IZVORNI ZNANSTVENI RAD / ORIGINAL SCIENTIFIC PAPER

Stability of water-soluble vitamins in formulations of liquid enteral nutrition

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Summary

Enteral formulas are special foods formulated to provide sufficient amounts of energy, macro- and micronutrients to meet the complete or supplementary nutritional needs of patients who cannot consume enough food to meet the body's daily energy and nutrient requirements. As part of our project, which aims to provide personalised nutritional solutions for people with serious health problems such as cancer and diabetes, three formulations of enteral nutrition containing proteins, fats, carbohydrates and fibre as well as essential vitamins and minerals were produced. The aim of this study was to investigate the effects of storage temperature and time on the chemical changes and degradation of water-soluble vitamins. The vitamin content was analysed in freshly prepared samples, three months after storage in temperature chambers at 37°C and after six months of storage at room temperature of 23°C. The results show a decrease in vitamin content, but different stability of the individual vitamins was observed depending on storage time and storage temperature. Generally, formulas showed a decline in the concentration of unstable vitamins C, B_1, and B_5, but also B_2, B_6, and B_7, which are considered more stable. The results are correlated with components and macronutrient composition of the formulas, changes in rheological properties, and thermal instability. This study emphasises the importance of carefully selecting the ingredients of the formula, especially the protein, fat and carbohydrate sources, as various interactions between the components can affect the vitamin content.

Sažetak

Enteralne formulacije su posebna hrana oblikovana da osigura dovoljne količine energije, makro- i mikronutrijenata za potpunu ili djelomičnu prehranu pacijenata koji ne mogu konzumirati dovoljno hrane za zadovoljenje dnevnih potreba organizma. U sklopu projekta, čiji je cilj pružiti personalizirana prehrambena rješenja za osobe s ozbiljnim zdravstvenim problemima poput raka i dijabetesa, pripravljene su tri formulacije enteralne prehrane koje sadrže proteine, masti, ugljikohidrate i vlakna te esencijalne vitamine i minerale. Cilj ovog istraživanja bio je istražiti učinke temperature i vremena skladištenja na kemijske promjene i razgradnju vitamina topivih u vodi. Sadržaj vitamina je analiziran u svježe pripremljenim uzorcima, tri mjeseca nakon čuvanja u temperaturnim komorama na 37°C i nakon šest mjeseci čuvanja na sobnoj temperaturi od 23°C. Rezultati pokazuju smanjenje sadržaja vitamina, ali uočena je različita stabilnost pojedinačnih vitamina ovisno o vremenu skladištenja i temperaturi skladištenja. Općenito, formulacije su pokazale pad koncentracije nestabilnih vitamina C, B_1 i B_5, ali i B_2, B_6 i B_7, koji se smatraju stabilnijima. Rezultati su razmatrani u kontekstu sastava makronutrijenata u formulacijama, uočenih promjena u reološkim svojstvima i temperaturne nestabilnosti pojedinih vitamina. Ova studija naglašava važnost pažljivog odabira sastojaka formulacija, posebice izvora proteina, masti i ugljikohidrata, jer različite interakcije između komponenata mogu utjecati na sadržaj vitamina.

Keywords: *enteral nutrition, malnutrition, water-soluble vitamins, vitamin stability*

Introduction

Enteral nutrition delivers essential macro- and micronutrients to individuals unable to meet their nutritional needs through oral intake. It is often required for conditions that affect swallowing, such as stroke, amyotrophic lateral sclerosis and Parkinson's disease, as well as in situations where swallowing is impaired due to mechanical ventilation or altered mental status. In addition, enteral nutrition is indispensable support in conditions with reduced food intake and increased nutritial requirements, for example in geriatric patients, and many chronic diseases like cancer, diabetes, renal, celiac and Crohn's disease. However, statistics show that 20-50% of patients admitted to hospital are affected by malnutrition caused by inadequate and untimely nutritional therapy (Cas and Charlton, 2022). This percentage increases during hospitalisation, leaving an estimated 33 million people at risk of malnutrition in Europe. Numerous studies have shown that timely and adequate nutritional therapy helps to reduce the number of hospital days and repeat hospitalisations, thus reducing healthcare costs.

The use of enteral nutrition for hospitalised, critically ill patients and patients receiving enteral nutrition at home has increased dramatically in recent decades (Yang et al., 2022). Enteral formulas belong to the group known as "food for special medical purposes". These are special foods formulated to provide adequate amounts of energy (calories), proteins, fatty acids, carbohydrates, minerals, and water- and fat-soluble vitamins to provide complete or supplemental nutrition to patients who, due to illness or inability to eat, cannot consume enough food to meet the body's daily energy and nutrient requirements (Aguilar-Nascimento and Kudsk, 2008; Reis et al., 2018; Delompré et al., 2019).

