

Kinetics of carbohydrate change during dehydration of d'Agen prunes

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The kinetics of changes in the four major simple carbohydrates (i.e. glucose, fructose, sucrose and sorbitol) present in d'Agen prunes are followed during dehydration. The effect of using different drying temperatures (70–90°C) is investigated. During dehydration of the fresh plum three major regimes may be identified corresponding to the onset of a particular type of reaction. Firstly, acid hydrolysis of sucrose occurs with a rapid decrease in the amount of sucrose in the plum. This happens over a period of 1.5–6 h, depending on the temperature, and corresponds to a moisture loss of 40–50%. During this time, the amounts of both fructose and glucose (hydrolysis products) increase proportionally. The second stage is characterised by degradation of fructose and glucose via Maillard-type reactions with nitrogen-containing compounds in the plum. At this point there is little change in the amount of sorbitol which, because of its structure, does not undergo Maillard reactions. The third stage (occurring after 8–10 h at 80°C) is characterised by loss of all three monosaccharides, including sorbitol. This is due to the onset of caramelisation reactions, which only occur at very low moisture contents in this temperature regime. At the same time it is likely that Maillard reactions are still occurring. The onset of caramelisation at higher temperatures is not as clearly delineated from the start of Maillard reactions as it is at 80°C or lower. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The d'Agen plum is the main variety of plum used in Australia and many other countries for prune production. It is typically smaller than eating varieties, has a more elongated shape and a yellow pulp. It is suitable for prune production because of its high sugar and solid contents. The sugar content and the chemical changes which occur during processing play a very important part in the final quality of the prune.

The d'Agen plum is known to contain three predominant sugars: the reducing monosaccharides glucose and fructose and the non-reducing disaccharide sucrose. The sugar content of plums varies with cultivar. Forni *et al.* (1992) analysed the sugar content of 13 cultivars of plums. Of the individual sugars, glucose and sucrose were always the highest, ranging from 2% to 6%. The fructose content ranged from 0.76% to 3.6%, with the exception of one variety which had a large amount of fructose. The total amount of free sugars ranged from 8% to 14.7%. Dako *et al.* (1970) reported a range in

total sugar content of 6.33–10.8%; Shchrkova and Vitanova (1977) obtained a range of 8.60–13.5%, while Wrolstad and Shallenberger (1981) reported a range of 5.25–13.2%. A preliminary study from this laboratory (Newman, 1994) found somewhat higher sugar concentrations with 'Autumn Giant', an eating variety, having a sugar content of 10–15%, and 'd'Agen' having a sugar content of 20–25%. Other than the above study, no published report has been found on the sugar content of the d'Agen variety.

The sugar alcohol sorbitol has also been reported to be present in the plum. Sorbitol is found in fruit of the family Rosaceae, such as pears, apples, cherries and prunes (Pigman, 1957). The presence of sorbitol in the plum has been noted by a number of workers; Forni *et al.* (1992) reported a range of 1–5.33%, and found there was about as much sorbitol present as fructose among the various cultivars of plums. No relationship was found between sucrose and sorbitol contents; however, the higher the total sugar content, the higher was that of sorbitol. Sorbitol content increases with ripening and is thought to begin to accumulate only after a certain sugar content has been reached (Forni *et al.*, 1992).

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Sorbitol is not a reactant molecule in the Maillard reaction since it lacks the necessary carbonyl group, and is not easily caramelised, thereby preventing excessive browning (Jacobs, 1951) during dehydration. The sorbitol content of the plum should be considered as one of the criteria for the production of a satisfactory dried product.

The aim of the present work was to monitor the changes in carbohydrate during the drying process. Little work on the kinetics of carbohydrate changes has previously appeared. The sugar and sorbitol contents of the d'Agen plum were also determined for the first time. Changes in sugar profiles and pH during drying are presented with a view to determining the type of degradation processes the plum undergoes. Three main types of reaction can be envisaged: acid hydrolysis of the non-reducing sugar sucrose; reactions of reducing sugars with nitrogen-containing compounds such as proteins or free amino acids (Maillard reactions); and thermal degradation (caramelisation) reactions. Sucrose is a non-reducing sugar and therefore does not participate in the Maillard reaction; however, hydrolysis liberates glucose and fructose which can then react by the Maillard scheme. Further investigation of sugar profiles during drying is needed to determine the thermal degradation processes occurring. The information may be useful in making recommendations as to the drying temperatures which could be employed to promote a high-quality dried product.

