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Application of the Cryo-Drying Technique in Maintaining Bioactive and Antioxidant Properties in Basil Leaves (*Ocimum basilicum*)

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Abstract: The objective of this work is to compare the levels of bioactive compounds in fresh and freeze-dried basil leaves (*Ocimum basilicum*), using methodological procedures that follow internationally recommended and accepted standards. The results show comparisons of bioactives between fresh and freeze-dried leaves, respectively, with results expressing the average levels of vitamin C (9.50–63.3 mg/100 g); total polyphenols (1.8–3.9 mgEAG/g); flavonoids (0.73–1.78 mg/g); chlorophyll a (2287.8–1003.8 µg/100 g); chlorophyll b (2606–2287 µg/100 g) and total carotenoids with averages of 16.71–20.6 mg/100 g). Regarding color, there was variation in the parameters L*, a*, and b* of the dry sample, but the tendency towards green e (a+) and yellow (b−) was maintained. Infrared analysis (FTIR) demonstrated the presence of functional groups related to cellulose, hemicellulose, and lignin. Thermogravimetry (TG/DTG) shows stability in the range of 234 °C, showing a more intense mass loss at 294.6 °C. Given the data, it is possible to infer that the application of freeze-drying produces few changes in bioactive compounds and chemical groups and maintains good thermal stability, proving to be a viable alternative to increasing the commercialization of basil leaves, as it prolongs their useful life, and increases the forms of food applications.

Keywords: phytochemical compounds; *Ocimum basilicum*; food drying

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1. Introduction

Basil (*Ocimum basilicum* L.), known in the Amazon region as "common basil", is an aromatic plant species widely used in the traditional diet of the northern region, and in formulations associated with traditional medicine for its antimicrobial, antioxidant, anti-inflammatory, anti-hyperglycemic, in addition to the potential for olfactory stimulation, given its richness in volatile compounds, are some of the pharmacological effects attributed to the different constituents of the *Ocimum* species [1–5].

In recent decades, Brazil and the world have been undergoing major social, cultural, economic, demographic, and technological transformations that result in changes in the health status and pattern of food consumption of the population, characterizing a process called epidemiological transition. In this scenario, the exacerbated intake of ultra-processed foods, which have high levels of preservatives, sodium, sugar, saturated fats, and several other substances, has been considered one of the risk factors that directly contribute to the increase in the rates of chronic non-transmissible diseases (CNCD) such as type 2 diabetes mellitus, cardiovascular diseases, obesity, and systemic arterial hypertension [1–3]. NCDs constitute seven of the ten main causes of death in the world, with cardiovascular diseases being the main causes of mortality [6].

In view of the influence that diet can have on the development of CNCDs, functional foods have gained prominence for their bioactive properties that have beneficial physiological and metabolic effects on the human body. Plants considered aromatic herbs have levels of bioactive compounds that act in the prevention of various systemic and neurodegenerative diseases, neoplasms, and inflammatory processes according to the various studies developed in recent decades [6–8]. Therefore, it is observed that aromatic herbs can act as functional foods and nutraceuticals, due to their bioactive properties acting by reducing the risk of health problems, and consequently, conferring/improving health and well-being [9].

The present study highlights the aromatic herb known as basil (*Ocimum basilicum* L.), also known as great basil or basil from the Amazon, which is cultivated in many countries where the climate is favorable [9]. Different parts of basil can be used, including the roots, stem, flowers, and seeds, however, it is popularly known for its green leaves that have a variety of shapes and colorful flowers. The leaves are commonly consumed in dried or fresh forms, which contain high levels of essential oils, flavonoids, and phenolic acids that are potent antioxidants, anti-inflammatory, antibacterial, and antiviral [10,11].

Several factors can change this composition of nutrients and the bioactive properties of foods such as edaphoclimatic characteristics, species, form of cultivation, harvesting, and thermal processing [12,13]. Another point of change is the drying processes; one of the most beneficial to products is lyophilization, which consists of freezing the product and, after that, dehydration by the sublimation process, which promotes the reduction of water content and, therefore, the minimization of the occurrence of most of the reactions that provoke the degradation of the product. Freeze-drying is considered one of the best drying methods since it maintains the nutritional and organoleptic properties of the food [14]. Cryo-drying (lyophilization) maintains its relationship with the perspective of maintenance/preservation of bioactive constituents with special attention to thermosensitive raw materials, through the application of the cold drying technique (sublimation), as can be seen in research with *Talinum triangulare* L leaves [15], *Xanthosoma taioba* [16], and *Acmella oleracea* [17].

