ELSEVIER

Food Chemistry 64 (1999) 89–93

Food Chemistry

# Nonenzymic browning reactions in boiled grape juice and its models during storage

Hüseyin Bozkurt [a], Fahrettin Göğüş [a], Sami Eren [a]*

[a]Gaziantep University, Department of Food Engineering, 27310 Gaziantep, Turkey

Received 26 September 1997; received in revised form 6 March 1998; accepted 6 March 1998

**Abstract**

Reaction orders, rate constants and activation energies were evaluated for 5-hydroxymethyl furfural (HMF) accumulation and brown pigment formation (BPF) in pekmez and its model systems stored at 55, 65 and 75°C over 10 days at pH 4.0. The model systems and their compositions were decided by considering the major pekmez components. Accumulation of HMF and BPF were the highest in pekmez and they were the smallest in model 1. ANOVA showed that the effects of temperature on both accumulation of HMF and BPF were significant ($p < 0.05$). Results obtained from non-linear regression analysis indicated that reaction order was 0.5 for 5-HMF accumulation and was zero for brown pigment formation in the model systems. Reaction orders were zero for both HMF accumulation and BPF in pekmez. Calculated activation energies for HMF and brown pigment formation were in the range of 49.7–103 kJ mol$^{-1}$ and 116–132 kJ mol$^{-1}$ respectively. 

## 1. Introduction

It has long been known that fruit juices darken during harvesting, transporting, processing and storage. Concentrates having more than 65% of total solids are normally stable to fermentation at any temperatures, but when stored at relatively high temperatures or for a long time, nonenzymic browning reactions take place.

Pekmez is the common name of boiled juice prepared from some fruits such as grape and mulberry in Turkey. It is produced by concentration of juice up to 70–80% soluble solids content. Grape pekmez contains 100 mg kg$^{-1}$ iron and 4000 mg kg$^{-1}$ calcium (Artik and Velioğlu, 1993).

The composition of grape juice is given in Table 1. Grape juice contains 18 amino acids. Eight of them, the highest ones in grape juice, are given in Table 1. The major ones are arginine, glutamine and proline (Drawert and Tressl, 1977). The juice contains high amounts of glucose and fructose in almost equal quantities, no sucrose and very small amounts of protein (Artik and Velioğlu, 1993).

Browning is the most common quality problem in pekmez, as in most concentrated fruit juices. Nonenzymic browning reactions of pekmez occur either by caramelization or the Maillard reaction. Caramelization in pekmez occurs by the decomposition of sugars at high temperatures (Hodge, 1953). The Maillard reaction takes place between amino acids and reducing sugars present in the pekmez. It results in undesirable colour, odour and flavour changes (Pribella and Betusawa, 1978; Toribio and Lozano, 1986) and is followed by the formation of intermediates such as 5-hydroxymethyl furfural (HMF), particularly under acidic conditions, (Lozano, 1991; Göğüş et al., 1998) and, finally, brown pigment formation (BPF) (Hodge, 1953).

The HMF is potentially a polymer building block and is an indicator of the Maillard reaction and of potential browning (Lee and Nagy, 1988). In fresh foods, the HMF level is close to zero (Babsky et al., 1986). However, it is found to be at a significant level in processed foods and so is often used as a quality indicator (Lee and Nagy, 1988; Cohen et al., 1994). HMF is also used as one of the quality parameters of pekmez (Göğüş and Eren, 1997). Maximum HMF limit for the first quality pekmez is 25 mg HMF per kg of pekmez (TS 3792, 1983).

The objectives of this work are: (1) to determine the HMF accumulation and BPF in pekmez and model systems during storage; (2) to determine any similarities or differences that exist between the pekmez and its

* Corresponding author.

