Changes in Sugars, Acids, and Amino Acids during Ripening and Storage of Apples (Cv. Glockenapfel)

Jörg Ackermann, Monica Fischer, and Renato Amadò*

Department of Food Science, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zurich, Switzerland

Changes in sugars, the principal acids, and amino acids were monitored in the ethanol-soluble matter of apples (cv. Glockenapfel) during a 6-month period between the beginning of July, while the apples were still on the tree, and January, after storage at 4 °C. Fructose was the principal sugar. Fructose, sucrose, and glucose increased at different rates till harvest, and then the levels remained constant after an initial drop. The sorbitol content did not vary much. Malic acid was the principal acid, and it decreased during growth and storage. Citric acid followed a similar trend. The amino acid content declined steadily during development. All of these changes can be related to the metabolic activity during fruit growth and maturation.

INTRODUCTION

The edible portion of apples consists, apart from water, of sugars, organic acids, cellulose, and pectic substances as well as several other minor constituents. The average content of these constituents is variable and depends not only on the varieties but also on the weather and location of the tree. Within the fruit itself the distribution of these components is not homogeneous.

The sugar and acid content have a marked influence on the sensory quality of the fruit. The main sugars present in apples are fructose, sucrose, and glucose. Sorbitol, which is present in lesser quantities in the fruit but in larger amounts in the leaves, plays an important role in the metabolism of sugar accumulation during development (Berüter, 1985).

Malic acid accounts for about 90% of the acid content of apples; citric, succinic, and traces of several other acids make up the rest (Hulme and Rhodes, 1971).

There is little information available on the changes in the acids during ripening on the tree and the ensuing storage, while more is known about the sugars. Most studies concentrate on the chemical composition at picking time or when the apples are ripe for consumption. In addition, the significant differences between apple varieties and cultivation conditions make it difficult to compare the values published, though the trends stay the same.

During an extensive study on the chemical changes of the pectic substances of apples (cv. Glockenapfel), a low molecular fraction was obtained after precipitation of the cell wall material with ethanol. The changes in these components and in the amino acid composition of this ethanol-soluble fraction were monitored during an interval of 30 weeks starting after the June drop and ending after a 3-month storage period.

The sugars and acids were analyzed with two independent methods so as to verify the results.

MATERIALS AND METHODS

Plant Material. Apples (cv. Glockenapfel) were picked from the tree at 3-week intervals from the beginning of July to the end of October. Each time 20 apples were collected from the same 5 trees, 4 apples per tree. At the end of October, which is the normal harvest time for these varieties, all of the apples were picked and stored at 4 °C at a relative humidity of 95% till the end of January, the apples from each tree being stored in separate containers. During the storage period samples were also collected at 3-week intervals; once again 20 apples were analyzed each time. The apples were provided by the Swiss Federal Research Station in Waedenswil.

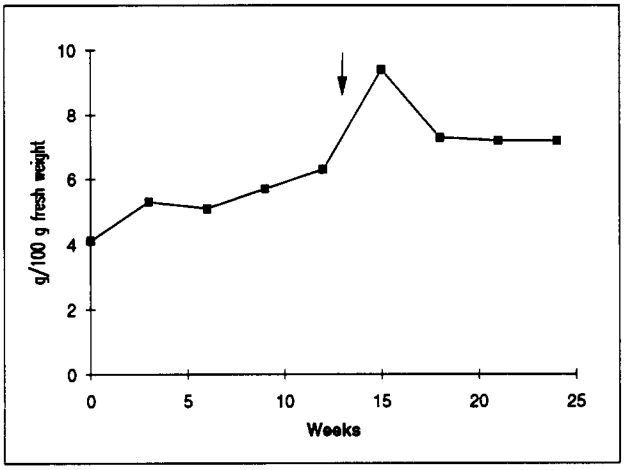


Figure 1. Evolution of the ethanol-soluble matter of apples (cv. Glockenapfel) during ripening and storage. The arrow indicates the time of harvest.

Preparation of the Extracts. The unblemished fruit was peeled, cored, and cut into small pieces which were plunged into boiling 96% ethanol (500 mL/200 g of fruit flesh) for 10 min and then blended for 5 min with a Sorvall Omni-Mixer (Du Pont, Newtown, CT). The slurry was then homogenized with a Polytron (Kinematica, Luzern, Switzerland) and filtered through a G3 glass filter. The residue was washed with 96% ethanol followed by acetone and diethyl ether and set aside for further examination. The ethanolic filtrates were collected and concentrated on an rotatory evaporator under reduced pressure at 40 °C and frozen until needed after addition of a few drops of 0.1% Merthiolate in ethanol to prevent microbial growth.