Therefore, it is observed that in addition to the intention of preserving food, it is important to observe the effects and changes that the freeze-drying process can generate on the levels of bioactive compounds in the leaves of basil from the Amazon. Thus, this research aims to analyze the bioactive compounds and the thermogravimetric and spectroscopic profile of basil leaves (*Ocimum basilicum*) from the Amazon.

2. Materials and Methods

2.1. Preparation of the Sample

The samples of manjericão (*Ocimum basilicum*) in nature referring to the 2024 harvest were obtained at the Ver-o-Peso market located in the Municipality of Belém, State of Pará, Brazil (geographic coordinates: latitude: −1.4648490 and longitude: −48.4564202). Subsequently, they were transported in low-density polyethylene (PEBD) plastic bags, and stored in the Food Science Laboratory of the Faculty of Nutrition of the Federal University of Pará, at a temperature of 25 °C. The leaves were selected, and the caules were removed. After that, the leaves were cleaned carefully and individually in running water and sanitized in containers with water and sodium hypochlorite with a dilution of 200 ppm (part per thousand) for 15 min and finally washed again in running water and dried at temperature atmosphere.

2.2. Biometric Characterization of the Leaves

The biometric characterization was performed according to the analytical methods of the Association of Official Analytical Chemists (1992) [18]. A sample of 50 leaves was manually defined, as they were individually weighed on a semi-analytical balance (Bel brand and model L303i) and sized using the following parameters: longitudinal and transversal compression.

2.3. Freeze-Drying Process and Physical Analyses

After sanitization, part of the fresh basil leaves were dried by lyophilization (Liotop SL 404, SOLAB, Piracicaba, Brazil) for 48 h and ground in a Reffinox mill (model TE650 Willye, Piracicaba, Brazil). Next, water activity analysis of fresh basil leaves (FBL) and post-freeze-drying basil leaves (PBL) was carried out through direct measurement, in triplicate, in the Labmaster–aw neo Series 3TE instrument from Novasina (Lachen, Switzerland), with internal control temperature at 25 °C. The humidity content was verified following the methodology nº. 934.06 (2016) [19]. To determine the pH, method number 981.12 of the Association of Official Analytical (AOAC, 2016) [19] was used, and measured with a bench pH meter (PG2000, GEHAKA, São Paulo, Brazil); The analysis of total titratable (ATT) acidity was performed according to the norms expressed by AIL (1985) [20], and the results were expressed in percentage (%) of citric acid (100 g^{-1}).

2.4. Analyses of Bioactive Compounds

Extract preparation

The extracts were prepared from fresh samples and lyophilized in ultrasound (Solid Steel São Paulo, Brazil), and the samples were suspended in a 70% (*w*/*v*) ethanol solution [21]. After sonication at a frequency of 20 kHz for 10 min at 20 °C, the material was subsequently centrifuged (Sigma 6-15H model, SIGMA, Osterode am Harz, Germany) at 3.900 rpm for 15 min. To obtain the crude extract, the supernatant was recovered, filtered, and concentrated in a rotary evaporator, Laborota 4000 model (Heidolph, Schwabach, Germany), under low pressure and controlled temperature (40 ± 5 °C). The extracts were stored in amber glass vials, added with nitrogen gas (N_2), hermetically closed, and stored at −18 °C until the moment of analysis.

2.4.1. Ascorbic Acid Content (AA)

Ascorbic acid Content was determined by titration with reduction in 2,6-dichlorophenol indophenol (DCFI) compound by ascorbic acid AOAC [22]. The freeze-dried leaves (5 g) were diluted with 40 mL of a 4% aqueous oxalic acid solution and mixed for 30 min at 3.900 rpm on a magnetic stirrer (Solab brand, model SL-91/3, SOLAB, Piracicaba, Brazil) in a dark room and then vacuum filtered. The filtered component was titrated with the addition of the 2,6-dichlorophenol indophenol solution until a pink color persisted. L-ascorbic acid was used to prepare the standard solution (0.5 mg/mL), and the concentration was calculated by comparison to the standard and expressed in mg/100 g of fresh mass.

2.4.2. Flavonoid Content

The flavonoid content was analyzed following the assay reported by Lees and Francis (1972) [23] using a UV–Vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 374 nm. Contents were extracted from 1 mL with 30 mL of 95% ethanol/1.5 M HCl (85:15, *v*/*v*) mixed for 15 min at 3.900 rpm on a magnetic stirrer (Solab, model SL-91/3). The extract was transferred to a 50 mL volumetric flask, completing the volume with ethanol–HCl (1.5 M) and stored for 12 h at 4 °C [22]. After filtration, the absorbance was measured in a UV–Vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 374 nm. The total flavonoid content was determined by applying the Lambert–Beer law and was calculated as mg/100 g using the following formula:

$$\text{Total flavonoids content} = \frac{A_{374}\ \text{X dilution factor}}{E^{1\%}_{1cm}374nm}$$

where A_{374} is the absorbance in the diluted sample and $E^{1\%}_{1cm}$cm, 374 is the value factor (76.6) of molar absorptivity for the acid–ethanol solvent measured in a 1 cm cell at 374 nm at a concentration of 1% (*w*/*v*).

