STUDIES ON DEVELOPMENT AND STORAGE STABILITY OF BYPRODUCTS INCORPORATED DOG BISCUITS FORTIFIED WITH CALCIUM AND SELENIUM

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
LIVESTOCK PRODUCTS TECHNOLOGY
(Minor Subject: Veterinary Public Health and Epidemiology)

By

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(L-2016-V-19-M)

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2018

CERTIFICATE – I

This is to certify that the thesis entitled, **"STUDIES ON DEVELOPMENT AND STORAGE STABILITY OF BYPRODUCTS INCORPORATED DOG BISCUITS FORTIFIED WITH CALCIUM AND SELENIUM"** submitted for the degree of **M.V.Sc.** in the subject of **Livestock Products Technology** (Minor Subject: **Veterinary Public Health and Epidemiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Khushpreet Singh Virk (L-2016-V-19-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, **"STUDIES ON DEVELOPMENT AND STORAGE STABILITY OF BYPRODUCTS INCORPORATED DOG BISCUITS FORTIFIED WITH CALCIUM AND SELENIUM"** submitted by **Khushpreet Singh Virk (L-2016-V-19-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.** in the subject of **Livestock Products Technology** (Minor Subject: **Veterinary Public Health and Epidemiology**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

This study was designed to optimize the incorporation level of poultry by-products viz., liver and gizzard along with calcium fortification for the development of dog biscuits. Poultry liver and gizzard were minced and air dried at 60 °C for 15 -16 hrs after that these were converted into powder. Three different levels of poultry liver and gizzard viz., 10%, 20% and 30% were incorporated separately in standardized dog biscuits formulation after replacing the refined wheat flour. All the treatment products were analyzed for sensory evaluation, dog acceptability test, texture profile and instrumental color profile. Incorporation of liver powder at 30% level was found most suitable for the preparation of dog biscuits. The developed product was analyzed for physico-chemical properties (pH and cooking yield), proximate composition (moisture, protein, fat and ash), mineral content (Ca, P and Se), instrumental color and texture profile analysis. The dicalcium phosphate was incorporated at 2% level in the 30% liver powder incorporated dog biscuits with acceptable physico-chemical and sensory qualities. The final product was packed under aerobic and MAP conditions and stored at ambient temperature (25°C) for 80 days. The samples were drawn at 20 days intervals and analyzed on 1, 20, 40, 60 and 80 days. The storage quality was evaluated on the basis of various physico-chemical (pH, water activity, moisture, TBARS, PV, FFA) microbiological (TPC, PC, coliforms count, yeast and mold counts), water activity and sensory analysis (5- point descriptive scale).The dog biscuits can be stored for 80 days without any marked loss in physico-chemical, microbiological and sensory qualities. The cost of production of developed dog biscuits was calculated and it was Rs. 210/Kg.

Keywords: Dog biscuits, liver, gizzard, sensory evaluation, texture profile, storage quality

Signature of Major Advisor **Signature of Student**

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ABBREVIATIONS USED

AOAC	:	Association of Official Analytical Chemists
APHA	:	American Public Health Association
APPA	:	American Pet Products Association
AAFCO	:	Association of American Feed Control Officials
a_w	:	Water activity
Ca	:	Calcium
CF	:	Crude Fibre
Cm^2	:	Square Centimeter
CP	:	Crude Protein
CFU	:	Colony forming unit
CO_2	:	Carbon dioxide
dL	:	Decilitre
DM	:	Dry Matter
EE	:	Ether Extract
FAO	:	Food and Agriculture Organization
FDA	:	Food and Drug Administration
FFA	:	Free fatty acids
Fig	:	Figure
g	:	Gram
hrs	:	Hours
H_2SO_4	:	Sulphuric acid
$HCLO_3$	:	Chloric acid
HNO_3	:	Nitric acid
i.e.	:	That is
Kcal	:	Kilocalorie
Kg	:	Kilogram
KWH	:	KiloWatt Per Hour
LDPE	:	Low density polyethylene
Lit	:	Litres
Lbs	:	Pounds
M	:	Molar
MAP	:	Modified Atmosphere packaging
Meq	:	Miliequivalent
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre

MT	:	Million tonnes
N	:	Newton
O_2	:	Oxygen
N_2	:	Nitrogen
NFE	:	Nitrogen Free Extract
°C	:	Degrees celsius
P	:	Phosphorous
PC	:	Psychrophilic Count
PET	:	Polyethylene Terephthalate
PSW	:	Poultry Slaughter Waste
PV	:	Peroxide Value
ROI	:	Return On Investment
SFV	:	Shear Force Value
Se	:	Selenium
SE	:	Standard error
SPC	:	Standard plate count
SPSS	:	Software Package for Social Sciences
TBARS	:	Thiobarbituric acid reactive substances
TBA	:	Thiobarbituric Acid
TCA	:	Trichloroacetic Acid
TPA	:	Texture profile analysis
USD	:	United States Dollar
w/v	:	Weight by volume
w/w	:	Weight by weight
Wt	:	Weight
%	:	Percentage
<	:	Less than
>	:	More than
µM	:	Micromolar

CHAPTER I

INTRODUCTION

According to FAO (2008) estimates 107 million livestock and more than 650 million poultry birds were slaughtered annually in India leading to production of 6.3 million tonnes meat. It leaves huge loads of by-products. On the basis of live weight of animal the by-products account for almost 60% and out of this 40% are edible and 20% are inedible (Chatli *et al* 2005). Efficient utilization of animal by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or under-utilization of by-products not only lead to loss of potential revenues but also lead to the added and increasing cost of disposal of these products along with major aesthetic and serious health problems.

It has been estimated that 11.4% of the gross income from beef and 7.5% of the income from pork come from the by-products. Previously, animal by-products were the favorite food in Asia, but health concerns have led to an increased focus on non-food uses, such as pet foods, pharmaceuticals, cosmetics and animal feed (Rivera *et al* 2000). Meat by-products are produced by slaughter houses, meat processors, wholesalers and rendering plant. These can be used as inputs to feeds for the poultry, fish and pets like dogs and cats. Presently in India, pet food production is more of cereal based and less of animal by-product based. Slaughterhouse waste or animal by-products such as liver, lung, kidney, brain, spleen and tripe has high nutritive value and these can be efficiently utilized for the production of pet foods as the animal proteins are the integral part of their diet.

Smaller families, decreasing birth rates, and many more factors are leading to the increasing adoption of pets in families, Pets are playing the role of companion in the modern society. They have three functions, namely reflective effect, healing effect and interpersonal interaction (Patricia, 2017). Globally, pet food and products were a $93 billion industry and the market is growing. In the United States, dog and cat food sales alone account for $14.5 billion with exports of nearly $1 billion. The global total for pet food and supplies for all pet animals is now approaching $40 billion annually. There are about 4.0 million pets in the Indian household with the population increasing by 26% every year due to rapid urbanization, changing lifestyles in the form of the rise in nuclear families and double income households. Pet food market

has huge potential in India with the growth rate of 10-15% per annum. Dogs, cats and other pet food contribute 80, 15 and 5% respectively to pet food market. With the increasing demand of pet foods, the incorporation of animal by-products into pet food may result into better returns to meat industry with controlling the environmental pollution along with supply of quality animal proteins, vitamins and minerals to pets at the low cost.

For many people, their dog is a member of the family and people like to give their pets- a balanced and complete diet. This could be possible by providing a dog with a healthy snack made with good ingredients specially of animal origin. Dry pet foods like biscuits are popular because they are easy to store, easy to feed and are economical. The popular dog biscuits are small, hard, bone-shaped product that is coloured to reflect its flavour. In addition to flavour variations, dog biscuits also are sold in different sizes: small biscuits for small dogs, large biscuits for large dogs. The shapes have also changed from the conventional shapes. Dog biscuits are usually used for training purposes and these may be used as regular diet for pets. Dog biscuits can be given not only as treats but also as ways to make the dog healthier. As a result of their low- moisture content, the biscuits resist mold growth and bacterial spoilage, these can be stored for the longer time at ambient temperature.

Meat and meat products are deficient of calcium, but in case of pet mineral requirement, calcium is required in the highest amount. Calcium requirement in pets during peak growth and lactation ranges from 1.0-1.8% of total diet on dry matter basis. Calcium is essential in the body for many functions including bone formation, blood coagulation, muscle contraction, and nerve impulse transmission. Calcium deficiency was once a more common disease. It resulted primarily from animals fed diets high in meat and organ meats, which are high in phosphorous and low in calcium. If the meat based dog foods are not fortified with calcium they may develop skeletal abnormalities often referred to as rickets. The bone could become soft or very thin and brittle. Increased calcium requirements during pregnancy and lactation, (eclampsia) may also cause calcium deficiency. Commercial products labeled “dicalcium phosphates” are industrial products resulting from the acidulation of rock phosphate, frequently with sulfuric acid, yielding phosphoric acid, which is neutralized with calcium carbonate after purification. These products are a mixture of varying amounts of dicalcium and monocalcium phosphates, phosphoric acid, calcium

carbonate, and impurities, depending on the origin of the raw material and procedures employed in its industrial production. It is commonly used as a calcium supplement, containing 40.0% soluble calcium by weight (DeLuca 2003).

Selenium is important trace mineral used to produce glutathione peroxidase, an enzyme that serves as a natural antioxidant. Selenium is also required for normal pancreatic function and lipid absorption. Glutathione peroxidase works with vitamin E to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals. Adequate amounts of selenium can spare vitamin E for other important physiological functions. Incorporation of sodium selenite as source of selenium in the pet biscuits will ensure that pets receive adequate amounts of both Vitamin E and selenium.

In the light of above discussion, to develop the dog biscuits based on the slaughter house byproducts and fortified with calcium and selenium, the present study was designed with the following objectives:

1. To optimize the incorporation level of different poultry by-products (liver, gizzard) etc. along with calcium and selenium fortification for development of dog biscuits.
2. To evaluate the storage stability of developed dog biscuits at ambient temperature and to calculate the production cost.

CHAPTER II

REVIEW OF LITERATURE

2.1 Pet foods

Pet foods are found in three basic forms i.e. dry, semi-moist and moist. The three form of pet foods have different moisture level; 3-11 per cent moisture in dry foods, 25-35 per cent moisture in semi-moist foods and 60-87 percent in moist foods (Hand *et al* 2010). Big national supermarket chains and pet retail outlets are famous with private label brands. The non-branded pet food products are available at lower prices for pet owners. The good brands are more likely to emphasize superior ingredients and nutrition. So, they are called premium and super premium foods. These products are found in different pet stores, pet superstores, veterinary hospitals and some farm/feed stores. The unique nutrient profile is contained in the veterinary therapeutic foods which are sold at veterinary practices or hospitals for treatment with specific disease states.

In advanced countries, Pet foods have a history of more than a hundred years. The first pet food was commercialized in human history by James Spratt in 1860 which leads to the development of pet food industry. Spratt developed the first dog biscuit which made by a mixture of wheat meals, vegetables, beetroot and beef blood. He was a successful businessman and set a new market demand and traded with English country gentlemen for sporting dogs.

Royal Canine, Propack, Eukanuba, Mars, Nutripet, Pedigree, Whiskas, Champ and Robust are some of the commercial pet food manufacturers. The ingredients like chicken, chicken byproducts, meat, meat and bone meal, meat byproducts, fish meal, ground wheat, Soya, corn gluten meal, rice flour, vegetable and animal fat, brewer's dried yeast, dried egg product, probiotics, minerals vitamins antioxidants and salt which are dominated in their products.

The pet food industry are mainly used two categories of ingredients which consisted of Grains and milling byproducts such as corn gluten meal and animal tissue byproducts from meat packing, fish-canning industries and poultry processing (Morris and Rogers, 1994).

The predominant ingredients of commercial dog food as reported by subcommittee on Dog Nutrition (1985) held at Washington D.C., National Academy Press is as follows:

Dry	Semi-moist	Canned/Moist
Corn grain	Corn grain	Meat byproduct
Soyabean meal	Meat byproduct	Meat
Animal fat	Soybean meal	Poultry byproduct
Meat and bone meal	Corn syrup	Soya flour

Philips (2004) reported that the people are really willing to spend extra money to provide healthy food for their pets. The pet food industry is considered by many to be "recession proof". Owners tend to buy food based on what their pet will eat and what is good for them, not on price and there is an increasing trend in today's market that people tend to think if a food is good and safe for them, that it should also be good and safe for their pet.

The feeding practices and blood metabolic profile of pet dogs reared on homemade diets were studied by Shakhar *et al* (2010). They found that homemade diets constitute the mainstay of feeding pet dogs in India, but these diets were nutritionally inadequate and imbalanced especially with respect to protein, energy and minerals.

With the increase in demand of pet foods, the market is rapidly growing in India. The trend driven access and awareness based nature of the nation make it an alluring target to capital rich multinationals, the recently interest in FDI's is a crucial aid to this process. In 2014 the market was valued at around $198.6 million dollars and is expected to grow at a CAGR of 13.9% to grow into a $434.3 million market by 2020 (India pet food market-growth ,trends and forecast 2015-2020).

The Indian pet food industry (July 2016) was valued at USD 130million and is expected to reach at USD 270 million in 2019 at a CAGR of 38%.Increased number of pet shops providing access to pet owners to pet products and increased awareness regarding access to pet foods instead of homemade food are main growth drivers.

According to a recently published report by TechSci Research, "India Pet Food Market Forecast and Opportunities, 2019", the pet food market in India is projected to cross USD270 million by 2019. Major factors driving the demand for pet food in the country include rising number of nuclear families particularly in urban areas; increasing pet ownership and rising per capita disposable income. In India, pet food predominantly includes packaged, ready-to-eat food products that are manufactured to provide complete nutrition to pets. Available in dry, wet and treat/snack form, these products are suitable for consumption by dogs, cats, birds and other pets. In India, dogs are more popular pets than other animals like cats, birds, fish, etc. This creates a significant opportunity for the pet food companies to come up with a wide range of food products for dogs. Dog food segment is expected to continue its dominance in the forecast period as the largest revenue contributor, followed by cat and fish food segments. Dry food is more popular in case of dog food segment due to general liking of dogs towards dry food, while cats prefer wet food. Treat/snacks are also popular among dogs, however, due to high prices, most of the pet owners prefer buying dry food.

In 2013, around 85% of the pets in India were dogs, with the northern region accounting for highest number of pets. Cats and fish, on the other hand, are more popular pets in the southern region of India .Mars International is the leading manufacturer and supplier of pet food products in the country. The company offers its low-cost products under Pedigree and Whiskas brand and premium quality products under Royal Canin brand. Other major pet food companies operating in the country include Provimi Animal Nutrition India, PetSetGo and Bharat International Pet Foods. "In terms of brand, Mars is dominating the pet food market in India, accounting for around 70% revenue share. However, other brands like Petsetgo, Drools, etc. are expected to increase their penetration in India by expanding their production or setting up new manufacturing units across the country over the next five years." said Mr. Karan Chechi, Research Director with TechSci Research, a research based global management consulting firm."India Pet Food Market Forecast and Opportunities, 2019" has evaluated the future growth

potential of India's pet food market and provides statistics and information on market sizes, shares and trends.

According to the report "Pet Food Ingredient Market by Ingredient (Cereals, Meat & Meat Products, Vegetables, Fruits, Fats, and Additives), Source (Animal-based, Plant-based, and Synthetic), Pet (Dog, Cat, and Fish), Form (Dry and Liquid), and Region - Global Forecast to 2023", The pet food ingredients market is estimated at USD 34.96 Billion in 2018 and is projected to reach a value of USD 45.44 Billion by 2023, at a CAGRs of 5.4%. The market is driven by factors such as increasing pet population and pet humanization.

According to the report of "Pet Food Market Size, Share & Trends Analysis Report By Product (Dry, Wet/Canned, Nutritious, Snacks/Treats), By Application (Dog, Cat, Others), By Region, Competitive Landscape, And Segment Forecasts, 2012 – 2022, the global pet food market size was valued at USD 76.53 billion in 2016 and is expected to register a CAGR of 4.3% in terms of revenue over the forecast period. Growing trend of pet adoption as a result of increasing number of nuclear families in emerging economies is expected to be a major growth driver over the coming years. With the increase in pet ownership, healthcare and spending, Asia's pet industry is expected to grow at 14.3 per cent annually from 2016 to 2021. Pet ownership as well as expenditure per pet is increasing (Asia-Pacific Veterinary Healthcare Market - Trends & Forecasts (2017-2022).

According to report of the "Pet care market: Southeast Asia industry analysis and opportunity assessment 2014-2020, Future Market Insights", few emerging markets are China,Japan and South Korea. In China, the pet food market accounts for 37 per cent of total industry revenue. In Japan, improved healthcare and rising awareness about pets' nutritional requirements has lead to longer pet life expectancy. Consumers in Japan have shown a willingness to take on ownership of new pets, particularly larger ones (Pet Care in Japan, Euromonitor, July 2017). In South Korea, the pet industry is forecasted to reach USD573 million in revenues by 2022. Online sales are driven by discounts and the hectic lifestyles of Koreans. The expected sale of pet food via the internet is around USD180 million in 2022.

The total expenditures and Ownership on dogs and cats is maximum in the United States and Europe but now it's growing fast in many emerging markets such as Brazil and China. In the United States, cats and dogs are the most popular pets with 45.3 million households owning cats and 56.7 million households owning dogs. Dog owners more pay per year on dogs, which about $304 a year on food and treats of dogs as compared with $239 a year on food and treats of cats (APPA, 2014).

2.2 Pets diet requirements

One characteristic that differentiates pet food from human food is that with pet food, one type of food has to satisfy all the daily nutritional requirement of a pet. To meet carbohydrates, protein, fat, and macronutrients requirements, a variety of ingredients such as grains, meat products, fat, and micro-ingredients (vitamin and minerals) are combined in a complex food matrix (Gibson and Alavi, 2013).

When considering protein requirements of pets, both dogs and cats need 22 amino acids to synthetize the various body protein structures. In dogs, 13 amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine are essential and must be present in the diet in a sufficient amount depending on the animal needs. Ten amino acids are considered essential for cats (Hussein, 2003a; Hussein, 2003b). Most of the commercial dog food products supply proteins above the minimum requirements. Thus, protein deficiencies in adult dogs is usually not expected (Kallfelz, 1989). However, in particular life stages such as gestation, lactation or during particular heavy work conditions, the choice of low quality and poor formulated diets can lead to protein deficiency (Case, 1999). Protein requirements can vary depending on factors such as protein quality, protein digestibility, energy intake, age, and reproduction status. For adult dogs, a minimum requirement of 18% crude protein (CP) on DMB was adopted by AAFCO for maintenance, and a minimum of 22% CP for growth and reproduction (AAFCO, 2016).

For adult cats, higher protein requirements are adopted by AAFCO: a minimum of 26% CP on DMB for maintenance, and a minimum of 30% CP on DMB for growth and reproduction stages. Although no specific requirements are adopted

for carbohydrate levels in dogs and cats diets, these pets can utilize carbohydrates when provided in a proper form. Most of the dog and cat food products contain carbohydrates in the form of starch from cereal grains such as corn, wheat, sorghum, barley, and rice. Glucose derived from carbohydrate digestion is utilized together to glucose produced endogenously (Hussein, 2003a; Hussein, 2003b).

Fats are important in pets' diets for several reasons such as because of their high energy density, because of their role as carriers of fat-soluble vitamins, the improvement of food palatability, and because they can provide desirable texture to the food. Low fat levels in the diets can lead to energy shortage and decreased palatability of diets with consequent intake reduction. A minimum level of 5% fat on DMB for dogs, and a minimum of 9% fat on DMB for cats, are recommended by AAFCO for maintenance (Hussein, 2003a; Hussein, 2003b).

From a nutritional perspective, if a product is well formulated in order to satisfy the nutritional requirements, the form of the food is not relevant. However, it might be relevant for the pet owner and have an influence on pet preferences (Dzanis, 2003).

2.3 Utilization of slaughter house byproducts in pet foods

The production and consumption of poultry products have been on the increase globally. With the large production of poultry meat worldwide, thousands of tons of organic by-products in the form of viscera, feet, head, bones, blood and feathers are generated (Zhu *et al* 2010). The viscera constitute about 30% of these wastes while feather could be up to 10% (Jamdar and Harikumar, 2005; Grazziotin *et al* 2007). It is a common practice to convert animal wastes into livestock feed and organic fertilizers. As a result, processes such as rendering, composting, chemical, microbial and thermal treatment of poultry and other animal wastes were developed and widely researched (Salminen and Rintala, 2002; Cai *et al* 1995; Kornilłowicz-Kowalska and Bohacz, 2011).

One of the traditional uses of raw and rendered animal byproducts has been its incorporation as ingredients in feeds and pet foods. These materials provide diets with adequate nutrients and good digestibility (Murray *et al* 1997). In fact, meat and bone

meal, blood meal, plasma meal, hydrolyzed feather meal, tallow or grease contain protein, fat, minerals and trace elements as well as B vitamins and some fatsoluble vitamins which are required in animal nutrition (Pearl, 2004). As mentioned for human food, the functions and usefulness of some organs may vary because of relevant fluctuations in the nutrient content depending on the animal species from which they are obtained.

Corbin (1992) stated that animal by-products have been a major contributor to the growth and expansion of the world's pet food industry and have supplied the majority of the proteins, fats, and minerals, and significant quantities of vitamins for pets through the years. Lowe (1989) stated that the variation in nutrient content of animal protein meals is much larger than that of protein of plant origin. Houpt and Smith (1981) reported that cats prefer fish, whereas the dogs prefer beef, pork and lamb to chicken liver and horse meat, and both strongly prefer to eat cereal diets. Dogs require higher protein and adequate concentration of essential amino acids in their diets.

Hubert *et al* (1994) reported that the primary protein source in dry commercial dog foods used are meat and bone meal (MBM), poultry byproduct meal (PBPM) other animal byproducts and soybean meal in various concentrations. Heinicke (2003) and Quigley *et al* (2004) reported that animal proteins are widely used in diets for companion animals due to their low cost, availability and nutrient value. Animal byproducts / poultry slaughter waste (PSW) are being used by almost all the pet food industries, as they are rich and cheap source of protein. Nutrient content and palatability are other desirable characteristics of poultry byproduct meal.

Murray (1997) studied the effects of raw and rendered animal by-products by incorporating into dog diets on nutrient digestion at the ileum and in the total tract. Diets fed contained various animal by-products including a rendered beef meat and bone meal (RMBM); fresh beef (FB); poultry by-product meal (PBPM); fresh poultry (FP); a plant-based control protein source, defatted soy flour (DS); and an animal-based control protein source, dehydrated whole egg (WE). The diets were extruded and kibbled. By-products varied widely in concentrations of OM, CP, amino acids,

and fat. Nutrient intakes were numerically higher for FB than for all other treatments. All nutrient intakes were higher ($P< 0.03$) for the FB treatment than for the RMBM treatment. Digestibility of DM, OM, CP, fat, and GE at the ileum were higher ($P< 0.06$) when dogs were fed diets containing FP than when fed diets containing PBPM. Amino acids were highly digestible at the ileum; however, digestibilities of all amino acids except cystine were higher ($P< 0.04$) for the diets incorporating FP vs PBPM. Total tract digestion was different among treatments for DM ($P < .02$), OM ($P< .01$), and GE ($P < .02$), and diets containing animal by-products were similar in total tract digestibility, greater than the DS control, and lower than the WE control.

Liu (2002) reported that many organ meats contain more polyunsaturated fatty acids than lean tissue. Brain, chitterlings, heart, kidney, liver and lungs have the lowest level of monounsaturated fatty acids and the highest level of polyunsaturated fatty acids. Hardy (2003) studied that more than 1 million metric tons of fish waste per year is generated from fish processing, most of which could be utilized in animal feed. Regenstein *et al* (2003) reported that fish waste supply high protein feed ingredients and palatability-enhancing agents for use in animal foods.

