A Rapid Method to Monitor Quality of Apple Juice During Thermal Processing

E. Cohen†, Y. Birk, C. H. Mannheim and I. S. Saguy*

E. Cohen, Y. Birk, I. S. Saguy: Institute of Biochemistry, Food Science and Nutrition and Faculty of Agricultural Food and Environmental Quality Sciences, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100 (Israel)
C. H. Mannheim: Department of Food Engineering and Biotechnology, Technion Israel Institute of Technology, Haifa 32000 (Israel)
(Received December 1, 1997; accepted March 2, 1998)

Quality deterioration of apple juice, thermally processed at constant temperatures ranging from 95 to 123 °C for 2 to 30 min, was measured by three quality indexes: nonenzymatic browning (NEBI), 5-hydroxymethylfurfural (HMF) and fluorescence relative index (FLRI). All three indexes followed an apparent zero-order reaction with activation energies of 148.6, 151.1 and 148.6 kJ/mole for NEBI, HMF and FLRI, respectively. FLRI correlated highly with HMF (R^2 = 0.98). The correlation between HMF and FLRI, obtained under constant temperatures, was verified under continuous process conditions of 85 to 135 °C and 30 to 180 s. FLRI was found to be a quantitative criterion, which could be applied for 'on-line' monitoring of the deleterious effects of thermal processing of apple juice.

Keywords: kinetics; browning; HMF; fluorescence

Introduction

Thermal processing of liquid foods is a widely utilized procedure in the food industry. Usually, the time and temperature required for a safe process depend upon the destruction of microbial spores and inactivation of enzymes. Typical deleterious quality changes occurring during thermal processing include nonenzymatic-browning, formation of undesired components, and degradation of vitamins and carbohydrate. The common approach to predict quality changes of a food system is to define a parameter, or an index of deterioration, that can be evaluated by a physical or chemical measurement whose decrease or increase correlates with quality (1). This index has to be sensitive enough to express the effect of the process on the quality. Nonenzymatic browning is considered as one of the major causes of quality loss and, therefore, a useful indicator of temperature abuse. Nonenzymatic browning reactions are of great significance in food stability and play an important role in most quality losses, e.g. discoloration, formation of off-flavour, nutrient losses (2–10).

Hydroxymethylfurfural (HMF) is an intermediate product in the formation of brown pigments during the Maillard reaction between hexoses and amino components, occurring during processing and storage (11–16). Before a visible brown colour develops, the early stages of the reaction are highly significant for gaining information necessary for predicting and controlling the extent of the browning reaction (17). The process of formation of fluorescence compounds is typically more sensitive than off-flavour, browning and colour development during processing and storage of foods, so the utilization of this index has been suggested for correlation with quality losses (18). However, only limited studies have monitored the formation of fluorescence compounds as an index to quality deterioration.

Several methods for quantifying the development of browning components have been suggested, e.g. high-performance liquid chromatography, colorimetric and spectrophotometric measurements (7, 19). Absorbency has been suggested as a quick and easy method for monitoring the extent of the reaction (18). These methods are laborious and time consuming. Our main objective was to develop a fast and easy-to-use fluorescence method that could be implemented for monitoring quality deterioration of apple juice during thermal processing.

†Current affiliation: Department of Hotel and Tourism Management, Ben-Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel.
*To whom correspondence should be addressed.

0023-6438/98/070612 + 05

Materials and Methods

Thermal processing

Commercially concentrated frozen apple juice was reconstituted to 13 °Bx, and thermally processed under various conditions which included both batch (nonflow) and continuous processes. Batch processing was carried out using stainless steel coils (1.0 m length, 6.3 mm outside diameter, 0.9 mm thickness) placed in a polyethylene glycol 400 bath. The coils were closed with screw caps after being filled with apple juice at 22 ± 2 °C, and a thermocouple wire (Omega Type T special, 0.5 mm diameter, ± 0.5 °C, Stamford, CT) connected to a data logger (Omega, type OM-481, TCV-16) was used to record time–temperature data. Thermal processing conditions were: 2 to 30 min, and holding temperatures were 95 to 123 °C (± 0.5 °C). Samples were withdrawn periodically, cooled immediately in an ice bath and analysed.

