Kinetics of Deterioration of Pineapple Concentrate

A.J. FONTANA, L. HOWARD, R.S. CRIDDLE, L.D. HANSEN and E. WILHELMSEN

ABSTRACT

Pineapple juice concentrate undergoes reactions leading to color formation and quality loss. Kinetics were studied calorimetrically, spectrally and by chemical analysis. Two exothermic processes occurred between 40° and 80°C, one independent of, and one dependent on O_2 concentration. The rate of the O_2 independent reaction decreased exponentially with time, (sucrose hydrolysis). The rate of the O_2 dependent process was constant with time, complex, and not defined. Heat producing reaction rates were not altered by concentrations of major sugars/organic acids. Development of color occurred later than heat production. Color development had a complex dependence on solids and O_2 concentration, and correlated with initial rate of 5-hydroxymethylfurfural (HMF) formation. CO_2, decrease in titratable amines and amino acids, and loss of reducing agents accompanied color development. Heat and color production and chemical changes correlated with sensory quality.

Key Words: pineapple, kinetics, juice concentrate, exothermic process, amino acids

INTRODUCTION

NONENZYMATIC BROWNING and quality degradation of pineapple juice and many other fruit juices occurs during concentration and storage (Hodge, 1953; Stadtman, 1948; Toribio and Lozano, 1984; Cornwell and Wrolstad, 1981; Clegg, 1964; Kanner et al. 1982). Color and flavor deterioration may develop from reactions between reducing sugars and amino acids (Maillard reactions), from formation and subsequent reactions of HMF, from Strecker type reactions, and other reactions (Reynolds, 1965). Understanding the time and temperature dependencies and nature of the reactions in the chemically complex concentrate could guide development of procedures for optimizing quality and costs. The specific reactions occurring in pineapple concentrate are not well characterized. Ascorbic acid loss and color changes were reported in pineapple slices packed in flexible, retortable pouches (Salunkhe et al., 1978) and browning of pineapple juice was retarded by sodium bisulfite and N-acetyl cysteine (Molnar-Perl and Friedman, 1990).

Our general objective was to develop information on the rate of degradation during processing of pineapple juice that would enable concentrate production with minimum quality loss. Direct sensory analysis is the best way to assess overall quality. Such tests are difficult and time consuming and not suited for product control. Chemical tests that could be quantitatively related to quality are therefore needed. Using these, predictive models could be developed to estimate changes in sensory attributes based on simply measured chemical changes.

The first phase of our study examined the kinetics of degradation by three independent descriptive techniques: (a) calorimetrically, to provide an overall characterization of kinetics of degradation and give insight into the nature of major chemical reactions; (b) colorimetrically, to define undesired pigmentation; and (c) sensorially, to relate processing time and temperature to overall quality. The second phase chemically identified pineapple concentrate components involved in reactions responsible for calorimetric response, color development and loss of overall quality. The combined data were used to define regression equations relating quality to measurable chemical properties.

Nearly all chemical reactions produce or absorb heat, thus calorimetry is used for measurement of kinetics in complex systems (Hansen and Criddle 1990; Hansen et al., 1989; Pikal and Dellerman, 1989; Brown and Galwey, 1989; Waters and Paddy, 1988; Raemy et al., 1987). It has proven particularly useful in systems where reactions are unknown. Our study employed two previously described calorimetric methods (Hansen and Criddle, 1990; Hansen et al., 1989) for characterization of degradation, but this is the first time such calorimetric methods were combined in one study. We combined a recently described method using 1 cm^3 volume, heat conduction DSC (Hansen and Criddle, 1990) with large volume (50 cm^3), isothermal, heat conduction calorimetry (Hansen et al., 1989). We used these to rapidly identify, respectively, the temperature range of interest and the kinetic rate law for decomposition. This combination has considerable potential for characterizing decomposition of complex organic materials.

Authors Fontana and Criddle are affiliated with the Dept. of Biochemistry & Biophysics, Univ. of California, Davis, CA 95616. Author Wilhelmsen is with Dole Packaged Foods Co., San Jose, CA 95131. Author Howard, formerly with Dole, is now with the Dept. of Horticultural Sciences, Texas A&M Univ., College Station, TX 77843. Author Hansen is with the Chemistry Dept., Brigham Young Univ., Provo, UT 84602.

MATERIALS & METHODS

ALL STUDIES were done on two lots of evaporatively concentrated (61°brix) commercial pineapple juice stored in small sealed glass containers and kept frozen until use.

Calorimetry

Isothermal calorimetry measurements were performed in a Hart Model 7708 heat conduction calorimeter (Hart Scientific, Pleasant Grove, UT) with a cylindrical 2.5 cm by 7.5 cm sample chamber using the methods of Hansen et al. (1989). Samples were sealed in glass ampules with appropriate atmosphere and additives. Generally, 1 to 9 mL aliquots of concentrate were used in each test. Samples were maintained at a selected test temperature while heat production rates were measured as a function of time. In some instances the heat rates were also followed in a Hart Model 7707 differential scanning calorimeter (DSC) operated in the isothermal mode (Criddle et al., 1989). In these experiments a pressure sensor was used to monitor changes in head-space gas pressure (Criddle et al., 1991). Measurement of rates of heat production as a continuous function of temperature employed a Hart model 7707 DSC using about 0.5g samples and the methods described by Hansen and Criddle (1990).

Color measurements

Concentrate samples were sealed in 5 mL glass ampules with appropriate atmosphere and additives, immersed for defined times in a Haake water bath controlled to ±0.1°C and stored frozen until measured. Samples were diluted to 1/3 starting concentration with distilled water and color development determined as a Hunter-L value, using a Hunter Lab Scan Model LS-5100 colorimeter operated in the transmission mode. Although Hunter-L, a, and b data were generated, only Hunter-L values were required to describe the change in color.