Karthikeyan *et al* (2002) studied the nutritional quality and palatability of pet food from poultry by-product meal. The studies were conducted to formulate 3 different foods containing 15% poultry by-products, meal in combination with leaker eggs. bakery waste. cereal and cereal by-products, edible oil permitted food additives, vitamin and mineral mixtures. The dough prepared by mixing these ingredients with water (1: 1 w/v) was moulded into rectangular biscuits of about 1.5 cm thickness and oven dried to moisture below 5%. The finished pet foods had high pepsin digestibility (69.7-71.4%) and were a good source of crude protein (22.9-23.7%), calcium (0.74-0.77%), phosphorus (0.67-0.70%), availablelysine (0.80-0.82%). Methionine (0.47-0.51 %) and ME (4.17-4.24 kcal/kg). Feeding 100g of these foods to adult pet dogs (10 kg) could meet 50 to 65% of their daily maintenance requirements for ME, CP, Ca, P, available lysine and methionine as prescribed by AAFCO (1993). The product was highly acceptable, when fed to pet dogs. The cost of ingredients utilized for pet food preparation worked out to about Rs 15/kg.

Fahey (2004) reported that a wide range of protein sources including meat and bone meals, poultry meals, poultry by-products meals, and soybean meal are traditionally used by the pet food industry. Willard (1990) reported that the lack of characterization of fish substrates products has led to their underutilization in commercial pet foods. Although the advantages of using these products as alternative protein sources are recognized in the pet food literature. Nutritionists are collecting and expanding the database on compositional analyses of fish substrates and are determining their bioavailability, palatability-enhancing traits and immunomodulatory role, in order to increase use of these alternative ingredients in dietary formulations for pets.

Krestel-Rickert (2001) developed the pet food product made up of the soft tissue (striated muscle, Viscera, and other organ tissue) of spent hens. The soft tissue of spent hen can be used by the pet food industry for the following reasons as it provides palatable material which is both a proven winner with pets and also provides good protein nutrition. It is also available at reasonable prices. Moreover, it has low ash and high quality protein. Pet food industry provides an environmentally friendly disposal method of light spent hens and it provides a solution to the spent hen disposal problem in the table egg industry.

Nie*et al* (2006) developed a pet treat with a rough surface texture and appearance is produced from a matrix binder and at least one granular filler having a particle size between 0.1 mm to 6 mm. The matrix binder may be selected from gelatinized starches, gelatinized flours, wheat gluten, soy protein, casein, caseinates, gelatin, hydrocolloids, gums and mixtures thereof. The granular filler may be selected from mill feeds, whole grains, distillers dried grains, pork grind, bird seed and mixtures thereof and extrusion shaping the pet treat.

Sunvold and Corrigan (2010) developed pet food in the form of coated kibbles. The coated kibble was made of a core and a coating. It was suggested that the core can be extruded and can have moisture, or water content less than 12 percent, the core can contain a coating. The coating can have a protein component from 50 percent to 95 percent of the coating and a binder component from 5 percent to 50 percent of

the pet coating. Cores can comprise the farinaceous material, proteinaceous material, and mixtures and combinations thereof. The carbohydrate source, or carbohydrate ingredient, or starch ingredient, can comprise cereals, grains, corn, wheat, rice, oats, corn grits, sorghum, grain sorghum/milo, wheat bran, oat bran, and amaranth. The protein source, or protein ingredient, can comprise chicken meals, chicken, chicken by-product meals, lamb meals, turkey, turkey meals, beef by-products, soybean meal, soy protein isolate, soy protein concentrate and com protein concentrate. The fat source, or fat ingredient, can comprise poultry fat, chicken fat turkey fat, pork fit, lard, beef fat, vegetable oils, corn oil, soy oil, cottonseed oil, palm oil, palm kernel oil and linseed oil.

Mahender *et al* (2013) conducted an experiment on a 4 x 4 LSD trial using adult Labrador dogs to find out the effect of incorporation of different levels of poultry slaughter waste (PSW) in dog biscuits, on dry matter (DM) intake, palatability and digestibility of nutrients. The treatment diets were No PSW (CON: n = 6); 5 percent PSW (PSW5; n =6); 10 percent PSW (PSW10: n =6) and 15 percent PSW (PSW15: n =6). The test diets were prepared by extrusion cooking process and fed in the form of differently shaped dry extruded type dog biscuits. The experiment lasted for 60 days. DM intake, DM intake as percent body weight and DM intake per kg metabolic body weight were significantly higher for the PSW5 group than other two PSW and control groups. The adult dogs consumed all test diets with equal preference to the control diet indicating, palatability of the PSW based dog diet biscuits was good and extrusion cooking of the diets had positive impact on the intake of the diets, while shape of the biscuit had no effect on the palatability.

Digestibility of OM, CP, CF and EE were significantly higher on FSW5 diet compared to control diet, while PSW10 and PSW15 diets we intermediate, indicating incorporation of PSW at 5 percent level in dog diet biscuits prepared by extrusion cooking had positive impact on digestibility of nutrients. The cost incurred on test diet PSW15 was 11 percent lesser when compared to control diet It was concluded that inclusion of PSW in the extruded dog diets improved palatability, DM intake and nutrient utilization and PSW could be incorporated in the adult dog diets at 15 percent level without any adverse effect on health of the animal while being cost effective.

Abdolghafour *et al* (2014) studied the effect of incorporation of buffalo meat by- products namely tripe meal and rice flour on development, quality evaluation and storage stability of pet foods under ambient condition. The quality of pet food was based on physicochemical characteristics namely moisture, ash, fat, protein content, pH, The Analysis using paired sample t-test for optimization. There was moisture content (8.211%), Ash content (3.571%), protein (17.85%), pH (6.688) and fat in the range (14.323%). The analysis model was found significant for protein, pH, ash and were not significant for moisture and fat.

Hill *et al* (2005) developed the pet chews made from flexible, tough rawhide that were impregnated with an emulsion and/or surfactant containing an ingestible, therapeutic ingredient suitable for releasing the particular therapeutic ingredient over the entire chew-life of the chew in order to help control, disrupt and remove biofilms, treatment of fetid breath, treating gum disease and treating other pet conditions. Deveau-Greene *et al* (2018) developed pet treats by processing cattle ears using high pressure processing. The cattle ears are covered with a liquid, such as water, and are exposed to high hydrostatic pressures for about one minute. The high hydrostatic pressures remove hair and implants from the cattle ears while preserving the texture and consistency of the ears, which are appealing to dogs and other pets.

Anderson *et al* (2001) developed a highly palatable and long lasting dog chew for pets. Pet chew is in form of roll or stick i.e. stick is made up of raw hide and its center is being filled with highly palatable meat based filling. Outside rawhide fraction is extremely tough and is chewy which makes the dog to consume chew for a long period of time. The inside meat filling is highly palatable, thus the animal maintains interest in the treat until nearly the entire chew has been consumed. The interior meat filling is preserved by reduced water activity to below 0.85 as a result of incorporating of salt, sugars and natural humectants. The filling is formulated and processed in such a manner that the water phase is bound within the filling and does not pass by capillary action to the outside rawhide fraction. This results in the outer rawhide shell maintaining a tough and chewable texture.

Dust *et al* (2005) reported that Chicken byproduct meal had the greatest total non-essential amino acid (TNEAA) concentrations (39.2%), and also high in glycine,

proline and hydroxyproline content. He also found chicken protein sources differed in concentrations of crude protein, acid hydrolyzed fat and total amino acids (TAA) by 20, 31 and 24 per cent, respectively and gross energy (GE) by 1.7 kcal/g. Karthikeyan (2004) found that recycling of poultry processing waste as valuable source of pet food is highly desirable as chemical analysis endorsed 94.43 DM, 53.46 CP, 33.12 EE, 0.24 CF, 2.75 NFE, 10.13 TA, 3.05 Ca, and 1.80 per cent P. Clapper *et al* (2001) analyzed the poultry meal for DM, OM, CP, fat, total dietary fiber (TDF) and for essential and non-essential amino acids and reported 96.2 DM, 90.4 OM, 74.5 CP, 15.0 fat and 2.7 per cent TDF.

Rivera *et al* (2000) studied the composition and protein fractions of different meat by-products used for pet food compared with mechanically separated chicken (MSC)Pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and mechanically separated chicken (MSC) were evaluated for proximate composition, protein distribution and connective tissue. Proximate composition varied among meat byproducts and MSC. Pork by-products contained the most crude protein ($p<0.05$). Low levels of high ionic strength soluble (HIS) proteins were obtained from meat by-products. Pork lungs and chicken viscera contained the greatest amounts of insoluble (IN) proteins ($p<0.05$). Total collagen values were positively correlated to IN proteins, intramuscular collagen (IMC) and elastin. Types I and III collagen could not be detected by SDS-PAGE for the different meat by-products though collagen solubility appeared to be significant. These results suggest functional property differences between specific by-products are likely when used in petfood product formulations.

2.3.1 Gizzard

The gizzards are muscular organ used for grinding and mixing of the food materials in preparation for digestion, thus replacing the mastication function of the teeth. The strength of the gizzard muscle and tough leather-like lining allow utilization of grit as well as the feed particles producing much friction in the grinding process. The physical breakdown of large feed particles increases their surface area, allowing more complete enzymatic digestion (Maiti and Ahlawat, 2011). The

increasing in the production of broilers followed by increasing in the quantities of offal's especially gizzards with high percentages of proteins and fats, can contribute for pet food production.

With the growing poultry production and processing activities, there would be an increased availability of the edible byproducts. Gizzard is one of the principal edible byproducts of poultry processing which is being marketed as variety meats along with dressed chicken. It forms nearly 3% of dressed chicken (Charonpong and Chen 1980) and as such it is less preferred by the consumer due to its peculiar flavour and texture. Gizzard contains approximately 20% proteins (Kondaiah and Panda 1987; Rao *et al* 1994) and has potential for using in cost effective, dog biscuits. Further, utilization of this byproduct would increase the profitability of broiler industry.

Abdelmageed *et al* (2013) conducted the study to explore the possibility of using broiler chicken's gizzard and abdominal fat in production of sausage. Five types of sausage with different level of gizzards meat (100%, 75%, 50%, 25%, and 0%) were processed. A taste panel was done for all types of sausage, and the 25% gizzards sausage was stored with the rest of meat and gizzard under freezing (-4oC) for 45days. Chemical and sensory analyses were done for all samples. There was non-significant difference ($P \leq 0.05$) between 25% gizzards sausage and commercial sausage in the appearance, tenderness, firmness, and overall acceptance. Storage of meat and meat products lead to increases in contents of moisture, ash, and pH, and decreases in contents of fat and protein. It is recommended to use gizzards and abdominal fat in sausage processing after good and quick cleaning with a percentage exceeds 25%.

Sulieman *et al* (2014) studied the effects of incorporating chicken's gizzards and abdominal fat in the quality of burger meat product. The effect of sex and weight of chicken on gizzards weight was determined. Two types of burger were processed, gizzard burger (GB) and beef burger (BB) and compared with Looli commercial burger (LCB). The quality of burger products was assessed using chemical and microbiological analyses as well as sensory evaluation. The results showed non-

significant difference ($P > 0.05$) between the two sexes on gizzards weight, while there was a significant difference ($P < 0.05$) due to the birds weight. Salmonella was not detected in the gizzards, while E.coli and Salmonella were present with high counts in beef fat. There was non- significant difference ($P > 0.05$) between gizzards and beef burger in their appearance, tenderness, firmness, taste and overall acceptance. Storage of meat and meat products lead to a significant increase in the total viable bacterial count. Storage increased moisture, ash, and pH, but decrease fat and protein. The study recommends utilization of gizzards and abdominal fat in burger production after proper and quick cleaning.

Sudheer *et al* (2011) developed restructured chicken product by incorporating optimum level of gizzard and fat and evaluated their quality attributes under refrigerated storage condition ($4 \pm 10^{o}C$). Incorporation of gizzard did not show any change in pH, but cooking yield reduced significantly ($P < 0.05$) at 40% level. There was a significant ($P < 0.05$) decrease in fat content and increase in protein and ash content with increase in the level of gizzard in the formulation. Significant ($P < 0.05$) improvement in the sensory attributes of chicken blocks was noticed by addition of gizzard. Incorporation of fat resulted in no change in pH, significant ($P < 0.05$) reduction in cooking yield, moisture and protein content and increase in fat and total ash content. Products incorporated with fat rated better than control for various sensory attributes. Significant ($P < 0.05$) increase in thiobarbituric acid reactive substance (TBARS), tyrosine value, stand plate count and psychrophilic count was observed during refrigerated storage ($4 \pm 10^{o}C$) in both control (no gizzard and fat) and test chicken blocks incorporated with gizzard and fat. The restructured chicken products were found acceptable up to 10 days under refrigerated storage condition ($4 \pm 10^{o}C$).

2.3.2 Liver

Liver is the major carbohydrate reservoir containing 50 percent of total carbohydrates found in the body. Carbohydrates are stored as glycogen and represent 2 to 8 percent of liver weight (Romans *et al* 1994). Measurement of pH and glycogen in liver was suggested as simple and reliable indicators of freshness of liver (Shelef,

1975; Hanna *et al* 1982; Herrero *et al* 1999). The higher content of glycogen (5%) in liver enables the production of lactic acid by enzymes and bacteria during storage. Therefore, glycogen content declines and lactic acid increases during storage (Shelef, 1975; Gill and Delacy, 1982). The pH values of fresh livers had been reported as 6.3 to 6.4 in beef (Shelef, 1975; Herrero *et al* 1999), 6.2 to 6.5 in pork (Hanna *et al* 1982) and about 6.21 to 6.63 in sheep livers (Gill and Delacy, 1982). These studies have also shown that liver pH gradually decreases during aerobic storage and pH values below 6.1 may be considered as indicative of liver spoilage. Liver exhibit large difference in protein content, distribution and vary widely in protein functionality and bind values as compared to lean meat (Rivera *et al* 2000). Liver and other edible offals influence the texture, emulsifying capacity, water holding properties and yield of the finished product. Meat by-products are underutilized and low priced because they are considered as an inferior source of protein compared to skeletal meat (Oliveros *et al* 1982). However, the increasing price of meat and processed meat products is causing the food industry to evaluate the utilization of all protein sources including edible offals (Gosaka *et al* 1988).

Composition and some functional properties of selected beef and pork by-products have been reported by different research workers (Oliveros *et al* 1982; Nuckles *et al* 1990; Kim *et al* 1991 and Rivera *et al* 2000). Functional properties of selected buffalo meatby-products have also been reported by Kondaiah *et al* (1986) and Krishnan and Sharma (1990).

Nuckles *et al* (1990) observed higher moisture, protein and lower fat in pork liver than other organs. They also observed that pork liver contained the highest quantity of low ionic strength soluble proteins. They reported that low levels of high ionic strength proteins in offals might lead to poor water and fat binding particularly when used in high moisture foods.

Liver contain low levels of myofibrillar and high levels of sacroplasmic and stroma proteins when compared to lean meat (Rivera *et al* 2000). Ramirez *et al* (1995) observed that liver proteins exhibited better emulsion capacity than heart but

produced weak gel. Rivera *et al* (2000) reported the moisture, protein ratio of 4.78 for pork edible offals and 3.6 to 3.8 for skeletal meat.

Liver and liver products are often more economic source of proteins, vitamins and minerals than retail meat cuts and are most nutritious of all meat items. Liver is also an excellent source of readily digestible haeme iron, provides vitamins particularly vitamin B12 and vitamin A (Romans *et al* 1994). Average composition reported for beef liver is water - 70%; Protein - 20%; Fat - 3.8%; Carbohydrate - 5.3%; Ash-1.3%; Ca - 8.0; Mg - 22.0; Phosphorus - 362; Iron - 6-12; sodium - 87; Potassium - 298 (mg/100 gm) (Shelef, 1975). Liver is, the only food source rich in vitamin A, riboflavin, niacin, vitamin B12 and iron content (Hedrick *et al* 1994). Liver is also a good source of vitamins D, E, K and other B complex vitamins. Liver is the principal storage site for glycogen, iron, foliate/folic acid and other minerals. Liver contains heme iron, which is easily absorbed as compared to nonhaeme iron present in skeletal muscle (Park and Attaie, 1988). Liver is considered as a good dietary source of foliates. The average content of foliate in beef liver is 650-700 µg/100 gm (Vatheristo *et al* 1996). Therefore, desiccated liver and liver extract have long been used as nutritional supplement in treating different types of anemia. It has also been shown that liver contains greater amount of polyunsaturated fatty acids as compared to meat (Lawrie, 1991).

Organ meats are known to contain higher amount of cholesterol than skeletal meat. Mustafa (1988) reported the value of 190 mg and 280 mg cholesterol per 100 gm of liver in beef and sheep, respectively. Anderson *et al* (1986) compared the liver cholesterol levels in different species are found the values of 214, 354, 280 and 300 mg cholesterol per 100 gm for goat, beef, sheep and pork liver,respectively. In another study, Park *et al* (1991) reported cholesterol level of 214 mg/100 gm in goat liver.

Liver incorporation in meat products has been avoided because of its poor binding quality, high collagen content and undesirable sensory qualities (color, flavor and texture) it imparts to the product (Nuckles *et al* 1990). Therefore a large percentage of this valuable protein source is discarded and not utilized for human

consumption (Oliveros *et al* 1982). Also liver is underutilized and low priced because of poor aesthetic appearance and shelf life.

Devatkal *et al* (2004) studied the physicochemical, functional and microbiological quality of buffalo liver and reported that buffalo liver is an important edible meat byproduct. However, in developing countries including India, it has a low commercial value and is underutilized. Proximate composition was: moisture – 71.92%, protein – 18.44%, fat – 5.60%, carbohydrate – 2.72%, total ash – 1.32% and total energy – 135 kcal. Mineral concentrations (mg%) in liver were: Na – 60.04, K – 274, Ca – 5.60, Mg – 6.20, Fe – 20.86 and Cu – 5.60. Mean glycogen (mg/g), total liver pigments (mg/g) and cholesterol (mg%) were 7.07,8.49 and 283.88, respectively. The mean pH values of buffalo liver was 6.42, WHC – 38 ml per 100 g and cooking yield was 73.15%. Protein extractability studies indicated that liver contains higher amounts of water-soluble proteins (20–40%) than salt soluble proteins (7–15%) and presence of high molecular weight proteins in salt soluble protein fractions. The average microbial counts (log10 cfu/g) for different organisms were APC – 6.10; psychrotrophs – 4.30; enterobacteriaceae counts – 4.97; staphylococcal counts 2.50 and total coliforms – 2.82.

2.4 Palatability testing of dog biscuits

Palatability can be described as the perception derived while the food is consumed. Palatability takes into account the perceived flavor and appearance by the animal, and the temperature, size,texture, and consistency of the food. Prior experience with a specific food can also play a role affecting palatability (Kitchell, 1978; Bradshaw, 2006). According to the National Research Council (NRC 2006) palatability is defined as "physical and chemical properties of the diet which are associated with promoting or suppressing feeding behavior during the pre-absorptive or immediate post absorptive period". Another aspect that needs to be taken into account when discussing palatability is the interaction between pet owners and pets (Aldrich and Koppel, 2015). Palatability tests can be consumption or non-consumption tests (Griffin, 1984). The consumption type of testing is more common and consists of measuring food intake by the animal. A consumption test can

investigate the acceptance of a specific food by the animal, such as with monadic or single-bowl (one-pan) test, or the preference of one food over another using two-bowl (two-pan, paired stimulus, split plate, or versus test) or forced choice preference test (Thombre, 2004; McArthur *et al* 1993; Tobie *et al* 2015).

2.4.1 Single Bowl Test

In the single bowl test food intake is measured, after a specified time interval, by the difference from the initial amount of food provided to the animal less the amount leftover. One or multiple feedings per day are planned and it is usually repeated for 5 or more days for a specific food. After a first sample is served, other samples can be presented to the animal for the same period of time. The different intake values relative to the different foods showed to the animal are then compared. This test resembles the type of feeding experience a pet would have in the household environment. As the name suggests, an acceptability test can help to understand whether particular samples are considered acceptable or unacceptable by the animal. But, it does not provide any information about preferences and degree of liking. So, this type of test is not recommended for situations that need to provide flavor direction when developing a product, or to collect data in order to back-up a marketing claim. The number of animals necessary for this type of test can be as low as 8-10. Acceptance tests can be performed using trained kennel pets or using untrained pets in a homeuse-test setting (HUT), and different breeds and pet size can be utilized (Aldrich and Koppel 2015). However, it must be noted that animals and in the home and kennel might not respond to foods in the same way (Griffin *et al* 1984).

2.5 Importance of calcium and selenium in pet nutrition

Calcium is known for its role in building strong bones, but it also performs several other functions in dog's body. Calcium helps keep dog's nails, teeth, and coat healthy. Calcium is required for digestion, blood clotting, squeezing and relaxing muscles, releasing hormones, and proper nerve function. Calcium even helps dog maintain a regular heartbeat. Dogs that do not have enough calcium can have hypocalcaemia. A dog is considered to be hypocalcemic when its total serum calcium is usually less than 7 mg/dL. Calcium deficiency can also cause general bone

and joint weakness, pain, and make a dog fracture prone. Dental health can also be compromised.Hypercalcemia in dogs can be very harmful. A dog is considered hypercalcemic when its total serum calcium is greater than 11.5 mg/dL. Dogs with abnormally high calcium levels may show signs of illness such as weakness, listlessness, increased drinking and urination, and loss of appetite. In some pets, prolonged hypercalcemia may contribute to formation of bladder or kidney stones

Cline (2012) reported that calcium and phosphorus are discussed together because of their close relationship, particularly in bone health. Most commercial pet foods contain calcium supplementation from a variety of sources. Naturally high sources of calcium include fishmeal (salmon meal), meat and bone meal, chicken or poultry meal, algae, kelp, many green leafy vegetables, bone meal, blood meal, and eggshells and whole eggs. Feral or wild populations of dogs and cats receive calcium from bones, and in small quantities from blood, but muscle is not an adequate source of calcium. Muscle is very high in phosphorus but low in calcium. Most commercial pet foods incorporate supplemental calcium into their rations to ensure the correct ratio of Ca:P. Animals fed all-meat diets are commonly affected with rickets because of the very low calcium levels as muscles is not an adequate source of calcium.

Meat and meat products are deficient of calcium, but in case of pet mineral requirement, calcium is required in the highest amount. Calcium requirement in pets during peak growth and lactation ranges from 1.0-1.8% of total diet on dry matter basis. Calcium is essential in the body for many functions including bone formation, blood coagulation, muscle contraction, and nerve impulse transmission. Calcium deficiency was once a more common disease. It resulted primarily from animals fed diets high in meat and organ meats, which are high in phosphorous and low in calcium. If the meat based dog foods are not fortified with calcium they may develop skeletal abnormalities often referred to as rickets. The bone could become soft or very thin and brittle. Increased calcium requirements during pregnancy and lactation, (eclampsia) may also cause calcium deficiency. Commercial products labeled “dicalcium phosphates” are industrial products resulting from the acidulation of rock phosphate, frequently with sulfuric acid, yielding phosphoric acid, which is

neutralized with calcium carbonate after purification. These products are a mixture of varying amounts of dicalcium and monocalcium phosphates, phosphoric acid, calcium carbonate, and impurities, depending on the origin of the raw material and procedures employed in its industrial production. DeLuca (2003) stated that calcium carbonate is commonly used as a calcium supplement, containing 40.0% soluble calcium by weight.

Schlesinger *et al* (2011) reported that with the introduction of pet foods in developed countries, chronic calcium imbalance had become uncommon. However, with the resurgence of homemade and raw-type diets for dogs and cats, there is an increased incidence of calcium, phosphorus, and vitamin D imbalance. Potchem (1977) investigated that chronic over supplementation with calcium, in combination with genetic and environmental factors, can lead to retarded bone remodeling and osteochondrosis**.** Lepine*et al* (1998) reported that pet food composition for large breed puppies contains about 0.75% to 0.95% by weight calcium and about 0.62% to 0.72% by weight phosphorous. The composition further includes a source of protein, fiber and fat. The pet food composition is fed to large breed puppies to provide optimum skeletal growth and reduce the incidence of skeletal abnormalities.

