















## Research Article

# Development of Drying Methodology for Intact Whole Buffalo Liver, Its Characterization, Shelf Life, and Evaluation of Palatability as Pet Treat

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Globally, large quantity of animal byproducts is generated from the slaughter of food animals, but there is lack of research articles related to drying of these byproducts and its use as pet food. Therefore, this study was conducted with the aim of utilization of intact whole buffalo liver by drying for pet treat, evaluating its shelf life and palatability. The intact liver surface was superficially sliced, and the surface was pierced. Thereafter, the livers were pretreated in 3% sugar and 4% salt solution (1:3 w/v) for 3 h followed by microwaving for 4 min and hot air drying at 60°C for 40 h (designated as T2L). The livers which were dried the same as T2L except surface piercing were referred as T1L, whereas the livers dried only using hot air oven were referred as control (CL). The moisture and protein contents of the dried CL and T2L were found to be 28.46% and 14.29% and 43.85% and 52.76%, respectively. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) image of T2L revealed the presence of few low as well as high molecular weight protein bands which were absent in CL and T1L indicating a comparatively lower level of protein degradation in T2L. The shelf life of T1L and T2L samples based on microbiological and lipid oxidation analyses was found to be more than 60 days at 25 and 4°C. Palatability studies using dogs showed that all dried samples were highly palatable. Thus, it could be concluded that intact buffalo liver could be dried using surface slicing, with/without piercing followed by salt and sugar pretreatment, 4 min microwaving, and hot air drying at 60°C for 40 h. Future study should focus on the sensory properties such as aroma, texture, and flavor and sensory analysis of the dried liver by human.

## 1. Introduction

Offals are the byproducts that are obtained from animal slaughter, and it includes all animal tissues that are not the part of the carcass [1]. The byproducts generated from the meat industry are considered valuable as many value-added substances can be generated from these byproducts [2]. A total of 7.5% and 11.4% of gross income can be generated from effective utilization of pork and beef byproducts, respectively [3].

A large amount of animal byproducts are generated by the meat industry globally, and each year, more than 18 million tons of meat byproducts are generated from the slaughter of approximately 330 million pigs, cattle, sheep, and goats in the European Union [4]. Pork byproducts can account for up to the level 30% live weight, whereas cattle byproducts account for up to 40% of the live weight [5]. Value addition of these byproducts is one of the most efficient ways of utilization of these materials for the reduction of disposal costs and generation of additional income [6]. According to the 20<sup>th</sup> Livestock Census of India, there are 109.85 million buffaloes in India and approximately 12.85 million buffaloes were slaughtered in the year 2020-2021 [7]. This provides estimation about the volume of buffalo liver produced in a year and the need to convert this byproduct into high-value pet food.

Different packaging systems like modified atmosphere packaging might be used to extend the shelf life of meat [8, 9]. Alternatively, meat drying can be considered as the most practical method of storage and preservation of meat in the case of absence of the proper cold chain [10]. It is well suited for the developing countries, especially those countries having a very humid and hot climate [10]. There are numerous drying methods that are used to dry various products, and the choice out of numerous drying methods that are used to dry various products depends on the convenience, economy of operation, value of the final product, market requirement, shelf life, and some other factors. Freeze drying is used to dry high-value products where the product is initially frozen, the pressure is lowered, and low heat is applied so as to sublime the frozen water directly from solid to gas phase. It retains the required color, aroma, density, and rehydration properties but is quite expensive because of high energy required to induce sublimation and maintaining vacuum [11]. In fluidized bed drying, the product is fluidized by passing of high velocity hot air to the product. Much higher drying rate is achieved than traditional drying but is unsuitable for products having high moisture content, high surface area to volume ratio, and rough surfaces [12]. Supercritical CO<sub>2</sub> drying uses carbon dioxide as a drying medium at low temperature which is normally close to ambient temperature [12]. Major advantages of this drying technique are low or absence of oxygen, less shrinkage, and better dehydration capacity. The important hindrances in this drying technique are high equipment and operating cost [13]. Infrared radiation (IR) drying technique is used for rapid contactless drying rate, easy construction, and operation [14]. In pulsed vacuum drying (PVD), successive change in the vacuum pressure in the drying chamber improves the transfer

of moisture during drying [15]. The nonthermal drying takes place under oxygen deficiency and thus reduces oxidation and browning of the product. Further, in PVD, tunnelling effect increases the micropore size and also interconnects them in the product [16] and additionally aids in moisture mass transfer. Low processing capacity and high cost are the two major limitations associated with pulsed vacuum drying [17]. Microwave drying due to its fast volumetric heating and deep penetration is good for quick dehydration with low energy consumption in shorter period of time compared to many other drying techniques [18]. Better rehydration, color, and reduced shrinkage are the advantages of the microwave-dried products [19]. However, nonuniform drying and overheating are the two major problems with microwave drying [20]. Additionally, microwave drying suffers from thermal runaway and the machine to arc [21]. On the other hand, convective hot air drying (HAD) takes longer time and energy to dry the products [22]. In spite of such lacunae, this drying method is extensively used on industrial scale and more than 80% of industrial dryers are convective HAD systems [23]. To prevail over the drawbacks of microwave and hot air drying, a combined drying system of microwave-assisted hot air drying is preferred to preserve the product quality, enhance the heating uniformity, and improve the overall drying efficiency [18, 24].

Pet food industry is an ever-growing sector, and while purchasing pet food, the owners mainly consider two factors such as the quality of ingredients used in the manufacture of pet food and the amount of animal protein source that are included during manufacture [25]. While manufacturing of pet foods, the major animal protein sources considered are poultry meat and offals, red meats, horse flesh, fish flesh, and other organ meats [1]. Among the organs, the intestines, liver, lungs, kidney, spleen, udder, condemned meat, etc., are commonly used along with cereals [1]. Moreover, animal byproducts in the form of raw or rendered materials are used in pet food since long [26]. These are good sources of proteins, essential amino acids, trace elements, fatty acids, and various vitamins [26]. Pet treats are considered as one of the fast-growing segments in the pet food industry [27]. Currently, in the pet food market, there are different kinds of pet treats available such as hard biscuits, edible manufactured bones, and soft treats [27]. Among the different varieties of dog treats, indulgent treats (meat products) are the largest segment, accounting for 35% of dog treats [28]. Considering the above factors, this study was designed to determine the suitable method of drying of whole buffalo liver, characterize it, find the shelf life of the dried liver, evaluate its palatability by dogs, and study the perception of the dog owners about the dried buffalo liver as pet treat.

## 2. Materials and Methods

*2.1. Chemicals.* Three-color prestained protein ladder (10-250 kDa) was procured from Genetix Biotech Asia Pvt. Ltd., New Delhi, India. SDS-PAGE-related chemicals were obtained from HiMedia, Thane (West), Maharashtra, India. Ehrlich's reagent was supplied by Sisco Research Laboratories (SRL) Pvt. Ltd., Andheri (East), Mumbai, Maharashtra,

India. Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were supplied by Central Drug House (CDH) Pvt. Ltd, Kolkata, West Bengal, India. All media used in microbiological analysis were obtained from HiMedia Thane (West), Maharashtra, India.