0308-8146/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved
PII: S0308-8146(98)00081-8

Table 1
Composition of grape juice

| Components | Composition |
|---|---|
| Free amino acids[a] | |
| Arginine | 1047 mg $l^{-1}$ |
| Proline | 449 mg $l^{-1}$ |
| Glutamine | 210 mg $l^{-1}$ |
| Alanine | 60 mg $l^{-1}$ |
| Glutamic acid | 58 mg $l^{-1}$ |
| Threonine | 49 mg $l^{-1}$ |
| Asparagine | 44 mg $l^{-1}$ |
| γ-Aminobutyric acid | 30 mg $l^{-1}$ |
| Sugars[b] | |
| Fructose | 98 g $l^{-1}$ |
| Glucose | 106 g $l^{-1}$ |
| Sucrose | 0 |
| Ash[c] | 0.3 g $kg^{-1}$ |
| Fat[c] | Trace |
| Protein[c] | 0.2 g $kg^{-1}$ |
| Minerals[c] | |
| Calcium, Ca | 11 mg $kg^{-1}$ |
| Iron, Fe | 0.3 mg $kg^{-1}$ |
| Sodium, Na | 2 mg $kg^{-1}$ |
| Phosphorus, P | 12 mg $kg^{-1}$ |
| Potassium, K | 116 mg $kg^{-1}$ |

[a] Drawert and Tressl (1977).
[b] Bielig et al. (1982).
[c] Karakaya and Artik (1990).

model systems according to their HMF accumulation and BPF; and (3) to determine the kinetic parameters for both HMF accumulation and BPF in pekmez and model systems.

## 2. Materials and methods

All chemicals used were of analytical grade. The amino acids, arginine and proline and the reducing sugars, glucose and fructose were purchased from Merck (Germany). Glutamine was purchased from Sigma Chemical Co. Ltd. (USA). The chemicals p-toluidine, glacial acetic acid, HCl, barbituric acid, HMF and 2-propanol (Merck, Germany), potassium ferrocyanide, zinc acetate and iodine (Reidel De-Haen, Germany) and starch (Pancreac, Spain) were used for HMF analysis.

### 2.1. Preparation of pekmez

Grapes (*Vitis vinifera*, L.; variety Sultana) were purchased from the local market (Gaziantep, Turkey). Grape juice was prepared by crushing and pressing the grapes. Prior to the concentration of the grape juice (pH 3.5) from 18 to 70°Brix, it was clarified by using bentonite (Sigma) and gelatine (Sigma) as clarifying agents and filtered through Whatman No. 41 filter paper. Filtered grape juice had a pH of ~4.0. A traditional heat treatment (concentration) method was applied for the preparation of the boiled grape juice (in open pan at 97–98°C for 3 h). The pH of boiled grape juice was pH 3.9 and was adjusted to pH 4.0 prior to the storage.

### 2.2. Preparation of model systems

Table 1 shows the major components of the grape juice. As seen, the glucose and fructose are the only sugars and proline, arginine and glutamine are major amino acids of grape juice and so pekmez. Thus, they were chosen to represent major components which are responsible for the nonenzymic browning reaction in pekmez. Model system 1 was prepared to determine the net effect of caramelization reaction, so only sugars (98 g $litre^{-1}$ fructose and 106 g $litre^{-1}$ glucose) which are present in the grape juice were used. Model system 2 was prepared to observe the effect of major Maillard reactants on the nonenzymic browning of grape juice during the processing and accelerated storage periods. It contained two sugars (98 g $litre^{-1}$ fructose and 106 g $litre^{-1}$ glucose) and three amino acids (1047 mg $litre^{-1}$ arginine, 210 mg $litre^{-1}$ glutamine and 449 mg $litre^{-1}$ proline). All the model solutions were adjusted to pH 4.0 by the addition of HCl (1 M) and they were concentrated by following the procedure as above.

The concentrates (70°Brix) of the models and pekmez with a pH of 4.0 were distributed into glass tubes in duplicate and closed tightly and held in a temperature-controlled laboratory oven (Nüve ES 500) at three different temperatures (55, 65 and 75°C). Tubes were removed periodically from the oven. The samples were analysed for both HMF accumulation and BPF and their pH was measured. Change in pH was not significant during storage of the samples.