In the preparation of enteral nutrition, nutrients are added to adapt the formula to the patient's needs and to compensate for losses in the processing of these products. The preparation of enteral nutrition involves several steps, including sterilisation and treatments, which can lead to various changes in the ingredients and bioavailability of the nutrients (García-Baños et al., 2005). In addition, formulas are often exposed to different storage conditions throughout the supply chain before consumption, which alters the composition (Frias and Vidal-

Valverde, 2001).

Vitamins, including water-soluble vitamins, are affected by various factors such as the type of packaging, storage time and storage conditions (e.g. oxygen, light and high temperatures). Vitamin C is sensitive to heat, oxygen and light (Guerra-Hernandez et al., 2002), but Baez et al. (2012) showed good stability of vitamin C in powdered enteral nutrition samples during storage when the temperature remains below 30°C. Fluctuations in pH and the presence of reducing agents for thiamine (vitamin B_1) have been shown to be factors contributing to loss of stability (Allwood and Kearney, 1998). Vitamin B_1 is stable below a pH of 5.5 but can be rapidly destroyed at a pH above 7.0 regardless of temperature (Yang et al., 2022). Frias and Vidal-Valverde (2001) analysed five commercial enteral nutrition formulas from a local pharmacy and reported that thiamine (vitamin B_1) was stable during 6 months of storage, especially at a temperature below 20°C. Ribeiro et al. (2011) reported that vitamins B_1, roboflavin (B_2) and pyridoxine (B_6) added to a neonatal parenteral nutrition containing a high concentration of calcium associated with organic phosphorus in the presence of oligoelements could be considered stable when stored between 4°C and 25°C for three days. In this study, the vitamin C showed a stability of 48 hours at 25°C, with or without light protection, so the authors suggest a short shelf life for the formulation under refrigeration. Albala-Hurtado et al. (2000) investigated the stability of vitamins in liquid and powdered form of infant milk and concluded, among other things, that the concentration of B-complex vitamins in infant milk does not change during storage at any temperature. In the study by Yang et al. (2022) the authors investigated the stability of vitamins A, E, C and thiamine during the storage of various powdered enteral formulations. For thiamine, they concluded that its content gradually decreased with increasing temperature or storage duration, while the vitamin C content remained stable.

As a part of our project aiming to deliver personalised nutritional solutions for people facing major health challenges, like cancer and diabetes, different formulations with innovative combinations of dietary fibres were prepared. The aim of this study was to investigate the effects of storage temperature and time on the chemical changes and degradation of water-soluble vitamins. Understanding these changes would make it possible to create models to estimate the stability of the product under different storage conditions (Penava et al., 2023).

Materials and methods

Formula preparation

The enteral nutrition samples were prepared in the following steps: dissolving and mixing the ingredients in water, followed by homogenisation, thermal treatment in an ultra-high temperature (UHT) process, cooling and aseptic filling into multilayer packaging (a combination of plastic, cardboard and aluminium layers) in a pilot-scale laboratory. All three formulas (F1, F2 and F3) are complete foods that contain all macronutrients (proteins, fats, carbohydrates and fibre) as well as essential vitamins and minerals. Basic composition of formulas and their macronutrient composition are given in **Table 1** and **Table 2**, respectively.

Standards and chemicals

All water-soluble vitamin standards, ascorbic acid (C), thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine hydrochloride (B_6), biotin (B_7), folic acid (B_9) and cyanocobalamin (B_{12}) were purchased from Sigma-Aldrich (St. Louise, USA). Acetonitrile and ethanol for sample preparation and mobile phase (HPLC grade) were purchased from J. T. Baker (Centre Valley, USA). High purity water was purchased from an in-house MilliQ system (Millipore, Bedford, MA, USA). Trifluoroacetic acid (TFA) was purchased from Carlo Erba Reagents (Emmendingen, Germany). The other reagents used were of analytical grade.

Storage conditions

Three model liquid enteral formulas (F1, F2 and F3) with different nutrient composition, were prepared to perform vitamin analyses and storage trials under different storage conditions. The vitamin content was analysed in freshly prepared samples after packaging at the beginning of the storage period, three months after storage of the packaging in temperature chambers at 37°C and also after six months of storage at room temperature of 23°C.

