Journal of Animal Research: v.13 n.05, p. 815-831. October 2023

DOI: 10.30954/2277-940X.05.2023.24

Enhancing Microbiota Analysis, Shelf-life, and Palatability Profile in Affordable Poultry Byproduct Pet Food Enriched with Diverse Fibers and Binders

Rishav Kumar*, Meena Goswami and Vikas Pathak

Department of Livestock Products Technology, College of Veterinary Sciences and AH, DUVASU, Mathura, U.P., INDIA

**Corresponding author: R Kumar; E-mail: rishavvet42@gmail.com*

Received: 19 Jan., 2023 **Revised:** 20 Aug., 2023 **Accepted:** 02 Sept., 2023

ABSTRACT

In this investigation, we examined the impact of incorporating fiber-rich vegetables and binders into pet food on microbiological aspects and storage stability under ambient conditions. Beyond achieving nutritional balance, assessing microbiological factors and storage durability is vital for ensuring the well-being of dogs. Varied levels of fiber-rich vegetables and binders were integrated into pet food formulations. Evaluation of pet food quality encompassed microbiological assessments, notably Coliform, Salmonella, yeast mold count, and total plate count, alongside physiochemical attributes like pH, TBARS, and FFA values. Pet food formulations containing poultry byproducts powder and a control group with chicken meat powder were stored at room temperature (25±1°C). Storage stability was assessed at 15-day intervals over 60 days. The highest mean pH values were observed in BP3>RG2=PO2>M3 treatments, while for TBARS, FFA values, and total plate count, the highest means were in BP3>M3>PO2>RG2. M3>BP3>PO2>RG2 exhibited the highest mean yeast and mold counts. Throughout storage, no Coliform or Salmonella counts were detected. PH, TBARS, FFA values, and microbiological counts significantly increased ($P<0.05$), while sensory attribute scores decreased ($P<0.05$) as storage progressed. RG2 demonstrated higher oxidation stability, lower microbiological counts, and significantly greater overall acceptability scores ($P<0.05$) compared to M3 and other treatments, lasting until the end of storage. The identified microorganism levels may have implications for prolonged consumption of contaminated food.

HIGHLIGHTS

- RG2 pet food excels in stability and oxidation resistance with varied fiber-rich ingredients during a 60-day storage.
- Monitoring pH, TBARS, and FFA is vital for ensuring pet food quality and safety during storage.
- RG2 excels in addressing nutritional and microbiological concerns in pet food production.

Keywords: Pet food, poultry byproducts powder, microbiology, storage stability

Pets have seamlessly integrated into modern society as cherished family members, both in urban and rural settings. Because of evolving lifestyles, improved economic conditions, and the companionship pets provide, pet feeding has become an integral facet of daily life. Pet food researchers are entrusted with the critical task of crafting well-balanced, nutritious pet food adhering to international standards set by AAFCO and NRC. The composition of pet food varies based on factors such as animal type, age, species, breed, and climatic conditions. Typically combining plant and animal ingredients, the pet food industry harmoniously aligns with slaughter, human food, and agricultural processing sectors, currently experiencing rapid growth. Ensuring safe pet food denotes that it poses no threat to animal health or the environment when prepared and consumed as intended (ISO 22,000:2018). Nonetheless, reports from the Rapid Alert System for Food and Feed (RASFF) in 2018 highlight pet food as a potential source of biological, physical, or chemical

How to cite this article: Kumar, R., Goswami, M. and Pathak, V. (2023). Enhancing Microbiota Analysis, Shelf-life, and Palatability Profile in Affordable Poultry Byproduct Pet Food Enriched with Diverse Fibers and Binders. *J. Anim. Res.,* **13**(05): 815-831.

Source of Support: None; **Conflict of Interest:** None

hazards that may adversely impact pets (Hill *et al*., 2009, Rolinec *et al*., 2016; Alvarenga *et al*., 2018; Meineri *et al*., 2019). The predominant type of pet food available is dry, often presented as biscuits due to its convenient storage and nutritional efficiency (Meineri *et al*., 2020). Consequently, assessing the quality of pet food remains essential, with numerous studies scrutinizing various aspects of dog food. This trend extends to the utilization of different ingredients, including plant-based components, in pet food formulations. The absence or presence of cereals is of particular interest in pet food, as it significantly influences the nutritional profile of the final product. The processing of pet food typically occurs at temperatures ranging from 80–160 °C under high pressure, aimed at reducing waste, enhancing product stability, and improving carbohydrate digestibility. These elevated temperatures additionally curtail the presence of pathogenic bacteria. However, it has been noted that thermal processes might not be effective if contamination occurs at later stages of production. Microbiological quality is a cornerstone of safe and healthy food production, alongside nutritional value. Pathogenic and non-pathogenic microorganisms are indicative of food hygiene standards, making their control imperative. Concerningly, pet food quality issues have raised concerns about their potential impact on both animal and human health. Incidents of pathogenic microorganism contamination, such as Salmonella, Listeria, and Escherichia, have been documented in pet food. Salmonella is a recognized hazard in animal feed, acting as a vector and reservoir (Maciorowski *et al*., 2006; Behravesh *et al*., 2010). Mycotoxins, produced by fungi, represent another hazard in pet food safety, necessitating vigilant monitoring. In recent years, instances of Salmonella and fungal contaminations in pet food have heightened awareness of potential risks to pets and humans alike. Thus, maintaining rigorous microbiological standards is essential for producing safe and nutritious pet food.

The primary objective of this study was to assess the microbiological safety and storage stability of different pet food formulations.

The aim of this study was to pet food for, with reference to evaluating their microbiological safety and storage stability.

MATERIALS AND METHODS

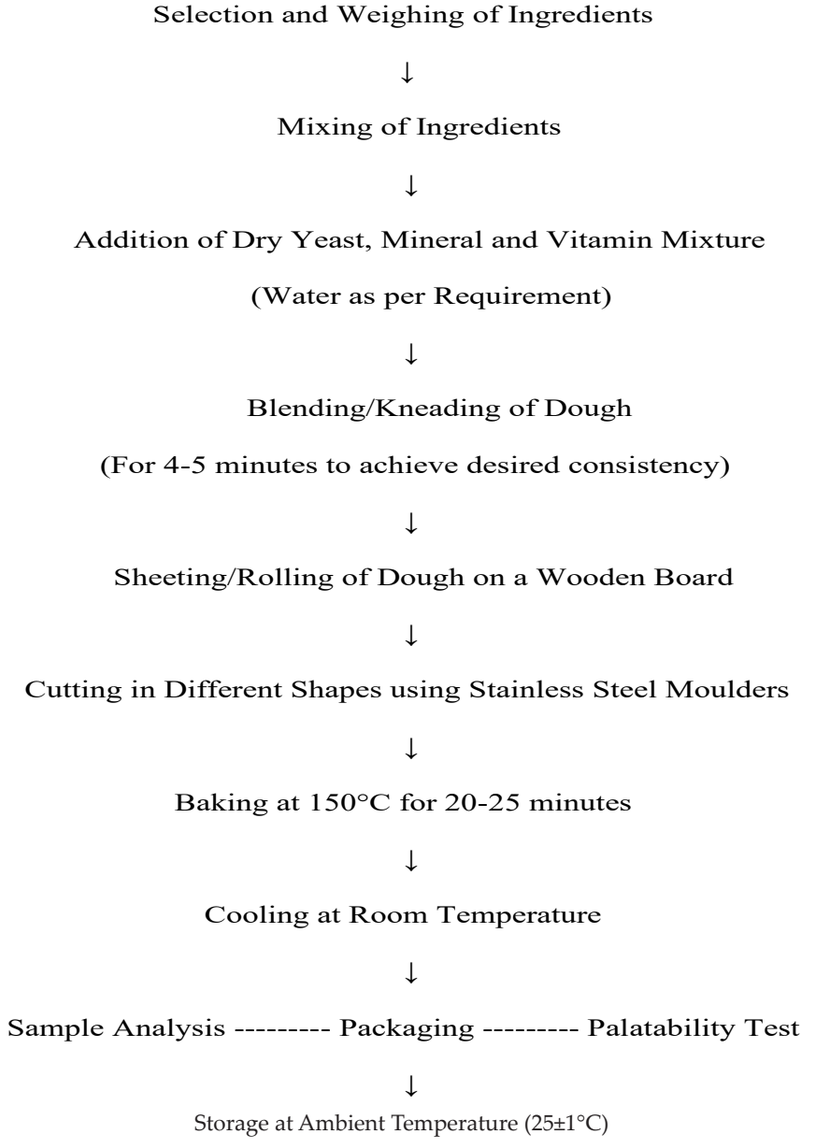
The experiment was carried out at the Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, U.P. Pt. Dean Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU) in Mathura, Uttar Pradesh, India, with the postal code 281001.

