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Investigating the Nutritional Value and Bioactive Components of Medicinal Herbs under Various Drying Conditions for Ruminants

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Abstract

The research was conducted to investigate the bioactive components and nutritional values of three medicinal herbs: moringa (*Moringa oleifera*), pineapple waste (*Ananas comosus*), and plantain (*Plantago lanceolata*), subjected to three drying methods: sun drying, shade drying, and freeze drying. The research aimed to identify the most effective drying technique for preserving specific bioactive components in these herbs. The experiment was carried out at Bangladesh Agricultural University in Mymensingh and the Bangladesh Livestock Research Institute in Savar. The herb's proximate components were determined. The total phenolic and flavonoid concentrations in the herbs were measured using a UV spectrophotometer. The specific bioactive compounds from each herb, moringa: kaempferol and myricetin; pineapple waste: gallic acid and catechin; and plantain: aucubin and acteoside, were quantified using ultra-high-performance liquid chromatography. Additionally, the herb's 24-hour *in vitro* gas production was also determined in ruminants. The herb's moisture, ether extract, and ash contents differed significantly ($P < 0.05$) across the drying methods, with freeze-drying yielding the most favorable results. Higher ($P < 0.05$) amounts of total phenolic and flavonoid compounds were detected in freeze-dried samples compared to those dried in shade and sun. The particular bioactive components in herbs were significantly ($P < 0.05$) more in freeze drying compared to shade and sun drying procedures. The freeze-dried herbs showed better *in vitro* GP_{24} production, dOM, and ME compared to the others.

It can be concluded that freeze-drying is a more effective method for preserving herbs compared to the other two methods for supplementing ruminant diet.

Keywords

Medicinal Herbs, Drying Methods, Bioactive Compounds

1. Introduction

People have relied on plants for their therapeutic qualities throughout history. Although this use has generally concentrated on improving human health, plants have also been and continue to be used to increase ruminant production and ethnoveterinary practice [1] [2]. Medicinal herbs contain biologically active substances such as phenolic compounds, flavonoids, glycosides, alkaloids, saponins, and tannins. These bioactive compounds are very sensitive and easily influenced by processing methods; thus, minimizing processing loss is necessary. Fresh herbs have enough moisture to degrade their quality over time. They can be processed to make herbs available in various forms throughout the year. The most popular and fundamental method for preserving medicinal plants after harvest is drying, which lowers moisture levels and gets rid of the microbial activity that initially degrades the quality of the herb. It also allows rapid preservation of medicinal properties of plant material in a complex manner. The drying method is a sensitive and crucial step in producing a product of superior quality [3]. Additionally, drying prolongs the shelf life of medicinal herbs by limiting the growth of microbes and preventing certain biochemical activities that could alter the organoleptic properties [4]. To improve the safety, quality, and consistency of finished products, as well as to enhance plant quality, it is imperative to implement high-quality, efficient agricultural and biomass processing systems [5]. There are several methods used for herb drying, such as sun drying [6], shade drying [7], freeze drying [8], and convection hot air drying [6]. It is impossible to forecast how a particular drying technique will affect the preservation of raw quality because it depends on the kinds of chemical compounds and plant types that are present [9]. Since it is widely known that fresh fruit deteriorates quickly, it is best to store it in a dry, cold environment to preserve most of its nutritional content. It is widely acknowledged that storage and processing conditions substantially influence the bioactive compounds in fruits and vegetables, especially phenolic antioxidants, vitamin C, and carotenoids [10]. The choice and application of an effective drying technique are essential for preserving bioactive chemicals in the manufacture of dried samples from natural sources. Yet, drying may adversely affect nutritional and phytochemical components [6] [11]-[13]. To our knowledge, there is scant evidence regarding the impact of various drying techniques on the bioactive compounds of selected medicinal herbs, specifically moringa, pineapple waste, and plan-

tain. Therefore, the study was undertaken to identify the most effective drying technique for preserving specific bioactive components of herbs.

2. Materials and Methods

The Bangladesh Livestock Research Institute and the Bangladesh Agricultural University were the sites of the experiment.

2.1. Collection of Herbs Materials

Plantains (*Plantago lanceolata*), whole aerial parts, pineapple waste (*Ananas comosus*), comprising peel and leaf, and moringa (*Moringa oleifera*) leaves, twigs, and branches were the three herb samples that were collected from the herb bank at the Shahjalal Animal Nutrition Field, Bangladesh Agricultural University. Following the removal of soil and other unnecessary materials, the obtained herb sample was manually cut into pieces measuring 3 to 4 centimeters.