Chemical analyses

HMF determinations were done with a Perkin-Elmer (Norwalk, CT) HPLC with LC-85 UV detector. An Aminex ion exclusion column (Bio-Rad HPX-87H, 300 × 7.8 mm i.d.) and Bio-Rad-Cation guard column (40 × 4.6 mm i.d.) were used. Diluted samples (13.5°brix) were filtered through Millipore-Millex HV filters prior to injection of 10 µL onto the column. The column was eluted with 9 mN sulfuric acid at 1.0 mL/min, 70°C and monitored at 284 nm. Quantification was based on comparison of peak areas with HMF standards. Total free amines were determined according to the formol index (AOAC, 1980). Amino acids were analyzed by ion exchange chromatography

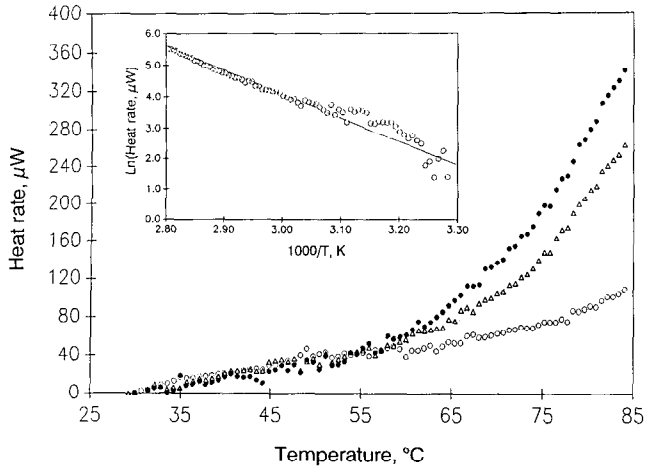

Fig. 1—Heat rates from degradation reactions as a function of temperature for 350 mg (●), 255 mg (Δ), and 140 mg (O) samples of pineapple concentrate measured in a Hart DSC by the method described in Hansen and Criddle (1990). Temperature was increased at 7°C/hr. The inset shows an Arrhenius type plot for the 350 mg sample.

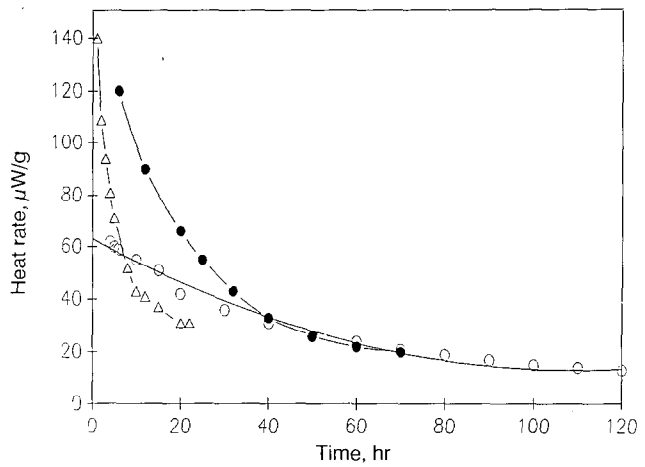

Fig. 2—Isothermal heat rates from identical samples of pineapple concentrate at 60 (O), 70(●), and 80°C (Δ) in air.

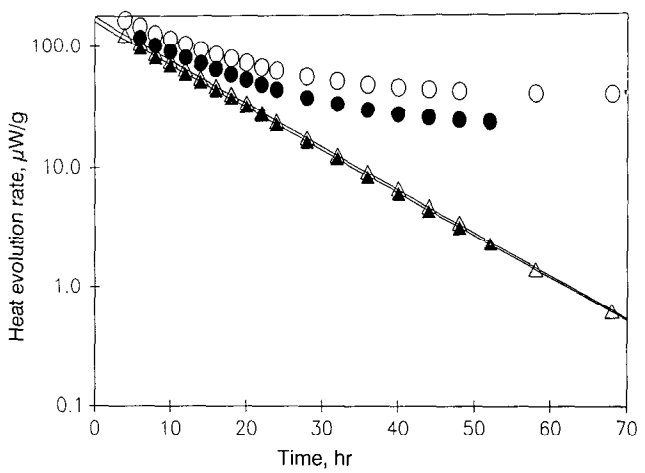

Fig. 3—Rates of pineapple concentrate degradation at 70°C in O_2 and N_2 atmospheres. Data are plotted as heat evolution rate (q_m) vs time in O_2 (O) and in N_2 (●), and as ($q_m + \Delta H_o k_o c_o$) vs time in (O_2 (Δ), and in N_2 (Δ).

at AA Laboratory, Mercer Island, WA. Headspace CO_2 was measured at 23°C with a Varian Model 920 GC (Walnut Creek, CA) equipped with thermal conductivity detector and CTR-I column (Alltech Associates Inc., Deerfield, IL). Helium was used as carrier gas at 60

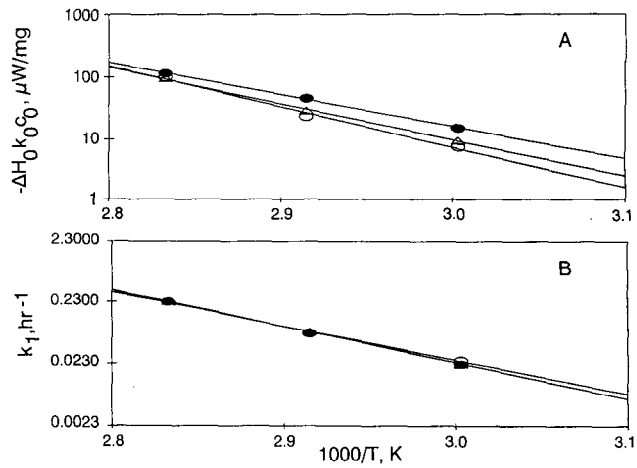

Fig. 4—Temperature dependence of the zero and first order exothermic reactions in pineapple juice concentrate. (A) Temperature effects on $-\Delta H_o k_o c_o$ in air (Δ), O_2 (●), and N_2 (O) atmospheres; (B) Temperature effect on the first order rate constant in air (O), O_2 (●), and N_2 (Δ).