Analytical methodologies

The samples of liquid enteral nutrition for vitamin analysis were analysed using a LCMS-2020 liquid chromatograph coupled to a single quadrupole mass spectrometer with electrospray ionisation (ESI) source (Shimadzu, Kyoto, Japan). Analyte separation was performed on a reversed-phase C18 column (Waters Atlantis™ dC18, 4.6 x 250 mm, 5 µm) thermostated at 25°C. The temperature of the desolvation gas was 250°C with a flow rate of 1.5 L/min. Capillary voltage was 3.5 kV. The mobile phase was 0.1% TFA and acetonitrile with gradient elution and the flow rate of the mobile phase was 1.4 mL/min. Signal response is monitored in SIM (Single Ion Monitoring) mode (**Table 3**).

Sample preparation

The stock solution of each vitamin was prepared as a 1 or 10 mg/mL solution in Mili-Q water and stored at -20°C in amber-coloured storage vials. The working mixture of water-soluble vitamins was prepared from the stock solutions as 1 mg/mL and 100 µg/mL in 0.1 M TFA and stored at 4°C until use, as were other necessary dilutions.

Among the different solvent mixtures, the best results for the extraction of all water-soluble vitamins from liquid enteral nutrition were obtained in an extraction protocol with a mixture of acetonitrile and ethanol

Table 1. *Basic composition of formulas.*

Formula	F1	F2	F3
Protein source	milk proteins, vegetable proteins	milk proteins	milk proteins
Fat source	vegetable oils	vegetable oils	vegetable oils, fish oil
Carbohydrate source	maltodextrins, sugars, starch	maltodextrins, sugars	maltodextrins, sugars
Added antioxidants[a]	yes	-	-
Added amino acids[b]	-	yes	-

[a] *Resveratrol and grape seed extract*

[b] *Amino acid mixture consisting of Leu, Val, Ile, Tyr, Phe, His, Trp, Thr and Lys*

Table 2. *Macronutrient composition data (per 100 mL of formula).*

Formulations	F1	F2	F3
Energy /kcal	150	150	150
Fat/g	7.5	5.6	5.4
Carbohydrates/g	14.2	16.2	14.6
Fibre/g	2.1	1.4	1.5
Proteins/g	7.6	10	10
Salt/g	0.19	0.16	0.24

followed by protein precipitation. 10 g of the liquid formulation sample was weighed into a 100 mL Erlenmeyer flask and 20 mL of the 1:3 ethanol/acetonitrile mixture was added. The respective sample was placed on a shaker for 30 minutes and on a magnetic stirrer (New Brunswick Scientific, Edison, New Jersey, USA) for 15 minutes. After mixing, the samples were centrifuged (Thermo Electron Corporation, Waltham, Massachusetts, USA) for 10 minutes, the supernatants were separated and the precipitates were washed with 20 mL of the ACN: MiliQ water mixture at a ratio of 70:30. The samples were mixed again on a magnetic stirrer for 15 minutes and centrifuged for 10 minutes. The supernatants were combined and a 20 mL aliquot was removed and evaporated to dryness on a rotary evaporator (Genevac SP Scientific, Warminster, Pennsylvania, USA). The precipitate was reconstituted in 1 mL of 0.1 M TFA and the mixture was filtered through a 0.45 µm filter. a 200 uL aliquot was transferred to an amber vial and further diluted with 102 µL of 0.1 M TFA (unspiked sample). The vitamin concentration in the samples was determined by the standard addition method by spiking one sample with the mixture of known vitamin concentration. The spiked sample was prepared by adding 102 µL of the spiking solution to the sample (200 µL) in which the mass concentration of vitamins was: C 437.09 µg/mL, B_1 4.97 µg/mL, B_2 4.30 µg/mL, B_3 3.31 µg/mL, B_5 16.56 µg/mL, B_6 3.31 µg/mL, B_7 2.65 µg/mL and B_9 1.66 µg/mL. 10 µL of each sample was injected into the device.

To check the linearity in the measurement range, a vitamin mixture was prepared in 0.1 M TFA, as C 1900 µg/mL, B_1 19 µg/mL, B_2 24 µg/mL, B_3 275 µg/mL, B_5 100 µg/mL, B_6 41 µg/mL, B_7 0.73 µg/mL and B_9 6 µg/mL and then diluted 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 times in 0.1 M TFA. 10 µL of each calibration solution was injected into the device.