Most authors report the pH of the plum to be in the region of 3–4. The pH varies between different cultivars. Barbanti *et al.* (1994) compared the pHs of 12 different cultivars of plums and found a range of 3.1–3.7. The d'Agen was not tested. However, the small fruits such as the d'Ente and French prune, to which the d'Agen is most similar, had pH values ranging from 3.3 to 3.6. No study in the literature has been found which examines whether the pH changes with drying. Caramelisation (Kroh, 1994) requires temperatures of $>120^{\circ}\text{C}$ or $9 < \text{pH} < 3$, while the Maillard reaction proceeds (Morton & MacLeod, 1982) effectively at temperatures $>50^{\circ}\text{C}$, depending on the nitrogen-containing compounds present, and is favoured at pH 4–7. Lower pH and high moisture increase the rate of browning in protein–sucrose systems by increasing the rate of sucrose hydrolysis, rather than any subsequent reactions. Determination of the pH therefore provides information on the likely mechanism for sugar degradation since the optimal conditions for non-enzymic browning reactions are known.

This work forms part of a larger project (Newman *et al.*, 1996) studying the prune dehydration process in order to improve the energy efficiency of the dehydration through an enhanced understanding of the factors influencing rate of moisture loss and chemical changes occurring during drying. At present, the Australian prune industry is keen to maintain its competitiveness

with overseas industries, many of which have the advantage of low-cost labour. Research into improved dehydration techniques with a view to producing a higher quality product is required to ensure the Australian prune drying industry can compete effectively with its overseas counterparts. A knowledge of the chemistry of the drying plum will contribute towards a revised protocol for the drying of the d'Agen plum in Australia in order to achieve a better quality dried product with a reduction in the energy costs involved.

MATERIALS AND METHODS

Fruit

The analyses were conducted on the d'Agen plum. The plums were obtained from the growing district at Young, NSW, during the 1995 season. Plums were stored in the refrigerator at $0\text{--}2^{\circ}\text{C}$ and frozen at -20°C . Also, commercial packaged 'Verity Prunes: Fancy d'Agen Australian Dessert Prunes (Large)' which were dried and packaged in Young, were analysed for their sugar content.

Preparation of plum juice for sugar analysis

The plums were destoned, the pulp weighed and diluted with Milli-Q water to give a pulp:water dilution ratio of 1:5. The pulp was mashed in the water and allowed to stand for 30 min before analysis to allow for thorough mixing of the sugars in the pulp with the water. Samples were filtered through a disposable Millipore $0.45\ \mu\text{m}$ filter unit prior to injection onto the chromatography column. The samples were diluted to prolong the lifetime of the column. The sugar composition of plums that had been frozen was determined following thawing overnight at room temperature.

Preparation of dried samples

Plums were dried in a Labec fan-forced oven, consisting of a temperature controller and a standard thermometer inserted in an outlet in the roof of the oven to monitor the drying temperature. Three drying temperatures were employed: 70°C , 80°C and 90°C . These temperatures were chosen to simulate temperatures used commercially. D'Agen plums were sorted into groups of eight and arranged on the trays. Groups of plums were removed at regular intervals during drying. The pulps of the eight plums of each group were combined and diluted by the method outlined for the sugar analysis of fresh samples. Drying of frozen plums was performed at 80°C after thawing overnight.

