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A B S T R A C T

The study comprises a systematic and quantitative evaluation of potential intrinsic and extrinsic factors that impact vitamin C degradation in a real food matrix. The supernatant of centrifuged apple purée was fortified in vitamin C, and degradation was followed without stirring. Model discrimination indicated better fit for the zero order model than the first order model which was hence chosen for determination of rate constants. pH influenced strongly vitamin C degradation in citrate-phosphate buffer but not in the apple purée serum. To get an idea of the impact of the food matrix, stability in apple purée serum was compared with that in carrot purée. In the latter, stability was slightly higher. Vitamin C degradation rates were not influenced by its initial concentration. The temperature effect was only marked in the temperature range 40–60 °C. In the range 60–80 °C, filling height of tubes had the greatest impact.

Keywords:
Vitamin C degradation
Fortification
Apple purée serum
Dehydroascorbic acid
Surface-to-volume ratio

1. Introduction

Vitamin C, consisting of ascorbic acid and dehydroascorbic acid, is an important vitamin in plant foods, and is characterized by its degradability in processing and food preparation. In spite of numerous studies, its degradation is not completely understood. Impact factors are often only known for model solutions (Aka, Courtois, Louarme, Nicolas, & Billaud, 2013; Kaack & Austed, 1998; Lee & Labuza, 1975; Oey, Verlinde, Hendrickx, & Van Loey, 2006; Rojas & Gerschenson, 1997, 2001; Wilson, Beezer, & Mitchell, 1995; Yamauchi, Nimura, & Kinoshita, 1993) but their importance, especially in a quantitative way, in real food products is lacking.

The predominant pathway of vitamin C degradation in aqueous liquid systems (water activity higher than 0.980) entails oxidation of ascorbic acid to dehydroascorbic acid (Fig. 1), which itself promptly degrades to 2,3-diketogulonic acid (Washko, Welch, Dhariwal, Wang, & Levine, 1992). By the hydrolysis of dehydroascorbic acid, the molecule looses its vitamin property. With increasing water activity (a_w) or moisture content, the degradation of ascorbic acid increases (Lee & Labuza, 1975). The reaction from ascorbic acid to its oxidized form dehydroascorbic acid and the following hydrolysis to 2,3-diketogulonic acid proceeds in water, without any oxidizers or reducing agents, at the same pace (Serpen & Gökmen, 2007). Fe^{3+} ions accelerate both reaction steps that is oxidation of ascorbic acid and following hydrolysis of dehydroascorbic acid. Cysteine in contrast enhances the reconversion of dehydroascorbic acid to ascorbic acid. During the oxidation of ascorbic acid, oxygen is not incorporated in the molecule itself but serves as acceptor of two electrons. Besides the aerobic degradation pathway via dehydroascorbic acid, ascorbic acid can also be degraded by an anaerobic pathway proceeding by hydrolysis (Schulz, Trage, Schwarz, & Kroh, 2007; Yuan & Chen, 1998). The latter is however much slower and occurs only to significant amounts over 120 °C (Dhuique-Mayer et al., 2007; Oey et al., 2006; Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey, 2013). Oxygen is therefore an indispensable reaction partner in the intermediate temperature range. When no headspace oxygen is available, degradation of ascorbic acid decelerates after an initial fast depletion of ascorbic acid which can be ascribed to consumption of oxygen as dissolved oxygen contents decrease concomitantly (Robertson & Samaniego, 1986; Verbeyst et al., 2013). However, changing initial oxygen contents in the range 0.41–3.74 mg/L does not impact the degradation rate of ascorbic acid at 36 °C (Robertson & Samaniego, 1986).

The experimental set-up concerning especially the airtightness and stirring of the system in which vitamin C degradation is followed is therefore indispensable to consider. These factors may explain the number of different models applied to describe vitamin C kinetics which range from zero, first and second order models to a biphasic and a Weibull model (Dhuique-Mayer et al., 2007; Eisonperchonok & Downes, 1982; Johnson, Braddock, & Chen,

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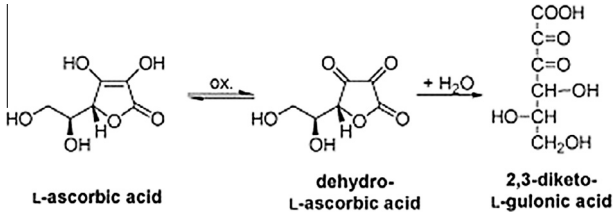

Fig. 1. First steps of ascorbic acid degradation via the oxidative pathway, adapted version from Schulz et al. (2007).

1995; Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992; Oey et al., 2006; Rojas & Gerschenson, 2001; Sapei & Hwa, 2014; Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickxx, 1998). In addition, most studies have been carried out only in model solution and behavior in real food products is lacking but indispensable to take into consideration as other components that are naturally present in plant material may interact and stabilize or impair respectively vitamin C stability.

Fructose and glucose enhance vitamin C stability in the temperature range 24–45 °C and diminish it in the range 70–90 °C (Rojas & Gerschenson, 2001). The effect in the storage temperature range has been related to diminished water activity and the one in the processing range to an enhanced non-enzymatic browning.