Table 1—Effects of additions of major components of pineapple concentrate on the rates of heat production at 60, 70 and 80°C

Sample wt, g	Atm	Additive	Additive wt, g	ln ($-\Delta H_1 c_1$, μW/g)	k_1, hr^{-1}	$-\Delta H k_o c_o$, μW/g	R^2
			80°C				
2.407	air	–	–	6.17	0.230	97	0.996
1.818	N_2	–	–	6.33	0.240	99	0.999
1.846	O_2	–	–	6.47	0.233	120	0.988
1.130	air	sucrose	0.3829	6.29	0.154	96	0.988
0.985	air	glucose	0.0990	5.99	0.230	118	0.998
1.132	air	fructose	0.1724	5.80	0.207	127	0.999
0.961	air	ascorbic acid	0.00035	6.17	0.187	144	0.997
0.879	air	malic acid	0.0017	5.94	0.184	145	0.999
1.126	air	citric acid	0.0160	6.59	0.341	122	0.999
			70°C				
4.925	O_2	–	–	5.16	0.083	38.9	0.999
4.673	N_2	–	–	5.07	0.082	21.2	0.999
8.445	air	–	–	5.50	0.091	25.5	0.949
8.422	air	BHT	0.0109	5.69	0.100	24.0	0.996
5.89	N_2	–[a]	–	5.00	0.075	35.0	0.996
8.567	air	S.S.	–[b]	5.16	0.079	23.5	0.996
8.957	air	EDTA	10mM	5.44	0.092	23.5	0.998
		dithiothreitol	10mM				
			60°C				
4.323	N_2	–	–	4.03	0.0256	7.3	0.997
3.356	air	–	–	4.19	0.0221	9.5	0.993
4.022	O_2	–	–	4.08	0.0221	14.4	0.999

[a] Sample was repeatedly evacuated and filled with N_2 at 40°C to remove dissolved O_2

[b] A coil of stainless steel wire was immersed in the sample.

mL/min. Quantification was based on comparison of peak areas with CO_2 standards. Reducing equivalents were determined by adding 2 mL of a 0.1% starch solution to 15 mL of concentrate and titrating to a faint blue endpoint with 0.1 N potassium triiodide solution (Skoog and West, 1980).

Sensory analysis

Sensory analysis was performed using a descriptive panel consisting of eight trained judges. Attributes scored on a scale of 1 = none to 9 = extreme were: color, pineapple flavor, oxidized flavor, cooked flavor. Overall acceptability was scored on a scale from 1 = extremely unacceptable to 9 = extremely acceptable. Samples were evaluated over three separate sessions. Each session included an untreated control. Replicates of select treatments were evaluated to insure that the panel was rating consistently from one session to the next.

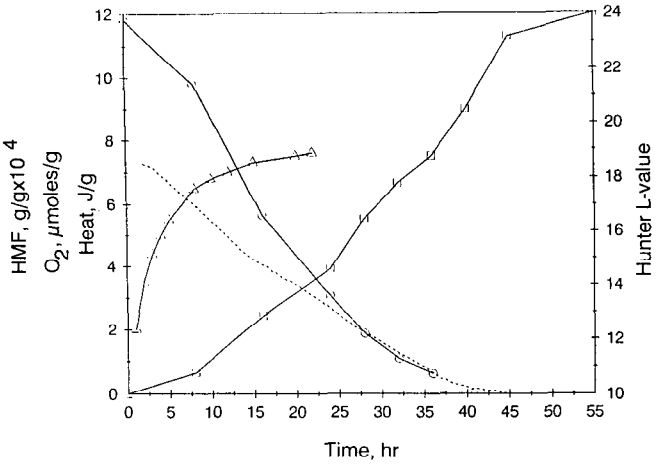

Fig. 5—Total heat production (Δ), oxygen use (–), color formation (O, Hunter-L), and HMF formation (□) with time of incubation of pineapple juice concentrate at 80°C.

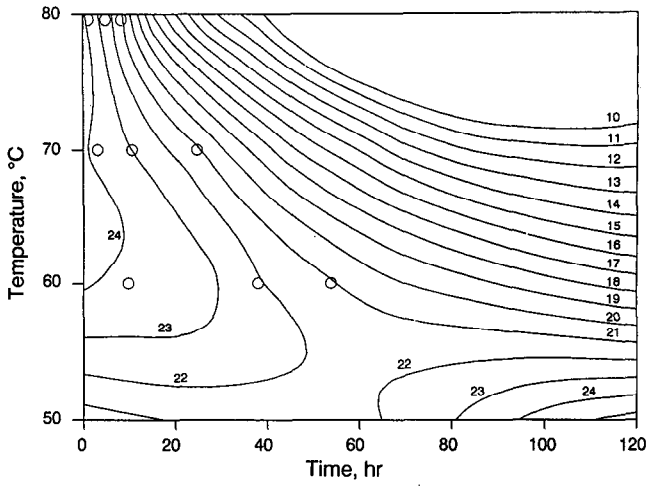

Fig. 6—Contour plot of the Hunter L-value as a function of time and temperature. Hunter L-values are indicated numerically on the contour lines.