Selenium**,** like the other trace minerals is necessary to sustain life and is essential for basic physiological functions in both animals and human. While the daily requirement for this mineral is obviously less, its importance and impact on the health and well being of livestock and humans are well documented in research. Schwarz and Foltz (1957) identified selenium as a micronutrient for bacteria, mammals and birds. Selenium can increase the health of the skin, potentially reducing dandruff and dry skin. Waters *et al* (2003) reported that selenium supplementation has shown to decrease DNA damage and increase epithelial cell apoptosis within the aging canine prostate.

Vleet (1975) studied the synergistic effect of selenium with vitamin E when administered together to Beagles. It was demonstrated that Beagles which were initially 5 to 8 weeks old, developed clinical signs of vitamin E-selenium deficiency after 40 to 60 days of consuming an unsupplemented semi synthetic

diet. Selenium can increase the health of the skin, potentially reducing dandruff and dry skin. It plays an important role in hair growth. Selenium can also improve the hair coat quality, making it more soft and shiny. As a result of a healthier coat, there is also the possibility of less shedding and hair loss. Yu *et al* (2006) reported that both low and high selenium in diet reduced hair growth in adult dogs.

Fish, meat, poultry, whole grains and dairy products are typical sources of selenium. AAFCO and the FDA have approved a selenium supplement to animal diets, most commonly in the form of sodium selenite for pet foods. Depending on the nature of ingredients used in pet food formulations, the selenium levels vary in all diets. Most dry and canned dog foods today use an inorganic type of selenium, sodium selenite or sodium selenate. In addition to selenium present in the pet food ingredients, additional selenium sources are added in commercial diet formulations. Forceville and Meaux (2007) reported that selenite had a pro-oxidant effect and thus, the use of selenate was preferred. Current selenium supplements are mainly dependent on inorganic sources like sodium selenite which are found to be less bioavailable and toxic. However, relative uses of selenium and its forms would be dependent on its nature of application and end use requirement. Keeping safety of the pet animals and environment as main focus areas, organoselenium compounds would be good and alternate prospective choices for research scientists working in pet animal nutrition.

2.6 Sensory quality and storage stability of pet food

Developed dog biscuits are dried products. Drying is a process in which water is removed from a material by evaporation or sublimation (Lewicki, 2004). The advantages of dried products are shelf stability, less storage space, ease of transport and most importantly, convenience and useful in natural disasters such as cyclones, floods earthquakes etc. These products are of much interest since they do not require refrigeration during marketing as well as storage. Both hydrolytic and oxidative rancidity development is a serious problem during the storage of meat products (Kowale *et al* 1996). Their importance can be assessed quantitatively on the basis of thiobarbituric acid value and peroxide index.

Lowe and Kershaw (1995) determined water activity and critical maximum moisture requirement over practically applicable ranges for several commercial pet diet components for stability and they found the same as aw = 0.60 and below spoilage by microorganisms and mites would not be a problem. Lin *et al* (1998) showed that the lipid oxidation rate constant of the extruded dry pet food was a function of fat types, added fat content and feed moisture content. Samples with poultry fat had a higher lipid oxidation rate than those with beef tallow addition in extruded dry pet food during storage. They also reported that the samples containing higher fat content or higher moisture content had lower rate of lipid oxidation.

Goswami *et al* 2015 developed the carabeef cookies by replacing refined wheat flour with carabeef powder. The carabeef cookies were sensory evaluated and baked at three different time–temperature combinations viz. 150-160°C for 35-40 minutes, 170-180°C for 25-30 minutes and 190-200°C for 15-20 minutes. Physicochemical properties, proximate analysis, color values, instrumental texture parameters were evaluated. Parameters like moisture, protein, fat, pH, diameter and ash percentage had no significant difference at higher baking temperature. Textural parameters such as hardness, shear force and adhesiveness values increased significantly. Whereas, mean cooking yield and thickness values showed decreasing trend, while spread ratio values showed increasing trend. Mean yellowness values had no notable difference, whereas mean redness, hue angle and chroma values increased in significant ($p<0.05$) manner with a higher baking temperature and lower time combinations. All sensory attributes scores decreased at a higher baking temperature. Carabeef cookies containing 50 per cent carabeef powder baked at 150-160°C for 35-40 minutes were selected as best treatment.

Jaiswal *et al* (2015) developed chicken meat biscuits by replacement of refined wheat flour with different levels (40%, 50% and 60%) of chicken meat powder prepared by mincing and dehydration of chicken meat and carried out the sensory evaluation and texture profile analysis at ambient temperature in aerobic packaging. Biscuits prepared with different levels of chicken meat were indicated as: (A) for control, (B) for biscuits containing 40% chicken meat, (C) for chicken meat

biscuits containing 50% and (D) chicken meat biscuits for 60%. They found that replacement of refined wheat flour with different levels of chicken meat powder (B, C, D) had significant effect ($P<0.05$) on work of shearing, appearance and colour, texture values, flavour, meat intensity, saltiness and overall acceptability in comparison with (A) control in aerobic packaging conditions. Replacement of refined wheat flour with different levels of chicken meat powder (B, C, D) shows highly significant effect ($P<0.01$) on shear force value and meat intensity compared to control (A) in aerobic packaging conditions.

Kumar *et al* (2016) prepared the fiber-enriched chicken meat biscuits incorporated with wheat bran and oat bran at three different levels i.e. 3%, 5% and 7% and evaluated them for quality attributes and storage stability at ambient temperature for 180 days. It was observed that bran incorporation showed increased trend in ash, moisture content and crude fiber content and decreased trend in pH values, fat content, water activity and calorific values. During storage, bran treated samples showed improved oxidative stability as measured by free fatty acids and thiobarbituric acid reactive substances value. Treated biscuits were recorded with significantly ($P<0.05$) lower pH value and higher water activity during entire storage. Oat bran incorporated chicken meat biscuit were found to have much maintained sensory characteristics than wheat bran incorporated meat biscuits and control throughout the storage study

Goswami *et al* (2017b) developed the fibre enriched carabeef cookies after replacing the refined wheat flour with orange pulp fibre (OPF) as Control (C) with no orange pulp fibre, OT1 (with 5% OPF), OT2 (with 10% OPF) and OT3 (with 15% OPF). The physico-chemical properties, textural parameters, color values and sensory attributes of fibre enriched carabeef cookies were evaluated. The significant ($P<0.05$) decrease in pH of cookies with OPF incorporation was found. In contrast to C, the cooking yield of product was significantly higher ($P<0.05$) in OT2 and OT3. The diameter and spread ratio decreased significantly ($P<0.05$) in OT3 than C, while thickness showed negligible significant difference. Mean diameter and spread ratio values had no significant difference between C, OT1 and OT2. Increased trend was

observed in mean moisture percentage whereas mean protein and fat content ($P<0.05$) were decreased in significant manner.

In carabeef cookies with incorporation of orange pulp fibre, significant ($P<0.05$) increase was seen in TDF, IDF and SDF percentage, in comparison to that in between C and OT1 for SDF percentage. Insignificant difference was observed in hardness, redness, yellowness, chroma and hue angle of carabeef cookies at any level of orange pulp fibre incorporation, but redness values were decreased with marginal increase in yellowness at higher level (OT3) of orange. OT3 showed decreased mean flavor, crispiness and after taste scores in comparison to C, however no significant difference was observed up to 10% level of incorporation in flavor and after taste scores. Sensory scores for all attributes were comparable with control up to 10% orange pulp fibre (OT2) level in cookies, therefore 10% orange pulp fibre incorporation in carabeef cookies was adopted as optimum.

Malav *et al* (2017) prepared the papad by utilizing spent hen meat powder and corn flour (Control, T-I), black gram flour (T-II) and combination of corn and black gram flour (T-III). They observed that when microwaving and frying cooking process was used, expansion percentage increased significantly ($P<0.05$) in T-I and T-II than the T-III products. In all the treatments, throughout the study, the sensory attributes increased significantly. The microwaved black gram flour added meat papad (T-II) was accepted as most acceptable on the basis of physico-chemical and sensory attributes and was further anlysed for textural properties, water activity and shelf life evaluation for the storage period of 45 days at room temperature (25°C). In contrast to control (T-I), selected product had significantly higher ($P<0.05$) hardness, fracturability and water activity values. It was concluded that meat papad could be stored at room temperature in LDPE pouches for 45 days, on the basis of physico-chemical (pH and TBARS value) and microbiological parameters (TPC, Coliform count and yeast and mould count), without marked deterioration in the quality.

The cookies were developed by Chauhan *et al* (2016) by utilizing amaranth flour and the effects of whole amaranth substitutions at various proportions were examined and evaluation of cookies baking behavior was carried out. Six types of

formulations of cookies were prepared with whole amaranth flour ranging from 20, 40, 60, 80, and 100%. The physical (thickness, diameter, spread ratio, and bake loss), textural, and organoleptic attributes of cookies were evaluated. In whole amaranth flour cookies, diameter and spread ratios were found to be higher i.e. 52.20 mm and 6.46, respectively, in comparison to other blends (20–80%) of cookies varying from 51.37 to 51.92 mm and 6.13 to 6.36, respectively. With the addition of amaranth flour, textural measurement showed that hardness of cookies decreased. Whole amaranth flour cookies required least snap force (72.4 N) compared to control (whole wheat flour) cookies (145 N). The amaranth cookies with up to 60% were acceptable as indicated by sensory data, however additional amaranth flour resulted in a decreased mean score for overall acceptability.

The potential of flaxseed as functional ingredient was studied by Ganorkar *et al* (2014) and cookies formulations was prepared with the substitution of refined wheat flour at 5%, 10%, 15%, 25% and 30% levels by incorporating roasted flaxseed flour (RFF). As flaxseed flour was increased, diameter and thickness of cookies increased. 15% flaxseed flour incorporated cookies were found to be well comparable with control in sensory evaluation. Up to 15% flaxseed flour incorporation, hardness and fracturability of experimental cookies increased and beyond this, these attributes showed decline trend. In comparison to control, the flaxseed flour cookies showed increase in moisture, ash, fat and protein content. Fiber content was nine times more in flaxseed flour cookies than the control. In optimized cookies, Alpha linolenic acid (ALA) content was increased from 0.19% (control) to 4.76%. The polyunsaturated to saturated fatty acid ratio showed improvement whereas ω-6 to ω-3 fatty acids were found to be decreased to well below maximum recommended value.

CHAPTER III

MATERIALS AND METHODS

3.1 SOURCE OF MATERIALS

3.1.1 Source of poultry by-products

Poultry byproducts i.e. liver and gizzard required for the experiments were collected from the Instructional poultry Processing Plant of Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. After slaughtering of poultry birds and gizzard were collected, cleaned and packed in LDPE bags and stored in freezer.

3.1.2 Refined vegetable oil

The refined soybean oil (Fortune, Adani Wilmar Ltd) was procured from local market of Ludhiana; Punjab, India was used in the formulation.

3.1.3 Refined wheat flour (Maida)

Refined wheat flour (Maida) was procured from local market of Ludhiana, Punjab, India was used in formulation.

3.1.4 Spice mix

The spices were procured from local market of Ludhiana, Punjab, India. After removal of extraneous matters, the spices were oven dried at 50±2°C for 2 hrs. The ingredients were ground mechanically and sieved through a fine (u.s.s. #30) mesh screen. The powders so obtained were mixed in suitable proportion to obtain a spice mix for pet biscuits. The spice mix was stored in a PET (polyethylene terephthalate) jar for subsequent use. The formulation of spice mix prepared is given in Table 1.

3.1.5 Table salt and baking powder

Table salt (Tata chemicals Ltd., Mumbai, India) and baking powder (Weikfield) used in product preparation were procured from local market of Ludhiana.

3.1.6 Dicalcium phosphate

Dicalcium phosphate used in product preparation were procured from the LobaChemie Pvt. Ltd, Mumbai, India.

Table 1: Composition of spice mix

Name of ingredients	Percentage (w/w)
Aniseed (Soanf)	10
Black pepper (Kalimirch)	10
Capsicum (Mirch powder)	6
Caraway seeds (Ajwain)	10
Cardamom dry (Badi Elaichi)	05
Cardamom dry (Chhoti Elaichi)	02
Cinnamon (Dalchini)	05
Cloves (Laung)	05
Coriander (Dhania)	17
Cumin seeds (Zeera)	15
Dry ginger powder (Soanth)	08
Mace (Javitri)	05
Nutmeg (Jaifal)	02
Total	**100**

3.1.7 Whole egg liquid and sugar

Chicken eggs were procured from poultry farm of Department of Animal Genetics and Breeding, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India and powdered sugar was procured from local market of Ludhiana.

3.1.8 Packaging materials

Low density polyethylene (LDPE) and LDPE/polyester/polyethylene laminated plastic bags in natural color were procured from reputed firms and used for packaging of dog biscuits.

3.1.9 Chemicals, media and standards

Analytical grade chemicals and media of high purity standards required for analyzing the products were procured from standard firms like SRL, Fisher Scientific, LobaChemie, Himedia, etc.

3.2 Formulation of control dog biscuits

Formulation and processing protocols of the dog biscuit was standardized on the basis of available literature and various preliminary trails conducted in laboratory. The standardized formulation is given in Table 2.

Table 2: Formulations of control dog biscuits:

S. No.	Ingredients	Percentage (w/w)
1.	Refined wheat flour (Maida)	55
2.	Vegetable oil	20
3.	Whole egg liquid	16
4.	Spice mix	3
5.	Sugar	3
6.	Table salt	2
7.	Baking powder	1

3.2.1 Processing of control dog biscuits

First of all, sugar powder was added in paddle mixture followed by vegetable oil and whole egg liquid in the paddle mixer then these ingredients are properly mixed till thick consistency was attained. After that all other ingredients were mixed. All the ingredients were used according to formulation and mixed in paddle mixture. Mixing of dough is carried out until uniform dispersion of ingredients is achieved. Biscuits were prepared by using stainless steel mold of different shapes and cooking is carried in baking oven at temperature of 180ºC for 10 minutes with in-between turning once.

3.2.2 Flow diagram for preparation of control dog biscuits:

Sugar powder, vegetable oil and whole egg liquid was mixed in paddle mixture

↓

Other ingredients are mixed

↓

Dough is prepared

↓

Mixing till uniform dough formation

↓

Filling of dough in stainless steel mold

↓

Cooking of biscuit at 180^0C for 10 minutes

↓

Packaging and storage of biscuits at room temperature

↓

Sensory evaluation and sample analysis

3.3 Experimental details

Experiment No. 1: Optimization of incorporation level of poultry by-products viz., liver and gizzard along with calcium and selenium fortification for the development of dog biscuits.

3.3.1. Preparation of dog biscuits

Poultry liver and gizzard were minced in the meat mincer (Mado Eskimo Mew-714, Mado, Germany). Minced poultry liver and gizzard were air dried at 60º C for 15 - 16 hrs in industrial tray dryer. After drying, the by-products were converted into powder form with help of grinder separately. These were stored in PET jar for the subsequent use.

For the preparation of dog biscuits, first of all, sugar powder is added in paddle mixer followed by vegetable oil and whole egg liquid. All other ingredients left are mixed. Ingredients are used according to formulation and mixed in paddle mixture and dough was prepared by mixing the ingredients. Mixing of dough is carried on until uniformity is achieved. Biscuit is made by filling dough in stainless steel mold of different shapes and cooking is carried in hot air oven at temperature of 180º C for 10 minutes. Both liver and gizzard incorporated biscuits were prepared separately.

3.3.2 Experimental design

Three different levels of poultry liver and gizzard were incorporated separately in pre-standardized dog biscuit formulation after replacing the refined wheat flour. The most suitable by-product and the final level of incorporation was selected on the basis of sensory evaluation (5 point scale), acceptability (response from pet owners, n=12), texture profile analysis and instrumental color profile. The selected level of incorporation was used for the development of dog biscuits.

Table 3: Different level of liver powder and gizzard powder in treatment dog biscuits

Ingredients	Control	T_1	T_2	T_3	T_4	T_5	T_6
Refined wheat flour	55	45	35	25	45	35	25
Liver powder	-	10	20	30	0	0	0
Gizzard powder	-	0	0	0	10	20	30

Where

$T_{1=}$ 10% Liver powder

T_2=20% Liver powder

T_3=30% Liver powder

T_4=10% Gizzard powder

T_5=20% Gizzard powder

T_6=30% Gizzard powder

These dog biscuits incorporated with most suitable by-product at optimum level were fortified with dicalcium phosphate on the basis of recommended dietary allowance of dogs. The final product was evaluated for its physico-chemical properties (pH and cooking yield), proximate composition (moisture, protein, fat and ash), mineral profile, instrumental color profile and texture profile analysis.

On the basis of sensory analysis and acceptability test i.e. single bowl test in dogs (n=12), the incorporation of 30% liver powder was found most suitable for the preparation of dog biscuits. 30% liver powder incorporated dog biscuits were analyzed for physico-chemical properties (pH and cooking yield), proximate composition (moisture, protein, fat and ash), mineral profile, instrumental color profile and texture profile analysis.

It was found that the selenium content in liver powder incorporated dog biscuits was near to recommended daily allowance (RDA) of dogs, so sodium selenite was not incorporated in the dog biscuits. As per the Association of American Food Control (AAFCO) recommended daily allowance (RDA) of Se for the dogs is 0.11 mg/Kg of food on DMB. Se content in chicken liver is 0.1 mg/100 gm of liver, So that RDA for Se can be fulfilled by the incorporation of liver powder.

The dicalcium phosphate was incorporated at 2% level in the 30% liver powder incorporated dog biscuits.

Experiment No. 2: Evaluation of the storage stability of developed dog biscuits at ambient temperature (25ºC)

Treatment dog biscuits selected from experiment no. 1 i.e. 2% dicalcium phosphate and 30% liver powder incorporated dog biscuits were packed under aerobic and MAP conditions and stored at ambient temperature (25ºC). The samples were analyzed on 1, 20, 40, 60 and 80 days. The storage quality was evaluated on the basis of

various physico-chemical (pH, TBARS, PV and FFA) and microbiological analysis (TPC, PC, coliforms count, staphylococcal count, yeast and mold count) and sensory analysis.

The cost of production of developed dog biscuits was calculated on the basis of cost of raw materials, depreciation in the equipment cost, overhead charges including wages or payment given to the workers. The final cost of production of finished product was estimated on the basis of cooking yield of the product.

3.4. Analytical procedures

3.4.1 pH

The pH of finely grounded pet biscuit was determined as per the method described by Trout *et al* (1992) with digital pH meter (FE-20-1-KIT, Mettler-Toledo India Pvt. Ltd., Mumbai) equipped with a combined glass electrode. Ten gram of sample was homogenized with 50 ml of distilled water for 1 min using pestle and mortar. The electrode was dipped into the suspension and the pH value of the sample was recorded.

3.4.2 Cooking yield

The weight of each product was recorded before and after cooking. The cooking yield was calculated and expressed as percentage by a formula:

$$\text{Cooking yield (\%)} = \frac{\text{Weight of baked product}}{\text{Weight of raw product}} \times 100$$

3.4.3 Single bowl test

To assess the acceptability of developed dog biscuits, this test was performed as described by Griffin *et al* (2003). In the monadic or single-bowl test a food is weighed and offered to the animal. Food intake is determined by difference from initial food on offer and orts (or leftovers) after a specified period of time. This can be monitored under normal feeding parameters singly or for multiple feedings per day and is repeated for several days, typically 5 days or longer. For experimental purposes multiple dogs or cats can be used and balanced by period in order to eliminate any environmental influences. The results indicate whether the animal, when presented with no choice, refuses a new food or with multiple periods consumes more or less of one food relative to the other. The use of 8–10 animals is typically sufficient to detect a trend.

3.4.4. Proximate composition

The moisture, protein, fat, and ash content of the product was estimated using automatic moisture analyzer, Kel plus, Socs Plus, and Muffle furnace, respectively following the method of AOAC (1995)

3.4.4.1 Moisture

The moisture content in dog biscuit was determined using automatic moisture analyzer (ESSAE and MAX-50). Finely ground pet biscuit (<5gm) were kept in sample plate and wait for 10-12 min for final reading.

3.4.4.2 Crude protein

The crude protein content was estimated as per method described in AOAC (1995) with suitable modifications using automatic digestion and distillation unit (Kel Plus-KES 12L, Pelican Industries, Chennai). Pre-weighed moisture free sample of approximately 0.2-0.3 g was digested in a Kjeldahl's digestion tubes after adding 10 ml of concentrated sulphuric acid and a pinch of digestion mixture (potassium sulphate and copper sulphate in 5:1 ratio) at 420ºC in the digestion unit. The appearance of clear green colored liquid indicated the completion of digestion. The sample was cooled and then diluted with distilled water (10-20 ml). Exactly 40 ml of 40 percent sodium hydroxide was added to the aliquot to make it alkaline. Distillation was carried automatically in the distillation unit. The ammonia liberated during the process gets collected in boric acid containing indicator (Toshiro's indicator) placed at the receiver end of the distillation unit. The distillate obtained was titrated against standard N/10 Hydrochloric acid to light pink end point. The percentage crude protein was calculated using the following formula. A parallel blank was run to eliminate the error.

$$\text{Nitrogen (\%)} = \frac{14.01 \times 0.1\ \text{N} \times (\text{TV} - \text{BV})}{\text{W} \times 1000} \times 100$$

Crude protein (%) = % Nitrogen x 6.25

Where: 14.01 = Molecular weight of ammonia

0.1N = Titration solution's normality

TV = Titer value

BV = Blank value

W = Sample weight in g

3.4.4.3 Fat content

The fat content in baked product was estimated by solvent extraction method as per AOAC (1995) using Socs Plus (SCS-6-AS, Pelican Industries, Chennai). Two gram of dried, ground sample was taken in an extraction thimble (Whatman No. 1 filter paper) fitted in a specially designed beaker. The initial weight of the empty beakers was noted (W_1). The thimbles with the samples were placed in the beakers containing around 80 ml of solvent (petroleum ether). The extraction was carried out automatically using 5 segments programme. After the process was over the beakers containing the fat residue were placed in hot air oven (100^{o}C) for 20-30 minutes. The beakers removed and cooled in desiccators. The final weight of the beakers was noted as W_2. The fat percentage in the sample was calculated using the following formula:

$$\text{Fat \%} = \frac{\text{Final weight of beaker } (W_2) - \text{Initial weight of beaker } (W_1)}{\text{Weight of sample in g}} \times 100$$

3.4.4.4 Ash content

The ash content in the dog biscuits was estimated as per AOAC (1995) method using muffle furnace. Around 2 g of moisture free sample was taken in pre-weighed moisture free crucibles. The crucibles were then placed on a hot plate for charring. After charring, the crucibles were transferred to muffle furnace set at 550 ^{o}C for around 6 hrs. After cooling of the furnace the crucibles were taken out in desiccators and final weight is recorded. The ash content was calculated using the formula:

$$\text{Ash (\%)} = \frac{\text{Final weight of the crucible - initial weight of the crucible}}{\text{Weight of the sample}} \times 100$$

3.4.4.5 Estimation of minerals

3.4.4.5.1 Calcium

5 g of sample after charring on hot plate was ashed in a muffle furnace for 4 hours at 600^o C. The ash was dissolved in 25 ml HCl (1+1) and transferred to 100 ml beaker, 5 ml of concentrated HCL added to it. The contents were heated to dryness on a water bath and heating was continued for another 30 minutes on the water bath to dehydrate the silica. 5 ml of concentrated HCl and 5 ml water was added to the

residue. It was again heated for a few minutes on the water bath and filtered through Whatman no. 42 filter paper and diluted to 250 ml with distilled water. A part of solution was kept for estimation of phosphorus.

50 ml of solution was taken in a beaker. Add 10 ml of saturated ammonium oxalate solution and boil. Two drops of methyl red indicator were added and contents were mixed thoroughly. The contents were neutralized with ammonia solution and boiled until the precipitate was coarsely crystalline. The mixture was allowed to settle for 4 hours and the precipitate was filtered through Whatman filter paper no. 42. The precipitates were washed with hot water until free from chlorides and oxalates were dissolved along with filter paper in 0.1 N sulphuric acid in the same beaker. The contents were heated to 60^0C and titrated with 0.05 N $KMnO_4$ to a pink colour end point. The calcium content was calculated from the amount of $KMnO_4$ used for titration.

$$\text{Calcium (\%)} = \frac{0.001 \times \text{ml } KMnO_4 \times 250}{50 \times \text{(g) sample}} \times 100$$

3.4.4.5.2 Phosphorous

This was determined by Ames method (1966).

