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Kinetic studies of L-ascorbic acid degradation in fruit juices for the improvement of pasteurization plants

Pasteurization especially high-temperature short time (HTST) heating is a widely used preservation method which inactivates microorganisms and enzymes, but also degrades compounds as L-ascorbic acid. For a gentle dimensioning of a pasteurization plant the knowledge of the kinetic figures is important. Activation energy, reaction order and pre-exponential factor of the L-ascorbic acid degradation in a model solution, apple, orange and black currant juice were determined. Lines of equal effects, which indicate different time-temperature combinations for the degradation, could be derived and compared with the lethal effect on microorganisms. The activation energies were located in the area of 25 to 44 kJ/mol for all samples except of orange juice (74 kJ/mol) in the range of 40–90 °C with a zeroth reaction order. Based on these values, the lines of equal effects showed a lesser degradation at higher temperatures and shorter holding times even in the typical setting range of pasteurization plants.

Descriptors: pasteurization, kinetic parameters, non-isothermal, line of equal effect, modelling

1 Introduction

1.1 Preservation of juice

The emerging demand for safe and nutritious juices has led to the development of various non-thermal preservation techniques such as pulsed electric fields (PEF), high pressure treatment (HP), pressure change technologies, ozone treatment, irradiation, manosonication and modified thermal processes as microwave or ohmic heating [1–5]. The new techniques are supposed to be gentler to heat sensitive substances like volatiles and phenolic compounds than thermal pasteurization [6]. In comparison with equivalent degree of microbial inactivation, which is necessary for healthy and unspoiled juices, there were only differences in residual enzyme activities [7]. Pasteurization is more effective on the inhibition of peroxidase than HP and PEF [7] and polyphenol oxidase requires temperatures more than 80 °C for inhibition [8]. The residual enzyme activities can cause amongst others a lower cloud stability, a decrease of polyphenols with a subsequent colour or odour change [9–11]. However, due to their limitations in plant capacity, type of package or because of inefficacies in damaging specific microorganisms the pasteurization particularly in terms of HTST is still the most common method in the industry for juice preservation [12]. Therefore, there is high interest in improving conventional pasteurization processes aiming for a gentle treatment and low quality losses. In practice, temperatures of 76.6 °C to 87.7 °C and time in the holding tube between 25 and 30 s are typically applied in HTST for fruit juices [13]. Since the lethal heat effect on microorganisms increases faster with raising temperature than some chemical reactions, which was mainly explored in dairy products [14], higher temperatures and shorter times could be favourable for a gentle pasteurization.

1.2 Quality of juice

Juice consists of a large number of potentially reactive compounds like sugars, organic and amino acids and vitamins as well as colour compounds and in a small amount phytochemicals [12, 15]. Several works listed in table 1 (see page 86) investigated kinetic figures of different compounds in food with an isothermal heat treatment and under different storage and heating conditions for the degradation of L-ascorbic acid. With the knowledge of the activation energy E_A and the pre-exponential factor k_0, the conversion of the compounds can be defined with the Arrhenius equation (Eq. 3) at different temperatures.

Non-isothermal methods were employed for the investigation of colour change kinetics of grape juice by *Rhim* et al. [16] or for dimethyl sulphide degradation in beer by *Huang* et al. [17]. In many cases the L-ascorbic acid degradation and colour change to an undesired colour impression were examined in regard to quality deterioration in juice [18]. Apart of juice, also model solutions were investigated to operate with a defined and reproducible system [19].

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Table 1 **Kinetic data reported for L-ascorbic acid degradation determined with isothermal methods**

Matrix	E_A [kJ/mol]	Thermal treatment [°C]	k_0 [min^{-1}]	Reaction order	Reference
Orange juice	52.74	65–90	$19.95*10^5$	pseudo 1	[5]
	71.0 ± 3.8	20–45	$(6.3 \pm 0.6)*10^2$	1	[49]
	56.02 ± 29.83	4–45	not specified	pseudo 0	[41]
Mango pulp	39 ± 14	80–150	$(1.3 \pm 0.5)*10^{-1}$	biphasic; 1	[50]
Model system	66.94†	61–105	not specified	0	[42]

† Data for $a_w = 0.9$

1.3 L-ascorbic acid

L-ascorbic acid is an important vitamin for human nutrition and frequently found in fruits [15]. In juices it can be added as an antioxidant, to stabilize the turbidity and to increase the viscosity [20]. The stability of L-ascorbic acid depends on ambient conditions as pH value, temperature, presence of metal ions and oxygen content [21–23]. At pH values lower than its pk_1 (4,04) it is more stable than above [24]. Heat treatment promotes the degradation generally but it is comparatively lower by HTST-treatment [24]. The degradation pattern of L-ascorbic acid differs depending on the oxygen content, whereby the reaction can be catalysed by metal ions during aerobic degradation [23, 24]. Due to rising temperature and larger °Brix-values, a lower oxygen solubility is expected [25].

Polyphenols can have an influence on the stability of L-ascorbic acid [26]. It was described that flavonoids are able to impair L-ascorbic acid degradation [12]. *Clegg* et al. showed that flavonols exhibit a higher potential for the protection of L-ascorbic acid against degradation than anthocyanins, which can even accelerate the oxidation [26].

The nutritional value of L-ascorbic acid consists among others of its effect as an antioxidant in human blood plasma for the protection against degenerative processes from oxidant stress [27], as an agent against scurvy [28] and for the improvement of iron absorption [29]. The loss of its vitamin activity occurs when L-ascorbic acid is degraded to 2,3-diketogulonic acid [24]. The degradation products of L-ascorbic acid can react with or without amino acids to browning products [24]. *Bharate & Bharate* showed a pathway for the degradation of L-ascorbic acid and the formation of brown pigments [30]. *Smuda & Glomb* showed the degradation products which incorporate in Maillard reaction [31]. *Shinoda* et al. revealed different compounds in a model solution of orange juice that affect the formation of several reaction products responsible for the non-enzymatic browning. As important chemical precursors an interaction of L-ascorbic acid, amino acids and different fruit sugars has been found whereas chelators and radical scavengers could inhibit the browning reactions [19].

Based on this importance for a consistent product quality, information about the temperature depending kinetic figures as reaction order, activation energy and pre-exponential factor of L-ascorbic acid degradation in juices is of great interest for the comparison with the microbial inactivation figures. With this information, optimized time-temperature parametrization can finally be elaborated with different time-temperature-combinations in a diagram of lines of equal effects on quality determining properties, which results in an improvement in current pasteurization practice.

