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# Effect of heat treatment on vitamin content during the manufacture of food products at industrial scale

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Received: December 1st, 2021. Received in revised form: June 16th, 2022. Accepted: August 16th, 2022.

**Abstract**
The molecular structure of vitamins makes them easily degradable under various conditions such as temperature, pressure, and pH. It is necessary to understand and determine the stability of vitamins during the processing of food products to ensure their presence at the time of consumption. In this study, the degradation of vitamins added was assessed before and after heat treatments during the manufacturing of food products. Vitamins of interest were quantified by HPLC. The results obtained allowed to establish that prolonged heating of the product caused water-soluble vitamins degradation. The greatest degradation was observed for vitamin B12, decreasing by 94% in a baby porridge product after pasteurizing at 85 °C for 15 s. In contrast, vitamin B1 showed the lowest reduction in a chocolate milk, decreasing by 4% after ultra-high-temperature (UHT) treatment at 139 °C for 5s.

*Keywords*: degradation; vitamins; heating; industrial scale.

# Efecto del tratamiento térmico sobre la concentración de vitaminas durante la fabricación de productos alimenticios a escala industrial

**Resumen**
La estructura molecular de las vitaminas las hace fácilmente degradables bajo diversos factores como temperatura, presión y pH. Es necesario determinar la estabilidad de las vitaminas durante la elaboración de productos alimenticios para garantizar su presencia al momento de consumo. Este estudio evaluó la degradación de vitaminas adicionadas en productos alimenticios antes y después de los tratamientos térmicos durante su fabricación a nivel industrial. Las vitaminas de interés fueron cuantificadas por HPLC. Los resultados obtenidos permitieron establecer que tiempos prolongados de calentamiento causaron una degradación drástica de vitaminas hidrosolubles. La mayor degradación se observó en la vitamina B12, decreciendo un 94% en un producto tipo papilla luego de pasteurizar a 85°C por 15 s. Por el contrario, la vitamina B1 presentó la menor reducción en una leche achocolatada decreciendo un 4% durante el proceso de ultra pasteurización (UHT) 139°C por 5 s.

*Palabras clave*: degradación; vitaminas; calentamiento; escala industrial.

## 1 Introduction

Vitamins are micronutrients required for proper body function (milligrams to micrograms per day). The lack of vitamins in the body can trigger health problems; therefore, they should be incorporated into a balanced diet. Vitamins are essential and not synthesised from biochemical reactions in the body, except for vitamin D. They are classified into two categories according to their solubility: (1) fat-soluble (Vitamins A, D, E, and K); and (2) water-soluble (Vitamin B complex and Vitamin C) [1].

These molecules are highly labile, been affected to a greater or lesser extent by a variety of factors such as temperature, light, oxygen, pH, reducing agents, oxidising agents, metal ions, etc. [2]. In the 80s, several studies were published describing these stability problems. It was concluded that vitamin C undergoes oxidation and copper catalyses this reaction. Thiamine is degraded by a reduction process caused by sodium metabisulphite—used as an antioxidant agent in many commercial amino acid solutions—and exposure to light [3]. In food processing, heat treatments and mechanical agitation generate lipid self-oxidation and photo-oxidation that degrade fat-soluble vitamins, and water-soluble vitamins are affected by leaching during heat treatments [1].

**How to cite:** Herrera-Ardila, Y.M., Orrego, D., Bejarano-López, A.F. and Klotz-Ceberio, B., Effect of heat treatment on vitamin content during the manufacture of food products at industrial scale. DYNA, 89(223), pp. 127-132, July - September, 2022.


Revista DYNA, 89(223), pp. 127-132, July - September, 2022, ISSN 0012-7353
DOI: https://doi.org/10.15446/dyna.v89n223.99775

Heat treatments are widely used in the food industry as a strategy for microbial control, looking to extend the shelf life of the products. However, extensive research has been done to evaluate the effect of these treatments on the stability of vitamins in dairy and non-dairy products. For instance, Bezie [4] reported losses of 20% of vitamin A after pasteurization and evaporation of condensed milk, but no reduction of vitamin E was observed after these two processes. Additionally, a 90% reduction of vitamin B12 in milk, after sterilization and evaporation, was also mentioned. In meat matrices like pork, Riccio et al. [5], found a strong decrease of B vitamins: 75% for thiamine, 42% for riboflavin and 35% for niacin. Vitamin content change in different matrices subjected to traditional heat treatments like water cooking, boiling, pressure cooking was reported by Lešková et al [6], showing that fat-soluble vitamins like A, D, E, K can be affected by these treatments, with reductions between 20% to 60% in different vegetable and meat products. Similarly, degradation of water-soluble vitamins was reported in meat and vegetable as well, with losses above 90% for labile vitamins like C and B1, after conventional heat treatments.

