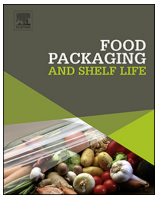
Food Packaging and Shelf Life 23 (2020) 100453

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl



Vitamin C loss kinetics and shelf life study in fruit-based baby foods during post packaging storage

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ARTICLE INFO

Keywords:
Fruit-based baby foods
Baby juices
Shelf life
Vitamin C loss kinetics
Ascorbic acid degradation

ABSTRACT

Simultaneous evolution of main components of L-ascorbic acid (AA) degradation route [AA, dehydro-L-ascorbic acid (DHA), and 2,3-diketogulonic acid (DKA)] were determined in three types of commercial fruit-based baby foods with different water or moisture content (MC) and stored over various periods of time (*t*) at different temperatures (T). The role of L-cysteine (Cys) on AA degradation was also studied. T, MC, and *t* were the main independent variables or factors affecting AA and DKG during post packaging storage. Similar pattern of AA degradation was observed in all products. Changes of AA during storage followed zero-order reaction kinetics. AA retention was higher (> 68 %) in those foods stored under refrigeration. Statistically significant relationship was estimated between time when maximum DKG was determined and T. Strong and moderately strong relationships were found between AA and Cys in 36 total trials at the 90 % or higher confidence level. Decreasing Cys promoted DKG formation and loss of biological activity and health benefits. Based on AA retention, a significant reduction of shelf life was determined for all products. Significance and implication of Cys supplementation of foods for infants and young children is discussed.

1. Introduction

As long as babies are drinking formula or breastfeeding, they are getting all the vitamin C that is needed, and no supplement is necessary. Until the age of six months, babies need 40 mg of vitamin C daily. This requirement increases to 50 mg per day when they are between the ages of six months to a year (Food and Nutrition Board, Institute of Medicine, & National Academies, 2011). The commercially available fruit-based baby food is an important food source in infant nutrition. Together with cereals, fruit-based baby foods are the first foodstuffs introduced in the diet of weaning infants, contributing to cover the vitamin requirements, especially those of vitamin C (Bosch, Cilla, García-Llatas, Gilabert, & Boix, 2013). L-ascorbic acid (AA) has multiple biochemical roles, though it is primarily a water-soluble antioxidant (Martí, Mena, Cánovas, Micol, & Saura, 2009) necessary for healthy skin and red blood cells, wound and bone healing, and infection prevention in human body (Mazurek & Pankiewicz, 2012).

It has been known for some time that AA is rapidly oxidized to dehydro-L-ascorbic acid (DHA) by an enzyme with oxidase activity in presence of oxygen (Mair & Grosch, 1979); or rather it is degraded non-enzymatically during handling, processing and storage of food (Cortés, Esteve, & Frigola, 2008; Gabriel et al., 2015). The chain of reactions continues with the transformation of DHA to 2,3-diketogulonic acid (DKG) and other breakdown products (Rojas & Gerschenson, 2001; Van Bree et al., 2012). The degradation of AA in food depends on the storage conditions: oxygen, light, temperature and time. The influence of food matrix (nutritional composition, the food form and structure) as well as of the packaging (material, colour and volume) on AA degradation must also be taken into account.

The mechanism which explains the degradation of AA (Fig. 1) is not fully known, although it seems that all authors agree about the existence of two parallel routes: aerobic and anaerobic (Khan & Martell, 1967a, 1967b; Lee & Nagy, 1988; Sakai, Watanabe, Takai, & Hasegawa, 1987). This is due to the presence of certain metals which accelerates

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https://doi.org/10.1016/j.fpsl.2019.100453
Received 1 April 2019; Received in revised form 25 November 2019; Accepted 13 December 2019

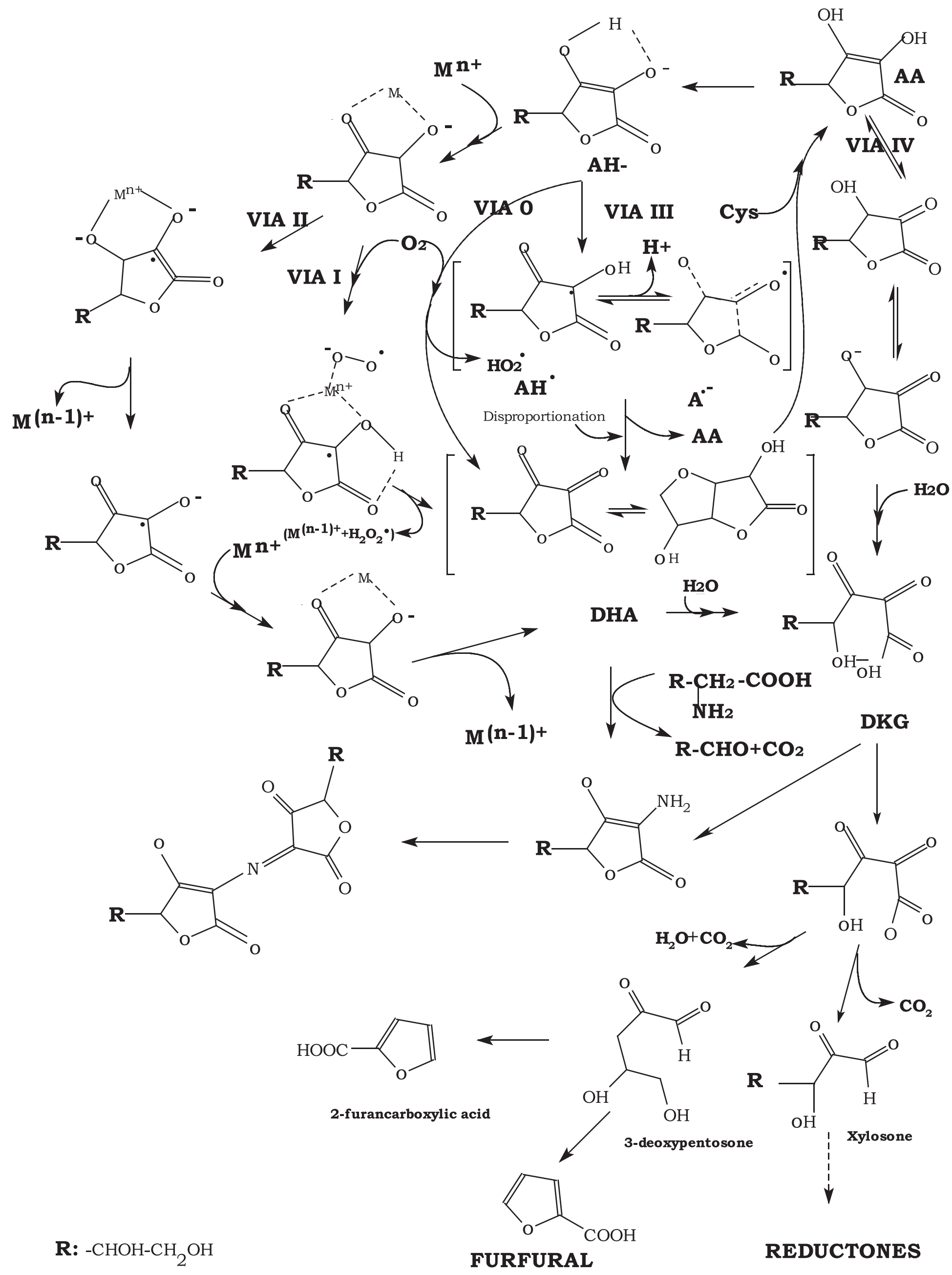

Fig. 1. L-ascorbic acid (AA) degradation scheme.