High-performance liquid chromatographic (HPLC) analysis

The chromatographic system consisted of a Knauer HPLC pump, a Rheodyne injector, a Waters differential refractometric detector (10 mV output, $\times 8$ attenuation) and a Shimadzu C-R6A Chromatopac integrator. A Waters 10 μm Silica-Pak, Radial-Pak cartridge (8 mm i.d. \times 100 mm) for carbohydrate analysis was used in a Waters RCM-100 radial compression module. A C18 precolumn was also connected to prevent sample build-up on the column. The mobile phase consisted of acetonitrile (LC grade), an amide modifier reagent (Waters SAM1), and Milli-Q water in the ratio 77:2:21, filtered and degassed under vacuum. The flow rate was 2 ml min^{-1} . Waters 'SAM1' reagent is designed to separate sugars and related compounds using Waters Silica-Pak chromatographic cartridges.

Standard sugar solutions of fructose, glucose, sucrose and sorbitol were prepared at 4000, 8000, 12000 and 16000 ppm. The standards were filtered using a disposable 0.45 μm filter unit prior to injection onto the column. The sample volume injected was 20 μl . The peak areas for the separated sugars were then obtained from the chromatogram, and three runs were performed at each concentration. This enabled the concentration of sugars in the samples to be determined. For the analysis of plums, the mean of three replications was taken and the sugar concentrations expressed as a percentage of fresh weight of plum. The sugar content of the dried plums was expressed as mg sugar g^{-1} fresh weight. The reasons for expressing the sugar levels in these terms rather than in the more conventional dry weight basis are explained later in the discussion.

pH determination

pH was measured using an Activon AEP312 pH probe and detector. This is a glass body combination pH probe with a Ag/AgCl refillable reference, with filling solution 4 M KCl with AgCl. The method that gave the most reproducible results was to split the plum in half, remove the stone and plunge the probe into one-half of the pulp. The probe was then inserted into the other pulp half and the pH taken as the mean of the two values. The pH was determined for fresh plums, frozen plums and plums dried to 50% weight loss. It was difficult to test the pH of plums dried further since there was not enough flesh to insert the probe into.

Silylation and gas chromatographic-mass spectrometric (GC-MS) analysis

Fractions were collected from relevant peaks of the HPLC trace. The solvent was removed by freeze-drying. Initially, a Dynavac high-vacuum FD-1 freeze-drier was used to lyophilise the bulk of the solvent. To complete the drying, a Savant SVC 100 Speedivac was utilised.

The material obtained was a waxy-looking gel, which probably still contained residual water.

Trimethylsilyl (TMS) derivatives were prepared by reaction with Sil-A reagent (Sigma), composed of trimethylchlorosilane, hexamethyldisilane and pyridine in a ratio of 1:3:9. Sigma Sil-A is used for the preparation of TMS derivatives of amino acids, sugars, alcohols, amines, steroids and phenols. The GC conditions were as follows: column, 30 m \times 0.25 mm coated with 0.25 μm SE-54; temperature programme, 70°C, hold 1 min, then 6°C min^{-1} to 280°C. The carrier gas was hydrogen. For GC-MS analysis the temperature programme was slightly different: 80°C, hold 1 min, then 6°C min^{-1} to 280°C using helium as carrier gas.

RESULTS AND DISCUSSION

Analysis of d'Agen plums: fresh and after storage

Figure 1 shows a sample chromatogram of a typical d'Agen plum with pulp diluted with water in the ratio 1:5. Peak 1 is the water peak, while peaks 2, 3, 4 and 5 are fructose, sorbitol, glucose and sucrose, respectively. The identity of individual sugars was confirmed by comparison with elution times of standard samples and by MS. The sugar content of the d'Agen plums as a function of refrigerated storage time is shown in Table 1. In the first 5 weeks, the changes in sugar content were minimal and no trends were noted during this period of storage. Glucose was found to be the main sugar present with a mean composition of 4.1%. The average sucrose and fructose contents were similar, although sucrose was higher in most plums analysed. The contents of sucrose and fructose were 2.89% and 2.48%, respectively. There was almost as much sorbitol present as glucose, with 4.04% sorbitol. This corresponds well with the range of 1–5.33% for sorbitol found in 13 cultivars of plums by Forni *et al.* (1992). The glucose/fructose ratio of 1.65 is also in accordance with the range of 0.9–5.8 reported by these workers. They also found a glucose/fructose ratio of 1.4–1.8 in d'Ente plums, which are the most similar to the d'Agen.