Reagents

a. Ascorbic acid 10%, this should be kept in the refrigerator and is good for about a month.
b. 0.42 percent ammonium molybdate 4 H_2O 1N sulphuric acid.
c. Solution C: mixed with solution (a) and (b) in 1:6 ratio to make solution c

Weighed sample (0.5-1.0 g) was digested by concentrated nitric acid and perchloric acid in 150 ml conical flask. Add 28.09 mg dipotassium hydrogen orthophosphate in 100 ml distilled water to get 50 mg of phosphorous per 100 ml to prepare stock solution .

Working solution was prepared by using 1 ml of stock solution and diluted to 100 ml with distilled water to give phosphorous concentration of 5 μg/ml. Then prepared standard curve with 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working solution, respectively.

Phosphorous was estimated by using 0.1 ml of test solution and volume was made to 0.1 ml with distilled water in a test tube. 4.5 ml solution C was added. After incubation for 45^0C temperature, optical density was measured at 760 nm. From standard curve, estimation of phosphorus for test solution was done.

3.4.4.5.3 Selenium

Weighed 1 gram of biscuit sample and transferred it to 250 ml Erlenmeyer flask. 15 -20 ml of triple acid mixture was added and the contents of the flask were mixed by swirling. Funnel was placed at the neck of the flask and was kept overnight. The flask was heated at low temperature on a hot plate. A vigorous reaction with evolution of brown fumes of nitrous oxide was observed immediately. After HNO_3 was boiled off, white fumes of $HCLO_3$ started coming out and the reaction was over within 1-2 minutes. The temperature was raised to the fullest around 400^oC so that the refluxing of H_2 SO_4 took place at the base of the neck of the flask. Heating was continuously done at this temperature till the contents of the flask gave a yellowish green appearance. The contents were cooled and diluted with 25 ml of distilled water. The solution was transferred to a 100 ml volumetric flask and volume was made up by giving washing to the Erlenmeyer flask. The solution was filtered through Whatman No. 1 filter paper and filtrate was used for determination of selenium in ICAP (Inductive Coupled Plasma) machine and reading was obtained.

3.5 Hardness and shear force value

Hardness and shear force value of dog biscuits were conducted using texture analyzer (TMS-PRO, Food Technology Corporation, USA) attached to software (texture expert).The test samples were placed on a platform in a fixture and compressed to 80% of their original height at a cross head speed of 2 mm per sec, using a 50 kg load cell and 75mm compression platen probe (P75). Six samples were analyzed under each treatment and the readings were averaged. Texture parameters computed were hardness (N) and shear force value (Kg/cm^2) and determined following descriptions by Bourne (1978) and interpreted as follows. Hardness (N) = maximum force required to compress the sample.

3.6 Instrumental color profile

Color profile was measured using Lovibond Tintometer (Lovibond house United Kingdom) set at 2^o of cool white light (d_{65}) and known as '*l*', *a*, and *b* values. '*l*' value

denotes (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/- blueness) values were recorded on/in a hundreds of pet biscuit kept in a plate. The instrument was calibrated using light trap (black hole) and white tile provided with the instrument. Then the above color parameters were selected. The instrument was directly put on the surface of pet biscuit at three different points. Mean and standard error for each parameter were calculated.

3.7 Storage study

3.7.1 2-thiobarbituric acid reacting substances (TBARS) value

The extraction method described by Witte *et al* (1970) was used with suitable modifications for the determination of TBARS values of dog biscuits. 10g of sample was triturated with pestle and mortar with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) prepared in 2M orthophosphoric acid solution for 2 min. The content was then transferred quantitatively to a beaker by rinsing with 25 ml of cold distilled water, well mixed and filtered through ashless filter paper (Whatman filter paper No.1). Then, 3 ml of TCA extract (filtrate) was mixed with equal amount of 2-thiobarbituric acid (TBA) reagent (0.005 M) in test tubes and placed in dark cabinet for 16 hrs. A blank sample was prepared by mixing 3ml of 10% TCA and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 to 533 nm using UV-VIS spectrophotometer (SL-159 Elico India Ltd., Mumbai). TBA value was calculated as mg malonaldehyde per Kg of sample by multiplying O.D. value with K factor of 5.2.

3.7.2 Free fatty acids (FFA)

The method as described by Koniecko (1979) was followed for quantification of free fatty acids. For this, 5 g of sample was blended into fine powder using anhydrous sodium sulphate and then mixed with 30 ml of chloroform for 2 min. The slurry was filtered through whatman filter paper no. 1 into a 100 ml conical flask. About 2 or 3 drops of 0.2 % phenolphthalein indicator solution were added to the chloroform extract, which was then titrated against 0.1N alcoholic potassium hydroxide to get the pink color end point. The quantity of potassium hydroxide required for titration was recorded and calculated as follows:

$$\text{Free fatty acid (FFA) \%} = \frac{0.1 \times \text{ml 0.1N alcoholic KOH} \times 0.282}{\text{Sample weight (g)}} \times 100$$

3.7.3 Peroxide value

The peroxide value was measured as per procedure described by Koniecko (1979) with suitable modifications. Five gram of biscuit sample was blended with 30 ml chloroform for 2 min in the presence of anhydrous sodium sulphate. The mixture was filtered through whatman filter paper no.1 and 25 ml aliquot of the filtrate was transferred to 250 ml conical flask to which 30 ml of glacial acetic acid and 2 ml of saturated potassium iodide solution were added and allowed to stand for 2 min with occasional shaking (swirling) after which 100 ml of distilled water and 2 ml of fresh 1% starch solution were added. Flask contents were titrated immediately against 0.1N sodium thiosulphate till the end point was reached (non-aqueous layer turned to colorless). The peroxide value (meq/kg of sample) was calculated as per the following formula:

$$\text{PV (meq/kg sample)} = \frac{0.1 \times \text{ml 0.1N sodium thiosulphate}}{\text{Wt. of sample (g)}} \times 100$$

3.8 Microbiological analysis

Standard Plate Counts (SPC), psychrophilic counts, Coliform counts and yeast and mold counts of the samples were enumerated following the methods as described by American Public Health Association (APHA 1992).

3.8.1 Preparation of sample and serial dilutions

The samples were opened in an inoculation chamber of laminar flow (RH-58-03, Rescholar equipments, Ambala) pre-sterilized by ultra-violet (UV) radiation. 10 g of sample from this was aseptically weighed and transferred to pre-sterilized mortar containing 90 ml of sterile 0.1% peptone water (RM001; Hi-Media Laboratories Pvt. Ltd., Mumbai). The sample was homogenized for 2 min using a sterile pestle and mortar for uniform dispersion and to get a 10^{-1} dilution of the sample. To prepare 10^{-2} dilution, 1 ml of this diluted solution was quantitatively transferred and then mixed uniformly in a test tube containing 9 ml of sterile 0.1% peptone water. Again 1 ml of 10^{-2} dilution was added to 9 ml 0.1% sterile peptone water and mixed to obtain 10^{-3} dilution and so on. Preparations of sample and serial dilutions were done near flame in a horizontal laminar flow apparatus observing all possible aseptic conditions. Serial dilutions were made as per requirement.

3.8.2 Standard Plate Counts

23.5 g of plate count agar (M091; Hi-Media Laboratories Pvt. Ltd., Mumbai) was suspended in 1000 ml glass of distilled water followed by boiling to dissolve the media completely and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min. Final pH of the medium was set at 7.0±0.2 at 25ºC. Pour plate technique was used. The plates were incubated at 35ºC for 48-72 hrs in an inverted position. Plates showing 30 to 300 colonies were counted manually. The average number of colonies was multiplied by reciprocal of the dilution and expressed as $\log_{10}$ cfu /g of sample.

3.8.3. Psychrophilic Count

The plate was prepared similar to that of standard plate count but incubated at 4-7°C for 15 days. The colonies were counted and expressed as $\log_{10}$ cfu/g.

3.8.4 Coliform counts

41.5 g of Violet Red Bile Glucose Agar procured from Hi-Media Laboratories Pvt. Ltd., Mumbai; (ME581) was suspended in 1000 ml of distilled water, boiled to dissolve the medium completely and cooled to 45^{o}C. The final pH of the medium was adjusted to 7.4±0.2 at 25°C. One ml in duplicate of suitable dilution was pipetted into the sterilized petridish. About 20 ml of the melted medium was poured over it, mixed slowly with rotating actions. The plates were allowed to stand for some time till the agar media got solidified. After solidification 4-5 ml of additional agar was added to form anaerobic layer and agar media was allowed to solidify. The plates were incubated at 35±2°C for 24 hrs. The numbers of red purple colonies with about 0.5 mm diameter surrounded by a zone of precipitated bile were counted. Colonies judged to be borderline cases were also counted. The average number of colonies was multiplied by the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

3.8.5 Yeast and mold counts

39 g of potato dextrose agar (MO96) obtained from (Himedia Laboratories Pvt. Ltd, Mumbai, India) was suspended in 1 litre distilled water, boiled to dissolve the media completely and sterilized by autoclaving at 15 lbs pressure (121^{o}C) for 15 min. The pH of sterilized medium was set to 3.5 by acidifying with 10 ml of 10% tartaric acid. Precaution was taken not to heat the medium after addition of acid. Pour plate technique was followed for inoculation of suitable sample dilution and plates

were incubated at 25^{o}C for 5 days. Black, white, red, greenish black colored colonies on the plates were counted and expressed as $\log_{10}$ cfu/g

3.9 Sensory evaluation

The experienced panel of teachers and postgraduate students of department of LPT, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab evaluated the samples for the sensory attributes viz. color and general appearance, meat flavour intensity and overall acceptability using 5-point descriptive scale where 5=excellent and 1=extremely poor.

3.10 Statistical analysis

The data will be analyzed statistically on "SPSS-16.0" (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran, 1994).

Duplicate samples will be drawn for each parameter and the whole set of experiment will be repeated three times to have total number of observations. The average values will be reported along with standard error. The statistical significance will be estimated at 5% level ($p<0.05$) and will be evaluated with Duncan's Multiple Range Test (DMRT).

Liver powder

Gizzard powder

Unbaked biscuit

Baked biscuit

CHAPTER IV

RESULTS AND DISCUSSION

The objectives of the present study were to develop the dog biscuits incorporated with most suitable poultry by-products viz., liver and gizzard thereafter calcium fortification of the developed dog biscuits were attempted. Thereafter, Evaluation of the storage stability of developed dog biscuits at ambient temperature (25 °C) under aerobic and modified atmosphere packaging conditions and to estimate the cost of production of developed products. These objectives were achieved by conducting three different experiments. The critical analysis of the results with suitable support of available literature to draw the inference, have also been attempted in the present chapter.

4.1 Experiment No. 1: Optimization of incorporation level of poultry by-products viz., liver and gizzard along with calcium and selenium fortification for the development of dog biscuits.

This experiment details the results obtained from the experiment carried out during our investigation in accordance with the above mentioned objectives are presented in the text with the support of statistically analyzed tables 4 to 9 and Figures 1 and 2.

4.1.1. Optimization of the incorporation levels of liver and gizzard powder for development of dog biscuits

Poultry liver and gizzard were minced in the meat mincer (Mado Eskimo Mew-714, Mado, Germany) separately. Minced poultry liver and gizzard were air dried at 60^{o}C for 15 -16 hr in industrial tray dryer. After drying, the by-products were converted into powder form with help of grinder separately. These were stored in PET jar for the subsequent use.

The chicken liver and gizzard powder were incorporated at 10, 20 and 30% separately in standardized dog biscuit formulation after replacing the refined wheat flour (Table 3). Six different types of dog biscuits were developed i.e. T1= 10% Liver powder, T2=20% Liver powder, T3=30% Liver powder, T4=10% Gizzard powder, T5=20% Gizzard powder and T6=30% Gizzard powder incorporated dog biscuits.

All the six treatment products were analyzed for sensory evaluation, dog palatability test (single bowl test), texture profile analysis and instrumental color profile. The most suitable by-product and the final level of incorporation was selected on the basis of above mentioned parameters.

4.1.1.1 Sensory evaluation, dog palatability test (single bowl test), texture profile analysis and instrumental colour profile of liver and gizzard powder incorporated dog biscuits

The results for Sensory evaluation on 5 point hedonic scale i.e. (General appearance, colour, meat flavour intensity, overall acceptability), dog acceptability test (Amount served/dog (g), Intake (g)/day/dog, Total amount served (g), Average Intake (g/day/dog), instrumental colour profile (Redness (a*), Yellowness (b*) and Lightness (L) and texture profile analysis (fracturability and Shear force value) are presented are presented in Tables 4 to 6 and Figures 1 and 2.

Table 4: Effect of different levels of incorporation of liver powder and gizzard powder on the Sensory parameters of dog biscuits

Sensory parameters	Liver and gizzard powder levels					
	T1 (10% liver)	T2 (20% liver)	T3 (30% liver)	T4 (10% Gizzard)	T5 (20% Gizzard)	T6 (30% Gizzard)
General appearance	2.95± 0.14^{c}	3.90± 0.12^{b}	4.33± 0.12^{a}	3.57± 0.12^{b}	3.08± 0.11^{c}	2.83± 0.10^{c}
Colour	2.82± 0.13^{c}	3.66± 0.14^{b}	4.19± 0.14^{a}	3.43± 0.14^{b}	2.99± 0.12^{c}	2.73± 0.12^{c}
Meat flavour intensity	2.68± 0.12^{d}	3.57± 0.16^{b}	4.39± 0.13^{a}	2.91± 0.19^{cd}	3.18± 0.17^{bc}	3.21± 0.17^{bc}
Overall Acceptability	2.84± 0.11^{c}	3.71± 0.14^{b}	4.48± 0.12^{a}	3.10± 0.17^{c}	3.13± 0.15^{c}	3.21± 0.15^{c}

N=21; T-1= 10% Liver powder, T-2= 20% Liver powder; T-3= 30% Liver powder; T-4= 10% Gizzard powder, T-5= 20% Gizzard powder; T-6= 30% Gizzard powder.

*Mean±S.E. with different superscripts row wise (small alphabets) differ significantly ($P<0.05$).

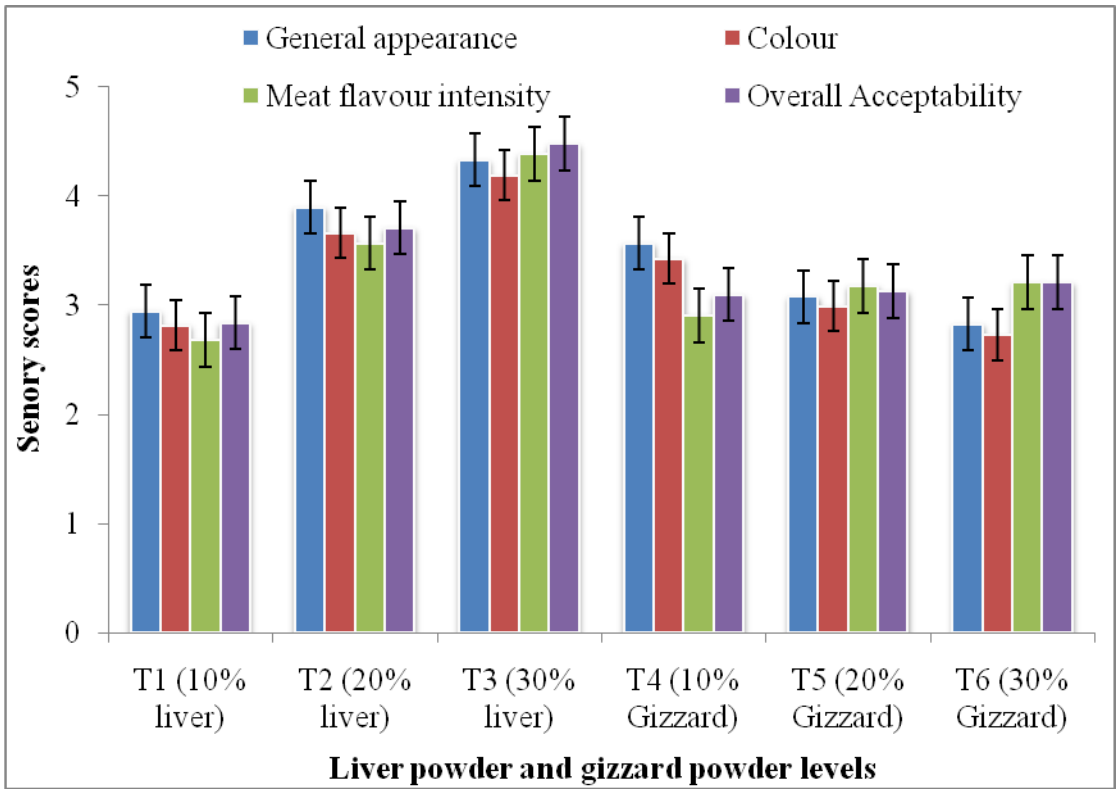


Figure 1: Effect of different levels of incorporation of liver powder and gizzard powder on the sensory parameters of dog biscuits

The results for sensory evaluation of all the liver and gizzard powder incorporated dog biscuits is presented in Table 4 and Figure 1.

The scores for all the sensory attributes showed significant ($P<0.05$) increasing trend with increasing level of chicken liver powder in the formulation whereas the scores for the gizzard powder incorporated dog biscuits showed the significant ($P<0.05$) decrease with the increasing incorporation level of gizzard powder which might be associated with increase in the dark colour of developed dog biscuits. Appearance scores for T_3 were significantly ($P<0.05$) higher than other treatment products which might be due to increase in redness value as shown in instrumental colour analysis.

Similarly, there was significant ($P<0.05$) increasing trend in colour scores with the increase in incorporation level of chicken liver powder but there was decreasing trend noticed on incorporation of gizzard powder. Colour score for T_3 were significantly ($P<0.05$) higher than T_1 and T_4 but the colour values for both these treatment products was significantly ($P<0.05$) higher thanT_1 , T_5 and T_6.

There was significant ($P<0.05$) increasing trend in meat flavour intensity scores with the increase in incorporation level of chicken liver powder and gizzard powder. Flavour scores for T_3 were significantly ($P<0.05$) higher than other treatment products which might be due higher level of chicken liver powder which was more liked by the sensory panelists. Meat flavour intensity scores for gizzard powder incorporated meat biscuits and T_2 were comparable to each other whereas the T_1 showed the lowest score among all the treatment products.

The sensory scores for all the parameters in the dog biscuits containing 30% chicken liver powder (T_3) were highest among all the treatment products and highly acceptable. The overall acceptability score were significantly ($P<0.05$) higher for the dog biscuits containing 30% chicken liver powder (T_3) compared to the other treatment products which is reflective of scores of other sensory parameters.

4.1.1.1.1 Dog acceptability test

The results for dog acceptability test (Amount of dog biscuits served for each treatment/dog (g), Intake (g)/day/dog, Total amount served (g) for all the treatment, Average Intake (g/day/dog) presented in Table 5.

To assess the acceptability of developed dog biscuits, single bowl test as described by Griffin *et al* (2003) was performed. In this test 12 dogs were selected having body weight of 4±2 Kg. 100 g of dog biscuits was served do the dogs for 5 days after their regular meals. Food intake is determined by difference from initial food on offer and orts (or leftovers). The same procedure was repeated for the other treatment dog biscuits. Under this test 500 g dog biscuits from each treatment was served to all the dogs. Analysis of data showed that intake of T_2 and T_3i.e 20% liver and 30% liver powder incorporated dog biscuits was highest in all the dogs. A total of six kg of each treatment biscuit was served to all the dogs. Statistical analysis of data revealed that consumption of T_2 and T_3 dog biscuits was significantly higher than the other treatment products. Among these treatment products, consumption of T3 was highest which might be due to the more meat flavour intensity as shown in the sensory analysis of dog biscuits.

Similarly Regenstein *et al* (2003) and Karthikeyan *et al* (2002) reported that incorporation of fish and animal waste in pet foods supply high protein feed ingredients and palatability enhancing agents in animal foods. Mahender *et al* (2013) also reported that incorporation of poultry slaughter waste (PSW) in dog biscuits results in increased in palatability of dog biscuits by conducting an experiment on adult Labrador dogs.

Table 5: Effect of incorporation of liver and gizzard powder on acceptability of developed biscuits by dogs

		Liver and gizzard powder levels					
		T1 (10% liver)	**T2 (20% liver)**	**T3 (30% liver)**	**T4 (10% Gizzard)**	**T5 (20% Gizzard)**	**T6 (30% Gizzard)**
Amount served/dog (g)		500	500	500	500	500	500
Intake (g)/day/dog	Dog 1	91.60 ± 2.29^{b}	94.00 ± 1.34^{ABa}	95.20 ± 0.80^{ABCa}	90.80 ± 2.13^{ABb}	90.40 ± 2.11^{ABb}	88.40 ± 2.98^{Ab}
	Dog 2	91.80 ± 2.60^{b}	93.00 ± 0.70^{BCb}	95.40 ± 1.21^{ABCa}	90.80 ± 2.48^{AB}	85.60 ± 1.63^{BCc}	81.60 ± 1.96^{ABd}
	Dog 3	91.80 ± 2.60^{b}	97.60 ± 0.93^{Aa}	97.60 ± 0.93^{Aa}	91.80 ± 2.60^{ABb}	87.80 ± 2.52^{ABbc}	83.80 ± 3.99^{ABc}
	Dog 4	91.60±2.29b	97.20 ± 1.07^{Aa}	97.20 ± 1.07^{ABa}	90.00 ± 1.60^{ABb}	90.00 ± 1.64^{ABb}	82.80 ± 4.35^{ABc}
	Dog 5	91.80 ± 2.60^{b}	94.00 ± 1.34^{ABa}	95.80 ± 1.53^{ABCa}	91.80 ± 2.60^{ABb}	87.80 ± 2.52^{ABc}	83.80 ± 3.31^{ABd}
	Dog 6	91.80 ± 2.60^{b}	94.60 ± 0.51^{ABa}	94.60 ± 0.51^{ABCa}	89.80 ± 1.59^{AB}	89.80 ± 1.59^{ABbc}	81.80 ± 2.78^{ABc}
	Dog 7	94.00 ± 0.71^{a}	93.00 ± 0.71^{BCa}	93.00 ± 0.71^{Ca}	93.40 ± 1.08^{Aa}	93.40 ± 1.08^{Aa}	91.40 ± 2.56^{Ab}
	Dog 8	91.80 ± 2.60^{b}	94.00 ± 1.34^{ABa}	94.00 ± 1.34^{BCa}	91.80 ± 2.60^{ABb}	86.80 ± 3.35^{ABCc}	85.40 ± 3.61^{ABc}
	Dog 9	91.60 ± 2.29^{b}	90.00 ± 2.30^{CDb}	95.20 ± 0.37^{ABCa}	90.00 ± 1.64^{ABb}	90.00 ± 1.64^{ABb}	84.00 ± 4.76^{ABc}
	Dog 10	89.20 ± 1.24^{b}	87.60 ± 0.93^{Db}	93.20 ± 1.28^{Ca}	85.80 ± 1.32^{Bb}	80.80 ± 1.93^{Cc}	75.40 ± 1.72^{Bd}
	Dog 11	90.80 ± 1.71^{b}	93.00 ± 0.71^{BCa}	94.00 ± 0.71^{BCa}	90.80 ± 1.71^{ABb}	89.80 ± 1.36^{ABb}	87.80 ± 3.10^{Ab}
	Dog 12	91.60 ± 2.29^{b}	94.00 ± 1.34^{ABb}	97.20 ± 0.97^{ABa}	91.60 ± 2.29^{ABb}	90.00 ± 1.64^{ABbc}	86.00 ± 4.32^{ABc}
Total amount served (g)		6000	6000	6000	6000	6000	6000
Average Intake (g/day/dog)		91.67 ± 1.39^{b}	93.50 ± 1.80^{a}	95.20 ± 1.27^{a}	90.70 ± 1.83^{b}	88.55 ± 1.19^{bc}	84.35 ± 2.34^{d}

N=12; T-1= 10% Liver powder, T-2= 20% Liver powder; T-3= 30% Liver powder; T-4= 10% Gizzard powder, T-5= 20% Gizzard powder; T-6= 30% Gizzard powder.