the auto-oxidation process of AA through an oxidative catalytic mechanism according to the sequence proposed by Martell (1982) (pathway I). Some metals such as Cu^{2+} and Fe^{3+} are catalytic independently from the presence of oxygen (pathway II). Moreover, AA can give DHA via its anion AH^{-} through the disproportion of the radical (pathway III) (Bielski, 1982). The indications of Kurata and Sakurai (1967a); Kurata & Sakurai, 1967b) (pathway IV) and the anaerobia via proposed by Tannenbaum, Archer, and Young (1985)) lead to DKG from AA without passing through DHA.

The effectiveness of L-cysteine (Cys) concentrations in inhibiting both non- and enzymatic browning in pear and loquat juices has been reported (Ding, Chachin, Ueda, & Wang, 2002; Montgomery, 1983). Friedman and Molnar-Perl (1990) demonstrated the inhibition of browning in fruits and juices by the presence of sulphur-containing amino acids and, more exactly, by the thiol groups of two Cys residues which can react in an oxidation reaction that yields a disulphide bond and prevents the oxidation of AA. Later, in a study carried out to investigate the kinetics of the wheat glutathione (GSH)-DHA oxidoreductase (Kaid, Rakotozafy, Potus, & Nicolas, 1997), it was found that Cys was not substrate but its addition to small amounts of GSH caused large activation of the enzymatic reduction of DHA to AA suggesting coupled oxidation of these thiol compounds.

Stable fruit-based baby foods are usually processed using heat treatment and can be stored for one or more years at ambient temperature. AA degradation in fruit-based baby foods during storage follows first-order kinetics, as reported in two studies: a homogenized fruit-based baby food made from apple puree stored for 60 weeks at 23, 30 and 37 °C (Palazón et al., 2009); and a fruit-based infant product with added AA containing pear fruit puree (22 %), tangerine juice (22 %), apple fruit puree (22 %), banana fruit puree (16.5 %), carrot puree (10 %), grape juice from concentrate, corn starch, and vitamin C stored for 32 weeks at 4, 25, 37, and 50 °C (Bosch et al., 2013). The changes of AA content during the storage of a commercially available apple/raspberry fruit baby food containing apple puree, water, raspberry puree (20 %), sugar, modified corn starch, citric acid, and AA stored for 19 days at 40, 55, 70, and 90 °C followed, however, a pseudo-first-order kinetics (Prchalová, Čížková, Ševčík, Hanušová, & Rajchl, 2016).

Taking into account the limited information available on AA degradation in fruit-based baby foods, the aim of the present study was to determine the simultaneous evolution of the main components of the degradation route of AA (AA, DHA, and DKG) in commercial fruit-based baby foods with different water content or moisture, over various periods of time and at different temperatures of storage. The role of the naturally present amino acid Cys on AA degradation was also studied. In this way, the great impact of the food matrix, understood as Cys concentration or water content, on AA degradation in the three types of fruit-based baby foods was highlighted. Finally, shelf life of fruit-based baby foods was determined based on post packaging AA loss kinetics and compared with that in labelling.

2. Materials and methods

2.1. Fruit-based baby food samples

Nine different fruit-based baby foods commercially available were manufactured by a Spanish company well positioned in the baby food market. The fruit-based baby foods were packaged into aluminium bags or glass bottles that preserved the hygienic, or other, qualities of foods (Codex Stan 73-1981, 2017Codex Stan 73-1981, 2017). The products were transported to the laboratory immediately after packaging for its storage at various temperatures. Fruit-based baby foods included three dehydrated fruit-based baby foods, three homogenized fruit-based baby foods, also named 'purees', and three baby juices. All dehydrated fruit-based baby foods (products D1, D2, and D3) had similar shelf life (32 months) and 2–3 % water or moisture content. Their nutritional composition as indicated by the labels of the containers was: proteins: 11.5–12.5 %; carbohydrates: 75.3–76.4 %; lipids: 9–10 %; minerals: 3.1–3.5 %, and fiber: 1.5–3 %. These products which are used frequently in feeding of infants from six months of age contained:

D1: Skimmed milk, apple and banana, pH 4.1 ± 0.2.
D2: Skimmed milk, peach, banana, apple and orange, pH 4.1 ± 0.2.
D3: Skimmed milk, carrot and beef, pH 5.6 ± 0.2.

The three homogenized fruit-based baby foods or 'purees' (products P1, P2, and P3) had higher MC (69–74 %) than dehydrated products and shorter shelf life (23 months). It contained:

P1: Carrot and rice, pH 6.1 ± 0.2.
P2: Banana, apple, pear and mandarin, pH 4.1 ± 0.2.
P3: Peach and banana, pH 4.1 ± 0.2.

The nutritional composition of 'purees' showed in labelling was: proteins: 0.6-0.8 %; carbohydrates: 9.1–28.2 %; lipids: 0.1-0.9 %; minerals: 0.2-0.6 %, and fiber: 1.8–14.5 %.

Baby juices (products J1, J2, and J3) were mainly characterized by high MC (85–87 %) and the shortest shelf life (14 months) among studied foods. The nutritional composition of baby juices showed in labelling was: proteins: 0.3-0.6 %; carbohydrates: 12.6–14.1 %; lipids: 0.1-0.4 %; minerals: 0.2-0.5 %, and fiber: 0.01-0.02 %. The juices contained:

J1: Carrot and grapes, pH 5.1 ± 0.2.
J2: Apple, pH 4.1 ± 0.2.
J3: Apple, pear, strawberry and blackcurrant, pH 3.6 ± 0.2.