Due to the chemical instability of most vitamins, one the biggest challenges for the food industry consists of reducing vitamins losses in processed food. Recently, consumers have developed a special interest on the nutritional quality of food. For this reason, measures were taken to promote the intake of vitamins in the diet through vitamin supplements and/or milk-based products, such as infant formulas, adult formulas, milk, yogurt, cheese, and fruit extracts [7].

Considering the importance of understanding the behaviour of vitamins during thermal processing at industrial scale, the present study aims to determine the behaviour of the vitamins added to four representative products from the portfolio of Alpina Productos Alimenticios S.A. BIC, including dairy and non-dairy products. Sampling points were specifically selected after heat treatments, including pasteurization (78-90 °C, 15 s), ultra-high-temperature (UHT) treatment (139 ºC, 5 s) and sterilization (125 °C, 20 min), for each product in an industrial set-up.

## 2 Materials and methods

### *2.1 Reagents*

The following reagents were used in this study: acetonitrile 99%, saturated NaCl solution, 99% ethanol, and 98% diethyl ether (Merck®); di-hydrogenated potassium phosphate 96%, 38% hydrochloric acid, sodium cyanide 99%, 85% orthophosphoric acid, and 99.5% acetone (PanReac®); 99% glacial acetic acid, methanol 99%, and KOH 98% (Sharlau®). The vitamins assessed were: B6; B9; B12; C; B1; B2; and B3 99% (Dr. Ehrenstorfer GmnH®).

### *2.2 Products and sampling*

Four representative dairy and non-dairy products with added vitamins were selected from the portfolio of Alpina Productos Alimenticios S.A. BIC: chocolate milk (Alpin), petit-suisse cheese (Alpinito), fruit beverage (Fruper) and baby porridge (Papilla Baby). Table 1 summarises the vitamins added to each product.

Table 1.
Vitamins added to the products selected for the study.

| Alpin | Alpinito | Fruper | Papilla Baby |
|---|---|---|---|
| Nicotinamide (B3) | Folic acid (B9) | Folic acid (B9) | Folic acid (B9) |
| Riboflavin (B2) | Cyanocobalamin (B12) | Cyanocobalamin (B12) | Cyanocobalamin (B12) |
| Thiamine (B1) Retinol (A) | Cholecalciferol (D3) | Ascorbic acid (C) | Pyridoxine (B6) |

Source: The authors

Table 2.
Amount of vitamins added during the manufacture of each product (g/ kg of product).

| Vitamin | Alpin | Alpinito | Fruper | Papilla Baby |
|---|---|---|---|---|
| **B9** | NA | 1.2 | 0.25 | 0.13 |
| **B12** | NA | 16.7 | 4.02 | 0.82 |
| **C** | NA | NA | 340 | NA |
| **ALP-UHT** | 100 | NA | NA | NA |
| **B6** | NA | NA | NA | 4.11 |
| **D3** | NA | 11.1 | NA | NA |

*Note.* NA = not added; ALP-UHT = Mixture of vitamins and sugars
Source: The authors

Table 2 illustrates the amounts of vitamins added at the beginning of the mixing process to each product. For Alpin, vitamin mix ALP-UHT consist of vitamin A (32.4%), vitamin B1 (2.08%), vitamin B2 (2.67%), vitamin B3 (31.41%), and sugar (31,45%) as dispersant material.

Samples for vitamin quantification were taken after heat treatments that can affect their content in final products. Fig. 1 shows each process for the products assessed and the selected manufacturing stages where samples were taken. Those samples were taken by triplicate in three different production batches and processed by extracting and quantifying each vitamin added from formulation.

### *2.3 Extraction*

The extraction of vitamins for each product was accomplished using the following methods: Vitamin A from Alpin and Alpinito was extracted according the method proposed by López [8] and Zhang et al. [9], with changes in the concentration of KOH added for saponification, ranging from 40 to 50% to obtain greater yield. Vitamins B1, B2, B3, B6, B9, and B12 from Alpin, Alpinito and Baby Porridge were extracted using the method proposed by Viñas et al. [10] and van Wyk et al. [11] with modifications at the end of the process, adjusting the final pH of the sample to 3.3 with 10 M KOH. Extraction of Vitamins B9 and B12 from Fruper followed the method proposed by Aslam et al. [2], with changes in the extraction temperature, lowering it to 60 °C for 40 minutes [12]. Finally, the extraction of vitamin C from Fruper was performed as described in the method proposed by Klimczak and Gliszczyńska-Świgło [13], modifying the centrifugation process by increasing the revolutions to 8500 rpm for 20 minutes to ensure better clarification of the sample [10-14].