All of the following analyses were performed in duplicate, and the coefficients of variation were usually less than 5%.

Dry Matter. The dry matter was determined gravimetrically by drying at 105 °C for 19 h after mixing with quartz sand according to AOAC Method 31.008 (AOAC, 1984).

Sample Preparation. For the enzymatic determination solutions containing 1 g of sample/L of distilled water were prepared; for HPLC the solutions contained 0.1 g of sample/L of distilled water.

Sugar Determination. *Enzymatic Determination.* The enzymatic determination of fructose, sucrose, glucose, and sor-

* Author to whom correspondence should be addressed.

0021-8561/92/1440-1131$03.00/0

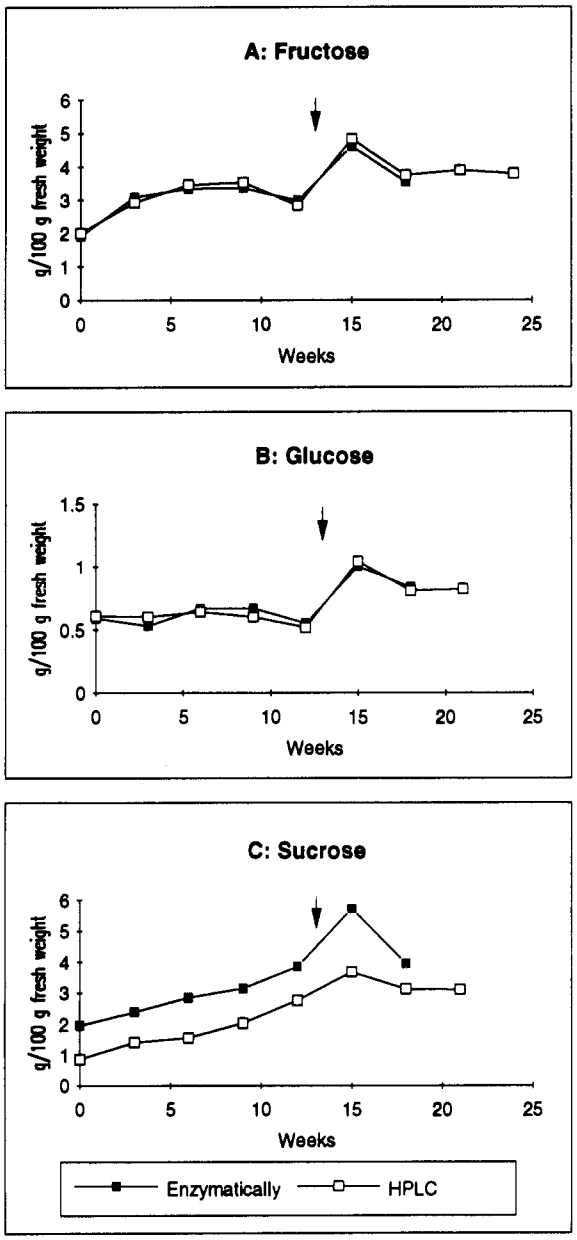


Figure 2. Changes in the sugar content of apples (cv. Glockenapfel) during ripening and storage. The arrow indicates the time of harvest.

bitol was done with test kits (Boehringer GmbH, Mannheim, Germany), and the absorptions were measured with a Clinicon 4010 photometer (Clinicon GmbH, Mannheim, Germany) at 340 nm. Glucose was determined by the hexokinase/glucose-6-phosphate dehydrogenase method. Fructose was determined afterward in the same sample by the hexokinase/phosphoglucose isomerase/glucose-6-phosphate dehydrogenase method. Sucrose was determined after hydrolysis by β-fructosidase (invertase) by difference between the glucose concentration before and after enzymatic inversion (Boehringer Mannheim, 1986). Sorbitol was determined as fructose after oxidation by sorbitol dehydrogenase. All of these enzymatic reactions led to the release of NADPH in stoichiometric quantities; the increase in NADPH was measured by its absorption at 340 nm.

Liquid Chromatography. The HPLC analysis of the sugars was carried out on a HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA) using an Aminex HPX-87C column (Bio-Rad, Richmond, CA) for the analysis of fructose and sorbitol and an Aminex HPX-87P (Bio-Rad) for sucrose and glucose. Twenty microliters of sample was injected at a flow rate of 0.6 mL/min using water as eluent and a column temperature of 85 °C. The components were detected with a HP-1037a refractive index detector (Hewlett-Packard).