Results and discussion

The development of a single method for the simultaneous determination of all water-soluble vitamins in fortified enteral nutrition is difficult due to the different structures and chemical properties of the vitamin compounds, the trace amounts of vitamins present, the complexity of the matrix, light instability and heat and solubility problems. In this study, different solvents and their mixtures were tested for the extraction of vitamins from enteral nutrition. The extraction of vitamins from a liquid food sample was performed with solid trichloroacetic acid (TCA), propanol, ethanol and acetonitrile. The best signal responses and recovery results were obtained with a mixture of acetonitrile and ethanol, followed by protein precipitation. In addition, a large amount of solvent was required to precipitate the proteins. Acetonitrile granulates the sample during processing, and after centrifugation three layers were present, making it difficult to analyse the sample. It was therefore necessary to select a solvent mixture that would ensure good sample processing, good analytical recovery and good signal behaviour of the compounds. Experiments were carried out with different solvent mixtures of ethanol/acetonitrile (10:90, 90:10, 25:75, 75:25 and 50:50) and monitored using UV chromatograms by observing the total response of the extracted sample at 254 nm. The best signal reactions and the best reproducibility of the extraction were achieved by extracting the liquid food sample in a solvent mixture of ethanol:acetonitrile in a ratio of 25:75.

The efficiency of the extraction was tested with different sample mixing times (30 minutes, 60 minutes, 3 hours, 24 hours). The signal responses of the compounds and the analytical recoveries were largely the same for all mixing times, with the only exception that the signal area for vitamin C decreased with time. For this reason, the sample mixing time was reduced to 30 minutes. After precipitation, the supernatant was evaporated and reconstituted in 0.1 M TFA. In addition, TFA caused the formation of ion pairs (Cai and Li, 1999). It was found that mixing TFA increased the retention time of polar analytes, possibly through the formation of ion pairs with the amino groups of the molecules. On the Waters Atlantis column, polar analytes were poorly retained without the use of an ion-pairing reagent.

A range of chromatographic columns and gradients were tested to achieve the best separation of the analytes. In addition to other columns (Zorbax, Kinetex) and chromatographic conditions (different run times and compositions of the mobile phases), the separation of the analytes was performed on a reverse C18 column (Waters Atlantis™ dC18, 4.6 x 250 mm, 5 µm), which was tempered at 25°C with a gradient elution for 45 minutes with 0.1% TFA in acetonitrile as mobile phases. Calibration curves were generated to check the linearity in the measurement range (**Figure 1)**, which resulted in a satisfactory correlation coefficient R^2 0.9921-0.9997. The concentration of vitamin B_{12} found the samples was too low to be detected by the SIM method, therefore the calibration curve for vitamin B_{12} was not generated. **Figure 2** shows SIM chromatograms of eight water-soluble vitamins that were contained in an enteral food sample in the study.

Stability tests were conducted on three different types of liquid formulas prepared in the pilot-scale laboratory (F1, F2, and F3). The samples kept in multi-layer opaque packaging (a combination of plastic, cardboard

Table 3. *LC/MS parameters for identification of water-soluble vitamins.*

LC parameters		MS parameters		Retention time/min
t / min	**eluent B / %**	**vitamin/MS ionization**	**m/z**	
0.01	0	B_3/ESI+	124	4.3
13.50	3	B_5/ESI+	220	15.7
15.00	15	B_6/ESI+	170	7.4
22.00	20	B_7/ESI+	245	21.0
25.00	100	B_1/ESI+	265	5.7
40.00	100	B_2/ESI+	377	21.7
40.01	0	B_9/ESI+	442	19.5
45.10	0	C/ESI-	175	3.0
		B_{12}/ESI+	1357	23.6

