Original paper

Biochemical study of the ripening of cider apple varieties

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Biochemische Untersuchung des Reifens von Cidreapfelsorten

Zusammenfassung. Die Änderung der Zusammensetzung der wichtigsten Zucker und organischen Säuren in fünf asturianischen Apfelsorten wurden während der Endphase des Reifens durch Hochleistungsflüssigchromatographie untersucht. Ebenfalls wurden im gleichen Zeitraum die Veränderungen im Stickstoff-, Polyphenol-, Zucker- und Stärkegehalt dieser Sorten bestimmt. Unterschiede ergaben sich bei den wichtigsten Zuckerarten (Fructose, Saccharose und Glucose) und Sorbitol der fünf Apfelsorten. Die größte Fructosemenge wurde in der Endphase des Reifens angereichert. Mit fortschreitendem Reifen nahm der Gesamtzuckergehalt zu, wohingegen der Gehalt an Apfelsäure, Stärke, Stickstoff und Polyphenolen abnahm.

Summary. Changes in the major sugars and organic acids in five Asturian apple varieties during the final stage of ripening were studied using HPLC. The changes in total nitrogen, polyphenols, sugars and starch in these varieties were also studied over the same period. Differences were noted among the major sugars (fructose, sucrose and glucose) and sorbitol for the five varieties studied. Fructose accumulated in a greater quantity in fruit during the final stage of ripening. The overall sugar content increased, while the malic acid and starch contents decreased in the final stage, which was preceded by a minimum in the nitrogen and polyphenol contents.

Introduction

The ripening of apples involves a series of biochemical transformations that affect their content of various chemical components such as sugars, organic acids, phenols and nitrogen-containing compounds, all of

which determine the sensory properties and nutritional value of apples and reach their optima on ripeness. This final stage of the ripening period also strongly influences the production of different apple derivatives and hence their organoleptic features.

The function, evolution and significance of the compounds mentioned above has been the subject of much research; they have been shown to influence both the ripening and growth of the fruit, and the synthesis of some of its components. Sorbitol is the main product photosynthesized in apple tree leaves [1], as well as the chief source of the carbon transferred to the fruit [2], where it is converted to simpler carbohydrates [3]. Sucrose is the carbon source on which young fruits grow, while fructose is the sugar that accumulates to the greatest extent during the ripening of the fruit. Organic acids are closely related to the ripening of climacteric fruits, which feature an interesting respiratory activity that takes up malic acid via oxidative decarboxylation [4]. Likewise, this acid can be used to form sugars in gluconeogenesis [5].

A number of parameters such as the internal ethylene concentration, the fruit consistency, the soluble solid concentration and the starch content are currently used to determine the optimum ripening period of fruit. The loss of consistency and starch and the increase in the soluble solids content are the parameters that most accurately indicate the best time for consumption of the fruit while fresh [6]. Other authors, such as Board and Woods [7], determine the degree of fruit ripeness from sugar/acidity ratios on account of the strong influence of these two components on the sensory properties of apples.

The above data prompted us to study the changes in each of the different components of apples throughout their ripening period. Thus, we monitored organic acids and sugars by HPLC over the ripening period of five apple varieties commonly grown in Northern Spain for their industrial use in obtaining juices and ciders. We also monitored the overall changes in phenol and nitrogen-containing compounds and the degradation of starch in order to determine the optimum degree of ripeness for industrial purposes.

Materials and methods

Apple varieties. We studied five spanish varieties with different organoleptic characteristics: Raxao (sharp), Collaos (mild sharp), Durón Arroes (sweet), Picona Rayada (bittersweet) and Meana (bittersharp).

Analysis of sugars and organic acids by HPLC. The chromatographic set-up used to analyse sugars consisted of a Waters (Milford, Mass., USA) 510 pump, a Wisp 712 automatic injector ($V_i = 10 \mu\text{l}$), a Waters 410 RI detector and Digital 380 data station. The column used was a Sugar Pak I model from Waters (300 mm long, 6.5 mm I.D.). The mobile phase was an aqueous solution containing 50 mg/l of the calcium salt of EDTA, which was degassed prior to use. The optimum working conditions for this HPLC system were found to be as follows: flow rate, 0.5 ml/min; column temperature, 80° C; detector temperature, 37° C; detector sensitivity, 32.