**2.2. Materials.** The whole buffalo liver was procured from the nearby meat market of Indian Veterinary Research Institute, Bareilly, India. While collection and transportation of the intact whole buffalo liver to the laboratory, all hygienic precautions were observed to avoid any contamination and quality deterioration. Ice box filled with ice cubes was used to carry the samples. For palatability test, commercial pet treat Meat Jerky (Grilled Liver Flavour) manufactured by Pedigree (Mars Petcare (Thailand) Co. Ltd., Nakhon Ratchasima, Thailand) were purchased from a local pet shop.

**2.3. Preparation of Liver and Its Pretreatment.** After procurement of the whole intact buffalo liver, it was cleaned under running tap water at room temperature until the remaining portion of the blood was cleared. The excessive amount of fat adhering to the offals was removed manually. The surface of the liver was sliced along the long axis approximately 1.5 cm deep. Parallel slices were made approximately 2.5 cm apart using a sharp pointed knife. Holes were made on the surface of whole buffalo liver using an iron bristled brush having 30 cm length and 5 cm breadth using a brush having iron bristles fixed 1 cm apart. The process of slicing and piercing the surface of offals was done to ensure deep penetration of the pretreatment solution during pretreatment and the heat during the drying process. After surface slicing and piercing, the whole liver was put in the pretreatment solution (4% salt and 3% sugar solution) at 1:3 w/v for 3 h.

**2.4. Drying of Intact Whole Buffalo Liver Using a Combination of Microwaving and Convective Hot Air Drying.** The whole buffalo liver after pretreatment was microwaved for a period of 4 minutes in a microwave oven (LG Microwave Appliance, Model No. MC-808WAR, Microwave 1350 W, manufactured by LG Electronics India Pvt. Ltd.). The whole buffalo liver after microwaving was dried in a food dryer oven (Ambay Biotech, Ambala, Haryana, India). Custom-made stainless steel wire mesh having 40 cm length and 27.5 cm breadth was used to dry the buffalo livers. The mesh size used was 3.5 cm × 3.5 cm. The drying was carried out at a temperature of 60°C.

The liver samples which were superficially sliced along its long axis, pierced, pretreated with salt and sugar, microwaved, and hot air dried were referred as T2L. T1L sample refers to the same as T2L but without surface piercing. Control liver (CL) samples were only hot air dried without slicing, piercing, pretreatment, and microwaving.

**2.5. Drying Time.** Trials were conducted for standardizing the time for convective hot air drying of whole intact buffalo liver. The most appropriate drying time was found to be 40 h for the whole intact buffalo liver. There was no further significant weight reduction in the samples after 40 h of hot

air drying. No further appreciable decrease in the weight with the drying time was taken as the end point of drying.

## 2.6. Analytical Procedures

**2.6.1. Proximate Composition.** The proximate composition of fresh and dried buffalo liver was analysed in three replicates using the methods in [29].

**2.6.2. Calorific Value.** Gross energy of the fresh and dried buffalo liver was calculated in triplicate by the following equation according to NRC [30].

$$\begin{aligned} \text{Gross energy (kcal/kg)} = & (5.7 \times \text{protein}) + (9.4 \times \text{fat}) \\ & + (4.1 \times (\text{NFE} + \text{crude fibre})). \end{aligned} \quad (1)$$

**2.6.3. Color Parameters.** Color score of the fresh and dried buffalo liver was measured using Hunter Lab (MiniScan EZ, Model No. 4500L, Virginia, USA). The samples were loaded inside the glass container, and the measurements were taken in three coordinates, namely,  $L^*$ ,  $a^*$ , and  $b^*$ , where  $L^*$  designates lightness (values ranging from 0 to 100),  $a^*$  indicates redness or greenness (values may be positive or negative), and  $b^*$  indicates yellowness or blueness (values may be positive or negative). Triplicate determination was carried out to arrive at the mean.

**2.6.4. Hydroxyproline and Collagen Content.** Hydroxyproline content of the fresh and dried buffalo liver was determined using the method of Balti et al. [31]. 50 mg of buffalo liver sample was mixed with 2 mL of 6N HCl. Sample was hydrolysed by autoclaving (Marang Scientific Works Pvt. Ltd., New Delhi, India) at 120°C for a time period of 1.5 h. After hydrolysis, 3 parts of the hydrolysed sample were taken out and were neutralised with 7 parts of 2N NaOH. 100 µL of the neutralised sample was pipetted out in a test tube, and to that, 200 µL of isopropane alcohol was added followed by 100 µL of freshly prepared oxidant solution. The contents in the test tubes were mixed well and incubated at room temperature for 4 min. Thereafter, 1.3 mL of Ehrlich's solution was added and incubated at 60°C for a period of 25 min in a water bath (MSW-275, MAC, Delhi, India). After cooling, the absorbance was measured using spectrophotometer (GENESYS 10S UV-VIS spectrophotometer, Model GEN10S UV-VIS Thermo Fisher Scientific, Madison, WI, USA) at the wavelength of 558 nm. The final concentration of hydroxyproline in the samples was expressed as milligrams of hydroxyproline per gram of sample. The analysis was conducted in three replicates to calculate the mean.

The hydroxyproline content of the fresh and dried buffalo liver was converted into collagen content by multiplying the hydroxyproline (mg/g) content of the dried liver sample with a factor of 7.54 [32].

$$\begin{aligned} \text{Collagen content of the sample (mg/g)} \\ = \text{hydroxyproline (mg/g)} \times 7.54. \end{aligned} \quad (2)$$

**2.6.5. Shear Force.** Shear force of the fresh and dried buffalo liver was determined by the Warner-Bratzler shear press (G-R Elec. Mfg. Co., Manhattan, KS, USA) using the method suggested by Berry and Stiffler [33] with modifications. The samples were accurately cut into 1 cm width and 1 cm height and enough length to hold in the blades of the shear press. The sample was placed perpendicularly to the blade and allowed to shear the sample. The shear force ( $\text{kg}/\text{cm}^2$ ) was noted down in three replicates by the deflection of the needle in the dial of the equipment.

**2.6.6. Water Activity ( $a_w$ ).** Water activity of the fresh and dried buffalo liver samples was estimated in triplicate using a hand-held portable digital water activity meter (AquaLab 4TE Dew Point Water Activity Meter, Decagon Devices, WA, USA).

**2.6.7. Determination of Protein Degradation by Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE).** SDS-PAGE of fresh and dried buffalo liver was performed using the method of Laemmli [34]. For sample preparation, 3 g of the sample was taken into which 9 mL of 0.05 M phosphate buffer (pH 7.4) was added at 1:3 ratio ( $w/v$ ). It was homogenized at 11000 rpm for 2 min using a tissue homogenizer (ULTRA-TURRAX T 25, Janke & Kunkel GmbH & Co. KG, IKA Labortechnik, Staufen, Germany). The homogenized samples were centrifuged at 5000 rpm for 2 min (REMI Laboratory Centrifuge, Model R-8C, Remi Equipments, Bombay, India). The supernatant was collected, and it was repeatedly centrifuged 2 times at 5000 rpm for 2 min. The clear supernatant was taken out for analysis.

For casting the gel, 12% of resolving gel and 4% of stacking gel were used. The sample preparation was done by mixing the sample with the sample buffer in the ratio of 1:2.5  $\mu\text{L}$  of prestained protein ladder was loaded followed by 15  $\mu\text{L}$  of the prepared samples. During electrophoresis, for the first 15 min, a current at the rate of 15 mA/gel was used, which was increased to 25 mA/gel. Reaching of bromophenol blue at the bottom of the gels indicated completion of the process, and it was followed by staining and destaining of the gels.