Changes do start occurring with extended cold storage times. The sucrose content of the d'Agen plums had decreased after 77 days of refrigerated storage, while the concentration of the other sugars remained relatively constant. After 94 days of storage, the sucrose level had decreased further, while the amounts

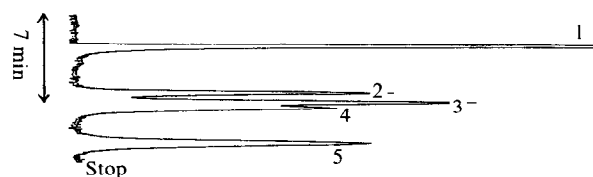


Fig. 1. Chromatogram showing carbohydrate peaks from a sample of a fresh d'Agen plum.

of fructose, glucose and sorbitol present were higher than the average concentrations found in the first 5 weeks.

Plums that had been stored in the freezer (for 20 weeks) were found to have the following composition: 2.78% fructose, 5.35% glucose, 1.37% sucrose, 4.22% sorbitol. The concentration of sucrose in the plum had decreased and in fact was absent from two of the samples analysed. This finding was anticipated because of the cell disruption that occurs upon freezing and subsequent liberation of enzymes such as invertase, which hydrolyses sucrose. The content of other sugars and sorbitol was very similar although slightly increased in comparison to the fresh samples. The increase in glucose and fructose is due to hydrolysis of the disaccharide sucrose into glucose and fructose. The glucose/fructose ratio of 1.94 was slightly elevated in comparison to that for fresh plums.

The packaged prunes were found to have the following sugar composition: 8.13% fructose, 12.32% glucose, 6.17% sorbitol. No sucrose was found in this produce. The total sugar (glucose and fructose) was 20.63%, while the total sugar plus sorbitol was 26.80%. Although the concentration of sugars in the flesh was greater due to loss of water, the glucose/fructose ratio of 1.53 was very similar to that found for fresh plums.

Some workers have reported that sorbitol is present in approximately the same concentration as sucrose; however, no relationship was found between the sorbitol and sucrose contents, in agreement with Forni *et al.* (1992). It was also found that plums with a high sugar content contain much more sorbitol. This was confirmed by the calculation of the linear regression, on 50 plums tested, between sorbitol and total sugars ($R^2 = 74.2\%$; $Y = 4.5770 + 1.1604X$) and between sorbitol and total sugars plus sorbitol ($R^2 = 90.9\%$; $Y = 4.5770 + 2.1604X$).

pH analysis

The pH of d'Agen plums was found to be in the general range reported by most authors of 3–4. The mean pH of the fresh plums tested was 3.63 (standard deviation 0.213) and the mean pH of frozen plums was 3.68 (± 0.11). Thus the process of freezing did not appear to affect the pH. In addition, drying for up to 5 h did not

alter the pH, with the mean pH of dried plums being 3.77 (± 0.13). Of all plums tested, the mean pH was found to be 3.7.

Analysis of sugar profiles during dehydration

Figure 2 shows a change in carbohydrate composition of plums dried at 80°C as a function of drying time. The sucrose completely disappeared from the sugar profile by 4–5 h. This corresponds (Newman *et al.*, 1996) to a moisture loss of 45–50%. Simultaneously with the decrease in sucrose concentration, there was an increase in both fructose and glucose. These two reducing sugars then gradually decrease in concentration over the drying time. Within the experimental error due to natural variation of sugars within the plum (see Table 1), the increase in the reducing sugars is consistent with the breakdown of sucrose into its constituent reducing sugars. This indicates that there is some form of thermal degradation of sucrose occurring, involving the hydrolysis of sucrose to yield glucose and fructose. Figure 2 also shows the change in sorbitol content of plums dried at 80°C as a function of drying time. Although there are fluctuations in the sorbitol content in the early stages of drying, these are probably due to differences in the plums themselves, and the sorbitol content remains relatively constant up until approximately 10 h of drying time, after which it decreases.