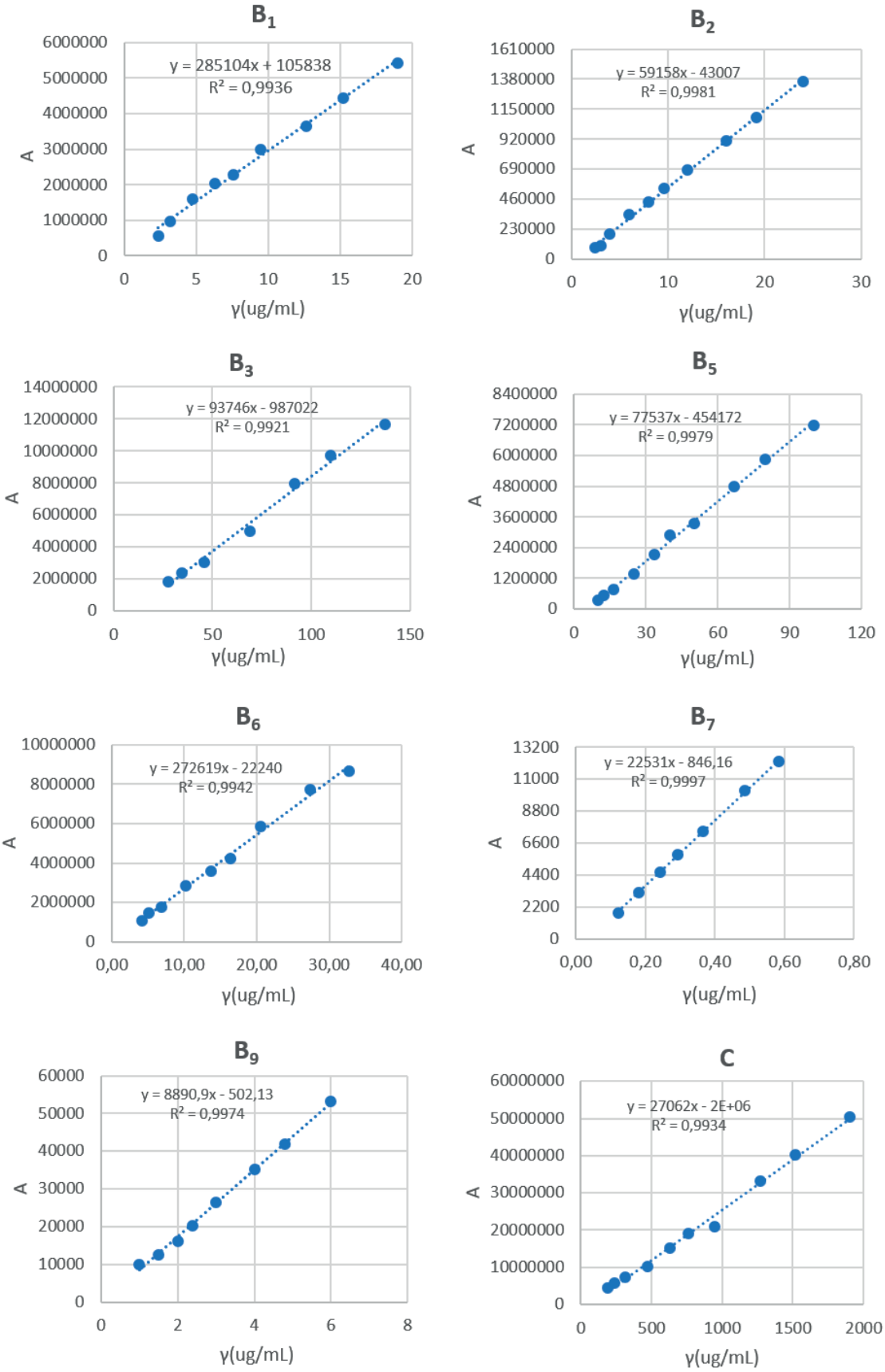

Figure 1. *Calibration curves of water-soluble vitamins.*

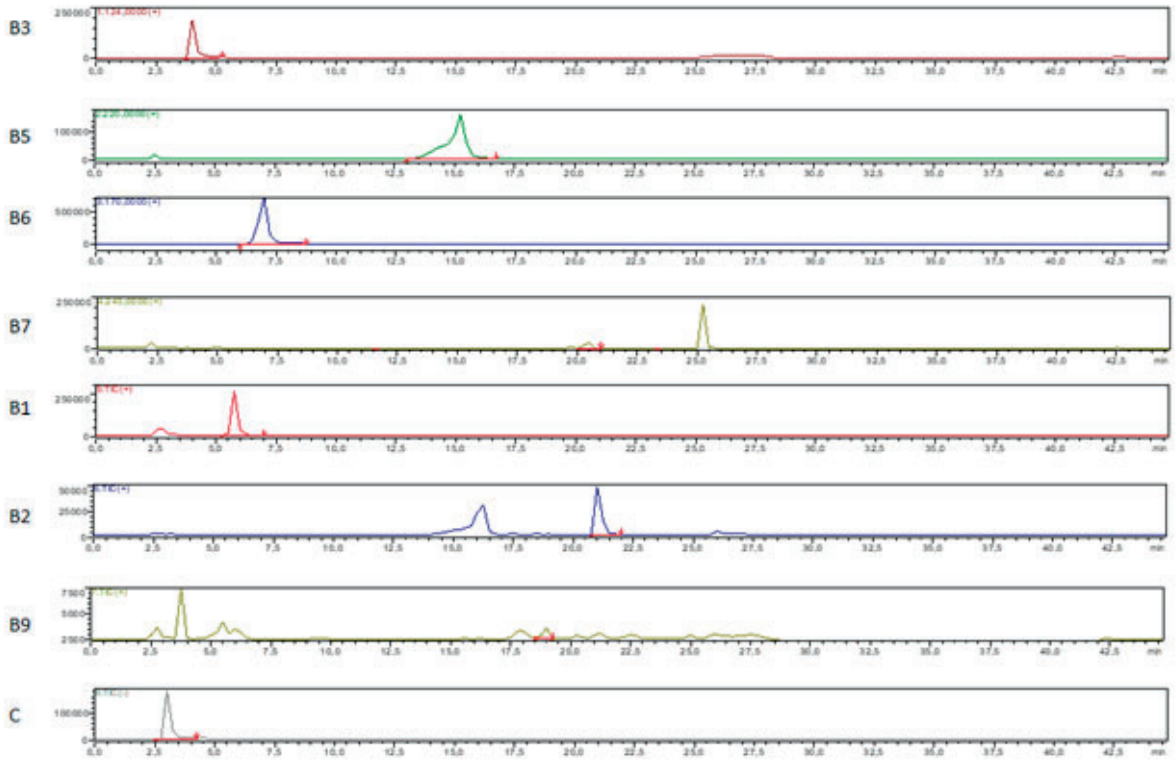

Figure 2. *SIM chromatograms of water-soluble vitamins in enteral food samples.*

and aluminium layers) were analysed immediately after preparation and the initial concentrations of water-soluble vitamins are given in **Table 4**. Vitamins were also analysed at the end of storage period of three months at 37°C and six months at 23°C. Samples stored at 37°C have a visibly more viscous texture compared to those stored at room temperature. In addition to the change in viscosity of the samples, there was also a change in colour, with the samples stored at 37°C being darker (**Figure 3**). The observed changes in the samples can be explained by biochemical and physical changes in the sample content. Over time, proteins in the formula may partially denature, especially at higher temperatures, and then aggregate to form larger complexes (Li and Zhu, 2025). Enteral formulas containing starch or soluble fibres can absorb water and swell. All these processes can increase the viscosity of the sample. Additionally, in formulas containing both proteins and reducing sugars, the Maillard reaction can occur slowly during storage, particularly at higher temperatures. This not only causes browning but also cross-links molecules, further increasing viscosity (El Hosry et al., 2025). The concentration of vitamins was determined using the standard addition method by adding the mixture of known vitamin concentration to a sample.

All three formulations showed a decrease in vitamin content, but different stability of the individual vitamins was observed depending on storage time and storage temperature (**Table 4, Figure 4 and Figure 5**). Vitamin C is generally very sensitive to light and heat due to complex oxidation and intermolecular rearrangement reactions (Yin et al., 2022; Ameye et al., 2025). Its decline in all formulae at both temperatures was not surprising, although the degradation in F1 was by far more