The impact of polyphenols depends on the kind of polyphenol. Anthocyanidins are described to be protected by vitamin C which acts as inhibitor of the oxidative degradation (Kaack & Austed, 1998). The described protective effect is probably due to reduction of the oxidized form of the polyphenol by ascorbic acid, which is in turn oxidized as has been found for chlorogenic acid and (−)-epicatechin (Aka et al., 2013). Flavonols in turn protect vitamin C (Clegg & Morton, 1968).

Vitamin C stability at 37 °C is higher in blackcurrant, orange juice and apple juice in comparison to water (Miller & RiceEvans, 1997). The effect has been supposed to be a consequence of protection by polyphenols in the fruit juices.

In addition, ascorbic acid oxidation follows a pH tendency in model solutions with higher stability at lower pH (Rojas & Gerschenson, 1997; Wilson et al., 1995; Yamauchi et al., 1993). The pH dependence of dehydroascorbic acid degradation in phosphate buffer comprising sucrose and EDTA at 23 °C follows the same trend. It degrades significantly faster at pH 7–8 than pH 3–5 (Bode, Cunningham, & Rose, 1990). The impact of concentration was studied by Oey et al. (2006) who stated that vitamin C stability increases with higher concentration and decreases with rising oxygen concentration. The effect of concentration may depend however on the conditions used as the stability of ascorbic acid is related to oxygen. Under the investigated conditions of Oey et al. (2006), a plateau was reached after an initial depletion that may be linked to oxygen consumption in the system. Higher stability was thus probably due to lower oxygen-vitamin C ratio with a constant amount of oxygen when ascorbic acid concentration was high. Dhuique-Mayer et al. (2007) reduced the initial dissolved oxygen content and could thus significantly reduce the degradation rate of ascorbic acid at 90 °C. The effect was however probably not exclusively due to a reduction of dissolved oxygen, as dissolved gases are liberated by heat, but a combination of dissolved oxygen and headspace oxygen depletion. Oxygen depletion in headspace reduces the degradation rate of vitamin C (Van Bree et al., 2012). In addition, an equilibrium between headspace and dissolved oxygen exists that is influenced by oxidations reactions in the food medium. Oxygen mass transfer affects crucially degradation rates under aerobic conditions (Mohr, 1980). Especially for viscous food products where mass transfers are driven by diffusion of molecules, the transfer of oxygen might be limiting for vitamin C degradation.

With temperature increase, dissolved oxygen levels decrease (Penicaud, Peyron, Gontard, & Guillard, 2012) which counteracts the acceleration of reaction rates with temperature increase described by the Arrhenius equation (Dhuique-Mayer et al., 2007; Manso, Oliveira, Oliveira, & Frias, 2001). Sugars also decrease the oxygen solubility (Joslyn & Supplee, 1949) leading to less oxygen availability for degradation of vitamin C.

The objective of the present study is to systematically investigate potential factors of vitamin C degradation in a quantitative way in order to evaluate their respective significance in apple purée serum. Factors that were studied comprised the intrinsic factors pH, food matrix composition and ascorbic acid concentration and the extrinsic factors temperature and the influence of filling height of experimental tubes. An intermediate temperature range was studied that can be encountered when food is reheated. As the bioactive form of vitamin C includes the oxidized form of ascorbic acid, dehydroascorbic acid, this concentration was incorporated in the modeling by considering the sum of these two molecules.

2. Material and methods

2.1. Chemicals

2,2′-Bipyridyl, ascorbic acid, trichloroacetic acid, dl-dithiothreitol, Na_2HPO_4, $NaH_2PO_4xH_2O$, *N*-Ethylmaleimide and citric acid monohydrate were purchased from Sigma-Aldrich (Deisenhofen, Germany). Ortho-phosphoric acid 85%, Iron(III)chloride hexahydrate were obtained from VWR (Leuven, Belgium). Ethanol was provided by Fisher Scientific (Fair Lwan, NJ, USA).

2.2. Supplementation

Apple purée was purchased at a local supermarket and carrot purée was produced by the project partner "Casamas" (Castelltercol, Spain). Both purées contained no added vitamin C. McIlvaine citrate-phosphate buffer at pH of 3.5, 5.5 and 7.5 was prepared for kinetics in buffer solutions. The apple purée serum was obtained by centrifugation of apple purée at 13,600 g for 10 min followed by filtration through a G3 sintered glass filter (Le Bourvellec et al., 2011). Supplementation mixtures were prepared in 50 mL corning tubes with screw caps (Dutcher, Brumath, France). Ascorbic acid was added to the respective medium to obtain a concentration of 3 mmol kg^{-1} (or 50 mg/100 g), under the study's reference conditions and additionally up to 2 and 5 mmol kg^{-1} (40 and 90 mg/100 g) when the impact of concentration was examined. When the pH effect was studied in the apple purée serum, adjustments of pH were carried out with sodium hydroxide (5 mol L^{-1}). pH was checked at the end of the thermal treatment and stayed constant. Mixtures were thoroughly vortexed and sonicated for better dissolution of ascorbic acid for approximately one minute. Solutions were transferred to 2 mL micro tubes (VWR, Leuven, Belgium) with caps and filled up to 1.5 mL under standard conditions. Under these conditions, a headspace containing the surrounding air remained. To study the effect of surface-to-volume-ratios, tubes were additionally filled up to 0.5 and 1 mL. Mixtures were immediately frozen after preparation and defrosted in a water bath before thermal treatment. Freezing and thawing did not affect vitamin C content significantly.