Development of byproduct powder

The preparation of poultry byproducts powder followed a method outlined by Kumar *et al.* (2023). Fresh and clean poultry byproducts were meticulously washed and drained to eliminate excess moisture. In the case of frozen poultry byproducts, they were defrosted under refrigeration over the course of a night to achieve normal consistency. The byproducts, which included the head, feet, and intestines, underwent autoclaving at 121°C for 15 minutes. After autoclaving, the material was allowed to cool and then minced using a meat mincer, passing it through 6 mm and 4 mm plates. The minced byproducts were evenly spread on stainless steel trays, ensuring uniform thickness, and subjected to drying in a hot air oven at 70±10°C for 16-18 hours, until a consistent weight was achieved. The resultant dried material was finely ground using a food processor (Inalsa Make), followed by sieving. Subsequently, the processed material was stored in pre-sterilized PET jars at a controlled temperature of 25±1°C.

Preparation of product

The formulation of pet food followed the approach outlined by Kumar *et al.* (2023), incorporating slight modifications (see Flow Diagram 1). The process entailed aligning nutritional requirements and ingredient selection with the guidelines set by AAFCO (American Association of Feed Control Officials) 2008 and NRC (National Research Council) 2006, specifically targeting the dietary needs of adult dogs in maintenance. Each ingredient was accurately weighed based on batch requirements. A predetermined quantity of poultry byproducts powder, alongside other components such as fiber-rich vegetables, iodised salt, vegetable oil, binder, wheat gluten, and white corn flour, were measured and placed in a plastic tub. Subsequently, the mixture underwent thorough blending for 1-2 minutes to ensure homogeneity. To this amalgam,



Flow diagram 1: Preparation of pet food

dry yeast, calcium carbonate, and mineral mixture were introduced into the plastic tub. The entire combination was uniformly mixed and kneaded for approximately 4-5 minutes, culminating in a well-blended dough exhibiting the desired consistency. The dough was then rolled out onto a wooden board using rolling pins, after which it was shaped according to preference through the use of stainless-steel food moulders. The newly shaped raw pet food was subjected to baking in a hot air oven at 150°C for approximately 20-25 minutes. Post-baking, the pet food was allowed to cool to room temperature, swiftly packaged within pre-sterilized LDPE bags, and finally stored in a cool, dry environment at ambient temperature. The development process for the fiber-rich vegetables pet food adhered to a similar procedure, which is elaborated in Flow Diagram 1.

Analysis of product

The quality evaluation of the pet food involved several standard procedures. The pH measurement was conducted following the method outlined by Trout *et al*., (1992). The determination of the TBARS (Thiobarbituric Acid Reactive Substances) value was carried out using the procedure described by Tarladgis *et al*. (1960). The assessment of the free fatty acid value was performed using a modified AOCS (American Oil Chemists' Society) method, as specified by Koniecko (1979). To microbiological studies, the preparation of samples and serial dilutions was executed according to the guidelines presented in APHA (American Public Health Association) 1992. Each sample was opened within an inoculation chamber of a laminar flow system (Model: RH-58-03, Science tech, India) that had been pre-sterilized using ultra-violet (UV) radiation. The determination of the total plate count was carried out in accordance with the procedure provided in APHA (1992) by employing Nutrient agar. In the case of yeast and mould count analysis, potato dextrose agar was utilized, with its pH adjusted to 3.5±0.1 using tartaric acid, following the method detailed in APHA (1992). The coliform count was determined following the protocol outlined by APHA (1992). The isolation of *Salmonella* spp. was conducted based on the procedure outlined in the OIE (World Organisation for Animal Health) Terrestrial Manual 2008. This procedure was derived from the ISO standard (6579:2002), with specific modifications implemented, including the incorporation of a Novobiocin supplement into Xylose Lysine Deoxycholate agar.

Palatability test

The assessment of palatability involved both the response of the pet dog and the observations of the owner, employing a questionnaire formulated in English according to the guidelines established by Ponmani (1997), Karthikeyan (2004), and Karthik *et al*. (2010). A 7-point descriptive scale was utilized for evaluating various characteristics of the pet food, with a score of 7 representing "extremely desirable" and a score of 1 indicating "extremely poor."The palatability test was conducted on a group of seven dogs, all belonging to the faculty and staff members of DUVASU, Mathura. These dogs were selected based on their matching age group and well-established breed. The test was conducted approximately 3-4 hours after their regular feeding time. The reactions of the dogs towards the pet food were carefully observed and recorded.

The observations were centred on the dog's behaviour towards the pet food, their interest in eating, and the way they consumed the food. These observations were systematically recorded within the prepared questionnaire. Additionally, the dog owners were asked to provide their opinions on various aspects of the pet food's characteristics. The score card included categories such as general appearance, color, intensity of the pet food, crispiness, consistency, odor, and overall acceptability. By combining both the dog's response and the owner's observations, the palatability assessment provided valuable insights into the acceptance and likability of the pet food, thereby contributing to a comprehensive understanding of its appeal to canine consumers.

STATISTICAL ANALYSIS

The collected data from the study were subjected to statistical analysis using the 'SPSS-20.0' software package. One-way ANOVA was conducted in line with the established methods outlined by Snedecor and Cochran (1995). Duplicate samples were taken for each parameter, and the entire experiment was replicated three times, resulting in a total sample size of six (n=6). For the palatability test, the assessment was carried out on the same set of seven dogs, and the test was repeated three times, yielding a total of 21 observations (n=21). The data obtained were subjected to one-way analysis of variance, along with a homogeneity test. To ascertain the differences between the means and identify the effects between various samples, Duncan's Multiple Range Test (DMRT) was employed. These statistical analyses were employed to draw meaningful conclusions from the collected data, providing insights into the effects and variations observed across different parameters and samples.

RESULTS AND DISCUSSION

Several preliminary trials were conducted to prepare poultry byproducts (head, feet, intestine) powder incorporated pet food to optimize the formulation based on method prescribed by Brindha and Rao (2017) and Kumar *et al.* (2024) with minor adjustments. Ultimately, the ideal formulation consisted of pet food containing 50% poultry byproduct powder. This mixture was then

baked in a hot air oven at 150°C for 20-25 minutes. The resulting nutrient content in the pet food aligned with the specifications outlined by the National Research Council (NRC) in 2006. Subsequently, the developed pet food was enriched with varying levels of ragi flour (5%, 10%, and 15%) and boiled mashed potato, replacing rice flour. After experimentation, it was determined that the most favourable compositions were 10% ragi flour (labelled as RG2) and 10% boiled mashed potato (labelled as PO2). For comparison, these selected treatments (RG2 and PO2) were pitted against a control pet food (BP3) and pet food containing poultry meat powder (M3). The evaluation included studies of microbiology, assessment of physical-chemical properties, and palatability testing.

pH

pH, a crucial intrinsic factor, holds a significant impact on microbial growth within meat products (ICMSF, 1980). In the case of M3, pH values were notably lower compared to the treatments on both the 0th and 15th days. However, from the 30th to the 60th day, no substantial differences were observed between M3 and the treatments. The initial lower pH values of the control in contrast to the treatments could be attributed to the slightly acidic pH of meat powder. Conversely, the higher pH values of the treatments might result from the substitution of lean meat powder (with a pH of 5.6-5.8) with poultry byproduct powder (ranging from 6.18 to 6.27), along with the inclusion of various functional ingredients possessing neutral or mildly alkaline pH. Similar findings were noted by Javeed and Khan (2016), who reported elevated pH values in extruded pet food containing meat byproducts and agricultural processing waste.

Throughout the storage period, no noteworthy differences surfaced among the treatments. Both the control and treatments exhibited a substantial rise ($P<0.05$) in mean pH values as storage progressed, attributed to bacterial activity liberating protein metabolites. However, despite these pH

Table 1: pH (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	6.23^{dB} ±0.04	6.26^{dB} ±0.06	6.33^{c} ±0.02	6.37^{b} ±0.03	6.41^{a} ±0.4	6.32±0.03
BP3	6.34^{dA} ±0.05	6.35^{dA} ±0.02	6.38^{c} ±0.04	6.42^{b} ±0.05	6.45^{a} ±0.04	6.38±0.04
RG2	6.35^{cA} ±0.06	6.36^{cA} ±0.04	638^{c} ±0.05	6.40^{b} ±0.03	6.42^{a} ±0.03	6.37±0.04
PO2	6.33^{dA} ±0.05	6.35^{cA} ±0.02	6.37^{c} ±0.04	6.41^{b} ±0.02	6.44^{a} ±0.03	6.37 ±0.03
Storage Mean	6.31 ±0.05	6.33 ±0.03	6.36 ±0.03	6.40 ±0.03	6.44 ±0.03	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly ($P<0.05$); n= 6 for each treatment.