RESULTS

Calorimetry

The scanning calorimetry method of Hansen and Criddle (1990) was used to rapidly determine the total range of temperature and concentrations appropriate for examination of pineapple concentrate by isothermal calorimetry. The rate of heat production resulting from reactions in the sample was measured on three different total amounts of concentrate while scanning temperature upward at 7°C/hr (Fig. 1). Above about 60°C heat rates and total heat production were proportional to sample mass. At the end of the upscan, the samples were held at 85°C for 1 hr and then scanned downward at 7°C/hr. During the 85°C period the heat rate from the sample decreased rapidly and reactions in the sample produced little heat during the downscan, thus indicating depletion of reactant(s) during the upscan and high temperature isothermal parts of the experiment. The scanning calorimetry data produced a linear Arrhenius plot above about 50°C with an approximate activation energy of 65 kJ/mol, (inset Fig. 1). The slope of the Arrhenius plot may be different below 50°C, possibly indicating a change in either the rate limiting step or the mechanism, but this is uncertain because of the low heat rates observed at low temperatures. Based on scanning calorimetry results, the temperature range from 60–80°C was selected for isothermal heat rate measurements on large samples. Reaction rates were too slow below 55°C and too fast above 80°C to conveniently be studied by isothermal heat conduction calorimetry.

The time dependence of the isothermal heat production rates from three identical concentrate samples incubated at 60, 70 and 80°C (Fig. 2) produced curves described by the sum of two rate equations, one first-order and the other zero-order in time.

The first-order equation giving the rate as a function of time was:

$$dn_1/dt = c_1\exp(-k_1t) \quad (1)$$

or in terms of heat rate

$$\Delta H_1 dn_1/dt = q_1 = -\Delta H_1 c_1 \exp(-k_1 t) \quad (2)$$

where n_1 is moles of reactant/g juice, t is time, c_1 is a constant for a given lot of juice (with units of mol s^{-1} g^{-1}), k_1 is the first order rate constant (with units of s^{-1}), ΔH_1 is the enthalpy change for the time dependent process (with units of J mol^{-1}), and q_1 is the rate of heat produced by the time dependent process (with units of W g^{-1} or J s^{-1} g^{-1}).

The zero order equation for rate as a function of time is

$$dn_0/dt = k_0c_0 \quad (3)$$

or in terms of heat rate

$$\Delta H_0 dn_0/dt = q_0 = -\Delta H_0 k_0 c_0 \quad (4)$$

where n_0 is moles of reactant/g juice, c_0 is a constant for a given lot of juice (with units of mol g^{-1}), k_0 is a zero order rate constant (with units of s^{-1}), ΔH_0 is the overall enthalpy change for the time-independent process (with units of J mol^{-1}), and q_0 is the rate of heat produced by the time independent process (with units of W g^{-1}). c_1 and c_0 are functions of the composition of the juice, and thus may vary from lot to lot or with addition of materials that alter the initial rates of these processes. Note that Eq. (1) and (3) are respectively first and zero order in time and should not be interpreted as representing kinetics of reactions which are first and zero order in concentrations. The derivation used here was similar to methods used in solid state kinetics where concentration has little meaning. This approach is particularly valuable when reactions are unknown.

The heat rate measured, q_m, was the sum of the heat rates from the two processes, i.e.

$$q_m = q_1 + q_0 = -[\Delta H_1 c_1 \exp(-k_1 t) + \Delta H_0 k_0 c_0] \quad (5)$$

which on rearranging and taking the natural logarithm gives

$$\ln(q_m + \Delta H_0 k_0 c_0) = \ln(-\Delta H_1 c_1) - k_1 t \quad (6)$$

Values for k_1 and $\ln(-\Delta H_1 c_1)$ can be obtained respectively from the slope and intercept of a plot of the left side of Eq. (6) against time if the data are accurately described by the equation. Making such a plot requires a prior value of $\Delta H_0 k_0 c_0$, however. We therefore determined the best value for $\Delta H_0 k_0 c_0$ by successive approximations, choosing the value of $\Delta H_0 k_0 c_0$ minimizing curvature in the plot as measured by the linear regression coefficient, R^2. Figure 3 shows plots of both $\ln(q_m)$ and $\ln(q_m + \Delta H_0 k_0 c_0)$ against time. The curvature in the plots of $\ln(q_m)$ demonstrate the necessity of including the zero order reaction in the analysis. The linearity of the plots including both first order and zero order reactions demonstrates the adequacy of the model to describe the data.

To determine whether or not O_2 was a reactant the heat rates were measured in O_2 and N_2. Figure 3 gives results at 70°C. The slopes and intercepts of the linear plots of $\ln(q_m + \Delta H_0 k_0 c_0)$ against time were the same, thus showing both k_1 and $\Delta H_1 c_1$ to be independent of O_2. The rate of the zero order reaction was increased or the products of the reaction were changed by O_2 as shown by the increase in q_m, especially at long times. The integral heat of the first order reaction at 70°C was determined to be 7.6 J/g pineapple concentrate from the area under the heat rate vs time curves corrected for the zero order reaction. The temperature dependencies of the first and zero order processes are illustrated in Fig. 4. Arrhenius plots

Table 2—Mean attribute scores and differences for pineapple concentrate samples exposed to various time and temperature combinations

	Control untrd	Control untrd	Control untrd	1 Hr 80°C	1 Hr 80°C	3 Hr 80°C	8 Hr 80°C	1 Hr 70°C	8 Hr 70°C	8 Hr 70°C	24 Hr 70°C	12 Hr 60°C	38 Hr 60°C	54 Hr 60°C	54 Hr 60°C	Sig. level
Color	5.21^{h}	5.36^{gh}	5.50^{gh}	5.79^{ef}	6.06^{ef}	6.44^{de}	7.75^{ab}	5.75^{fg}	6.71^{d}	6.50^{de}	8.13^{a}	6.37^{de}	7.25^{c}	7.31^{bc}	7.62^{bc}	99.9%
Pa flavor	4.36^{ab}	4.25^{ab} cdef	4.57^{a}	4.14^{ab} bcde	4.12^{ab}	3.94^{ab} bcdef	2.94^{de} abcd	4.44^{ab}	4.00^{ab} cdef	3.67^{bcd} bcde	2.56^{e}	3.75^{bc} bcdef	2.83^{e}	3.06^{cde}	2.94^{de} bcde	99.9%
Oxidized	2.93^{ef}	3.37	3.00^{ef}	3.64	3.25^{efd}	3.50	4.00	2.64^{f}	3.36	3.75	4.64^{a}	3.50	4.17^{abc}	4.33^{ab}	3.57	99.9%
Cooked	3.14^{de}	3.29^{cde}	2.42^{e}	3.86^{cd}	3.14^{de}	4.19^{cd}	6.07^{ab}	2.71^{e}	4.07^{cd}	4.25^{c}	6.62^{a}	4.14^{cd}	5.25^{b}	6.14^{ab}	5.75^{ab}	99.9%
Overall acceptability	5.33^{a}	5.36^{a}	5.86^{a}	5.14^{ab}	5.06^{ab}	4.50^{b}	3.12^{cd}	5.19^{ab}	5.21^{ab}	4.42^{b}	2.50^{d}	4.44^{b}	3.08^{cd}	3.44^{c}	3.37^{c}	99.9%

