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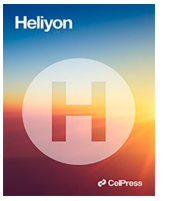

Research article

Degradation kinetics of vitamins in different enteral feeding formulas during storage at different temperatures

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ABSTRACT

Vitamin degradation may be affected differently by various food matrices. In this study, the kinetics of vitamin A, B_1, and C degradation were directly compared in two types of enteral feeding formulas (EFFs) with different energy densities over a nine-month storage period at 4, 25, and 30 °C. The content of vitamins A, B_1, and C was measured in the initial and stored formulas. The results justified the finding that the content of these vitamins was gradually decreased with storage time or temperature increases during the period. At each temperature during storage, the degradation of vitamins A, B_1, and C followed first-order kinetics, and the rate constants calculated indicated the degradation of vitamins was temperature-dependent. The EFF-B exhibited a higher activation energy for vitamin degradation than that in the EFF-A, and the activation energy indicated an inverse relationship with the fat content of EFFs. The outcomes might provide a reference for the development and application of EEFs.

1. Introduction

In recent decades, the advantages of enteral nutrition support for the treatment and rehabilitation of patients with different diseases have become more and more obvious. Enteral feeding formula (EFF) provides essential macronutrients and micronutrients to meet the daily nutrition requirements of different patients, ranging from the critically ill and hypermetabolic to the stable and receiving home enteral nutrition [1,2]. Commercial EFFs are mainly composed as follows: from animal or vegetal protein; from intact or hydrolyzed carbohydrates; and from lipids such as vegetal or animal fat. Vitamins, minerals, and fiber are frequently used as formula ingredients [3,4].

Vitamins are vital for the improvement in the nutritional status of patients as well as playing significant roles in many physiological functions [5]. Therefore, they are usually fortified in sufficient amounts to supply sufficient micronutrients for patients daily intakes of 1500–1800 kcal when the EFFs are designed and manufactured [6]. EFF is frequently processed by commercial sterilization methods of ultra-high temperature (136–142 °C, 3–5s) or autoclaving (121 °C, 15–20 min) for long-term storage. In addition, after their manufacture, they are ordinarily stored under uncontrolled environmental conditions throughout the entire supply chain [7]. The stability of vitamins can be affected by many kinds of factors, for instance, storage time, storage conditions (temperature, oxygen, light, and humidity), packaged form, and the nature of the food matrix [8]. Taking everything into account, the vitamin content in enteral feeding formulas deserves more attention.

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The degradation of vitamins during storage is the major problem contributing to the nutritional quality decrease in EFF, which makes it very important for the EFF manufacturers to ensure reasonable processing and storage of the EFF under appropriate conditions. Meanwhile, because the addition of vitamins to enteral feeding formulas is designed to provide nutrients to patients and the vitamin content is constrained by product labeling laws, it is of great importance to control the stability and degradation of vitamins in EFFs. Given all that, it is necessary to understand the degradation reaction of vitamins by studying the kinetics of vitamin degradation in EFFs during storage. Kinetics models of degradation could be applied not only to assess the food quality objectively, rapidly, and economically but also be used to predict the effect of a few experimental variables on the key nutritional quality [9]. Different studies on degradation kinetics in previous literature have attempted to estimate the degradation rate constants of vitamins in formula food. A few degradation kinetic models, for example, zero-order, first-order, and second-order kinetic reactions, have been successfully employed to evaluate the loss of vitamins. While a good deal has been learned about the stability of vitamins in common foods, including dairy products, dietary supplements, and powdered or liquid infant formulas in recent years [10–13], even fewer comprehensive reports on the degradation kinetics of vitamin loss in enteral feeding formulas during storage are available in the literature.

Vitamin degradation may be affected differently by various EFF formulas. The primary targets of the present paper were: firstly, to investigate the content of vitamins A, B_1, and C in two kinds of freshly prepared EFFs and compare the producers' declaration on the label with the concentrations found in the EFFs; secondly, to assess the stability and the degradation kinetics of the vitamins A, B_1, and C during storage at 4, 25, and 30 °C for 1, 2, 3, 6, and 9 months.

2. Material and methods

2.1. Samples and storage

Eenteral feeding formulas were freshly prepared in our pilot factory according to the commercial EFF manufacturing process as well as immediately used for studies on storage under different conditions, which were normocaloric formula (EFF-A) and hypercaloric formula (EFF-B), respectively. Table S1 indicated the main constituent of each formula on the labels described. The EFFs were homogenized, packaged in light-inhibiting containers, and finally sterilized by autoclaving (121 °C for 15 min).

All samples were kept in their original sealed packaging (a light-inhibiting glass container) in a constant temperature box at 4, 25, and 30 °C for 1, 2, 3, 6, and 9 months during the experimental period. In our study, temperatures of 4, 25, and 30 °C simulated the refrigerated condition, normal ambient condition, and extreme conditions without air conditioning in the summer in markets and food stores, respectively. The vitamin content was measured in triplicate (parallel samples from three different original packagings) promptly after production, with each detection time extending until 9 months of storage. The vitamins were analyzed during the first week of each month.

2.2. Standards and chemicals

Both methanol and n-butyl alcohol were of high-performance liquid chromatography (HPLC) grade and obtained from Oceanpak (Stockholm, Sweden). The ultra-pure water was produced by an in-house YouPu system (Sichuan, China). The standards for vitamins were purchased from First Standard (Tianjing, China). The concentrations of working solution used to construct a calibration curve for vitamins A, B_1, and C were 0.5–10.0, 0.1–3.0, and 0.3–3.0 μg/mL, respectively. These solutions were maintained at −20 °C before use, and all other reagents were of analytical grade or better, such as diethyl ether, petroleum ether, and potassium ferricyanide.