Table 2: TBARS values (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	0.19^{e} ±0.02	0.29^{d} ±0.04	0.39^{cB} ±0.04	0.48^{bB} ±0.01	0.67^{aB} ±0.04	0.40 ±0.03
BP3	0.18^{e} ±0.02	0.27^{d} ±0.04	0.45^{cA} ±0.05	0.57^{bA} ±0.02	0.76^{aA} ±0.05	0.44 ±0.03
RG2	$0.\ 16^{e}$ ±0.04	0.25^{d} ±0.05	0.32^{cC} ±0.03	0.38^{bC} ±0.05	0.51^{aC} ±0.01	0.32 ±0.03
PO2	0.17^{e} ±0.01	0.26^{d} ±0.01	0.36^{cB} ±0.04	0.46^{bB} ±0.02	0.65^{aB} ±0.02	0.38 ±0.02
Storage Mean	0.17 ±0.02	0.26 ±0.03	0.38 ± 0.04	0.47 ±0.02	0.64 ±0.03	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly ($P<0.05$); n= 6 for each treatment.

shifts, the palatability attributes and intake ratios of the dogs consuming the pet food remained unaffected. This aligns with Pandey's observations (2012) of increased pH in dry pet food derived from buffalo offals during a 60-day storage at ambient temperature. Similarly, Karthikeyan *et al.* (2010) documented a significant (P<0.05) pH increase in dry pet food derived from poultry byproducts, with values shifting from 5.44 to 5.82 over 112 days of aerobic storage.

TBARS (Thiobarbituric Acid Reactive Substances)

Mean TBARS (Thiobarbituric Acid Reactive Substances) values showed no significant differences between the control and treatments on days 0 and 15. However, on days 30, 45, and 60, the mean TBARS values of BP3 were notably higher (P<0.05) than those of M3 and the other treatments. The elevated TBARS values in BP3 could be attributed to a higher initial microbial load, potentially leading to increased lipid oxidation in comparison to the other treatments. Similar outcomes were noted by Pandey (2012) and Virk *et al*. (2019) in pet food containing buffalo offals and dog biscuits containing liver meal, respectively. Although no significant differences were observed between M3 and PO2 over the storage period, RG2 exhibited significantly (P<0.05) lower mean TBARS values from day 30 to day 60. This reduction in TBARS values within RG2 could be attributed to the higher phenolic and flavonoid content present in ragi flour, which possess antioxidant properties beneficial for the pet food. McDonough and Rooney (2000) identified ferulic, p-coumaric, and cinnamic acids as major phenolics in finger millet. Additionally, Subba and Muralikrishna (2002) reported that around 70% of finger millet phenolic acids existed in a free form, with ferulic acid (18.60 mg/100g) being the prominent bound form and protocatechuic acid (45.0 mg/100g) as the primary free phenolic acid. Throughout storage, both control and treatment products displayed

Table 3: FFA values (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	0.19^{d} ±0.04	0.28^{dA} ±0.03	0.34^{cA} ±0.05	0.38^{bA} ±0.06	0.47^{aA} ±0.05	0.33 ±0.04
BP3	0.23^{d} ±0.02	0.31^{dA} ±0.01	0.39^{cA} ±0.03	0.48^{bA} ±0.05	0.59^{aA} ±0.02	0.40 ±0.02
RG2	0.15^{d} ±0.02	0.21^{dC} ±0.04	0.24^{cC} ±0.06	0.28^{bC}±0.04	0.33^{aC} ±0.01	0.24 ±0.03
PO2	0.17^{d} ±0.03	0.24^{dB} ±0.02	0.30^{cB} ±0.03	0.36^{bB} ±0.05	0.42^{aB} ±0.01	0.29 ±0.02
Storage Mean	0.18 ±0.01	0.26 ±0.02	0.31 ±0.04	0.37 ±0.05	0.45 ±0.02	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05); n= 6 for each treatment.

Table 4: Total plate count (log10cfu/g) (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	1.28^{e} ±0.05	1.42^{dB} ±0.04	1.72^{cB} ±0.04	2.12^{bB} ±0.06	2.78^{aB} ±0.04	1.86 ±0.03
BP3	1.25^{e} ±0.08	1.57^{dA} ±0.07	1.98^{cA} ±0.03	2.51^{bA} ±0.04	3.23^{aA} ±0.06	2.10 ±0.05
RG2	$1.\ 19^{e}$ ±0.10	1.27^{dC} ±0.04	1.52^{cC} ±0.04	1.85^{bC} ±0.03	2.19^{aC} ±0.05	1.60 ±0.05
PO2	1.24^{e} ±0.04	1.39^{dB} ±0.06	1.64^{cB} ±0.08	2.08^{bB} ±0.04	2.64^{aB} ±0.06	1.79 ±0.05
Storage Mean	1.24 ±0.05	1.41 ±0.05	1.71 ±0.04	2.14 ±0.03	2.71 ±0.04	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05); n= 6 for each treatment.

a significant increase (P<0.05) in TBARS values due to the permeability of packaging material to oxygen, which facilitated lipid oxidation (Raja *et al*., 2014). However, all products remained within the 1–2 mg/kg threshold levels for meat product rancidity, as reported by Watts (1962). Warris (2000) suggested that TBARS values exceeding 0.5 indicate some oxidation in fresh meat samples, while values surpassing 1.0 indicate a potentially unacceptable level. Karthik *et al*. (2010) also noted a significant (P<0.05) increase in TBARS values in pet food containing 20% spent hen meal, rising from 0.41 mg/kg to 2.52 mg/kg over a 45-day storage period. Similarly, Pame *et al*. (2018) observed a significant (P<0.05) increase in TBARS values in pet kibbles containing animal byproducts during room temperature storage for up to 60 days.

Free fatty acid content (FFA)

Free fatty acid content serves as an indicator of hydrolytic rancidity in fats, providing an estimation of the free oleic acid present in the product. Initially, there were no significant differences in mean FFA values between the control and treatments on day 0. However, starting from day 15 and extending to day 60, the mean FFA values of M3 and BP3 were notably higher (P<0.05) than those of RG2 and PO2. The heightened FFA values in BP3 could be attributed to elevated microbial lipase activity, possibly resulting from the higher microbial load in the pet food containing poultry byproduct powder. This microbial load remained significantly (P<0.05) higher throughout storage, as also observed in the total plate count in the present study. Similarly, higher FFA values in M3 could be linked to its higher moisture and fat content, which might accelerate lipid oxidation and the release of free fatty acids. In contrast, RG2 displayed significantly (P<0.05) lower FFA values compared to the control and other treatments from day 15 to day 60 of storage. This reduction in FFA values in RG2 could be attributed to the superior antioxidant properties of ragi flour. This trend in free fatty acid content mirrored the trend observed for TBARS values,

Table 5: Yeast and mould count (log10cfu/g) (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	0.00	0.00	0.98^{cA} ±0.08	1.42^{bA} ±0.13	1.76^{aA} ±0.07	0.83 ±0.08
BP3	0.00	0.00	0.85^{cA} ±0.15	1.38^{bA} ±0.12	1.63^{aA} ±0.06	0.77 ±0.06
RG2	0.00	0.00	0.37^{cB} ±0.11	1.19^{bB} ±0.10	1.38^{aB} ±0.09	0.58 ±0.06
PO2	0.00	0.00	0.43^{cB} ±0.06	1.26^{bB} ±0.04	1.45^{aB} ±0.07	0.62 ±0.04
Storage Mean	0.00	0.00	0.65 ±0.15	1.31 ±0.09	1.55 ±0.07	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05); n= 6 for each treatment.

Table 6*: Coliform* count (log10cfu/g) (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	ND	ND	ND	ND	ND	---
BP3	ND	ND	ND	ND	ND	---
RG2	ND	ND	ND	ND	ND	---
PO2	ND	ND	ND	ND	ND	---
Storage Mean	---	---	---	---	---	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; n= 6 for each treatment.

Table 7: *Salmonella* count (log10cfu/g) (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1oC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	ND	ND	ND	ND	ND	---
BP3	ND	ND	ND	ND	ND	---
RG2	ND	ND	ND	ND	ND	---
PO2	ND	ND	ND	ND	ND	---
Storage Mean	---	---	---	---	---	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; n= 6 for each treatment.