2.2. Preparation of Dried Samples

With certain modifications, fresh herb samples were desiccated to a consistent weight utilizing three distinct drying techniques (sun, shade, and freeze), following the methodology outlined by Nguyen *et al.* [14]. Three sets of 300 g of waste from moringa, pineapple, and plantain were weighed for sun, freeze, and shade drying. During the sun-drying process, herb samples were exposed to direct sunlight from 9:00 am to 5:00 pm each day under an average temperature of 30.3˚C and relative humidity of 74.9% for seven consecutive days. In the shade drying process, herb samples were placed in a well-ventilated room, spread evenly in an aluminum tray, and dried at 30˚C ± 5˚C and 50% - 70% relative humidity. For consistent drying, the materials were rotated every hour. Shade drying was carried out under natural airflow for a period of 14 days. Freeze drying was conducted at the Feed Safety and Phyto Nutrition Lab of Bangladesh Agricultural University, using a Heto Drywinner freeze drier (Heto-Holten A/S, Allerod, Denmark) to freeze herbs before placement at −50˚C for a duration of 2 to 3 days. After drying, the samples were stored in airtight plastic containers and maintained at room temperature in the nutrition laboratory until analysis was performed.

2.3. Determination of Proximate Components of Herbs Materials

To evaluate the proximate constituents of the three herbs subjected to three distinct drying techniques, samples were desiccated for 24 hours at 105˚C in a forced-air oven and then ground into powder using 1 mm sieve for proximate evaluation. According to the method of the Association of Official Analytical Chemists [15], analysis techniques specified that dry matter was assessed by drying at 105˚C for 24 hours, in addition to the examination of crude protein, ether extract, and ash. The technique employed to ascertain acid detergent fiber and neutral detergent fiber was developed by [16].

2.4. Extraction of Herbs Materials

With certain modifications, the method of [17] was used to extract the moringa herb, pineapple waste, and plantains. A 500 mg sample of ground herb was measured into a test tube. Ten milliliters of 80% aqueous methanol were added, and the suspension was gently stirred. The tubes were vortexed for one minute and centrifuged at 1500 g for ten minutes, after which the supernatants were collected. Precipitates were subjected to re-extraction. The combined supernatants served as extracts. The concentrated extracts were subjected to lyophilization and subsequently weighed.

2.5. Determination of Total Phenolic Content

The Folin-Ciocalteu technique was used to determine the total phenolic content of herbal extracts [18]. To put it briefly, 1 milliliter of extract was carefully combined with 2.5 milliliters of 10% Folin-Ciocalteu reagent. Three milliliters of 2% Na_2CO_3 were added to the mixture after it had settled for three minutes. The mixture was incubated for two hours at 25˚C and 125 rpm in the dark, and its absorbance at 760 nm was measured using a UV-VIS spectrophotometer. The total phenolic content was measured in milligrams of gallic acid equivalents (GAE) per gram of dry matter.

2.6. Determination of Total Flavonoid Content

The aluminum chloride method was used to measure the total flavonoid concentration [19]. To put it briefly, a volumetric flask measuring 10 milliliters was filled with 1 milliliter of the extracted sample and 4 milliliters of water. Five minutes later, add 0.3 mL of 10% aluminum chloride and 0.3 mL of 5% sodium nitrite. One milliliter of 1 M sodium hydroxide was added to the mixture after it had been incubated for six minutes at room temperature. Distilled water was immediately added to reach the ultimate volume of 10 milliliters. A UV spectrophotometer was used to measure the sample's absorbance at 510 nm compared to the blank. Using milligrams of quercetin equivalent per gram of dry weight, quercetin was used as the reference. The experiment was conducted thrice to ensure accuracy.

2.7. Determination of Bioactive Compounds

Ultra-high-performance liquid chromatography was used to identify two specific bioactive compounds in each of three herbs. The bioactive compounds of moringa herbs (kaempferol, myricetin) were quantified using the methodologies outlined by Shervington *et al.* [20], the compounds in pineapple waste (gallic acid, catechin) were assessed following the procedures established by Li *et al.* [21], and the bioactive constituents of plantain herbs (aucubin and acteoside) were analyzed according to the techniques described by Al-Mamun *et al.* [22].

