 80% RH. The last three weeks were characterised by pronounced degradation processes resulting in drying and rotting of the fruits. The pH was at first stable and then increased from 2.5 to a final value of 2.8 (Figure 2F). Both density and viscosity of the juice increased due to a concentration of solutes in the juice (Figure 2G and 2H, respectively). The nephelometric turbidity increased (Figure 2I). The average increasing opacity of the juice was in agreement with estimates of juice turbidity and the appearance of the fruits. The content of soluble solids in the juice increased from 8.2 to 10.2 °Brix on average (Figure 2J). Titratable acidity remained stable during the complete period of study (Figure 2K). The content of vitamin C and phenolic compounds developed in a similar way (Figures 2L and 2M), first it increased and then it decreased, a trend more pronounced for vitamin C than for phenolic compounds.

The overall adulteration of the juice became significant after the first month of storage. This suggests that if fruits are kept at 5 °C and 80% RH, a high quality juice can be obtained after a maximum of four weeks storage of fruits.

Further studies on postharvest storage of chaenomeles fruits should comprise the use of modified atmospheres, other techniques to reduce respiration and treatments to decrease the negative effects of storage fungi (Di Venere *et al.* 1998, Johnson & Colgan 1998, Gorris *et al.* 1998, Roller *et al.* 1998, Kader 2000).

CONCLUSION

The characteristics and biochemical composition of chaenomeles fruits were clearly influenced by the site of cultivation. A milder climate stimulated both fruit size and accumulation of secondary metabolites. Fruits kept at 1 °C and 85% RH could be stored significantly longer without severe loss of internal quality compared to fruits kept at 5 °C and 80% RH. The characteristics of fresh fruit were well preserved at 1 °C, even during two months of storage, but then a significant loss in juice yield was observed. This was accompanied by browning of the fruit skin, an increase in juice turbidity, changes in content of vitamin C and organic acids and a modification of aroma. At 5 °C pronounced changes were observed in all parameters analysed during the complete period of storage. Using 1 °C as the storage temperature, fruits could

be preserved for nine weeks maximum. By contrast fruits could be kept only for four weeks at 5 °C without significant loss in internal quality.

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