Table 8: Overall acceptability scores (Mean±SE) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1oC)

Treatment	0 Day	15 Day	30 Day	45 Day	60 Day	Treatment Mean
M3	6.68^{aA} ±0.06	6.59^{bA} ±0.03	6.33^{cB} ±0.04	6.26^{dB} ±0.05	6.12^{eB} ±0.06	6.39 ±0.04
BP3	6.69^{aA} ±0.06	6.54^{bA} ±0.08	6.37^{cB} ±0.04	6.28^{dB} ±0.09	6.15^{eB} ±0.03	6.40 ±0.06
RG2	6.53^{aB} ±0.08	6.47^{abB} ±0.06	6.44^{bcA} ±0.03	6.36^{cA} ±0.07	6.25^{dA} ±0.04	6.41 ±0.05
PO2	6.47^{aC} ±0.07	6.37^{bC} ±0.04	6.31^{bcB} ±0.04	6.25^{cdB} ±0.06	6.14^{dB} ±0.08	6.30 ±0.05
Storage Mean	6.59 ±0.06	6.49 ±0.05	6.36 ±0.03	6.28 ±0.06	6.16 ±0.05	

M3-- (control) pet food prepared with 50 % chicken meat powder. BP3 – poultry byproducts incorporated pet food with 10% cruciferous vegetables powder, RG2- – poultry byproducts incorporated fiber fortified pet food with 10% ragi flour, PO2- – poultry byproducts incorporated fiber fortified pet food with 10% boiled potato mash; Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05); n= 6 for each treatment.

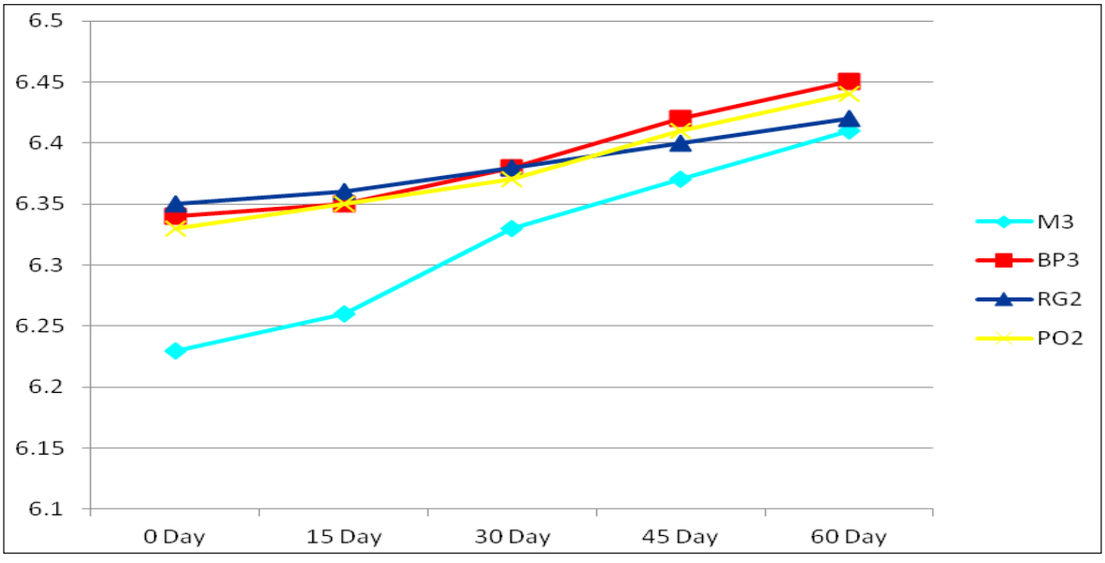


Fig. 1: pH values of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

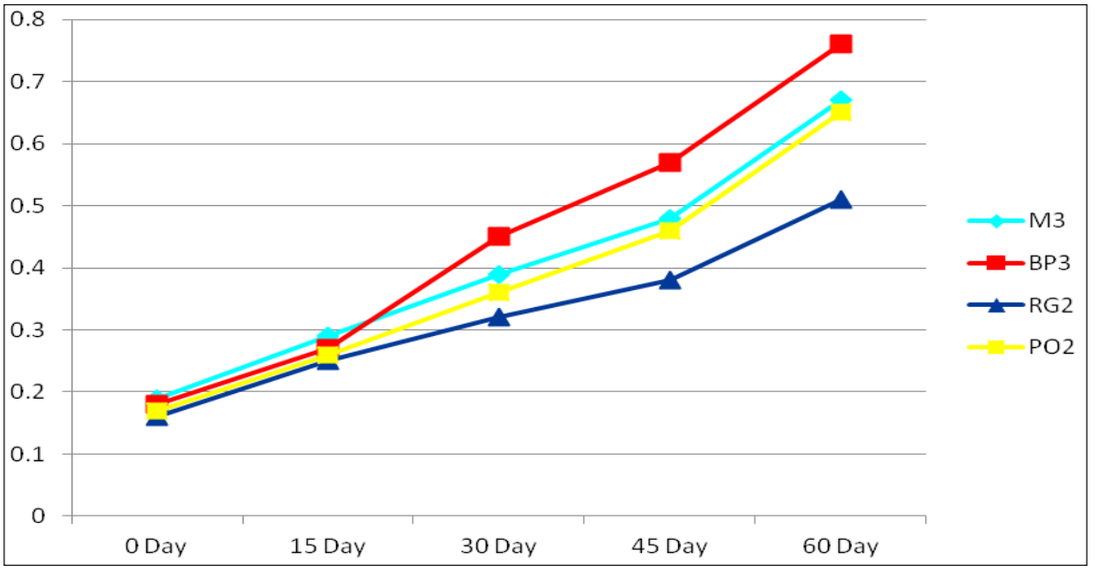


Fig. 2: TBARS values of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

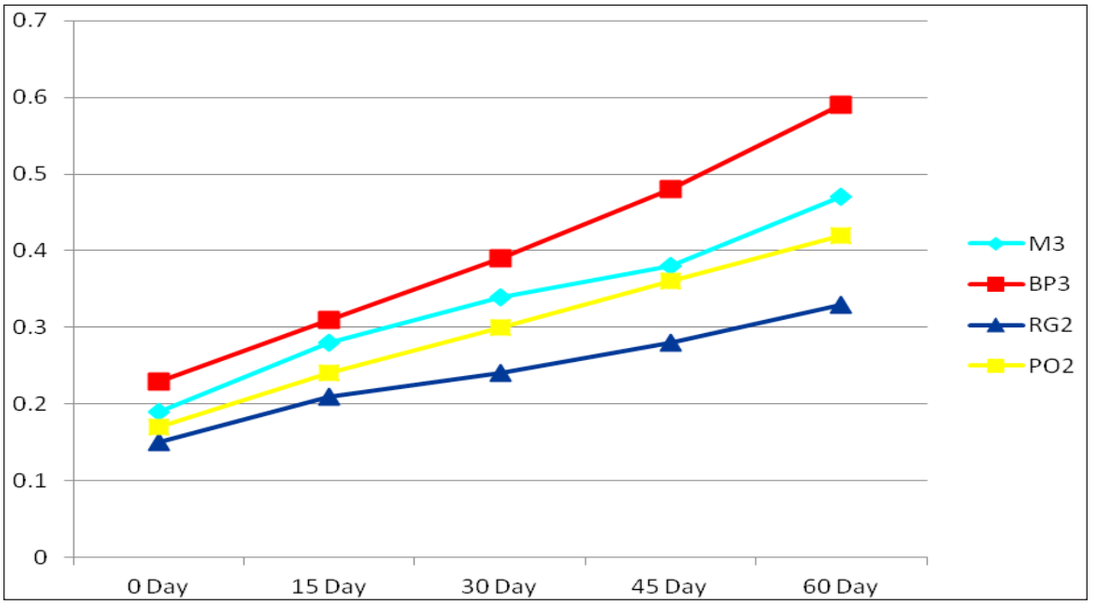


Fig. 3: FFA values of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

demonstrating a positive correlation between the two. This correlation can be attributed to both parameters being related to fat oxidation (Reitznerová *et al*., 2017). Brindha and Rao (2017) also reported significantly (P<0.05) lower FFA values in pet food incorporating cauliflower waste and slaughterhouse byproducts, compared to control and other treatments, during 30 days of room temperature storage. As storage progressed, FFA values for both control and treatment groups showed a significant increase (P<0.05). Mahesh (2018) similarly observed a significant (P<0.05) increase in TBARS and FFA values in canine food incorporating porcine origin variety meats, stored

at room temperature for up to 30 days. In line with these findings, Virk *et al*. (2019) reported a significant (P<0.05) increase in free fatty acid values in dog biscuits containing buffalo offals, with values rising from 0.10 to 0.21 during an 80-day room temperature storage period.

Total plate count (TPC)

There were no significant differences in the total plate count (TPC) between the control and treatments on day 0 due to the high baking temperature and hygienic processing of the pet food. However, from day 15 to day 60, the TPC of BP3 was notably higher (P<0.05) than that of M3 and the other treatments. This could be attributed to the availability of nutrients and more favourable conditions for microbial growth in BP3. Conversely, no significant differences were observed between M3 and PO2 throughout the storage period. However, RG2 had significantly (P<0.05) lower mean TPC than M3 and the other treatments from day 15 to day 60.

