Influence of Storage on the Composition of Clarified Apple Juice Concentrate

N.E. Babsky, J.L. Toribio, and J.E. Lozano

ABSTRACT

The effect of storage on apple juice concentrate was determined by following changes in composition during a period of 111 days at 37°C. Results showed that storage caused an 87% loss in the total free amino acids, which was mostly due to decreases in glutamic acid, asparagine and aspartic acid. The formol titration method was inadequate for determining the amino compounds involved in Maillard-type reactions. Sucrose was hydrolyzed under these conditions at a rate corresponding to a first order process. The reducing sugars increased at a rate determined by the inversion of sucrose; no consumption attributable to browning reaction was detected. Reduction of organic acids was 9% while apparent phenolic compounds increased from 0.149 to 0.215 g/100g. A maximum accumulation of HMF was observed after 100 days of storage.

INTRODUCTION

DURING STORAGE apple juice is exposed to temperatures which have an adverse influence on quality due to the so-called nonenzymatic browning reaction. Intensive studies of the problem have helped to clarify some of the involved chemical mechanisms and allowed Hodge (1953) to present a lucid, integrated scheme of some of the reactions known to play a role in Maillard-type browning. As knowledge has unfolded, it has become apparent that nonenzymatic browning during storage of apple juice concentrate at relatively high temperatures (Toribio and Lozano, 1984) is highly significant and worthy of study to gain the knowledge necessary to predict and control the color deteriorative process.

In these fruit juices the major constituents believed to be involved in browning are the reducing sugars, amino acids, polyphenols and organic acids (Joslyn, 1956; Cornwell and Wrolstad, 1981). The purpose of the present work was to evaluate changes in the composition of clarified apple juice concentrate during prolonged storage at 37°C. Accumulation of hydroxymethylfurfural (HMF) was also studied to determine the mode and extent of build-up of this product of hexose degradation under the above conditions of storage.

MATERIALS AND METHODS

APPLE JUICE CONCENTRATE (AJC) (72° Brix, pH = 3.51) was obtained from Ind. Cipolletti S.A. (Cipolletti, Rio Negro, Argentina). The juice was stored in aseptic glass vials without head space in the dark at 37 ± 0°C and 40-50 mL samples were extracted each week and stored at -20°C until analyzed.

Compositional analysis

Total phenolics were determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Total acids (titrable acids) were determined by direct titration in accordance with the method reported by the International Federation of Fruit Juice Producers (IFFJP, 1974). Hydroxymethylfurfural (HMF) was also quantitatively determined following the procedure described by the IFFJP (IFFJP, 1974) which is based on the colorimetric reaction between barbituric acid, p-toluidine and HMF.

Table 1—Changes in total acidity, amino-N compounds, total phenolics and sugars during storage of apple juice concentrate

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Acidity index</th>
<th>Total phenolics</th>
<th>Reducing sugars</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100g</td>
<td>g/100g</td>
<td>moles/100g</td>
<td>moles/100g</td>
</tr>
<tr>
<td>0</td>
<td>2.64</td>
<td>0.149</td>
<td>0.286</td>
<td>0.0390</td>
</tr>
<tr>
<td>7</td>
<td>2.58</td>
<td>0.163</td>
<td>0.291</td>
<td>0.0376</td>
</tr>
<tr>
<td>14</td>
<td>2.47</td>
<td>0.157</td>
<td>0.290</td>
<td>0.0319</td>
</tr>
<tr>
<td>21</td>
<td>2.52</td>
<td>0.169</td>
<td>0.291</td>
<td>0.0291</td>
</tr>
<tr>
<td>28</td>
<td>2.52</td>
<td>0.164</td>
<td>0.299</td>
<td>0.0260</td>
</tr>
<tr>
<td>38</td>
<td>2.47</td>
<td>0.164</td>
<td>0.306</td>
<td>0.0287</td>
</tr>
<tr>
<td>45</td>
<td>2.41</td>
<td>0.174</td>
<td>0.233</td>
<td>0.0180</td>
</tr>
<tr>
<td>60</td>
<td>2.41</td>
<td>0.178</td>
<td>0.313</td>
<td>0.0222</td>
</tr>
<tr>
<td>70</td>
<td>2.41</td>
<td>0.186</td>
<td>0.326</td>
<td>0.0268</td>
</tr>
<tr>
<td>84</td>
<td>2.41</td>
<td>0.196</td>
<td>0.338</td>
<td>0.0200</td>
</tr>
<tr>
<td>104</td>
<td>2.35</td>
<td>0.211</td>
<td>0.332</td>
<td>0.0163</td>
</tr>
<tr>
<td>111</td>
<td>2.41</td>
<td>0.215</td>
<td>0.329</td>
<td>0.0160</td>
</tr>
</tbody>
</table>

* Units per 100g apple juice concentrate. Average of two determinations.