**2.6.8. Microstructure Analysis.** The microstructure analysis of fresh and dried buffalo liver samples was analysed using a scanning electron microscope (Cube-II, EMCRAFTS, Gyeonggi-do (12814), Republic of Korea). The samples were processed according to Běhalová et al. [35] with slight changes. The samples were cut in the dimension of 1 mm thickness, 1 cm length, and 0.5 cm breadth followed by overnight fixation in 10% formaldehyde. The formaldehyde fixed samples were then put in ascending grades of ethanol in the order of 10%, 30%, 50%, 70%, 90%, 100%, and again in 100% for 5–10 minutes each. The processed samples were then fixed onto the stubs using adhesive tape followed by gold coating (G20 Ion Sputter Coater, GSEM Co., Ltd., Suwon City, Gyeonggi-do, South Korea). The gold-coated samples were visualised at 1000x.

**2.6.9. Weight Loss Percentage of Dried Buffalo Liver.** The weight loss percentage of dried whole buffalo liver that occurred during the process of drying of fresh whole buffalo

liver was calculated in three replicates using the following equation:

$$\begin{aligned} \text{Weight loss percentage} \\ = \frac{\text{weight of fresh liver} - \text{weight of dried liver}}{\text{weight of fresh liver}} \times 100. \end{aligned} \quad (3)$$

**2.6.10. Shrinkage Percentage of Dried Buffalo Liver.** The shrinkage percentage of dried whole buffalo liver was estimated using the water displacement method. The volume of fresh liver before drying and dried liver (after wrapping in plastic cling film) was noted separately by dipping it in the volumetric beaker filled with water, and the volume of displaced water was noted and recorded as the volume of samples. The shrinkage percentage was calculated in three replicates using the following formula:

$$\begin{aligned} \text{Shrinkage percentage} \\ = \frac{\text{volume of fresh liver} - \text{volume of dried liver}}{\text{volume of fresh liver}} \times 100. \end{aligned} \quad (4)$$

**2.6.11. Rehydration Ratio (RR) of Dried Buffalo Liver.** The rehydration ratio of dried buffalo liver was determined by the method described by Aksoy et al. [36] with some modifications. 5 g of dried buffalo liver sample was weighed and put it into a beaker containing 50 mL of distilled water which was maintained at 30°C. The sample was taken out and weighed every 30 min until it reached a constant weight. Once the constant weight reached, the sample was taken out and the excess water was removed from the surface using a blotting paper and weighed. The rehydration ratio was calculated in triplicate using the following equation:

$$\text{Rehydration ratio (RR)} = \frac{\text{weight of rehydrated sample}}{\text{weight of dried sample}}. \quad (5)$$

## 2.7. Storage Study of Dried Buffalo Liver

**2.7.1. Microbiological Analysis.** The microbiological parameters (total plate count, coliform count, and yeast and mold count) of dried liver were analysed using FSSAI [37] method. Two sets of dried liver (CL, T1L, and T2L) were packed in LDPE bags and sealed. One set was stored at room temperature ( $25 \pm 1^\circ\text{C}$ ), and the second set was stored at  $4 \pm 1^\circ\text{C}$ . For microbiological analysis, total plate count, coliform count, and yeast and mold count were recorded at every two weeks (0, 15, 30, 45, and 60 days) of storage. All plates were made in triplicate, and the microbial count was recorded as log CFU/g.

**2.7.2. Lipid Oxidation Index.** Lipid oxidation was determined by analysing thiobarbituric acid reactive substance (TBARS) value and free fatty acid values over a period of 60 days with two-week intervals. Similar experimental design



as used for the microbiological studies was also utilized in three replicates to determine the lipid oxidation index of stored dried buffalo liver samples.

(1) *Thiobarbituric Acid Reactive Substance (TBARS) Value.* TBARS values of the dried buffalo offals during the storage study were determined using the method described by Witte et al. [38]. To 10 g of dried buffalo liver, 25 mL of 20% pre-cooled trichloroacetic acid (TCA) solution was added. Triturated samples were transferred to a beaker, and the content was filtered using a Whatman No. 1 filter paper, and the filtrates were considered as TCA extract. 5 mL of TCA extract was mixed with 5 mL of 5 mM thiobarbituric acid (TBA) solution in a test tube. The test tubes were incubated at 70°C for a period of 30 min in a water bath (MSW-275, MAC, Delhi, India). The absorbance was measured at 523 nm and was converted into milligrams of malonaldehyde per kilogram of sample by multiplying the absorbance value with 5.2. The mean values were determined by carrying out the experiment in triplicate.

(2) *Free Fatty Acid.* The free fatty acid value of dried buffalo offals during the storage study was determined using the titrimetric method [29]. 50 mL of 95% ethanol was added to 5 g of grounded dried buffalo liver sample. Four drops of 1% phenolphthalein indicator were added and titrated against 0.1 N KOH solution. The end point was noted by the appearance of pink color which persisted for at least 10 seconds. Free fatty acid value was determined in triplicate using the following equation:

$$\text{Free fatty acid as oleic acid} = \frac{28.2 \times N \times V}{W}, \quad (6)$$

where  $W$  is the weight of sample taken (g),  $N$  is the normality of KOH used for titration (0.1 N), and  $V$  is the volume of 0.1 N KOH used during titration (mL).

**2.8. Palatability Evaluation of Dried Whole Buffalo Liver.** Palatability evaluation of liver samples and commercial dog treats was conducted by acceptance and preference tests. All feeding trials were carried out on the experimental dogs according to the approved guidelines put forward by IAEC/CPCSEA (Protocol No. IAEC/07.07.2022/L2). Tests were conducted on 5- to 6-year-old four dogs (one Beagle, two Dalmatians, and one Spitz) kept in the Experimental Animal Shed, Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India. The feeding trials were performed in a Latin square design. Three days wash off period was given after every set of feeding trials. The whole dried buffalo liver was cut into pieces measuring 1.5 cm length, 1 cm breadth, and 1 cm thickness. The level of feeding was fixed at 10% of the total calorie intake of the dog.

**2.8.1. Acceptance Test for Dog.** The acceptance test was done by single-bowl test according to Griffin [39] with some alterations. The required amount of food was weighed and provided to the dog in a bowl. The time in seconds taken for

the dog to consume the sample was noted. For comparison, a commercial pet treat was also tested in dogs. The time taken for consuming the commercial pet treat was also noted. The test was carried out three times to determine the mean time.

**2.8.2. Preference Test for Dogs.** Preference test was done by two-bowl test according to Hutton [40] with slight changes. The required amount of sample was taken in one bowl, and the commercial pet treat was taken in the second bowl at the same time. The dogs were first allowed to sniff both bowls, and after that, the bowls were provided to the dogs at the same time. The dogs were freely allowed to choose the food according to its preference. The bowl from which the dog consumed first was noted. The test was repeated three times to arrive at the final conclusion.