2.4.3. Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu assay as reported by Aliakbarian et al. (2011) [24] and was measured at 725 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The TPC results were standardized against gallic acid equivalents per 100 g (mg GAE/100 g). The method was based on the linear equation $Y = 0.0017X$, where Y is mg GAE/100 g and X is the read absorbance, with $R^2 = 0.9966$.

2.4.4. Antioxidant Activity Was Evaluated Using the ABTS [2,2'-Azinobis 3-ethylbenzthiazoline-6-sulfonic acid] Radical Scavenging Methodology

The absorbance was measured at 734 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The antioxidant capacity was calculated in triplicate against a calibration curve of ethanolic solutions of known Trolox concentrations (Rufino et al., 2010) [25]. The determination was based on the linear equation $Y = -0.3364X + 0.6239$ ($R^2 = 0.997$).

2.4.5. Carotenoids

The total carotenoid content was quantified by the method proposed by Godoy and Rodriguez-Amaya (2001) [26], and submitted to reading in a UV–Vis spectrophotometer (Kasuaki, model IL-592) at 450 nm. The calculation of the total carotenoid content was performed using the specific molar absorptivity coefficient for β-carotene in petroleum ether ($A\frac{1\%}{1\ \mathrm{cm}} = 2592$) (Davies, 1976) [27] and the results were expressed in mg/100 g of the sample.

$$\mathrm{CT} = \mathrm{Abs} \times 10^4 \times \mathrm{F} \times (\mathrm{V}\ /\ \mathrm{M}) \times 2592$$

where CT = total carotenoid content (mg/100 g); Abs = absorption at 450 nm; F = dilution factor (dimensionless); V = extract volume before dilution (mL); M = sample mass (g), and 2592 = coefficient of molar absorptivity of β-carotene in petroleum ether.

2.4.6. Chlorophyll Content

The percentage of chlorophyll was determined by the Nagata and Yamashita (2022) method [28]; an aliquot (1 g) of leaves was macerated with 10 mL acetone solution (80%) (v/v) until all pigmentation was extracted and then centrifuged at 4000 rpm for 10 min (Sigma model 6-15H). The supernatant was transferred to a 25 mL volumetric flask. The volume was completed with acetone solution (80%) (v/v). Absorbance readings were performed using a UV–Vis spectrophotometer (UV-1800, Kasuaki, model IL-592) with 647 nm and 663 nm wavelengths. P.A. acetone was used as a negative control. Each sample was analyzed in triplicate. The results were expressed in mg of chlorophyll by 100 g of sample. The analysis of chlorophyll a and b contents was obtained according to the equations.

$$\text{Chlorophyll Contents a } (\mu g/g) = -0.999A663 + 0.989A645$$

$$\text{Chlorophyll Contents b } (\mu g/g) = -0.328A663 + 1.77A645$$

2.5. Instrumental Color

The instrumental color parameters were analyzed in a digital colorimeter (Chroma Meter CR-300, Konica Minolta, Tokyo, Japan) using the CIELAB with the following operating conditions: diffuse lighting/0° viewing angle and D65 light source. System to assess the chromaticity coordinates (L* for luminosity, a* for red color intensity, b* for yellow color intensity). The chromaticity (C*) was calculated according to McLellan et al. (1995) [29].

2.6. Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) analyses were carried out using a Perkin Elmer spectrometer, Frontier 98737 model (Waltham, MA, USA) at ambient temperature in the 4000–400 cm^{-1} range. The spectra were registered by averaging 40 scans with

a resolution of 4 cm^{-1} in transmission mode. The samples were analyzed as potassium bromide (KBr) disks.

2.7. Thermogravimetric Analysis

Thermogravimetric analysis (TG) was used to investigate the thermal stability of lyophilized leaves under an air atmosphere in a TA instrument, model Q-500 (New Castle, DE, USA). Approximately 10 mg of sample was heated from 25 °C to 700 °C at a rate of 10 °C/min. Derived thermogravimetric curves (DTG) were used to measure and compare peak temperatures.

2.8. Statistical Analysis

The results of physical analyses and bioactive compounds were performed in triplicate (mean ± standard deviation) and submitted to the Statistica software version 7.0 (Statistica, Hamburg, Germany, 2000) [30].