2.8. *In Vitro* Gas Production Kinetics

2.8.1. Animal and Diet

One bull with rumen fistulation, which was fed *ad libitum* Napier grass, was used

to obtain fresh rumen fluid. The concentrate mixture accounted for 1.5% of its body weight and contained 33% wheat bran, 20% khesari bran, 15% rice bran, 10% crushed maize, 10% crushed wheat, 5% soybean, 2% vitamin and mineral premix, 1% DCP, 1% salt, and 3% molasses. All the bulls have access to drinking water.

2.8.2. Collection of Rumen Fluid

On the research farm of the Bangladesh Livestock Research Institute, rumen fluid was extracted from an animal that had previously been fistulated. The collected rumen liquid was promptly combined, transferred to a preheated 39˚C vacuum flask, purged with CO_2, and then quickly capped to preserve anaerobic conditions. The flasks were conveyed to the laboratory in a thermostatic container to maintain a temperature of 39˚C. Four layers of pre-sterilized cheesecloth were used to filter the rumen fluid, and CO_2 was continuously flushed to reduce oxygen (O_2) exposure.

2.8.3. *In Vitro* Gas Production Measurement

Using an automated gas production measuring method created by Ankom Technology® (Macedon, NY, USA; ANKOMRF gas production system), the gas production (GP) kinetics of various moringa, pineapple waste, and plantain herbs were assessed [23]. This method tracks gas pressure in several modules, enabling the assessment of ruminal fermentation kinetics.

The system may support up to 50 distinct modules, each of which sends data to a computer via radio frequency. ANKOM pressure sensor modules, which include microchips and radio transmitters (pressure range: −69 to +3447 kPa; resolution: 0.27 kPa; precision: 0.1% of observed readings), are included in each module. This module is housed in a septa glass bottle with a 313-milliliter real capacity. The measured gas pressure was converted into moles of gas using the "ideal" gas law (Equation (1)) and into milliliters (mL) of gas using Avogadro's equation (Equation (2)).

$$\text{Number of moles of gas (n)} = p(V/RT) \quad (1)$$

where: n is the amount of gas created in moles (mol), p is the pressure in kilopascals (kPa), V is the headspace volume in the glass container in liters (L), T is the temperature in Kelvin (K), and R is the gas constant (8.314472 L·kPa/K/mol).

$$\text{Gas production in milliliters (mL)} = n \times 22.4 \times 1000 \quad (2)$$

The *in vitro* fermentation experiment was performed in triplicate for each treatment, with a control module incorporated into the system. Rumen fluid was collected aseptically from a cannulated animal and maintained at 39˚C until use. The same batch of rumen fluid was used for all treatments within each experimental run to minimize variation. The incubation of the target sample was carried out in a module containing 200 mg of respective feedstuff, 80 mL of buffer medium, and 20 mL of rumen fluid as inoculum. As stated by Goering and Van Soest (1970) [24], the buffer medium's composition included *in vitro* buffer solution

(NH_4HCO_3, $NaHCO_3$), resazurin 0.1% (w/v) solution, *in vitro* micro mineral solution ($CaCl_2 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$), KH_2PO_4 anhydrous, *in vitro* macro mineral solution (Na_2HPO_4 anhydrous, $MgSO_4 \cdot 7H_2O$), and reducing solution (Cysteine HCl, 1N NaOH, $Na_2S \cdot 9H_2O$). All of the glassware, solution, and inoculum were maintained at 39˚C before placing them into the respective module. Finally, all the materials (target sample, buffer medium, and inoculum) were placed into their respective modules, purged with CO_2 further, and then placed in a shaking incubator with a temperature of 41˚C and a rotation speed of 100 rpm. The fermentation was carried out until the cumulative gas pressure reached a stationary phase.

According to [25], equations for analyzing nutrients and estimating digestible organic matter and metabolizable energy were based on the gas production (GP) value after 24 hours.

$$\text{Percentage of digestible organic matter} = 15.38 + 0.8453\ GP_{24} + 0.0595\ CP + 0.0675\ CA$$

$$\text{Metabolizable energy (MJ per kilogram DM)} = 2.43 + 0.1206\ GP_{24} + 0.0069\ CP + 0.0187\ CF$$

where: GP_{24} denotes gas production (mL per 200 mg DM) within 24 hours of incubation, while CP, CA, and CF stand for crude protein, crude ash, and crude fat, respectively, at g per kilogram DM.