*Mean±S.E. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly (P<0.05).

4.1.1.1.2 Instrumental colour profile and texture profile analysis

The results for instrumental colour profile Redness (a*), Yellowness (b*) and Lightness (L) and texture profile (fracturability and Shear force value) of all the liver and gizzard powder incorporated dog biscuits is presented in Table 6 and Figure 2.

Redness (*a** value) is an indicator of freshness of the meat and criteria for quality evaluation by the consumers. Redness (*a**) value showed the pattern T-1>T-2>T-3>T-6>T-4>T-5 amongst treatments and followed a decreasing trend in liver powder incorporated dog biscuits. Redness (*a**) value of dog biscuits incorporated with chicken gizzard powder was significantly ($P<0.05$) lower than the liver incorporated dog biscuits. The yellowness (b*) of T3 and T6 dog biscuits was significantly ($P<0.05$) lower than the other treatment products. Lightness (*L** value) of chicken liver powder incorporated dog biscuits was significantly ($P<0.05$) higher than the chicken gizzard powder incorporated dog biscuits.

Several authors have studied the colour of meat and meat products and reported that meat oxidation causes a decrease in *a** value (Lavieri and Williams 2014; Kumar *et al* 2015). Realini *et al* (2015) also reported decrease in *b** values of beef patties containing Acerola fruit extract. Selgas *et al* (2009) reported same trend for *a** and *b** values in hamburgers containing dried tomato powder. Similar observations were recorded in oxidative stability of pork emulsion containing tomato products and pink guava pulp during refrigerated (4± 1ºC) aerobic storage by Joseph *et al* (2014).

The results for the texture profile analysis revealed that values for both fracturability and shear force showed declining trend with increase in the incorporation level of liver and gizzard powder, which might be due to the replacement of refined wheat flour with by-products powder having low starch content and lower gelatinization on cooking of the developed biscuits. Similarly, Malav *et al* (2017) also reported significantly higher ($P<0.05$) hardness, fracturability values in spent hen meat papad incorporated with black gram flour. The highest values for fracturability was observed for the dog biscuits incorporated with 10% gizzard powder, whereas the highest values for shear force was observed for the dog biscuits incorporated with 10% liver powder.

Table 6: Effect of different levels of incorporation of liver and gizzard powder on colour and textural parameters of dog biscuits

Parameters	Liver and gizzard powder levels					
	T1 (10% liver)	T2 (20% liver)	T3 (30% liver)	T4 (10% Gizzard)	T5 (20% Gizzard)	T6 (30% Gizzard)
Redness (a*)	10.73± 0.09^{a}	10.69± 0.06^{a}	9.04± 0.13^{b}	6.77± 0.05^{c}	6.68± 0.15^{c}	7.03± 0.20^{c}
Yellowness (b*)	25.84± 0.08^{a}	26.07± 0.15^{a}	25.83± 0.29^{b}	25.89± 0.17^{a}	26.04± 0.25^{a}	22.83± 0.29^{b}
Lightness (L)	45.49± 0.22^{b}	46.71± 0.23^{ab}	45.62± 0.73^{c}	47.54± 0.27^{a}	47.65± 0.22^{a}	47.47± 0.67^{a}
Fracturability (N)	1.85± 0.03^{c}	1.60± 0.03^{d}	1.13± 0.06^{f}	2.27± 0.06^{a}	2.18± 0.03^{b}	1.49± 0.05^{e}
SFV (Kg/cm^{2})	3.71± 0.08^{a}	3.36± 0.06^{b}	2.87± 0.05^{c}	3.56± 0.07^{a}	3.30± 0.07^{b}	2.89± 0.08^{c}

N=6; T-1= 10% Liver powder, T-2= 20% Liver powder; T-3= 30% Liver powder; T-4= 10% Gizzard powder, T-5= 20% Gizzard powder; T-6= 30% Gizzard powder.
*Mean±S.E. with different superscripts row wise (small alphabets) differ significantly (P<0.05).

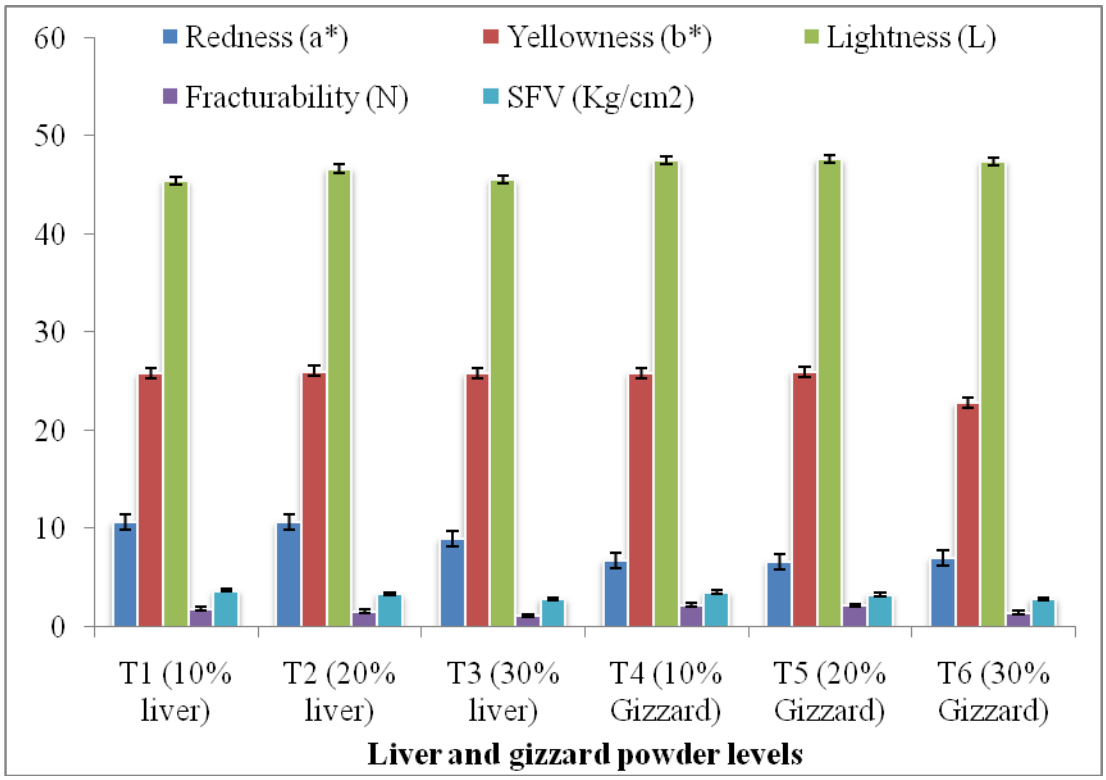


Figure 2: Effect of different levels of incorporation of liver and gizzard powder on colour and textural parameters of dog biscuits

On the basis of results of sensory evaluation, dog palatability test instrumental colour profile and texture profile analysis, the incorporation of chicken liver powder at 30% level (T3) was adjudged best for the development of dog biscuits. T_3 product was selected for further studies.

4.1.1.2 Analysis of chicken liver powder

The results for proximate composition and mineral content of liver powder is presented in Table 7. In the present investigation following results were obtained Moisture (5.65±0.55 %), Protein (74.82±0.45%), Fat (14.16±0.31%), Ash (4.08± 0.12%), Calcium (24±0.06 mg/100 g), Phosphorus (234±0.09 mg/100 g), Selenium (0.11±0.001 mg/100 g). Similarly Devatkal *et al* (2004) studied the physicochemical, functional and microbiological quality of buffalo liver on fresh weight basis and reported the following results: Proximate composition was: moisture - 71.92%, protein - 18.44%, fat - 5.60%, carbohydrate - 2.72%, total ash - 1.32% and total energy - 135 kcal. Mineral concentrations (mg%) in liver were: Na - 60.04, K - 274, Ca - 5.60, Mg - 6.20, Fe - 20.86 and Cu - 5.60. Mean glycogen (mg/g), total liver pigments (mg/g) and cholesterol (mg%) were 7.07, 8.49 and 283.88, respectively.

Table 7: Proximate composition and mineral content of liver powder

Parameters	Chicken liver powder
Moisture (%)	5.65±0.55
Protein (%)	74.82±0.45
Fat (%)	14.16±0.31
Ash (%)	4.08±0.12
Calcium (mg/100 g)	24±0.06
Phosphorus (mg/100 g)	234±0.09
Selenium (mg/100 g)	0.11±0.001

N=6; Mean±S.E.

It was found that the selenium content in liver powder incorporated dog biscuits was near to recommended daily allowance (RDA) of dogs, so sodium selenite was not incorporated in the dog biscuits. As per the Association of American Food Control (AAFCO) recommended daily allowance (RDA) of Se for the dogs is 0.11 mg/Kg of food on DMB. Se content in chicken liver is 0.1 mg/100 gm of liver, So that RDA for Se can be fulfilled by the incorporation of liver powder

4.1.1.3 Fortification of calcium and selenium in the dog biscuits

The developed dog biscuits incorporated with most suitable by-product i.e. chicken liver powder at optimum level i.e. 30% were fortified with dicalcium phosphate on the basis of recommended dietary allowance of dogs. The dicalcium phosphate was incorporated at 2% level after replacing the refined wheat flour in the standardized formulation.

It was found that the selenium content in liver powder incorporated dog biscuits was near to recommended daily allowance (RDA) of dogs, so sodium selenite was not incorporated in the dog biscuits. As per the Association of American Food Control (AAFCO) recommended daily allowance (RDA) of Se for the dogs is 0.11 mg/Kg of food on DMB. Se content in chicken liver is 0.1 mg/100 gm of liver, So that RDA for Se can be fulfilled by the incorporation of liver powder.

The results for physico-chemical and proximate composition of liver powder incorporated and calcium fortified dog biscuits is presented in Table 8 and Figure 3. Analysis of data revealed that the pH values increased significantly ($P<0.05$) upon incorporation of 2% dicalcium phosphate. The addition of dicalcium phosphate showed a significant effect ($P<0.05$) on the cooking yield of liver incorporated dog biscuits. Cooking yield of dog biscuits decreased significantly ($P<0.05$) with the incorporation of 2% level of dicalcium phosphate in the formulation. Decrease The dog biscuits developed after the incorporation of liver powder at 30% level had higher percentage of protein (35.37±0.31), fat (20.33±1.45) and ash (4.93±0.20) that might be due to replacement of refined wheat flour having low protein, fat and ash content with the chicken liver powder having highprotein, fat and ash content.

Table 8: Physico-chemical properties and proximate analysis of developed dog biscuits

Parameters	T-1 (30% liver powder)	T-2 (30% liver powder + 2% Dicalcium phosphate)
pH	5.05 ± 0.13^{b}	6.19 ± 0.14^{a}
Cooking Yield (%)	92.28 ± 0.42^{a}	90.88 ± 0.22^{b}
Moisture (%)	4.75±0.26	4.78±0.66
Protein (%)	35.37±0.31	34.82±0.39
Fat (%)	20.33±1.45	19.76±2.10
Ash (%)	4.93 ± 0.20^{b}	5.41 ± 0.19^{a}

N=6; T-1= 30% liver powder incorporated dog biscuits, T-2= 30% liver powder and 2% Dicalcium phosphate incorporated dog biscuits. *Mean±S.E. with different superscripts row wise (small alphabets) differ significantly ($P<0.05$).

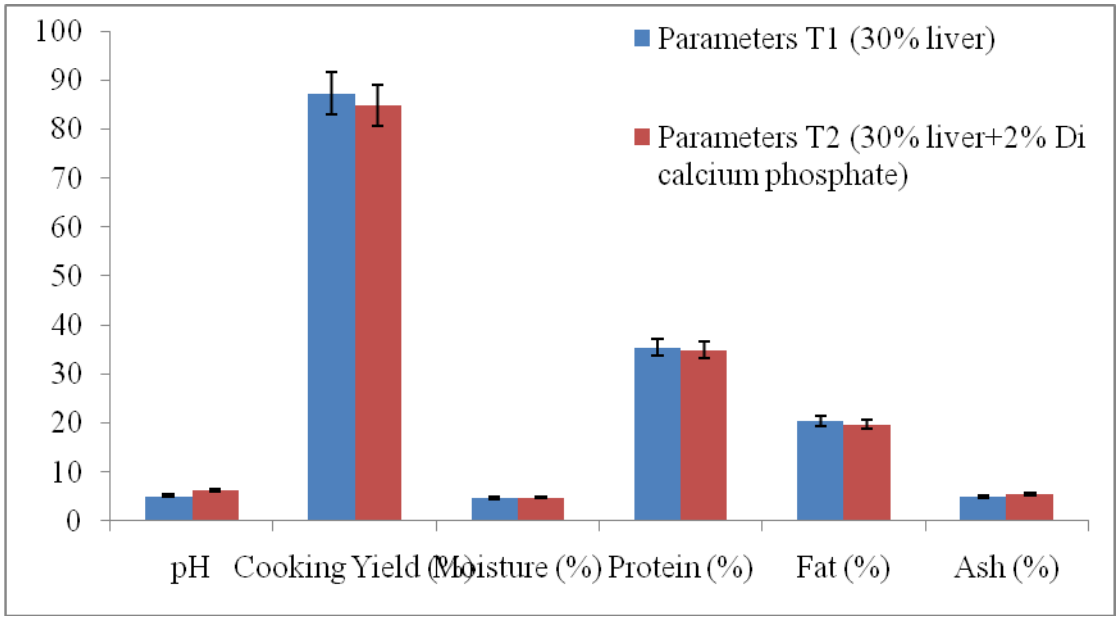


Figure 3: Physico-chemical properties and proximate analysis of developed dog biscuits

The developed dog biscuits had very low moisture content (4.75±0.26 %) and the values for moisture, protein, fat content in T-1 and T-2 dog biscuits were comparable to each other but the incorporation of 2% dicalcium phosphate significantly ($P<0.05$) increased the ash content of T-2.

The results for mineral content, instrumental colour profile and texture profile analysis of liver powder incorporated and calcium fortified dog biscuits is presented in Table 9 and Figure 4. Statistical analysis of data revealed that incorporation of 2% dicalcium phosphate significantly ($P<0.05$) increased the calcium and phosphorus content of developed dog biscuits. Se content was comparable among both the treatment products. Se content of 30% chicken liver powder incorporated dog biscuits was 0.024 mg/100 g. the reason behind the higher values of Se content in the chicken liver powder.

The redness (a*), yellowness and lightness of T-1 (30% liver powder)dog biscuits was significantly ($P<0.05$) higher than the T-2. The lower values of redness for the T-2 dog biscuits might be due to dilution darker colour of chicken liver powder. Both fracturability and shear force showed increasing trend with the incorporation of 2% dicalcium phosphate. This increase was significantly ($P<0.05$) in fracturability whereas the increase in shear force value was insignificant.

Table 9: Mineral content, instrumental colour profile and texture profile analysis of developed dog biscuits

Parameters	T-1 (30% liver)	T-2 (30% liver+2% Dicalcium phosphate)
Calcium (%)	0.37±0.02[b]	1.55±0.05[a]
Phosphorus (%)	0.22±0.01[b]	0.63±0.03[a]
Selenium (mg/100 g)	0.024±0.001	0.023±0.001
Redness (a*)	10.73±0.09[a]	8.30±0.23[b]
Yellowness (b*)	25.79±0.12[a]	22.35±0.28[b]
Lightness (L)	45.49±0.22[a]	42.02±0.88[b]
Fracturability (N)	1.24±0.17[a]	2.27±0.36[b]
SFV (Kg/cm^2)	3.78±0.14	3.89±0.18

N=6; T-1= 30% liver powder incorporated dog biscuits, T-2= 30% liver powder and 2% Dicalcium phosphate incorporated dog biscuits. *Mean±S.E. with different superscripts row wise (small alphabets) differ significantly (P<0.05).

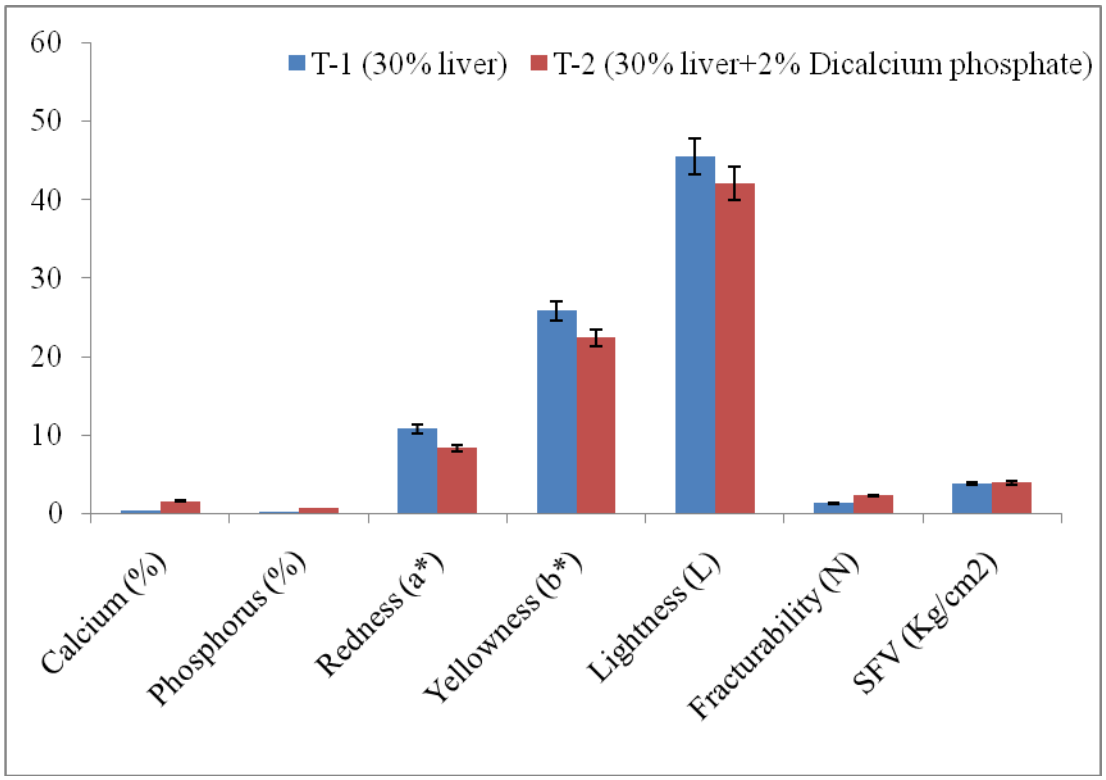


Figure 4: Mineral content, instrumental colour profile and texture profile analysis of developed dog biscuits

4.2 Experiment 2: Evaluation of the storage stability of developed dog biscuits at ambient temperature (25 °C).

Dog biscuits selected from experiment No. 1 i.e. 2% dicalcium phosphate and 30% chicken liver powder incorporated dog biscuits were packed under aerobic and MAP conditions and stored at ambient temperature (25 °C).

A total of two treatments viz. 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Aerobic packaging (T_A), 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Modified Atmospheric Packaging (T_{MAP}) were prepared. MAP was carried out with a combination of 50% CO_2 and 50% N_2 flushing using packaging machine (Roschermatic, Type 19/S/CL) after sterilizing the bags in the laminar flow (Model: RH-58-03; Rescholar equipment's, Ambala) with ultra-violet radiation.

All the packaged samples were stored at ambient temperature for 80 days. The samples were drawn at 20 days intervals and analyzed on 1, 20, 40, 60 and 80 days. The storage quality was evaluated on the basis of various physico-chemical (pH, water activity, moisture, TBARS, PV, FFA) microbiological (TPC, PC, coliforms count, yeast and mold counts), water activity and sensory analysis (5-point descriptive scale). The results are presented in statistically analyzed (Tables 10-13) and Figures 5-8. The results were critically reviewed and discussed in the light of objectives.

4.2.1 Physico-chemical quality

The results for the physico-chemical parameters such as pH, Water activity, Moisture, Thiobarbituric acids reacting substances (TBARS) and free fatty acids **(**FFA) are presented in statistically analysed Table 10 and Figure 5.

Significant ($P<0.05$) changes were noticed in all the parameters under physico-chemical quality of both the treatment products with advancement of storage period and also between the two packaging systems.

4.2.1.1 pH

The pH value (Table 10) followed a significant ($P<0.05$) decreasing trend with the advancement of storage period for both the treatment products under different packaging conditions. The linear decreasing trend of pH value might be due to production of acid by fermentation of sugar. pH value of MAP packaged product has significant ($P<0.05$) lower values than the aerobically packaged products during the entire storage period. The increase in the pH of MAP packaged products was at slower rate than products of Aerobic packaging, which might be due to buffering by carbonic

acid produced due to the influx of CO_2 gas in the package (Ashie *et al* 1996). Singh *et al* (2011) also reported gradual decrease in the pH of chicken snacks stored in laminated pouches at ambient temperature. Similar observations were recorded by Jairath*et al* (2013) in enrobed goat meat bites and Singh *et al* (2013) in chicken lollypop.

Similarly, Singh *et al* (2011) and Kumar *et al* (2016) also reported gradual decrease in the pH of chicken snacks and chicken meat biscuits respectively stored in laminated pouches at ambient temperature. Kumar and Sharma (2004) also observed a declining trend in pH of vacuum packed low fat pork patties prepared with 4% barley flour stored under refrigeration (4±1°C) for a period of 35 days. Similar decrease in pH during refrigerated storage was also reported by Verma *et al* (2013) in sheep meat nuggets on incorporation of guava powder and Banerjee *et al* (2012) in goat meat nuggets on incorporation of broccoli powder extract. Verma *et al* (2012a) also observed a decrease in pH of low-fat chicken nuggets on sodium chloride replacement and incorporation of bottle gourd. Verma *et al* (2012b) also reported a decrease in pH of low-fat chicken nuggets on sodium chloride replacement and incorporation of chickpea hull flour.

4.2.1.2 Water activity (a_w)

In general, a_w followed significantly ($P<0.05$) increasing trend in both the dog biscuits of both packaging methods during gradual advancement of storage study, but the rate of increase of a_w was comparatively lower in MAP. It might be attributed to the packaging material which is less moisture permeable laminates (polyester/ polyethylene) used in MAP as compared to LDPE bags used in aerobic packaging. Increase in a_w of both the treatment dog biscuits in both packaging systems was due to increase in moisture content of products during storage. On 1^{st} day of storage, a_w of aerobic and MAP packaged products were comparable, however MAP packaged products recorded significantly ($P<0.05$) lower a_w on 20^{th}, 40^{th}, $60^{th}$and 80^{th} day of storage.

4.2.1.3 Moisture (%)

Similar to the water activity, moisture content of stored dog biscuits also followed significantly ($P<0.05$) increasing trend in both treatment dog biscuitsin both packaging methods during gradual advancement of storage study, but the rate of increase of moisture content was comparatively lower in MAP. It might be attributed to the packaging material which is less moisture permeable laminates (polyester/

polyethylene) used in MAP as compared to LDPE bags used in aerobic packaging. The increase in moisture content of products during storage was reflected in the increase in the values of water activity. On 1st day of storage, moisture content of aerobic and MAP packaged products were comparable, however MAP packaged products recorded significantly ($P<0.05$) lower moisture content in the subsequent storage period.