2.2. Storage conditions

Samples of dehydrated fruit-based baby foods, homogenized fruit-based baby foods and baby juices were stored immediately after packaging (time 0) at four different isothermal conditions (5, 25, 30, and 40 °C) in temperature programmable control incubators (Sanyo MIR-153, Sanyo Electric Co. Ltd., Osaka, Japan). The main components of the degradation route of AA (AA, DHA, and DKA) and the Cys content were determined at zero time and after 25, 45, 70, 95, 120, 145, 160, 185, and 200 days of storage. Samples were maintained in their original containers until analysis. All trials were tripled. One thousand eighty data for each chemical parameter [total data: 9 (foods) × 4 (temperatures) = 36 × 3 (replicates) = 108 × 10 (times) = 1080 × 4 (analytes) = 4,320] were determined.

2.3. Analytical determinations of AA, DHA, and DKG

First, dehydrated fruit-based baby foods were reconstituted by dilution in water, following the instructions on how to use indicated by the manufacturer. From each one of the three types of fruit-based baby food, 25 mL were taken and clarified by adding 200 μL of Ultrazym 100 G (5 g/L) (Novozymes, Bagsvaerd, Denmark). The samples were stored in hermetic containers for 9 h at 8 °C since the refrigerated storage temperature slows the degradation rate of AA (Burdurlu, Koca, & Karadeniz, 2006; Uddin, Hawlader, Ding, & Mujumdar, 2002). Later, 3 mL of solution was filtered through a C18 Sep-pak cartridge (Waters Corporation, Milford, Massachusetts, USA) conditioned with water and methanol. The first millilitre of the solution was discarded and then 40 μL of the solution was injected into an HP Agilent 1100 Series HPLC System (Hewlett-Packard, Palo Alto, CA, USA). The mobile phase was water:EDTA (97.5:2.5 v/v) with 15 mL/L thiodiglycol (Fluka AG, Buchs, Switzerland), pH adjusted to 7.0 with metaphosphoric acid. The flow used was 0.5 mL/min. A Spherisorb ODS2 (25 × 4.6 mm) column (Tracer Analítica S.L., Sant Cugat del Valles, Spain) was used. The post column reaction was carried out with 300 mg/L 1,4-dithio-DL-threitol (Fluka AG) in phosphate buffer at pH 6.5. The elution was transported through a tephlon tube of 1/16" internal diameter and 0.010" external diameter, interwoven in a special way to favour the mixture, and submerged in water at 80 °C. The function of the post column reaction was DHA reduction to AA in order to detect it at the measurement wavelength, 267 nm. This method was considered as effective for the simultaneous detection of AA and DHA (Ziegler, Meier, & Sticher, 1987). Standard AA and DHA were bought from Merck (Darmstadt, Germany). The DKG pattern was obtained by adding NaOH 0.5 N to a solution of DHA until pH 7, as described by Doner and Hicks (1981).

2.4. Cys content determination

The measurement of free amino acids was carried out through HPLC of dansylated derivatives with ultraviolet detection at 254 nm (De Jong, Hughes, Wieringen, & Wilson, 1982). Briefly, 25 *g* sample were adjusted to pH 8.5 and were balanced out to 50 mL with acetone. To 1 mL of supernatant fraction, 1 mL of internal standard and 2 mL of derivative solution (2 ppm of dansyl chloride in acetone) were added. The internal standard consists in 70 mg/L of ornithine chlorhydrate in 50 mM pH 9 borate buffer. The mixture was maintained at 37 °C in darkness for 8 h. Later, it was centrifuged and filtered through 0.45 μm. The column used was Novapack C18 3.9 × 150 mm (Waters Corporation). The mobile phase was acetonitrile:20 mM phosphate buffer pH 6.5; initial gradient 5:95 for 5 min increasing 1 % every minute until reaching 45 % acetonitrile. The quantification was carried out using ornithine as internal standard due to its absence in the product; it was calculated according to the relative response factors of each amino acid respect to ornithine.

2.5. Statistical analysis

In order to study the influence of factors such as T, MC, and *t* on the evolution of the analysed compounds (AA, DHA, DKG, and Cys) in all tested fruit-based baby foods (dehydrated baby foods, 'purees', and baby juices), a multifactorial analysis of variance (ANOVA) was applied. Statgraphics® Plus for Windows 3.0 (Statistical Graphic Corp. and Graphic Software Systems Inc., Rockville, Maryland, USA) was used for Statistical Analysis of data. The method used to discriminate among the means was Fisher's least significant difference (LSD) procedure ($P \le$

Table 1
Statistical significance of main factors affecting L-ascorbic acid degradation in commercial fruit-based baby foods.

Factors	AA	DHA	DKG	Cys
T	***	NS	***	NS
MC	**	NS	***	***
Pd	***	NS	***	***
t	***	NS	***	**

AA, L-ascorbic acid; **DHA**, dehydro-L-ascorbic acid; **DKG**, 2,3-diketogulonic acid; **Cys**, cysteine; **T**, storage temperature; ***t***, time; **MC**, moisture content; **Pd**, commercial fruit-based baby food product; **NS**, non-statistically significant ($P > 0.05$); **,*** statistically significant ($P \leq 0.05$) or ($P \leq 0.001$), respectively.

0.05) and Regression Analysis to describe the relationship between variables.

3. Results and discussion

3.1. Main factors affecting AA degradation throughout storage

All products showed similar DHA values and statistically significant differences on AA, DKG and Cys content independently of T, MC, and *t* (Table 1). Six homogenous groups were estimated for AA. The products P2, P3, and J3 exhibited the highest AA mean concentration (3449.12 μmol/L) whereas products D3 and P1 contained the lowest (1,451.03 μmol/L), probably owing to the exclusive presence of carrot as vitamin C source. After storage an increase in DKG content was observed in all products subjected to analysis. Three homogenous groups were estimated for DKG. The products P2 and P3 which contained high AA levels showed the maximum DKG mean value (118.17 μmol/L). Products such as D1, D2, D3, J1, J2, and J3 (means between 13.19 and 46.16 μmol/L) did not show statistically significant differences at the 95 % confidence level. Considering the presumptive relationship between AA retention during storage and amount of Cys (Ding et al., 2002; Friedman & Molnar-Perl, 1990) in fruit-based baby food, changes of Cys content were also determined. Means of all dehydrated fruit-based baby foods (D1, D2, and D3) differed significantly among them (787.74, 870.14, and 260.44 μmol/L) and from those estimated by 'purees' and baby juices which formed a big homogenous group with the lowest Cys content (between 16.41 and 28.46 μmol/L).