[a-h] Within each attribute, means with different superscripts are significantly different.

Table 3—Some chemical and compositional changes as affected by headspace atmosphere and 75°C storage for 24 hr

Atmosphere	Sucrose decrease	Glucose increase	Fructose increase	Net Carbohydrate decrease[a]	Total free amines decrease	HMF increase
	µMoles/g/24 hr					
Oxygen	471	402	454	85	25.0	2.33
Air	488	411	468	92	15.2	2.38
Nitrogen	480	408	461	90	16.0	2.20
		Mole Ratios				
		A	B	C		
Oxygen		3.4	36.5	10.7		
Air		6.1	38.6	6.7		
Nitrogen		5.6	40.9	7.3		

[a] Calculated as: [2(µMole/g Sucrose decrease) - (µMole/g Fructose increase + µMole/g Glucose increase)]. A: Net Carbohydrate Decrease/Total Free Amine Decrease; B: Net Carbohydrate Decrease/HMF Increase; C: Total Free Amine Decrease/HMF Increase.

give all three E_a values near 92 kJ/mole for the first order process in air, O_2, and N_2 (plot of k_1), and for the zero order process values of 80 kJ/mole in O_2, 85 in air, and 100 kJ/mole in N_2.

The effect of each of the major constituents in pineapple concentrate (sucrose, glucose, fructose, ascorbic acid, malic acid, and citric acid) on the exothermic reaction rates were examined by doubling the initial concentration by adding the solid component to the concentrate. Kinetics of heat production in the altered samples were measured at 80°C (Table 1). Doubled concentrations of sucrose, malic acid and ascorbic acid all significantly decreased k_1. Glucose and fructose had negligible effect on k_1; citric acid increased k_1. Glucose, fructose and all three acids increased $\Delta H_0 k_0 c_0$. None of the additives had a significant effect on $\ln(-\Delta H_1 c_1)$.

Effects of potential catalysts or inhibitors were determined by adding the material to the concentrate and measuring the heat rates at 70°C. Neither the first order nor the zero order rate was altered in the presence of 0.13% BHT, 10 mM EDTA, 10 mM dithiothreitol, or coiled stainless steel wire. In the case of BHT, low solubility may have prevented detection of an antioxidant effect. Dithiothreitol, which would quench free radical reactions and remove peroxides, prevented browning in the sample. This observation separated the first order heat producing reaction from color production and confirmed the result that the first order heat producing reaction did not involve oxygen or oxidizing agents. The oxygen dependent, zero order heat rate may monitor some early step of browning reactions, but not the color producing reaction.

To measure O_2 uptake rate, the gas pressure inside the calorimeter ampule was monitored simultaneously with heat rate in some of the isothermal experiments in the DSC. There was a slow decrease in pressure with time. This pressure change in a constant volume ampule (0.55g juice, 0.54 mL air in headspace) at constant temperature was related to and plotted as a change in µmoles of gas. The pressure change represents the sum of oxygen depletion and CO_2 production in the headspace gas. CO_2 production was determined calorimetrically in parallel experiments using the methods of Criddle et al. (1991) so that the rate of O_2 depletion could be plotted in Fig. 5. The pressure change appeared as two approximately constant, sequential slopes with rates of -0.369 (<15 hr) and -0.265 (>15 hr) µMoles gas hr^{-1} g^{-1} concentrate. A net pressure decrease in the ampule indicated the rate of O_2 consumption exceeded the rate of CO_2 production. All O_2 in the headspace of the calorimeter ampule was consumed within 42 hr at 80°C.

Colorimetry

Color development at 60, 70 and 80°C was followed by measurement of Hunter-L values. Hunter-L values are not a linear function of concentration and can not be used directly to rigorously produce a rate expression, but enable general examination of the time course of color development. Figure 5 shows changes in Hunter-L values of concentrate at 80°C and a contour plot summarizing the Hunter-L values as a function of time and temperature was developed (Fig. 6). Color development was accelerated by increasing O_2 pressure, but the shape of the curve was unaffected. Color development was not significantly altered by addition of BHT, EDTA, stainless steel shavings, increasing sucrose 1.5 fold or doubling fructose, glucose, citric acid (or sodium citrate), ascorbate, or malate.

The sigmoidal shape of the Hunter-L curve (Fig. 5) was observed at each of the test temperatures. These curves plus isothermal cross sections of Fig. 6 suggest the reactions leading to color development may be autocatalytic and introduce the possibility that some intermediate may be accumulating that stimulates color formation. This hypothesis was tested by heating concentrate at 80°C for 3, 6, and 9 hr and then adding an aliquot of highly colored material to untreated concentrate and incubating at 60°C. In the mixed samples there was an initial color change due to addition of the darkly colored preheated sample, but there was no change in kinetics of subsequent color development. Thus, products developed during the lag phase appear to be integral components of developing colored products rather than catalytic components necessary for initiating further color development.