3. Results and Discussion

3.1. Biometric Analysis of Leaves Fresh

Leaves are plant organs that interact with various environmental factors and have essential functions for the proper development of plants, including capturing sunlight to carry out the photochemical process of obtaining energy (photosynthesis) and promoting gas exchange with the atmosphere, sweating, and breathing [31]. Figure 1 presents the leaf biometric parameters such as weight, and transverse and longitudinal length of the basil sample in the present study.

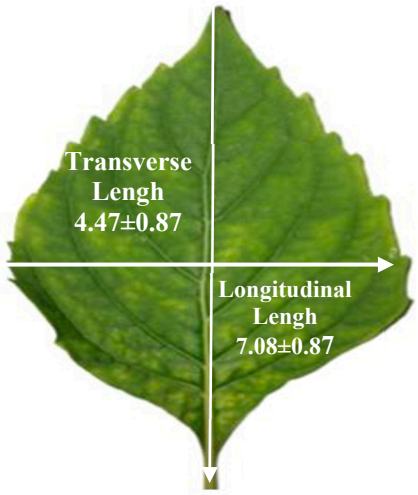

Figure 1. Biometric characterization of fresh basil leaves.

The mean weight found was 0.39 g (±0.184), with a longitudinal length of 7.08 cm (±0.877) and a transverse length of 4.47 cm (±0.872). Significant dispersion was observed in the standard deviation values between the longitudinal and transversal length averages, inferring that the leaves have varied sizes.

In the study by Prinsi et al. (2020) [32], which analyzed some varieties of *Ocimum basilicum*, including the classic Italian (green), grown in pots under greenhouse conditions, an average weight of 0.53 g was obtained for the green variety, which is greater than obtained in the current search. This may be related to the fact that there are anatomical differences between the varieties and the cultivation method since the basil in the cited study was exposed to a source of controlled sunlight and the one in the present study to direct sunlight in the field. This type of production directly influences the weight (fresh mass) and size of the leaves of *Ocimum basilicum*, given that there is better control of solar radiation and, consequently, optimization of photosynthetic and metabolic processes,

providing greater growth and development of the plants that are cultivated in this type of system [33].

Edaphoclimatic factors such as the availability of incident solar radiation can result in anatomical and ultrastructural variations, which demonstrates that this species has phenotypic plasticity, that is, the ability to change its morphology and physiology according to the environmental conditions in which it is exposed [34].

3.2. Evaluation of the Physical Characteristics

Table 1 shows the values obtained in the evaluation of the physicochemical characteristics of the fresh basil leaves (FBL) and post-freeze-drying (PBL).

Table 1. Physicochemical characteristics of the (FBL) and (PBL).

Basil	Water Activity	Humidity (%)	pH	ATT (g/100 g Citric Acid)
FBL	0.98 ± 0.0004	87.9 ± 0.388	6.89 ± 0.105	0.160 ± 0.064
PBL	0.33 ± 0.001	5.2 ± 0.568	6.64 ± 0.043	2.078 ± 0.036

Results expressed as mean ± standard deviation. Analysis performed in triplicate.

When analyzing the water activity parameter of the FBL, a value of 0.98 was verified, which is much higher than that of the PBL, which presented 0.33. The available water content in aerial parts of plants, such as leaves, is very high because it plays a number of important roles in plant physiology, such as serving as a basic substrate for photosynthesis.

The drying of aromatic herbs fresh is widely used to increase the quality and stability of these foods. According to research by Silva et al. (2016) [35] who carried out a physical-chemical analysis of the leaves after the drying process of *Plectranthus barbatus Andrews* (known as "Brazilian boldo"), which is a vegetable from the same family as basil (*Lamiaceae*), observed an aw of 0.324, which is similar to the value obtained from the present research, which highlights the influence of this process on the water content present in the vegetables that undergo this processing.

It is noted that the FBL has a high susceptibility to chemical and microbiological alterations in relation to the PBL since they presented aw > 0.80. Therefore, dehydration is a good tool for the conservation of aromatic herbs because it removes the free water present in plant tissues, reducing their perishability, speed of enzymatic reactions, and growth and development of deteriorating and pathogenic microorganisms [36].

The FBL humidity result obtained in the present research was 87.9%, which was similar to the values observed in the research by Soares et al. (2021) [37], which carried out a U% analysis of different spices of the *Lamiaceae* family fresh and dehydrated, including basil, rosemary (*Rosmarinus officinalis*), mint (*Mentha Spicata*), and oregano (*Origanum vulgare*), which presented U% fresh of 85.45%, 73.90%, 91.03% and 88.36%, respectively. These spicy herbs, including basil, have a high humidity content, which is one of the extrinsic factors that influence the sensory quality, composition, stability, and conservation of food.