2.3. Thermal treatment

After thawing, tubes were transferred to floating tube racks and immersed in a heated water bath ED-19 from Julabo (Seelbach,

Germany). The impact of temperature in the range 40–80 °C was studied. The aimed temperature was reached in the tubes after approximately three minutes. The first point of each time curve was excluded from heat treatment and directly refrozen. After withdrawal, tubes were put in an ice bath and immediately frozen (⩽18 °C). Standard conditions of this study corresponded to heat treatment at 80 °C, an added vitamin C concentration of 3 mmol kg^{-1} and a filling volume of tubes of 1.5 mL.

2.4. Determination of moisture content

A weighing boat was put in a drying cabinet for 2 h at 70 °C. After cooling down to room temperature and weighing, 3 g of sample were put onto it. The vessel stayed for 4 days at 70 °C and was weighed again. The dry matter (%) was determined by dividing the sample weight after, by the sample weight before drying, and multiplying by 100. The moisture content (%) was calculated by subtracting the dry matter from 100%.

2.5. Determination of soluble solids (°BRIX)

The content of soluble solids was measured with a digital refractometer (PR-101 ATAGO, Norfolk, VA) and expressed in °BRIX at 20 °C.

2.6. Polyphenol analysis

Analysis of polyphenols was carried out by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) – Diode Array Detection (DAD) after thioacidolysis as recently reported by (Le Bourvellec et al., 2011).

2.7. Ascorbic acid and dehydroascorbic acid analysis

Ascorbic acid and dehydroascorbic acid quantification was performed as reported by Stevens, Buret, Garchery, Carretero, and Causse (2006). Firstly, dehydroascorbic acid is reduced to ascorbic acid by dithiothreitol for determination of the total vitamin C amount that is the bioactive amount consisting of the sum of ascorbic acid and dehydroascorbic acid. Fe^{3+} ions are then added in excess which oxidize ascorbic acid and are reduced in turn to Fe^{2+} ions. The thus generated Fe^{2+} ions react with 2,2′-Bipyridyl to a colored complex. Absorbance was measured at a wavelength of 525 nm on a spectrophotometer (Safas Xenius, Monaco). A calibration line was freshly prepared before each assay.

No vitamin C was detected in the apple purée serum when not supplemented. The non-fortified apple purée serum was then taken as control to verify if coloration or oxidizable molecules generated during heat treatment might lead to a signal increase. The apple purée serum was therefore, as the apple purée serum with added vitamin C, heated at 80 °C. A kinetic during 640 minutes was gathered, but no signal increase over time was detected (data not shown).

To avoid bias of modeling as a consequence of differing starting points, the difference in percent of the initial, analyzed vitamin C concentrations of each kinetic curve to the aimed concentration was calculated. The resulting percentage of the difference was used to correct all curve points of the corresponding time curve.

2.8. Kinetic modeling

A zero order model (1) and first order model (2) were fitted to the kinetic of vitamin C determined under the standard condition of this study.

$$c = k_{app} * t + c_0 \quad (1)$$

$$c = c_0 * e^{-k_{app} t} \quad (2)$$

c corresponds to the concentration of vitamin C that is the sum of ascorbic acid and dehydroascorbic acid, c_0 to the initial vitamin C concentration, t to the heat treatment duration and k_{app} to the apparent rate constant.

Model discrimination has been conducted through comparison of Akaike information criteria (AIC) indicating the model with the best predictive precision (van Boekel, 2009) and comparison of distribution of residuals to verify homoscedasticity of variance. The functions AIC and residuals provided by Software R were used.

2.9. Software

RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/ has been applied to carry out linear and non-linear regression to experimental data, for determination of AIC values, plotting residuals, for calculation of rate constants, graphical illustration of scattering plots and for model fittings.

3. Results and discussion

3.1. Matrix simplification

Apple purée is a viscous matrix and needs to be thoroughly mixed to ensure homogeneity. With a view to facilitate sample preparation and especially limit doubts on sample homogeneity, a matrix was searched with similar composition but easier to homogenize. Le Bourvellec et al. (2011) characterized the phenolic and polysaccharidic composition of the liquid supernatant of centrifuged apple purée and observed that the liquid part contains still a considerable amount of polyphenols and low amounts of soluble fibers. With a view to survey if the supernatant of apple purée is suitable for this study; the polyphenol, sugar and moisture content of apple purée and its serum (Table 1) were analyzed as they might potentially enhance or impair respectively vitamin C (Miller & RiceEvans, 1997; Rojas & Gerschenson, 2001). Furthermore initial vitamin C concentrations in apple purée and its serum were determined.