Organic acids were analysed using an LKB (Bromma, Sweden) high performance liquid chromatograph furnished with a Model 2152 pump, a Rheodyne 7125 injection valve (20- μl loop), an LKB 2140 fast spectral detector and an IBM data station. The column was a Spherisorb ODS-2 model (250-mm long, 4 mm I.D.) and was packed with 5- μm particles (Tracer, Barcelona, Spain). The mobile phase was a 0.01 mol/L potassium dihydrogen phosphate buffer adjusted to pH 2.25 with phosphoric acid and degassed with helium prior to use. The working conditions used were as follows: flow-rate, 0.5 ml/min; column temperature, 25° C; final UV wavelength, 206 nm.

Other analytical methods. The total sugar content was determined by reduction of an alkaline copper tartrate solution with the formation of cuprous oxide. This was oxidized to cupric ion with a sulphuric-ferric solution that is reduced to ferrous ion and, finally, determined permanganometrically (Bertrand method). Sucrose was previously hydrolysed in 10% HCl at 75° C for 20 min [8]. – The total nitrogen content was determined by distillation of ammonia over boric acid and subsequent titration with hydrochloric acid (Kjeldahl method) [9].

The starch-iodine test was implemented according to Lezec and Babin [10] by using solutions containing 6.6 and 3.3 g/l of KI and I_2 , respectively. – Phenolic compounds were analysed colorimetrically at 720 nm after reacting the samples with the Folin-Ciocalteu reagent in an alkaline medium at 70° C for 10 min [11].

Reagents. All reagents used were analytical-grade chemicals and were employed as supplied, without further purification. Organic acids and sugars were purchased from Merck (Darmstadt, FRG) and Sigma (St. Louis, Mo., USA). Solvents used in the mobile phase were of HPLC grade and were also employed as supplied.

Sample preparation. Samples of about 1 kg whole apples were selected. One-quarter of each apple was excised and placed in a blender (sample weight, 250 g). The fruit was covered with an adequate volume of ethanol (1250 ml absolute ethanol) and HCl (1.25 ml) was added to adjust the final alcohol and acid contents to 80 w/w and 0.1% v/v, respectively. The fruit and ethanol were blended for 2 min at high speed. The resulting slurry was refluxed while being stirred under a nitrogen atmosphere for 2 h. The extract was then filtered through a no. 3 gooch crucible and the residue was washed twice with 600 ml 80% ethanol. The combined extracts were concentrated to approximately 250 ml in a rotary evaporator and finally made up to 500 ml with 80% ethanol. – Prior to injection, apple extracts were filtered through a 0.45- μm Millex membrane; the analysis for sugars also required filtering through a Sep Pak C_{18} cartridge (Waters) pretreated with methanol to retain coloured pigments.

Recovery. Acids and sugars were quantified by the external standard method on the basis of peak areas. Each apple extract was analysed in triplicate before and after adding known amounts of both types of product. Three concentration levels of spiked sugars and acids

were analysed. The average recoveries obtained ranged between 91% and 105% for sugars and were always close to 100% for all acids, which testifies to the accuracy of the method used to analyse these solutes. The coefficients of variation for the three replicates of each sample were usually less than 6%.

Results and discussion

We monitored the changes in each of the major sugars (fructose, glucose and sucrose) and sorbitol by HPLC, as well as that of total sugars over the final stages of ripening of five varieties of apples grown in Northern Spain and chosen for their diverse organoleptic properties and industrial significance in the production of juices and ciders. As can be seen from Fig. 1, fructose, sucrose, glucose and total sugars tended to accumulate at the end of the ripening period.

As fructose is the major sugar of apples, the changes in total sugars were similar to that of this sugar. Likewise, the sucrose started to degrade after the optimum ripening time. However, the evolutionary profile of each sugar had its own characteristics. Thus, the Raxao variety showed a fructose maximum 15 days before the optimum processing time, which was determined by fruit consistency, starch content and protopectin/water-soluble pectin ratio; this was also the case with the total phenolic content. On the other hand, the greatest variation in the sugar contents in the Durón Arroes variety was marked by a significant change in the sucrose content in the last stage of ripening. In contrast to the other four varieties, the sucrose content of Meana was always higher than that of glucose. During three consecutive harvests, we observed the same sucrose pattern in the Meana and Durón Arroes varieties. The different pattern of these varieties can be assigned to a variety characteristic linked to less enzymic cleavage activity of sucrose, acid invertase, alkaline invertase and/or sucrose synthase. As can be seen in Fig. 1, no significant changes in the sorbitol concentration were observed during the final stage of ripening.