BPF was followed by measuring the colour as absorbance. After the proper dilution of 1 g sample with triple distilled water, the absorbance was read at 420 nm by spectrophotometer (Spectronic-Bausch 20).

HMF was determined quantitatively following the procedure described by the IFFJP (1964) based on the colorimetric reaction between barbituric acid, p-toluidine and HMF forming a red coloured complex. The intensity of red colour is dependent upon the amount of HMF. HMF analysis was performed by measuring the red colour at 550 nm with a Spectronic-Bausch 20 spectrophotometer. A calibration curve of HMF was utilized to quantify HMF concentrations. All measurements were made in duplicate.

Regression analyses (Sigma Plot 41) were performed to determine the reaction orders and the rate constants for both HMF accumulation and BPF.

Statistical analyses using ANOVA and multiple range test (LSD) were carried out by using the Statgraph statistical package within the 95% confidence interval. Statistical analyses were performed for the systems during the 10 day period with respect to their HMF accumulation and BPF for all temperatures.

## 3. Results and discussion

Figs. 1 and 2 show the accumulation of HMF and BPF in model systems and pekmez at 75°C. As mentioned above, model 1 contained only sugars to identify the effect of caramelization on the total rate of browning in more complicated systems (model 2 and pekmez). Two-way ANOVA (temperature×time) and multiple range test (LSD) were applied to see the net effect of temperature on both HMF accumulation and BPF rate during the 10 day period at three different temperatures (55, 65 and 75°C). ANOVA showed that the effect of temperature on accumulation of both HMF and BPF was significant ($p < 0.05$). Results of the multiple range test showed that increasing the temperature from 55 to 75°C significantly affected ($p < 0.05$) the rates of both HMF accumulation and BPF. The effect of caramelization on the rate of HMF accumulation, in model 1, at 55°C was not significant ($p > 0.05$) at 95% confidence interval. However, it was significant ($p < 0.05$) at 65 and 75°C. This means that the net effect of caramelization reaction on HMF accumulation was not so high at 55°C and its effect increased with increasing temperature. The presence of the three amino acids with two reducing sugars, model 2, that are the major reactants of the Maillard reaction increased both HMF accumulation and BPF. The increase in HMF accumulation and BPF in this model was significant ($p < 0.05$) at all temperatures (55, 65 and 75°C). Therefore, it can be concluded that the contribution of the Maillard reaction to the formation of brown pigments and accumulation of HMF was greater than the caramelization reaction.

As seen in Figs. 1 and 2, the accumulations of HMF and BPF in pekmez are higher than those in models although they contain major components which are responsible for the browning of pekmez. The differences between their HMF accumulations and BPF rates may be explained by the minor components in pekmez. It contains high amounts of some metal ions, such as iron (Karakaya and Artik, 1990) and phenolic substances (Cheynier et al., 1988). The metal ions and phenolic substances were found to have an accelerator effect on the browning reaction (Labuza and Baisier, 1993, Cheynier et al., 1988). In addition to this, HMF amounts formed during the processing of the models and pekmez are highly different (Fig. 1). This reactive intermediate accelerates the rate of browning reaction during the further storage period (Figs. 1 and 2). Concentration of these products (HMF and BP) were the highest in boiled grape juice and they were the smallest in model 1.