Rodriguez *et al*. (2007) highlighted the antimicrobial activities of compounds such as gallic acid, protocatechuic acid, phydroxybenzoic acid, vanillic acid, p-coumaric acid, syringic acid, ferulic acid, trans-cinnamic acid, and quercetin present in finger millet flour against various bacterial strains including *E. coli*, *B. cereus*, *S. aureus*, *Y. enterocolitica*, and *L. monocytogenes*. Sinha and Dua (2016) similarly demonstrated the antimicrobial potential of methanolic extracts from potato peels against both gram-positive (*Bacillus amyloiquefaciens* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) through an agar well diffusion method. The mean TPC of both the control and treatments increased significantly (P<0.05) as storage progressed. This gradual rise in microbial count indicated post-processing or handling contamination, in line with the findings of Fischer *et al*. (2007) who emphasized the importance of hygienic practices despite dry extruded pet food serving as a less conducive substrate for microbial development. Brindha and Roa (2017) also noted a significant (P<0.01) increase in the total viable count of pet food incorporating poultry slaughterhouse byproducts, from log 3.46 to 5.90 cfu/gram over 50 days of room temperature storage. Similar trends were observed by Kumar *et al*. (2007) in chicken meat patties, reporting significant (P<0.05) increases in total plate count at each storage interval in both control and treatment samples. Karthik *et al*. (2007) further supported these findings, reporting a significant

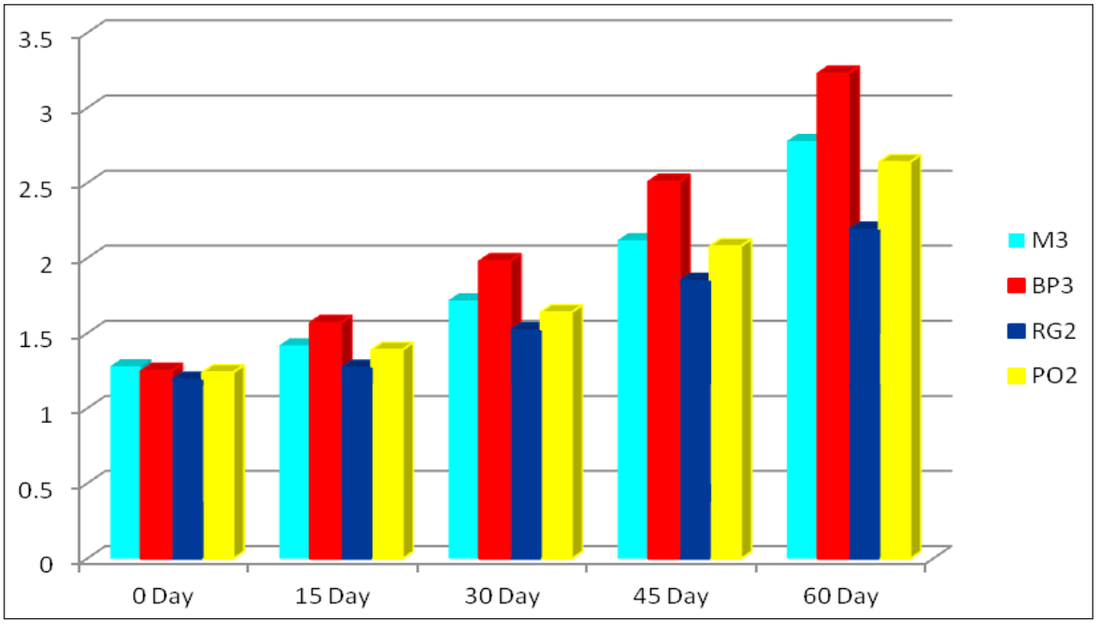


Fig. 4: Total plate count (log10cfu/g) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

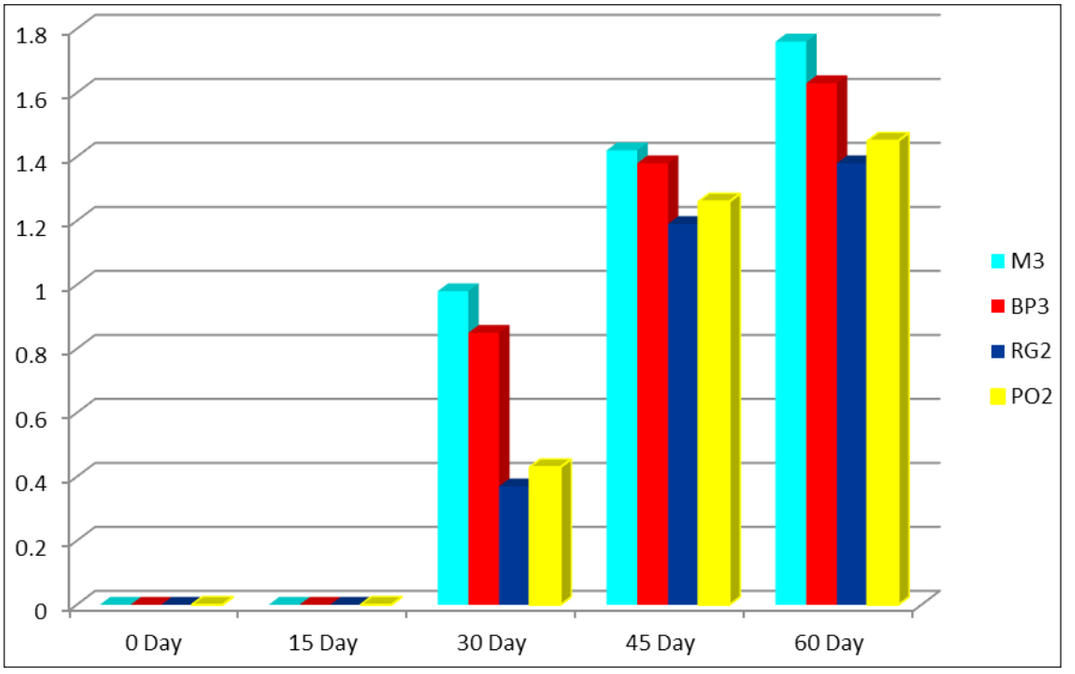


Fig. 5: Yeast and mould count (log10cfu/g) of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature

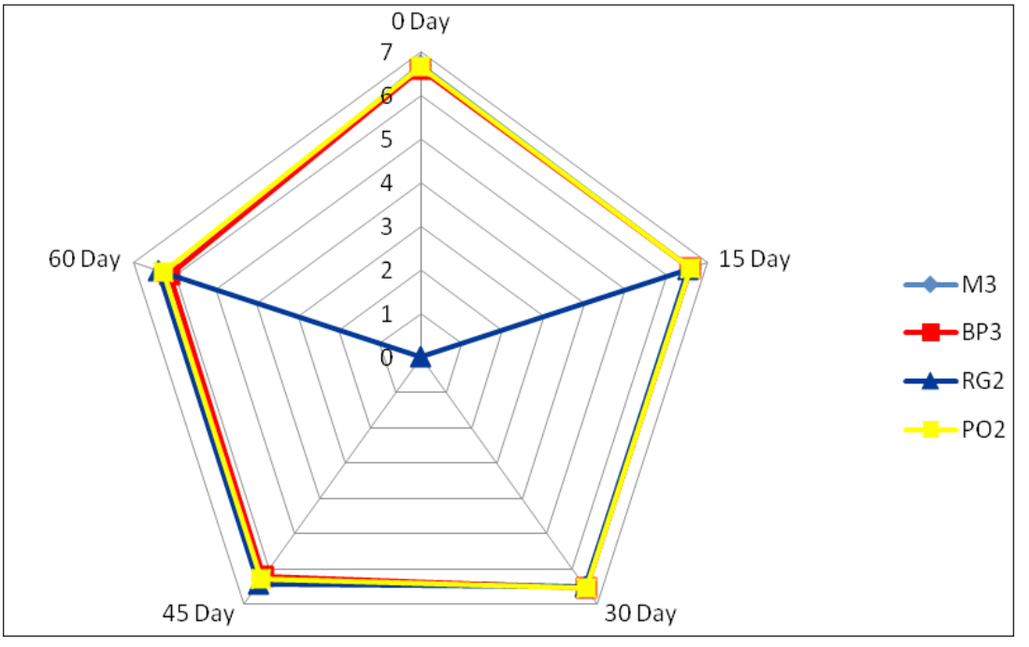


Fig. 6: General appearance scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