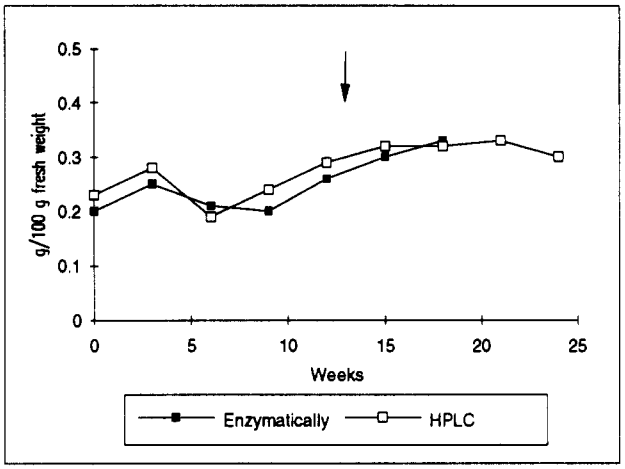


Figure 3. Changes in the sorbitol content of apples (cv. Glockenapfel) during ripening and storage. The arrow indicates the time of harvest.

The peaks were quantified by the external standard method.

Acid Determination. *Enzymatic Analysis.* For the enzymatic determinations of L-malic acid and citric acid test kits (Boehringer) were used and the absorptions measured at 340 nm with a Clinicon 4010 photometer (Clinicon). Malic acid was determined by the L-malate dehydrogenase method and citric acid by the citrate lyase method (Boehringer Mannheim, 1986). All of these enzymatic reactions led to the release of NADPH in stoichiometric quantities; the increase in NADPH was measured by its absorption at 340 nm.

Liquid Chromatography. The HPLC determination of L-malic acid was carried out on a HP 1090 liquid chromatograph (Hewlett-Packard) using an Aminex HPX-87C column (Bio-Rad). Ten microliters was injected at a flow rate of 0.6 mL/min using as eluent 96% 0.01 N H_2SO_4, 0.5 mM $CaSO_4$, and 4% CH_3CN. The column temperature was 85 °C. L-Malic acid was detected with a diode array detector at a wavelength of 210 nm.

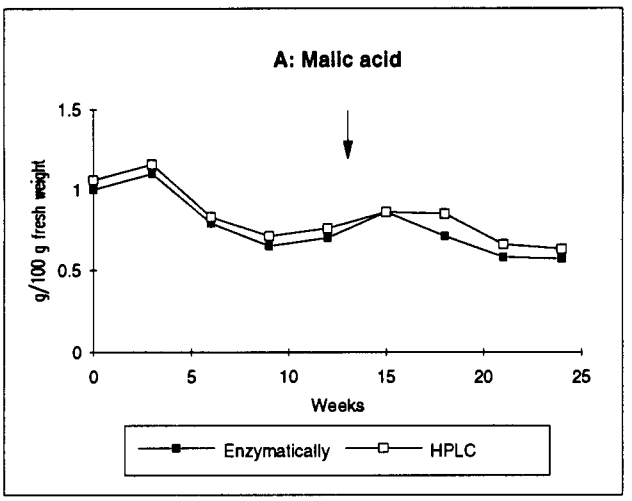
The peaks were quantified by the external standard method.

Amino Acid Determination. The amino acid composition of the samples was determined after hydrolysis in 6 N hydrochloric acid at 110 °C for 24 h by automated amino acid analysis on an Alpha Plus amino acid analyzer 4151 (Pharmacia LKB, Uppsala, Sweden).

RESULTS AND DISCUSSION

As can be seen in Figure 1 the dry matter of the ethanol-soluble fraction (ES) increased regularly during the first 14 weeks while the apples were on the tree; during storage it remained relatively constant. The jump visible after 15 weeks occurs approximately 1 week after harvest. After the June drop, the cell division phase is terminated and the cell growth phase begins, which could explain these observations. During this expansion, metabolites accumulate in the cells, which accounts for an increase in the dry matter. In contrast to the ES there was a decline in the ethanol-insoluble matter (EI) during the first 20 weeks; the levels then remained stable till senescence (Fischer and Amadò, unpublished data). This points to the fact that during development synthesis of cell wall polysaccharides is slower than water intake and cell expansion. As expected, the sum of the water content, ES, and EI was close to 100%.