The total phenolic concentration decreased from 6.9 mmol kg^{-1} in whole apple purée to 4.8 mmol kg^{-1} in the liquid supernatant. The concentration of polyphenols remained thus in the same order of magnitude as that of ascorbic acid planned as reference concentration that is 3 mmol kg^{-1}. The polyphenol loss was in accordance to those reported previously for apple serum of twelve different apple varieties (Le Bourvellec et al., 2011). All polyphenol classes were still represented in the supernatant albeit the content of procyanidins and the degree of polymerization of flavan-3-ols decreased. Procyanidins decreased from 2.0 mmol kg^{-1} to 9.8 mmol kg^{-1} and the degree of polymerization from 3.9 to 2.8. The contents of (+)-catechin, (−)-epicatechin, phloretin-2-xyloglucoside, phloridzin, 5′-caffeolyquinic acid, para-coumaroylquinic acid and total flavanols did not decrease at all. Concerning the sugar content, the same °BRIX was found in the liquid part (16.1 ± 0.1 °BRIX) as in the whole apple purée (15.9 ± 0.4 °BRIX), and thus the potential impact of sugars was considered to be the same in both matrices. The water content in both apple matrices was high but not different (serum: 80.1 ± 0.9; apple purée: 78.0 ± 1.6). Fernández-Salguero, Gómez, and Carmona (1993) determined a moisture content of apples of 88.5 ± 0.3 % and a corresponding water activity (a_w) of 0.988 ± 0.002.

The concentration of vitamin C before supplementation was 0.2 mmol kg^{-1} in apple purée, under the limit of detection in the apple purée serum and thus negligible compared to the

Table 1
Vitamin C and polyphenol contents (in μmol kg^{-1}) and BRIX content (in °) of apple purée and the apple purée serum (Vit. C: Vitamin C, CAT: (+)-catechin, EC: (−)-epicatechin, PCA: Procyanidins, DPn: average degree of polymerization of flavan-3-ols (catechin + procyanidins), XPL: phloretin-2-xyloglucoside, PLZ: phloridzin, CQA: 5′-caffeoylquinic acid, pCoQA: para-coumaroylquinic acid, TotalFl: total flavonols, TotalP: total polyphenols, s.d.: standard deviation, n.d.: not detectable), [a]Quantified as phloridzin, [b]Quantified as *p*-coumaric acid, [c]Quantified as quercetin.

	Vit. C	CAT	EC	PCA	DPn	XPL[a]	PLZ	CQA	pCoQA[b]	TotalFl[c]	TotalP	BRIX
Apple purée	216	73	254	2044	4	200	686	551	39	86	6943	16
s.d.	34	1	3	168	0.1	2	3	4	0.4	1	55	0
Apple purée serum	n.d.	77	254	976	3	202	691	559	40	71	4809	16
s.d.	n.d.	3	11	22	0.02	7	24	20	1	2	9	0

supplementation concentrations that were studied that is 2 mmol kg^{-1}, 3 mmol kg^{-1} and 5 mmol kg^{-1}.

To validate that the slight compositional change did not impact the pace of vitamin C degradation, both matrices were carefully intermixed with ascorbic acid. The stability of vitamin C in the respective matrix was then examined under the study's reference condition. No difference was observed between the whole apple purée and its serum. A rate constant k_{app} of $30 \pm 2 \times 10^{-4}$ mmol kg^{-1} min^{-1} was determined for whole apple purée and of $31 \pm 2 \times 10^{-4}$ mmol kg^{-1} min^{-1} for the apple purée serum (in the following Section 3.2 is explained). The apple purée serum was retained for further studies as allowing more reliable homogenization and presenting only small differences in potentially impairing or protecting compositional properties.

3.2. Model discrimination and determination of rate constants

In literature, zero and first order models are often used to describe linear and exponential relationships of time dependent curves. With a view to gather quantitative data and classifying the significance of different factors, experimental data was fitted to the zero and first order model. Model discrimination was carried out at the study's reference conditions by comparison of Akaike information criteria (AIC). A degradation percentage of at least 70%, as recommended by van Boekel (2009) for accurate model discrimination, was respected. Akaike information criteria were of −7.3 for the zero order model and 2.4 for the first-order model. This indicated better fit for the zero order model since the value is inferior to that of the first order model. The experimental data (points), the corresponding model (line) and the respective residuals for the two models are depicted in Fig. 2. The fitting of a zero order model looks visually acceptable although the fit at the beginning is not ideal but still admissible. Acceptable fit is corroborated by equally distributed residuals. In contrast, for the first order model, the fit at the end of the kinetics was not good and the residuals were not equally distributed.