**2.8.3. Perception of Pet Owners.** The perception of pet owners towards dried buffalo liver was evaluated according to Koppel [41]. For the evaluation, twenty-two dog owners and eighteen cat owners were selected. They were given with three packets of dried buffalo liver samples consisting of CL, T1L, and T2L to be fed according to the body weights of their pets (for dog owners, the samples were cut into 1.5 cm length, 1 cm breadth, and 1 cm thickness; for cat owners, the samples were cut into 1 cm length, 0.5 cm breadth, and 0.5 cm thickness). The level of feeding was advised to the owners (10% of total calorie intake), and they were allowed to give the dried liver samples to their pets kept at their homes (one type of sample for three consecutive days, once in a day, and 2 h after the regular feeding). The responses from pet owners were collected using the questionnaires provided to them in a printed format.

**2.9. Statistical Analysis.** For each experiment, three sets of trials were conducted with duplicates in each set to ensure the consistency of results. Variance and least significant difference (LSD) of the results were analysed as per Snedecor and Cochran [42]. The means were compared with one-way and two-way ANOVA followed by Duncan's multiple range test [43] in IBM® SPSS® software version 26.

### 3. Results and Discussion

**3.1. Proximate Composition, Hydroxyproline, and Collagen Content of Fresh Buffalo Liver.** The proximate composition and physicochemical properties of fresh buffalo liver are given in Table 1. Wazir et al. [44] reported that the fresh cattle liver contains 71.94 to 71.90% moisture, 18.32 to 20.10% protein, 4.90 to 7.01% fat, and 1.23 to 1.31% of ash. Biel et al. [45] reported that the liver of beef cattle maintained in an organic production system was having an energy value of 136 kcal/100 g. These literatures agree with the results obtained in this study.

The collagen and hydroxyproline content in the fresh buffalo liver was found to be 2.18% and 0.29%, respectively. Babicz et al. [46] reported 1.77% collagen in porcine liver (Polish Landrace breed).

TABLE 1: Proximate composition and physicochemical parameters of fresh (FL) and dried buffalo liver. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Parameters/liver samples	FL	CL	T1L	T2L
Moisture (%)	69.90 ± 0.79	28.46 ± 0.19 <sup>a</sup>	17.37 ± 0.12 <sup>b</sup>	14.29 ± 0.16 <sup>c</sup>
Crude fat (%)	7.63 ± 0.23	17.33 ± 0.11 <sup>b</sup>	19.79 ± 0.29 <sup>a</sup>	20.28 ± 0.26 <sup>a</sup>
Crude protein (%)	18.46 ± 0.47	43.85 ± 0.16 <sup>c</sup>	50.57 ± 0.13 <sup>b</sup>	52.76 ± 0.20 <sup>a</sup>
Ash (%)	1.34 ± 0.05	3.19 ± 0.02 <sup>c</sup>	3.71 ± 0.05 <sup>b</sup>	3.88 ± 0.01 <sup>a</sup>
Total carbohydrates (%)	2.67 ± 0.12	7.17 ± 0.11 <sup>b</sup>	8.56 ± 0.33 <sup>a</sup>	8.77 ± 0.28 <sup>a</sup>
Gross energy (kcal/kg)	1878.88 ± 49.99	4422.60 ± 15.19 <sup>c</sup>	5093.88 ± 20.70 <sup>b</sup>	5273.74 ± 10.30 <sup>a</sup>
Color				
<i>L</i> *	15.37 ± 0.24	10.87 ± 0.26 <sup>a</sup>	7.51 ± 0.14 <sup>b</sup>	6.00 ± 0.09 <sup>c</sup>
<i>a</i> *	2.70 ± 0.20	2.70 ± 0.26 <sup>a</sup>	2.05 ± 0.06 <sup>b</sup>	2.62 ± 0.13 <sup>ab</sup>
<i>b</i> *	4.93 ± 0.08	3.48 ± 0.16 <sup>c</sup>	4.11 ± 0.05 <sup>b</sup>	4.69 ± 0.22 <sup>a</sup>
Hydroxyproline (%)	0.29 ± 0.001	0.33 ± 0.001 <sup>c</sup>	0.36 ± 0.001 <sup>b</sup>	0.40 ± 0.002 <sup>a</sup>
Collagen (%)	2.18 ± 0.10	2.53 ± 0.10 <sup>c</sup>	2.76 ± 0.005 <sup>b</sup>	3.03 ± 0.02 <sup>a</sup>
Shear force (kg/cm <sup>2</sup> )	1.23 ± 0.01	1.32 ± 0.03 <sup>c</sup>	1.47 ± 0.02 <sup>b</sup>	2.77 ± 0.03 <sup>a</sup>
Water activity	0.991 ± 0.0001	0.864 ± 0.001 <sup>a</sup>	0.715 ± 0.002 <sup>b</sup>	0.592 ± 0.004 <sup>c</sup>
Weight loss (%)	NA	58.71 ± 0.38 <sup>c</sup>	67.70 ± 0.47 <sup>b</sup>	72.20 ± 0.47 <sup>a</sup>
Shrinkage (%)	NA	52.34 ± 0.47 <sup>c</sup>	62.25 ± 0.64 <sup>b</sup>	68.17 ± 0.44 <sup>a</sup>
Rehydration ratio	NA	1.23 ± 0.0002 <sup>c</sup>	1.31 ± 0.002 <sup>a</sup>	1.24 ± 0.002 <sup>b</sup>

$n = 6$ ; mean ± SE; mean values between columns with different superscripts (a, b, and c) are significantly different ( $P < 0.05$ ). NA refers to not applicable.

### 3.2. Proximate Composition and Physicochemical Properties (Hydroxyproline, Collagen, Shear Force, Water Activity, Weight Loss, Shrinkage, and Rehydration Ratio) of Dried Buffalo Liver

**3.2.1. Proximate Composition of Dried Buffalo Liver.** The proximate composition of dried whole buffalo liver (CL, T1L, and T2L) is represented in Table 1. To the best of the authors' knowledge, there are no literatures regarding the proximate composition of dried liver of buffalo. The significantly ( $P < 0.05$ ) lower moisture content in T2L and T1L compared to CL was due to the effect of microwaving, deeper heat penetration, and easy moisture loss during convective hot air drying due to surface slicing and piercing. The crude fat percentage of T2L and T1L (20.28 and 19.79%, respectively) was found to be significantly ( $P < 0.05$ ) higher than the crude fat percentage of CL (17.33%). Crude protein of T2L (52.76%) was significantly ( $P < 0.05$ ) higher than that of T1L (50.54%) and CL (43.85%). Total ash of T2L (3.88%) was significantly ( $P < 0.05$ ) higher compared to T1L (3.71%) and CL (3.19%).

**3.2.2. Color of Dried Buffalo Liver.** The color parameters of dried buffalo liver (CL, T1L, and T2L) are given in Table 1. No corresponding literature was found to correlate the obtained results. The  $L^*$  value of CL (10.87) was significantly higher compared to that of T1L (7.51) and T2L (6.00). The lower  $s$  value of T2L indicated its darker color. The  $a^*$  value of CL (2.70) was observed to be significantly higher ( $P < 0.05$ ) than that of T1L (2.05). The  $b^*$  value of T2L (4.69)

was significantly ( $P < 0.05$ ) higher than that of T1L (4.11) and CL (3.48) suggesting its higher yellowness than the other two treatment samples.