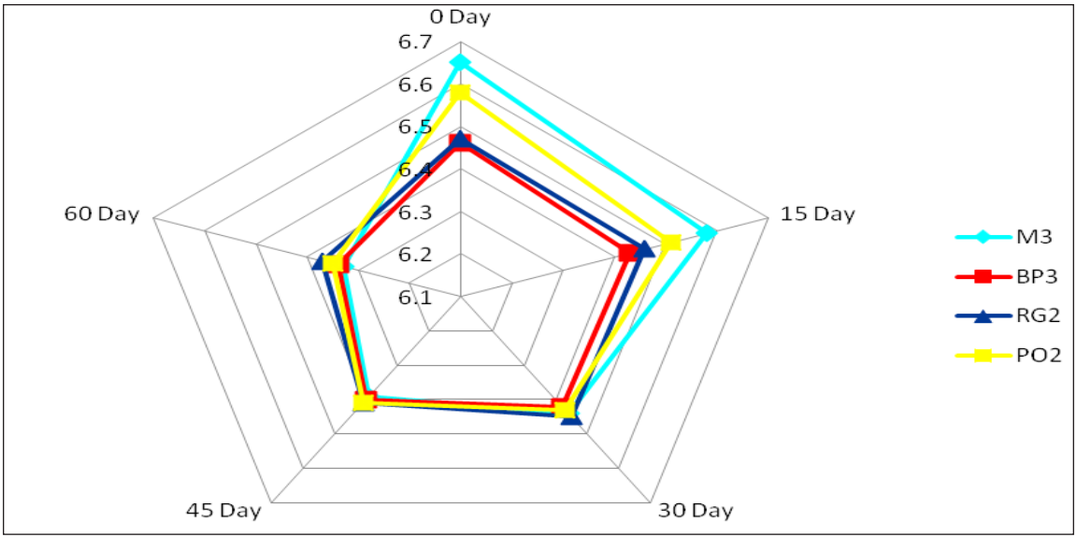


Fig. 7: Color scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

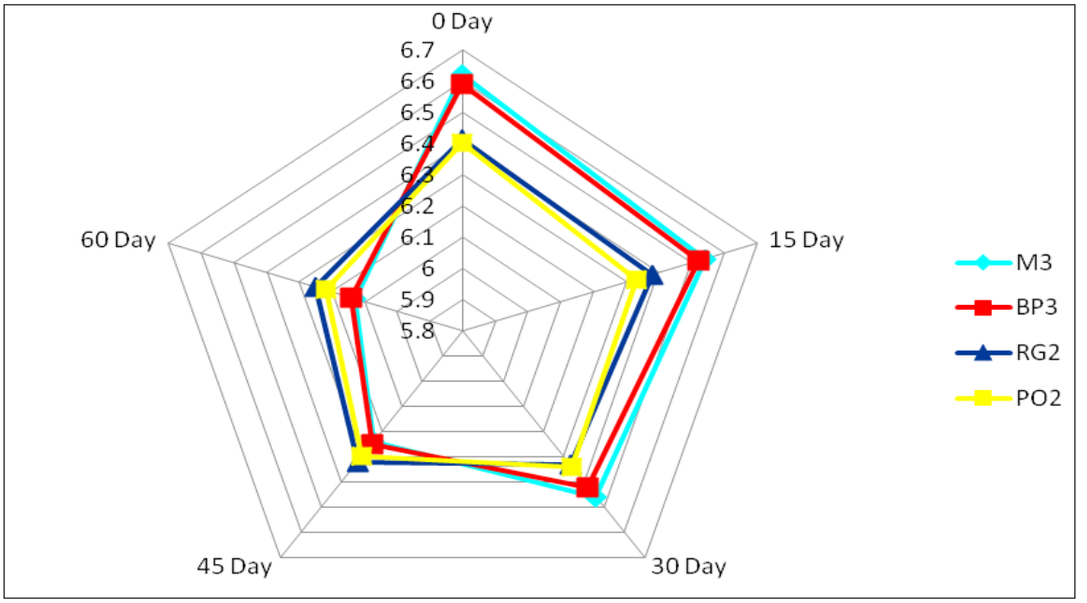


Fig. 8: Odour scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

(P<0.01) increase in the total viable count from log 2.62 to log 10.19 cfu/gram in pet food over 90 days of room temperature storage.

Yeasts and moulds Count

Yeasts and moulds were not detected in any of the products on days 0 and 15. However, from day 30 to day 60, the yeast and mold count of M3 and BP3 was significantly (P<0.05) higher than that of RG2 and PO2. Martins *et al.* (2003) identified Aspergillus (58.3%), Penicillium (38.3%), and Mucor (38.3%) in commercially available dry pet food for dogs, cats, and birds, albeit at low contamination levels (101 to 102 cfu/g). There were no significant differences

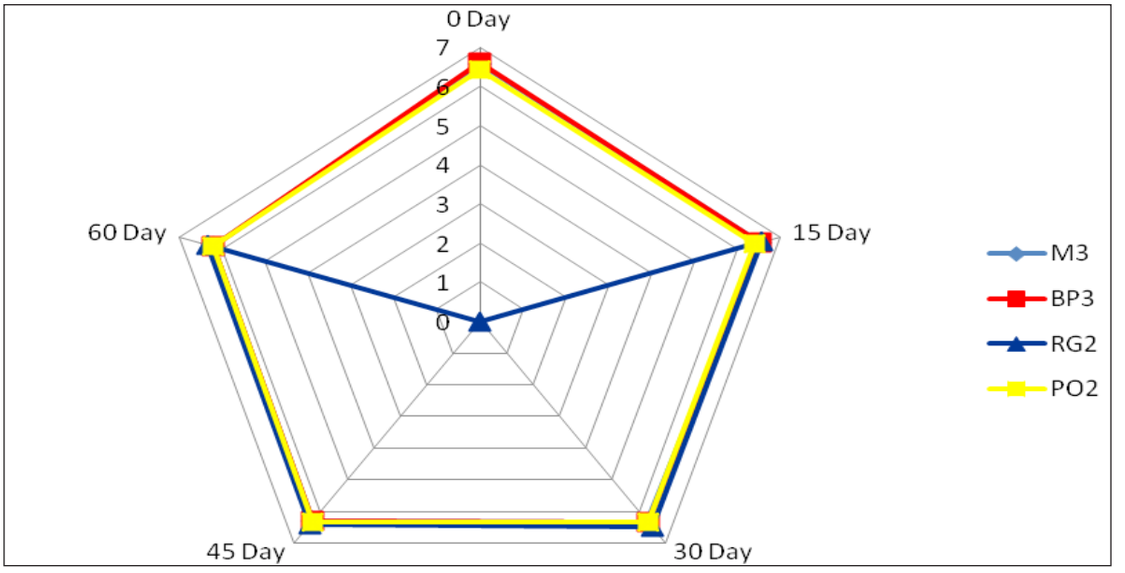


Fig. 9: Crispiness scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

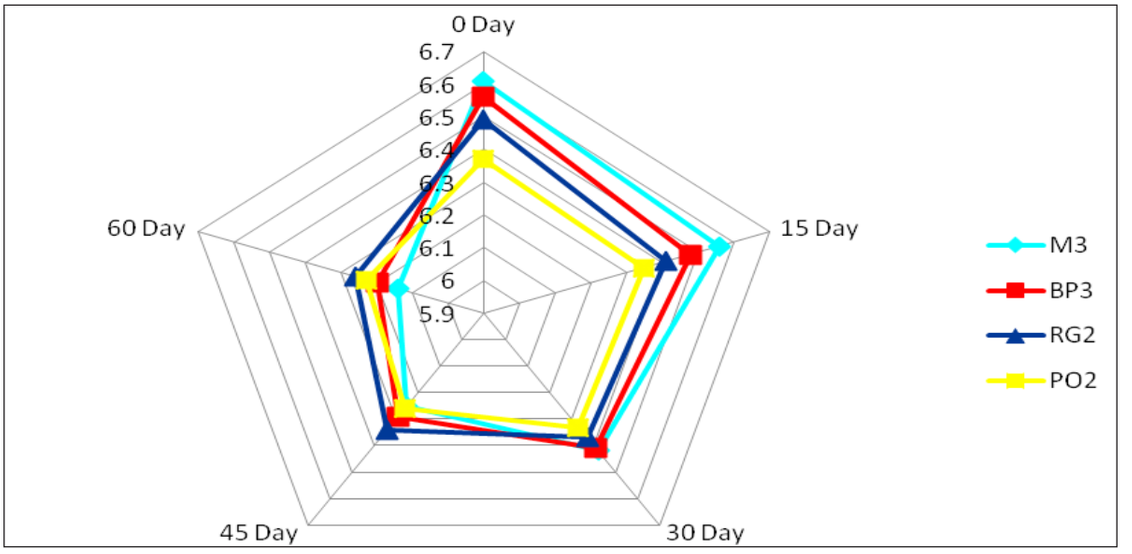


Fig. 10: Consistency scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

between M3 and BP3, as well as between RG2 and PO2, throughout the storage period. The lower yeast and mold count in RG2 and PO2 in comparison to M3 and BP3 could be attributed to the antimicrobial activities of ragi and boiled potato mash. Gamit *et al*. (2020) similarly reported significantly ($P<0.05$) lower yeast and mold counts in chicken meat cutlets containing ragi flour compared to the control during a 9-day storage period under refrigeration. The lower microbial count might also be associated with the lower fat content in PO2 and RG2 in comparison to M3 and BP3. Wirth (1972) noted that low-fat products often have a better shelf life than full-fat products due to greater heat penetration in low-fat products. The yeast and mold count increased significantly ($P<0.05$) as storage