4.2.1.4 Thiobarbituric acids reacting substances (TBARS) value

Results in Table 10 clearly revealed that TBARS values followed a significantly ($P<0.05$) increasing trend throughout the storage period for both aerobic and MAP dog biscuits incorporated with liver powder and dicalcium phosphate. Duration of the storage affected the overall TBARS formation of the developed dog biscuits significantly ($P<0.05$). The amounts of malonaldehyde (MDA) increased during ambient temperature storage with significant difference ($P<0.05$). The increase in TBARS values on storage might be attributed to oxygen permeability of packaging material that led to lipid oxidation (Raja *et al* 2014). Ratanatriwong *et al* (2011) and Kumar *et al* (2016) reported gradual increase in the TBARS values in fish and chicken snacks and chicken meat biscuits respectively stored at ambient temperature. From start of storage study the highest TBARS values were found in aerobically packaged products as compared to MAP packaged products indicating a low degree of lipid oxidation in MAP products. However, TBARS values in MAP products increased significantly ($P<0.05$) at slower rate than aerobic products. Modified atmosphere packaged treatment dog biscuit with high CO_2 might have reduced the rate of lipid oxidation during storage study.

The TBARS values during the entire storage period was well below the critical value of 3 mg/kg (Jouki *et al* 2012) at which rancidity is detected. The results for the TBARS values are also reflected in the scores of meat flavour intensity under sensory evaluation as there was no off flavour were detected even on the last day i.e. 80th day of storage. Other workers have also reported progressive increase in lipid oxidation of meat products during storage period (Kumar and Sharma 2003 and Malav *et al* 2017). Bhattacharyya *et al* (2013) reported similar observations in Duck sausages and Ilayabharathi *et al* (2012) in spent chicken sausages. Such an increase in TBARS value during storage due to oxidative rancidity has been reported by many workers (Prabhakara Reddy and Rao, 1997 and 2000; Nag *et al* 1998; Tan and Shelef 2002 and Biswas *et al* 2011).

4.2.1.5 Free fatty acids value

Free fatty acids are the products of enzymatic and microbiological degradation of lipids, indicator of fat stability during storage. Free fatty acid content also followed a similar trend as shown in the TBARS values, there was positive correlation between TBARS values and free fatty acids values since both were related with fat oxidation. Free fatty acid (FFA) value in the developed dog biscuits significantly ($P<0.05$) increased with storage time. It can be attributed to progressive oxidation of lipids and lipase action in meat products during storage. The increase in FFA value might be due to formation of lipid peroxides during storage (Umesha *et al* 2014). Similar findings have also been reported by Singh *et al* (2015) during storage of chevon cutlets and Rathour *et al* (2017) in chevon rolls. Earlier several researchers have also reported the increasing trend of FFA content during storage of buffalo meat (Rao and Kowale, 1988), goat meat patties (Das *et al* 2006) and Kale (2009) (chicken snack sticks). In general, FFA followed a significant ($P<0.05$) increasing trend in all the products during storage, but the rate of increase of FFA was significantly ($P<0.05$) lower in MAP than aerobic packaging. It may due to the packaging material which was low permeable films/laminates (polyester/ polyethylene) and CO_2 used in MAP than LDPE bags used in aerobic packaging.

4.2.1.6 Peroxide value

Peroxide value (PV) of developed dog biscuits followed an increasing trend in control and treatments as that of TBARS values and FFA values. The Peroxide value (PV) of MAP packaged dog biscuit was significantly ($P<0.05$) lower than the aerobically packaged dog biscuits. It might be due to the formation of hydroperoxides during storage than their degradation into secondary oxidation products. Similar increase in peroxide value of stored meat products was also reported by Birla (2018) and Rathour *et al* (2017).

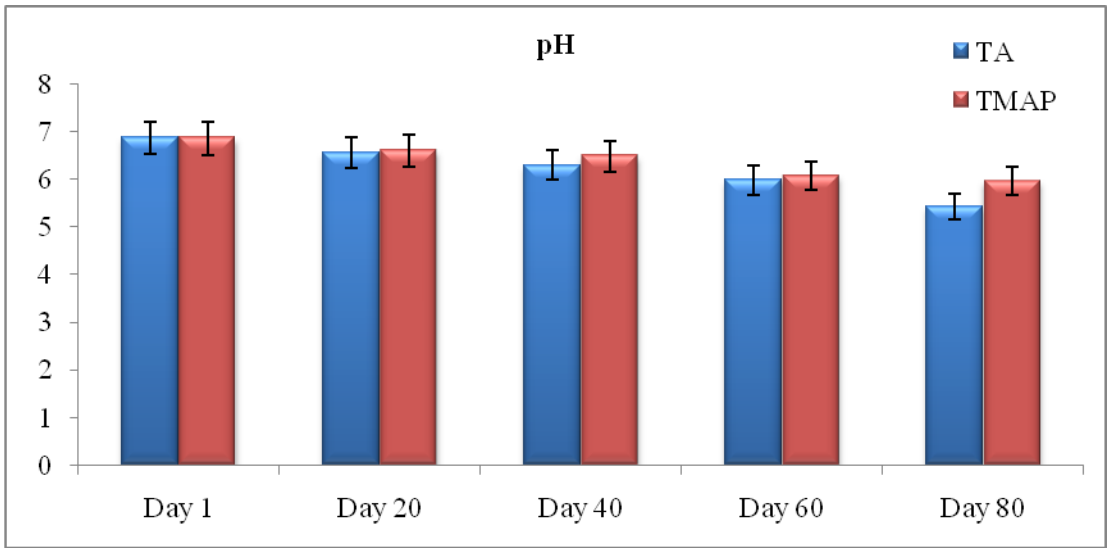
Rhee *et al* (1999) also reported the steady increase in PV of meat exudates stored at 37°C under aerobic conditions for 4 months. These results are in consonance with Botsoglou *et al* (2014) who studied effect of olive leaf (*Oleaeuropea* L.) extracts on protein and lipid oxidation of frozen n-3 fatty acids-enriched pork patties. Qi *et al* (2015) while studying on Lychee (*Litchi chinensis* Sonn.) seed water extract as an antioxidant in meat products observed similar increase in PV during aerobic storage period of 15 days.

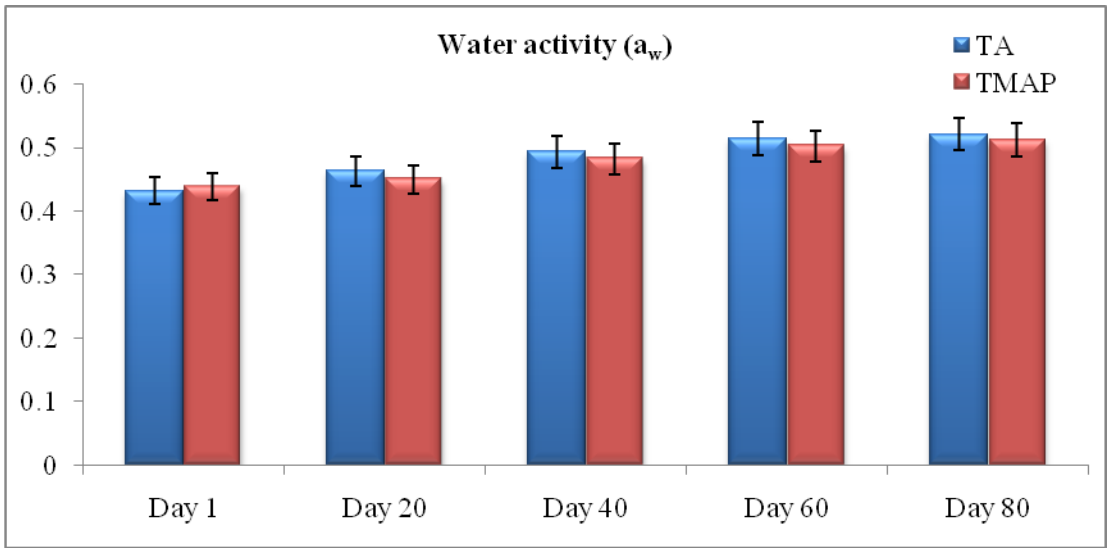
Table 10: Physico-chemical parameters of developed dog biscuits during ambient temperature storage under aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*

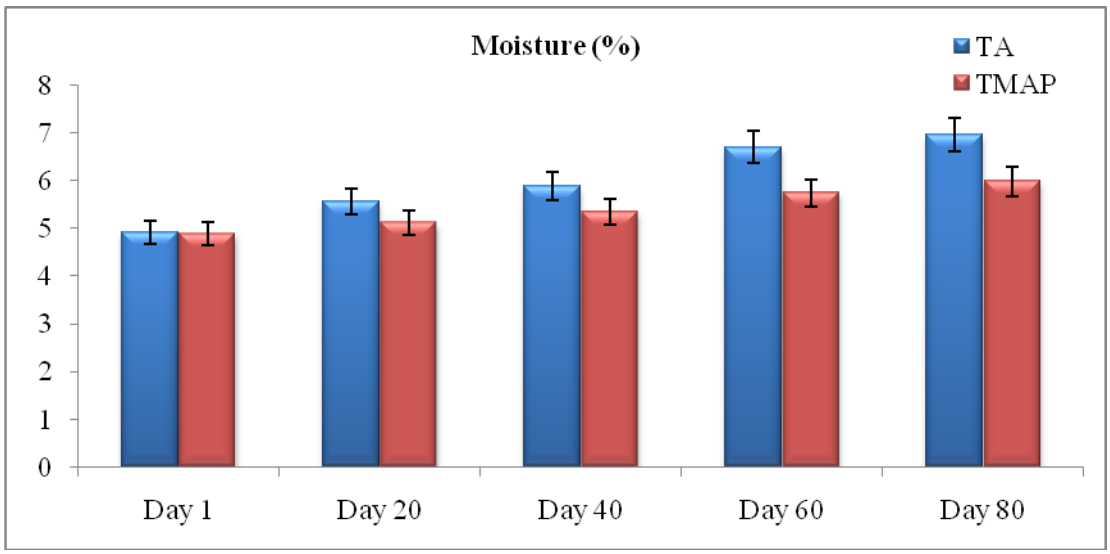
Treatments / Days	Storage period (Days)				
	Day 1	Day 20	Day 40	Day 60	Day 80
			pH		
T_A	6.87± 0.02^{a}	6.56± 0.02^{bB}	6.30± 0.01^{cB}	5.98± 0.03^{dB}	5.43± 0.04^{eB}
T_{MAP}	6.86± 0.04^{a}	6.60± 0.02^{bA}	6.48± 0.01^{cA}	6.07± 0.03^{dA}	5.96± 0.04^{eA}
			Water activity (a_w)		
T_A	0.432± 0.005^{e}	0.463± 0.002^{dA}	0.493± 0.001^{cA}	0.514± 0.002^{bA}	0.521± 0.002^{aA}
T_{MAP}	0.438± 0.003^{e}	0.450± 0.003^{dB}	0.482± 0.001^{cB}	0.502± 0.002^{bB}	0.512± 0.002^{aB}
			Moisture (%)		
T_A	4.91± 0.18^{c}	5.56± 0.18^{bA}	5.88± 0.29^{bA}	6.70± 0.25^{aA}	6.96± 0.23^{aA}
T_{MAP}	4.88± 0.11^{c}	5.11± 0.07^{bB}	5.34± 0.17^{bB}	5.74± 0.13^{aB}	5.98± 0.16^{aB}
			***TBARS value*(*mg malonaldehyde/Kg)**		
T_A	0.16± 0.002^{e}	0.35± 0.022^{dA}	0.45± 0.008^{cA}	0.64± 0.013^{bA}	0.84± 0.012^{aA}
T_{MAP}	0.15± 0.002^{e}	0.29± 0.022^{dB}	0.38± 0.008^{cB}	0.49± 0.013^{bB}	0.68± 0.012^{aB}
			Free fatty acid (% oleic acid)		
T_A	0.10± 0.004^{e}	0.14± 0.004^{d}	0.17± 0.003^{c}	0.19± 0.007^{b}	0.21± 0.002^{a}
T_{MAP}	0.09± 0.003^{e}	0.11± 0.002^{d}	0.13± 0.001^{c}	0.16± 0.004^{b}	0.18± 0.002^{a}
			Peroxide value (meq/Kg)		
T_A	4.20± 0.10^{e}	5.00± 0.15^{dA}	6.17± 0.14^{cA}	7.20± 0.15^{bA}	8.03± 0.19^{aA}
T_{MAP}	4.15± 0.10^{e}	4.62± 0.11^{dB}	5.11± 0.17^{cB}	5.59± 0.10^{bB}	6.13± 0.14^{aB}

N=6; T_A= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Aerobic packaging; T_{MAP}= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Modified atmospheric packaging.

*Mean±S.E. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly (P<0.05)


pH
TA
TMAP
8
7
6
5
4
3
2
1
0
Day 1
Day 20
Day 40
Day 60
Day 80


Water activity (a_w)
TA
TMAP
0.6
0.5
0.4
0.3
0.2
0.1
0
Day 1
Day 20
Day 40
Day 60
Day 80


Moisture (%)
TA
TMAP
8
7
6
5
4
3
2
1
0
Day 1
Day 20
Day 40
Day 60
Day 80

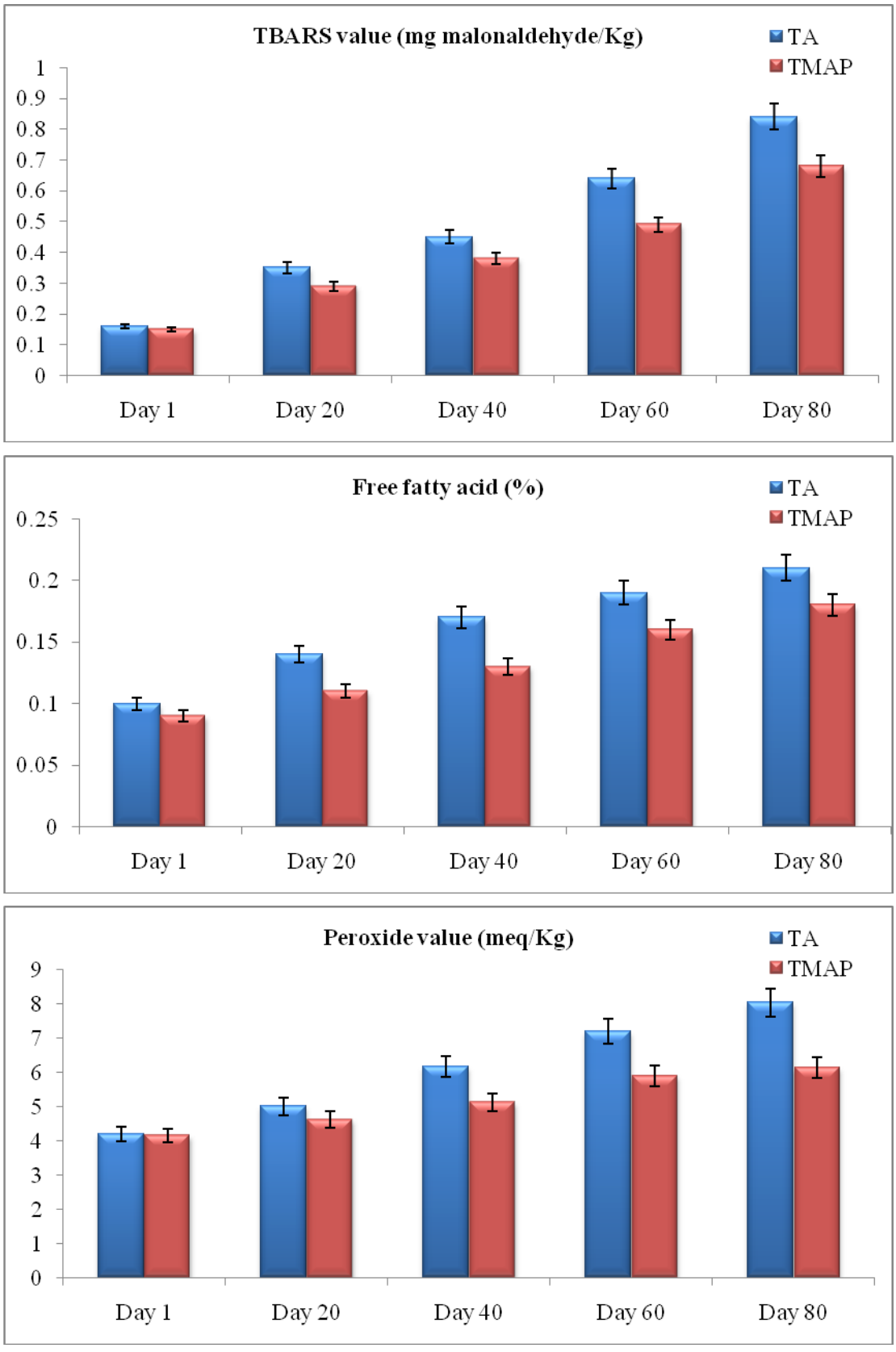


Figure 5: Physico-chemical parameters of developed dog biscuits during ambient temperature storage under aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*

4.2.2 Instrumental colour and texture parameters

The results of instrumental colour profile and texture parameter of developed dog biscuits incorporated with 30% liver powder and 2.0% dicalcium phosphate are presented in Table 11 and Figure 6.

Incorporation of liver powder affected the lightness (*L** value) redness value (*a** value) and yellowness (*b** value) of dog biscuits, where the decreasing trend was observed in all these colour values. The rate of decrease in the lightness scores was lower for the MAP packaged products. Sang-Keun *et al* (2015) observed the similar findings in the emulsion-type pork sausage incorporated with the *Caesalpinia sappan* extract.

Redness (*a**) value showed significantly ($P<0.05$) declining trend for both the products during the storage which might be due to gradual oxidation of myoglobin and accumulation of metmyoglobin with storage time (Mancini and Hunt 2005). But the *a** value was higher in MAP packaged dog biscuits during entire period of storage. Similar results were reported by Moroney *et al* (2015) on quality indices of fresh pork after feeding polysaccharide (laminarin and fucoidan) extracts from brown seaweed (*Laminaria digitata*).

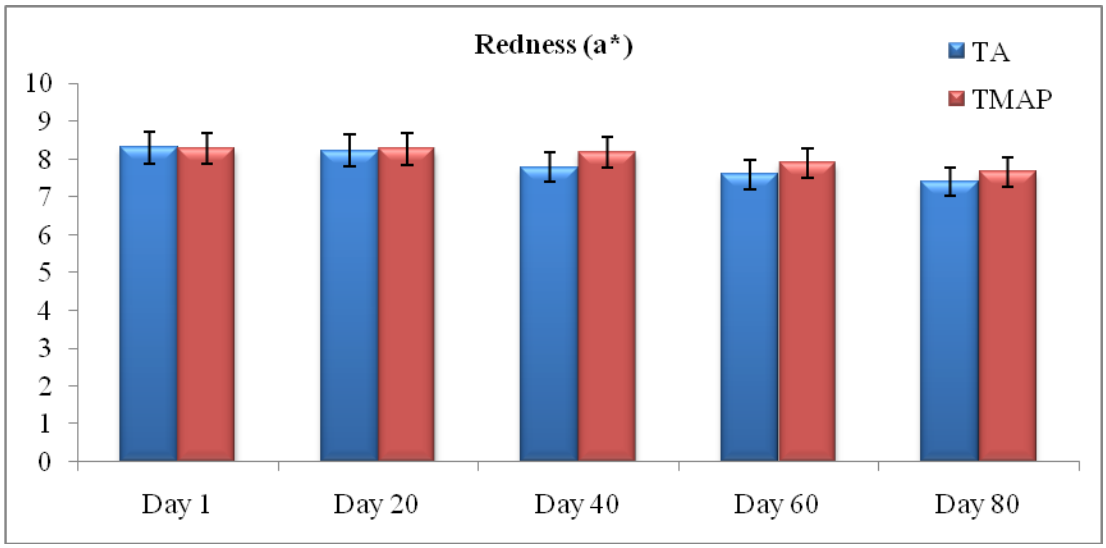
Incorporation of 30% liver powder and 2.0% dicalcium phosphate affected the yellowness (*b** values) and the rate of decrease was significantly ($P<0.05$) lower for all the treated samples throughout the storage period. This could be attributed to the fact of the development of the metmyoglobin brown pigment (Lin *et al* 2015). Realini*et al* (2015) also reported a decrease in *b** values of beef patties containing Acerola fruit extract. High final pH also affected the colour stability of the raw pork, because it affects enzyme activity and the rate of oxygenation. Reducing enzymes are necessary to convert metmyoglobin back to oxymyoglobin, which is well known to contribute to the colour of fresh meat (Pietrasik *et al* 2006).

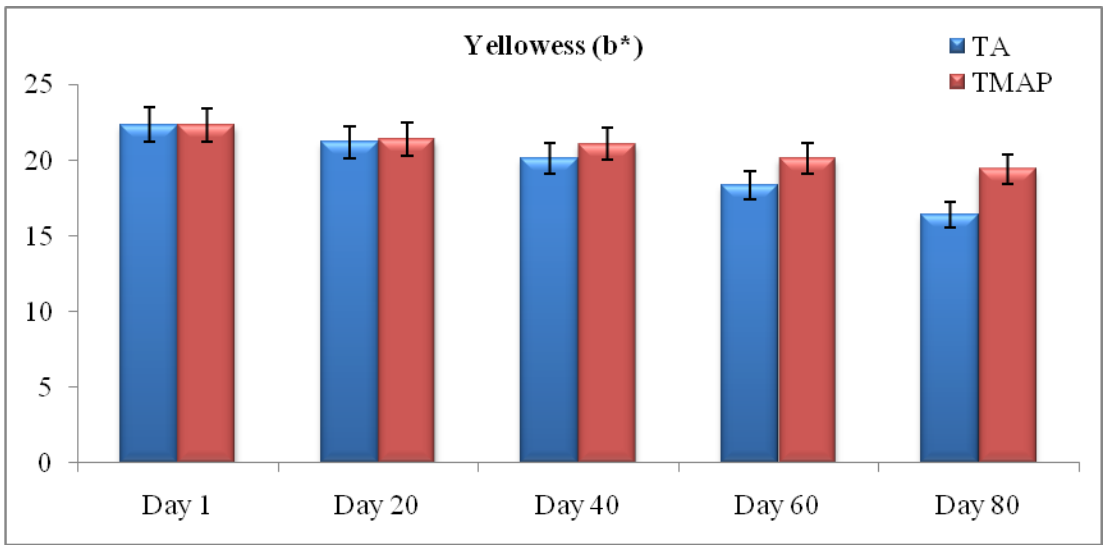
Table 11: Instrumental colour and texture parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