2.3. Determination of vitamins

Vitamin A was determined following the national standard and previous literature [14]. The samples were saponified at room temperature overnight using absolute ethanol, potassium hydroxide solution, and ascorbic acid as an antioxidant. After saponification, the retinol was extracted with a 200:200 vol ratio between diethyl ether and petroleum ether. And then this mixed solution was washed and condensed; the resulting volumes were then supplemented by methanol and filtered through a nylon syringe filter (0.22 μm) after thorough shaking. Finally, the sample was injected into the HPLC system (Agilent, NYSE.A, Palo Alto, California, USA) consisting of a quaternary pump (1260 Quat Pump VL), an ultraviolet detector (1260 VWD), a column oven (1260 TCC), an injector with a 20 μL loop, and Agilent OpenLAB ChemStation software. A Shimsen VD C30 column (250 × 4.6 mm) was used for separations in an acclimatized room (20 °C). Methanol-water (96:4 v/v) was used as the mobile phase and was pumped at a flow rate of 0.8 mL/min. The wavelength selected was optimum for vitamin A detection ($\lambda = 325$ nm).

Vitamin B_1 was measured according to China [15] and Zeeb et al. [16]. Thiamine was extracted with acid hydrolysis and then derivatized with potassium ferricyanide. Then the sample was made up the volume with n-butyl alcohol and filtrated through a 0.45 μm nylon syringe filter after shaking thoroughly and ready to inject into the HPLC system (Agilent, NYSE.A, Palo Alto, California, USA). This system consisted of a quaternary pump (1260 Quat Pump VL), a fluorescence detector (1260 FLD), a column oven (1260 TCC), an injector with a 20 μL loop, and Agilent OpenLAB ChemStation software. An Agilent 5 TC C18 column (250 × 4.6 mm) was used for separations in an acclimatized room (30 °C). Methanol-sodium acetate solution (35:65, v/v) was used as the mobile phase, and the flow rate was 0.8 mL/min. The wavelength selected was optimum for vitamin B_1 detection ($\lambda ex = 375$ nm, $\lambda em = 435$ nm).

Vitamin C was determined based on the fluorospectrophotometry method [17,18]. Ascorbic acid was used for the calibration of the standard curve for vitamin C. About 5 g of sample was dissolved with metaphosphate/acetic acid solution and subsequently

Table 1
Analysis of vitamin A content in two different enteral feeding formulas.

	Temperature (K)	Storage time (month)	Vitamin A (μg RE/100 mL)	Label (μg RE/100 mL)	Losses (%)	Adequacy (%)	Vitamin A (μg RE/100 kcal)
EFF-A	277(4 °C)	0	121.28 ± 4.61[a]	85.0	0.00	142.68	121.28 ± 4.61
		1	118.30 ± 2.65 [ab]		2.46	139.18	118.30 ± 2.65
		2	116.11 ± 0.28 [abc]		4.26	136.60	116.11 ± 0.28
		3	114.48 ± 1.13 [bc]		5.61	134.68	114.48 ± 1.13
		6	113.08 ± 0.87 [bc]		6.76	133.04	113.08 ± 0.87
		9	110.36 ± 0.87[c]		9.00	129.84	110.36 ± 0.87
	298(25 °C)	0	121.28 ± 4.61[a]		0.00	142.68	121.28 ± 4.61
		1	115.97 ± 2.85 [b]		4.38	136.44	115.97 ± 2.85
		2	112.50 ± 2.16 [b]		7.24	132.35	112.50 ± 2.16
		3	100.74 ± 2.37[c]		16.94	118.52	100.74 ± 2.37
		6	91.30 ± 1.81 [d]		24.72	107.41	91.30 ± 1.81
		9	82.46 ± 2.34[e]		32.01	97.01	82.46 ± 2.34
	303(30 °C)	0	121.28 ± 4.61[a]		0.00	142.68	121.28 ± 4.61
		1	108.25 ± 1.78 [b]		10.74	127.35	108.25 ± 1.78
		2	104.35 ± 3.71 [b]		13.96	122.76	104.35 ± 3.71
		3	96.10 ± 2.32[c]		20.76	113.06	96.10 ± 2.32
		6	89.31 ± 2.99 [d]		26.36	105.07	89.31 ± 2.99
		9	76.98 ± 1.15[e]		36.53	90.56	76.98 ± 1.15
EFF-B	277(4 °C)	0	116.74 ± 1.09[a]	84.0	0.00	138.98	97.28 ± 0.91
		1	115.96 ± 0.43[a]		0.67	138.05	96.63 ± 0.36
		2	116.02 ± 0.30[a]		0.62	138.12	96.68 ± 0.25
		3	115.35 ± 1.20 [ab]		1.19	137.32	96.13 ± 1.00
		6	114.86 ± 0.92 [ab]		1.61	136.74	95.72 ± 0.77
		9	113.45 ± 0.82 [b]		2.82	135.06	94.54 ± 0.68
	298(25 °C)	0	116.74 ± 1.09[a]		0.00	138.98	97.28 ± 0.91
		1	114.93 ± 3.02[a]		1.55	136.82	95.78 ± 2.52
		2	108.25 ± 2.29 [b]		7.27	128.87	90.21 ± 1.91
		3	106.76 ± 0.96 [bc]		8.55	127.10	88.97 ± 0.80
		6	103.35 ± 1.80[c]		11.47	123.04	86.13 ± 1.50
		9	93.33 ± 1.63 [d]		20.05	111.11	77.78 ± 1.36
	303(30 °C)	0	116.74 ± 1.09[a]		0.00	138.98	97.28 ± 0.91
		1	114.14 ± 1.15[a]		2.23	135.88	95.12 ± 0.96
		2	109.81 ± 1.06 [b]		5.94	130.73	91.51 ± 0.88
		3	102.74 ± 1.59[c]		11.99	122.31	85.62 ± 1.33
		6	91.17 ± 2.22 [d]		21.90	108.54	75.98 ± 1.85
		9	86.45 ± 1.40[e]		25.95	102.92	72.04 ± 1.16

Values are the relative content and the mean of three determinations±standard deviation. Values in the same column for each physiochemical characteristic with the same superscript are not significantly different ($p \geq 0.05$).
μg RE/100 mL = μg RE/100 kcal × Energetic destiny.

determined by the fluorospectrophotometer RF 6000 (Shimadzu, Japan). The optimum wavelength selected for detection of vitamin C (λex = 350 nm, λem = 430 nm) and the concentration of sample was calculated by the standard curve of vitamin C.