Nonenzymatic browning index

Ethyl alcohol (5 mL; AR 950 g/kg), was added to 5 mL of an apple juice sample, centrifuged (10 min; 7800 × *g*), and the absorbency of the supernaant was read at 420 nm (Ultraspec 4050, LKB, Uppsala, Sweden). The value obtained was considered as the nonenzymatic browning index NEBI (20).

5-Hydroxymethylfurfural

The Keeney and Bassette method (17) was utilized as follows: 2 mL of the supernatant (from the NEBI test described above), 2 mL (120 g/kg) 3-chloroacetic acid (TCA) and 2 mL 0.025 mol/L thiobarbituric acid (TBA) were mixed in 16-mL screw-cap test tubes. The test tubes were placed in a water bath at 40 ± 0.5 °C, heated for 50 min and then cooled immediately with tap water to approximately 25 °C. The absorbency measured at 443 nm (Ultraspec 4050) was defined as the quality index utilized to quantify HMF. The actual HMF was read from a calibration curve of HMF (Sigma, St Louis, MO) ranging from 0 to 20 mg/kg.

Fluorescence relative index

The fluorescence relative index (FLRI) was measured on the processed samples by use of a fluorescence spectrophotometer (Model 4800, SLM Instruments Inc., Urbana, IL) utilizing maximum emission and excitation wavelengths measured at 493 and 400 nm, respectively. Since fluorescence could be affected by various factors including variety, maturity, natural compounds, growing and processing conditions, freshly reconstituted apple juice was used as the reference (RLFI = 1).

Continuous flow process

A continuous flow system was utilized for thermally treating small volumes of liquid, and obtaining short come-up time, high temperature conditions, instant cooling without flashing and multiple time–temperature data collection (21). Processing conditions were, a holding temperature of 85–135 °C and a total processing time (heating, holding and cooling) of 30 to 180 s. In order to prevent boiling inside the tube, the system was held under a positive pressure of ca. 4×10^5 Pa of nitrogen.

Kinetics

The changes in the quality index at constant temperature were described for a zero order (21)

$$C = C_0 + t\{ k_{ref} e^{[-\frac{Ea}{R}(\frac{1}{T}-\frac{1}{T_{ref}})]} \} \qquad \text{Eqn [1]}$$

where C is the concentration at time t; C_0 is the initial concentration at time t = 0; k_{ref} is the reaction rate constant at the reference temperature, T_{ref} (353 °K); *Ea* is the activation energy; R is the ideal gas constant; and T is the absolute temperature at the given time t.

Statistical analysis

The reaction rate constant of each quality attribute was derived through linear regression (Statistix, ver. 4.0, Analytical Software, FL). The activation energy and the rate constant at the reference temperature (i.e. T_{ref} = 353 °K) were estimated by a nonlinear regression method (BMDP AR Statistical Software, 1990, CA), using all the data (22). All samples were analysed in duplicate.

Results and Discussion

Kinetic models are often utilized to describe the quality changes in foods during thermal processing and storage. Typically, kinetic data of foods exposed to different processes and heat treatments at constant temperatures can be fitted to a zero- or a first-order reaction (3, 4, 15). As the formation of fluorescence compounds is quite complex, kinetic data expressing their formation are limited. Efforts have focused on the development of adequate procedures for deriving fluorescence and brown pigments in glucose–glycine model systems. It was assumed that, owing to the complex nature of the reactions, the unknown composition of the various pigments and their wide molecular weight distribution, an apparent zero-order reaction is applicable (18).