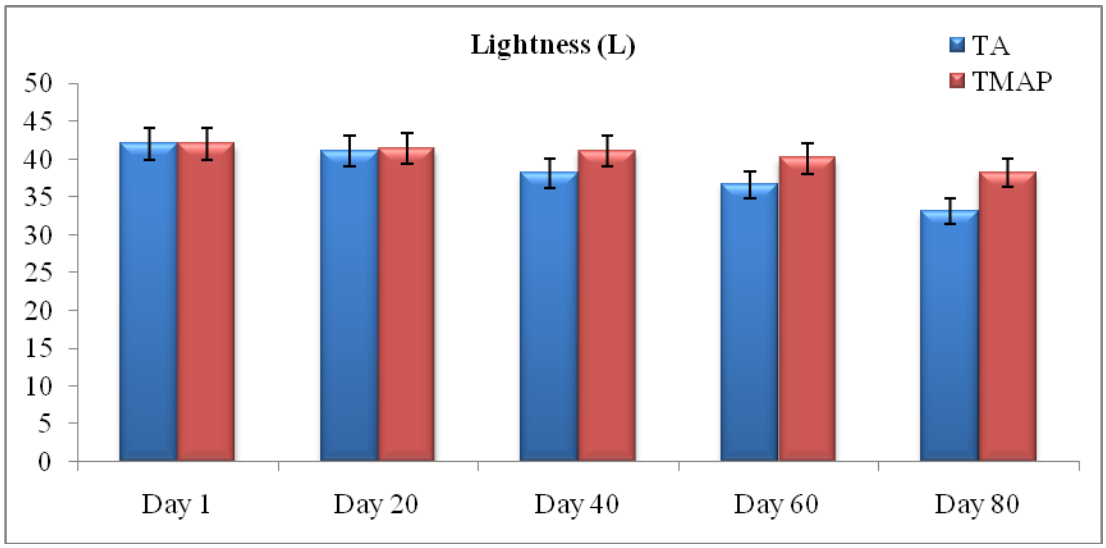
Treatments/ Days	Storage period (Days)				
	Day 1	Day 20	Day 40	Day 60	Day 80
			Redness (a*)		
T_A	8.30± 0.23^{b}	8.23± 0.21^{b}	7.78± 0.34^{a}	7.59± 0.29^{a}	7.41± 0.31^{a}
T_{MAP}	8.28± 0.23^{b}	8.26± 0.19^{b}	8.18± 0.14^{ab}	7.89± 0.15^{a}	7.65± 0.22^{a}
			Yellowness (b*)		
T_A	22.35± 0.28^{e}	21.19± 0.17^{d}	20.13± 0.20^{cB}	18.33± 0.19^{bB}	16.38± 0.22^{aB}
T_{MAP}	22.31± 0.19^{a}	21.37± 0.11^{d}	21.05± 0.18^{cA}	20.12± 0.17^{bA}	19.38± 0.22^{aA}
			Lightness (L)		
T_A	42.02± 0.88^{d}	41.11± 0.47^{d}	38.13± 0.51^{cB}	36.59± 0.49^{bB}	33.11± 0.54^{aB}
T_{MAP}	42.00± 0.88^{b}	41.37± 0.39^{d}	41.07± 0.58^{cA}	40.11± 0.54^{bA}	38.18± 0.46^{aA}
			Fracturability (N)		
T_A	2.30± 0.06^{a}	2.20± 0.03^{a}	2.08± 0.06^{a}	1.80± 0.10^{b}	1.59± 0.08^{b}
T_{MAP}	2.28± 0.06^{a}	2.25± 0.05^{a}	2.11± 0.08^{a}	1.99± 0.11^{b}	1.71± 0.06^{b}
			Shear Force Value (Kg/cm^2)		
T_A	3.89± 0.18^{a}	3.65± 0.09^{a}	3.32± 0.16^{b}	3.11± 0.13^{b}	2.89± 0.09^{c}
T_{MAP}	3.80± 0.16^{a}	3.71± 0.11^{a}	3.59± 0.18^{a}	3.42± 0.15^{ab}	3.13± 0.08^{b}

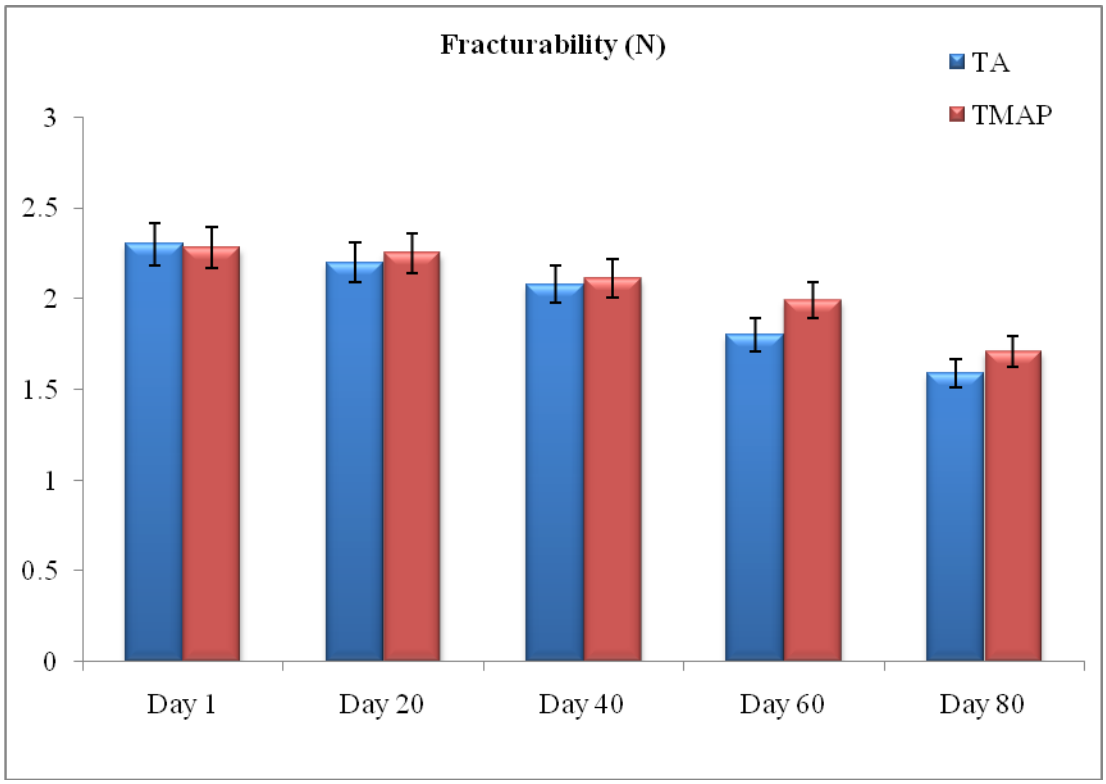
N=6; T_A= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Aerobic packaging; T_{MAP}= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Modified atmospheric packaging.

*Mean±S.E. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($P<0.05$)


Redness (a*)
TA
TMAP
10
9
8
7
6
5
4
3
2
1
0
Day 1
Day 20
Day 40
Day 60
Day 80


Yellowess (b*)
TA
TMAP
25
20
15
10
5
0
Day 1
Day 20
Day 40
Day 60
Day 80


Lightness (L)
TA
TMAP
50
45
40
35
30
25
20
15
10
5
0
Day 1
Day 20
Day 40
Day 60
Day 80



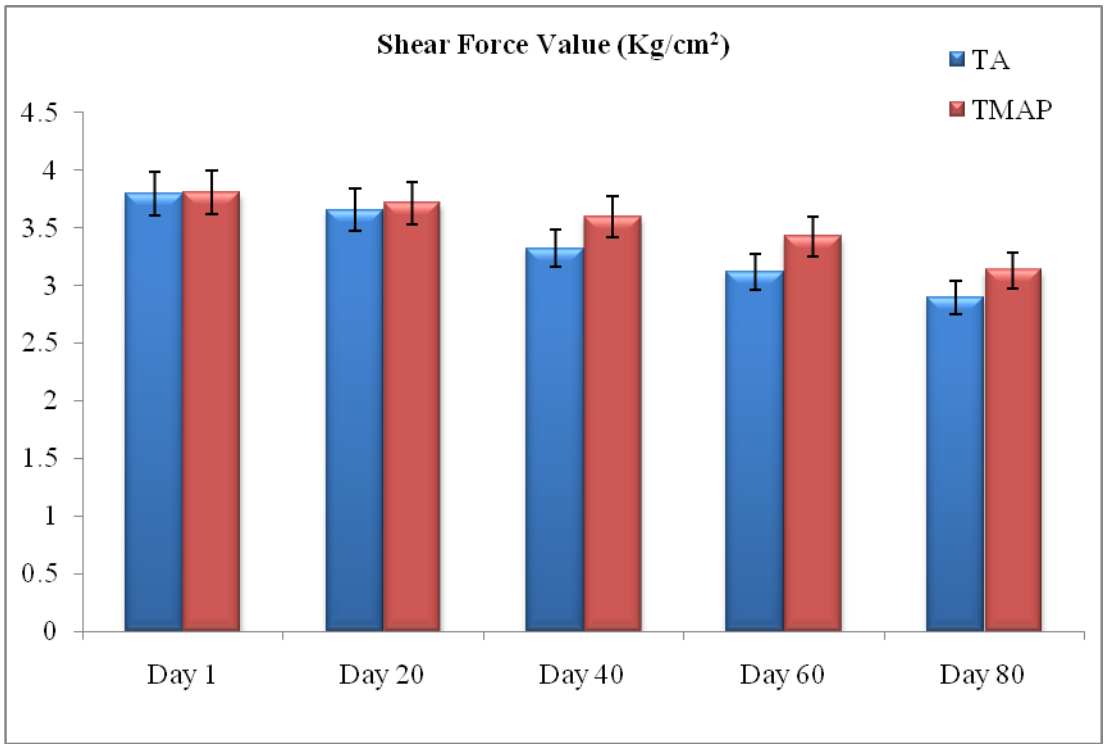


Figure 6: Instrumental Colour and texture parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

4.2.3 Microbiological Quality

The mean values for changes in microbial profile of aerobically and MAP packaged dog biscuits incorporated with 30% liver powder and 2.0%dicalcium phosphate as detected on 1, 20, 40, 60 and 80 days of ambient temperature storage are presented in statistically analysed Table 12 and Figure 7.

The microbial counts increased significantly ($P<0.05$) with advancement of storage period. There was no significant difference in the mean microbiological counts of aerobically and MAP packaged dog biscuits between the two packaging systems at the start of storage; however, significant differences were found after 20^{th} day of storage.

4.2.3.1 Total Plate Count (TPC)

The mean values for Total plate count (TPC) for aerobically and MAP packaged dog biscuits increased significantly ($P<0.05$) at each subsequently storage interval. However it remained below the threshold level for spoilage (Cremer and Chipley 1977) till 80^{th} day of storage for both the dog biscuits. This increase could be due to the availability of the nutrients and more favourable conditions for microbial growth. Though TPC increased significantly ($P<0.05$) during storage, it was significantly ($P<0.05$) lower in MAP than aerobic packaged products except on day one of storage. The lower microbial load in MAP might be due to the addition of 50% CO_2 gas in the package, which act on the lag phase of bacterial growth cycle and inhibits the growth of bacteria (Ashie *et al* 1996). Similar findings were reported by Singh (2013) in chicken lollypops and Kumar and Sharma (2004) in pork patties.

Singh *et al* (2011) also reported an increase in TPC at each storage interval in meat snacks. Similar findings were reported by Kumar *et al* (2007) in chicken meat patties who also reported an increase in total plate count at each storage interval both in control and treatment samples. Anandh (2014) reported that the microbial counts increased significantly ($P<0.01$) with increasing storage period. Reddy and Rao (2000), Chidanandaiah *et al* (2009), Kumar and Tanwar (2011) Bhat *et al* (2011) and Malav *et al* (2017) observed a similar increase in TPC while studying different meat products stored at refrigeration temperature.

4.2.3.2 Coliform count

Coliforms were not detected in all the control and treatment products during entire storage period. It reflects the hygiene conditions followed during the preparation of the product as well as the high treatment employed during cooking (Kumar and Sharma 2004). Similarly, Frazier and Westhoff (2008) also reported that cooking of the products at high temperature leads to the destruction of major microflora. Similar finding was observed by Sharma (2013) in MAP packed chicken meat croquets and Jairath (2013) in enrobed goat meat bites. Singh *et al* (2011) have reported similar findings in chicken snacks at ambient temperature. Similar results were reported by Kumar and Sharma (2004) in pork patties.

4.2.3.3 Psychrophilic count

Table 12 revealed that the psychrophilic count (PC) was not detected during the entire storage period in both aerobically and MAP packaged dog biscuits which might be due to the storage of product at ambient storage.

4.2.3.4 Yeast and Mold count (YMC)

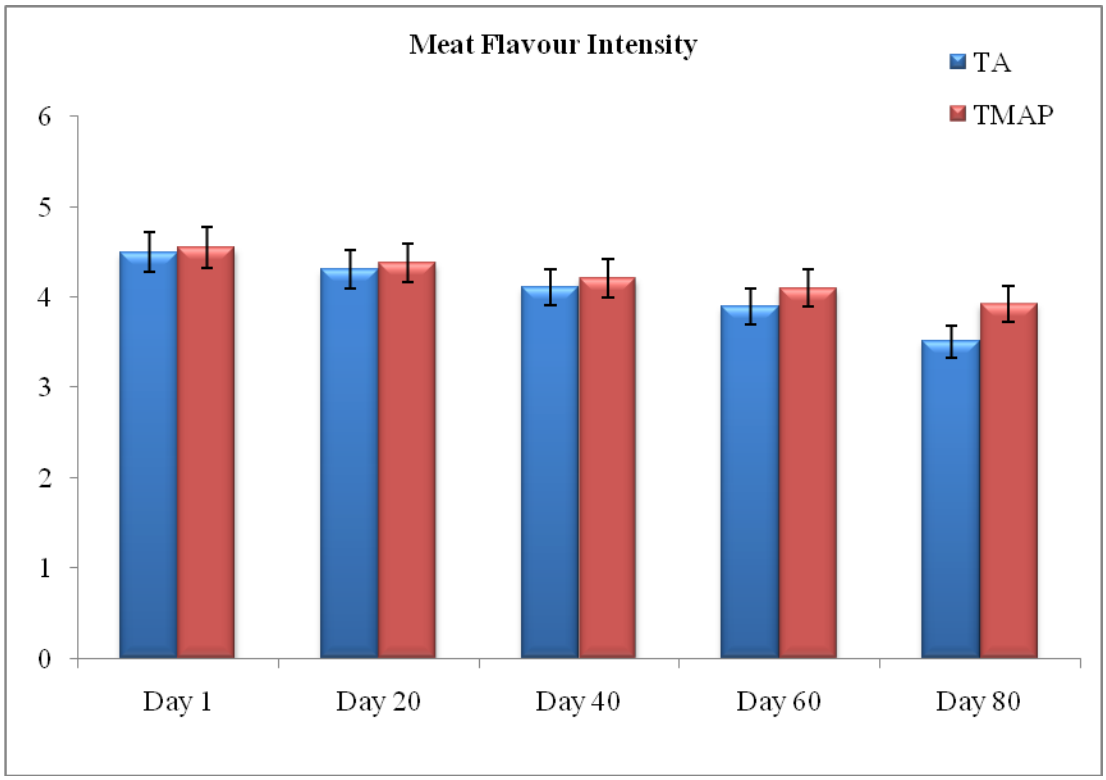
Yeast and mold were detected in both the treatment products. Yeast and mold increased significantly ($P<0.05$) at each subsequently storage interval for both Aerobic and MAP packaged products. However, significantly ($P<0.05$) lower YMC were noticed for MAP dog biscuits. It could be attributed to antimicrobial effect of infused gases in the packages. Similarly, Singh *et al* (2011) reported that yeast and mold appeared during the last day of storage of chicken snacks due to the availability of nutrients in meat. Further there were no visible Yeast and Mold spores/mycelia and off-odor on treatment products on the last day of storage for both the aerobically and modified atmospherically packaged products. Das *et al* (2013) and Singh *et al* (2011) also reported similar results in chicken nuggets and chicken snacks, respectively.

Table 12: Microbiological parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

Treatments/ Days	Storage period (Days)				
	Day 1	**Day 20**	**Day 40**	**Day 60**	**Day 80**
	Standard Plate Count (log_{10}cfu/g)				
T_A	1.11 ± 0.07^{e}	1.97 ± 0.08^{dA}	2.93 ± 0.06^{cA}	3.59 ± 0.04^{bA}	5.15 ± 0.07^{aA}
T_{MAP}	1.10 ± 0.05^{e}	1.31 ± 0.06^{dB}	1.58 ± 0.09^{cB}	2.21 ± 0.07^{bB}	3.29 ± 0.09^{aB}
	Coliform Count (log_{10}cfu/g)				
T_A	ND	ND	ND	ND	ND
T_{MAP}	ND	ND	ND	ND	ND
	Psychrophilic Count (log_{10}cfu/g)				
T_A	ND	ND	ND	ND	ND
T_{MAP}	ND	ND	ND	ND	ND
	Yeast and Mold Count (log_{10}cfu/g)				
T_A	1.04 ± 0.04^{e}	1.29 ± 0.03^{dA}	1.72 ± 0.04^{cA}	2.34 ± 0.04^{bA}	2.90 ± 0.07^{aA}
T_{MAP}	1.02 ± 0.04^{e}	1.09 ± 0.03^{dB}	1.32 ± 0.04^{cB}	1.94 ± 0.04^{bB}	2.37 ± 0.07^{aB}

N=6; T_A= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Aerobic packaging; T_{MAP}= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Modified atmospheric packaging.

*Mean±S.E. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($P<0.05$)



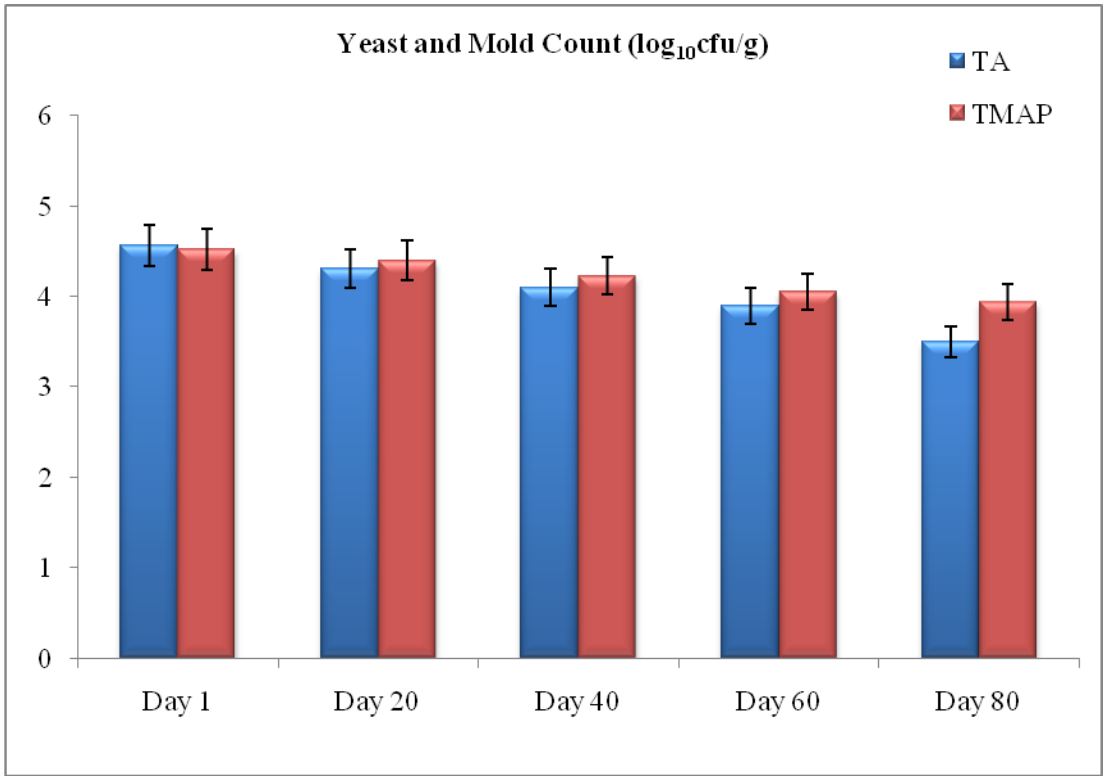


Figure 7: Sensory parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

4.2.4 Sensory quality

The results for sensory evaluation of developed dog biscuits incorporated with 30% chicken liver and 2% dicalcium phosphate stored at ambient temperature under aerobic and modified atmosphere packaging (MAP) conditions are presented in statistically analyzed Table 13 and Figure 8.

4.2.4.1 General appearance and colour

The appearance scores showed significant ($P<0.05$) decline in products of both aerobic and MAP with the progress of storage period but decline in the scores for MAP products was significantly ($P<0.05$) slower than aerobic packaging. The most probable cause of decrease in appearance score might be attributed to non-enzymatic browning reaction between lipid oxidation products and amino acids. Appearance scores for control and treatment products of both packaging methods were comparable on 1^{st}, 20^{th} and 40^{th} day of storage, however on 60^{th} and 80^{th} day of storage the appearance scores for aerobic packaged dog biscuits was significantly ($P<0.05$) lower than MAP packaged products. The gradual decrease in color and appearance scores on storage might be due to pigment and lipid oxidation resulting in nonenzymatic browning (Kumar *et al* 2010). The color and appearance of liver powder incorporated dog biscuits were found to be acceptable by all sensory panelists, throughout the storage period.

Decreasing trend of appearance scores was also reported in chicken meat kachori by Poodari (2017) and spent hen meat papad by Malav *et al* (2018). A similar decrease in appearance scores of chicken and fish snacks with increase in storage period was also reported by Singh *et al* (2011) and Ratanatriwong *et al* (2011) respectively. The color and appearance of liver incorporated dog biscuits were found to be acceptable by all sensory panellists throughout the storage period.

4.2.4.2 Meat flavour intensity

The Meat flavour flavor intensity scores also showed a significant ($P<0.05$) decreasing trend as the days of storage advanced for both aerobic and MAP products. The flavor scores of control and treatment products of both packaging methods were comparable on 1^{st}, 20^{th} and 40^{th} day of storage, however on 60^{th} and 80^{th} day of storage the appearance scores for aerobic packaged products was significantly ($P<0.05$) lower than MAP packaged products. Greene and Cumuze (1982)

documented that there was a correlation between sensory scores and TBARS values in cooked ground meat. Decline in flavor scores were significantly lower in MAP products than aerobic products. It might be correlated to rate of increase in TBARS, FFA values and peroxide values during storage period. The lipid peroxidation deteriorates the nutritive value, produces toxic substances (viz. malondaldehyde, peroxides, and oxysterols) and compromises the organoleptic properties due to production of off-odors, changes in color and appearance (Chatli and Joseph 2014).

Raja *et al* (2014) noted that the reduction in Meat Flavour Intensity could be attributed to the increased lipid oxidation, liberation of fatty acids and increased microbial load. A gradual decline of flavor might also be due to the expected loss of volatile flavor components from spices and condiments on storage of meat products. The progressive decrease in flavor could be correlated to increase in TBARS value of meat products microbial growth (Tarladgis *et al* 1960) stored under aerobic conditions. Decline in flavor scores of meat snacks during storage was also reported by Singh *et al* (2011). The decrease in flavor and meat flavor scores with the advancement of the storage period might also be due to dilution in meaty flavour. Similar reports have been published by Padda *et al* (1989), Kumar and Sharma (2004).

4.2.4.3 Overall acceptability

As the days of storage advanced, scores for overall acceptability showed significantly ($P<0.05$) declining trend. Continuous decrease in overall acceptability scores might be due to decrease in other sensory parameters namely colour and appearance, flavor, texture, meat flavor intensity and crispiness. The sensory panelists rated the developed dog biscuits under aerobic and MAP acceptable even on day 80^{th} i.e. end of storage period.

Decrease in overall acceptability with increasing storage period was also reported by Devatkal and Mendiratta (2001) in pork rolls. Similar findings have also been reported by Kumar and Sharma (2004), Bhat and Pathak (2009), Bhat *et al* (2013a) and Bhat *et al* (2013b) for various meat products. Sensory quality of both the dog biscuits was significantly ($P<0.05$) influenced by MAP over aerobic under ambient temperature storage condition. The overall acceptability scores for the chicken liver incorporated dog biscuits followed the same pattern as observed for other sensory attributes.