2.4. Kinetic calculations

The reaction rate expression of degradation kinetics with time could be written by a mathematical equation as follows [19,20]:

$$-d[C]/dt = k\,[C]^n \quad (1)$$

here, "k" is the reaction rate constant, "n" is the order of reaction, and "C" is the quantitative content of the analyte considered with time. Integration of equation (1) for a first-order equation could be represented as equation (2):

$$\ln C_t/C_0 = -kt \quad (2)$$

where "C_0" is vitamin content at time "0", "C_t" is vitamin content at time "t" and "t" is storage time by the months. The half-life values were calculated using the following equation (3):

$$t_{1/2} = 0.693/k \quad (3)$$

The relationship of degradation rate constant to temperature was quantified by the Arrhenius equation (4):

$$K_T = k_0 \exp(-E_A/RT) \quad (4)$$

where "k_0" is the pre-exponential constant, "R" is the gas constant [8.3144 J/(mol K)], "T" is the absolute temperature (K = 273+°C) as the temperature used in the Arrhenius equation must be in degrees Kelvin, "E_A" is the activation energy in kJ/mol. E_A is calculated as a

Table 2
Analysis of vitamin B_1 content in two different enteral feeding formulas.

	Temperature (K)	Storage time (month)	Vitamin B_1 (mg/100 mL)	Label (mg/100 mL)	Losses (%)	Adequacy (%)	Vitamin B_1 (mg/100 kcal)
EFF-A	277(4 °C)	0	0.46 ± 0.02^{a}	0.32	0.00	143.75	0.46 ± 0.02
		1	0.45 ± 0.01 ^{ab}		2.17	140.63	0.45 ± 0.01
		2	0.44 ± 0.01 ^{ab}		4.35	137.50	0.44 ± 0.01
		3	0.44 ± 0.00 ^{ab}		4.35	137.50	0.44 ± 0.00
		6	0.43 ± 0.01 ^{ab}		6.52	134.38	0.43 ± 0.01
		9	0.42 ± 0.01 ^{b}		8.70	131.25	0.42 ± 0.01
	298(25 °C)	0	0.46 ± 0.02^{a}		0.00	143.75	0.46 ± 0.02
		1	0.45 ± 0.00 ^{ab}		2.17	140.63	0.45 ± 0.00
		2	0.45 ± 0.01 ^{ab}		2.17	140.63	0.45 ± 0.01
		3	0.44 ± 0.01 ^{b}		4.35	137.50	0.44 ± 0.01
		6	0.42 ± 0.01^{c}		8.70	131.25	0.42 ± 0.01
		9	0.41 ± 0.01^{c}		10.87	128.13	0.41 ± 0.01
	303(30 °C)	0	0.46 ± 0.02^{a}		0.00	143.75	0.46 ± 0.02
		1	0.45 ± 0.01 ^{ab}		2.17	140.63	0.45 ± 0.01
		2	0.43 ± 0.02 ^{bc}		6.52	134.38	0.43 ± 0.02
		3	0.41 ± 0.01^{c}		10.87	128.13	0.41 ± 0.01
		6	0.38 ± 0.00 ^{d}		17.39	118.75	0.38 ± 0.00
		9	0.33 ± 0.01^{e}		28.26	103.13	0.33 ± 0.01
EFF-B	277(4 °C)	0	0.44 ± 0.01 ^{ab}	0.34	0.00	129.41	0.37 ± 0.01
		1	0.45 ± 0.01^{a}		−2.27	132.35	0.37 ± 0.00
		2	0.43 ± 0.01 ^{ac}		2.27	126.47	0.36 ± 0.00
		3	0.42 ± 0.01 ^{cd}		4.55	123.53	0.35 ± 0.01
		6	0.42 ± 0.02^{c}		4.55	123.53	0.35 ± 0.01
		9	0.41 ± 0.01 ^{d}		6.82	120.59	0.34 ± 0.00
	298(25 °C)	0	0.44 ± 0.01^{a}		0.00	129.41	0.37 ± 0.01
		1	0.43 ± 0.00 ^{ab}		2.27	126.47	0.36 ± 0.00
		2	0.43 ± 0.01 ^{ab}		2.27	126.47	0.36 ± 0.01
		3	0.42 ± 0.01 ^{b}		4.55	123.53	0.35 ± 0.00
		6	0.42 ± 0.00 ^{b}		4.55	123.53	0.35 ± 0.00
		9	0.39 ± 0.01^{c}		11.36	114.71	0.33 ± 0.00
	303(30 °C)	0	0.44 ± 0.01^{a}		0.00	129.41	0.37 ± 0.01
		1	0.43 ± 0.01^{a}		2.27	126.47	0.36 ± 0.01
		2	0.44 ± 0.00^{a}		0.00	129.41	0.37 ± 0.00
		3	0.41 ± 0.02 ^{b}		6.82	120.59	0.34 ± 0.01
		6	0.36 ± 0.01^{c}		18.18	105.88	0.30 ± 0.01
		9	0.32 ± 0.02 ^{d}		27.27	94.12	0.26 ± 0.01

Values are the relative content and the mean of three determinations±standard deviation. Values in the same column for each physiochemical characteristic with the same superscript are not significantly different ($p \geq 0.05$).
mg/100 mL = mg/100 kcal × Energetic destiny.

product of a gas constant R and the slope of the graph obtained by plotting ln k against 1/T.

2.5. Data statistics and analysis

Chromatographic data were acquired and analyzed with OpenLAB ChemStation (Agilent, NYSE.A, Palo Alto, California, USA). Data of others were presented as mean ± standard deviation SD (n = 3) and obtained using software of SPSS 22.0 (SPSS Inc., Chicago IL, USA). Excel 2010 and Origin 8.0 (Origin Lab, Hampton, AK, USA) were used to analyze kinetic data by regression analysis, and $p \leq 0.05$ was regarded as statistically significant.

3. Results and discussion

3.1. Vitamins A, B_1 and C in samples freshly prepared

The initial value (time 0) of vitamins A, B_1, and C in two different EFFs (EFF-A and EFF-B) that were freshly prepared is shown in Tables 1–3. At the beginning of the study, in EFF-A (time 0), the vitamins A, B_1, and C had values of 121.28 μg RE/100 mL, 0.46 mg/100 mL, and 30.18 mg/100 mL, respectively. While the levels according to the label were 85.0 μg RE/100 mL, 0.32 mg/100 mL, and 15.0 mg/100 mL, respectively, this therefore represented an adequacy value of 142.68 %, 143.75 %, and 201.20 %, respectively. In addition, in EFF-B, the initial contents of 116.74 μg RE/100 mL, 0.44 mg/100 mL, and 35.42 mg/100 mL were found, respectively, as well as the label values of 84.0 μg RE/100 mL, 0.34 mg/100 mL, and 17.4 mg/100 mL, which meant adequacy values of 138.98 %, 129.41 %, and 203.56 %, according to the label statements.