Figure 1 shows changes of FLRI with processing time at constant temperature. It is obvious that the reaction followed an apparent zero-order reaction, and the derived rate constants are listed in **Table 1**. Applying an Arrhenius equation for expressing the effect of temperature, and utilizing all the data in a one-step method (22), the derived activation energy was 148.6 ± 6.7

kJ/mol. A similar approach was utilized to obtain NEBI and HMF reaction rates (**Table 2**), indicating again an apparent zero-order reaction with activation energies of 148.6 ± 13.4 and 151.1 ± 4.2 kJ/mol, respectively. It is worth noting that the rate of formation of HMF in concentrated apple juice has also been reported to resemble a second-order autocatalytic reaction (23). However, this kinetic behaviour is applicable for significantly higher HMF concentrations.

Typical activation energies of 66.9–125.5 and 141.4–195.8 kJ/mol have been reported for NEBI and HMF, respectively (18). These values depend on the specific food system, and the pertinent process conditions. An activation energy of 113 kJ/mol for NEBI in apple juice exposed to thermal conditions in the range of 37 to 130 °C was reported (6), and is significantly lower compared to the value obtained in this work. Possible explanations for this discrepancy are a mechanism change occurring most probably when both low and high processing temperatures are combined, and the method applied for the estimation of the energy of activation (21). On the other hand, the activation energy for HMF found here agrees well with previously reported values calculated at typical thermal processing condition (15).

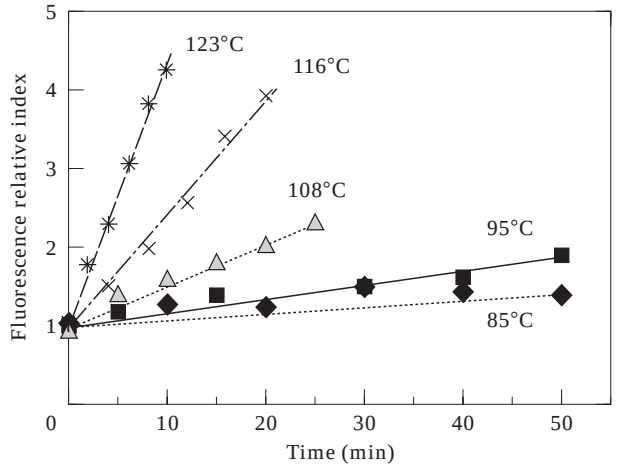

Fig. 1 Change of fluorescence relative index (FLRI) of apple juice with processing time at constant temperature

It is worth noting that the application of one- or two-step methods for deriving the kinetic parameters could yield different values and, thus, special caution is required when comparing reported data. The one-step method provides a narrower confidence interval (22), and could also possible changes in the reaction mechanism.

Linear regression of the quality indexes determined in apple juice samples (n = 26), processed at various constant time–temperature conditions, showed a significant correlation (R^2 = 0.98, P < 0.01) between HMF and FLRI. Since, many other compounds (such as Schiff's base derivatives) nitrogenous compounds in browning reactions are known to develop fluorescence but not furfurals, and fluorescence compounds appear in food products before undesirable colour or off-flavour is noticeable. Therefore FLRI could furnish an early indication for temperature abuse or quality deterioration.

To demonstrate the applicability of FLRI as a quality indicator, an additional set of apple juice samples (n = 81) was treated under typical processing conditions of time and temperatures used in aseptic juice lines. **Figure 2** depicts HMF and FLRI data obtained under these conditions. Linear regression analysis furnished the following relationship:

$$HMF = 7.14 * \text{FLRI} - 6.94 \quad R^2 = 0.97 \qquad \text{Eqn [2]}$$

This relationship indicates that HMF concentration (in mg/kg) could be derived from the known FLRI, with high accuracy (P < 0.001). Residual analysis of the calculated vs. experimental data showed a random variation and a zero mean, indicating a good fit of the data with the linear model.

HMF content in apple juice concentrate (72 °Bx), properly produced and stored, was reported to be considerably lower than 10 mg/100 g (23). Common

Table 1 Zero-order reaction rate constants of quality indexes for thermally processed apple juice at different temperatures

Quality index	k ([C]/min)×10^3			
	95°C	108°C	116°C	123°C
Nonenzymatic browning index (A_{420})	0.9±0.3	1.9±0.3	6.4±1.0**	14.2±1.1**
5-Hydroxymethylfurfural (A_{443})	9.1±0.6*	65.4±5.7**	157.6±4.2**	334.1±24.4**
Fluorescence relative index	15.0±4.3*	49.9±3.4**	151.5±6.9**	332.1±13.4**

*P < 0.05: **P < 0.01.
Values given are means and standard deviations for n = 10.