3.3. pH impact

Firstly, pH dependence of vitamin C degradation was tested in citrate-phosphate buffer at pH 3.5, 5.5 and 7.5 (Fig. 3A, Table 2). Ascorbic acid was the most stable at pH 3.5 with a rate constant of $32 \pm 2\ 10^{-4}$ mmol kg^{-1} min^{-1} and the least stable at pH 5.5 with a rate constant of 70 ± 5 mmol kg^{-1} min^{-1}. Small difference between pH 3.5 and 7.5 was observed visually with slightly less stability at pH 7.5. There was however no difference when rate constants were compared ($32 \pm 2\ 10^{-4}$ mmol kg^{-1} min^{-1} vs. $35 \pm 3\ 10^{-4}$ mmol kg^{-1} min^{-1}). Wilson, Beezer, and Mitchell (1995) observed no difference between rate constants in ethanoic buffer at pH 1.0 and 2.0 but when pH was changed to 3.9, 5.0 or 9.0. This can be supposed to be due to higher susceptibility of the monoionic form of ascorbic acid, dominant at pHs between its first pK_a (4.3) and its second pK_a (11.8). However, Rojas and Gerschenson (1997) observed that the stability of ascorbic acid depends on the used acid. They reported that ascorbic acid degrades faster in model solution acidified with phosphoric acid than with citric acid at 80 °C. Different interaction between trace metals, ligands and oxygen might therefore have been responsible (Harel, 1994). The result of this study may thus have been a consequence of the citrate-phosphate buffer. The highest stability was observed at pH 3.5 which can be ascribed to dominance of the fully protonated form of ascorbic acid at a pH under its first pK_a. In addition, the proportion of citric and phosphate molecules changed with pH. The difference between pH 5.5 and 7.5 might thus be due to a change of ligands and their respective complex formation with trace metals and oxygen.

To get a better idea of vitamin C stability as a function of pH in a real food matrix, the pH of the apple purée serum was increased by sodium hydroxide. Degradation curves and rate constants at natural pH of the apple purée serum and at a pH of 7.5 were subsequently compared (Fig. 2B, Table 2). Uncertainty of values was quite high at pH 7.5 with a rate constant of 29 ± 4 mmol kg^{-1} min^{-1} and on this basis, no difference was observed, neither visually nor by comparing rate constants.

In carrot purée, exhibiting a natural pH of 5.5 (Fig. 3C, Table 2), vitamin C was slightly more stable than in the apple purée serum which was observed visually as well as by comparing rate constants. The rate constant in carrot purée was 24 ± 2 mmol kg^{-1} min^{-1}. However, the origin for this difference is difficult to ascertain. Comparison with buffer solutions to elucidate the effect of pH is not reliable as the effect of pH, as shown above, depends enormously on the used buffer. Additionally, this origin might not exclusively arise from pH differences but also from the composition of the matrix. The stability is influenced by sugars, especially fructose, the prevalent sugar in many apples (Rojas & Gerschenson, 1997; Suni, Nyman, Eriksson, Bjork, & Bjorck, 2000). Polyphenols are reported to exhibit a protective effect at least at 37 °C compared to drinking water (Miller & RiceEvans, 1997). Antagonistic effects are probable, that is a protective and impairing effect of other naturally present components in food matrices. Observed degradation rates in apple purée serum and carrot purée were very close at 80 °C in spite of the different compositions.

3.4. Concentration effect

Supplementation concentration depends on particular needs of consumer groups. Establishing the effect of concentration changes on degradation rates is therefore crucial. As expected from a zero order reaction, no difference was observed between the rate constants that were obtained by starting with different initial concentrations (Fig. 3D, Table 2). In contrast to this result, Oey et al. (2006) have reported on a concentration dependence of aerobic and anaerobic rate constants determined by applying a biphasic model which was based on a first order model and differentiated between aerobic and anaerobic degradation. Under their conditions, the degradation of vitamin C was fast at the beginning and then stagnated. Since they used glass vials with rubber septum oxygen availability in the system may have been limited and