Total amino acids were determined according to the formol index (AOAC, 1980). Individual free amino acids were quantified by INTI Laboratories (Miguelete, Buenos Aires, Argentina) with a Beckman amino acid analyzer. Method: As described in Beckman Manual (118/119 BL/CL IM2, 1977). Analysis of physiological fluids.

Soluble sugars were quantified using a Waters model ALC 244 (Waters Associated Inc., Milford, MA) liquid chromatograph equipped with a differential refractometer R401 Unit, Model U6K injector and Model 600A solvent delivery system under the following conditions: ambient temperature, refractive index detector, mobile phase acetonitrile/water (80:20) at 1.5 mL/min and chart speed 0.25 cm/min. Samples were diluted (1.000g concentrate to 12.000g) with double distilled water, filtered through Sep-pak C18 and Millipore 0.45 μM, injected in 20 μL aliquots and quantified by the external standard method. Since the chromatographic system used in this work was unable to separate glucose from sorbitol, the values of glucose reported here should be interpreted as including sorbitol.

RESULTS & DISCUSSION

Acidity

Results presented in Table 1 show that total acidity as malic acid did not significantly change during storage. The role of organic acids appears to be essentially catalytic (Reynolds, 1965). The slight decrease in acidity might be partly due to copolymerization of organic acids with products of the browning reactions. Lewis et al. (1949) also suggested that organic acids can react with reducing sugars to produce brown pigments.

Total phenolic compounds

Phenolic compounds present in fruit products may react to form brown polymeric compounds (Abers and Wrolstad, 1979). If this reaction plays any role in the color development of apple juice, total phenolics content should not increase during storage as Table 1 shows. Cornwell and Wrolstad (1981) proposed that reductone compounds present in the juices interfere with the Folin-Ciocalteau reagent increasing the apparent phenolics contents.
Table 2—Free amino acid composition of apple juice concentrate. Variation during storage

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Storage time, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>40.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>9.6</td>
</tr>
<tr>
<td>Serine</td>
<td>296.8</td>
</tr>
<tr>
<td>Asparagine</td>
<td>8.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>57.6</td>
</tr>
<tr>
<td>Proline</td>
<td>0.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.8</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.7</td>
</tr>
<tr>
<td>Valine</td>
<td>4.3</td>
</tr>
<tr>
<td>Orn, Tyr, Phe, Lys, His, Arg..</td>
<td>trace</td>
</tr>
<tr>
<td>TOTAL</td>
<td>371.2</td>
</tr>
</tbody>
</table>

% Retention

Amino acids

The amino acids composition of apple juice concentrate is shown in Table 2. The major constituents were asparagine (asn), aspartic acid (asp) and glutamic acid (glu). These values are similar to those found by Burroughs (1957) and Czapski (1975). However, Bielig and Hofsommer (1982), working with about 90 samples of apples, apple juices and concentrates found that every apple juice has a characteristic amino acids spectrum and no mean value can be specified. The other individual amino acids amounted to less than 10%. The concentration changes of total amino acids during storage were very large (Table 2). Asp and glu decreased more markedly than all the other amino acids.

Joslyn (1956) indicated that of the many amino acids present in orange juice lysine (lys) and glu were more likely to be involved in browning. Several studies (Warmbier et al., 1976; Spark, 1969; Eichner and Karel, 1972; Reyes et al., 1982) reported Maillard browning of reducing sugars with only one or two amino acids other than asp, asn and glu. Wolfrom et al. (1974) and Ashoor and Zent (1984) studied the influence of different amino acids in model systems. None of these studies have shown glu and asn to be very high browning producing compounds.

The experimental data were fitted to an exponential equation:

\[ AA = 493.23 \exp(-0.0162t) \]  

where: \( AA \) = total amino acids content, mg/100g concentrate; \( r^2 = 0.986 \).

Figure 1 shows the AA value reduction, expressed as percentage of the initial total amino acids content, compared with the nonenzymatic browning (NEB) development, also expressed in a relative way for the same juice at the same conditions (Toribio and Lozano, 1984).

Carbohydrates

Figure 3 shows the hydrolysis of sucrose after 111 days at pH 3.55 and 37°C. It is well known (Glasstone, 1946) that the rate of hydrolysis is a function of the concentration of reactants, temperature and acid-catalyst concentration. However, if excess water is present the rate of disappearance of sucrose can be represented by a pseudo-first order reaction rate equation:

\[ S = S_0 \exp(-Kt) \]  

where: \( S_0 \) = initial sucrose concentration, moles/100g concentrate; \( S \) = sucrose concentration at time \( t \); \( K \) = rate constant, \( t \) = time, min. The experimental data were fitted to this equation, resulting in a value of \( K = 0.00822 \text{ day}^{-1} \). Hydrolysis, also called inversion because it is accompanied by an inversion of the angle of polarization, yields the two simple
COMPOSITION CLARIFIED APPLE JUICE CONC. . .