2 Materials and methods

2.1 Materials

The model solution (MS) was prepared immediately before the experiment started in order to diminish a previous reaction of the

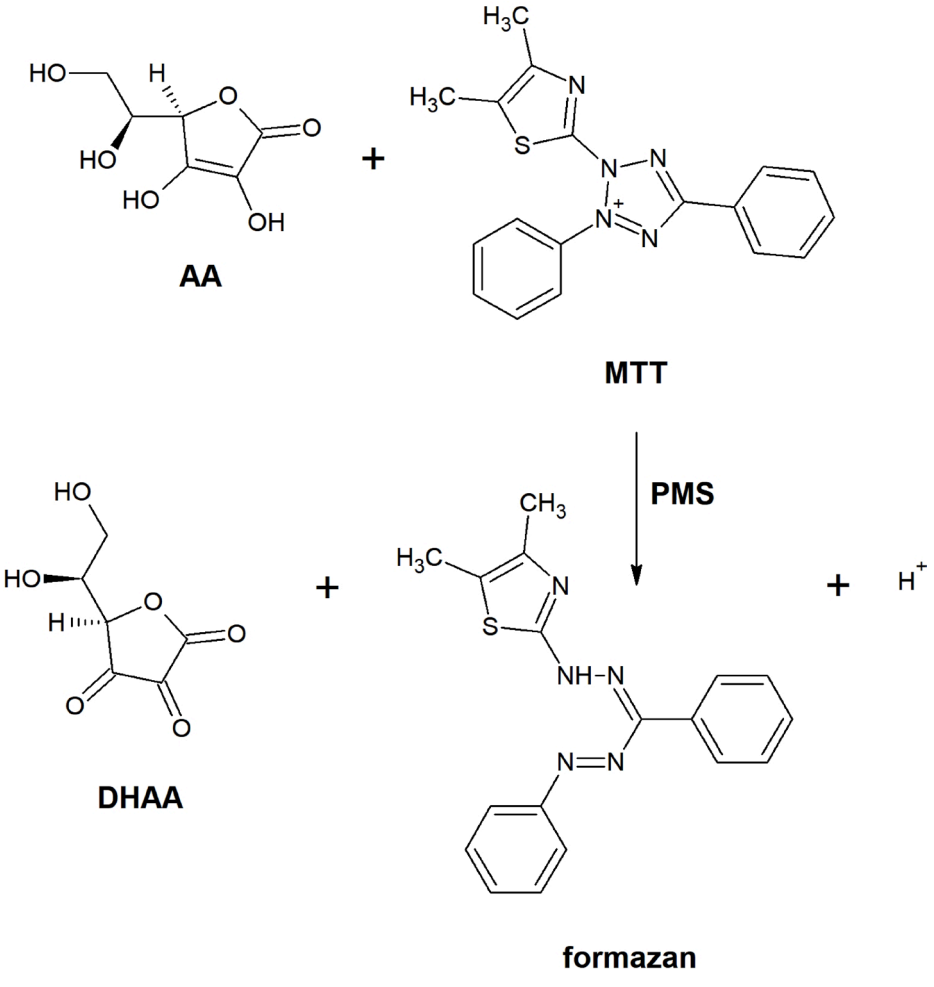

Fig. 1 **Reaction of L-ascorbic acid with MTT [33]**

ingredients. The compounds of the model solution were adapted to the composition of apple juice [32]. The solution consisted of 17 g/L sucrose (Südzucker, Mannheim), 64 g/L fructose (Danisco, Kotka) and 26.4 g/L dextrosemonohydrate (Cargill, Krefeld). The pH-value was adjusted to 3.5 with malic acid (Merck, Darmstadt).

Clear and cloudy apple juice (AJ) (Lagenser Fruchtsäfte™), black currant juice (BCJ) (dm™), fresh oranges (OJ) (Valensina®) and fresh apples (variety 1: Elstar; variety 2: orchard) were purchased in a local supermarket. For the fresh juices, fruits were cut, pressed, filtered and all apple juices were spiked with 350 mg/L L-ascorbic acid (Chemsolute, Renningen). The content of soluble solids content (SSC) was measured with the refractometer J157 (Rudolph Research Analytical, Hackettstown), the pH-value with the pHSensor SE 101-MS (Knick, Berlin).

2.2 Sugar and organic acids

Sugar and organic acid composition was determined by liquid chromatography (Perkin Elmer Flexar LC, Waltham) equipped with an UV-VIS (210 nm) and RI detector. A Nucleogel Sugar 810 H column (7.8 mm ID, 300 mm; MachereyNagel, Düren) was used with an isocratic mobile phase (25 mmol/L sulphuric acid in water; Fluka, Seelze) at a flow rate of 0.6 mL/min and a temperature of 35 °C. Samples of 5 µL were injected directly after a filtration with a 0.25 µm syringe filter.

2.3 Determination of L-ascorbic acid

The automatic photometer Gallery (Thermo Scientific, Newington) was used for the photometric measurement of L-ascorbic acid (AA). Cloudy samples were centrifuged (10 min, 5450 g) prior further analysis. As shown in figure 1 the photometric method is based on the reduction of tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.1 mmol/L) by means of the electron carrier phenazine methosulfate (PMS; 1 mmol/L) to a coloured formazan by L-ascorbic acid and other reducing substances [33]. The formazan was measured at a wavelength of 575 nm. For the blank value L-ascorbic acid were oxidized by ascorbate oxidase (10 KU/L) to dehydroascorbic acid (DHAA) and only the remaining reducing substances in the analysis solution were measured in order to calculate the L-ascorbic acid content [33]. Stock solution of L-ascorbic acid (Chemsolute, Renningen) was prepared in 1.5 % w/v meta-phosphoric acid (Roth, Karlsruhe) with a pH-value of 3.5 [34] and a test kit (Thermo Fisher Scientific, Vantaa) was used for quantification.

2.4 Determination of the kinetic figures

2.4.1 Reaction order

The reaction order is required for the calculation of the activation energy and pre-exponential factor [35]. Therefore, isothermal experiments were carried out. According to *Fink*, the term $\left(\frac{c_t}{c_0}\right)^{1-n}$ comprising the initial concentration c_0, the concentration at a variable time c_t and the reaction order n was plotted against time t [36]. The highest coefficient of determination (R^2) expresses the most appropriate reaction order.

2.4.2 Activation energy

For the calculation of the activation energy, a non-isothermal method according to *Coats & Redfern* with a linear heating rate was applied. This method requires only one defined heating rate [35]. The conversion of a component in chemical reactions can be described with equation 1 [35]

$$\frac{d\alpha}{dt} = k(1-\alpha)^n \qquad \text{(Eq. 1)}$$

with the reaction rate constant k. α describes the fraction of the decomposed component (Eq. 2) [37] where c_{End} is 0 mg/L.

$$\alpha = \frac{c_0 - c_t}{c_0 - c_{End}} \qquad \text{(Eq. 2)}$$

The temperature dependency of chemicals reactions can be described with the Arrhenius equation 3 with the frequency factor k_0 and the activation energy E_A [35]

$$k = k_0 e^{-\frac{E_A}{RT}} \qquad \text{(Eq. 3)}$$

The linear heating rate β for non-isothermal experiments can be described by equation 4 [35]

$$\beta = \frac{dT}{dt} \qquad \text{(Eq. 4)}$$

The combination of the equations 1, 3 and 4 leads to equation 5 [35]

$$\int_0^\alpha \frac{d\alpha}{(1-\alpha)^n} = \frac{k_0}{\beta}\int_0^T e^{-\frac{E_A}{RT}}dT \qquad \text{(Eq. 5)}$$

The integral on the right hand side has no explicit solution but it can be replaced with an approximation shown in Coats & Redfern as well as Huang et al. [17, 35]. The calculation of the activation energy can be carried out for n ≠ 1 with equation 6 [17].

$$ln\left[\frac{1-(1-\alpha)^{1-n}}{T^2(1-n)}\right] = ln\left[\frac{k_0 R}{\beta E_A}\left(1-\frac{2RT}{E_A}\right)\right] - \frac{E_A}{RT} \qquad \text{(Eq. 6)}$$

A pre-processing for smoothing was applied. The experimentally obtained data were plotted in a concentration-time diagram and a function was obtained by a quadratic polynomial fit. More complex fitting function such as higher polynomials or exponential function did not provide better results in a here relevant extent. With this function, the concentration can be calculated for any time-temperature combination and experimental fluctuations can be compensated.