Sensory analysis

Samples were selected for sensory analysis based on kinetics of color development. Nine time-temperature points (shown by the open circles) were chosen to represent the topographic surface in Fig. 6 with Hunter-L values >19. A Hunter-L value of 19 is the limit for sensory analysis studies because the colors of samples with lower values are too dark to distinguish. Mean attribute scores for pineapple concentrate samples exposed to the nine time-temperature combinations were compared (Table 2).

Chemical analysis

Changes in concentrations of sucrose, glucose, fructose, free amine and HMF in pineapple concentrate incubated at 75°C for 24 hr in N_2, air and O_2 were summarized (Table 3). Sucrose disappearance by hydrolysis was nearly complete. Fructose and glucose both increased from sucrose hydrolysis, but there

Table 4—Free amino acid content of pineapple juice concentrate stored at 75°C

Amino acid[a]	2 hr storage			12 hr storage			30 hr storage		
	O_2	Air	N_2	O_2	Air	N_2	O_2	Air	N_2
	μMoles								
Alanine	2.58	2.58	2.44	2.17	2.25	1.97	2.41	2.31	1.90
Arginine	0.44	0.44	0.43	0.29	0.36	0.31	nd	nd	nd
Aspartic acid	1.91	nd	1.93	1.94	2.05	2.10	1.97	2.01	1.91
Cystine	0.11	0.11	0.11	tr.	0.08	tr.	nd	nd	nd
Glutamic acid	0.02	nd	nd	nd	nd	nd	nd	nd	nd
Glycine	1.07	1.02	0.97	0.87	0.90	0.79	0.56	0.50	0.63
Histidine	0.54	0.53	0.52	0.31	0.31	0.30	0.20	0.22	0.20
Isoleucine	0.24	0.23	0.21	0.18	0.18	0.15	0.11	0.11	0.10
Leucine	0.52	0.53	0.47	0.42	0.35	0.31	0.31	0.32	0.30
Lysine	0.60	0.59	0.60	0.38	0.40	0.44	0.13	0.15	0.12
Methionine	1.36	1.39	1.33	1.01	1.06	0.94	0.54	0.58	0.55
Phenylalanine	0.38	0.39	0.35	0.31	0.32	0.28	0.21	0.21	0.21
Proline	0.71	0.59	0.48	0.37	0.63	0.47	0.36	0.45	0.43
Serine	3.90	4.00	3.73	3.03	3.11	2.69	2.29	2.20	2.00
Threonine	0.71	0.70	0.66	tr.	0.41	0.33	0.45	0.36	0.29
Tryptophan	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tyrosine	0.05	0.05	0.47	0.40	0.41	0.36	0.27	0.27	0.26
Valine	0.29	0.71	0.72	0.27	0.43	0.42	0.27	tr.	0.43
Asparagine	13.7	14.6	11.1	9.31	9.75	8.46	6.43	6.15	5.33
Glutamine	1.29	1.22	0.85	nd	nd	nd	nd	nd	nd
Gamma-amino butyric acid	1.61	1.66	1.60	1.21	1.37	1.18	0.66	0.67	0.65
Ethanolamine	1.02	1.07	1.03	1.03	0.84	0.75	0.39	0.38	0.35
Total Amino Acids	33.7	32.8	30.1	23.3	25.3	22.2	17.6	17.0	15.7

[a] Average error in determinations is < ±2% except for proline with error < ±5%.

Table 5—Some chemical changes during storage of pineapple concentrate as affected by headspace atmosphere[a]

Atm.	Temp (°C)	Reducing equiv. decrease	Free amine decrease	Carbon dioxide increase	Mole ratio: Reducing equiv/CO_2	Mole ratio: Free amine/CO_2
		μMoles/g in 24 hr*				
Oxygen	75	15.1	14.2	0.94	16.2	15.1
Air	75	11.5	14.2	0.68	16.9	20.8
Nitrogen	75	12.4	14.5	0.53	23.6	27.6
Oxygen	65	5.7	3.7	0.61	9.5	6.1
Air	65	4.4	3.6	0.32	13.7	11.3
Nitrogen	65	4.6	4.1	0.16	29.4	26.5
Oxygen	55	2.7	2.0	0.22	12.2	8.8
Air	55	2.7	1.9	0.14	19.2	13.1
Nitrogen	55	2.1	1.7	0.09	23.3	18.8
Oxygen	45	0.93	0.50	0.05	18.1	9.8
Air	45	0.85	0.54	0.03	24.3	15.5
Nitrogen	45	0.74	0.64	0.03	25.4	16.6

[a] Average of two determinations.

Table 6—Effects of temperature and headspace atmosphere on Q_{10} values for degradation reactions in pineapple concentrate

Atmosphere	Q_{10} Values: Temperature, °C 45–55	55–65	65–75
	Loss of Free Amines		
Oxygen	3.96	1.86	3.85
Air	3.48	1.94	3.89
Nitrogen	2.61	2.46	3.53
	Loss of Reducing Equivalents		
Oxygen	3.26	2.09	2.71
Air	3.24	1.63	2.51
Nitrogen	2.80	2.11	2.63
	CO_2 Production		
Oxygen	4.40	2.77	1.54
Air	4.67	2.29	2.13
Nitrogen	3.00	1.78	3.31

was a net carbohydrate decrease. HMF formed in relatively high concentrations. None of the changes was affected by changing the partial pressure of oxygen in contact with the sample. To aid in comparison of changes, mole ratios of changes in carbohydrate, amino acids and HMF were also compared (Table 3).

Changes in concentrations of individual free amino acids in concentrate incubated at 75°C in N_2, air, and O_2 were compared (Table 4). The decrease in free amino acids during 30 hr incubation was 15 to 25 μMoles/g pineapple concentrate, a value quite similar to the loss of total free amine (Table 3).