**3.2.3. Hydroxyproline and Collagen Content of Dried Buffalo Liver.** Collagen content and hydroxyproline content of dried liver (CL, T1L, and T2L) are given in Table 1. The authors could not find supporting research publications to corroborate the results. The hydroxyproline content of T2L was 0.40%, and it was significantly ( $P < 0.05$ ) higher compared to T1L (0.36%) and CL (0.33%).

**3.2.4. Shear Force of Dried Buffalo Liver.** The shear forces of dried buffalo liver samples (CL, T1L, and T2L) are given in Table 1. Shear force of T2L was 2.77 kg/cm<sup>2</sup>, and it was significantly ( $P < 0.05$ ) higher than that of T1L (1.47 kg/cm<sup>2</sup>) and CL (1.32 kg/cm<sup>2</sup>). The highest shear force of T2L sample might be due to the toughness produced by the greater moisture loss and shrinkage of T2L compared to other samples.

**3.2.5. Water Activity of Dried Buffalo Liver.** Growth of spoilage microbes is inhibited if the water activity is less than 0.60 [47]. But the water activity level itself will not eliminate all microbes. During the storage, if some favourable condition arises, the microbes will flare up and cause spoilage of the food. Reduction of moisture content in a food will result in the reduction of water activity also, but the moisture content and water activity are not directly proportional [48]. The least water activity of T2L sample compared to other samples was due to the less moisture content.

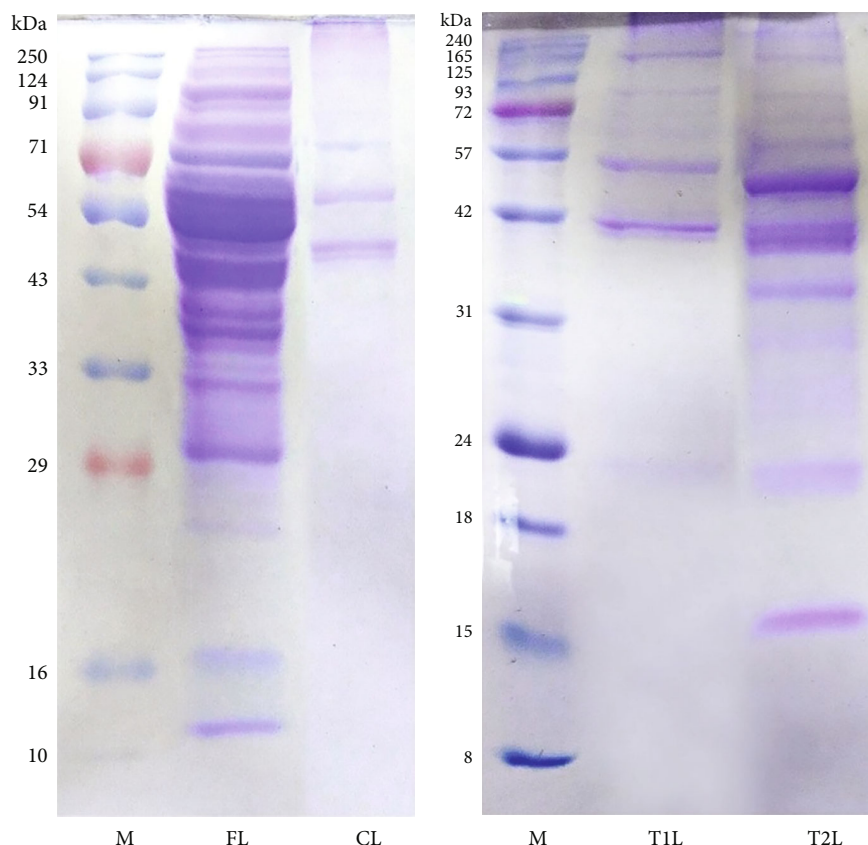


FIGURE 1: SDS-PAGE profile of fresh and dried buffalo liver samples. FL is a fresh liver sample. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying. M denotes the ladder (protein marker).

**3.2.6. Weight Loss, Shrinkage, and Rehydration Ratio of Dried Buffalo Liver.** The more weight loss percentage of T2L and T1L samples was attributed to the microwaving, surface slicing, and piercing of the samples that helped in easy moisture removal. Higher shrinkage of T2L may be attributed to the greater moisture loss in the T2L sample during drying.

Rehydration ratio of dried liver samples can be correlated with the microstructure of the samples discussed in Section 3.4. More rehydration ratio in T1L sample compared to T2L might be due to the more porous structure of T1L sample compared to that of T2L sample. As the porosity increases, the rehydration ratio also increases due to the trapping of water into the pores inside the samples during the process of rehydration [49]. The lowest rehydration ratio of CL might be due to the presence of more moisture content in CL samples.

**3.3. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Fresh and Dried Buffalo Offals.** The electrophoretic profiles of fresh and dried buffalo liver samples are given in Figure 1. The SDS-PAGE image of FL revealed many protein bands ranging from 240 kDa to 10 kDa, whereas in the three samples (CL, T1L, and T2L), few protein bands particularly lower than 33 kDa were absent indicating the degradation of these lower molecular

weight protein chains by heat and/or microwave treatments. The dried samples CL, T1L, and T2L showed faint band intensities of protein bands of molecular weight 124, 91, and more than 71 kDa compared to fresh liver sample (FL) due to breakdown of these proteins during the process of drying. The CL revealed the lowest molecular weight protein bands ranging between 54 and 43 kDa, and thereafter, only smear bands were visible. Similar pattern was found in T1L except that one faint band was visible near 29 kDa. Compared to CL and T1L, T2L samples showed protein chains of lower molecular weight also in addition to higher molecular weight protein chains, signifying the less harsh effect of heat on protein degradation due to surface slicing and piercing in T2L samples. The authors were unable to find similar work to further support their findings.

**3.4. Microstructure Analysis of Fresh and Dried Buffalo Liver.** The scanning electron microscopic images of FL, CL, T1L, and T2L are given in Figure 2. The FL sample was observed to be having relatively more porosity compared to the dried liver samples (CL, T1L, and T2L). As a result of drying, the samples shrunk and the structure became more solid. Among the dried liver samples, CL was found to be less porous than FL, T1L was having less porosity than CL, and T2L was having the least porosity. Giri and Prasad [49] compared different methods of drying of mushrooms and found out that air-