The reason for plotting the change in sugar levels on a mass of fresh plum basis, rather than the more usual dry weight, is because it is clear from this work (and other recent work from this laboratory) that, at temperatures of 80°C and above, weight losses other than by evaporation occur. For example, at 90°C the weight loss at 18 h is over 74%, whereas the initial moisture content of these plums is known to be 68% (Sabarez *et al.*, 1996). It was therefore thought best to compare the carbohydrate levels on a fresh weight basis. The weight loss curves are included to enable comparison of the sugar changes with the overall drying process.

Figures 3 and 4 show the drying profiles of plums at temperatures of 70°C and 90°C, respectively. The sucrose has completely disappeared by 6–7 h at 70°C (40% moisture loss) and by 2 h at 90°C (30% moisture loss). However, with the disappearance of sucrose, the same coincident rise in glucose and fructose occurs as

Table 1. Changes in carbohydrates of d'Agen plums during refrigerated storage

Storage days	Fructose (%)	Glucose (%)	Sucrose (%)	Sorbitol (%)	Total sugars and sorbitol (%)
15	2.47 (0.86)	3.71 (0.96)	2.92 (1.54)		
24	2.30 (0.45)	4.02 (0.65)	3.40 (1.99)		
28	2.84 (0.92)	4.98 (1.09)	2.25 (1.34)	3.91 (2.02)	14.0
38	2.29 (0.63)	3.69 (0.55)	2.97 (0.73)	4.17 (1.24)	13.1
77	2.72 (0.94)	4.41 (1.25)	1.44 (1.09)	3.81 (2.40)	12.4
87	2.16 (0.24)	3.97 (0.37)	1.77 (0.59)	3.37 (0.30)	11.3
94	3.05 (0.82)	4.92 (1.15)	1.29 (1.12)	4.56 (0.97)	13.8

Numbers in parentheses represent the standard deviation from the mean.

was observed at 80°C, although at the higher temperature the sorbitol clearly starts to decrease much earlier in the profile, after 3–4 h (equivalent to a moisture loss of about 50%).

Following the disappearance of sucrose from the drying profile, a degradation peak appeared in the chromatogram, which eluted shortly after the normal elution time of sucrose. Figure 5 shows a chromatogram of a plum dried for 6 h. Comparison of this chromatogram with Fig. 1 shows that peaks 1–4 are the same components, peak 5 (the sucrose peak) is absent and there is a large, wide peak in the profile eluting just after the sucrose elution time. This degradation peak was also present in the sugar profile of the packaged 'Verity Prunes', although it was smaller. This may be due to the degradation of these components themselves with time.

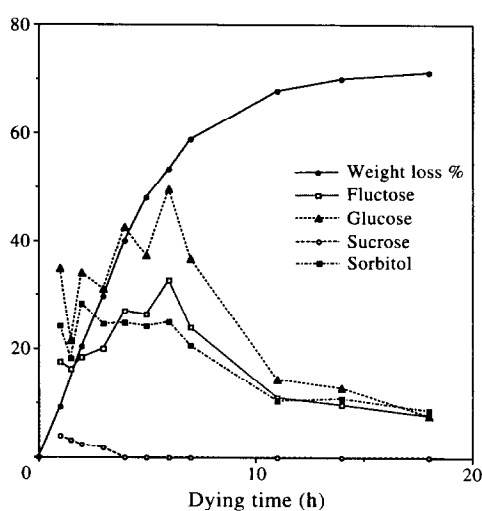


Fig. 2. Changes in amounts of carbohydrate and fruit weight in d'Agen plums dried at 80°C with drying time: □, fructose; ▲, glucose; ○, sucrose; ■, sorbitol (all in mg g⁻¹ fresh plum); ●, weight loss (% of plum).