These results indicate that modelling of the food systems is quite difficult and the models prepared do not show the same characteristics as in the original food. Some modelling studies of the food systems related to

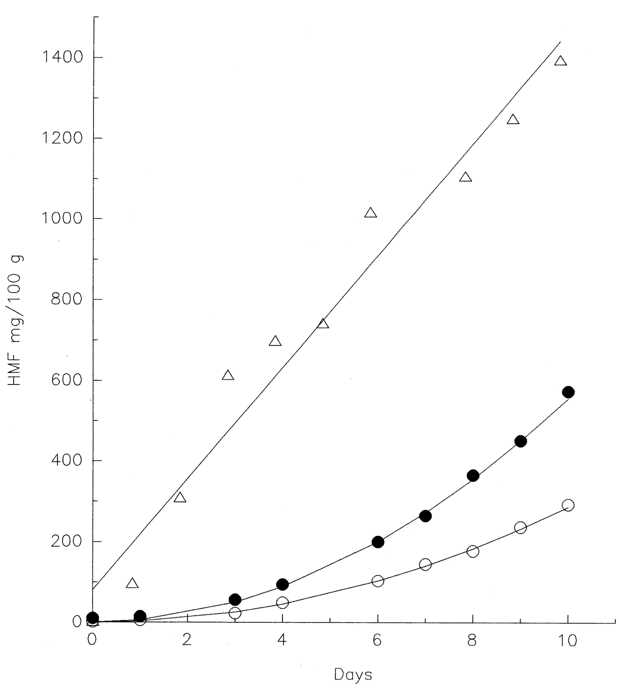


Fig. 1. Accumulation of HMF in pekmez and its model systems at 75°C and pH 4.0 (symbols refer to the experimental data, the lines refer to the predicted model). △, Pekmez; ○, Model 1; ●, Model 2.

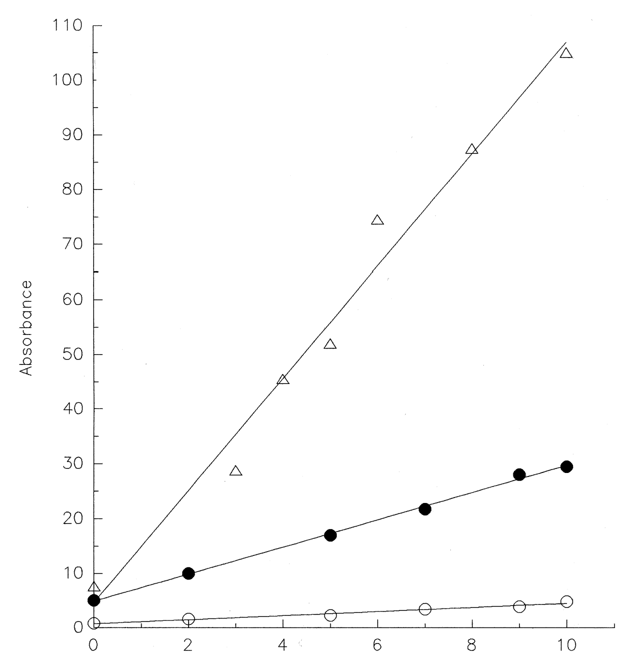


Fig. 2. Brown pigment formation in pekmez and its model systems at 75°C and pH 4.0 (symbols refer to the experimental data, the lines refer to the predicted model). △, Pekmez; ○, Model 1; ●, Model 2.

the browning reactions are reported in the literature. The model(s) used in these studies have been selected according to the major component(s) of the foods represented. In most of these modelling studies, only sugars (Buera et al., 1987a., Lee and Nagy, 1990), or sugars and amino acids (Buera et al., 1987b; Wong and Stanton, 1989) were used as the components. Furthermore, the concentrations of the selected components in these models are not realistic to simulate real food products (Tsai et al., 1991; Lee et al., 1979). As a result, it can be concluded that the model systems with few components of the food products are not suitable to represent the nonenzymic reactions occurring in the real food systems because the presence of a minor component which is not available in the model system can accelerate or decelerate the reaction rate in the food during processing or storage.

### 3.1. Reaction kinetics of HMF accumulation and BPF

HMF accumulation in different systems was followed as a function of pH (Cerrutti et al., 1985), temperature (Lozano, 1991) and water activity (Petriella et al., 1985). It was found that the temperature effect was very strong on the accumulation of HMF. Hodge (1953) explained, in his classical scheme for Maillard reactions, that the pathway giving the intermediates leading to HMF favours low pH.