Chinese legislation (GB 29922-2013) established upper and lower limit requirements for each vitamin in enteral formulas (vitamin A: 39.0–225.0 μg RE/100 kcal, vitamin B_1: 0.07-N.S. mg/100 kcal and vitamin C: 5.6-N.S. mg/100 kcal, respectively) [21], and the

Table 3
Analysis of vitamin C content in two different enteral feeding formulas.

	Temperature (K)	Storage time (month)	Vitamin C (mg/100 mL)	Label(mg/100 mL)	Losses (%)	Adequacy (%)	Vitamin C (mg/100 kcal)
EFF-A	277(4 °C)	0	30.18 ± 0.89^{a}	15.0	0.00	201.20	30.18 ± 0.89
		1	29.95 ± 0.06^{a}		0.76	199.67	29.95 ± 0.06
		2	29.39 ± 0.40 ^{ab}		2.62	195.93	29.39 ± 0.40
		3	28.97 ± 0.28 ^{bc}		4.01	193.13	28.97 ± 0.28
		6	28.65 ± 0.71 ^{bc}		5.07	191.00	28.65 ± 0.71
		9	28.04 ± 0.39^{c}		7.09	186.93	28.04 ± 0.39
	298(25 °C)	0	30.18 ± 0.89^{a}		0.00	201.20	30.18 ± 0.89
		1	29.87 ± 0.22^{a}		1.03	199.13	29.87 ± 0.22
		2	28.68 ± 0.72 ^{b}		4.97	191.20	28.68 ± 0.72
		3	27.90 ± 0.17 ^{b}		7.55	186.00	27.90 ± 0.17
		6	26.60 ± 0.37^{c}		11.86	177.33	26.60 ± 0.37
		9	24.85 ± 0.61 ^{d}		17.66	165.67	24.85 ± 0.61
	303(30 °C)	0	30.18 ± 0.89^{a}		0.00	201.20	30.18 ± 0.89
		1	28.92 ± 0.47 ^{ab}		4.17	192.80	28.92 ± 0.47
		2	26.96 ± 0.91 ^{b}		10.67	179.73	26.96 ± 0.91
		3	24.52 ± 0.65^{c}		18.75	163.47	24.52 ± 0.65
		6	22.66 ± 0.16 ^{d}		24.92	151.07	22.66 ± 0.16
		9	21.52 ± 0.43 ^{d}		28.69	143.47	21.52 ± 0.43
EFF-B	277(4 °C)	0	35.42 ± 0.37^{a}	17.4	0.00	203.56	29.52 ± 0.31
		1	34.95 ± 0.06 ^{ab}		1.33	200.86	29.13 ± 0.05
		2	34.39 ± 0.40 ^{bc}		2.91	197.64	28.66 ± 0.33
		3	33.97 ± 0.28^{c}		4.09	195.23	28.31 ± 0.24
		6	32.65 ± 0.71 ^{d}		7.82	187.64	27.21 ± 0.59
		9	32.08 ± 0.80 ^{d}		9.43	184.37	26.74 ± 0.67
	298(25 °C)	0	35.42 ± 0.37^{a}		0.00	203.56	29.52 ± 0.31
		1	33.87 ± 0.22 ^{b}		4.38	194.66	28.23 ± 0.19
		2	32.68 ± 0.72 ^{b}		7.74	187.82	27.23 ± 0.60
		3	29.60 ± 0.59^{c}		16.43	170.11	24.67 ± 0.50
		6	20.36 ± 0.52 ^{d}		42.52	117.01	16.97 ± 0.43
		9	18.45 ± 0.70^{e}		47.91	106.03	15.37 ± 0.58
	303(30 °C)	0	35.42 ± 0.37^{a}		0.00	203.56	29.52 ± 0.31
		1	33.45 ± 0.32 ^{b}		5.56	192.24	27.87 ± 0.26
		2	31.18 ± 0.67^{c}		11.97	179.20	25.98 ± 0.56
		3	30.16 ± 1.09^{c}		14.85	173.33	25.13 ± 0.91
		6	20.18 ± 0.19 ^{d}		43.03	115.98	16.82 ± 0.16
		9	17.60 ± 0.70^{e}		50.31	101.15	14.67 ± 0.58

Values are the relative content and the mean of three determinations±standard deviation. Values in the same column for each physiochemical characteristic with the same superscript are not significantly different ($p \geq 0.05$).
mg/100 mL = mg/100 kcal × Energetic destin

European Society for Clinical Nutrition and Metabolism (ESPEN) recommended values shall be provided by enteral nutrition (vitamin A: 60.0–100.0 μg RE/100 kcal, vitamin B_1: 0.1–0.2 mg/100 kcal and vitamin C: 6.7-N.S. mg/100 kcal, respectively) [5]. In the present study, vitamin A, B_1, and C indicated an initial content greater than the minimum recommended value for GB 29922-2013 and the recommended value for ESPEN.

3.2. Kinetic studies of vitamin degradation

Generally, the kinetics of different vitamins degradation in foods during manufacture and storage have been calculated through regular monitoring of the content losses under several constant-temperature conditions [20]. These degradation kinetics of most vitamins comply with first-order kinetic reactions and a classical Arrhenius model can be established [10–13,22]. Therefore, we assumed the presence of first-order kinetics of vitamins degradation in current research. The degradation of vitamins was investigated under different storage conditions by plotting ln "(C_t/C_0)" versus time "t" (months). The rate constant "k" was the slope of these graphs, and the half-life value ($t_{1/2}$) (months) was calculated by equation (3). Fig. 1a–c showed the kinetics of vitamin degradation at 4, 25, and 30 °C in both different EFFs. The rate constant, half-life value, and correlation coefficient (R^2) of each vitamin in both different EFFs were demonstrated in Table 4. Except for the plot of vitamin B_1 of EFF-B at 4 °C ($R^2 = 0.81$), the plots all complied with first-order kinetics and the correlation coefficients were greater than 0.90.