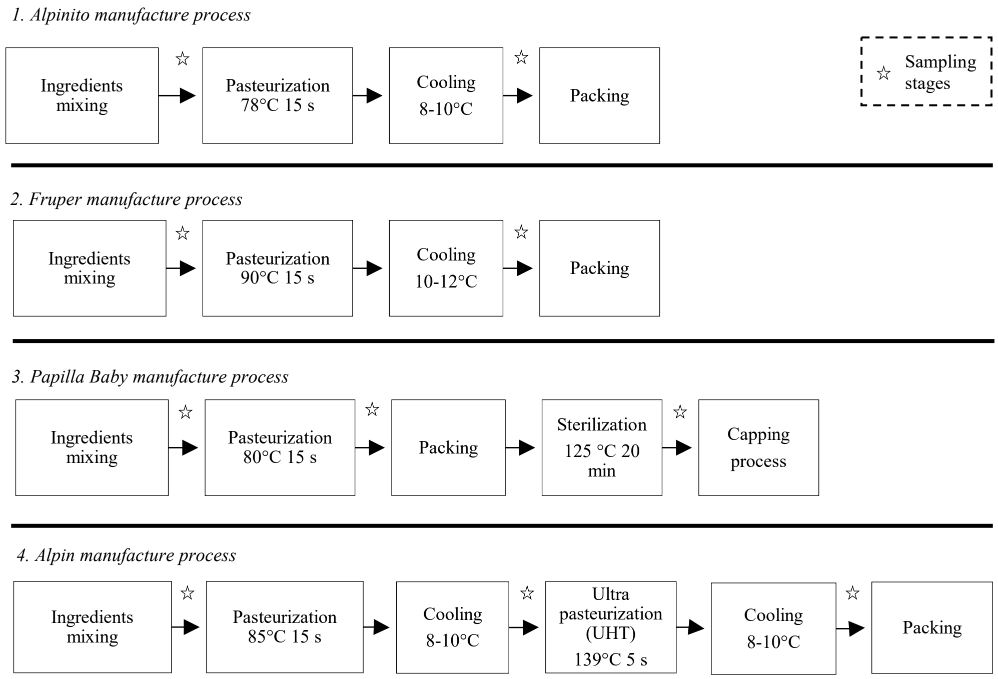

Figure 1. Process diagrams and sampling stages
Source: The authors

### 2.4 *Quantification*

Quantification of vitamins was achieved using high-performance liquid chromatography (HPLC) technique using a PERKIN ELMER® HPLC equipment coupled with diode array (DAD) and light scattering (LDD) detectors. Chromatographic conditions applied: oven temperature = 30 °C; flow = 1.0 mL/min; injection volume = 100 µL; detection = 210 nm (B6), 283 nm (B6, B9, B12, C), 361 nm (B12), 325 (A), and 265 (D3). All measurements were made by triplicate. Two different chromatographic systems were applied: one for fat-soluble vitamin A and the other for water-soluble vitamins (B1, B2, B3, B6, B9, B12, and C). The first one used an isocratic method (99% methanol: HPLC water), while the second one included an acetonitrile ramp: 25 mM phosphate buffer, pH 3.32.

### 2.5 *Statistical analysis*

The analysis of variance (ANOVA) was performed using Excel (Microsoft). Tukey´s multiplate range test (HSD) was used to compare means of the vitamins content between heat treatments for each of the products mentioned before. Significant difference was defined as p <0.05.

## 3 Results and analysis

The results obtained for each selected processing stage assessed during the manufacture of the four products (Alpin, Alpinito, Fruper, and Papilla) are illustrated in Fig. 2. The results are expressed as mean ± standard deviation. Means with different letters on the same graph were significantly different ($p$ <0.05).