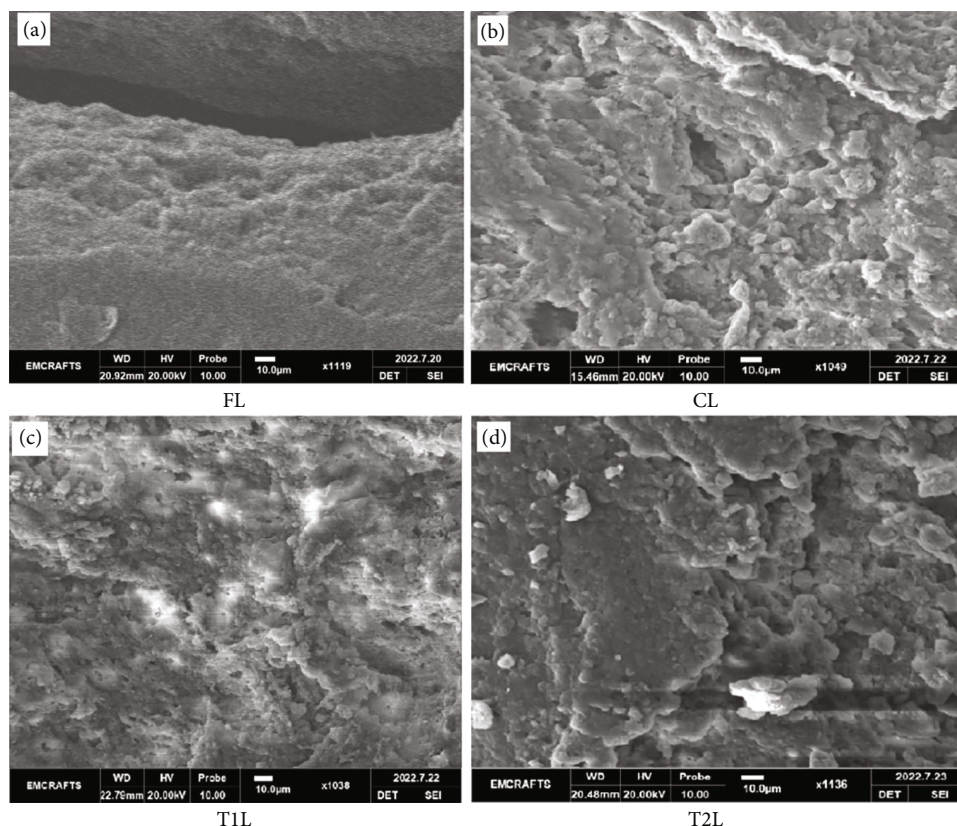


FIGURE 2: Scanning electron micrograms of fresh and dried buffalo liver samples: (a) FL is a fresh liver sample. (d) T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. (c) T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. (b) CL refers to buffalo liver dried simply by hot air drying.

dried mushroom samples were having less porosity and open structure compared to the microwave-vacuum dried samples due to the shorter drying time and lesser drying temperature in the microwave-vacuum drying process. Witrowa-Rajchert and Rżęca [50] used different drying methods for apple slices and stated that convective hot air drying of apple slices resulted in less porosity and high density of samples compared to the combination of microwave-convective hot air drying method which resulted in greater porous structure and low density of the samples. As T2L and T1L samples were having less porosity compared to CL, this finding contradicted the finding of Witrowa-Rajchert and Rżęca [50].

### 3.5. Storage Study of Dried Buffalo Liver

#### 3.5.1. Microbiological Analysis

(1) *Total Plate Count (TPC) of Dried Buffalo Liver Stored at Room Temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$ .* The total plate count of dried buffalo liver (CL, T1L, and T2L) stored at room temperature and  $4^\circ\text{C}$  is represented in Table 2. Kukier et al. [51] suggested the upper permissible limit of total plate count in animal feeds as  $10^6$  CFU/g (6 log CFU/g). The higher microbial load in CL samples might be attributed to the higher moisture content in the sample. None of the samples (CL, T1L, and T2L) crossed the upper limit for total plate count even at the

60 days of analysis as suggested by Kukier et al. [51]. At day 60, CL, T1L, and T2L showed corresponding TPC of 4.42, 4.22, and 4.10 log CFU/g and 4.18, 4.03, and 4.00 log CFU/g when stored at  $25 \pm 1$  and  $4 \pm 1^\circ\text{C}$ , respectively. Akhtar [52] studied the storage stability of vacuum-packed and conventional packed dried poultry meat stored at room temperature and reported that during storage, the TPC of dried conventionally packed products were 2.556 log CFU/g on 0 day and it increased to 4.539 log CFU/g on 60 days of analysis.

(2) *Coliform Count of Dried Buffalo Liver Stored at Room Temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$ .* Throughout the period of study, coliforms were not detected in any of the samples in the three treatment groups (CL, T1L, and T2L) at both room temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$  of storage. This might be due to the hygienic practices followed during the processing stage.

(3) *Yeast and Mold Count of Dried Buffalo Liver Stored at Room Temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$ .* The yeast and mold counts of dried buffalo liver (CL, T1L, and T2L) stored at room temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$  are given in Table 3. GMP [53] suggested that the upper limit of total yeast and mold count in animal feed should not exceed  $10^4$  CFU/g (4 log CFU/g). The upper limit for CL (as suggested by GMP [53] for yeast and mold count) crossed on day 45 of analysis



TABLE 2: The total plate count (log CFU/g) of dried buffalo liver stored at room temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4 \pm 1^\circ\text{C}$  at different time intervals. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Samples/intervals	0 day	15 days	30 days	45 days	60 days
Storage at room temperature ( $25 \pm 1^\circ\text{C}$ )					
CL	$2.63 \pm 0.01^{eA}$	$3.29 \pm 0.001^{dA}$	$3.80 \pm 0.001^{cA}$	$4.31 \pm 0.001^{bA}$	$4.42 \pm 0.001^{aA}$
T1L	$2.12 \pm 0.01^{eB}$	$2.88 \pm 0.001^{dB}$	$3.67 \pm 0.05^{cB}$	$4.07 \pm 0.002^{bB}$	$4.22 \pm 0.01^{aB}$
T2L	$1.77 \pm 0.03^{eC}$	$2.67 \pm 0.004^{dC}$	$3.62 \pm 0.01^{cC}$	$3.87 \pm 0.001^{bB}$	$4.10 \pm 0.002^{aC}$
Storage at $4 \pm 1^\circ\text{C}$					
CL	$2.63 \pm 0.01^{eA}$	$3.28 \pm 0.003^{dA}$	$3.75 \pm 0.001^{cA}$	$4.12 \pm 0.001^{bA}$	$4.18 \pm 0.001^{aA}$
T1L	$2.12 \pm 0.01^{eB}$	$2.79 \pm 0.002^{dB}$	$3.58 \pm 0.001^{cB}$	$3.94 \pm 0.002^{bB}$	$4.03 \pm 0.002^{aB}$
T2L	$1.77 \pm 0.03^{eC}$	$2.12 \pm 0.01^{dC}$	$3.53 \pm 0.01^{cC}$	$3.81 \pm 0.001^{bC}$	$4.00 \pm 0.01^{aC}$

$n = 6$ ; mean  $\pm$  SE; mean values between columns with different superscripts (a, b, c, d, and e) are significantly ( $P < 0.05$ ) different. Mean values between rows with different superscripts (A, B, and C) are significantly different ( $P < 0.05$ ).