2.9. Statistical Analysis

The experiment was conducted using a factorial arrangement of treatments consisting of herb type and drying method. Each herb × drying method combination was replicated independently (n = 3) times.

Data were analyzed using a two-way Analysis of Variance (ANOVA) with the following statistical model:

$$Y_{ijk} = H_i + D_j + (HD)_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the observed response variable is the overall mean, H_i is the effect of the ith herb, D_j is the effect of the jth drying method, $(HD)_{ij}$ is the herb × drying method interaction effect, and ε_{ijk} is the residual error. The data were examined using SPSS 20.0, a statistical program. Additionally, the treatment averages for several parameters were compared using Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Nutrient Composition of Herbs at Different Drying Methods

The nutritional composition of herbs, derived from various drying techniques, is presented in Table 1. The findings indicated that the amount of dry matter of plantain herbs, moringa, and pineapple waste was considerably ($P < 0.05$) lower in samples that were freeze-dried as opposed to those that were dried in the sun or shade. Crude protein, ADF, and NDF did not significantly differ ($P > 0.05$) across all herbs under any of the various drying methods. The Ash content of pineapple waste herb was considerably ($P < 0.05$) lower in freeze drying, followed

by shade and sun drying. The ether extract content of plantain herb was ($P < 0.05$) in freeze drying, followed by shade and sun drying. There was a significant herb × drying method interaction effect on the dry matter (DM) content of all herbs ($P < 0.05$).

Table 1. Nutritional composition of herbs under three drying methods and the associated herb × drying method interactions.

Herb type	Drying method			SEM	H	D	H × D
	Sun	Shade	Freeze		P-value		
Moringa							
DM	8.62[b]	10.94[a]	7.72[c]	0.482	0.001	0.001	0.001
CP	17.05	17.75	17.86	0.240	0.001	0.405	0.842
ADF	32.78	32.88	32.75	0.180	0.001	0.974	0.904
NDF	49.27	48.40	47.93	0.310	0.001	0.229	0.342
EE	3.58	3.39	3.26	0.070	0.001	0.247	0.991
Ash	8.51	8.41	8.27	0.170	0.001	0.883	0.747
Pineapple waste							
DM	8.31[b]	9.9[a]	7.76[c]	0.320	0.001	0.001	0.001
CP	6.94	7.06	7.24	0.060	0.001	0.157	0.842
ADF	31.05	30.79	30.69	0.070	0.001	0.095	0.904
NDF	59.66	58.41	57.97	0.340	0.001	0.108	0.342
EE	2.88	2.69	2.55	0.060	0.001	0.056	0.991
Ash	8.62[a]	8.17[ab]	8.07[b]	0.040	0.001	0.040	0.747
Plantain							
DM	9.67[a]	10.33[a]	8.53[b]	0.280	0.001	0.002	0.001
CP	13.94	14.50	14.83	0.231	0.001	0.231	0.842
ADF	26.26	26.03	26.13	0.09	0.001	0.645	0.904
NDF	37.41	37.33	37.40	0.09	0.001	0.952	0.342
EE	2.94[a]	2.72[b]	2.66[b]	0.040	0.001	0.006	0.991
Ash	14.07	14.16	13.99	0.06	0.001	0.586	0.747

DM: Dry matter; CP: Crude Protein; ADF: Acid detergent fibre; NDF: Neutral detergent fibre; H: Herb; D: Drying method; SEM: Standard error of the mean; a - c: Means with different superscripts in the same row differed significantly at $P < 0.05$.

3.2. Herbs Total Phenolic and Flavonoid Content at Three Different Drying Methods

The total phenolic and flavonoid concentration in herbs subjected to various drying techniques is presented in Table 2. The present findings showed that the concentration of total phenolics and flavonoids in moringa, pineapple waste, and plantain herbs was considerably ($P < 0.05$) greater in freeze drying compared to shade and sun drying. The results showed that the interaction between herb type and drying method had a significant effect on total phenolic and total flavonoid content of all herbs ($P < 0.05$).

Table 2. Total phenolic and flavonoid contents of herbs as affected by drying method and herb × drying method interaction.