Table 3.
Vitamin reduction (%) after thermal processes.

| | | B1 | B2 | B3 | B6 | B9 | B12 | A | C | D3 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Alpin** | 1st | 0.3 | 13.4 | 9.4 | | | | 0.3 | | |
| | 2nd | 9.6 | 1.7 | 0.3 | | | | 0.1 | | |
| **Alpinito** | | | | | | 17.0 | 55.1 | | | 43.3 |
| **Fruper** | | | | | | 9.1 | 89.0 | | 47.7 | |
| **Papilla** | 1st | | | | 59.4 | 10.4 | 94.1 | | | |
| **Baby** | 2nd | | | | 11.8 | 1.1 | 0.0 | | | |

*Note.* 1st: first treatment; 2nd: second treatment.
Source: The authors

Regarding Alpin, Fig. 2.a shows a statistically significant decrease of vitamins B2, B3 and A after the pasteurization step. Vitamin B1 was not affected by pasteurization. As shown in Table 3, highest vitamin reductions occurred after pasteurization for vitamin B2 (13.4% reduction), and after UHT for vitamin B1 (9.6% reduction). Despite the significant statistical difference found for all vitamins, the reductions in concentration did not alter the nutritional value of the product according to national regulations, in fact, is possible to notice that bars in Fig. 2.a, for Alpin, do not change abruptly between heat treatments for any of the vitamins. The study conducted by Fulias et al. [15] on the thermal behaviour of vitamins B1, B2, and B6 determined that the three compounds had relatively high thermal stability. In addition, the authors observed that B2 had been the most stable vitamin.

For vitamin A, there was no perceptible degradation between the initial concentration and that in the finished product. Cox et al. [16] studied the stability of vitamin A in pasteurized whole milk and skim milk. They found less degradation of vitamin A in whole milk than in skim milk, and suggested that natural antioxidants, such as tocopherols found in milk fat, had improved the stability of vitamin A. These results may explain the stability of this vitamin in Alpin, considering that it is manufactured with whole milk.

For Alpinito, vitamin B12, showed the highest concentration reduction in the mixture after pasteurization, in comparison to vitamins B9 and D3 (Table 3, Fig. 2.b). The

stability of vitamin B12 is linked to pH, being unstable in acidic media (pH < 7) [17]. According to the previous statement and given that the pH value in Alpinito was 4.5, vitamin B12 was significantly affected by the heat treatment. In the case of vitamin D3, there was a significant decrease during the thermal treatment of the product, and according to studies conducted by Mahmoodani et al. [17], this fact can be explained by the lipoxidation of fatty acids, which could be one of the processes that cause the degradation of vitamin D3. These authors found that the generation of lipid oxidation products formed by heat treatments was associated with the degradation of vitamin D3.

The behaviour of vitamins during the manufacture of Fruper are illustrated in Fig. 2.d, indicating a significant decrease in vitamins C (47.7%) and B12 (55.1%) after pasteurization. Vitamin B9 showed the lowest reduction after the heat treatment (9.1%). According to some studies [18-20], losses in ascorbic acid can range from 10 to 90%, depending on the amount of oxygen present in the product at the time of the heat treatment. During oxidative degradation, ascorbic acid is oxidized to dehydroascorbic and, by cleavage of the lactone ring, gives 2,3 diketogulonic acid, which no longer has biological activity. For vitamin B9, studies [21,22] have indicated that the degradation resulting from pasteurization was within the range of 10 to 20%, similar to the result obtained in this study (9.1%). Also, folic acid (B9) losses were associated with oxygen content in the product and the level of ascorbic acid, which has a protective influence. On the other hand, contrary to the stability of vitamin B9, it was observed that 55% of vitamin B12 degraded after the heat treatment. Other studies have indicated that the degradation of this vitamin after thermal pasteurization and UHT processes had ranged from 10 to 40% [23].

Finally, for Papilla, it was observed a considerable reduction of vitamins B6 and B12 (Fig. 2.c), decreasing 59.4% and 94.1% after pasteurization (Table 3), respectively. For vitamin B9, the reduction after the first heat treatment was 10.4%. In the manufacturing, the product is subjected to an autoclave sterilization process, which implies an additional thermal treatment that leads to another stage where vitamins can be significantly degraded. In the case of Papilla Baby, a similar behaviour of vitamins was observed. For example, vitamin B9 had high stability in comparison to vitamin B12, which was significantly degraded after the thermal processes. Similarly, a degradation of Vitamin B6 after pasteurisation was noticeable, with a decrease of 60%. Studies have found that heat treatments degraded vitamin B6 from 30 to 40% [1]. The highest degradation observed in this product can be attributed to the additional autoclave stage, in which the product is subjected to a second heat treatment.