extensive; after 3 months at 37°C only 14% of vitamin C was still present and after 6 months at 23°C 1%. As for the B vitamins, the lowest stability in all three formulas after 3 months at 37°C was observed for vitamins B_2 and B_6 (**Table 4, Figure 4**). B_1 showed the best stability in F3, B_3 in F1 and F2, while B_9 in F1 and F3. After a storage period of 6 months at 23°C, a strong degradation of B_2, B_6 and B_7 was observed, with B_1 being most stable in F3, B_3 in F1 and F2 and B_9 in F1 and F3 (**Table 4, Figure 5**). Ameye et al. (2025) reported a comprehensive study on the effects of physical state, temperature, pH, protective atmosphere, protein hydrolysis, fats, fibre, packaging, and flavour on nutrient degradation by analysing the results of shelf-life studies of 1400 recipes of food for special medical purposes. The study highlights that liquid format, temperature, and pH are primary drivers of vitamin degradation. Thus, vitamins C, B_1, B_5, and B_9 are among the most vulnerable under typical storage conditions, especially in liquid, acidified, high-temperature settings, while B_2, B_3, B_6, and B_7 are much more stable. Acidification of food can protect vitamin B_1 from degradation, as found in fruit products and energy drinks (Voelker et al., 2018). However, when pH is closer to neutral, as in our formulas, or slightly alkaline, greater loss of vitamin B_1 was observed. At this pH, the free base of vitamin B_1 is protonated, making it more susceptible to hydrolysis and cleavage to produce 4-methyl-5-hydroxyethyl thiazole and 2-methyl-4-amino-5-hydroxymethyl pyrimidine (Pachapurkar and Bell, 2005). The lower stability of vitamin B_5 can be attributed to the presence of an amide bond, which undergoes slow hydrolysis in liquid formulas at elevated temperatures, while vitamin B_9 is prone to oxidation and thermal degradation, for example during UHT treatment. As a general trend, our studied formulas showed a decline in the concentration of unstable vitamins C, B_1, and B_5, but also B_2, B_6, and B_7, which are considered