In the study by De Martino et al. (2022) [38], who evaluated the influence of drying on the quality of the aerial parts of *Ocimum basilicum*, including the convective heating oven for 24 h at 50 °C, a post-drying U% of 10.52% was observed, this being greater than that obtained in the present study, which was 5.2%. This inequality of values may be related to the drying temperature, in which the one in the present study was 55 °C, proving to be more efficient in removing the free water present in the sample.

Regarding pH, a value of 6.89 was verified for the FBL sample and 6.64 for PBL, demonstrating that both are in the pH range close to neutrality. The study by Henrique et al. (2017) [39], who analyzed fresh organic basil leaves found a pH value of 6.43, which is within the range of neutrality, which corroborates the finding of the present study. It is observed that drying did not significantly impact the pH, since herbs such as basil are naturally framed in the pH range close to neutrality.

The ATT values obtained in the FBL sample were 0.160 g/100 g citric acid and in the PBL sample were 2.078 g/100 g citric acid, demonstrating that drying increased the content of organic acids present in the dried sample. This fact can be explained by the decrease in water content and the respective concentration of acids present.

3.3. Bioactive Compounds and Vitamin C

Table 2 expresses the values of vitamin C and bioactive compounds: chlorophyll a and b, total polyphenols, flavonoids, and antioxidant activity obtained in the studied samples.

Table 2. Bioactive compounds and vitamin C in (FBL) and (PBL).

Bioactive Compound	FBL	PBL
Chlorophyll a (μg/100 g)	2287.8 ± 0.02	1003.8 ± 0.03
Chlorophyll b (μg/100 g)	2607.4 ± 0.23	2287.8 ± 0.02
Vitamin C (mg/100 g)	95.0 ± 0.50	63.30 ± 0.70
Total polyphenols (mg EAG/g)	1.80 ± 0.01	3.90 ± 0.57
Flavonoids (mg GAE/g)	0.73 ± 1.20	1.78 ± 0.01
Antioxidant activity (μg TE/g)	9.75 ± 1.30	12.35 ± 1.07
Total carotenoids (mg/100 g)	16.71 ± 0.92	20.60 ± 0.97

Results expressed as mean ± standard deviation. Analysis performed in triplicate.

The chl a and b values obtained in the analyses of pigments present in *Ocimum basilicum* in the FBL sample were 2287.8 μg/100 g and 2607.4 μg/100 g, respectively. In the PBL sample, values of chl a 1003.8 μg/100 g and chl b 2287.8 μg/100 g were obtained. Note that the chlorophyll contents were higher in the FBL sample, with the chlorophyll b content being greater than that of a in both samples, confirming that the chl b is less unstable to temperature elevation than the chl a.

Chlorophyll (Chl) is a pigment widely used by industries as a natural dye in the production of cosmetics and food. In addition, it is considered an important factor for the acceptance and acquisition of vegetables by consumers, given that the green color is widely used as a quality parameter both for fresh edible vegetables and for dry spices [40–42].

Regarding the vitamin C content, it was possible to observe that the FBL sample (95.0 mg/100 g) has a higher vitamin C content compared to the PBL (63.3 mg/100 g). Comparing with the research by Othman et al. (2021) [43], who analyzed several compounds, such as ascorbic acid, in microgreens of fresh samples of green basil (*Ocimum basilicum* L.) and red basil (*Ocimum basilicum* P'urpurascens') grown in a climatic chamber, ascorbic contents of 65.68 mg/100 g, 105.87 mg/100 g were observed, respectively. These are similar values to the current research, and small variations can be explained by the particularity of the samples since microgreens are smaller plants that are already developed, and the diversity of species analyzed and the methodology used for quantification (use of metaphosphoric acid and high-performance liquid chromatography (HPLC)) were different.

According to Pham et al. (2018) [44], ascorbic acid is a vitamin subject to oxidative degradation after prolonged exposure to factors such as heat and light, among others, where the enzyme ascorbate oxidase released from cell membranes is ruptured during the drying process. The vitamin C value of the freeze-dried sample was slightly lower due to possible variables such as crushing and homogenization carried out after cryo-drying, at ambient temperatures in the northern region of Brazil, inducing thermosensitivity of this constituent, also leading to the activation of action processes enzymatic (oxidases) in the presence of oxygen, added to the action of light (photosensitivity). These environmental constituents degrade the chemical structure of vitamin C, which is sensitive to these variables that are imposed when preparing the sample for analysis [45,46].