3.2.1. Vitamin A degradation kinetics

From the results in Table 1, When the samples were held at 4 °C for 9 months of storage, the vitamin A in EFF-A recorded a content of 110.36 μg RE/100 mL, which represented a degradation of 9.00 % relative to the initial level and an adequacy of 129.84 % on the basis of the label. EFF-B recorded a value of 113.45 μg RE/100 mL under the same conditions, which represented a degradation of 2.82 % compared with the initial content and an adequacy of 135.06 % on the basis of the label. At 25 °C, after 9 months of storage, EFF-A showed content of 82.46 μg RE/100 mL, which represented a decrease of 32.01 % and a label adequacy of 97.01 %, while EFF-B

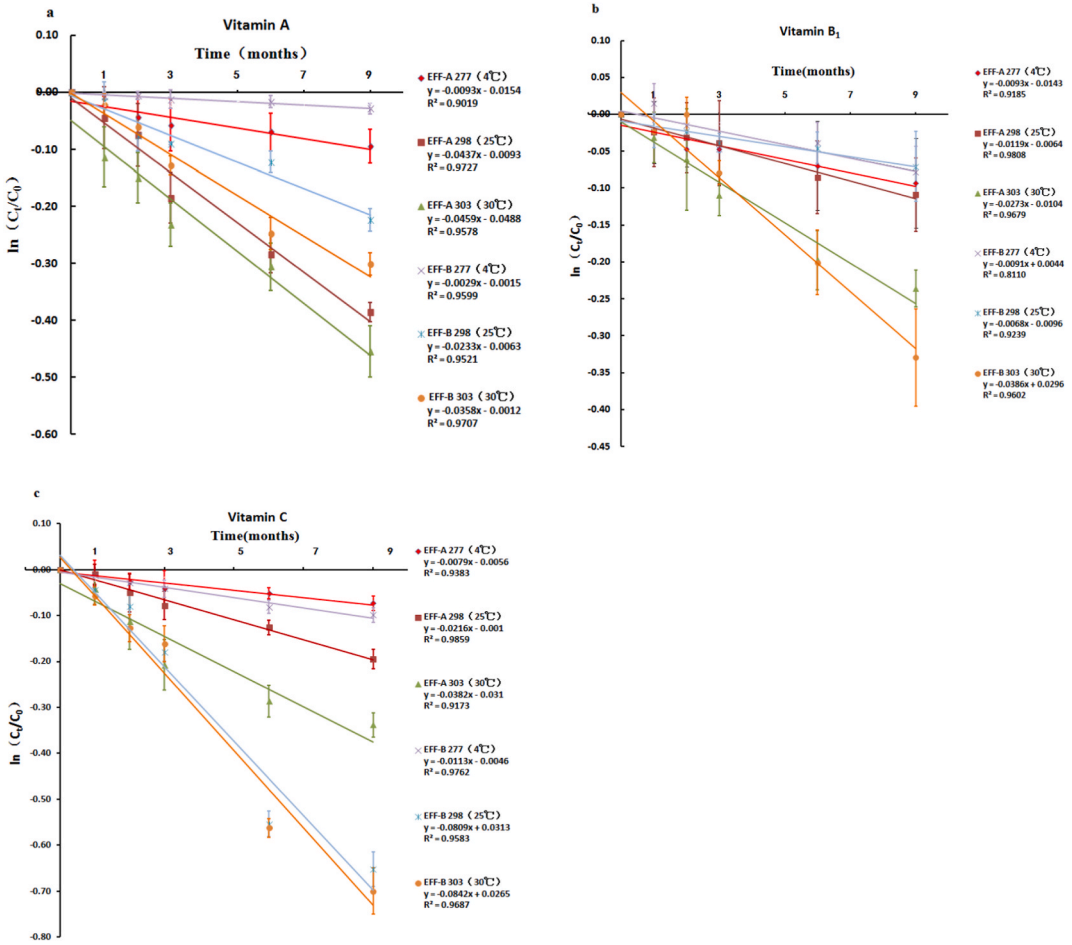

Fig. 1. Degradation kinetics of vitamins A (Fig. 1a), B_1 (Fig. 1b) and C (Fig. 1c) in two different enteral feeding formulas (EFF-A and EFF-B) at 4 °C, 25 °C and 30 °C. C_t: Concentration of vitamin at time t, C_0: Concentration of vitamin at time 0, t: Storage time in months.

Table 4
Rate constant (k) and half-time (months) of vitamin degradation in two different enteral feeding formulas.

Temperature (K)		Time of half destruction and determination coefficients of first-order								
		Vitamin A			Vitamin B_1			Vitamin C		
		$t_{1/2}$(month)	R^2	k	$t_{1/2}$(month)	R^2	k	$t_{1/2}$(month)	R^2	k
EFF-A	277(4 °C)	74.52	0.9019	0.0093	74.52	0.9185	0.0093	87.72	0.9383	0.0079
	298(25 °C)	15.86	0.9727	0.0437	58.24	0.9809	0.0119	32.08	0.9859	0.0216
	303(30 °C)	15.10	0.9578	0.0459	18.48	0.9916	0.0375	18.14	0.9173	0.0382
E_A, (kJ/mol)				45.24			28.34			39.38
EFF-B	277(4 °C)	238.97	0.9599	0.0029	76.15	0.8110	0.0091	61.33	0.9762	0.0113
	298(25 °C)	29.74	0.9521	0.0233	64.17	0.9239	0.0108	8.57	0.9583	0.0809
	303(30 °C)	19.36	0.9707	0.0358	17.95	0.9602	0.0386	8.23	0.9687	0.0842
E_A, (kJ/mol)				67.65			28.52			57.13

indicated content of 93.33 μg RE/100 mL, indicating a reduction of 20.05 % and a label adequacy of 111.11 %. When the formulas were stored at 30 °C for 9 months, EFF-A showed a content of 76.98 μg RE/100 mL, which represented a reduction of about 36.53 % and a label adequacy of 90.56 %, while EFF-B indicated a value of 86.45 μg RE/100 mL, indicating a reduction of 25.95 % and a label adequacy of 102.92 %. Although significant losses of vitamin A levels in EFF-A and EFF-B were seen at all the storage temperatures after 9 months, the label adequacy was more than 80 % and complied with the food labeling regulations [23].