The changes in fructose, glucose, and sucrose during ripening and storage are presented in Figure 2. Similar results were found both enzymatically and by HPLC except for sucrose, where a constant and significant difference was found. This observation has been made before under similar conditions (Pfeiffer and Radler, 1985; Tomlins et al., 1990), and because of its higher specificity the enzymatic method seems to be more trustworthy. Fructose



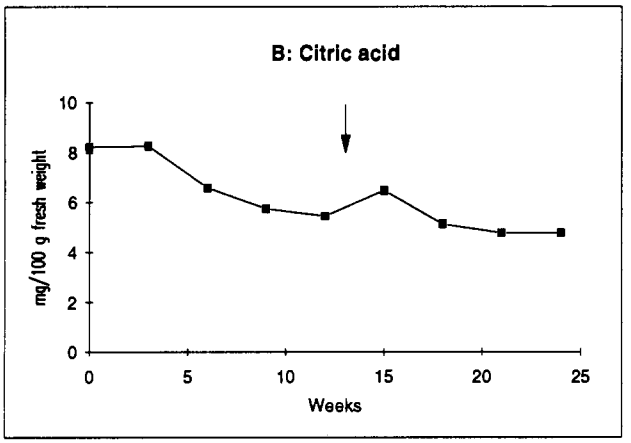


Figure 4. Changes in the acid content of apples (cv. Glockenapfel) during ripening and storage. The arrow indicates the time of harvest. (Citric acid was only determined enzymatically.)

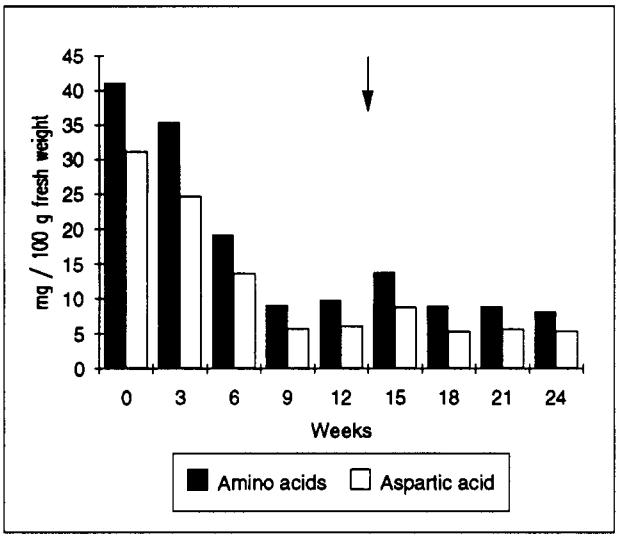


Figure 5. Changes in the amino acid content of the ethanol-soluble fraction of apples (cv. Glockenapfel) during ripening and storage. The arrow indicates the time of harvest.

is the most prominent sugar, with levels between 3.9 and 5.7% on a fresh weight basis in the ripe apple. The sucrose content oscillated between 3.5 and 4.6% and the glucose content between 0.8 and 1.0%. Sucrose increased steadily until harvest; fructose and glucose, on the contrary, fluctuated around the same value till just before harvest, when they suddenly increased in content. The highest sugar concentration was reached around picking time and

Table I. Amino Acid Content of the Ethanol-Soluble Fraction of Unripe and Ripe Apples (Cv. Glockenapfel)

amino acid	week 0 (mg/100 g of fresh weight)	week 24 (mg/100 g of fresh weight)
aspartic acid/asparagine	31.135	3.066
threonine	0.365	0.000
serine	1.279	0.193
glutamic acid/glutamine	4.649	0.804
proline	0.000	0.000
glycine	0.569	0.123
alanine	0.693	0.069
cysteine	0.000	0.000
valine	0.336	0.078
methionine	0.057	0.000
isoleucine	0.217	0.062
leucine	0.349	0.062
tyrosine	0.000	0.000
phenylalanine	0.927	0.033
lysine	0.229	0.053
histidine	0.115	0.025
arginine	0.131	0.021

declined rapidly to a more or less constant level during the first few weeks of storage. The accumulation of the sugars is linked to the conversion of sorbitol translocated from the leaves to the fruit during cell expansion, fructose and sucrose being the favored conversion products (Berüter, 1985). The rapid increase in the glucose concentration just before harvest can also be related to starch synthesis and hydrolysis (Osterloch, 1980). The sudden drop of all three sugars at the beginning of the storage period can be explained by the fact that the apples were harvested just before the climacteric. This phase is characterized by a period of increased respiration during which the sugars and acids are rapidly used as substrates in the metabolic processes. Then, once the cell growth period is terminated, the sugar content does not vary much anymore.