As can be seen, the levels of total polyphenols present in FBL (1.8 mg EAG/g) were lower than those found in PBL (3.9 mg EAG/g). In the research by Prinsi (2020) [47], the leaves fresh of three different varieties of *O. basilicum* showed levels of phenolic compounds

of 5.57 mg GAE g^{-1} in the classic Italian cultivar, 7.11 mg GAE g^{-1} in the cultivar Red Rubin, and 6.07 mg GAE g^{-1} in dark opal, these results are higher than those found in the present research. This difference can be explained by the conditions of cultivation in a greenhouse (which offers better conditions for leaf development), disparity in the morphology of the varieties studied, and the state of maturation, since the leaves in the cited research were harvested in the vegetative phase (characterized by explosive growth), since the stage of development may be one of the factors that affect the content of phenolic compounds [48].

Thus, it was noticed that in the present work, drying increased the bioaccessibility of the total polyphenols in the PBL compared to the levels present in the FBL, and this fact may have occurred due to the influence of the use of freeze-drying on the plant structures of the PBL sample, which may have enabled the release of these compounds into the extracellular environment and allowed the extraction of higher levels in the sample. Thus, ingestion of dehydrated basil proves to be a good way of ingesting polyphenols, since these are important antioxidant and anti-inflammatory agents.

Regarding the flavonoid contents, the PBL sample (1.78 mg GAE/g) showed a higher content than the FBL sample (0.73 mg GAE/g). Several recent studies described in the scientific literature demonstrate that Ocimum and its species have significant antioxidant and anti-inflammatory activities due to their high levels of polyphenols and flavonoids [30,33,48,49]. In research by Anusmith et al. (2020) [50], which aimed to evaluate the phytochemical composition and antioxidant, anti-inflammatory, anticancer, and genoprotective properties of extracts of different species of Ocimum (dry) extracted by ultrasound-assisted methods, it was observed total flavonoid contents of 662 mg GAE/g, 65.7 mg GAE/g, 54.3 mg GAE/g, 54.7 mg GAE/g, 55.2 mg GAE/g and 65.6 mg GAE/g for *O. gratisssimum*, *O. basilicum*, *O. canum*, *O. kilimandscharicum*, *O. tenuiflorum* and *O. citriodorum*, respectively.

These findings differ from those found in the present study, both due to the difference in the extraction method, as well as due to edaphic factors and the variety of species studied. Another study carried out by Ullah et al. (2022) [51] evaluated the flavonoid content in methanolic extracts (obtained by the cold extraction method) of *O. sanctum*, which presented 2.016 mg/g and *O. basilicum* 2.034 mg/g, emphasizing the presence of considerable levels of flavonoids in the extracts of these basil species.

Comparing the FBL and PBL samples, it was found that freeze-drying increased the flavonoid content by approximately 6.75%. This result can be justified by the fact that these compounds are present mainly in the aerial parts of plants, such as leaves, which, when applied to drying methods on the raw material, cause changes in the structural integrity of the cellular matrix, a breakdown of plant tissues, and a reduction in water content by mass transfer, which, consequently, can increase the concentration of these compounds [52].

As for the potential antioxidant activity, the fresh sample showed an average of 11.75 (µg/mL), and in the freeze-dried sample 17.35 (µg/mL), showing that drying by freeze-drying, which uses low temperatures based on the sublimation process, concentrate the antioxidant activity expressed in the ABTS radical. When compared with the studies by Baskaran et al. (2023) [53] with different extracts of Indian basil dried in hot air, they showed values of 12.4 and 16.5 µg/mL, with values close to the fresh material evaluated in this research, but lower than the lyophilized material, added to the fact that this research did not produce its extracts with organic solvents, maintaining the principles of green chemistry.

Looking at the carotenoid data in Table 3 and comparing it with the studies by Cvitković et al. (2021) [54], who analyzed the antioxidant properties and pigments of dried herbs considered condiments and medicinals in the Mediterranean, their TC contents 9.68 mg 100 g^{-1}, 9.43 mg 100 g^{-1}, 14.24 mg 100 g^{-1}, 9.26 mg 100 g^{-1}, 6.02 mg 100 g^{-1} for *Myrtus communis* L., *Pistacia lentiscus* L., *Thymus vulgaris* L., *Salvia officinalis* L., and *Laurus nobilis* L., respectively, are lower than the current research, demonstrating that basil is a good source of carotenoids in the diet, especially when consumed in dehydrated form.

Table 3. Colorimetric analysis of fresh (FBL) and freeze-dried (PBL) basil leaves.