The degradation of vitamin A is primarily caused by oxidation and isomerization under the action of oxygen, light, storage time, temperature, and so on [24]. As shown in Table 4, the vitamin A rate constant in EFF-A raised from 0.0093 per month at 4 °C to 0.0459 per month at 30 °C, and the half-time value ($t_{1/2}$) reduced from 74.52 to 15.10 months as the temperature went up from 4 to 30 °C. The rate constant of vitamin A in EFFB increased from 0.0029 per month at 4 °C to 0.0358 per month at 30 °C, and the half-time value ($t_{1/2}$) reduced from 238.97 to 19.36 months as the temperature rose from 4 to 30 °C. There were strong correlations with linear regressions of the natural log of the retention percent of vitamin A over time for different EFFs at different temperatures in this research (R^2 = 0.90–0.97). The first-order kinetics model of vitamin A degradation identified in the present research was consistent with previous studies on enteral feeding formulas or infant milk formulas [11,25]. The kinetics of vitamin A degradation at different storage temperatures for EFF-A and EFF-B were assessed from the plot of ln "(C_t/C_0)" versus time "t" (months) (Fig. 1a). The results indicated that vitamin A stability in those samples was significantly influenced by storage temperature, the amount of vitamin A remaining decreased with increasing temperature, and the degradation rate of vitamin A was accelerated gradually with storage temperature. Frias & Vidal-Valverde [26] found similar results for enteral feeding formulas Ia (EFF Ia, the fat content was 3.6 g/100 mL) and IIa (EFF IIa, the fat content was 3.9 g/100 mL) kept at 4, 20, and 30 °C for 3, 6, and 9 months, respectively. The vitamin A in these formulas showed a slight change at 4 °C after 3-months of storage, and reductions of 45–46 % and 84–85 % were found after storage of 6 and 9 months, respectively. While when the products were held at 20 and 30 °C, losses in vitamin A were also obtained: 2–5%, 53–58 %, and 89–98 % for EFF Ia, and 6–8%, 76–80 %, and 99 % for EFF IIa. The vitamin A degradation of these formulas was significantly higher than our samples.

In addition, it was clearly seen from Fig. 1a that there was a sharper reduction in the vitamin A content of EFF-B under the same storage condition compared to EFF-A, which might indicate that a hypercaloric formula with more fat content is beneficial for the stability of vitamin A during storage. They were in line with the results from Vidal-Valverde et al. [27] and Szlagatys-Sidorkiewicz et al. [28], who all observed that fat might have a protective effect on the stability of vitamin A. The chemical form of vitamin A (acetate or palmitate esters) added to the formulas might be another reason for high degradation. Chávez-Servín et al. [29] observed that when powdered infant formulas fortified with retinyl acetate and palmitate, respectively, were kept at normal temperature after 70 days, losses of retinyl acetate (4–37 %) were greater than those of retinyl palmitate (0.3–18.7 %). Similar results were obtained in this study when retinyl acetate was added as a fortifier to the formulas.

3.2.2. Vitamin B_1 degradation kinetics

The vitamin B_1 content of the EFFs is given in Table 2. As can be seen, after 9 months of storage at 4 °C, the vitamin B_1 levels of the EFFs at 4 °C were 0.42 and 0.41 mg RE/100 mL for EFF-A and EFF-B, respectively. Compared with the initial content, the results represented losses of 8.70 % and 6.82 %. And an adequacy of 131.25 % and 120.59 %, according to the label. The variations in vitamin B_1 content at 25 °C were 0.41 and 0.39 mg/100 mL for EFF-A and EFF-B, respectively. The reductions of 10.87 % in EFF-A and 11.36 % in EFF-B compared with the initial content, respectively. According to the label, the measured value of EFF-A represented an adequacy value of 128.13 %, and the measured value of EFF-B represented an adequacy value of 114.71 %. While those values for 30 °C were 0.33 and 0.32 mg/100 mL for EFF-A and EFF-B, respectively. Compared with the initial content, the degradation was 28.26 % in EFF-A and 27.27 % in EFF-B, respectively. According to the label, the measured value represented 103.13 % and 94.12 % of the stated label for EFF-A and EFF-B, respectively. Significant losses of vitamin B_1 levels in EFF-A and EFF-B were seen at all the storage temperatures after 9 months, but the label adequacy complied with the food labeling regulations [23], which was more than 80 %. And also, the degradation rates of vitamin B_1 in both formulas were almost the same under identical conditions.

It has been widely reported that factors such as storage temperature, time, pH, moisture, and so on, could result in vitamin B_1 degradation during storage. [22]. Table 4 demonstrates that the rate constant of vitamin B_1 in EFF-A raised from 0.0093 per month at 4 °C to 0.0375 per month at 30 °C, and the half-time value ($t_{1/2}$) reduced from 74.52 to 18.48 months as the temperature went up from

4 to 30 °C. The rate constant for vitamin B_1 in EFF-B increased from 0.0091 per month at 4 °C to 0.0386 per month at 30 °C, and the half-time value ($t_{1/2}$) reduced from 76.15 to 17.95 months as the temperature rose from 4 to 30 °C. In this study, we obtained high correlations ($R^2 = 0.81–0.99$) with linear regressions of the natural log of the retention percent of vitamin B_1 over time for both kinds of EFFs at different temperatures. The kinetics for vitamin B_1 degradation at different storage temperatures for EFF-A and EFF-B were obtained from the plot of ln "(C_t/C_0)" versus time "t" (months) (Fig. 1b). The results indicated that the degradation of vitamin B_1 in EFFs also followed first-order kinetics. As expected, vitamin B_1 stability was also significantly affected by storage temperature, and the kinetic reactions developed faster as the temperature went up. Frias & Vidal-Valverde [26] obtained several similar results and found that the content of vitamin B_1 in EFF Ia and EFF IIa showed important changes when these formulas were held at 4 °C for 9-months. A significant degradation (35 %) was observed. Similar effects occurred when these formulas were stored at 20 °C for 9 months, but the decrease in vitamin B_1 level was more sharp (47–53 %). When they were stored at 30 °C for 9 months, the reduction was most noticeable (52 %).