Table 2 Reaction rate constants at reference temperature (353°K) and activation energy of quality indexes for thermally processed apple juice

Quality index	k_{ref} (353°K)×10^4 ([C]/min)	Activation energy (kJ/mole)
Nonenzymatic browning index (A_{420})	0.55±0.25	148.6±13.4
5-Hydroxymethylfurfural (A_{443})	13.02±1.85	151.1±4.2
Fluorescence relative index	13.32±3.12	148.6±6.7

Values given are means and standard deviations for n = 26.

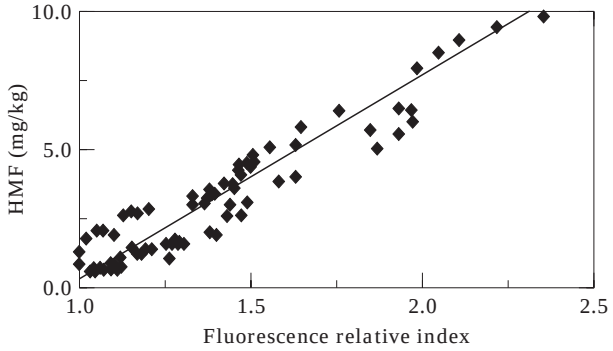

Fig. 2 Relationship of 5-hydroxymethylfurfural (HMF) and the fluorescence relative index (FLRI) of thermally processed apple juice

industry practice for acceptable HMF concentration of a high-quality thermally processed apple juice is generally below 5 mg/kg. Hence predicting a high concentration of HMF is probably of insignificant interest to the manufacturer. Although a wide range of HMF concentrations was used for deriving the regression model (Eqn [2]), the maximum discrepancy was 0.5 mg/kg due to the confidence interval of the regression analysis. Hence, for practical 'on-line' quality monitoring FLRI could be utilized.

The correlation derived between FLRI and NEBI was significant but relatively low (R^2 = 0.61). These data are not surprising, as NEBI is spectrophotometrically detectable only when sufficient brown pigments have accumulated. The lag phase makes NEBI of less importance as a quality criterion for monitoring on-line changes occurring during the initial stages of quality loss.

The determination of FLRI could be utilized for monitoring apple juice quality without any preparatory treatment. The simplicity of this method circumvents time-consuming procedures, and it is well suited as a routine method for monitoring quality processing or storage. To account for the natural variability in initial apple juice quality the exact relationship between FLRI and HMF (Eqn [2]) should be derived.

In conclusion, the determination of the concentration of fluorescence compounds in thermally processed apple juice is a rapid and easy-to-use method, and could be adopted as a routine technique to monitor quality. The linear correlation found between FLRI and HMF could be used to circumvent time-consuming HMF measurements. FLRI utilization to estimate HMF requires quantification to account for variation in initial apple juice quality.

References

1 Saguy, I. and Karel, M. Index of deterioration and simulation of quality losses. In: Kapsalis, J. G. (Ed.), *Objective Methods in Food Quality Assessment*. Boca Raton, FL: CRC, pp. 233–260 (1987)

2 Baisier, W. M. and Labuza, T. P. Maillard browning kinetics in a liquid model system. *Journal of Agricultural and Food Chemistry*, **40**, 707–713 (1992)

3 Garza, S., Giner, J., Martin, O., Costa, E. and Ibarz, A. Colour, sugars and HMF evolution during thermal treatment of apple juice. *Food Science and Technology International*, **2**, 101–110 (1996)

4 Ibarz, A., Martin, O. and Barbosa-Canovas, G. V. Nonenzymatic browning kinetics of concentrated pear juice. *Food Science and Technology International*, **3**, 213–218 (1997)