Parameters	FBL	PBL
ΔL*	34.05 ± 5.052	23.10 ± 0.890
Δa*	−14.95 ± 1.490	−3.22 ± 0.058
Δb*	21.76 ± 2.393	13.05 ± 0.270
ΔC*	26.40	13.44
ΔE*	18.57	

Results expressed as mean ± standard deviation. Analysis obtained in triplicate. L* = luminosity or brightness; a* = green color coordinate (negative a*); b* = color coordinate yellow (b* positive); C* = chroma ΔE* = total color difference.

According to the data, the carotenoids were not affected by the freeze-drying process, not negatively inferring in plant structures, and the carotenoid–protein complexes allowed a better extraction of carotenoids from the dried vegetable raw material by lyophilization [55,56].

3.4. Analysis of Colorimetric Variation

The color parameters and their variations imposed by freeze-drying are shown in Table 3.

According to the results observed in Table 2, all values of color coordinates of the PBL sample changed in comparison with the FBL sample. It was found that the color coordinate L* showed a decrease, going from 34.05 in the FBL to 23.10 in the PBL, demonstrating that there was a darkening of the raw material. Regarding the a* coordinate, the result was negative for both samples, demonstrating a tendency towards green coloration, since they are leaves, and it was also verified that convection caused a reduction in this parameter due to the possible action on the pigments (chlorophyll). The b* coordinate, on the other hand, showed positive values indicating the prevalence of the yellow hue to the detriment of the blue color, with a significant reduction in the PBL sample.

The results of the C* coordinate were 26.40 and 13.44 for the FBL and PBL samples, respectively. It is inferred that the FBL sample demonstrates that the purity or intensity of the color of this sample is greater compared to the PBL sample, meaning that the fresh leaves have greater saturation (which is directly linked to the concentration of the coloring element and represents an attribute quantitative for intensity), that is, they are brighter and more vivid in human visual perception, compared to the dehydrated sample that presented a darker tone.

Regarding the total color difference, it was observed that the PBL sample suffered a color loss of 18.257 (ΔE*), inferring that the application of lyophilization on basil causes significant color changes. Therefore, this alteration can influence the moment of acquisition of the dry product since the consumer generally associates greater palatability with foods with brighter and brighter colors to the detriment of darker and less bright foods [57].

3.5. Analysis of Chemical Clusters by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum, due to analytical requirements, can only be performed on properly dried material. Therefore, chemical groups are presented only in the PBL sample (shown in Figure 2). The application of this tool allows for obtaining information related to functional chemical groups and their vibrational states according to interactions and changes in the structure and composition of materials [58].

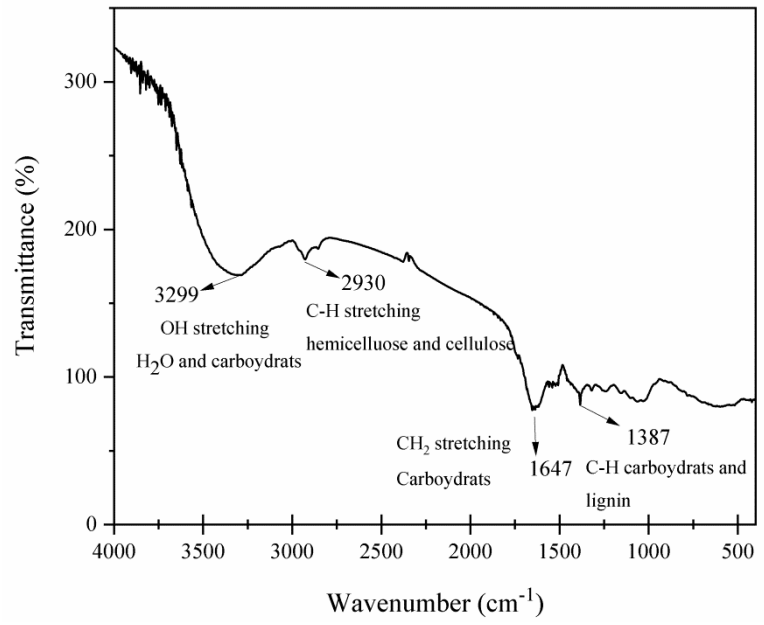

Figure 2. Spectrum pattern of the PBL sample.

The chemical groups presented in the broadest spectral bands between 3299 cm^{-1} to 1387 cm^{-1} are related to stretching vibrations of -OH groups present in water and cellulose membranes [16,17,59]. The presence of the band at 2930 cm^{-1} and 1387 cm^{-1} may be related to CH_2 bending in hemicellulose and CH vibrations, which commonly appear in carbohydrates and lignans present in large amounts in leafy materials, such as basil [59]. These findings may be related to the presence of fibers in the material, reinforcing the functional aspects of freeze-dried basil leaves.