Fewer studies on the kinetics model for vitamin B_1 degradation in enteral feeding formulas have been conducted during storage. However, the first-order degradation kinetics of vitamin B_1 in other foods have been widely reported in previous studies [12,20,30,31]. A number of foods, including fruit products and energy drinks, could provide acidic environments to protect the stability of vitamin B_1 [22]. However, many other food sources of vitamin B_1 are closer to neutral or slightly alkaline, such as different kinds of dairy products, dietary supplements, and powdered or liquid infant formulas. The higher the pHs in the above foods, the more loss of vitamin B_1 might contribute during storage [22]. In this system, the free base of vitamin B_1 is protonated, which makes it easy to hydrolyze and cleavage to produce 4-methyl-5-hydroxyethyl thiazole and 2-methyl-4-amino-5-hydroxymethyl pyrimidine [32]. In both enteral feeding formulas closed to neutral pH (6.5–6.8), significant degradation of vitamin B_1 was observed at all temperatures during the 9-month study period. The results in this paper were in line with previous studies.

Another possible reason for vitamin B_1 degradation could be the chemical form of vitamin B_1 (thiamine mononitrate (TMN) or thiamine chloride hydrochloride (TClHCl)) added in the formulas. The stability of TMN and TClHCl in different solutions with storage time was directly compared by Adrienne et al. [22]. They found first-order kinetic reactions for both TMN and TClHCl. The degradation of TMN was all obviously faster than that of TClHCl at certain concentrations and storage temperatures when the samples with different concentrations were kept at 25, 40, 60, 70, and 80 °C until 6 months. The similar situation was also obtained from our study when TClHCl was used as the source of vitamin B_1.

3.2.3. *Vitamin C degradation kinetics*

Table 3 demonstrates that when these formulas were stored at 4 °C for 9 months, the measured value of vitamin C in EFF-A was 28.04 mg/100 mL (which represented 186.93 % of the stated label and 7.09 % loss), and the recorded value of EFF-B was 32.08 mg/100 mL (which represented 184.37 % of the stated label and 9.43 % loss). At 25 °C for 9 months, EFF-A showed a value of 24.58 mg/100 mL, which indicated a reduction of 17.66 % and a label adequacy of 165.67 %. EFF-B represented the level of 18.45 mg RE/100 mL, showing a reduction of 47.91 % and a label adequacy of 106.03 %. After 9 months of storage at 30 °C, the recorded values of EFF-A and EFF-B were 21.52 and 17.60 mg/100 mL, respectively, which represented the adequacy values of 143.47 % and 101.15 % with respect to the label information. Compared with the initial content, the degradation was 28.69 % in EFF-A and 50.31 % in EFF-B. Although significant losses of vitamin C content in EFF-A and EFF-B were seen at all the storage temperatures after 9 months as well, the label adequacy was more than 80 %, which complied with the food labeling regulations [23].

Vitamin C is an essential nutrient as well as an antioxidant, but under less desirable conditions it is prone to decompose. Vitamin C degradation includes aerobic and anaerobic pathways, which are dependent on oxygen, light, heat, storage temperature, and time. The oxidative degradation of vitamin C usually appears during processing, while anaerobic degradation usually occurs during storage [33]. In the case of vitamin C, Table 4 indicates that the rate constant in EFF-A increased from 0.0079 per month at 4 °C to 0.0382 per month at 30 °C, and the half-time value ($t_{1/2}$) decreased from 87.72 to 18.14 months as the temperature rose from 4 to 30 °C. The rate constant in EFF-B increased from 0.0113 per month at 4 °C to 0.0842 per month at 30 °C, and the half-time value ($t_{1/2}$) reduced from 61.33 to 8.23 months as the temperature went up from 4 to 30 °C. High correlations in linear regressions of the natural log of percent vitamin C remaining over time for different EFFs at different temperatures were obtained ($R^2 = 0.92–0.99$) in this research. The kinetics of vitamin C degradation in both EFFs was assessed from the plot of ln "(C_t/C_0)" versus time "t" (months) (Fig. 1c) at different storage temperatures. When the vitamin C values of both EFFs were plotted versus storage time, the best fit model for vitamin C degradation was also first order, which was similar to the other studies on different formulas [10,13,34,35]. The results indicated vitamin C content in both EFFs decreased significantly over time, and the degradation rate depended upon storage temperature. The finding of Li et al. [36] was in line with the current study. They found the decreased rates of vitamin C in an enteral feeding formula were 38.90 % and 64.77 % at 25 °C and 40 °C for 30 days of storage, respectively, which was far more than our samples under the same condition.

However, the reduction of vitamin C was faster in EFF-B than in EFF-A, which was also clearly observed from the half-time values of 4 months in EFF-A and EFF-B at 30 °C. Although vitamin C degradation was slower in EFF-A than EFF-B, it did go up with storage temperature. This might be due to the fact that EFF-B contained more fat and was more easily oxidized, while vitamin C acted as an antioxidant in this system but degraded much more rapidly. Wang et al. [37] reported the content of vitamin C in a powdered enteral formula stored at 37 °C for 180 days and 25 °C for 720 days. Their results indicated the loss rates for vitamin C were 30.7 % and 21.7 %, respectively, which were significantly lower than those in this study. It was probably related to easier vitamin C degradation in solution. Also, the effect of light on vitamin C was greater than keeping it in a dark place. Li et al. [36] observed that the vitamin C loss rate under 25 °C light conditions (38.90 %) was increased by 7.0 % compared with light avoidance conditions (31.91 %). That was why the formulas were kept in their original, sealed packaging (a light-inhibiting glass container) in the present research.

3.3. Arrhenius plot for vitamin degradation

Activation energy is an important measure of the sensitivity of the degradation reaction of vitamins to temperature, which was calculated by using Arrhenius plots. In order to confirm the influence of storage temperatures on vitamin A, B_1, and C degradation, the graphs of "ln k" versus "1/T" were drawn to get Arrhenius plots for EFF-A and EFF-B (Fig. 2a–c). The nature of the line for the aforementioned plots showed the losses of vitamins over storage time to follow the Arrhenius equation. We could obtain the degradation rate constants (k_T) of the vitamins by calculating the slopes of the graphs and then using the k_T to calculate the activation energies of different vitamins in both formulas. The values of activation energy were given in Table 4.