We also monitored the changes in malic, quinic and shikimic acid throughout the ripening of the five apple varieties and found the greatest variations to occur in the concentrations of the major acids (malic and quinic). Shikimic acid, occurring at much lower concentrations than the other two, underwent only slight changes. We could not determine the presence of citric acid as it was found in small concentrations and was only detected when malic acid occurred at greater concentrations, thereby confirming that changes in both acids follow a similar pattern.

The changes in malic acid (Fig. 2) during the final stage of ripening was marked by a sharp decrease in its concentration, which was concomitant with the final accumulation of sugars (Fig. 1). This seemingly indicates that this acid plays a major role in gluconeogenesis, as reported for other fruits [5]. Likewise, the quinic acid content decreased during the fruit ripening (Fig. 2). This decrease may be the result of the synthesis of chlorogenic acid from quinic and caffeic acid, the latter is previously activated by coenzyme A [12].

Fig. 1. Changes in the total sugar (○), fructose (◇), sucrose (△), glucose (□) and sorbitol (*) contents throughout the ripening of the five apple varieties studied: arrows optimal processing time

We also monitored the polyphenol and total nitrogen contents over the ripening of all five apple varieties. The variation in the total polyphenol content (Folin index) was similar in all of them (Fig. 3), the phenolic compound concentration reaching a minimum prior to the final accumulation of sugars with the exception of the Raxao variety, which, as stated above, showed a maximum coinciding with that of sugars 15 days prior to the end of the ripening period, after which it started to decrease.

This decrease in the polyphenol content appears to arise from the conversion of phenolic compounds of low molecular mass, mainly flavanols, to flavonols of high molecular mass, the extracted level of which under the working conditions should be lower, as reported by Goldstein and Swain [13] for other non-aqueous solvents such as methanol. Likewise, the cinnamic acid ester con-

centrations decrease during the ripening of apples, which also adds to the decrease in the Folin index during that period.

In relation to the changes in total nitrogen content in the five varieties studied (Fig. 3), there was a minimum preceding the final accumulation of sugars.

Finally, the results yielded by the starch-iodine test (Fig. 3) were used to determine the degradation of starch during the fruit ripening period. As can be seen, the starch index decreased as the fruit ripened. Values between 2 and 1 are indicative of strong degradation, the final stage of ripening, and therefore suggest the need for prompt processing of the fruit.

In short, the profile reflecting the changes in the parameters studied was similar for all five apple varieties in the period immediately preceding the optimum degree of

Fig. 2. Changes in the malic (○) and quinic acid (◇) contents throughout the ripening of the five apple varieties studied

ripeness. In addition, the final degradation of starch, the accumulation of sugars and the decrease in malic acid content was preceded by a decrease in the levels of phenol and nitrogen-containing compounds (Fig. 3).

Conclusion

The results obtained in the HPLC monitoring of the sugar and organic acid contents of five apple varieties in their final ripening stage showed the characteristic behaviour of the major sugars, of which fructose was the one most extensively preserved and accumulated at the end of the ripening period. The changes in malic acid and the final accumulation of sugars were also found to be interrelated. The changes in the overall parameters (nitrogen

and polyphenols) over the ripening period was similar for all five varieties, showing a minimum before the optimum ripening period of the fruit. Finally, the starch content was found to decrease in the final stage of ripening, something that is related to the decrease in malic acid and the accumulation of sugars and can thus be considered an accurate indicator of the degree of ripeness of each variety. All the compounds studied underwent significant changes close to the optimal degree of ripeness, so the processing date can significantly condition the production and quality of the derivatives obtained.

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Fig. 3. Changes in the nitrogen (Δ), polyphenol (○) and starch (□) contents throughout the ripening of the five apple varieties studied

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