2.4.3 Pre-exponential factor

Theoretically, the pre-exponential factor can be calculated from the intercept of the y-axis of the linear equation 6. If the coefficient of determination of the linear equation showed a non-linear course ($R^2 < 1$) the resulting error can lead to a deviation. Therefore, the

pre-exponential factor can be calculated as shown in equation 8 with the help of the rate law of the zeroth reaction order (Eq. 7), the Arrhenius equation (Eq. 3), taking the isothermal measured data for the change of the degraded L-ascorbic acid Δc in a time interval Δt [14].

$$-\frac{dc}{dt} = k \qquad \text{(Eq. 7)}$$

$$k_0 = \frac{\Delta c}{\Delta t e^{-\frac{E_A}{RT}}} \qquad \text{(Eq. 8)}$$

Here $\Delta c = c_1 - c_2$ and $\Delta t = t_2 - t_1$ ($t_2 > t_1$).

2.4.4 Lines of equal L-ascorbic acid degradation

A diagram of lines with equal effects represents different time-temperature-combinations for the degraded amount of L-ascorbic acid and the microbiological lethal effect. The comparison of microbiological and chemical lines of equal effects allows the identification of temperature-time combinations with the lowest nutritive losses. The working area of pasteurization must be located on or above the line of applied pasteurization units and below the accepted quality decline. A schematic graph for illustration is shown in figure 2. The time-temperature-combinations for the microbiological lethal values can be calculated via the Pasteurization units (PU) with equation 9 [38].

$$PU = t(min)10^{\frac{T-T_B}{z}} \qquad \text{(Eq. 9)}$$

The applied temperature is T, the reference temperature T_B for fruit juices is by convention 80 °C [39]. The z-values indicates the required temperature increase that is necessary to obtain the same effect in a tenth of the time [14]. Exemplary selected z-values of fruit juice relevant spoiling microorganisms can be obtained from table 2. To calculate a line of equal effect for chemical degradation, a concentration change must be defined. For this particular concentration change, e.g. L-ascorbic acid degradation, for any temperature the corresponding time that is required can be determined with equation 8 with the previous calculated kinetic values of E_A and k_0. If this temperature-time combination is used as reference point (T_C, t_C), all temperature-time combinations at a specific degraded concentration can be calculated using equation 10 and plotted in a diagram as a line of equal effects.

$$\ln(t) = \ln(t_C) - \frac{E_A}{R}\left(\frac{1}{T_C} - \frac{1}{T}\right) \qquad \text{(Eq. 10)}$$

2.4.5 Thermal processing

The isothermal and non-isothermal experiments were performed in a 500 mL-double-walled beaker glass tempered by a thermostatic water bath (Versacool 7, Thermo Scientific, Newington) mixed with a magnetic stirrer (300 rpm) and controlled by a temperature-measuring sensor (Pt-100) as shown in figure 3. The temperature applied for the isothermal experiments was 85 °C for 8000 s and the non-isothermal experiments were conducted with a linear heating rate of 0.25 °C/min with the temperature range of 40 to 90 °C. Before the experiments were carried out, the heating rate was tested to be linear and the constancy of the temperature was verified for the isothermal experiments. The temperature range and heating rate were chosen in a way that an adequate oxidation of L-ascorbic

Table 2 **z-values for the inactivation of selected microorganisms in model solution (MS), apple juice (AJ) and orange juice (OJ)**

Microorganism	Matrix	cell conditions	pH-value	Brix °Brix	z-value °C	Reference
Saccharomyces cerevisiae	MS	vegetative cells	2.8	13.5	5.8	[51]
Escherichia coli O157:H7	AJ	vegetative cells	3.5	11.7	5.6†	[52]
Alicyclobacillus acidoterrestris	OJ	bacterial spores	3.5	11.7	7.8	[53]

† Calculated from logD against temperature [51]

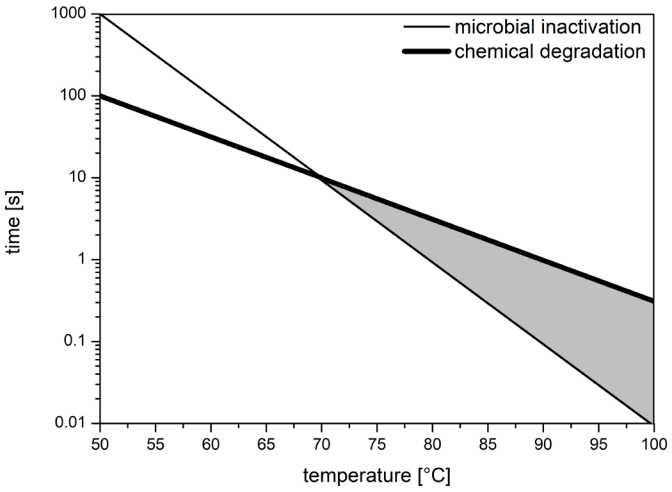

Fig. 2 **Exemplary schematic graphs of lines with equal effect for typical chemical degradation and typical microbial inactivation, grey area represents the preferred pasteurization area**

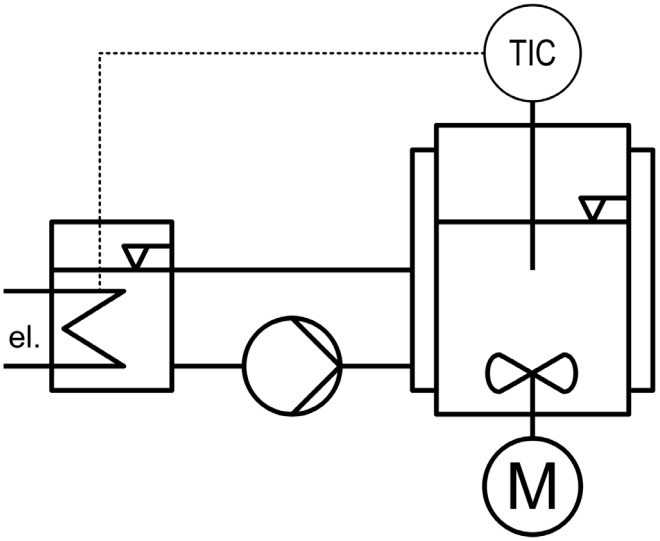

Fig. 3 **Schematic drawing of the experimental setup**

Table 3 **Composition of the model solution (MS) and juices (AJ-apple, OJ-orange, BCJ-black currant) in the non-isothermal experiments (n = 3)**