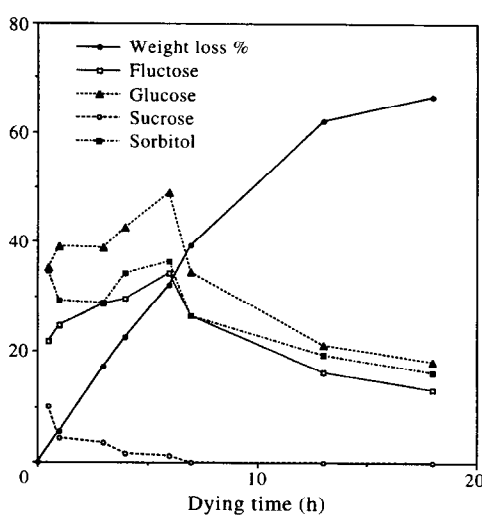


Fig. 3. Changes in amounts of carbohydrate and fruit weight in d'Agen plums dried at 70°C with drying time: □, fructose; ▲, glucose; ○, sucrose; ■, sorbitol (all in mg g⁻¹ fresh plum); ●, weight loss (% of plum).

In the commercial manufacture of these prunes they may be stored for up to 2 years after dehydration before being rehydrated and packaged, after which the shelf-life can be another 2 years.

This poorly defined HPLC peak is probably a complex mixture of degradation products. An attempt was made to investigate it by making silylated derivatives from fractions collected from the HPLC and performing GC-MS analysis. Two of the major peaks were found to correspond to xylose (a pentose sugar) and aspartic acid. The latter is known to be a major amino acid present in plums (Jacobs, 1951). Because of the complex nature of the matrix, a greater number of products from this broad peak might be expected; however, the presence of these two compounds is very informative. Xylose elutes before fructose under the HPLC conditions used, and not after sucrose from where the fractions were collected. The implication, therefore, is that xylose is not present here in its free form but as an adduct. Given the presence of aspartic acid and the nature of the process, it is possible that an adduct between the sugar and the amino acid has been detected. However, the fact that the GC-MS analysis revealed the free silylated species is indicative that the process of silylation has destroyed the adduct. Silylation is a relatively benign procedure, and therefore if the adduct was formed it

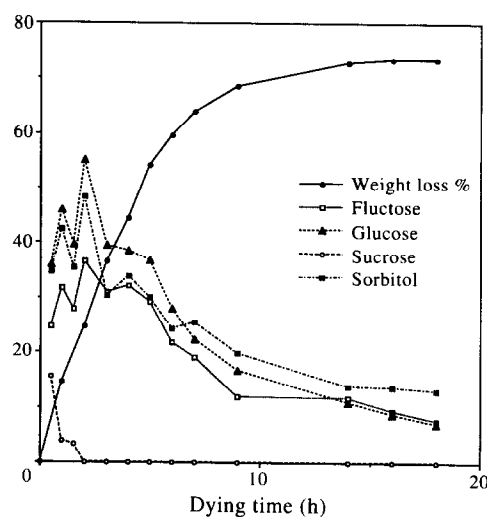


Fig. 4. Changes in amounts of carbohydrate and fruit weight in d'Agen plums dried at 90°C with drying time: □, fructose; ▲, glucose; ○, sucrose; ■, sorbitol (all in mg g⁻¹ fresh plum); ●, weight loss (% of plum).

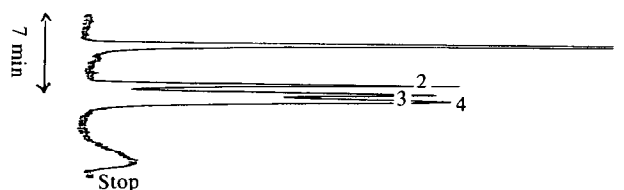


Fig. 5. Liquid chromatogram showing changes in carbohydrate concentration and the appearance of an unknown peak in a plum dried for 6 h at 80°C.

may only be present as part of an equilibrium process. It is likely that what has been formed is a Schiff base adduct (Fig. 6), which is an early step in the Maillard reaction. This process is known to be a reversible equilibrium. Consequently, the silylation would disturb the equilibrium by removal of reactants. In addition, it is known that aspartic acid (Herz & Shallenberger, 1960) reacts with reducing sugars at relatively lower temperatures (around 60°C) compared with other amino acids found in plums. This may explain why this particular amino acid is found in the GC analysis. This evidence, although speculative, does suggest that the rapid drop in glucose and fructose in the drying plum after the disappearance of sucrose is due to the onset of Maillard reactions, Schiff base formation being an early product in this complex chain.