The following equation was used to fit both HMF accumulation and BPF data by using non-linear regression analysis (two-parameter fitting of $n$ and $k$).

$$n \neq 1 C^{(1-n)} - C_0^{(1-n)} = (1-n)kt$$

where $C$ is the concentration at time $t$, $C_o$ is the concentration at time zero, $n$ is the reaction order, $k$ is the rate constant and $t$ is the time. $C_o$ values were calculated to check the suitability of the model. It was found that the calculated $C_o$ values were in agreement with the experimental results.

The reaction orders, rate constants, their correlation coefficients ($R^2$) and 95% confidence intervals for HMF accumulation and BPF are given in Tables 2 and 3 respectively. As seen in Table 2, reaction orders for HMF accumulation were found to be 0.5 and zero for models and pekmez, respectively. Reaction order for BPF was zero for all systems. These results are in agreement with those of previous studies (Cohen et al., 1994; Buera et al., 1987a).

It was reported in the literature that the reaction order of HMF accumulation was found to be changing from 0 to 2 depending on the reactants and the experimental conditions (Peleg et al., 1992; Shallenberger and Mattik, 1983; Körmandy et al., 1994). The results found previously suggest that the reaction order for HMF is flexible. Reaction order for BPF was found to be zero for monosaccharides (Buera et al., 1987a) and for sugar-amino acid systems (Buera et al., 1987b).

The Arrhenius relationship was applied to find the activation energies (Ea). Table 4 shows the calculated activation energies, their 95% confidence intervals and correlation coefficients.

The activation energies for BPF in the systems were in the range of 116–132 kJ mol$^{-1}$ which is within the range (105–210 kJ mol$^{-1}$) of typical Ea for nonenzymic browning of foods reported by Saguy and Karel (1980). It was found that the activation energy for caramelization of glucose + fructose (model 1) was 116 kJ mol$^{-1}$

Table 2
The reaction orders, rate constants (mg HMF/100 g sample/days) with their 95% confidence intervals and $R^2$ values for HMF accumulation in the systems

| | HMF Accumulation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 55°C | | | 65°C | | | 75°C | | |
| Systems | $n$ | $k$ | $R^2$ | $n$ | $k$ | $R^2$ | $n$ | $k$ | $R^2$ |
| Pekmez | 0 ± 0.01 | 15.5 ± 1.05 | 0.98 | 0 ± 0.01 | 50.4 + 21.34 | 0.99 | 0 ± 0.01 | 136 ± 9.53 | 0.98 |
| Model 1 | 0.59 ± 0.02 | 1.21 ± 0.13 | 0.99 | 0.55 ± 0.01 | 1.59 ± 0.04 | 1.00 | 0.54 ± 0.01 | 3.47 ± 0.26 | 0.99 |
| Model 2 | 0.57 ± 0.01 | 1.75 ± 0.16 | 0.99 | 0.56 ± 0.01 | 2.75 ± 0.08 | 1.00 | 0.55 ± 0.01 | 5.43 ± 0.14 | 0.99 |

Table 3
The reaction orders, rate constants (OD/days) with their 95% confidence intervals and $R^2$ values for BPF in the systems

| | Brown pigment formation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 55°C | | | 65°C | | | 75°C | | |
| Systems | $n$ | $k$ | $R^2$ | $n$ | $k$ | $R^2$ | $n$ | $k$ | $R^2$ |
| Pekmez | 0 ± 0.01 | 0.63 ± 0.079 | 0.97 | 0 ± 0.01 | 2.72 ± 0.170 | 0.99 | 0 ± 0.01 | 10.2 ± 0.66 | 0.99 |
| Model 1 | 0 ± 0.01 | 0.03 ± 0.001 | 0.99 | 0 ± 0.01 | 0.15 ± 0.012 | 0.97 | 0 ± 0.01 | 0.38 ± 0.029 | 0.99 |
| Model 2 | 0 ± 0.01 | 0.19 ± 0.015 | 0.98 | 0 ± 0.01 | 0.57 ± 0.039 | 0.98 | 0 ± 0.01 | 2.49 ± 0.058 | 0.99 |