5 Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., Warner, F. P. and Jumel, K. Studies on non-enzymatic browning in orange juice using a model system based on freshly squeezed orange juice. *Journal Science and Food Agriculture*, **52**, 85–95 (1997)

6 Labuza, T. P. and Saltmarch, M. The kinetics of nonenzymatic browning reaction as affected by water in foods. In: Rockland, L. B. and Stewart, G. F. (Eds), *Water Activity: Influence on Food Quality*. New York, NY: Academic Press, p. 605 (1981)

7 Lee, H. S. and Nagy, S. Quality changes and nonenzymatic browning intermediates in grapefruit juice during storage. *Journal of Food Science*, **53**, 168–172, 180 (1988)

8 Lozano, J. E. Kinetics of non enzymatic browning in model systems simulating clarified apply juice. *Lebensmittel-Wissenschaft und-Technologie*, **24**, 355–360 (1991)

9 Nagy, S., Lee, H., Rouseff, R. L. and Lin, J. C. C. Nonenzymatic browning of commercially canned and bottled grapefruit juice. *Journal of Agricultural and Food Chemistry*, **38**, 343–346 (1990)

10 Saguy, I., Kopelman, I. J. and Mizrahi, S. Extent of nonenzymatic browning in grapefruit juice during thermal and concentration processes: kinetics and prediction. *Journal of Food Processing and Preservation*, **2**, 175–184 (1978)

11 Askar, A. Flavor alterations during production and storage of fruit juices. *Füssiges Obst*, **51**, 564–569, 610–614 (1984)

12 Ooghe, W. and Dresselaerts, D. Quality parameters for pineapple juice. *Fruit Processing*, **5**, 11–12, 14–17 (1995)

13 Rassis, D. and Saguy, I. S. Kinetic of aseptic concentrated orange juice quality changes during commercial processing and storage. *International Journal of Food Science and Technology*, **30**, 191–198 (1995)

14 Tonelli, S. M., Errazu, A. F., Porras, J. A. and Lozano, J. E. Prediction of 5-HMF formation in industrial apple juice evaporator. *Journal of Food Science*, **60**, 1292–1294 (1995)

15 Toribio, J. L. and Lozano, J. E. Formation of 5-hydroxymethyl-furfural in clarified apple juice during heating and elevated temperatures. *Lebensmittel-Wissenschaft und-Technologie*, **20**, 59–63 (1987)

16 Lee, H. S. and Nagy, S. Relative reactivities of sugars in the formation of 5-hydroxymethyl sugar catalyst model systems. *Journal of Food Processing and Preservation*, **14**, 171–178 (1990)

17 Keeney, M. and Bassette, R. Determination of intermediate compounds in the dairy stages of browning reaction in milk products. *Journal of Dairy Science*, **43**, 945–960 (1959)

18 Labuza, T. P. and Baisier, W. M. The kinetics of nonenzymatic browning. In: Schwartzberg, H. G. and Hartel, R. W. (Eds), *Physical Chemistry of Foods*. New York: Marcel Dekker Inc., pp. 595–649 (1992)

19 Kessler, H. G. and Fink, R. Changes in heated stored milk with interpretation by reaction kinetics. *Journal of Food Science*, **51**, 1105–1111, 1155 (1986)

20 Meydav, S., Saguy, I. and Kopelman, I. J. Browning determination in citrus products. *Journal of Agricultural and Food Chemistry*, **25**, 602–604 (1977)

21 Cohen, E., Birk, Y., Mannheim, C. H. and Saguy, I. S. Kinetic parameters for quality change during continuous

thermal processing of grapefruit juice. *Journal of Food Science*, **59**, 155–158 (1994)

22 COHEN, E. AND SAGUY, I. Statistical evaluation of Arrhenius model and its applicability in prediction of food quality losses. *Journal of Food Processing and Preservation*, **9**, 273–290 (1985)

23 BABSKY, N. E., TORBIO, J. J. AND LOZANO, J. E. Influence of storage on the composition of clarified apple juice concentrate. *Journal of Food Science*, **51**, 564–567 (1986)