TABLE 3: Yeast and mold count (log CFU/g) of dried buffalo liver stored at room temperature ( $25 \pm 1^\circ\text{C}$ ) at different time intervals. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Samples/intervals	0 day	15 days	30 days	45 days	60 days
Storage at room temperature ( $25 \pm 1^\circ\text{C}$ )					
CL	0	$3.84 \pm 0.01^{dA}$	$3.97 \pm 0.01^{cA}$	$4.09 \pm 0.002^{bA}$	$4.14 \pm 0.003^{aA}$
T1L	0	$3.49 \pm 0.01^{dB}$	$3.84 \pm 0.01^{cB}$	$3.94 \pm 0.01^{bB}$	$3.99 \pm 0.002^{aB}$
T2L	0	$3.41 \pm 0.01^{dC}$	$3.75 \pm 0.04^{cC}$	$3.85 \pm 0.01^{bC}$	$3.91 \pm 0.01^{aC}$

$n = 6$ ; mean  $\pm$  SE; mean values between columns with different superscripts (a, b, c, d, and e) are significantly different ( $P < 0.05$ ). Mean values between rows with different superscripts (A, B, and C) are significantly different ( $P < 0.05$ ).

when stored at room temperature. At room temperature storage, T1L and T2L samples never crossed the upper limit even on 60 days of analysis. Yeast and mold counts were found to be 4.14, 3.99, and 3.91 log CFU/g for CL, T1L, and T2L, respectively, stored at  $25 \pm 1^\circ\text{C}$  on 60 days of analysis. Akhtar [52] studied the storage stability of vacuum-packed and conventional packed dried poultry meat stored at room temperature and reported that during storage, the yeast and mold counts of dried conventionally packed products were not detected on 0 day and it increased to 1.306 log CFU/g on 60 days of analysis. Yeast and mold were not detected in CL, T1L, and T2L stored at  $4^\circ\text{C}$  throughout the storage period from day 0 to day 60. This might be due to the lower temperature of storage. The microbial quality of buffalo *keema* (minced meat) samples stored at refrigeration ( $4 \pm 1^\circ\text{C}$ ) and ambient temperature ( $37 \pm 1^\circ\text{C}$ ) for 3 days was studied [54]. They observed that yeast and mold were not detected on the 3<sup>rd</sup> day of storage at  $4 \pm 1^\circ\text{C}$  whereas a highly significant ( $P < 0.01$ ) growth of yeast and mold ranging from 1.05 to 1.10 log CFU/g was detected in the samples stored at  $37 \pm 1^\circ\text{C}$ .

### 3.5.2. Lipid Oxidation Index

(1) TBARS of Dried Buffalo Liver Stored at Room Temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$ . TBARS of dried buffalo liver (CL, T1L, and T2L) stored at room temperature and  $4^\circ\text{C}$  are given

in Table 4. So far, there are no standards or upper limits regarding the TBARS value of pet foods. Jouki and Khazaei [55] suggested that TBARS value of 3 mg malonaldehyde per kilogram can be considered as the critical limit of meat containing products intended for human consumption. TBARS threshold limit of 1-2 mg of malonaldehyde per kilogram of fresh meat was suggested by Karthik et al. [56]. As the final product in this study was a processed product and it was not intended for human use, the higher threshold limit of 3 mg malonaldehyde per kilogram as suggested by Jouki and Khazaei [55] could be taken into consideration.

All samples stored at room temperature and  $4^\circ\text{C}$  never crossed the critical limit of TBARS value of 3 mg of malonaldehyde/kg of sample as suggested by [50] throughout the storage study. Aksu and Kaya [57] studied the shelf life of pastirma, a cured dried meat product produced from beef, and reported that pastirma stored at  $4^\circ\text{C}$  with modified atmospheric packaging showed a TBARS value of 0.48 mg malonaldehyde per kilogram on 0 day and the value increased to 1.48 mg malonaldehyde per kilogram on 60 days.

(2) Free Fatty Acid of Dried Buffalo Liver Stored at Room Temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4^\circ\text{C}$ . The free fatty acid values of dried buffalo liver (CL, T1L, and T2L) stored at room temperature and  $4^\circ\text{C}$  are represented in Table 5. There are no standards or upper limit values of free fatty acids in pet

TABLE 4: TBARS values (milligrams of malonaldehyde/kilogram of sample) of dried buffalo liver stored at room temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4 \pm 1^\circ\text{C}$  at different time intervals. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Samples/intervals	0 day	15 days	30 days	45 days	60 days
Storage at room temperature ( $25 \pm 1^\circ\text{C}$ )					
CL	$0.54 \pm 0.003^{eA}$	$0.99 \pm 0.004^{dA}$	$1.13 \pm 0.006^{cA}$	$1.29 \pm 0.005^{bA}$	$1.72 \pm 0.003^{aA}$
T1L	$0.52 \pm 0.003^{eB}$	$0.94 \pm 0.003^{dB}$	$1.04 \pm 0.001^{cB}$	$1.20 \pm 0.003^{bB}$	$1.41 \pm 0.001^{aB}$
T2L	$0.50 \pm 0.005^{eC}$	$0.68 \pm 0.004^{dC}$	$0.79 \pm 0.002^{cC}$	$0.88 \pm 0.002^{bC}$	$1.29 \pm 0.004^{aC}$
Storage at $4 \pm 1^\circ\text{C}$					
CL	$0.54 \pm 0.003^{eA}$	$0.65 \pm 0.002^{dA}$	$0.77 \pm 0.004^{cA}$	$1.05 \pm 0.002^{bA}$	$1.44 \pm 0.001^{aA}$
T1L	$0.52 \pm 0.003^{eB}$	$0.63 \pm 0.004^{dB}$	$0.76 \pm 0.002^{cB}$	$0.91 \pm 0.005^{bB}$	$1.23 \pm 0.005^{aB}$
T2L	$0.50 \pm 0.005^{eC}$	$0.62 \pm 0.001^{dC}$	$0.70 \pm 0.002^{cC}$	$0.79 \pm 0.001^{bC}$	$1.03 \pm 0.004^{aC}$

$n = 6$ ; mean  $\pm$  SE; mean values between columns with different superscripts (a, b, c, d, and e) are significantly different ( $P < 0.05$ ). Mean values between rows with different superscripts (A, B, and C) are significantly different ( $P < 0.05$ ).

TABLE 5: Free fatty acid values (% oleic acid) of dried buffalo liver stored at room temperature ( $25 \pm 1^\circ\text{C}$ ) and  $4 \pm 1^\circ\text{C}$  at different time intervals. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Samples/intervals	0 day	15 days	30 days	45 days	60 days
Storage at room temperature ( $25 \pm 1^\circ\text{C}$ )					
CL	$0.62 \pm 0.02^{eA}$	$2.22 \pm 0.01^{dA}$	$3.23 \pm 0.01^{cA}$	$3.48 \pm 0.01^{bA}$	$3.84 \pm 0.04^{aA}$
T1L	$0.49 \pm 0.01^{eB}$	$1.94 \pm 0.01^{dB}$	$2.97 \pm 0.01^{cB}$	$3.20 \pm 0.01^{bB}$	$3.52 \pm 0.02^{aB}$
T2L	$0.41 \pm 0.01^{eC}$	$1.32 \pm 0.01^{dC}$	$2.69 \pm 0.01^{cC}$	$2.91 \pm 0.01^{bC}$	$3.20 \pm 0.01^{aC}$
Storage at $4 \pm 1^\circ\text{C}$					
CL	$0.62 \pm 0.02^{eA}$	$1.65 \pm 0.01^{dA}$	$2.41 \pm 0.01^{cA}$	$2.86 \pm 0.01^{bA}$	$3.42 \pm 0.02^{aA}$
T1L	$0.49 \pm 0.01^{eB}$	$1.43 \pm 0.01^{dB}$	$2.22 \pm 0.01^{cB}$	$2.78 \pm 0.01^{bB}$	$3.20 \pm 0.03^{aB}$
T2L	$0.41 \pm 0.01^{eC}$	$1.00 \pm 0.01^{dC}$	$1.90 \pm 0.01^{cC}$	$2.35 \pm 0.02^{bC}$	$2.74 \pm 0.01^{aC}$

$n = 6$ ; mean  $\pm$  SE; mean values between columns with different superscripts (a, b, c, d, and e) are significantly different ( $P < 0.05$ ). Mean values between rows with different superscripts (A, B, and C) are significantly different ( $P < 0.05$ ).

food. During the storage study of pet food incorporated with 20% of spent hen meal, the free fatty acid value ranged from 2.8% oleic acid on 0 day to 7.3% oleic acid on 60 days of storage [56]. Compared to this study [56], the present study was showing less free fatty acid values varying from 0.41 and 0.41% oleic acid (in T2L) on 0 day to 3.84 and 3.42% oleic acid (in T2L) on 60 days when stored at room temperature and  $4^\circ\text{C}$ , respectively.