	pH	SSC	Glucose	Fructose	Sucrose	Malic acid	Citric acid	L-ascorbic acid
	–	°Brix	[g/L]	[g/L]	[g/L]	[g/L]	[g/L]	[mg/L]
MS	3.15–3.53	9.73–12.75	23.63–24.11	63.21–65.59	15.77–16.67	n.d.	–	290–374†
clear AJ	3.38–3.48	10.96–13.36	25.28–26.61	66.85–70.59	12.03–12.52	7.31–7.50	n.d.	341–365†
cloudy AJ	3.35–3.51	11.68–13.41	23.47–24.58	65.20–67.46	15.42–15.93	6.58–6.75	n.d.	556–584€
fresh AJ (var. 1)	3.98–4.02	15.51–15.63	21.45–21.84	73.83–74.18	51.32–52.99	6.35–6.50	n.d.	333–367†
fresh AJ (var. 2)	3.28–3.38	13.72–14.05	20.78–21.43	68.96–70.00	37.16–38.31	8.26–9.30	n.d.	233–281†
OJ	3.76–3.85	11.95–12.40	21.75–23.03	22.40–23.59	54.68–57.22	2.17–2.26	8.83–9.06	423–460§
BCJ	2.88–2.99	16.61–17.97	44.01–45.21	65.65–67.23	n.d.	9.38–10.09	31.06–31.69	928–991§

n.d. not determinable †spiked €spiked & native content §native content

acid could be achieved. However, also a realistic pasteurization temperature range was considered. The experimental design with a double-walled reaction vessel was chosen to achieve a uniform temperature distribution through permanent stirring and to perform accurate temperature control directly in the heating medium.

The samples were immediately cooled in ice water to decelerate chemical reactions and were subsequently analysed. Temperature, time and the L-ascorbic acid concentration were recorded for the kinetic calculation.

2.4.6 Statistical analysis

The experiments were carried out in triplicate. The values were given as an arithmetic mean with the minimum-maximum span. In order to determine the differences between the different matrices, a one-way analysis of variance (ANOVA) was carried out. Least Significant Difference test (LSD; $p < 0.05$) was used to determine the significant difference between the juices.

2.4.7 Software

Microsoft Excel was used for kinetic and the statistical data calculation. Origin was used for diagrams and regression analysis, RI-CAD for the process flowsheet and Chemsketch for the chemical equation.

3 Results and Discussion

3.1 Composition of juices

Table 3 shows the composition of the different juices. A similar concentration of L-ascorbic acid was added to each of the spiked juices (target minimum 350 mg/L). Because of setting time of the temperature in the thermostat and the dissolution time of the L-ascorbic acid, a variation of the initial content was unavoidable.

3.2 Reaction order

For the calculation of the kinetic figures, the reaction order has to be known. Regarding the literature, for the L-ascorbic acid degradation, a zeroth- or more frequently first-order reaction is assumed (Table 1). Own experiments, obtained from the isothermal trials at 85 °C, tended clearly to the zeroth order. Figure 4 sums up the coefficients of determination (R^2) in dependency on the reaction order for all samples, except black currant juice (BCJ) due to the

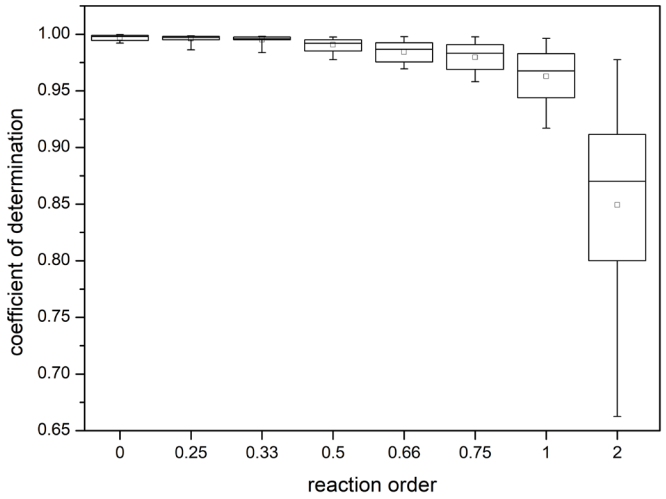

Fig. 4 **Coefficients of determination R^2 for all matrices (without BCJ), used for the selection of the reaction order; Boxplot with arithmetic mean, median, 25/75 % quartile and span**

Table 4 **R^2 selection of the reaction order in whole numbers for the L-ascorbic degradation with the help of spans; isothermal experiments at 85 °C and 8000 s (MS-model solution, AJ-apple juice, OJ-orange juice, BCJ-black currant juice, var.-variety, n = 3)**

	0	1	2
	R^2		
MS	0.9948–0.9996	0.9171–0.9439	0.6626–0.7752
clear AJ	0.9957–0.9991	0.9219–0.9763	0.676–0.8999
cloudy AJ	0.9973–0.9992	0.9645–0.9762	0.8568–0.9116
fresh AJ (var. 1)	0.9970–0.9993	0.9432–0.9873	0.8000–0.9505
fresh AJ (var. 2)	0.9923–0.9990	0.9656–0.9828	0.8150–0.9041
OJ	0.9933–0.9989	0.9469–0.9847	0.8066–0.9347
BCJ	0.9274–0.9932	0.9448–0.9965	0.9586–0.9976

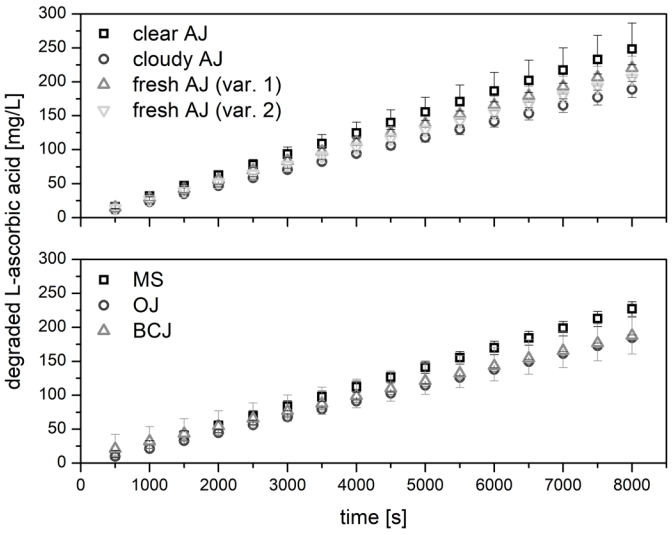

Fig. 5 Degraded L-ascorbic acid during isothermal processing at 85 °C, arithmetic mean with span (n = 3)