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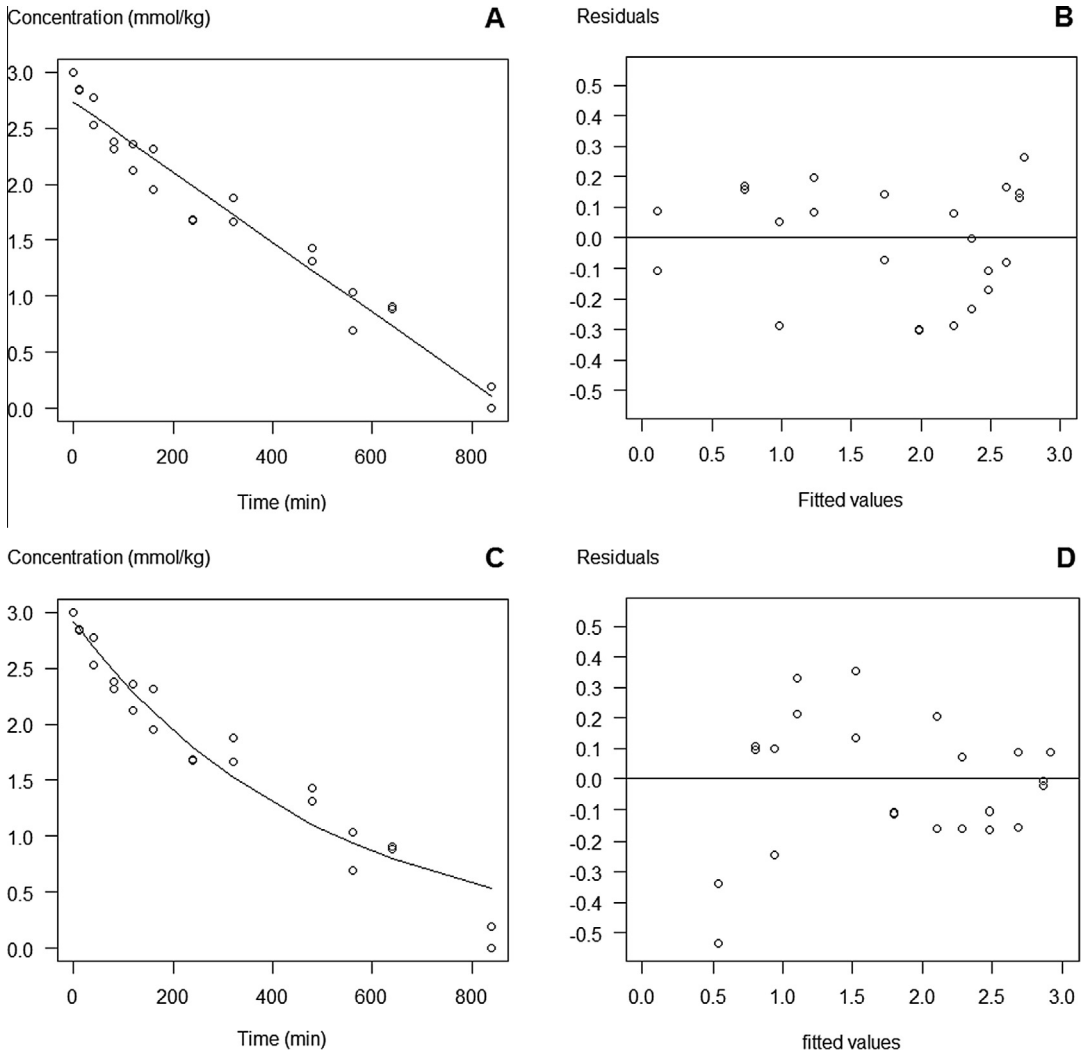

Fig. 2. Modelling discrimination: model fit (line) to experimental data (points) of (A) zero-order model and (C) first-order model and (B) corresponding residuals for zero-order model and (D) first-order model of kinetics at the study's reference conditions (apple purée serum, 3 mmol kg^{-1} vitamin C, 80 °C, 1.5 mL filling volume).

explain the diverging results. A limited amount of oxygen and consequently a lower oxygen-to-ascorbic acid concentration-ratio when ascorbic acid concentration was high may explain the concentration dependence that they observed. A mathematical artifact may also have been involved, as they compared C/C_0, which mechanically increased the slopes for lower C_0. In addition vitamin C degraded linearly (concentration vs. time) in this study, which can be assumed to be due to constant oxygen contents in the system for transformation of ascorbic acid. Coherently, perforation of tubes caps did not lead to an enhancement of degradation. Wilson et al. (1995) determined rate constants by applying a first order model on graphs where the heat flow (transformed by the natural logarithm) was plotted against time. These rate constants were not affected by the concentration of ascorbic acid. As they worked at 25 °C dissolved oxygen availability is high and the chemical conversion might depend only on the activation energy.

3.5. Temperature dependence

Temperature dependence was studied between 40 °C and 80 °C (Fig. 4A). For reheating and warm-keeping of food especially the range 60–80 °C is important to ensure food safety that is to limit microbial growth. In this temperature range, rate constants did not differ (Table 2) in contrast a marked influence of temperature below 60 °C. Arrhenius equation was not employed as rate constants stayed constant between 60 and 80 °C. Dhuique-Mayer et al. (2007) determined an activation energy of 36 kJ mol^{-1} in citrus juice in for the temperature range 50–100 °C. Difference between the curves at 70 °C and 80 °C was however small and degradation was only followed at the very beginning with a limited number of points. In strawberries and raspberries, ascorbic acid degradation is only slightly temperature dependent in the range 80–90 °C (Verbeyst et al., 2013). The apparent stagnation of rate constants in the present study may hence indicate that energy supply was not the limiting factor but probably oxygen availability.