T, MC, and *t* were the main independent variables or factors affecting AA and DKG content in commercial fruit-based baby foods. The refrigerated storage of fruit-based baby food led to significant differences on AA content (3,217.71 μmol/L) in comparison with those products stored at 25 − 30 °C (2613.55 μmol/L) and 40 °C (2,105.54 μmol/L). On the contrary, the storage at high temperatures (30 − 40 °C) induced greater DKG formation (85.93 μmol/L) than at mild and low

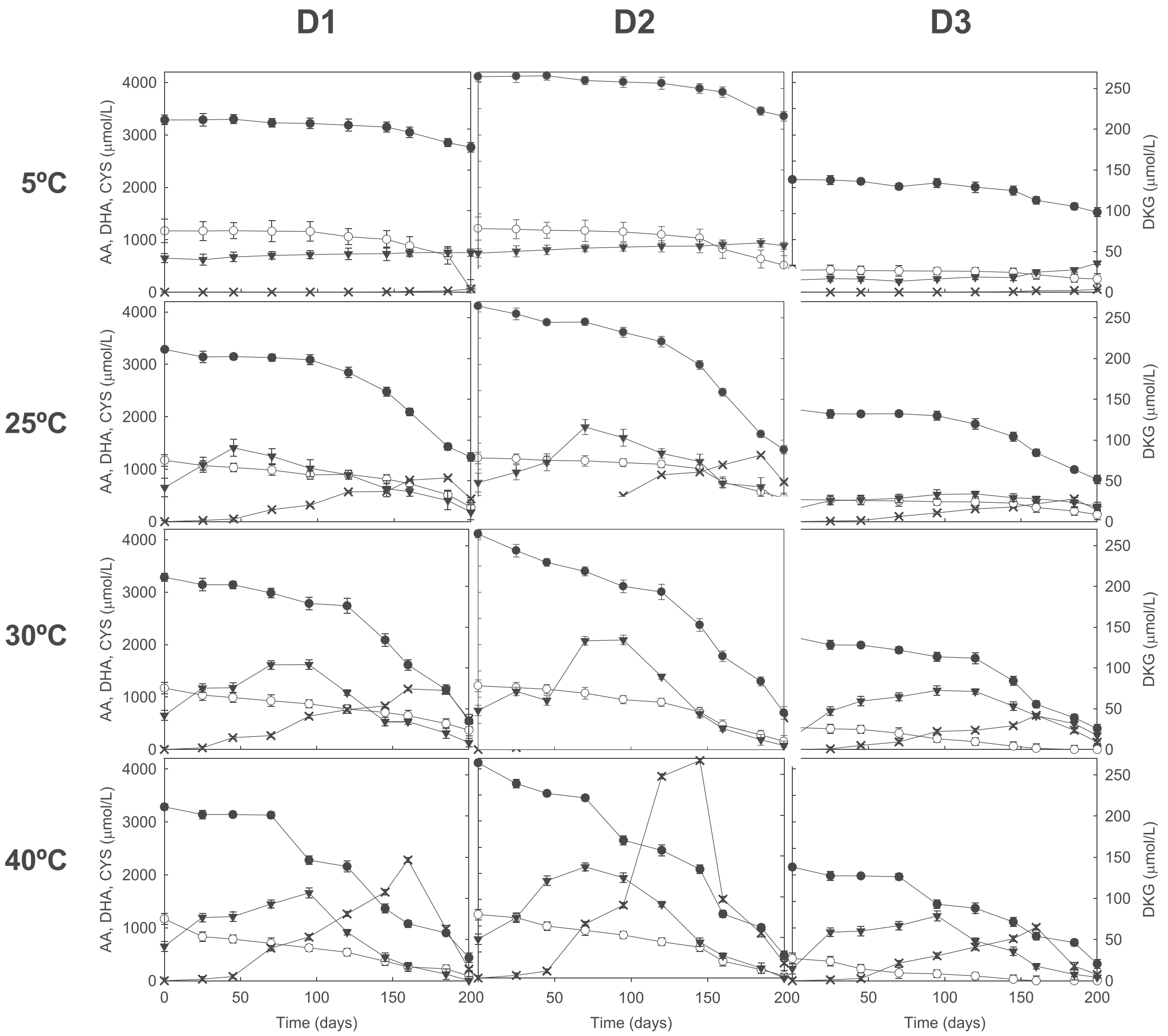

Fig. 2. Evolution of Lascorbic acid (●, AA), dehydro-L-ascorbic acid (▼, DHA), 2,3-diketogulonic acid (×, DKG), and cysteine (o, Cys) values at 5 °C, 25 °C, 30 °C, and 40 °C for different dehydrated fruit-based baby food samples (n = 3). **D1**: Skimmed milk, apple and banana; **D2**: Skimmed milk, peach, banana, apple and orange; **D3**: Skimmed milk, carrot and beef.

temperature (35.43 and 1.01 μmol/L, respectively).

Changes on AA and DKG content were also influenced by MC. Dehydrated fruit-based baby foods (2–3 % MC) showed statistically significant differences on AA content (2402.97 μmol/L) compared to baby juices (85–87 % MC) (2838.09 μmol/L) at the 95 % confidence level. 'Purees', however, exhibited significantly higher DKG formation (96.30 μmol/L) than dehydrated fruit-based baby foods and baby juices (29.97 μmol/L).

t provided also significant differences on AA and DKG content. A total of seven homogenous groups were estimated for AA. High retention of AA was observed until 70 days (3102.86 μmol/L) and then gradually decreased until the end of the storage period. However, no statistically significant differences were estimated between 185 (1774.33 μmol/L) and 200 (1406.31 μmol/L) days of storage. A time- and temperature-dependent decrease in AA content was determined at all tested temperatures in disagreement with Bosch et al. (2013) who reported no AA losses at 4 °C using samples of similar nature. In contrast, AA degradation in dried kiwifruits and freeze-dried guava as function of time and temperature (30 – 50 °C) was also reported by Uddin, Hawlader, and Zhou (2001)) and Uddin et al. (2002). The formation of DKG showed four points of increase with respect to *t*, i.e.: at 25 (6.21 μmol/L), 95 (37.73 μmol/L), 120 (74.83 μmol/L), and 145 (119.88 μmol/L) days. The highest DKG content (124.28 μmol/L) frequently occurred after 160 days of storage except for those products kept in refrigeration (5 °C) which reached the maximum at the end of storage period (200 days).

3.2. Evolution of degradation products of AA during storage

Figs. 2–4 show the simultaneous evolution of AA, DHA, DKG, and Cys parameters at different T for all dehydrated fruit-based baby foods, 'purees', and baby juices, respectively. A similar pattern of AA degradation was observed in all commercial fruit-based baby products with independence of their initial MC, AA, and Cys content. Degradation of AA in fruit-based baby products during storage was favoured by the increase of T and *t*. The maximum losses of AA after storage of the three types of fruit-based baby foods for 200 days at 5, 25, 30, and 40 °C were 32 %, 60 %, 85 %, and 98 %, respectively. Vitamin C loss was much smaller in the case of fruit-based baby foods stored under refrigeration (Figs. 2–4). The formation of DKG reached its maximum levels in the final stretch of storage, between 145 and 200 days, as the temperature drops. The amount of Cys decreased continuously, even becoming undetectable in some cases. The DHA values showed irregular and indefinable trend.