Table 4. *Initial concentrations (mg/100 mL) of water-soluble vitamins in formulas **F1**, **F2** and **F3**.*

	C	B_1	B_2	B_3	B_5	B_6	B_7	B_9
F1	27.63	0.35	0.09	0.76	0.77	1.82	0.022	0.031
F2	19.73	0.11	0.58	0.81	0.56	0.40	0.040	nd
F3	13.86	0.10	0.15	1.26	0.752	0.77	nd	0.042

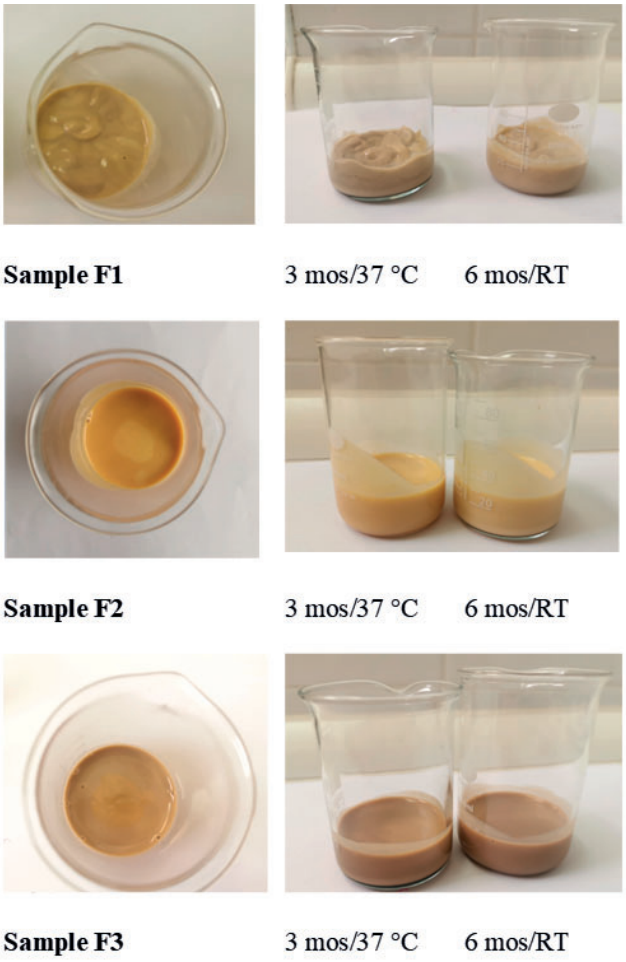

Figure 3. Samples F1, F2 and F3 at the beginning of the stability test (left) and after the storage period.

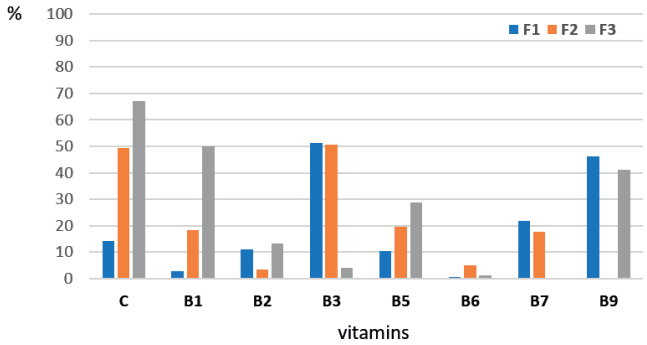

Figure 4. Stability of vitamins in enteral food samples F1, F2 and F3 over a storage period of 3 months at 37 °C.

more stable. It was, however, reported that hydrolysed proteins or amino acids can influence the stability of certain vitamins, possibly *via* interactions, binding, or catalytic degradation.