Comparing the data from this research with powder acmella oleracea [17] and taioba leaves under thermal processing [16] it is possible to notice the similarities of chemical constituents based on high-intensity bands at 3400 cm^{-1} and 1063 cm^{-1}, related to spectral vibrations of chemical groups common to plant materials, without losses related to processing and drying, these bands being associated with the presence of constituent material of cellulose, hemicellulose, lignin, and organic acids, constituents of carbohydrate groups, with prevalence in fibrous materials. These data are similar to the findings of the present research.

Another parameter of great relevance evaluated is the behavior of the basil leaf when subjected to a progressive increase in temperature, as shown in Figure 3.

The results show the behavior of the basil leaf when a progressive increase in temperature is imposed. The TGA and DTG curves (Figure 3) show the thermal events of the samples between 41.2 and 700 °C and their first derivative (DTG), which is a mathematical tool used in conjunction with TGA to obtain more accurate information about events imposed by the increase in temperature in the samples.

The thermograms obtained for basil leaves showed two stages of mass decomposition, respectively. The first event evidenced by the TGA curves close to 100 °C corresponds to weight loss due to water evaporation (sample dehydration). The most prominent mass loss occurred during the second thermal stage, evidenced by the TGA curve at a temperature range of 234 °C with a maximum peak at 294.6 °C, which may be related to the beginning of the thermal decomposition of carbohydrates, proteins, and vegetable fibers. with intense losses, until almost the entire consumption of the sample close to 550 to 600 °C probably resulting in only inorganic compounds such as minerals.

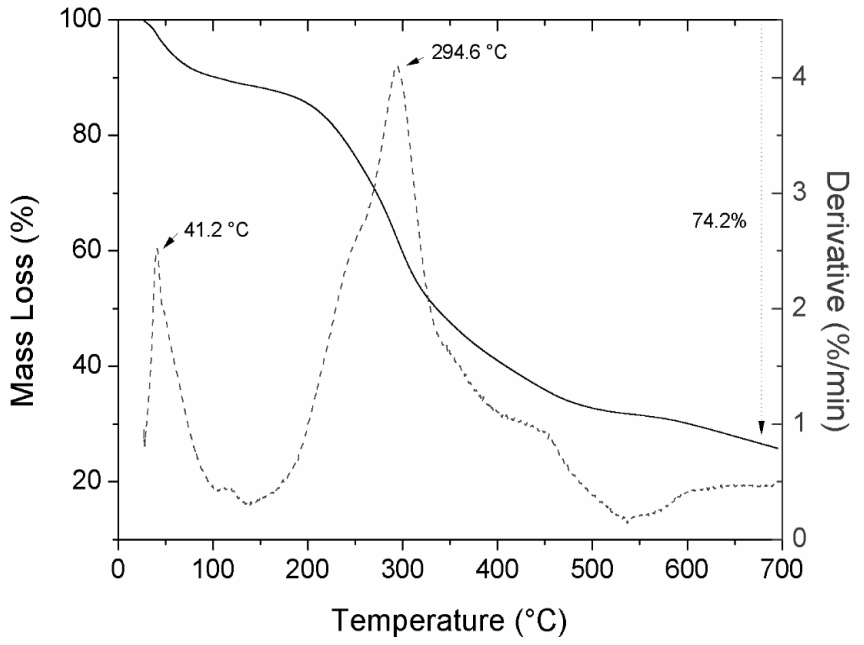

Figure 3. Shows the thermal behavior of freeze-dried basil leaves.

4. Conclusions

The comparison of the stability parameters of fresh basil leaves and their freeze-dried powder, as expected, showed low water activity, humidity, and pH, which points to a reduction in their perishability in relation to fresh leaves, demonstrating that the method of cryo-drying is an important tool to increase the shelf life and applicability of powdered basil. However, there was an influence of the freeze-drying process on the levels of bioactive compounds, with increases in phenolic compounds, flavonoids, and carotenoids (higher concentration) and less action on chlorophylls a and b, vitamin C, and antioxidant capacity, showing that the development of basil powder had small changes in these constituents compared to their fresh consumption.

Regarding the analysis of chemical groups, the FTIR spectrum indicated the presence of material comprising cellulose, hemicellulose, lignin, and organic acids. Due to its behavior in the face of progressive temperature rise, thermogravimetry showed stability in the temperature range suitable for most working conditions in the food industry.

Thus, it was found that both fresh leaves and leaves processed by freeze-drying (powder) are potential sources of bioactive compounds. The application of the freeze-drying method adds greater durability and high resistance to degradation. Finally, it is suggested that new research be carried out on the action of bioactive constituents in vitro and in vivo as an alternative for preventing the development of chronic non-communicable diseases.

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