The sorbitol (Figure 3) content is very low and does not change much, though it shows a slight increasing trend after harvest. Since the sorbitol produced during growth on the tree is not accumulated but continuously converted into fructose, sucrose, and glucose, its actual concentration in the fruit would seem to remain constant. The slight increase observed during storage can be attributed to the anaerobic conversion of fructose (Osterloch, 1980).

Malic acid, with concentrations between 0.4 and 1.0%, is the principal acid; only traces of citric and succinic acid were observed. The results are shown in Figure 4; succinic acid levels were of the order of 2 mg/100 g of fresh weight and are not reported. The acid concentration in the fruit flesh declined during the development, though, once again, a jump was observed just before harvest. The decrease can be attributed to a dilution effect caused by mass increase during the cell growth phase. After storage, increased respiration is also responsible for the decline since malic acid is the principal metabolic substrate together with the sugars.

The amino acids determined in this study are those in the ES fraction; they are present as free amino acids or low molecular weight peptides. The changes in this amino acid fraction are presented in Figure 5. This fraction represented about 1% of the ripe apple. During the first 10 weeks there was a dramatic drop in the amino acid content of the fruit flesh, after which time the values stayed more or less constant. The variation is probably related to the processes of protein synthesis and degradation during maturation as well as to the dilution effect. Aspartic acid and asparagine were determined together since asparagine is converted to aspartic acid during hydrolysis. They are the principal amino acids found and account

for about 70% of the amino acid content, followed by glutamic acid/glutamine, serine, glycine, and phenylalanine. Table I presents the data for the amino acid content of the ES of unripe and ripe apples.

As already mentioned, because of the influence of the varieties but also because of factors such as the weather or the ratio of leaves to fruit, it is very difficult to compare the composition of different apples. The stage of development at which the composition is determined also has an influence, measurements being usually carried out after harvest when the apples are table ripe. The values determined here around harvest time are consistent with those for other varieties presented by different authors (Berüter, 1985; Will and El-Ghetany, 1986; Chan et al., 1972). Only the sucrose content is consistently higher than that given in the literature, but this could be a characteristic of the variety Glockenapfel.

ACKNOWLEDGMENT

We thank the Swiss Federal Research Station in Waedenswil for supplying the apples.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984.

Berüter, J. Sugar accumulation and changes in the activities of related enzymes during development of the apple fruit. *J. Plant Physiol.* **1985**, *121*, 331–341.

Boehringer Mannheim. In *Methods of Biochemical Analysis and Food Analysis*; Boehringer Mannheim GmbH: Mannheim, 1986; pp 20–22, 78–79, 96–98, 100–103.

Chan, W. W.; Chong, C.; Taper, C. D. Sorbitol and other carbohydrate variation during growth and cold storage of McIntosh apple fruits. *Can. J. Plant Sci.* **1972**, *52*, 743–750.

Hulme, A. C.; Rhodes, M. J. C. In *The Biochemistry of Fruits and their Products*; Hulme, A. C., Ed.; Academic Press: London, 1971; Vol. 2, Chapter 10.

Osterloch, A. In *Obstlagerung*; VBF Deutscher Landwirtschaftsverlag: Berlin, 1980; Chapter 2.

Pfeiffer, P.; Radler, F. Determination of Organic Acids, Sugars, Glycerol and Ethanol in Wine by High Performance Liquid Chromatography with a Cation Exchange Resin. *Z. Lebensm. Unters. Forsch.* **1985**, *181*, 24–27.

Tomlins, K. I.; Baker, D. M.; McDowell, I. J. HPLC method for the analysis of organic acids, sugars, and alcohol in extracts of fermenting cocoa beans. *Chromatographia* **1990**, *29* (11/12), 557–561.

Wills, R. B. H.; El-Ghetany, Y. Composition of Australian foods. 30. Apples and pears. *Food Technol. Aust.* **1986**, *38* (2), 77–82.

Received for review December 3, 1991. Revised manuscript received April 1, 1992. Accepted April 24, 1992.

Registry No. Fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; sorbitol, 50-70-4; L-malic acid, 97-67-6; citric acid, 77-92-9; aspartic acid, 56-84-8; asparagine, 70-47-3; threonine, 72-19-5; serine, 56-45-1; glutamic acid, 56-86-0; proline, 147-85-3; glycine, 56-40-6; alanine, 56-41-7; cysteine, 52-90-4; valine, 72-18-4; methionine, 63-68-3; isoleucine, 73-32-5; leucine, 61-90-5; tyrosine, 60-18-4; phenylalanine, 63-91-2; lysine, 56-87-1; histidine, 71-00-1; arginine, 74-79-3; glutamine, 56-85-9.