To explain the results obtained, we turned to the differences in the components and macronutrient composition of the formulas (**Table 2**). Despite similar calorie values, F1 contains less protein and more fat than F2 and F3. F1 also has a slightly higher fibre content than the other two. Formula F2 contains only soluble fibre, while the other two also contain a small amount of insoluble fibre. In addition to the complex carbohydrates contained in all three formulations, F1 also contains starch components. Starch is a plant energy source consisting mainly of amylose and amylopectin (Ledezma, 2018). Amylose tends to form left-handed single helices with six residues per turn, whereas

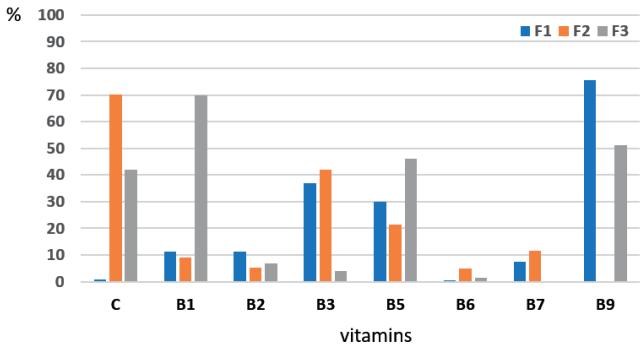

Figure 5. Stability of vitamins in enteral food samples F1, F2 and F3 over a storage period of 6 months at 23 °C.

amylopectin is organised in clusters as packed double helices. Starch is known to form inclusion complexes with ligands, mainly alcohols, ketones, esters, aldehydes and acids, through non-covalent bonds and has a strong tendency to form supramolecular structures (Goubet et al., 1998; Tan and Kong, 2019). The question arises as to whether the low concentration of certain vitamins, contained in F1, is due to degradation or entrapment in the starch matrix, making extraction from liquid enteral nutrition less efficient. The same applies to other vitamins containing polyhydroxy and carboxylic acids. Starch-based delivery systems are used for the controlled release of various food additives (Tian et al., 2022) and are capable of preserving vitamin C during a long storage period (Borrmanna et al., 2013). Therefore, it is possible that modification of extraction protocol is required to release trapped vitamins when starch is used as a carbohydrate source in complex liquid enteral nutrition.

Next, formula F3 contains fish oil as well as vegetable oil. Marine oils are a rich source of omega-3 fatty acids, which are among the most popular dietary supplements due to their potential metabolic benefits and promise in preventing cardiovascular and inflammatory diseases (Calder 2004; Calder 2013). In contrast to plant sources of omega-3 fatty acids, marine oils contain the long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are highly susceptible to oxidation during storage due to their large number of double bonds and their position within the fatty acid chain (Albert et al., 2013). In the presence of various initiators, light, temperature and oxygen, a lipid radical is formed and a reaction chain is set in motion that generates lipid peroxides and other radicals. Peroxidation can impair the bioactivity of omega-3 fatty acids, but can be reduced by the addition of antioxidants (e.g. vitamin E). However, radicals are highly reactive chemical species and, once formed, can be harmful to various dietary components, including proteins and vitamins, especially B_1, B_2 and B_6 (Liu et al., 2025). In addition, the source of minerals can also affect the stability of vitamins. Studies have shown that vitamins C, B_2 and B_6 are significantly lost at 37°C and 20°C in the presence of metal sulphates, but not amino acid chelates as a mineral source (Marchetti et al., 2000). Three commercially available mineral mixtures were used for three formulas (supplementary materials), but all contain a combination of organic and inorganic salts, making it difficult to draw conclusions about the possible interaction of minerals and the vitamins analysed. Finally, apart from matrix interactions, we must consider the thermal instability of many vitamins and the degradation that can begin during UHT treatment.

Conclusions

Eight water-soluble vitamins in enteral food samples were analysed and quantified using the HPLC-MS method. The stability of the vitamins in the samples depends on the storage conditions, the storage time and the storage temperatures. During the storage period, a visibly more viscous texture was observed in the samples stored at 37°C compared to the samples stored at room temperature. The results show that vitamin concentration decreases with time, but the observed changes in rheological properties, especially viscosity, affect the effective analysis of vitamins in liquid enteral nutrition samples. In addition, differences in the stability of certain vitamins were correlated with changes in the composition of the three formulas.

The results of this study emphasise the importance of carefully selecting the ingredients of the formulation, especially the protein, fat and carbohydrate sources. The interactions between the components are still poorly understood, but can significantly influence the vitamin profile. These results can serve as a basis for further adjustments to the formulation in terms of vitamin content, the amount of excess vitamin added and modification of the production process. They also help to assess the impact on other changes that occur in the samples over time (e.g. changes in rheological properties) to ensure that an appropriate vitamin content is maintained throughout the shelf life of the product.

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