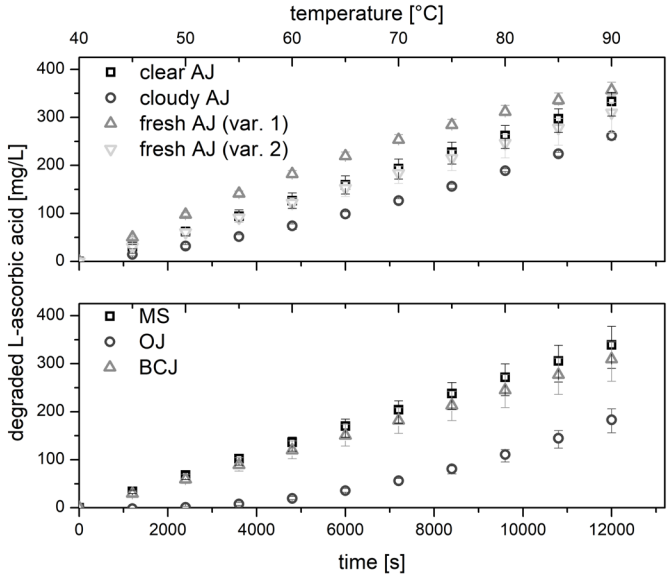

Fig. 6 Degraded L-ascorbic acid during non-isothermal heat treatment, arithmetic mean with span (n = 3)

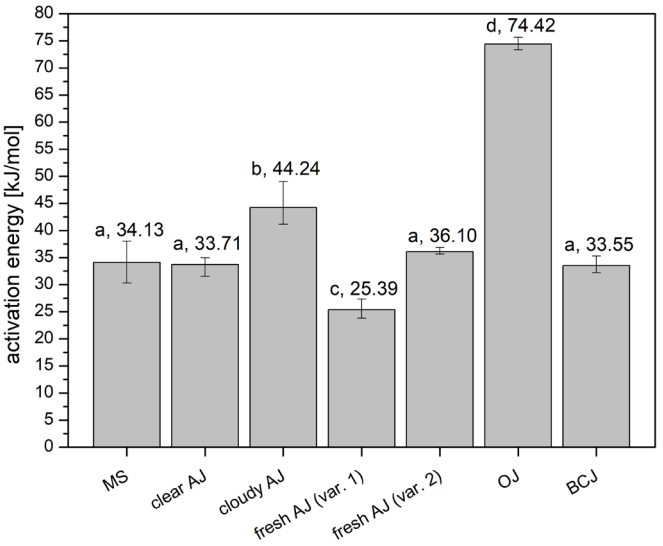

Fig. 7 Activation energy for the L-ascorbic acid degradation in different juices, arithmetic mean with span (n = 3). Labels indicate the mean value [kJ/mol] and different letters the significant differences by LSD test (p < 0.05)

deviation from the other juices, which would result in a wider range. Apparently, the L-ascorbic acid degradation behaved similarly in all matrices. With increasing reaction order, the R^2-values changed for the worse and had a wider spreading. Table 4 shows, here including BCJ, the evaluation of the reaction order broken down for whole numbers (0, 1, and 2). The results revealed that for all samples with exception of the BCJ the zeroth reaction order (n = 0) was most applicable. The BCJ however seem so far to be an exception. The R^2 for the BCJ differed only slightly for the different reaction orders. A reason was the high native content of L-ascorbic acid (cf. Table 3). While the absolute amount of degraded L-ascorbic acid c_t is in a similar magnitude for all juices (Fig. 5), the amount in relation to the initial amount c_0 is comparably small in case of the BCJ. The reaction order cannot be determined with sufficient accuracy because of the similar course for the different reaction orders for $\frac{c_t}{c_0} = f(k * t)$ at low conversion rates [14]. As intermediate result, the zeroth reaction order was selected for the further calculations which corresponds to the results reported by several works [40–42].

3.3 Activation energy

The kinetic values obtained by non-isothermal heating are expected to be more accurate due to the lesser individual errors of the method [43]. In figure 6, the amount of L-ascorbic acid loss over the reaction time and the time related temperature increase is displayed for the selected juices with non-isothermal treatment. The interrelation of degradation and time was rather linear. Exceptions were the cloudy apple juice and even more clearly in case of the orange juice. In addition, it is visible that L-ascorbic acid degradation was more enhanced in orange juice at higher temperatures compared to other juices. Figure 7 shows the activation energy for the L-ascorbic acid degradation ($R^2 > 0.9$). The activation energy in orange juice was significantly higher than in the other juices but coincided nearly with the values given in table 1. The higher activation energy indicates the different behaviour of the degradation in orange juice with increasing temperature explained by the Arrhenius equation. Therefrom derived equation 11 [44] can be used to calculate the rate constant k_2 for any temperature T_2 when activation energy and rate constant k_1 is known for one defined temperature T_1.

$$k_2 = e^{\frac{E_A(T_1 - T_2)}{R * T_2 T_1}} * k_1 \quad \text{(Eq. 11)}$$

With a larger activation energy, the rate constant increases at higher temperatures. This means that a larger amount of L-ascorbic acid will be degraded at higher temperatures. This effect was identifiable for orange juice in the concentration-time diagram (Fig. 6).

According to *Miller & Joslyn*, the L-ascorbic acid oxidation is impaired by an increasing sugar content and a decreasing pH-value [45]. However, no correlation of the degradation rate of L-ascorbic acid with the sugar content nor with the pH-value could be found.

Orange juice contains a high amount of the flavanone-glycoside hesperidin [15] which could be a reason for significantly higher activation energy due to the protective function against the oxidation of L-ascorbic acid. In contrast, black currant juice contains mainly anthocyanins [26] and apple juice contains hydroxycinnamic acids [46]. There was a significantly higher activation energy for cloudy apple juice compared to clear apple juice. The lower content of polyphenols in clear apple juice compared to cloudy apple juice [46] hardly seems to have any influence on the hindrance of the degradation, since the activation energy of the model solution is on the same level. In case of the self-made (fresh) juices, a higher oxygen absorption could have catalysed the degradation. It is visible that the freshly pressed apple juices had a different activation energy. According to *Kahle* et al., there are significant differences in the polyphenol content between dessert apples (var. 1) and cider apples (var. 2) [46]. This difference could explain the deviant activation energy. Purchased juice are assumed to have a lower polyphenol content than freshly produced [46], but other factors such as metal ions and oxygen input during production can have an impact on the stability of L-ascorbic acid [21, 23].

Disadvantageous for the determination of the activation energy could be an oxygen input whilst stirring and production. The oxygen concentration has an influence on the reaction mechanism and may thus affect the found reaction order [24]. At lower oxygen concentrations (< 0.63 %), a zeroth reaction order is expected and with a higher oxygen content a faster oxidation is evident [21].

Figure 8 shows the correlation of degraded L-ascorbic acid in the defined experimental set-up and the activation energy. The plot allows the assumption of an exponential relation confirming the applicability of the Arrhenius equation.

3.4 Pre-exponential factor

The pre-exponential factor is necessary for the calculation of any time-temperature combinations related to an arbitrarily selected concentration change (Eq. 8) and for the calculation of the reaction rate constant k. Unlike the activation energy, the pre-exponential factor cannot be satisfactorily determined with the non-isothermal Coats & Redfern method. The reason is the need of an extrapolation to find an axis intercept. The extrapolation is highly sensitive to even minor deviation occurring in the non-isothermal experiments. Therefore, as previously for the identification reaction order, the isothermal method was used with equation 8. Figure 9 reveals exemplary evidently better suitability of the isothermally found axis intercept (representing the pre-exponential factor) compared with non-isothermal method since this corresponds approximately to the course of the measuring points. The isothermally calculated k_0-values for all matrices are shown in table 5. These figures coincide fairly with literature data shown in table 1. The pre-exponential factor is temperature-dependent, but for practical reasons it is assumed to be constant in the Arrhenius equation [44].