Table 4
Activation energies of the systems with their 95% confidence intervals and $R^2$ values

| | HMF accumulation | | Brown pigment formation | |
|---|---|---|---|---|
| Systems | Ea (kJ $mol^{-1}$) | $R^2$ | Ea (kJ $mol^{-1}$) | $R^2$ |
| Pekmez | $103 \pm 3.2$ | 0.99 | $132 \pm 1.6$ | 0.99 |
| Model 1 | $49.7 \pm 3.1$ | 0.92 | $116 \pm 1.9$ | 0.99 |
| Model 2 | $53.5 \pm 2.8$ | 0.98 | $122 \pm 2.9$ | 0.99 |

for BPF. This activation energy value is in accordance with the published data for this type of reaction (Lozano, 1991). The results found for Maillard reactions of glucose + fructose with triple amino acids (model 2) were slightly higher than those of the caramelization. It was also noted by Buera et al. (1987b) that the activation energies of caramelization reactions are similar to those of Maillard reactions in single sugar and amino acid systems.

## 4. Conclusions

Accumulation of HMF and BPF were the highest in pekmez. The effects of temperature on both HMF accumulation and BPF in pekmez and its model systems were significant ($p < 0.05$). Reaction orders of models and pekmez were 0.5 and zero, respectively, for HMF accumulation. Reaction order of all systems was zero for BPF. The activation energies were in the range of 116–132 kJ $mol^{-1}$ for BPF and in the range of 50–103 kJ $mol^{-1}$ for HMF accumulation in the systems.

## References


Artik, N. and Velioğlu, S. (1993) Bazi pekmez örneklerinin standarda uyğunluğunun belirlenmesi üzerine araştirma. *Standard* **32**, 51–54.

Babsky, N. E., Toribio, J. L. and Lozano, J. E. (1986) Influence of storage on the composition of clarified apple juice concentrate. *J. Food Sci.* **51**, 564–567.

Bielig, H. J., Fethe, W., Koch, J., Wallrauch, S. and Wucharpfennig, K. (1982) Richtwerte und schwankunsbreiten bestimter kennzahlen (RSK-werte) für Apfelsaf, Traubensaf und Orangesaft. *Flüss Obst.* **49**, 188–199.

Buera, M. P., Chirife, J., Resnik, S. L. and Lozano, R. D. (1987a) Nonenzymatic browning in liquid model systems of high water activity. Kinetics of color changes due to caramelization of various single sugars. *J. Food Sci.* **52**, 1059–1062.

Buera, M. P., Chirife, J., Resnik S.L. and Wetzler, G. (1987b) Nonenzymatic browning in liquid model systems of high water activity. Kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. *J. Food Sci.* **52**, 1063–1067.

Cerrutti, P., Resnik, S. L., Seldes, A. and Fontan, C. F. (1985) Kinetics of deteriorative reactions in model food systems of high water activity: glucose loss, 5-hydroxymethylfurfural accumulation and fluorescence development due to nonenzymatic browning. *J. Food Sci.* **50**, 627–630.

Cheynier, V., Osse, C. and Rigaud, J. (1988) Oxidation of grape juice phenolic compounds in model solutions. *J. Food Sci.* **53**, 1729–1732.

Cohen, E., Birk, Y., Mannheim, C. H. and Saguy, I. S. (1994) Kinetic parameter estimation for quality change during continuous thermal processing of grapefruit juice. *J. Food Sci.* **59**, 155–158.