**3.6. Shelf Life of the Dried Whole Buffalo Liver.** The shelf life of dried whole buffalo liver was estimated on the basis of microbiological analysis and lipid oxidation index during the storage study. At room temperature ( $25 \pm 1^\circ\text{C}$ ), the shelf life was found to be 30 days for CL and more than 60 days for both T1L and T2L. At refrigeration temperature ( $4^\circ\text{C}$ ), the shelf life was found to be more than 60 days for all the samples (CL, T1L, and T2L).

### 3.7. Palatability Evaluation of Dried Buffalo Liver

**3.7.1. Acceptance Test (Single-Bowl Test) and Preference Test (Two-Bowl Test) of Dried Buffalo Liver.** The results of single-

bowl test conducted for dried buffalo liver (CL, T1L, and T2L) are given in Table 6. All four dogs took the least time for consuming dried buffalo liver samples compared to the commercial pet treat indicative of more liking of the dogs towards dried buffalo liver samples. The mean time taken by the four dogs to consume the commercial pet treat was 212.87 s, whereas for CL, T1L, and T2L samples, it was recorded to be 21.97, 18.86, and 15.62 s, respectively.

In the two-bowl test, it was observed that the dried livers (CL, T1L, and T2L) were invariably preferred by all four dogs over the commercial pet treat procured from the market provided in the second bowl.

**3.7.2. Perception of Pet Owners.** Among the pet owners considered for the analysis, 77.27% of the dog owners and 66.67% of cat owners were already providing a type of treat to their pets. During the survey, 63.63% of the dog owners and 55.53% of the cat owners responded that their pets liked the dried buffalo liver samples very much. 50% and 44.44% of dog and cat owners, respectively, gave a five-star rating for the dried buffalo liver samples provided to them. 70.59% of

TABLE 6: Time taken in seconds (s) by the four dogs to consume dried buffalo liver and commercial treats. T2L refers to the buffalo liver dried by surface slicing, piercing, pretreatment, microwaving, and hot air drying. T1L denotes buffalo liver dried in the same way as T2L except without surface slicing and piercing. CL refers to buffalo liver dried simply by hot air drying.

Dogs	Time taken to consume commercial treat (s)	Time taken to consume CL (s)	Time taken to consume T1L (s)	Time taken to consume T2L (s)
DOG-1	241.22 ± 1.64 <sup>a</sup>	21.91 ± 0.54 <sup>b</sup>	19.20 ± 0.35 <sup>c</sup>	16.38 ± 0.46 <sup>d</sup>
DOG-2	126.64 ± 1.41 <sup>a</sup>	18.04 ± 0.44 <sup>b</sup>	15.52 ± 0.35 <sup>c</sup>	11.86 ± 0.47 <sup>d</sup>
DOG-3	363.60 ± 1.78 <sup>a</sup>	31.08 ± 0.66 <sup>b</sup>	27.47 ± 0.72 <sup>c</sup>	24.24 ± 0.61 <sup>d</sup>
DOG-4	120.02 ± 1.56 <sup>a</sup>	16.84 ± 0.86 <sup>b</sup>	13.27 ± 0.70 <sup>c</sup>	10.01 ± 0.84 <sup>d</sup>
Means ± SE	212.87 ± 1.60	21.97 ± 0.62	18.86 ± 0.53	15.62 ± 0.59

$n = 6$ ; mean ± SE; mean values between columns with different superscripts (a, b, c, and d) are significantly different ( $P < 0.05$ ).

dog owners and 75% of the cat owners were willing to change from the usual pet treat that they were providing to the dried buffalo liver.

#### 4. Conclusions

The drying methodology for intact whole buffalo liver was optimized, and it was found that surface piercing pretreatment for 3 h in 3% sugar and 4% salt solution (1:3  $w/v$ ) and microwave treatment for 4 min followed by convective hot air drying at 60°C for 40 h dried the liver satisfactorily having shelf life of over 60 days of storage at room temperature (25 ± 1°C) and 4°C. The moisture content, TBARS value, free fatty acid value, and TPC (at 4°C) in T2L (14.29%, 1.03 mg malonaldehyde/kg of sample, 3.20% oleic acid, and 4.00 log CFU/g, respectively) were significantly ( $P < 0.05$ ) lower compared to control and T1L. T2L also exhibited significantly ( $P < 0.05$ ) lower water activity (0.592) and higher water loss (72.20%) than the control and T1L. SDS-PAGE images revealed the presence of lower molecular weight protein bands in T2L similar to control and T1L. Besides, T2L possessed more number of higher molecular weight bands than control and T1L, indicating the lesser effect of drying treatment in T2L due to surface slicing and piercing of liver. All three dried buffalo liver samples were highly palatable and acceptable by the experimental dogs, pet dogs, and cats. Considering all above facts, it was concluded that T2L sample was superior for long-term storage of dried liver than the control and T1L.

#### Data Availability

Data will be made available on request.

#### Additional Points

**Animal Welfare Statement.** The authors confirm that the ethical policies related to animal welfare were adhered to and the appropriate ethical review committee approval has been received from CPCSEA, Ministry of Fisheries, Animal Husbandry and Dairying, New Delhi, India, vide letter no. V-11011(13)/9/2022-CPCSEA-DADF dated 06/09/2022. All feeding trials were carried out on the experimental dogs according to the approved guidelines put forward by IAEC/CPCSEA (Protocol No. IAEC/07.07.2022/L2).

#### Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that can affect the study.

#### Authors' Contributions

Anand T S carried out the experiments, data collection, result analysis, and original draft writing. Tanbir Ahmad secured the fund, designed the experiments, supervised the experiments, and edited the original manuscript. Devendra Kumar was involved in writing the original manuscript draft, reviewing, and editing it. I. Prince Devadason made available different resources, reviewing, and editing. Sanjod K. Mendiratta conceptualized the idea and reviewed the research process. Akhilesh Kumar Verma was tasked with data analysis and reviewing and editing of manuscript. Ashim Kumar Biswas helped in carrying out some of the experiments and supervised the research work. Suman Talukder helped in design of experiments, data analysis, and editing of manuscript. Zunjar B. Dubal contributed in the designing of the experiments and helping in experiments related to microbiology and data analysis. Asit Das was involved in managing the experimental dogs, its maintenance, carrying out the dog feeding experiment, data collection, and its analysis. Yasotha Thirupathi and Aditya D. Deshpande helped in getting SEM images and inferring the images. Aruna. T S and A. R. Sen were involved in data analysis and result interpretation.

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