3.6. Impact of the surface-to-volume ratio

The influence of oxygen accessibility was studied by comparing three different filling volumes of experimental tubes and thus three different surface-to-volume ratios. Changing the surface-to-volume ratios had a major impact on vitamin C degradation rate in the temperature range 60–80 °C (Fig. 4. B, Table 2). The rate constant was approximately doubled by decreasing the filling volume of the tube from 1.5 to 0.5 mL. As whole apple purée contains more soluble fibers, potentially influencing diffusion of ascorbic acid and oxygen in the medium and limiting convection, the filling volume was also changed for kinetics in whole apple purée (Table 2). Idem,

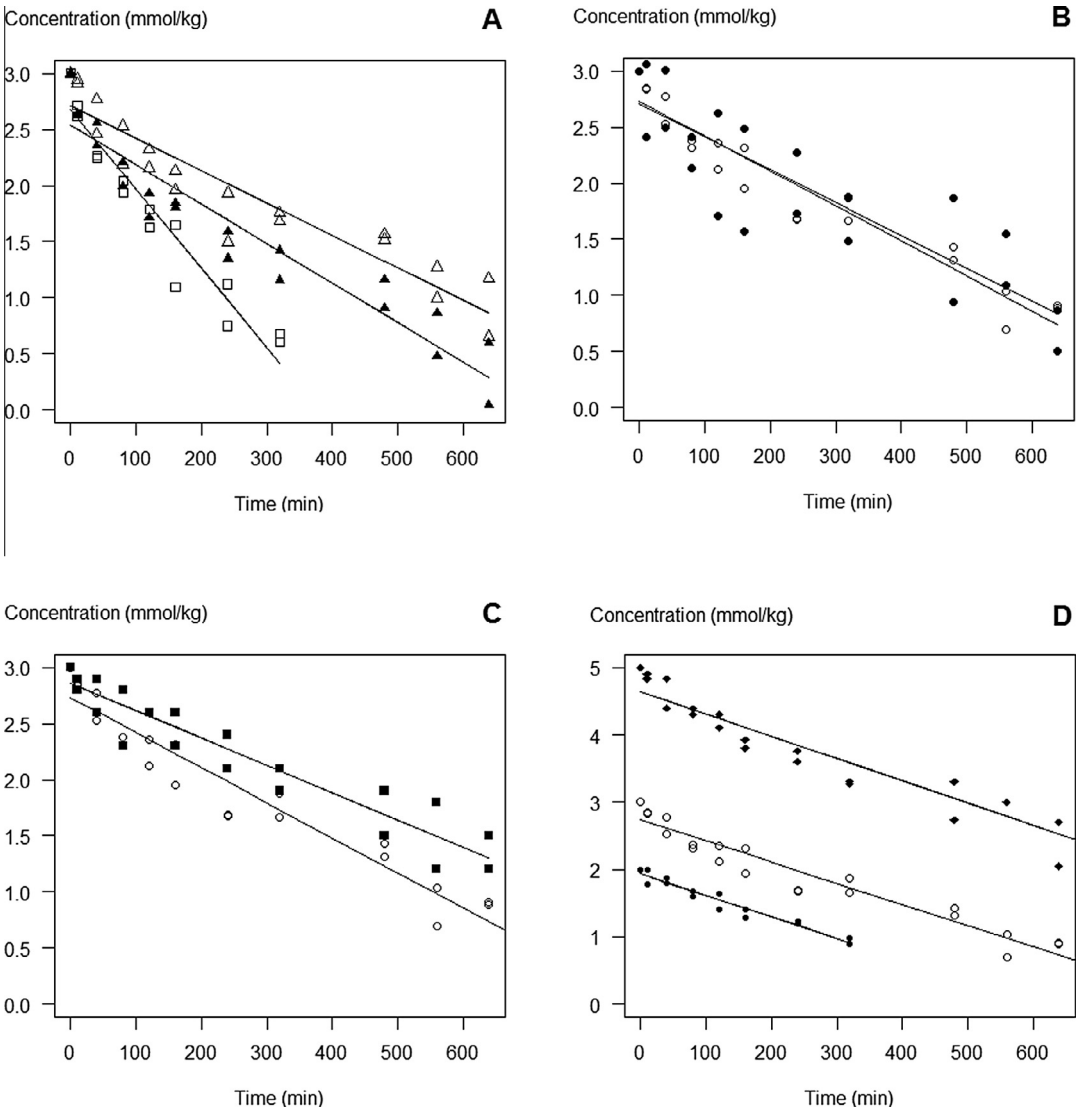

Fig. 3. Effect of changing intrinsic factors on degradation of vitamin C: A: Model solution: △pH 3.5, □pH 5.5 vs. ▲pH 7.5; B: Apple purée serum: ○ pH 3.5 vs. ● pH 7.5; C: ○ apple purée serum vs. ■ carrot purée; D: Different initial ascorbic acid concentration in apple purée serum: ● 2 mmol kg^{-1}, ○ 3 mmol kg^{-1} vs. ◆ 5 mmol kg^{-1}.

Table 2
Rate constants obtained by applying zero order model on vitamin C degradation kinetics under different extrinsic and intrinsic condition (k_{app}: apparent rate constant, conc.: concentration, temp.: temperature).