It is known that sorbitol does not undergo the Maillard reaction, so the fact that the amount of sorbitol in the drying plum remains constant (Fig. 2) during the decrease in glucose and fructose is consistent with this. After 8 h of drying at 80°C, the sorbitol starts to decrease. At this stage the water loss is about 60%, and it is likely that the water activity within the prune is sufficiently low to allow caramelisation reactions to proceed even at 80°C. At the highest temperature used (90°C), sorbitol decreases much earlier in the profile, starting after 3–4 h. This indicates that thermal degradation reactions such as caramelisation are occurring. There is, therefore, a less clearly defined onset of the different mechanisms for sugar loss at 90°C.

The kinetics of sugar changes during drying of d'Agen plums in the temperature range considered may thus be best summarised by defining three regimes. These three phases in the drying cycle are very clearly delineated at 80°C, which is close to the conditions used commercially. Up to a water loss of 40–45%, the predominant reaction is acid hydrolysis of sucrose (plum

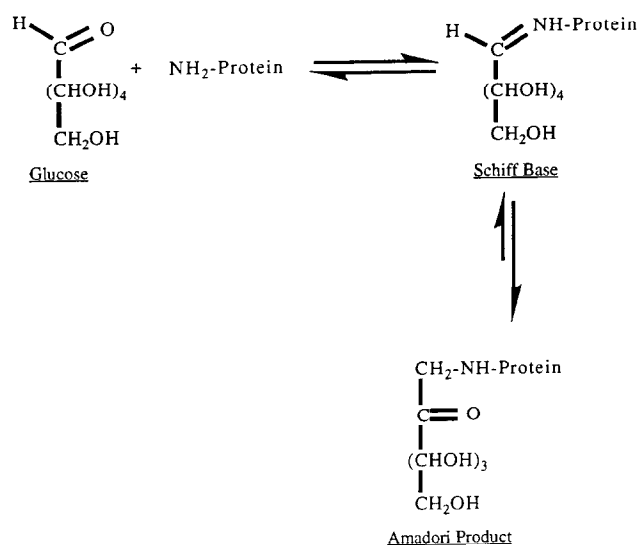


Fig. 6. Formation of a Schiff base—part of the Maillard reaction scheme.

pH of 3.6) exhibited by a loss of sucrose and a corresponding increase in fructose and glucose. During this early stage, there is little evidence of other sugar degradation reactions. At about 45–50% water loss, Maillard reactions start to occur, evidenced by the rapid loss of fructose and glucose (the constant amount of sorbitol) and the appearance of an additional peak in the HPLC trace. This second regime continues to dominate proceedings until at a water loss of about 60% (after 8–10 h at 80°C) conditions are sufficiently extreme to allow caramelisation to commence (third phase). At higher temperature, the divisions tend to merge.

CONCLUSIONS

The changes in sugars have been followed during drying of d'Agen plums. The drying seems to be best described as a three-regime process. The delineation between these regimes is clear at 80°C and less so at higher temperatures. Commercial driers use temperatures close to 80°C. There are a number of alternative reactions that the sugars present may undergo. Perhaps the most important factor in deciding which reaction might dominate during any stage is the pH. The pH found in the d'Agen plums is similar to that obtained by Barbanti *et al.* (1994) for d'Ente prunes. The pH of the plums is not in the range optimal for either Maillard or caramelisation reactions. However, low pH and high moisture, present in the early stages of drying, favour the hydrolysis of the disaccharide sucrose, liberating glucose and fructose. This glucose and fructose subsequently undergo Maillard degradation reactions. In the later stages of drying, the loss of glucose, fructose and sorbitol is quite rapid. Although caramelisation generally requires higher temperatures and sorbitol does not react readily, these reactions may occur in the later stages of drying when the concentration of water in the plum is very low.

It would be interesting to investigate the effect of plum pH upon the changes in carbohydrate levels during drying. This is in progress.

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