Herb type	Drying method			SEM	H	D	H × D
	Sun	Shade	Freeze		P-value		
Moringa							
Total phenolic (mg/gdw)	3.56^{c}	4.92^{b}	5.96^{a}	0.346	0.001	0.001	0.001
Total flavonoid (mg/gdw)	3.39^{c}	4.29^{b}	5.33^{a}	0.279	0.001	0.001	0.001
Pineapple waste							
Total phenolic (mg/gdw)	5.20^{c}	6.62^{b}	6.95^{a}	0.270	0.001	0.001	0.001
Total flavonoid (mg/gdw)	5.92^{c}	6.71^{b}	7.00^{a}	0.16	0.001	0.001	0.001
Plantain							
Total phenolic (mg/gdw)	6.15^{c}	8.19^{b}	11.15^{a}	0.73	0.001	0.001	0.001
Total flavonoid (mg/gdw)	4.13^{c}	6.59^{b}	8.61^{a}	0.647	0.001	0.001	0.001

SEM: Standard error of the mean; a - c: Means with different superscripts in the same row differed significantly at $P < 0.05$.

3.3. Moringa Herb Bioactive Compounds

The bioactive components of the moringa herb are displayed in Table 3. The results indicated that the bioactive component kaempferol was considerably ($P < 0.05$) elevated in freeze-dried herbs compared to those subjected to shade and sun drying. The bioactive component myricetin was considerably ($P < 0.05$) increased in freeze-dried moringa herb compared to shade and sun drying.

Table 3. Herbs-specific bioactive compounds at different drying methods.

Herbs	Bioactive compound	Drying method			SEM	P-value
		Sun drying	Shade drying	Freeze drying		
Moringa	Kaempferol (mg/g)	6.28^{c}	7.20^{b}	7.95^{a}	0.240	<0.001
	Myricetin (mg/g)	0.13^{c}	0.15^{b}	0.19^{a}	0.009	<0.001
Pineapple waste	Gallic acid (mg/g)	5.16^{c}	6.06^{b}	6.74^{a}	0.229	<0.001
	Catechin (mg/g)	5.40^{c}	6.16^{b}	6.86^{a}	0.212	<0.001
Plantain	Aucubin (mg/g)	3.66^{c}	4.46^{b}	6.29^{a}	0.390	<0.001
	Acteoside (mg/g)	22.90^{c}	25.83^{b}	30.84^{a}	1.160	<0.001

SEM: Standard error of the mean; a – c: Means with different superscripts in the same row differed significantly at $P < 0.05$.

3.4. Pineapple Waste Herb Bioactive Compounds

The pineapple waste herb bioactive compounds are shown in Table 3. The results indicated that the bioactive compound gallic acid was considerably ($P < 0.05$) elevated in freeze-dried pineapple waste herbs compared to those subjected to shade and sun drying. The bioactive component catechin was considerably ($P < 0.05$) elevated in freeze-dried herbs compared to those subjected to shade and sun drying.

3.5. Plantain Herb Bioactive Compounds

The plantain herb bioactive compounds are presented in Table 3. The results indicated that the bioactive component aucubin was considerably ($P < 0.05$) elevated in freeze-dried plantain herb, followed by shade-dried and sun-dried herbs. The bioactive compound of acteoside was also considerably ($P < 0.05$) greater in herbs that were freeze-dried as opposed to those that were shade and sun-dried.

3.6. *In Vitro* Gas Production Kinetics of Herbs at Different Drying Methods

The kinetics of *in vitro* gas production for herbs subjected to various drying processes are illustrated in Table 4. Regarding moringa, the freeze-drying method produced the highest amount of gas at 24 h (25.7 mL), followed by 24.1 mL for shade drying and 14.0 mL for sun drying, which was the lowest ($P < 0.001$). The digestible organic matter (53.3%) and metabolizable energy (7.35 MJ/kg DM) of the freeze-drying method for moringa were considerably ($P < 0.05$) superior to those of the other drying techniques. A similar scenario was observed regarding the pineapple waste, where the freeze-drying method was better with GP24, dOM, and ME compared to the other two drying methods. The dOM and ME of plantain using the freeze-drying method were more effective (42.8% & 5.76 MJ per kg DM, respectively) compared to shade drying (42.3% & 5.70 MJ per kg DM, respectively) and sun drying (39.9% & 5.42 MJ per kg DM, respectively) ($P < 0.01$).