3.3. Reaction order estimation of AA degradation

The order of reaction was estimated graphically (Marfil, Santos, & Telis, 2008). AA degradation curves were obtained for each T and fruit-based baby food (Fig. 5Sa-d). Experimental data were fitted to a linear model and the quality of the adjustment was evaluated through the statistical coefficient of determination R^2 (Remini et al., 2015). Thirty six (36) plots were obtained with R^2 values ranged between 0.724 and 0.9845.

This degradation kinetic model suggested a zero-order reaction which is expressed by Eq. (1):

$$C_t = C_0 - kt \tag{1}$$

where C_t is the AA content at a certain time t, C_0 is the AA concentration at time zero, and k is the zero-order kinetic reaction constant. As the storage temperature increased this constant increased and reached, e.g., values of -2.9045 ± -0.6506, -9.8532 ± -3.3236, -12.4732 ± -3.6306, and -14.0443 ± -4.6627 at 5 °C, 25 °C, 30 °C, and 40 °C for dehydrated fruit-based baby foods. In the case of ‘purees’, k values were -0.4989 ± -0.3243, -5.4169 ± -3.1353, -9.7147 ± -5.1679, and -13.5472 ± -6.9510, whereas for baby juices were -4.6285 ± -2.2436, -8.9196 ± -1.5364, -13.2927 ± -3.1462, and -18.7583 ± -5.5978. Soares and Hotchkiss (1999) early mentioned zero-order kinetics for AA degradation in de-aerated orange juice. Zero-order kinetics have been also successfully used to describe AA degradation for headspace oxygen concentrations lower than 0.63 % in a model fruit juice (Van Bree et al., 2012), as well as in fresh strawberry juices (Sapei & Hwa, 2014). In contrast, the first-order kinetic model is the most widely used describing the oxidative degradation of AA (Bosch et al., 2013; Burdurlu et al., 2006; Palazón et al., 2009; Polydera, Stoforos, & Taoukis, 2005; Serpen & Gökmen, 2007).

3.4. T dependence of AA loss

AA loss in each fruit-based baby food was graphically represented as a funtion of T (Fig. 5). A clear and fast trend of AA degradation was observed when T increased. The relationships between AA and T were adequately fitted to a linear model (y = a – bx) in nine (9) plots with R^2 values ranged between 0.9064 and 0.9844. Therefore, AA loss could be expressed by Eq. (2):

$$C_T = C_0 - kT \tag{2}$$

where C_T is the AA content at a certain T after 200 days of storage, C_0 is the initial AA concentration of product, and k is the kinetic reaction constant for each fruit-based baby food. This constant reached values of -65.053 ± -26.1112, -73.4017 ± -36.4498, and -75.68 ± -14.1184 for dehydrated fruit-based foods, 'purees' and baby juices, respectively.

3.5. Relationship between time when maximum DKG content was found and T

The degradation of AA to DHA affects the vitamin C stability since the biological activity of AA is lost when DHA is further degraded to DKG irreversibly. DHA possesses a similar biological activity as AA in humans (Tsujimura et al., 2008). The knowledge of the DKG content at a certain time would be, hence, a good approximation to lowering of vitamin C content at that particular moment. It would really be of great interest to know when the loss of vitamin C is maximum in long-term stored fruit-based baby foods under specific conditions. A statistically significant relationship was found between time when maximum DKG content was determined and T ($P =$ 0.0000; $r =$ -0.9173) at the 99 % confidence level (Fig. 6). It can be described accurately by Eq. (3).

$$t_{\mathrm{DKGmax}} = 208.65 \text{ -}1.3462 \cdot \mathrm{T} \tag{3}$$

The statistic R^2 indicated that the model as fitted explains 84.14 % of the variability in t_{DKGmax}.

3.6. Effect of Cys content on AA degradation

The relationships between the Cys parameter and each of the main components of the degradation route of AA (AA, DHA, and DKG) in commercial fruit-based baby foods were adequately fitted to a linear model (y = a + bx). A statistically significant positive correlation was found between AA and Cys at the 99 % confidence level ($P =$ 0.0003; $r =$ 0.1912). In contrast, DKG was negatively correlated with Cys content ($P =$ 0.0005; $r =$ -0.1823). No statistically significant relationship between DHA and Cys at the 90 % or higher confidence level was estimated ($P =$ 0.6405).

The low correlation coefficient value ($r =$ 0.1912) indicated a relatively weak relationship between AA and Cys. Taking into account the great differences in AA and Cys content among the analyzed products, this relationship was further studied in a particular way for each baby food stored at all the temperatures. Table 2 summarizes the correlation coefficients and *P*-values for the relationship between AA and Cys in fruit-based baby foods stored at different temperatures. Strong