Medium	pH	k_{app} (10^{-4} mmol kg^{-1} min^{-1})	Conc. (mmol kg^{-1})	Temp. (°C)	Filling volume (mL)
Phosphate-citrate buffer	3.5	32+/−2	3	80	1.5
Phosphate-citrate buffer	5.5	70+/−5	3	80	1.5
Phosphate-citrate buffer	7.5	35+/−3	3	80	1.5
Apple purée	3.5	30+/−2	3	80	1.5
Apple purée serum	3.5	31+/−2	3	80	1.5
Apple purée serum	7.5	29+/−4	3	80	1.5
Carrot purée	5.5	24+/−2	3	80	1.5
Apple purée serum	3.5	32+/−2	2	80	1.5
Apple purée serum	3.5	33+/−2	5	80	1.5
Apple purée serum	3.5	6+/−0	3	40	1.5
Apple purée serum	3.5	11+/−1	3	50	1.5
Apple purée serum	3.5	29+/−3	3	60	1.5
Apple purée serum	3.5	28+/−1	3	70	1.5
Apple purée serum	3.5	61+/−3	3	80	0.5
Apple purée	3.5	61+/−5	3	80	0.5
Apple purée serum	3.5	42+/−2	3	80	1.0

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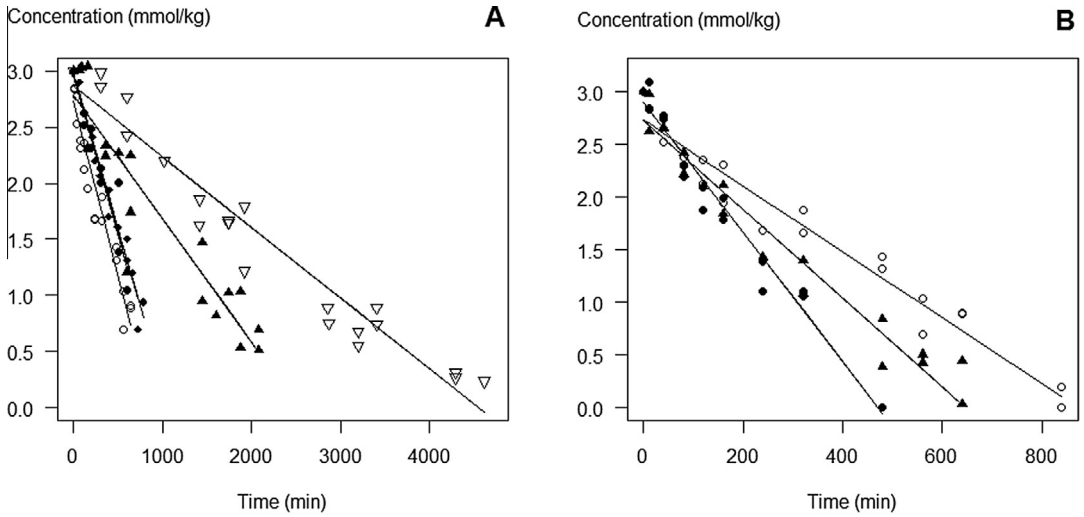

Fig. 4. Effect of changing extrinsic factors on degradation of vitamin C: A: Temperature dependence: ▽ 40 °C, ▲ 50 °C, ● 60 °C, ◆ 70 °C vs. ○ 80 °C; B: Filling volume change: ● 0.5 mL, ▲ 1.0 mL vs. ○ 1.5 mL.

vitamin C degraded faster when filling volume was smaller. Furthermore, no difference between the apple purée serum and the whole apple purée was observed for the smaller filling volume either. The causal origin of the impact of surface-to-volume ratio can arise only from different oxygen availabilities as other factors remained equal. Perforating tubes when filling volume was high did not increase the degradation rate in this study. Oxygen replenishment could thus be excluded. Ascorbic acid at 20 °C degrades faster near the surface in agar gel, which was associated to higher amounts of dissolved oxygen in regions near the surface (Penicaud, Broyart, Peyron, Gontard, & Guillard, 2011). Kinetics of vitamin C obtained by withdrawal of aliquots at the bottom and the surface of the same tube were not different. Longer diffusion distances to the surface when filling volume was high may be a possible explanation for the observed effect. Surface exposition appeared to be of crucial importance.

4. Conclusion

Comparison of vitamin C degradation in different conditions emphasized the crucial rule of extrinsic factors in a real food matrix. The surface-to-volume ratio was the factor that had the greatest impact if only the temperature range 60–80 °C, which is usually sighted for warm holding of food, was considered. Adapted geometry of recipients might hence reduce losses during reheating. Temperature had only an impact on degradation in the range 40–60 °C implying a mechanism change above 60 °C. Oxygen availability in the medium decreases with temperature and oxidation reactions and might have been the limiting factor in apple purée serum between 60 and 80 °C, as further supply of energy did not increase anymore the degradation pace. The common used Arrhenius equation to determine the activation energy (E_A) was consequently not applicable. Only a slight difference has been observed between apple purée and carrot purée as supplementation matrix and thus both matrices are likewise appropriate for supplementation. pH seems to play a minor role in real food matrices. Furthermore, degradation rate of ascorbic acid appeared independent of initial concentration making concentration adaptions easily predictable if other factors stay equal.

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