Table 4. *In vitro* gas production kinetics of herbs at different drying methods.

Herbs	Drying method	GP_{24}	dOM (%)	ME (MJ/kg DM)
Moringa	Sun	14.0^{c}	43.1^{c}	5.94^{c}
	Shade	24.1^{b}	52.0^{b}	7.18^{b}
	Freeze	25.7^{a}	53.3^{a}	7.35^{a}
	SEM	0.08	0.05	0.008
	P-value	<0.001	<0.001	<0.001
Pineapple waste	Sun	15.8^{c}	38.7^{c}	5.34^{c}
	Shade	25.6^{b}	46.7^{b}	6.49^{b}
	Freeze	26.6^{a}	47.6^{a}	6.60^{a}
	SEM	0.051	0.033	0.201
	P-value	<0.001	<0.001	<0.001
Plantain	Sun	12.4^{c}	39.9^{c}	5.42^{c}
	Shade	14.7^{b}	42.3^{b}	5.70^{b}
	Freeze	15.2^{a}	42.8^{a}	5.76^{a}
	SEM	0.43	0.45	0.53
	P-value	<0.001	<0.001	<0.001

GP_{24}: *In vitro* gas production at 24 h; dOM: Digestible organic matter; ME: Metabolizable energy; SEM: Standard error of the mean; a - c: Means with different superscripts in the same column differed significantly at $P < 0.05$.

4. Discussion

4.1. Nutrient Composition of Herbs at Different Drying Methods

Drying is the most commonly employed method for prolonging the shelf life of leafy greens. Numerous losses transpire during the drying process, including alterations in the chemical, physical, and nutritional makeup of the leaves. In this study, the amount of dry matter of all herbs was greater in freeze-drying compared to sun and shade drying procedures. The authors [26] stated that drying methods significantly affected the moisture content, antioxidant activity, and concentrations of phenolic and flavonoid compounds in herbs. This outcome aligned with the findings of [27], who determined that the dry matter content of shade-dried moringa herb samples exceeded that of sun and cabinet drying methods. However, [28] reported that Cabinet dried moringa samples were better than others, and they had the highest nutrient retention, followed by shadow, sun drying and oven dried samples. The impact of several drying techniques, including oven, sun, shade, and freeze-drying, was examined. It was observed that freeze-drying was the most efficient method for nutrient retention and moisture removal, whereas sun drying was the least effective method [29]. Regarding the crude protein, ADF, NDF, and ash content of herbs, the results of the current investigation showed no significant differences ($P > 0.05$) across the various drying methods. The researcher of [30] [31] indicates that elevated temperatures and extended exposure can affect protein content through the denaturation of protein structures. Additionally, they observed a notable reduction in protein alongside an elevation in fiber and ash content in the moringa plant with extended duration and elevated temperature. The authors also stated that the drying of vegetables leads to the breakdown of nutrients [32].

4.2. Herbs Total Phenolic and Flavonoid Content at Three Different Drying Methods

Phenolic molecules in plants are linked to antioxidant and anticancer activities [33] [34]. Consequently, preserving phenolic chemicals during the drying, extraction, and isolation processes is crucial. The overall flavonoid and phenolic flavonoid content of herbs in the current investigation was greater in freeze-drying compared to shade and sun-drying procedures. The findings of the present study align with earlier studies on persimmon and pomegranate peel, indicating that the freeze-drying method preserves a greater concentration of total phenolic, flavonoid, and antioxidant capacity compared to conventional drying techniques [8] [35]. Freeze-drying was the most promising approach for retaining the nutraceutical qualities of moringa leaf when compared to sun drying and oven drying [36]. Different drying procedures dramatically changed the phytoconstituents (phenolics, flavonoids) of herbs. This is consistent with the results of [37], who found that freeze-dried samples of Moringa stenopetala leaf exhibited superior total phenolic and flavonoid concentration than samples prepared using other techniques. The drying process was significantly influenced by the total phenolic and flavonoid

concentration of *Moringa oliefera* leaf [38]. The quality of moringa powder derived from shade drying exceeded that obtained from sun drying and oven drying [39]. For plantain herbs, TPC and TFC of Plantago lanceolata leaf were significantly altered by the various drying methods (sunlight, thermostatic oven, and shade), whereas shade-drying was preferred to the others concerning the phytochemical content of dried samples [40]. The processing treatments and environmental factors, including temperature and light intensity, can influence phenolic content, while the authors recommended oven drying as a superior method for preserving phenolic compounds in albedo [41]. The TPC and TFC of *Allium hirtifolium* slices were found to be highest in the freeze-dried samples [42]. The investigation found that the total phenolic concentration in moringa leaf was lower, as stated by [43] [44], which were 32.90 mg per gram and 45.81 mg per gram, respectively.