3.4.1 Lines of equal L-ascorbic acid degradation

The heat load of real pasteurization conditions provides only a rather minor extent of L-ascorbic acid degradation. In figure 10 (see page 92) a technically possible heating range is shown. The steeper curves represent the lines of equal inactivation of different and juice relevant microorganisms in an extent of 10 PU. The flatter curves are the lines of equal degradation (20 mg/L) of L-ascorbic acid. The different steepness is a result of different activation energies and z-values respectively.

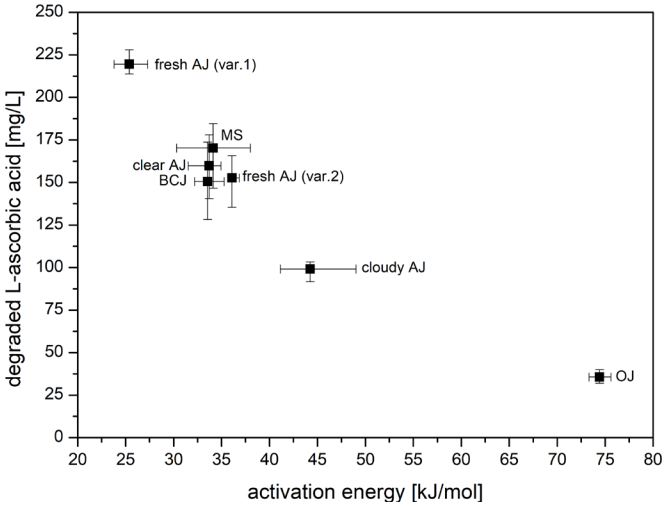

Fig. 8 Absolute amount of degraded L-ascorbic acid at 6000 s, obtained from the defined non-isothermal test arrangement versus activation energy, arithmetic mean with span (n = 3)

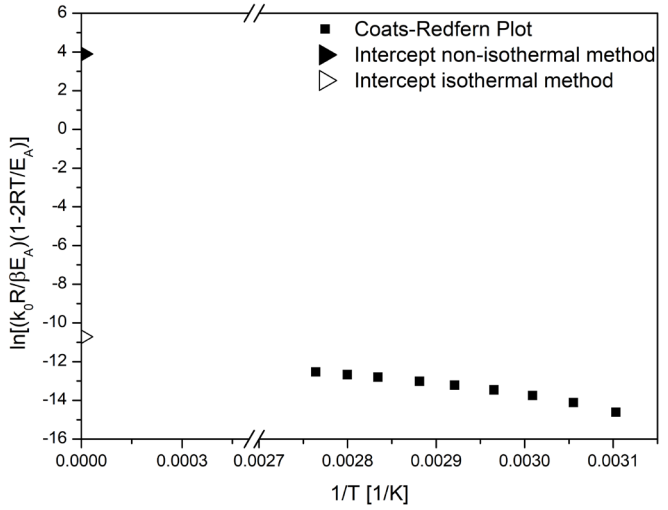

Fig. 9 Coats and Redfern Plot of cloudy apple juice with non-isothermal and calculated isothermal intercept

Table 5 Pre-exponential factor k_0 of L-ascorbic acid degradation in different juices, arithmetic mean and span at 85 °C (n = 3), time span 8000 s with intervals of 500 s

Matrix	k_0 [molL^{-1}s^{-1}]
MS	(1.52; 1.31–1.56)*10^{-2}
clear AJ	(1.50; 1.49–1.57)*10^{-2}
cloudy AJ	(3.75; 3.67–3.76)*10^{-1}
fresh AJ (Var. 1)	(8.15; 8.02–8.83)*10^{-4}
fresh AJ (Var. 2)	(2.77; 2.74–2.94)*10^{-2}
OJ	(9.47; 8.18–9.71)*10^{3}
BCJ	(1.20; 1.06–1.91)*10^{-2}

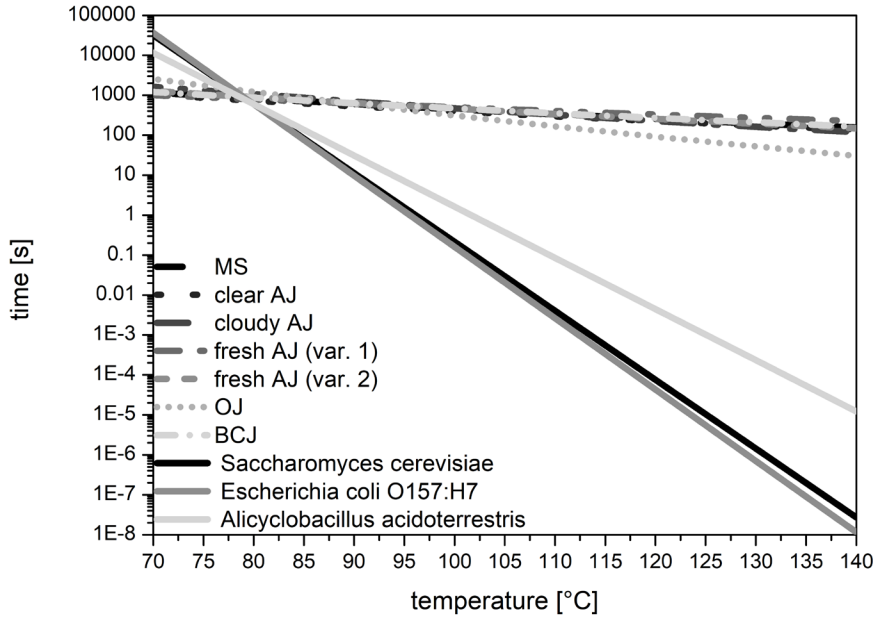

Fig. 10 Lines of equal L-ascorbic acid degradation (20 mg/L) and microbial inactivation with 10 pasteurization units

In spite of the comparably small contribution for L-ascorbic acid protection, it seems nonetheless worth to improve the current pasteurization practice. At conventional pasteurization temperatures of 76.6–87.7 °C [13], there is a higher product damage due to the loss of L-ascorbic acid than at higher temperatures and further deterioration is more probable due to the reaction of the degradation products of L-ascorbic acid with other juice ingredients.

According to *Labuza & Riboh*, the extrapolation for the shelf life prediction is influenced by different factors such as analytical precision, selection of an appropriate reaction order or the preference of different reactions at different temperatures [47]. Therefore, the course shown in figure 10 should be seen as an approximation and not as an exact calculation for the degraded amount of L-ascorbic acid.

In addition, it is more advantageous for the sensory quality of juices that the enzymes get inactivated at higher pasteurization temperatures, since e.g. polyphenol oxidase is only inactivated at temperatures above 80 °C [8–11]. Due to the small differences during pasteurization, subsequent effects on the pasteurized product, such as filling, transport and storage, are important influencing factors in order to maintain the quality.