The time course of HMF formation is shown in Fig. 5. The kinetics of HMF formation were similar to those for color development also shown. However, the rate of increase in HMF was not altered by changes in oxygen partial pressure while that of color development was. The time course of HMF formation at all temperatures measured showed a slow rate of formation during the first several hours, followed by an accelerated rate of production. At 70°C for example, about 100 ppm HMF accumulated within the first 15 to 20 hr and up to 1000 ppm after 50 hr. The rate of formation of HMF was unaffected by addition of glucose, sucrose, fructose, ascorbate, malate, BHT and EDTA. Addition of citric acid, but not sodium citrate, accelerated HMF formation.

Addition of 10 ppm HMF to pineapple concentrate reduced, but did not eliminate, the time of initial slow rate of color development. This effect of HMF was blocked by addition of 0.1% $NaHSO_3$, suggesting that HMF or other reactive aldehydes may be an important precursor in color development. Note, however, that while HMF continued to increase throughout the time course of the reaction (Fig. 5), any promotional effects of HMF must have been saturated at low levels, as evidenced by the results of experiments with mixed aged and fresh concentrate showing the rate of color development was independent of intermediates.

Changes in reducing equivalents, carbon dioxide and free amine during incubation for 24 hr at 45–75°C in N_2, air and O_2 atmospheres were compared (Table 5). Oxygen increased loss of reducing equivalents and CO_2 production, but had no effect on loss of free amine. Relative magnitudes of changes in reducing equivalents and free amines are presented as ratios to CO_2 produced. Increasing O_2 significantly reduced those ratios in most experiments. Changes in Q_{10} values (Q_{10} = rate at T+10°C/rate at T°C) with different reaction temperatures and atmospheric compositions were compared (Table 6) for reactions involving loss of amines and reducing equivalents, and for production of CO_2. The generally lower Q_{10} values

Table 7—Correlation analysis relating quality to chemical change

	Accept	Color	Pa	Cooked	Sucrose	Glucose	Fructose	RED	L	HMF
Accept	*1.00000 0.0									
Color	−0.96401 0.0001	1.00000 0.0								
Pa	0.98538 0.0001	−0.96857 0.0001	1.00000 0.0							
Cooked	−0.95505 0.0001	0.98906 0.0001	−0.96701 0.0001	1.00000 0.0						
Sucrose	−0.94267 0.0001	0.98892 0.0001	−0.96062 0.0001	0.98689 0.0001	1.00000 0.0					
Glucose	−0.93175 0.0003	0.97909 0.0001	−0.93554 0.0002	0.97239 0.0001	0.98762 0.0001	1.00000 0.0				
Fructose	−0.89390 0.0012	0.94729 0.0001	−0.87605 0.0020	0.94113 0.0002	0.95724 0.0001	0.98004 0.0001	1.00000 0.0			
RED	−0.78470 0.0123	0.77193 0.0148	−0.82700 0.0060	0.79551 0.0104	0.78118 0.0129	0.69110 0.0392	0.65871 0.0537	1.00000 0.0		
L	−0.88952 0.0013	0.90235 0.0009	−0.87672 0.0019	0.89853 0.0010	0.85671 0.0032	0.83788 0.0048	0.78917 0.0114	0.67611 0.0456	1.00000 0.0	
HMF	−0.82625 0.0060	0.86085 0.0029	−0.78626 0.0120	0.83375 0.0052	0.80101 0.0095	0.79546 0.0104	0.80250 0.0092	0.68184 0.0431	0.90846 0.0007	1.00000 0.0

* Correlation coefficient/P value

above 55°C in the presence of oxygen suggest changes in reaction mechanisms or rate limiting steps between the lower (45–55°C) and higher (<55°C) temperatures.

Relating quality and chemical changes

Sensory data were generated for pineapple juice reconstituted from thermally treated concentrate. A simple regression analysis was used to examine all possible linear-models-with-intercepts describing the sensory attributes as a function of changes in sucrose, glucose, fructose, reducing equivalents, Hunter-L and HMF (Table 7). In this matrix, all variables strongly correlated, only fructose vs reducing equivalents had $p > 0.05$, with most $p < 0.01$. This degree of correlation sets an important limit on interpretation of these data, as discussed below. Using the correlation coefficient R^2 as selection criterion, equations 7 and 8 are two of the best three-variable models.

$$\text{"Acceptability"} = 6.0 - 1.6(\text{Glucose Change}) - 0.6(\text{Reducing Loss}) - 1.2(\text{Hunter-L Loss}) \quad (7)$$

$$\text{"Cooked"} = 2.4 + 2.5\,(\text{Glucose Change}) + 0.7(\text{Reducing Loss}) + 1.2(\text{Hunter-Loss}) \quad (8)$$

"Acceptability" and "Cooked" are the averages of attributes scored by the sensory panel. Glucose Change, Reducing Loss, and Hunter-L Loss are fractions between 0 and 1 of the maximum observed change in glucose concentration, reducing equivalents, and Hunter-L, respectively. R^2 for Eq. (7) and (8) were 0.93 and 0.99, respectively. Sucrose disappearance was the best variable for all single-variable-with-intercept models, with R^2 values ranging from 0.88 to 0.98. Loss of sucrose indicated the time-temperature integral of the sample rather than being a direct indicator of quality loss.

DISCUSSION

DIFFERENTIAL SCANNING CALORIMETRY was used to follow changes in rates of decomposition reactions as a continuous function of temperature applying procedures used to follow plant metabolic rate changes (Hansen and Criddle, 1990). This allowed rapid characterization of the temperature range of interest for study of degradation reactions in the juice. Such technique to study degradation reactions is a novel means of characterizing temperature ranges for foods or other commodities. Measurable reaction rates became significant above about 50°C.