Fig. 3—Extent of sucrose hydrolysis in apple juice concentrate as a function of time of storage at 37°C: ○ Experimental data; (—) Full line represents Eq. (1).

Fig. 4—Dependence of first order rate constant on pH for sucrose hydrolysis at 37°C: ○ Value of K, this study; (—) Full line represents Schoebel et al. (1969) correlated data.

Fig. 5—Increase of reducing sugars as a function of time of storage: ○ Experimental data; (—) Full line represents Eq. (2).

sugars, D-glucose and D-fructose. The rate of appearance of total reducing sugars is described by Eq. (3):

\[
R = 2S_0 \left(1 - e^{-Kt}\right) + R_0 \tag{3}
\]

where: \(R = \) reducing sugars (glucose + fructose) concentration at time \(t\) moles/100g concentrate; \(R_0 = \) reducing sugar concentration at \(t = t_0\); and \(t = \) time, min.

Experimental data obtained by Schoebel et al. (1969) on the dependence of the first order reaction rate on pH, ranged from 1.7-2.76, were fitted to the exponential curve \(K = a \cdot b^pH\) \((r^2 = 0.996)\) and extrapolated to pH = 4. Figure 4 compares this information with the \(K\) value obtained in this work.

Figure 3 shows the development of total reducing sugars during storage, which increased in concentration in accordance with the predicted kinetics (Eq. 3). Hence, looking at Fig. 3 to 5, hydrolysis appeared to be the major cause of sucrose reduction (and reducing sugars increase) at a rate determined by pH and temperature.

Akhavan and Wrolstad (1980) verified that slight losses (6%) in total sugars occur after 112 days of storage at 37°C of pear concentrate. Stadtmann (1948) considered the possibility that relatively small chemical changes are required to produce brown pigment of intense color. If this is the case, the changes in reducing sugars necessary to produce large changes in color might be hard to be detectable. Beveridge and Harrison (1981) detected no loss of reducing sugar after heating a 72.5° Brix pear juice at temperatures up to 80°C for 2 hr. Reyes et al. (1982) found that glucose undergoes more browning than fructose with glycine at 60°C and pH 3.5. Any detectable variation in the fructose/glucose ratio may indicate unbalanced consumption of these reducing sugars due to nonenzymatic browning reaction. No significant variation was recorded in this study. Mean value was F:G = 1.53 ± 0.05 (w:w) where the range shown is the standard deviation.

HMF

Formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from amino acids and hexoses or from the acidic degradation of hexoses has been widely recognized (Scallet and Gardner, 1945; Reynolds, 1965; Schallenberger and Mattick, 1983). The HMF increase during storage of foods containing hexoses was positively identified and quantified (Keeney and Bassette, 1958; Driilieau and Prout, 1971; Resnik and Chirife, 1978).

Figure 6 shows the HMF increase of the concentrated apple juice at 37°C during 111 days of storage. The rate of accumulation can be divided into three period. First period is characterized as an induction time of approximately 2 wk. During the second period the rate showed a rapid increase of HMF with a maximum at 50 days. After that maximum the rate of formation diminished rapidly, and the HMF production approached a plateau of 44 mg/100g concentrate at approximately 100 days. A similar behavior attributable to a second order autocatalytic reaction (Frost and Pearson, 1961) was also recognized by Schallenberger and Mattick (1983) during the acidic degradation of hexoses. It would appear that after 50 days of storage under the present conditions, HMF started to form brown pigments (melanoidins) at such a rate that 40-50 days...
The chromato graphic observations reported in this work indicated that the amino acids present in apple juice were involved in browning reaction. However, results indicated that they reacted at different rates. For example, glutamic acid and asparagine were reduced 20 times and 10 times, respectively, whereas only half of the initial glycine, leucine and proline were consumed during the storage.

Carbohydrate analysis indicated inversion of sucrose under acidic conditions. No loss of reducing sugars due to nonenzymatic browning was detected.

Results of the present study indicate that in an apple juice concentrate properly produced and stored the HMF content is considerably lower than 10 mg/100g but increases with storage and more than 40 mg/100g were found after 100 days at 37°C.

Rate of formation of HMF resembled that of a second order autocatalytic reaction which was shown to be characteristic of sugar decomposition. In this case simultaneous formation and consumption of HMF by reaction with other compounds seems to have occurred. It could be inferred that HMF stopped accumulating when the rates of formation and disappearance were equal.

REFERENCES


MS received 8/1/85; revised 10/21/85; accepted 11/25/85.

Volume 51, No. 3, 1986—JOURNAL OF FOOD SCIENCE—567