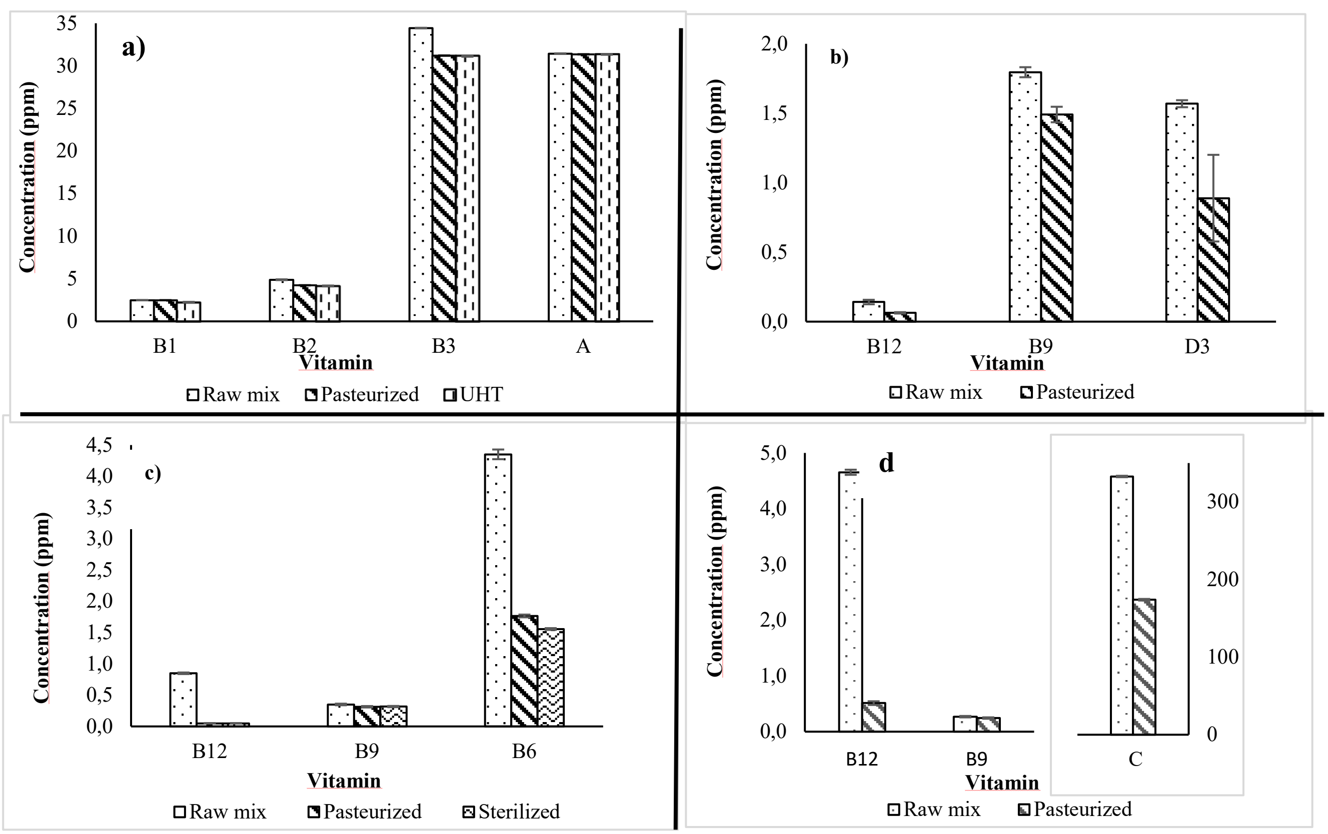


Figure 2. Vitamin concentration after each heat process. a) Alpin b) Alpinito c) Papilla Baby d) Fruper.
Source: The authors

## 4 Conclusions and recommendations

The assessment of the different processes involved in the manufacture of the products selected from the portfolio of Alpina Productos Alimenticios S. A. BIC allowed us to conclude that the temperature factor present in all the processing stages was critical for some vitamins. The greatest degradation of vitamins was observed for vitamin B12 in Fruper and vitamin B6-B12 in Papilla Baby. On the other hand, vitamins added in Alpin were more stable during thermal processes, comparing to the other products evaluated. The results obtained allowed to establish that prolonged heating of the product caused water-soluble vitamins degradation (as found in B6, B9, B12 and C) while lipo-soluble vitamins are more stable during thermal processes (vitamin A). However, this study evidence that water-soluble vitamins as B1, B2 and B3 are more stable than B6, B9, B12 and C. Additionally, the interaction between all compounds in the different matrices could affect the behaviour of vitamins content. Further studies could assess vitamin interactions with other components in different matrices (dairy, non-dairy) to corroborate the results obtained in this research.

Degradation factors from heat treatments found in this study can help in future formulations for product development, minimizing random testing and targeting the appropriate vitamin content in the final product. Furthermore, the resultant vitamin content for each product after thermal processes coincide with the concentrations reported on each nutritional table.

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