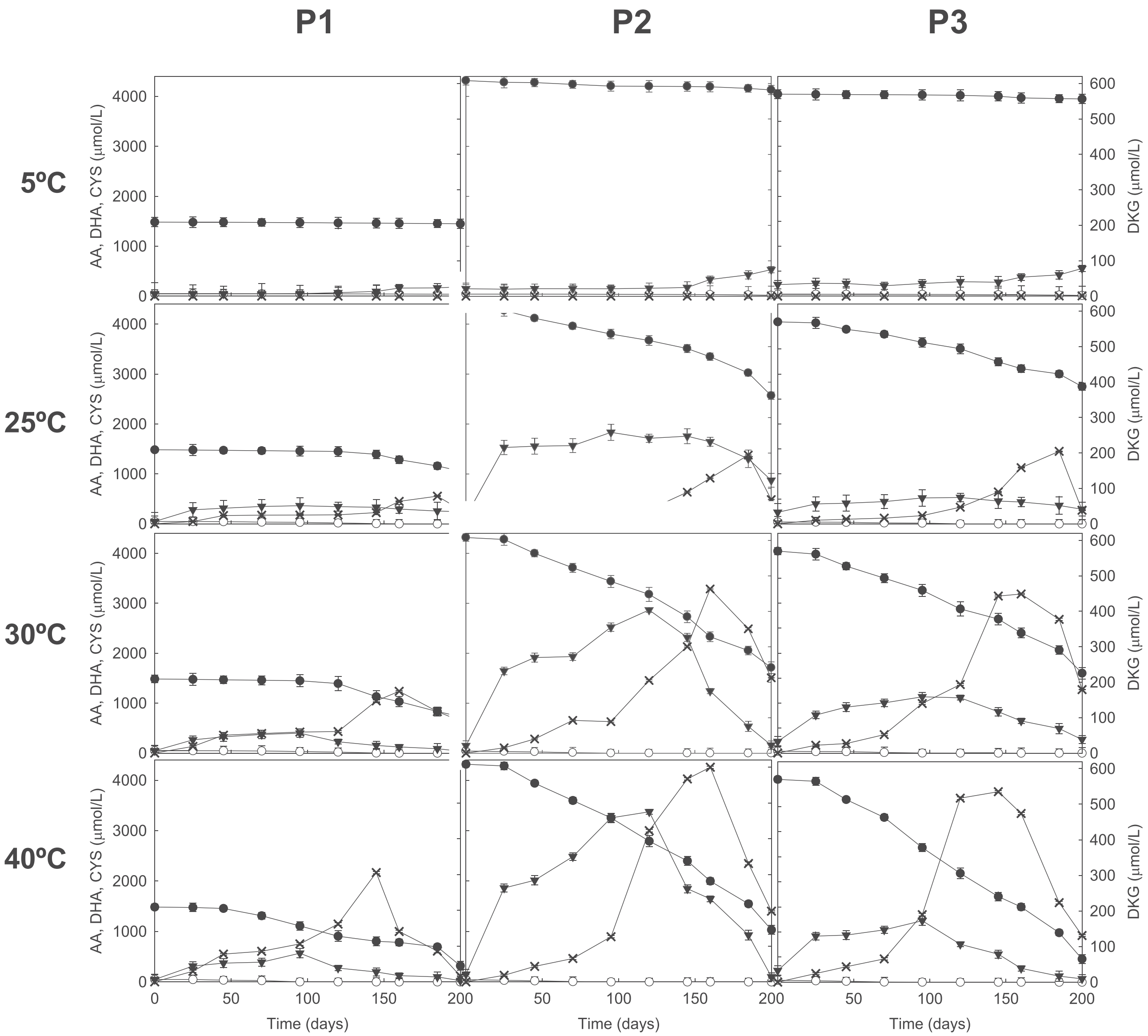

Fig. 3. Evolution of Lascorbic acid (●, AA), dehydro-L-ascorbic acid (▼, DHA), 2,3-diketogulonic acid (×, DKG), and cysteine (o, Cys) values at 5 °C, 25 °C, 30 °C, and 40 °C for different homogenized fruitbased baby food samples (n = 3). **P1**: Carrot and rice; **P2**: Banana, apple, pear and mandarin; **P3**: Peach and banana.

relationships between the parameters were found at the 99 % confidence level in most cases (33/36).

The amino acid Cys could destabilize DHA and contribute to the formation of Strecker aldehydes (Hidalgo, Alcón, & Zamora, 2013) in fruit-based baby foods. In the Strecker degradation the α-dicarbonyl group from DHA would react with Cys to form an aroma-active aldehyde (García-Torres, Ponagandla, Rouseff, Goodrich-Schneider, & Reyes-De-Corcuera, 2009). The results, however, do not support this hypothesis. On the contrary, oxygen can promote browning by way of AA oxidation to DHA with further formation of decomposition products such as furfural, hydroxymethylfurfural (HMF) and methylfurfural (MF), all related to browning in stored citrus juices. Sulphur-containing amino acids can be used to inhibit the aforementioned browning. Friedman and Molnar-Perl (1990), and Molnar-Perl and Friedman (1990) reported the inhibition of browning by Cys and acetylcysteine in both an amino acid-glucose model system and fruit juices. It has been also known that sulfhydryl compounds such as homocysteine (Hcy), Cys, and GSH have the potential to reduce DHA to AA (Eitenmiller, Landen, & Ye, 2016; Park, 2001). Therefore, a possible explanation of our results suggests that the Cys reduced DHA to AA quicker than DHA was degraded to DKG by other substances. As a consequence the concentration maximums of DKG coincided with low levels of Cys or its total disappearance from the fruit-based baby foods stored at 25, 30 and 40 °C (Figs. 2–4). Thus strong and moderately strong relationships ($-0.5033 \leq r \leq -0.9989$) between the variables were found at the 90 % or higher confidence level in thirty-five from thirty-six total trials (35/36). Decreasing of Cys content during storage promoted DKG formation and loss of vitamin C activity.

In summary, a high Cys content significantly increased AA retention in fruit-based baby foods during storage. Now we must ask ourselves. What is the significance and implication of this finding? According to Institute of Medicine (2005), there is no evidence that amino acids found in usual or even high intakes of protein from food present any risk. So, can baby foods receive high concentrations of Cys found in dietary protein and amino acid supplements? Certainly caution may be warranted since data on the adverse effects of high levels of amino acid intakes from dietary supplements are limited. Moreover, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food states that the use of Cys in foods for infants and young children for technological purposes is of no safety concern (European Food Safety Authority (EFSA), 2006). The Panel considers also that the proposed use of Cys is not incompatible with previous