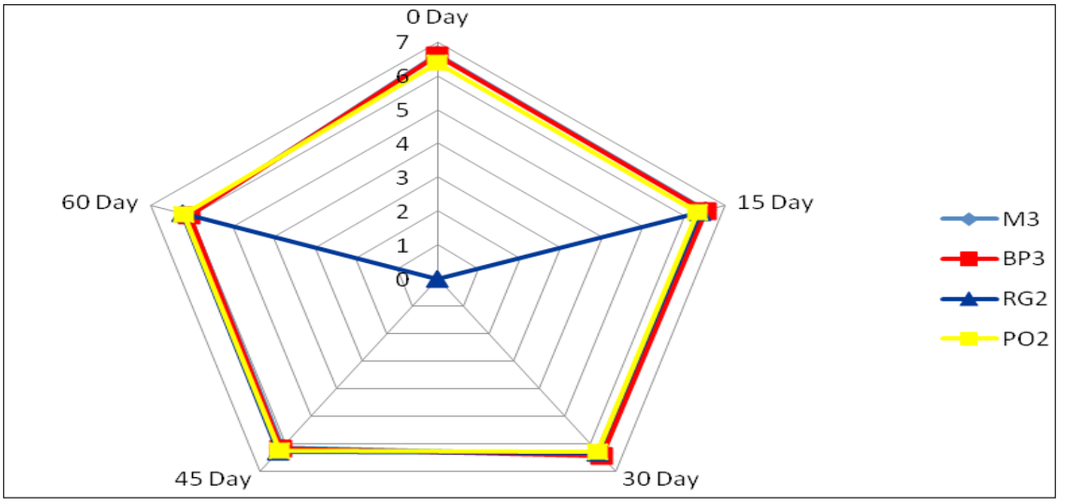


Fig. 11: Meat flavor intensity scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

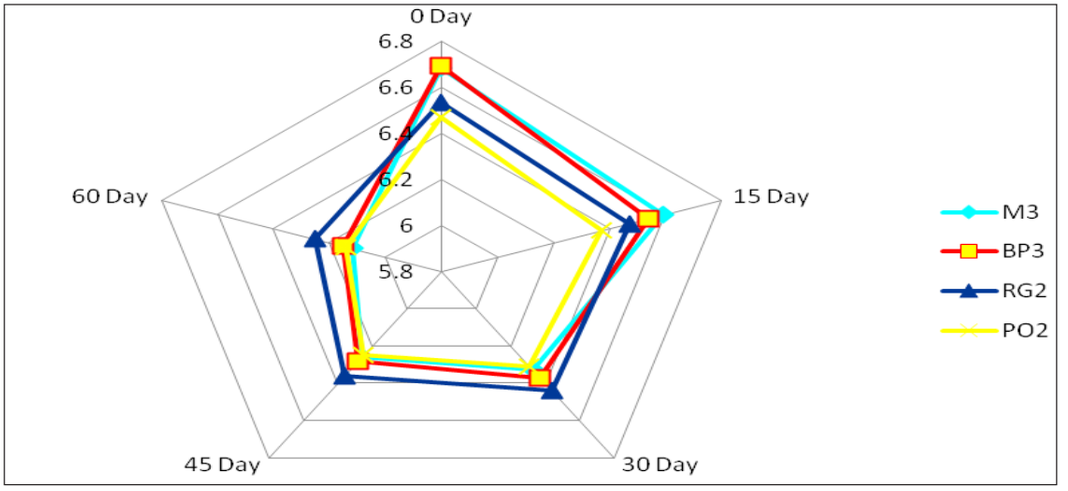


Fig. 12: Overall acceptability scores of poultry byproducts incorporated fiber fortified pet food during storage at ambient temperature (25±1ºC)

progressed in both the control and treatment groups. Pame *et al*. (2017), Virk *et al*. (2019), Singh *et al*. (2011), and Pandey (2012) also reported similar findings of increased yeast and mold counts in pet food, pet biscuits, chicken snacks, and buffalo offals-incorporated dry pet food during aerobic storage at ambient temperatures.

Coliform count

Throughout the storage period, no Coliform growth was detected in the control or treatment products. The absence of Coliforms could be attributed to the baking process, where pet food was baked at 150°C for 20-25 minutes, well above their death point (57°C). Hygienic conditions were maintained during processing, storage, and analysis of the products, further contributing to the absence of Coliform growth. Frazier and Westhoff (2008) also reported that cooking meat products at high temperatures led to the destruction of major microflora.

Salmonella count

Regarding Salmonella growth, it was not detected throughout the storage period in the control or treatment products. The high baking temperature and hygienic handling during processing and storage likely prevented Salmonella growth. Ahn *et al*. (2007) observed similar results, noting no Salmonella growth in cooked beef incorporating various plant extracts stored aerobically under refrigeration.

Salmonella is recognized as a potential pathogen in pet food and can present health risks to both pets and humans. The absence of Salmonella growth in the study's products is a positive indicator of safe processing and handling practices.

Palatability Score

No significant differences were observed between M3 and treatments on days 0, 15, and 30. However, from day 45 to 60, M3 and BP3 had significantly ($P<0.05$) lower general appearance scores compared to RG2 and PO2. This decline in appearance scores in M3 and BP3 could result from non-enzymatic browning between lipid oxidation products and amino acids. In contrast, RG2 and PO2 scored higher due to the antioxidant properties of ragi flour and boiled potato mash, slowing myoglobin oxidation. Over time, all scores decreased significantly ($P<0.05$) due to pigment and lipid oxidation causing non-enzymatic browning.

M3 scored significantly ($P<0.05$) higher in meat odour on days 0 and 15, while no significant differences were seen from days 30 to 60. This could be due to the rich meaty flavour and higher fat content in M3 and BP3. However, RG2 and PO2 scored higher from days 30 to 60, attributed to their antioxidant and antimicrobial properties. Crispiness scores were higher for M3, BP3, and RG2 compared to PO2 on days 0 and 15, with RG2 surpassing M3 and others from days 30 to 60. PO2 scored lower due to moisture absorption, resulting in undesirable softness. Consistency scores were initially higher in M3, but differences disappeared from days 30 to 60 due to slower oxidation and bacterial growth.

RG2's higher scores were attributed to lower oxidation and microbial activity. Meat flavour intensity scores of M3 and BP3 exceeded RG2 and PO2 due to meaty flavours and fat content. However, RG2 and PO2 scored higher from days 30 to 60 due to antioxidants and antimicrobial properties. Similarly, overall acceptability scores were higher for M3 and BP3 initially, but RG2 outscored M3 and others from days 30 to 60 due to better attributes.

CONCLUSION

The chosen experimental treatments (RG2 and PO2), alongside poultry byproducts powder-incorporated pet food (BP3) and the control (M3) containing chicken meat powder, underwent storage assessment at ambient room temperature (25±1°C) over a duration of two months, with evaluations conducted at 15-day intervals. The pH values exhibited the following order in treatment means: BP3>RG2=PO2>M3. For indicators of oxidative stability, the treatment means displayed the sequence: BP3>M3>PO2>RG2, in the context of TBARS (thiobarbituric acid reactive substances) and FFA (free fatty acid) values. In terms of microbiological examination, the highest treatment mean for total plate count was observed in BP3>M3>PO2>RG2, while for yeast and mould count, the sequence was M3>BP3>PO2>RG2. Throughout the storage period, Coliform and Salmonella counts remained absent in both control and treatments. As time progressed, pH, TBARS, FFA values, and microbiological counts for all instances increased significantly ($P<0.05$), whereas sensory attribute scores consistently declined significantly ($P<0.05$). Notably, RG2 displayed superior oxidation stability and lower microbiological counts, alongside markedly higher overall acceptability scores ($P<0.05$) in comparison to M3 and other treatments. This is attributed to RG2's maintenance of desirable attributes, including meaty odour, aroma, and consistency throughout storage. The relatively lower production cost of the prepared pet foods compared to commercially available options underscores its promising market viability.

ACKNOWLEDGEMENTS

The authors are also thankful to Vice-chancellor, U.P. Pandit Deen Dayal Upadhyayay Pashu Chikitsa Vigyan Vishwavidhyalaya Ewam Gau Anusandhan Sansthan Mathura, 281001 (U.P.), for providing infrastructural facility to review this study.