4.3. Bioactive Content of Herbs at Different Drying Methods

Since freeze-drying extracts more bioactive components than other drying methods that employ heat, it is thought to be one of the most accurate methods for maintaining the nutrients and color quality of food products [45] [46]. The current findings of kaempferol, bioactive compound of freeze-dried moringa leaf extract, were slightly lower than the findings by several researchers [47] [48]. The bioactive constituents of moringa plants had concentrations of myricetin and kaempferol that varied from 406 - 2699 mg per kilogram & 1730 - 3440 mg per kilogram, each, and this was greater than the present findings. These may be due to the origin of moringa herbs from different geographical locations. The findings of [20] indicated that refluxing moringa leaves with a solution of 0.10 M hydrochloric acid for 24 hours yielded flavonols, specifically kaempferol (133 mg/kg) and myricetin (292 mg/kg), with myricetin being marginally higher and kaempferol lower than the current results. The content of bioactive compounds in pineapple waste was found to increase after drying [49]. Researchers have compared the drying of freeze, microwave, and infrared with traditional techniques like hot air, solar, tray, oven, and vacuum drying, examining the phytochemical losses in fruits during the drying process [50]-[53]. The drying process can significantly influence the quality of herbs by affecting their physicochemical properties and bioactive compounds [54]. The best drying techniques for maintaining or boosting phytochemicals in dried fruits were found to be infrared and freeze drying. Heat sensitivity is a well-known characteristic of numerous phytochemicals, including some polyphenols. The current findings corroborated those of a prior study on the bioactive substances in narrow-leaf plantains, which were sensitive to various drying methods [55]. The iridoid glycosides catapol and aucubin of Plantago lanceolata dropped by 50% and 25%, respectively, after the leaves were dried for 8 hours at 60˚C in comparison to the fresh biomass. The best method for maintaining the maximum concentration of bioactive compounds in African eggplants is freeze drying [56]. Freeze-drying is superior to oven-drying because the latter was

discovered to cause the degradation of bioactive substances in plants due to the impacts of heat treatment [57].

4.4. *In Vitro* Gas Production Kinetics of Herbs at Different Drying Methods

For assessing the fermentation characteristics of different feedstuffs, including herbs, *in vitro* gas production is a crucial technique. The present study observed the highest gas production volume in freeze-drying herbs, followed by shade and sun drying. The specific bioactive compounds of different herbs were also found to be higher in freeze drying than in shade and sun drying methods. The various processing techniques may have influenced their chemical composition and, consequently, their digestibility, which could be responsible for the variation in gas production. The authors obtained that starch and gas generation correlated positively, while NDF content and gas production were negatively correlated [58]. *In vitro* gas production and associated parameters are known to be influenced by the nutritional composition of feedstuffs [59]. Moreover, cumulative gas generation may also vary due to variations in botanical fractions (*i.e.*, tops, leaves, stems) and levels of anti-nutritional constituents, such as tannins [60] [61]. Freeze-drying preserves phenolic components such as tannins and flavonoids in herbs more effectively than shade or sun-drying. These chemicals create tannin-protein complexes that are resistant to breakdown in the rumen, hence enhancing the availability of digestible bypass protein for animal feeding [62].

5. Conclusion

It can be concluded that freeze-drying is a more effective method for preserving herbs compared to the other two methods, as it exhibits higher levels of total phenolic, flavonoid, and bioactive compounds. It also shows superior *in vitro* digestibility in ruminants. It is recommended that an *in vivo* study be required to validate the *in vitro* data on supplementing herbs in the ruminant diet, which will increase feed efficiency and diminish greenhouse gas (GHG) emissions.

Ethical Approval

The Institutional Committee for Animal Use and Ethics at the Bangladesh Livestock Research Institute approved all experimental protocols related to animal research (Memo No. T-4/(Part-6) 2015/1799; Date: 13/12/2020).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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