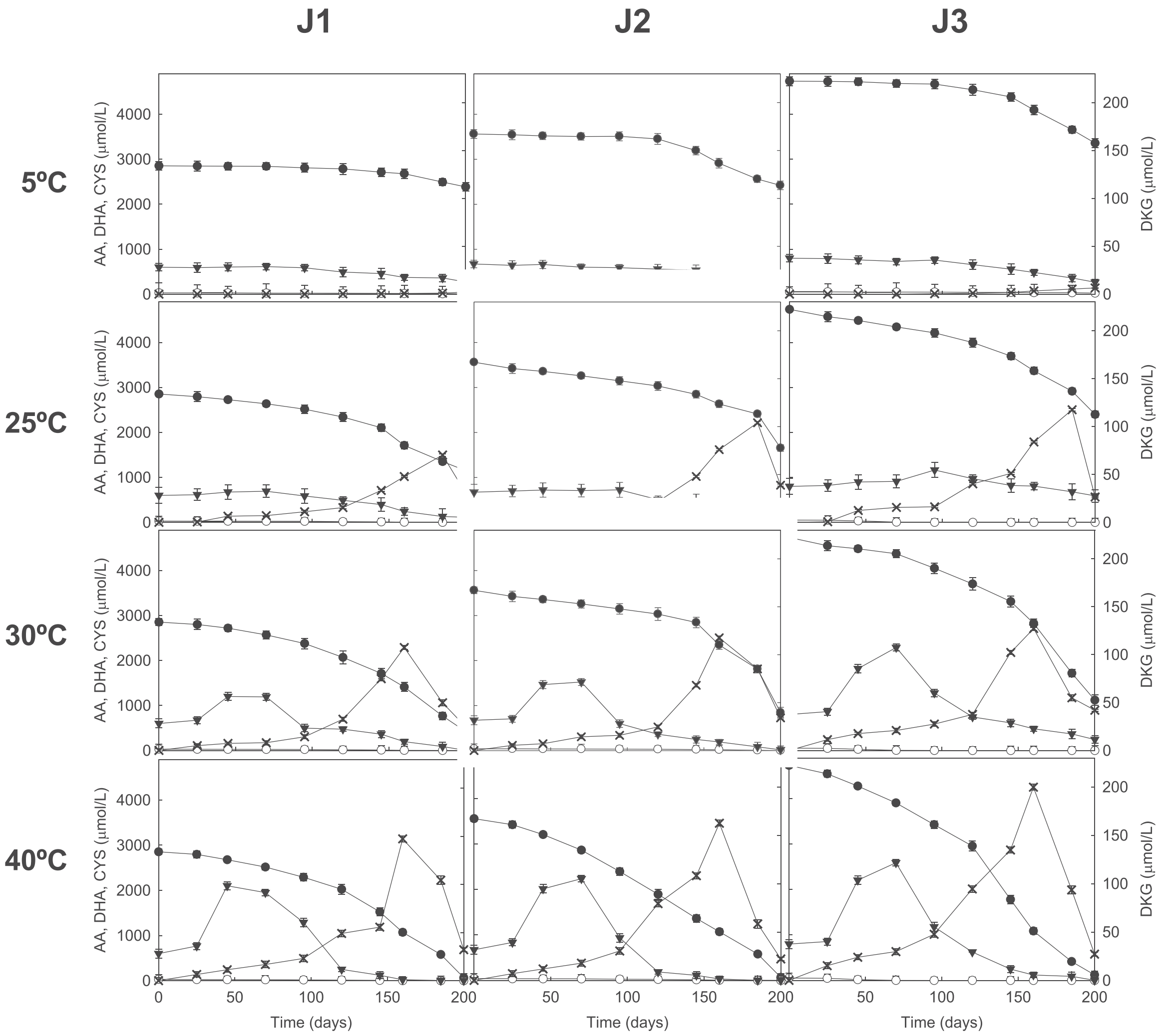

Fig. 4. Evolution of Lascorbic acid (●, AA), dehydro-L-ascorbic acid (▼, DHA), 2,3-diketogulonic acid (×, DKG), and cysteine (o, Cys) values at 5 °C, 25 °C, 30 °C, and 40 °C for different baby juices (n = 3). **J1**: Carrot and grapes; **J2**: Apple; **J3**: Apple, pear, strawberry and blackcurrant.

advice of the EC Scientific Committee for Food (SCF) that the addition of amino acids to foods intended for infants and young children should only be permitted for the purpose of improving the nutritional value of the foodstuff.

3.7. *Shelf life check*

AA abounds in citrus fruits such as orange and mandarin present in some of the baby foods studied. Decrease of AA concentration to levels unacceptable by industrial practice often defines orange juice shelf life. According to the AIJN-European Fruit Juice Association Code of Practice (CoP) for the evaluation of fruit and vegetable juices, AA content has to be more than 20 mg/100 mL (~1135.59 µmol/L) orange juice at expiration date (AIJN-European Fruit Juice Association, 2008). On the other hand, regulation in force regarding the labelling and presentation of baby food products require that the information on the product's shelf life be included using the concept of minimum duration or preferred consumption date. 'Use by date' indicates that the manufacturer guarantees the nutrient content and the general acceptability of the quality of the infant formula up to that date.

In accordance with all above mentioned, shelf life of fruit-based baby foods stored at ambient temperature (25 °C) was calculated through Eq. (1), substituting C_t with 1135.59 µmol/L and k with the estimated graphically value for each type of product (Table 3). Based on AA retention, decrease of shelf life of fruit-based baby foods compared to labelling ranged from 76 % to 84 % for dehydrated foods, from 35 % to 73 % for 'purees', and from 8 % to 52 % for baby juices. Estimated reduction in shelf life of products P1 and J1 differs markedly from the calculated for other similar products, since the initial concentration of AA in them is also significantly lower.

4. Conclusions

Simultaneous changes of AA, DHA, DKG, and Cys content in commercially available fruit-based baby foods with different water or moisture content during storage at various temperatures were determined. T, MC, and *t* were the main independent variables or factors affecting AA and DKG concentration changes. Similar pattern of AA degradation was observed in all products with independence of their initial MC, AA, and Cys content. The changes of AA content during the storage followed zero-order reaction kinetics. Retention of vitamin C (> 68 %) was much higher in the case of fruit-based baby foods stored under refrigeration (5 °C). A statistically significant relationship between t_{DKGmax} and T ($P =$ 0.0000; r = -0.9173) was estimated. Likewise, positive correlation between AA and Cys was found at the 99 % confidence level ($P =$ 0.0003; r = -0.1912) whereas DKG was negatively correlated ($P =$ 0.0005; r = -0.1823). Decreasing of Cys content during storage promoted DKG formation and loss of biological activity and health benefits. A significant reduction of shelf life based on AA retention was determined for all fruit-based baby foods used in