REFERENCES

AAFCO, 2008. Official publication of the Association of American Feed Control Officials. Inc., Atlanta.

Ahn, J., Grun, IU. and Mustapha, A. 2007. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiol.,* **24**: 7-14.

Alvarenga, I.C., Ou, Z. and Thiele, S. 2018. Effects of milling sorghum into fractions on yield, nutrient composition, and their performance in extrusion of dog food. *J. Cereal Sci.,* **82**: 121–128.

APHA, 1992. American Public Health Association. Compendium of methods for the microbiological examination of foods. Washington, DC.

Brindha, N. and Rao, V.A. 2017. Cost Effective Utilization of Poultry and Cruciferous Vegetable Waste as a Raw Material to Develop a Shelf-stable Pet Food. *J. Anim. Res. Nutr.,* **2**(7): 67-71.

Fischer, A.R., De Jong, A.E., Van Asselt, E.D., De Jonge, R Ferrer., L.J. and Nauta, M.J. 2007. Food safety in the domestic environment. An interdisciplinary investigation of microbial hazards during food preparation. *Risk Anal.,* **27**: 1065-1082.

Frazier, W.C. and Westhoff, D.C. 2008. Food Microbiology. 4th Edn. Tata McGraw Hill Publication Limited, New Delhi.

Gamit, M., Gupta, S. and Savalia, C.V. 2020. Quality characteristics of chicken meat cutlets incorporated with finger millet *(Eleusine coracana)* flour. *J. Anim. Res.,* 10**(1)**: 111-116.

Hill, R.C, Choate, C.J, Scott, K.C. and Molenberghs, G. 2009. Comparison of the guaranteed analysis with the measured nutrient composition of commercial pet foods. *J. Am. Vet. Med. Assoc.,* **234**: 347–351.

ICMSF, 1980. Reduced water activity in microbiological foods. Vol. 1. Factors affecting life and death of microorganisms. *Academic press. New York.*, 76.

ISO, 22000:2018. Food safety management systems — Requirements for any organization in the food chain International Organization for Standardization Switzerland.

Javeed, A. and Khan, M.A. 2016. Extruded pet food development from meat byproducts using extrusion processing and its quality evaluation. *J. Food Sci. Technol.,* **7**(1): 245-252.

Karthik, P., Kulkarni, V.V. and Sivakumar, K. 2010. Preparation, storage stability and palatability of spent hen meal-based pet food. *J. Food Sci. Technol.,* **47**(3): 330-334.

Karthik, P. 2007. In studies on the preparation of spent hen meal and its utilization in pet dogs. MVSc Thesis thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

Karthikayan, J., Kumar, S., Anjaneyulu, A.S.K. and Rao, K.H. 2000. Application of hurdle technology for development of caprine keema, and its stability at ambient temperature. *Meat Sci.,* **54**: 9-15.

Karthikeyan, N. 2004 Effect of Processing Methods in Nutritional Quality and Storage Stability of Poultry by Product Meat Based Pet Foods. PhD Thesis submitted to Indian Veterinary Research Institute (IVRI), Bareilly (Uttar Pradesh), India.

Koniecko, R. 1979. Handbook of meat Chemists. A very Publishing Group, Inc. Wayne, New Jersy., **23**: 53-55.

Kumar, R., Goswami, M., Pathak, V., Verma, A.K. and Rajkumar, V. 2023. Quality improvement of poultry slaughter house byproducts based pet food with incorporation of fiber-rich vegetable powder. *Explor. Anim. Med. Res*., **13**(1): 54-61.

Kumar, R., Goswami, M., Pathak, V., Bharti, S.K., Verma, A.K., Rajkumar, V. and Patel, P. 2023. Utilization of poultry slaughter byproducts to develop cost effective dried pet food. *Anim. Nutr. Technol.,* **23**: 165-174.

Kumar, R., Thakur, A. and Sharma, A. 2023. Comparative prevalence assessment of subclinical mastitis in two crossbred dairy cow herds using the California mastitis test. *J. Dairy Vet. Anim. Res*., **12**(2): 98–102**.**

Kumar, R.R., Sharma, B.D, Kumar, M. and Chidanandaiah, Biswas, A.K.2007. Storage quality and shelf-life of vacuum-packaged extended chicken patties. *J. Muscle Foods*, **18**(3): 253-63.

Kumar, R., Goswami, M., Pathak, V. and Singh, A. 2024. Effect of binder inclusion on poultry slaughterhouse byproducts incorporated pet food characteristics and palatability. *Anim. Nutr. Technol.,* **24**: 177-19.

Maciorowski, K.G., Herrera, P., Kundinger, M.M. and Ricke, S.C. 2006 Animal Feed production and contamination by foodborne Salmonella. *J. Für Verbraucherschutz Leb.,* **1**: 197–209.

Mahesh, B. 2018. Development and Evaluation of Canine Food from Variety Meats (Heart, Liver & Kidney) of Porcine Origin. MVSc Thesis submitted to Sri Venkateswara Veterinary University Tirupati, (Andra Pradesh) India.

Martins, M.L., Martins, H.M. and Bernardo, F. 2003 Fungal flora and mycotoxins detection in commercial pet food. *Revista Portuguesa de Ciências Veterinárias.*, **32**: 179-184.

McDonough, C.M. and Rooney, L.W.2000. The millets. In: Kulp, K., Ponte, Jr., J.G. (Eds.), *Handbook of Cereal Science and Technology. Marcel Dekker Inc, New York.*, **78**: 177–201.

Meineri, G., Candellone, A. and Bello, F.D.2020. Gluten contamination of canned and dry grain-free commercial pet foods determined by HPLC-HRMS. *Italian J. Anim. Sci.,* **19**: 253–261.

Meineri, G., Peiretti, P.G. and Tassone, S. 2019. Nutritional value of extruded dog food with mechanically separated chicken meat or meat by-products. *BIOLOGY,* **12***:* 45-48.

NRC, 2006. In Nutrient Requirements of Dogs and Cats. Animal Nutrition Series. National Research Council of the National Academies; National Academy Press: Washington, DC, USA.

Pame, K., Sathu, T., Vasudevan, V.N., Prajwal, S. and Gunasekaran, P. 2017. A study on the effect of physicochemical characteristics, palatability and storage quality of kibbles incorporated with slaughterhouse byproducts for canine. *Int. J. Livest. Prod.,* **7**: 228-237.

Pandey, A. 2012. Development of dry pet food by incorporation of buffalo offals. PhD Thesis submitted to Department of Livestock products technology, IVRI, Izatnagar. Uttar Pradesh, India

Raja, W.H., Kumar, S., Bhat, Z.F. and Kumar, P. 2014. Effect of ambient storage on the quality characteristics of aerobically packaged fish curls incorporated with different flours. *Springerplus,* **3**(1): 1-10.

Ratanatriwong, P.A., WongSa-Ngasri, W., Suwansri, S. and Thanasukarn, P. 2011. The 12th Asean Food Conference. *In:* Optimization of drying-process efficiency and shelf-life extension by active packaging in crispy minced-fish snacks. Bangkok, Thailand. BITEC Bangna.

Reitznerová, A., Šuleková, M., Nagy, J., Marcinčák, S., Semjon, B., Čertík, M. and Klempová, T. 2017. Lipid peroxidation process in meat and meat products: a comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Molecules.,* **22**(11): 1988.

Rolinec, M., Bíro, D. and Gálik, B. 2016. The nutritive value of selected commercial dry dog foods. *Acta Fytotech Zootech.,* **19**: 25–28.

Singh, V.P., Sanyal, M.K., Dubey, P.C. and Mendirtta, S.K. 2011. Quality assessment of vacuum packaged chicken snacks stored at room temperature. *J. Stored Prod. Postharvest Res.,* **2**(6): 120-126.

Sinha, N. and Dua, D. 2016. Evaluation of antioxidant and antimicrobial properties of potato *(Solanum tuberosum*) peels. *Indian J. Agric. Biochem.,* **29**(1): 23-27.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical methods. First east west press edition, New Delhi.

Subba, Rao. and Muralikrishna, G. 2002. Evaluation of the antioxidant properties of free and bound phenolic acids.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. and Dugan, L.R. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Sic.,* **37**: 44–48.

Virk, K.S., Malav, O.P., Chatli, M.K., Metha, N., Kumar, P. and Wagh, R.V. 2019. Development of calcium fortified biscuits incorporated with slaughterhouse byproducts and evaluation of their palatability in dogs. *Indian J Anim Sci.,* **36**: 77-82.

Watts, B.M. 1962. Symposium on Foods: Lipids and their oxidation. Schultz, H.W., Day EAR, Sinnhuber RP (Ed.), AVI Pub Co, Westport, Connecticut, 202.

Wirth, F. 1972. The technology of low-fat meat products. *Fleishwirtschaft.,* **52**(11): 1375-1376.