The low degradation refutes the assumption that pasteurization is highly damaging process for L-ascorbic acid. Nevertheless, a further optimization is possible by including the heat impact of the recuperation zones in the calculation of the pasteurization units and using higher temperatures with a shorter or no holding time due to the short holding times at high temperatures. This has to be evaluated for each beverage individually since certain reactions occur only at higher temperatures, such as caramelization above a temperature of 120 °C [48]. For this optimized pasteurization practice the lines of equal effects have to be flatter for the chemical reactions than the lines of equal microorganism inactivation and the range of least deterioration must be selected.

4 Conclusion

The activation energy of L-ascorbic acid degradation in juices can be sufficiently calculated using the non-isothermal Coats & Redfern method with a zeroth reaction order. For different types of juice, significantly different activation energies are calculated. However, in relation to the large difference to the microbial inactivation figures, the activation energy variations among the juices have a comparatively small influence when comparing with the lines of equal effects. As expected by the initial hypothesis, the comparisons of the lines of equal effect reveal a potential for a process improvement in terms of a gentler process. However, the results show also that the usual pasteurization conditions in practice may not cause severe damages to the product, but it is still possible to optimize the process by setting higher temperatures and shorter holding times and considering the recuperation zones with their heat load contribution. This knowledge can also be used in the beer pasteurization in general or for beer-based mixed drinks. An application in the development of quality-influencing reaction products or aroma and undesired colour components is also conceivable. HMF or Thiobarbituric Acid Index might be exemplary indicators. An important precondition for this is that the lines of equal effects are flatter compared to the lines of equal inactivation of microorganisms for a successful application of higher pasteurization temperatures.

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Conflict of Interest

The Authors declare that there is no conflict of interest.

5 References

1. Mañas, P. and Pagán, R.: Microbial inactivation by new technologies of food preservation, Journal of applied microbiology, **98** (2005), no. 6, pp. 1387-1399.
2. Patil, S.; Bourke, P.; Frias, J. M.; Tiwari, B. K. and Cullen, P. J.: Inactivation of Escherichia coli in orange juice using ozone, Innovative Food Science & Emerging Technologies, **10** (2009), no. 4, pp. 551-557.
3. Aschoff, J. K.; Knoblauch, K.; Hüttner, C.; Vásquez-Caicedo, A. L.; Carle, R. and Schweiggert, R. M.: Non-Thermal Pasteurization of Orange (Citrus sinensis (L.) Osbeck) Juices Using Continuous Pressure Change Technology (PCT): A Proof-of-Concept, Food and Bioprocess Technology, **9** (2016), no. 10, pp. 1681-1691.
4. Gentry, T. S. and Roberts, J. S.: Formation kinetics and application of 5-hydroxymethylfurfural as a time–temperature indicator of lethality for continuous pasteurization of apple cider, Innovative Food Science &

Emerging Technologies, **5** (2004), no. 3, pp. 327-333.
5. Lima, M.; Heskitt, B. F.; Burianek, L. L.; Nokes, S. E.U.E. and Sastry, S. K.: Ascorbic acid degradation kinetics during conventional and ohmic heating, Journal of Food Processing and Preservation, **23** (1999), no. 5, pp. 421-443.
6. Aguilar-Rosas, S. F.; Ballinas-Casarrubias, M. L.; Nevarez-Moorillon, G. V.; Martin-Belloso, O. and Ortega-Rivas, E.: Thermal and pulsed electric fields pasteurization of apple juice: Effects on physicochemical properties and flavour compounds, Journal of Food Engineering, **83** (2007), no. 1, pp. 41-46.
7. Vervoort, L.; van der Plancken, I.; Grauwet, T.; Timmermans, R. A.H.; Mastwijk, H. C.; Matser, A. M. et al.: Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice, Innovative Food Science & Emerging Technologies, **12** (2011), no. 4, pp. 466-477.
8. Krapfenbauer, G.; Kinner, M.; Gössinger, M.; Schönlechner, R. and Berghofer, E.: Effect of thermal treatment on the quality of cloudy apple juice, Journal of agricultural and food chemistry, **54** (2006), no. 15, pp. 5453-5460.
9. Castaldo, D.; Laratta, B.; Loiudice, R.; Giovane, A.; Quagliuolo, L. and Servillo, L.: Presence of Residual Pectin Methylesterase Activity in Thermally Stabilized Industrial Fruit Preparations, LWT - Food Science and Technology, **30** (1997), no. 5, pp. 479-484.
10. Murata, M.; Noda, I. and Homma, S.: Enzymatic Browning of Apples on the Market: Relationship between Browning, Polyphenol Content, and Polyphenol Oxidase, The Japanese Society for Food Science and Technology (Nippon Shokuhin Kagaku Kogaku Kaishi), **42** (1995), no. 10, pp. 820-826.
11. Komthong, P.; Katoh, T.; Igura, N. and Shimoda, M.: Changes in the odours of apple juice during enzymatic browning, Food Quality and Preference, **17** (2006), no. 6, pp. 497-504.
12. Schobinger, U.: Frucht- und Gemüsesäfte: Technologie, Chemie, Mikrobiologie, Analytik, Bedeutung, Recht ; 99 tables, 3., rev. ed., Ulmer, Stuttgart, 2001.
13. Nelson, P. E. and Tressler, D. K. (Eds.): Fruit and vegetable juice processing technology, 3d ed., AV I Pub. Co, Westport, Conn., 1980.
14. Kessler, H.-G.: Lebensmittel- und Bioverfahrenstechnik: Molkereitechnologie ; with 109 tables, 4., rev. and extended ed., 2. reprint, Verl. A. Kessler, München, 2011.
15. Belitz, H.-D.; Grosch, W. and Schieberle, P.: Food chemistry: With 634 tables, 4., rev. and extended ed., Springer, Berlin, Heidelberg, 2009.
16. Rhim, J. W.; Nunes, R. V.; Jones, V. A. and Swartzel, K. R.: Kinetics of Color Change of Grape Juice Generated using Linearly Increasing Temperature, Journal of Food Science, **54** (1989), no. 3, pp. 776-777.
17. Huang, Y.; Tippmann, J. and Becker, T.: Non-Isothermal Kinetic Models of Degradation of S-Methylmethionine, Journal of Food Process Engineering, **39** (2016), no. 6, pp. 573-580.
18. Rawson, A.; Patras, A.; Tiwari, B. K.; Noci, F.; Koutchma, T. and Brunton, N.: Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances, Food Research International, **44** (2011), no. 7, pp. 1875-1887.
19. Shinoda, Y.; Komura, H.; Homma, S. and Murata, M.: Browning of model orange juice solution: Factors affecting the formation of decomposition products, Bioscience, biotechnology, and biochemistry, **69** (2005), no. 11, pp. 2129-2137.
20. Kolniak-Ostek, J.; Oszmiański, J. and Wojdyło, A.: Effect of l-ascorbic acid addition on quality, polyphenolic compounds and antioxidant capacity of cloudy apple juices, European Food Research and Technology, **236** (2013), no. 5, pp. 777-798.
21. van Bree, I.; Baetens, J. M.; Samapundo, S.; Devlieghere, F.; Laleman, R.; Vandekinderen, I. et al.: Modelling the degradation kinetics of vitamin C in fruit juice in relation to the initial headspace oxygen concentration, Food Chemistry, **134** (2012), no. 1, pp. 207-214.
22. Hsieh, Y. H. P. and Harris, N. D.: Effect of sucrose on oxygen uptake of ascorbic acid in a closed aqueous system, Journal of Agricultural and Food Chemistry, **41** (1993), no. 2, pp. 259-262.
23. Khan, M. M. and Martell, A. E.: Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation, Journal of the American Chemical Society, **89** (1967), no. 16, pp. 4176-4185.
24. Fennema, O. R. (Ed.): Principles of food science: Part 1 Food chemistry, Marcel Dekker Inc., New York, Basel, 1976.
25. Sadler, G. D.; Roberts, J. and Cornell, J.: Determination of Oxygen Solubility in Liquid Foods Using a Dissolved Oxygen Electrode, Journal of Food Science, **53** (1988), no. 5, pp. 1493-1496.
26. Clegg, K. M. and Morton, A. D.: The phenolic compounds of blackcurrant juice and their protective effect on ascorbic acid, International Journal of Food Science and Technology, **3** (1968), no. 3, pp. 277-284.
27. Frei, B.; England, L. and Ames, B. N.: Ascorbate is an outstanding antioxidant in human blood plasma, Proceedings of the National Academy of Sciences of the United States of America, **86** (1989), no. 16, pp. 6377-6381.
28. Weinstein, M.; Babyn, P. and Zlotkin, S.: An orange a day keeps the doctor away: scurvy in the year 2000, Pediatrics, **108** (2001), no. 3.
29. Sayers, M. H.; Lynch, S. R.; Jacobs, P.; Charlton, R. W.; Bothwell, T. H.; Walker, R. B. et al.: The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya, British journal of haematology, **24** (1973), no. 2, pp. 209-218.
30. Bharate, S. S. and Bharate, S. B.: Non-enzymatic browning in citrus juice: Chemical markers, their detection and ways to improve product quality, Journal of food science and technology, **51** (2014), no. 10, pp. 2271-2288.
31. Smuda, M. and Glomb, M. A.: Maillard degradation pathways of vitamin C, Angewandte Chemie (International ed. in English), **52** (2013), no. 18, pp. 4887-4891.
32. Senser, F. and Souci, S. W. (Eds.): Der kleine Souci-Fachmann-Kraut: Lebensmitteltabelle für die Praxis, 3., rev. and extended ed., Wiss. Verl.-Ges, Stuttgart, 2004.
33. Deneke, U.; Michal, G. and Beutler, H. O.: Neue Methode zur Bestimmung von Vitamin C in Lebensmitteln, Deutsche Lebensmittel-Rundschau, **74** (1973), no. 11, pp. 400-403.
34. Klimczak, I. and Gliszczyńska-Świgło, A.: Comparison of UPLC and HPLC methods for determination of vitamin C, Food Chemistry, **175** (2015), pp. 100-105.
35. Coats, A. W. and Redfern, J. P.: Kinetic Parameters from Thermogravimetric Data, Nature, **201** (1964), no. 4914, pp. 68-69.
36. Fink: Über lagerungsbedingte Veränderungen von UHT-Vollmilch und deren reaktionskinetische Beschreibung, Dissertation, Technische Universität München, 1984.
37. Vyazovkin, S.; Chrissafis, K.; Di Lorenzo, M. L.; Koga, N.; Pijolat, M.; Roduit, B. et al.: ICTAC Kinetics Committee recommendations for collecting experimental thermal analysis data for kinetic computations, Thermochimica Acta, **590** (2014), pp. 1-23.
38. Dammann, A.; Schwarzer, K.; Müller, U. and Schneider, J.: Flash Pasteurization of Beer – a Critical Review, BrewingScience - Monatsschrift für Brauwissenschaft, **64** (2011), no. 3/4, pp. 32-40.
39. Schneider, J.; Müller, U.; Schwarzer, K.; Becker, B. and Wilhelm, P.:

Lemgo Database for D- and z-values: useful for beverage spoilage microorganisms, Brauwelt International, **28** (2010), no. 5, pp. 252-256.
40. Sapei, L. and Hwa, L.: Study on the Kinetics of Vitamin C Degradation in Fresh Strawberry Juices, Procedia Chemistry, **9** (2014), pp. 62-68.
41. Kaanane, A.; Kane, D. and Labuza, T. P.: Time and Temperature Effect on Stability of Moroccan Processed Orange Juice during Storage, Journal of Food Science, **53** (1988), no. 5, pp. 1470-1473.
42. Laing, B. M.; Schlueter, D. L. and Labuza, T. P.: Degradation kinetics of ascorbic acid at high temperature and water activity, Journal of Food Science, **43** (1978), no. 5, pp. 1440-1443.
43. Dammann, A.; Schwarzer, K.; Vullriede, T.; Müller, U. and Schneider, J.: Determination of the Kinetic Parameters of a Time-Temperature Integrator for the Flash Pasteurization, BrewingScience - Monatsschrift für Brauwissenschaft, **65** (2012), 9/10, pp. 134-139.
44. Westphal, G.; Buhr, H. and Otto, H.: Reaktionskinetik in Lebensmitteln, Springer Berlin Heidelberg, Berlin, Heidelberg, 1996.
45. Miller, J. and Joslyn, M. A.: Effect of sugars on oxidation of ascorbic acid; influence of pH and type of buffer, Food research, **14** (1949), no. 4, pp. 354-363.
46. Kahle, K.; Kraus, M. and Richling, E.: Polyphenol profiles of apple juices, Molecular nutrition & food research, **49** (2005), no. 8, pp. 797-806.
47. Labuza, T. P. and Riboh, D.: Theory and Application of Arrhenius Kinetics to the Prediction of Nutrient Losses in Foods, Food Technology, **36** (1982), no. 10, pp. 66-74.
48. Kroh, L. W.: Caramelisation in food and beverages, Food Chemistry, **51** (1994), no. 4, pp. 373-379.
49. Manso, M. C.; Oliveira, F. A.R.; Oliveira, J. C. and Frias, J. M.: Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions, International Journal of Food Science and Technology, **36** (2001), no. 3, pp. 303-312.
50. Hiwilepo-van Hal, P.; Bosschaart, C.; van Twisk, C.; Verkerk, R. and Dekker, M.: Kinetics of thermal degradation of vitamin C in marula fruit (*Sclerocarya birrea* subsp. *caffra*) as compared to other selected tropical fruits, LWT - Food Science and Technology, **49** (2012), no. 2, pp. 188-191.
51. Shearer, A. E. H.; Mazzotta, A. S.; Chuyate, R. and Gombas, D. E.: Heat resistance of juice spoilage microorganisms, Journal of Food Protection, **65** (2002), no. 8, pp. 1271-1275.
52. Splittstoesser, D. F.; McLellan, M. R. and Churey, J. J.: Heat resistance of Escherichia coli O157:H7 in apple juice, Journal of Food Protection, **59** (1996), no. 3, pp. 226-229.
53. Silva, F. M.; Gibbs, P.; Vieira, M. C. and Silva, C. L. M.: Thermal inactivation of Alicyclobacillus acidoterrestris spores under different temperature, soluble solids and pH conditions for the design of fruit processes, International Journal of Food Microbiology, **51** (1999), 2-3, pp. 95-103.

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