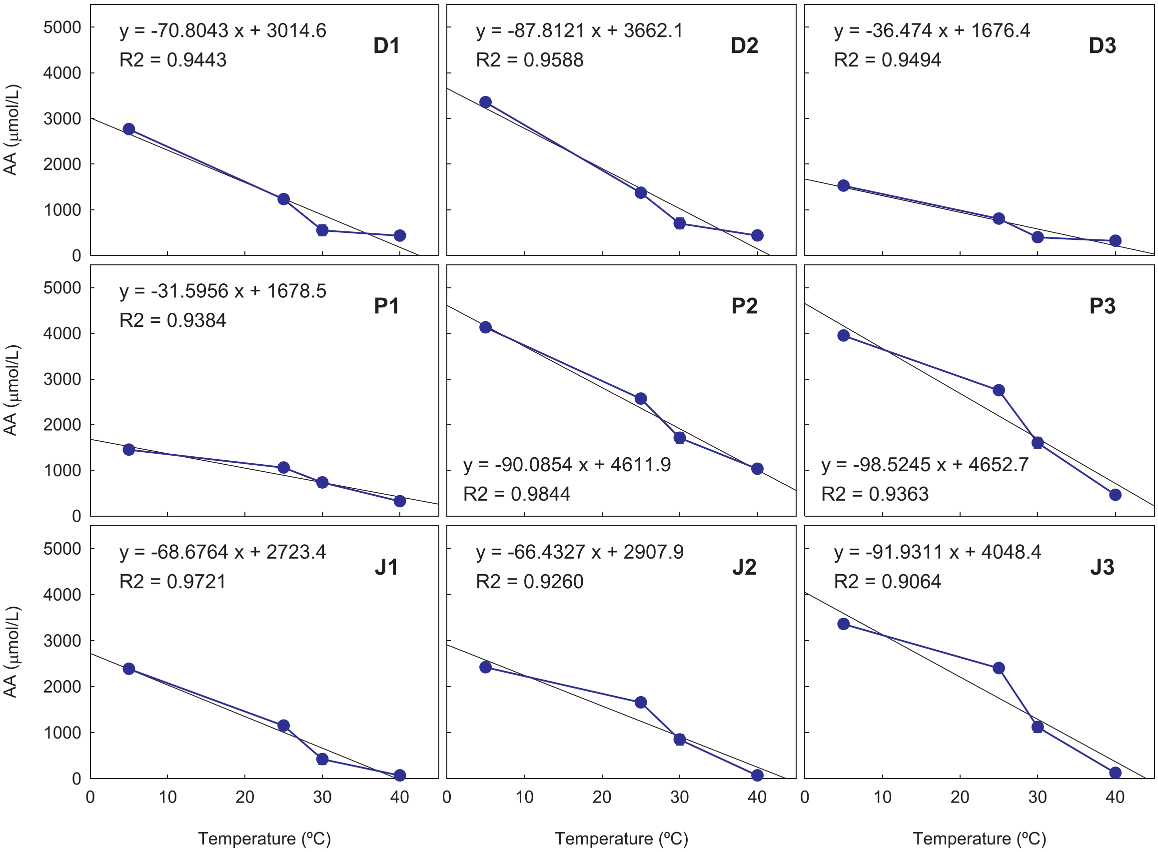

Fig. 5. Temperature dependence of L-ascorbic acid (AA) loss as a function of temperature. **D1-D3**: Dehydrated fruit-based baby foods; **P1-P3**: Homogenized fruit-based baby foods; and **J1-J3**: Baby juices.

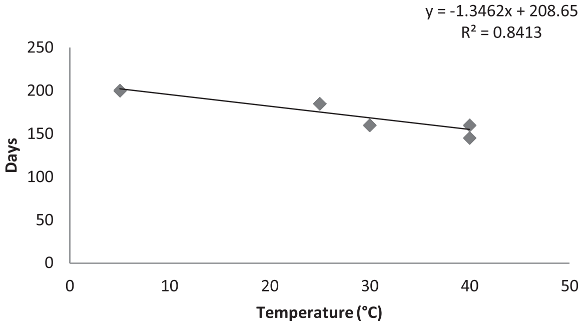

Fig. 6. Linear relationship between time when maximum 2,3-diketogulonic acid (DKG) content was determined and storage temperature (T).

Table 2
Correlation coefficients (r) and P-values estimated for the relationship between L-ascorbic acid and cysteine content in three types of fruit-based baby foods preserved at different temperatures.

	Temperature							
	5 °C		25 °C		30 °C		40 °C	
Food	r	P	r	P	r	P	r	P
D1	0.9342	< 0.01	0.9659	< 0.01	0.9637	< 0.01	0.9460	< 0.01
D2	0.9832	< 0.01	0.9928	< 0.01	0.9950	< 0.01	0.9919	< 0.01
D3	0.9843	< 0.01	0.9854	< 0.01	0.9146	< 0.01	0.8664	< 0.01
P1	0.9703	< 0.01	0.8041	< 0.01	0.8941	< 0.01	0.8557	< 0.01
P2	0.9126	< 0.01	0.8530	< 0.01	0.8127	< 0.01	0.7868	< 0.01
P3	0.9641	< 0.01	0.9008	< 0.01	0.8397	< 0.01	0.7932	< 0.01
J1	0.9361	< 0.01	0.8985	< 0.01	0.9759	< 0.01	0.9552	< 0.01
J2	0.9763	< 0.01	0.9842	< 0.01	0.9834	< 0.01	0.9864	< 0.01
J3	0.9510	< 0.01	0.6780	< 0.05	0.6146	< 0.10	0.6681	< 0.05

D1, dehydrated skimmed milk, apple and banana; **D2**, dehydrated skimmed milk, peach, banana, apple and orange; **D3**, dehydrated skimmed milk, carrot and beef; **P1**, homogenized carrot and rice; **P2**, homogenized banana, apple, pear and mandarin; **P3**, homogenized peach and banana; **J1**, carrot and grapes juice; **J2**, apple juice; **J3**, apple, pear, strawberry and blackcurrant juice.

the present study.

CRediT authorship contribution statement

José A. Cánovas: Investigation, Methodology. **Sara Gea-Botella:** Investigation, Methodology. **Fernando Borrás:** Formal analysis, Data curation. **Nuria Martí:** Conceptualization, Validation. **Manuel Valero:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Project administration. **Domingo Saura:** Conceptualization, Validation, Project administration. **María C. Martínez-Madrid:** Visualization, Supervision. **José Laencina:** Investigation, Supervision.

Acknowledgements

The authors gratefully acknowledge the excellent technical assistance and financial support from Mitra Sol Technologies S.L.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fpsl.2019.100453.

Table 3
Study on L-ascorbic acid (AA) loss kinetics and shelf life in fruit-based baby foods stored at ambient temperature (25 °C).

	C_o (μmol/L)	C_{exp} (μmol/L)	C_o-C_{exp} (μmol/L)	k (μmol/L·d)	*t* (d)	SL (d)	Red (d)	%Red
D1	3285.81	1135.59	2150.22	9.9525	216	960	744	77.5
D2	4111.56	1135.59	2975.97	13.1260	227	960	733	76.4
D3	2149.77	1135.59	1014.18	6.4810	156	960	804	83.8
P1	1483.79	1135.59	348.20	1.8805	185	690	505	73.2
P2	4316.69	1135.59	3181.10	7.8564	405	690	285	41.3
P3	4044.39	1135.59	2908.80	6.5139	447	690	243	35.2
J1	2853.57	1135.59	1717.98	8.4971	202	420	218	51.9
J2	3561.57	1135.59	2425.98	6.2940	385	420	35	8.3
J3	4735.71	1135.59	3600.12	10.6230	339	420	81	19.3

$\mathbf{C_o}$, AA concentration at time zero; $\mathbf{C_{exp}}$, AA concentration at the expiration date; **k**, the zero-order kinetic reaction constant; ***t***: time; **SL**, commercial labeled shelf life; **Red**, time shelf life reduction; **%Red**, percentage of shelf life reduction.

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