The activation energies derived from the Arrhenius relationship for vitamins A, B_1, and C in various food matrices have been reported. The activation energies of vitamin A were 45.24 and 67.65 kJ/mol for EFF-A and EFF-B, respectively, in the present study. They were close to the result of 43.88 kJ/mol that Baéz et al. [10] studied in a powdered enteral formula, while far lower than those previously reported by our team, that the activation energies of vitamin A were 122.94 kJ/mol and 125.29 kJ/mol in two different powdered enteral formulas [38]. Also, Albalá-Hurtado et al. [25] studied the activation energy of vitamin A, which was 92.05 kJ/mol in liquid infant milks. Juan Francisco et al. [11] found activation energy in growing-up milk at 36.4 kJ/mol. The range of associated activation energies for vitamin B_1 was between 33 and 124 kJ/mol, and the temperature-dependence of the rate constant could be represented using the Arrhenius equation [20,22,30]. In the current study, the activation energies of vitamin B_1 were 28.34 kJ/mol for EFF-A and 28.52 kJ/mol for EFF-B, respectively. The results were significantly lower than previous papers, which was presumably due to the difference in food matrices. The activation energies for vitamin B_1 were 123.48 kJ/mol and 105.98 kJ/mol in two different powdered enteral formulas, as reported by our team in a previous paper [38]. Moreover, the R^2 values of vitamin B_1 determined by the Arrhenius calculations had far lower correlations (0.52–0.57) than vitamins A and C in this study. This was mainly because vitamin B_1 had a similar degradation rate when both EFFs were kept at the same temperature for 9 months of storage. In addition, the activation energies for vitamin C were 39.38 kJ/mol for EFF-A and 57.13 kJ/mol for EFF-B, respectively. The range of vitamin C activation energies in this study is consistent with previous studies. Jiang et al. [13] found that the value of activation energy for vitamin C was 74.67 kJ/mol in milk formula. Baéz et al. [10] found that the value of activation energy was 51.96 kJ/mol in powdered enteral formula. In this present work, the EFF-B had a higher activation energy of vitamin degradation than that in the EFF-A during storage; reactions with high activation energy are more temperature sensitive [39], and the activation energy indicated an inverse relationship with the fat content of EFFs.

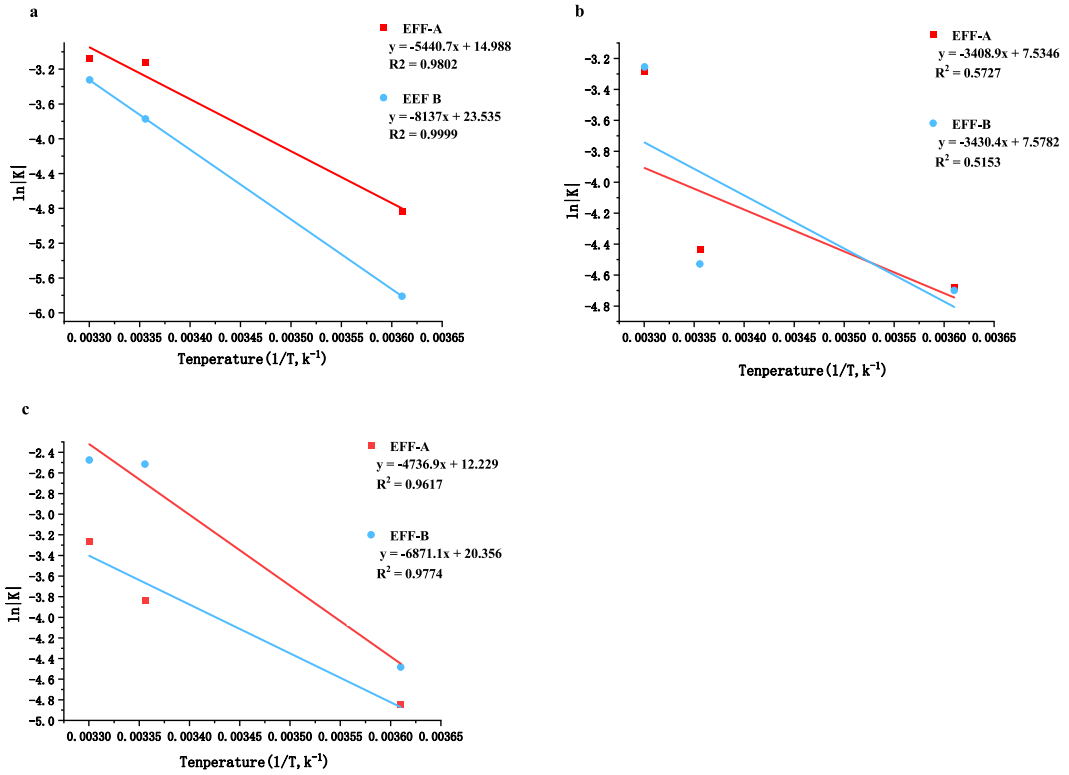

Fig. 2. Arrhenius plots for degradation of vitamins A (Fig. 2a), B1 (Fig. 2b) and C (Fig. 2c) in two different enteral feeding formulas (EFF-A and EFF-B). k: Rate constant for vitamin degradation in two different enteral feeding formulas, T: Storage temperature (K).

4. Conclusion

The two kinds of enteral feeding formulas with different energy densities were kept under different storage conditions up to 9 months. The results justified the finding that the content of vitamins was progressively decreased with storage time or temperature increases during the period. Vitamin C degradation was higher than that of the other studied vitamins during storage. Under the same conditions, EFF-A degraded less than EFF-B in terms of vitamins B_1 and C, while EFF-B degraded more in terms of vitamin A. The degradation of vitamins A, B_1, and C followed first-order kinetics at each temperature during storage. The rate constants calculated indicated that the degradation of vitamins was temperature-dependent. The EFF-B had a higher activation energy for vitamin degradation than that in the EFF-A, and the activation energy indicated an inverse relationship with the fat content of EFFs. The stability of vitamins in other types of EFFs still requires further study. If there is enough available information on the degradation of vitamins in different EFFs during storage, it could provide a reference for the development and application of EEFs.

Ethics statement

No ethical approval is needed for this review article.

Data availability

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Hong Yang: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ling Hou:** Writing – original draft, Software, Formal analysis, Data curation. **HongMei Sun:** Writing – original draft, Software, Formal analysis, Data curation. **ShuHong Ye:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29653.

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