Temperature dependencies of the rates of heat production, losses of amines and reducing power, and CO_2 production suggested that some major change in degradation mechanism occurs below about 50°C (Fig. 1 and Tables 5 and 6). The Q_{10} values (3 to 4) over 45–55°C indicate a very great change in reaction rates over that range. This also points to 50°C as a key temperature in processing pineapple juice. Minimizing the time at which juice is maintained above that temperature should greatly decrease degradation. However, degradative reactions become so slow below 50°C, that little additional quality improvement would be gained by rapidly cooling below that point.

The isothermal calorimetric data showed that the major degradative reactions above 50°C could be described by the sum of two rate equations, first order and zero order in time. The first order process was deduced to be hydrolysis of sucrose because the decrease in sucrose paralleled the heat production rate, and the total heat/g concentrate from the first order reaction (about 7.6 J/g) corresponded closely to the total heat expected for nearly complete hydrolysis of sucrose, i.e. (0.42 mmol sucrose/g)(18 J/mmole) = 7.6 J/g sucrose. Also, no other component altered by heating was present in sufficient quantity to produce the observed heat by oxidative or nonoxidative reactions. Finally, the reaction was oxygen independent and not altered by additives such as dithiothreitol that would change the course of oxidation reactions. No other reaction is consistent with the chemistry or amount of heat released. The observation that the first order reaction was slowed by addition of sucrose appeared to contradict this conclusion. However, the hydrolysis rate of sucrose is also dependent on water activity which would be decreased by sucrose addition to the concentrate (Monsalve et al., 1990; Toribio et al. 1984; Cerrutti et al., 1985). Inversion of sucrose to glucose and fructose is unimportant to overall quality, although it increases the pool of glucose available for further degradation.

The zero order, exothermic process probably included most or all of the degradative reactions leading to color and quality loss above 50°C. The rate of these reactions was increased by increasing O_2 partial pressure, and the zero order rate of heat release generally paralleled the rate of O_2 uptake. From that rate (0.27–0.37 μMole/hr/g) and heat rate (120 μW/g) measured at 80°C in isothermal measurements, the heat produced/mole of oxygen consumed was 1200 to 1600 kJ/mole O_2. The heat/mole O_2 for combustion or oxidation of organic molecules is -455 ± 15 kJ/mole (Erickson, 1987; Thornton, 1917). Thus, less than half the heat from the zero order reaction was derived directly from reaction of O_2. The zero order heat production must have resulted from a complex of (O_2 dependent and O_2

independent reactions leading to a variety of products, including those related to color development.

Color formation in pineapple concentrate at high temperatures paralleled HMF formation (Fig. 5), but was not entirely dependent on it. The reaction of glucose at high temperatures and acid pH to form HMF is well documented (Heimlich and Martin, 1960; Cerrutti et al., 1985). Reactions in simple glucose solutions show time courses similar to those for HMF formation in the pineapple concentrate. HMF formation is independent of O_2, but HMF is known to undergo further oxygen dependent reactions to form colored products (Heimlich and Martin, 1960). However, addition of dithiothreitol blocked color formation without altering the rate of zero order heat production, and therefore HMF reactions with oxygen could not be a major pathway for pigment formation.

Addition of colorless HMF to the concentrate accelerated color development, but the rate of color formation was not linearly related to HMF concentration. HMF involvement in development of other colored products requires reaction with some other components(s) (X) produced more slowly in reactions that are rate limiting to total color development. The observed sigmoidal rate of color formation requires only the postulate that the reaction product of X and HMF continues to react with ever increasing levels of HMF in some type of sequential reaction that produces colored components. Active aldehydes other than HMF could also participate in condensation reactions (Wolfrom et al., 1948). Inhibition of color development could be achieved by removing oxygen, by trapping HMF or by limiting HMF formation.

Color forming reactions unrelated to HMF could also occur at high temperatures in the concentrate. The Maillard reaction of aldehydes, such as glucose, with free amines to form colored products is suggested by the loss of free amines in heated concentrate. However, the Maillard reaction is slow at the pH of concentrate (3.3) and little browning is likely to occur through this reaction (Whistler and Daniel, 1985; Ashoor and Zent, 1984). The Strecker reaction, in which decarboxylation leads ultimately to colored products, is suggested by CO_2 formation, loss of reducing equivalents, and decrease in ascorbate concentration. The observed decrease in formol titratable amines may have resulted partially from Strecker type reactions that consume amino residues, release CO_2 and form products that can react further with O_2 to form colored compounds. However, the ratio of free amine decrease/CO_2 production ranges from 6 to 28, depending on temperature and head space atmosphere, (Table 5) suggesting that Strecker reactions did not account for a large portion of the loss of amines. Ascorbic acid degradation in aerobic conditions can also produce CO_2 and result in pigment formation (Browerman, 1963). Color could develop from many other reactions in the chemically complex concentrate.

Degradation of ascorbic acid probably accounted for a major portion of the loss of reducing equivalents. This reaction in pineapple concentrate is also responsible for some of the CO_2 release. However, the rate of loss of reducing equivalents in the concentrate was 10 to 30 times the rate of production of CO_2 (Table 5). Production of CO_2 has a high dependence on O_2 concentration, changing by a factor of more than three between O_2 and N_2 atmospheres. The rate of loss of reducing power was only slightly increased (about 25%) by increasing the partial pressure of O_2 from 0 to 1 atm. Other studies have found about 80% of the loss of ascorbic acid was by reactions not requiring O_2. In addition, intermediates in the breakdown of ascorbate may be involved in reactions with other concentrate components without loss of CO_2.

The relations between chemical changes and quality indices could be used to form hypotheses about effects of processing changes on quality. However, correlation among effects of all degradative reactions on quality preclude postulation of cause and effect relationships between specific reactions and quality. A detailed analysis of individual and integrated reaction kinetics is required for guiding improvement of production processes. Calorimetry, colorimetry, and chemical analysis have provided information on rates and magnitudes of major deteriorative reactions in pineapple juice concentrate. Future studies of kinetics of degradation must focus on inhibition or acceleration of individual reactions to facilitate understanding of mechanisms.

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