Drawert, F. and Tressl, R. (1977) Moderne physikalic chemsche methoden und ihre anwerdung auf probleme der biogenese von aromastoffen. *Ernahrungs-Unschau.* **17**, 392–400.

Göğüş, F. and Eren, S. (1997) Pekmez imalati esnasinda esmerleşme reaksiyonlarinin hizinin belirlenmesi. *Gida Teknoldjisi* **2**, 34–39.

Göğüş, F., Bozkurt, H. and Eren, S. (1998) Nonenzymic browning reactions in multi sugar and amino acid systems. *J. Food Process. Preser.* (in press).

Hodge, J. E. (1953) Dehydrated foods. Chemistry of browning reactions in model system. *J. Agric. Food Chem.* **1**, 928–943.

IFFJP (International Federation of Fruit Juice Producers Methods) (1964) Analysen-analyses; Fruit-Union Suisse Assoc. *Svizzera Frutta Zug Switzerland* **12**, 1–4.

Karakaya, M. and Artik, N. (1990) Zile pekmezi üretim tekniği ve bileşim unsurlarinin belirlenmesi. *Gida* **15**, 151–154.

Körmandy, I., Patkai, Gy., Senyi, J. and Gion, B. (1994) Examination of the usefulness of emprical kinetic equations for describing the formation of 5-HMF in grapefruit juice. *J. Food Eng.* **23**, 519–531.

Labuza, T. P. and Baisier, W. M. (1993) The kinetics of nonenzymatic browning. In *Physical Chemistry of Foods*, eds. H. G. Schwartzberg and R. W. Hartel, pp. 595–649. Marcel Dekker Inc., New York.

Lee, H. S. and Nagy, S. (1988) Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technol.* **42**, 91–97.

Lee, H. S. and Nagy, S. (1990) Relative reactivities of sugars in the formation of 5-hydroxymethyl furfural in sugar-catalyst model systems. *J. Food Process. Preser.* **14**, 171–178.

Lee, C. M., Lee, T. C. and Chichester, O. C. (1979) Kinetics of the production of biologically active Maillard browned products in apricot and glucose-L-tryptophan. *J. Agric. Food Chem.* **27**, 478–482.

Lozano, J. E. (1991) Kinetics of non enzymatic browning in model systems simulating clarified apple juice. *Lebensm.-Wiss. und-Technol.* **24**, 335–360.

Peleg, H., Naim, M., Zehavi, U., Rouseff, R. L. and Nagy, S. (1992) Pathway of 4-vinylguayacol formation from ferulic acid in model solutions of orange juice. *J. Sci. Food Agric.* **40**, 764–767.

Petriella, C., Resnik, S. L., Lozano, R. D. and Chirife, J. (1985) Kinetics of deteriorative reactions in model systems of high water activity: color changes due to nonenzymatic browning. *J. Food Sci.* **50**, 622–626.

Pribella, A. and Betusawa, M. (1978) Veranderungen in gehalt on sticktoffaltingen soften bei der lagerung von obstsaft-konzentraten. *Fruchtsaft-Industrie* **9**, 15.

Saguy, I. and Karel, M. (1980) Modelling of quality deterioration during food processing and storage. *Food Technol.* **37**, 78–85.

Shallenberger, R. S. and Mattik, L. R. (1983) Relative stability of glucose and fructose at acidic pH. *Food Chem.* **12**, 159–165.

Toribio, J. L. and Lozano, J. E. (1986) Heat induced browning of clarified apple juice at high temperatures. *J. Food Sci.* **51**, 172–175.

Tsai, C. H., Kong, M. S. and Pan, B. S. (1991) Water activity and temperature effects on nonenzymic browning of amino acids in dried squid and simulated model system. *J. Food Sci.* **56**, 665–677.

TS 3792 (1983) *Boiled grape juice*. Turkish Standard Institute.

Wong, M. and Stanton, D. W. (1989) Nonenzymic browning in kiwifruit juice concentrate systems during storage. *J. Food Sci.* **54**, 669–673.