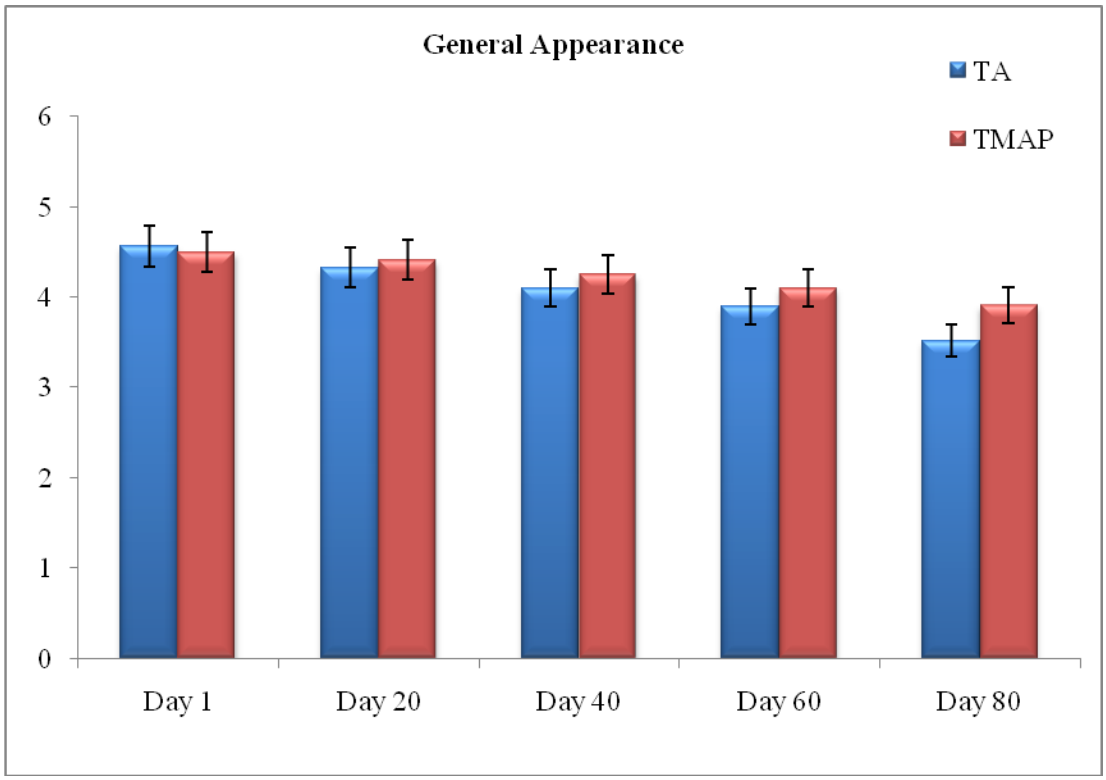
Hence, it can be concluded that at ambient temperature storage, 30% chicken liver and 2% dicalcium phosphate incorporated dog biscuitscan be successfully stored up to 80 days under aerobic packaging and MAP conditions without any marked loss in physico-chemical quality, microbiological quality and acceptable sensory attributes within permissible limits.

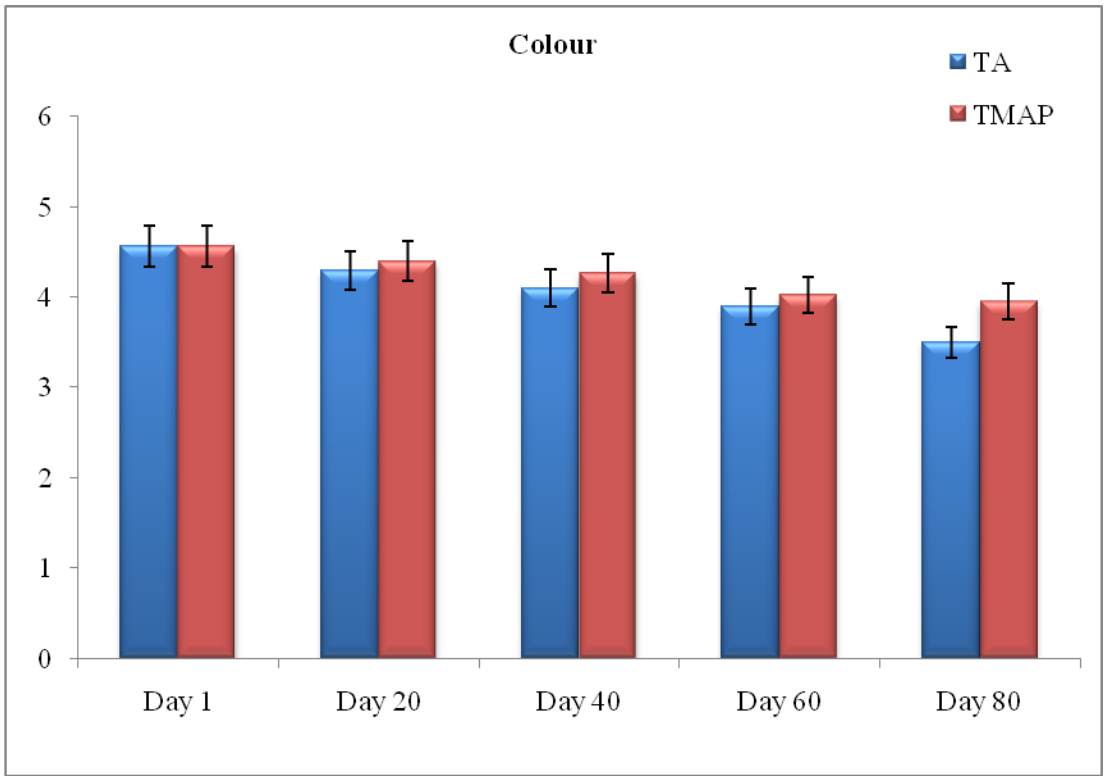
Table 13: Sensory parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

Treatments/ Days	Storage period (Days)				
	Day 1	Day 20	Day 40	Day 60	Day 80
			General Appearance		
T_A	4.56± 0.08^{c}	4.33± 0.10^{bc}	4.10± 0.13^{b}	3.90± 0.10^{bB}	3.52± 0.11^{aB}
T_{MAP}	4.50± 0.09^{c}	4.41± 0.07^{bc}	4.25± 0.11^{b}	4.10± 0.06^{bA}	3.91± 0.10^{aA}
			Colour		
T_A	4.57± 0.11^{d}	4.29± 0.10^{c}	4.10± 0.14^{cb}	3.90± 0.09^{b}	3.50± 0.09^{aB}
T_{MAP}	4.56± 0.06^{c}	4.40± 0.08^{b}	4.26± 0.11^{b}	4.02± 0.06^{a}	3.95± 0.05^{aA}
			Meat Flavour Intensity		
T_A	4.50± 0.09^{a}	4.31± 0.09^{a}	4.11± 0.09^{a}	3.90± 0.08^{aB}	3.51± 0.13^{aB}
T_{MAP}	4.55± 0.08^{c}	4.38± 0.05^{bc}	4.21± 0.07^{b}	4.10± 0.04^{bA}	3.93± 0.08^{aA}
			Overall Acceptability		
T_A	4.57± 0.05^{a}	4.31± 0.09^{a}	4.10± 0.11^{a}	3.90± 0.08^{aB}	3.50± 0.13^{aB}
T_{MAP}	4.52± 0.07^{c}	4.40± 0.05^{bc}	4.23± 0.09^{b}	4.05± 0.07^{bA}	3.94± 0.11^{aA}

N=21; T_A= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Aerobic packaging; T_{MAP}= 30% liver and 2% Dicalcium phosphate incorporated dog biscuits in Modified atmospheric packaging.

*Mean±S.E. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($P<0.05$)


General Appearance
TA
TMAP
6
5
4
3
2
1
0
Day 1
Day 20
Day 40
Day 60
Day 80


Colour
TA
TMAP
6
5
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1
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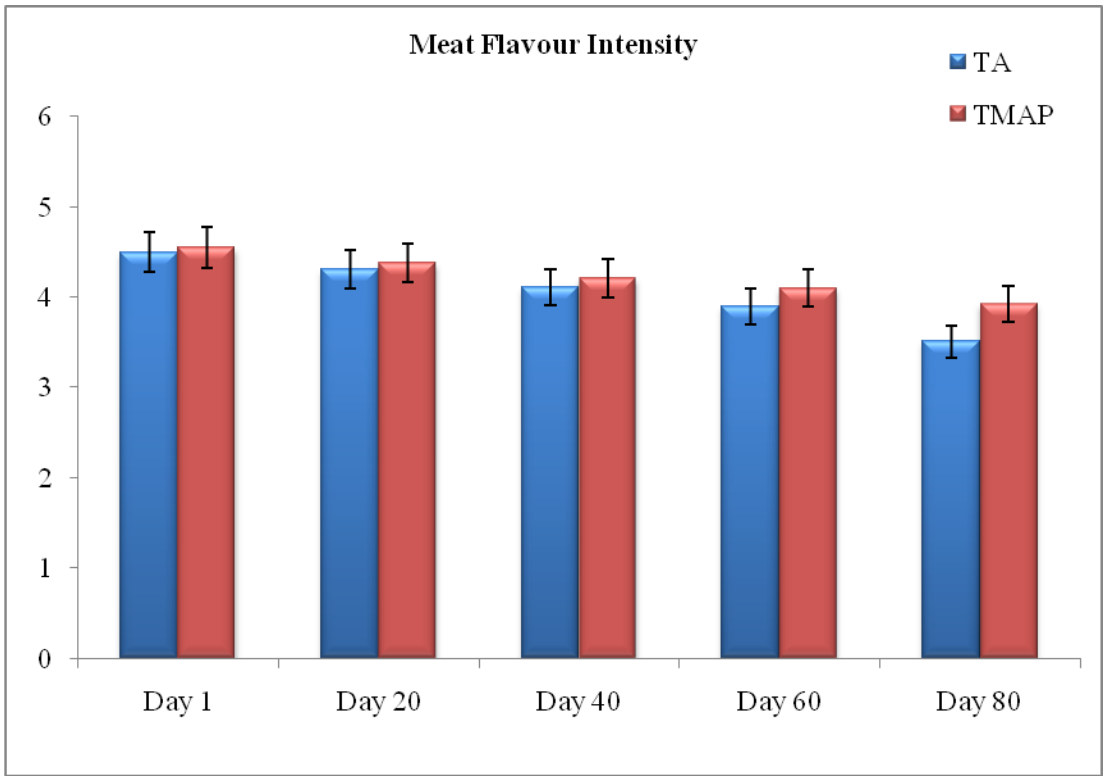
Day 1
Day 20
Day 40
Day 60
Day 80



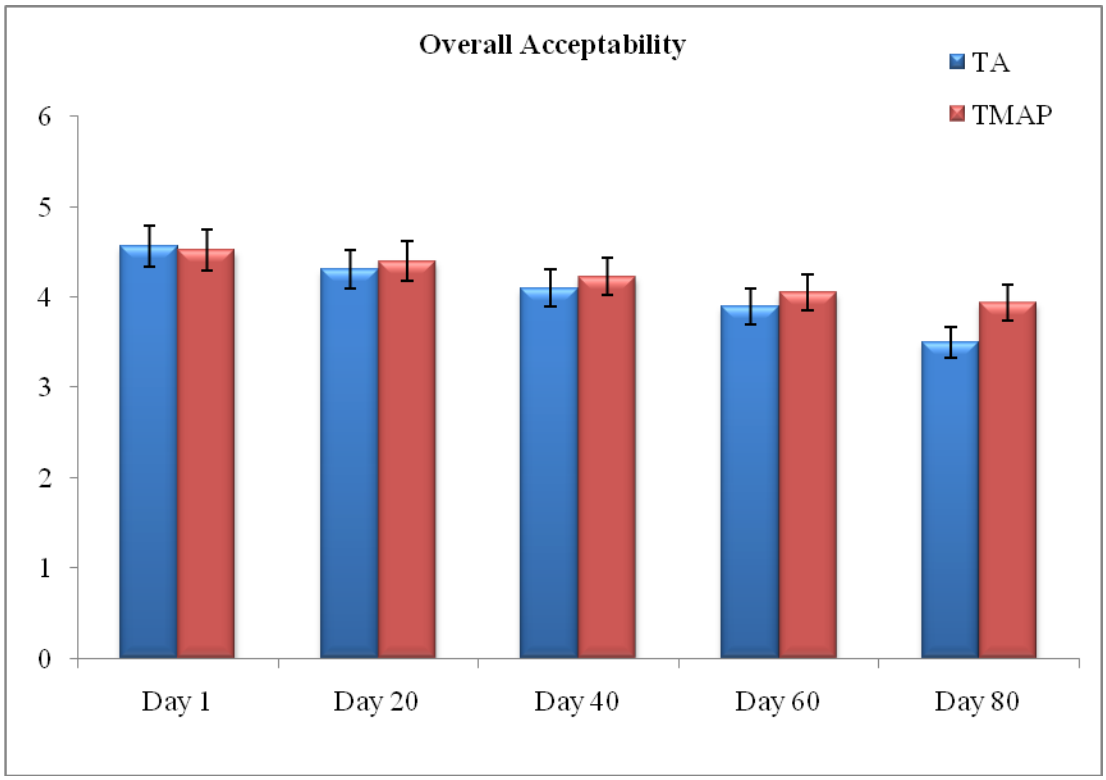


Figure 8: Sensory parameters of developed dog biscuits during ambient temperature storage under Aerobic and Modified Atmosphere Packaging conditions (Mean±S.E.)*.

4.3 Experiment No. 3: Economics of production of dog biscuits incorporated with 30% chicken Liver powder

Development of any technology can be identified as successful until it is used for the benefit of the society. Technology for food products depends not only on its general appearance, color, aroma etc but also on its nutritive value and cost of production. For this the economics including the cost of production of dog biscuits was worked out taking the assumption that per day production of dog biscuits was 50 kg. To estimate accurate cost of production of dog biscuits under commercial conditions, the expenditure incurred in terms of recurring items, labour charges, water and electricity charges, depreciation on machineries, rent paid and maintenance cost was taken under consideration.

4.3.1 Raw material cost

Raw materials are the basic ingredients in the manufacture of dog biscuits. The raw materials required for preparation of dog biscuits are refined wheat flour, table salt, spices mixture, powdered sugar, baking powder, vegetable oil and whole egg liquid. In addition, poultry liver powder and dicalcium phosphate were also utilized in present work. The retail prices for these ingredients are relatively stable in our marketing system. However, the cost of these ingredients can be lowered if purchased in bulk quantities from distributors/whole sale agents.

Table 14: Cost of production of spice mix

Name of ingredients	Quantity (gm)	Rate (Rs/Kg)	Cost (Rs)
Aniseed (Soanf)	100	150.00	15.00
Black pepper (Kalimirch)	100	750.00	75.00
Caraway seeds (Ajwain)	100	200.00	20.00
Cardamom dry (BadiElaichi)	50	1200.00	60.00
Cardamom dry (ChhotiElaichi)	20	900.00	18.00
Cinnamon (Dalchini)	50	180.00	9.00
Cloves (Laung)	50	1100.00	55.00
Coriander (Dhania)	150	150.00	22.50
Cumin seeds (Zeera)	150	200.00	30.00
Capsicum powder (mirch powder)	80	140.00	11.20
Dry ginger powder (Soanth)	80	300.00	24.00
Mace (Javitri)	50	1200.00	60.00
Nutmeg (Jaifal)	20	900.00	18.00
Total	1000		417.70 (≈418)

Table 15: Cost of formulation for dog biscuits

Ingredients	Rate Rs/kg	Control dog biscuit		Liver powder incorporated dog biscuit (30%)	
		Qt. (Kg)	Amount (in Rs)	Qt. (Kg)	Amount (in Rs)
Poultry liver powder	200.00			15	3000
Baking powder	40	0.5	20.00	1	40.00
Spice mix	418.00	1.5	627.00	1	418.00
Refined oil	100.00	10	1000.00	10	1000.00
Salt	20.00	1	20.00	1	20.00
Refined wheat flour	40	27.5	1100.00	10.5	420.00
Sugar	40	1.5	60.00	1.5	60.00
Whole egg liquid	100.00	8	800.00	8	800.00
Dicalcium phosphate	360.00	--		2	720
Total (Rs)	--		**3627**	--	**6478**

4.3.2 Cost of processing equipments

The essential equipments and accessories required for preparation of dog biscuits and approximate cost of processing and other machineries required for the preparation of 50 kg dog biscuits.

Table 16: Cost of processing equipments

Equipments	No. required	Cost (in Rs)
Tray drier	1	1,00,000
Meat mincer	1	5,000
Paddle mixer	1	30,000
Refrigerator (500 L)	2	50,000
Cooking oven	1	50,000
Impulse sealer	2	6,000
Geyser(50 L)	1	25,000
Weighing balances	2	5,000
Deep freezer(360 L)	1	60,000
Furniture and utensils (steel table, knives, vessels etc.)	-	50,000
Total (Rs)		**3,31,000.00**

Depreciation rate = 10% per annum 331,000.00

i.e. = **Rs** 33100/annum

i.e. = **Rs** 91.6 ~**Rs 92.00**/day

4.3.3 Cost of electricity

A processing plant requires electricity for the operation of various equipments and adequate illumination of the working space. The electricity charges presently are approximately **Rs8/**KWh under industry category use. The cost of electricity incurred for production of 50 kg of dog biscuits can be calculated.

Table 17: Cost of electricity

Equipments	**Watt × hr**	**KWh**
Tray drier	1000 × 3	3.0
Meat mincer	1000 × 3	3.0
Paddle Mixer	500 x 3	1.5
Geyser	500 x 4	2.0
Refrigerator	150 × 2 × 20	6.0
Deep freezer	150 x 1 x 20	3.0
Lights, fans, weighing balance etc.	500 × 10	5.0
Cooking oven	1000 x 2	2.0
Total unit		**25.50**

Therefore the cost of electricity = 25.50 KWh × **Rs** 8/ KWh = Rs **204 /day**

4.3.4 Packaging cost

Dog biscuits should be properly packaged in LDPE bags. About 2 kg LDPE bags, each with a capacity of holding 250 gm finished product would be needed. 1 kg of printed LDPE bag would cost, **Rs 200**/kg. Thus cost of packaging material would be **Rs 200**/day. Cartons would be also required for bulk packaging, storage, transportation and distribution so the additional cost is around **Rs 100**/day. So total packaging cost will be **Rs 300**/day.

4.3.5 Labour cost

The labour cost of skilled person and unskilled person would be **Rs 400**/day and **Rs 300**/day respectively. For preparation of 50 kg dog biscuits, one skilled and two unskilled labours would be required per day.

So, the labour cost can be calculated as

Skilled staff = 400 × 1 = **Rs 400** /day

Unskilled staff = 300 × 2 = **Rs 600** /day

Total labour cost = **Rs 1000** /day

4.3.6 Cost of water

It is the most essential ingredient in food processing plant. For processing of 50 kg, around 500 litre of water would be required.

Water charges (500 Lit.) = 500×0.10 = **Rs 50**

4.3.7 Premises rent

Properly constructed building is the basic infrastructure required for processing plant. A building in a peri urban area / locality which has sufficient space to hold the entire processing unit for setting up a small scale meat processing unit with all facilities would cost around **Rs 30,000** per month. Therefore, rent per day **Rs 1,000 /day.**

4.3.8 Maintenance cost

The daily use materials like telephone, detergent, soap, sanitizer etc. that are required to maintain the equipments, building and premises hygienically would cost approximately **Rs 300 per day.**

4.3.9 Total expenditure

The sum of all the above costs (6.1 to 6.8) account for total cost for the production of 50 kg dog biscuits.

Table 18: Total expenditure for preparation of 50 kg dog biscuits

Parameter	**Control**	**Liver incorporated dog biscuits (30%)**
Raw materials cost	3627.00	6478.00
Cost of machineries (depreciation cost)	92	92
Cost of electricity	204	204
Packaging cost	300	300
Labour cost	1000	1000
Cost of water	50	50
Premises rent	1000	1000
Maintenance cost	300	300
Total expenditure	**6573.00**	**9424.00**

4.3.10 Product yield

The product yield was around 90% for control and treatment.

4.3.11 Retail cost of dog biscuits

Total expenditure for the preparation of 50 kg dog biscuits was **Rs 6573.00** for control and **Rs 9424.00** for treatment. The product yield (Kg) was 90 for control and treatment.

$$\text{Cost of 1 kg product} = \frac{\text{Total expenditure}}{\text{Product yield}}$$

Therefore, the cost of 1 kg product was **Rs 146.00** for control and **Rs 209.42** for treatment.

Assuming Selling price of developed dog biscuits is Rs 300/Kg

Total income for 50 kg = Total sale price of 50 kg– Total cost of production of 50 kg = **Rs 15,000-10471= Rs. 4529 (for 50 kg)**

4.3.12 Total Profit

=Total income - Commission to retailer Rs 2/ packet (50 Packs×2= Rs.100 /day)

Total Profit/day (Developed biscuit) =Rs.4529-Rs.100= Rs.4429 (for 50 packet-1kg each)

Total Profit /month (Developed biscuit) = Rs.4429 x 25= Rs.1,10,725

I. Variable cost = Rs.10471 x 25 = Rs.2,61,775

II. Fixed cost = Rs.3,31,000

4.3.13 Total project cost = Rs. 5,92,775.00

Say, loan amount of `6,0,000.00 @ 12% interest per annum for 12 months term = Rs.6,00,000+Rs.72,000=Rs.6,72,000

Amount of loan repayment per month = Rs.6,72,000.00/12 =Rs.56000 /- (for 12 months only)

Net Profit/month= Rs1,10,725- Rs.56000= Rs.54725

4.3.14 Break Even Point

$$\text{Break Even Point (sales in Rs.)} = \frac{\text{Fixed Cost x selling price}}{\text{Total sales – Variable cost}}$$

$$= \frac{3,\,31,000 \times 300}{15000-10471}$$

$$= \textbf{Rs. 21,925/-}$$

4.3.15 Cost benefit ratio

$$\text{Cost benefit ratio} = \frac{\text{Total profit}}{\text{Total cost of production}}$$

$$= \frac{4429}{\mathbf{10471}}$$

$$\mathbf{= 0.42}$$

4.3.16 Return on investment (ROI)

$$\text{ROI} = \frac{\text{Net profit per year}}{\text{Working capital+ Fixed cost}}$$

$$= \frac{54725\times 12}{10471\times 25\times 12+ 33100}$$

$$= 0.2068 \text{ or } 21\ \% \text{ for the first year}$$

CHAPTER V

SUMMARY AND CONCLUSIONS

According to FAO (2008) estimates 107 million livestock and more than 650 million poultry birds were slaughtered annually in India leading to production of 6.3 million tonnes meat. It leaves huge loads of by-products. On the basis of live weight of animal the by-products account for almost 60% and out of this 40% are edible and 20% are inedible. Efficient utilization of animal by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or under-utilization of by-products not only lead to loss of potential revenues but also lead to the added and increasing cost of disposal of these products along with major aesthetic and serious health problems. Pet food market has huge potential in India with the growth rate of 10-15% per annum. Dogs, cats and other pet food contribute 80, 15 and 5% respectively to pet food market. With the increasing demand of pet foods, the incorporation of animal by-products into pet food may result into better returns to meat industry with controlling the environmental pollution along with supply of quality animal proteins, vitamins and minerals to pets at the low cost. Meat and meat products are deficient of calcium, but in case of pet mineral requirement, calcium is required in the highest amount. If the meat based dog foods are not fortified with calcium they may develop skeletal abnormalities often referred to as rickets. The bone could become soft or very thin and brittle. Development of dog biscuits incorporated with poultry slaughter house byproducts and calcium can be a promising approach.

Experiment No. 1: Optimization of incorporation level of poultry by-products viz., liver and gizzard along with calcium and selenium fortification for the development of dog biscuits.

The chicken liver and gizzard powder were incorporated at 10, 20 and 30% separately in standardized dog biscuit formulation after replacing the refined wheat flour. Six different types of dog biscuits were developed i.e. T1= 10% Liver powder, T2=20% Liver powder, T3=30% Liver powder, T4=10% Gizzard powder, T5=20% Gizzard powder and T6=30% Gizzard powder incorporated dog biscuits. All the six treatment products were analyzed for sensory evaluation, dog palatability test (single bowl test), texture profile analysis and instrumental color profile. The most suitable by-product and the final level of incorporation was selected on the basis of above

mentioned parameters. The scores for all the sensory attributes showed significant (P<0.05) increasing trend with increasing level of chicken liver powder in the formulation whereas the scores for the gizzard powder incorporated dog biscuits showed the significant (P<0.05) decrease with the increasing incorporation level of gizzard powder. The overall acceptability score were significantly (P<0.05) higher for the dog biscuits containing 30% chicken liver powder (T_3) compared to the other treatment products which is reflective of scores of other sensory parameters.

Redness (*a** value) is an indicator of freshness of the meat and criteria for quality evaluation by the consumers. Redness (*a**) value showed the pattern T-1>T-2>T-3>T-6>T-4>T-5 amongst treatments and followed a decreasing trend in liver powder incorporated dog biscuits. Redness (*a**) value of dog biscuits incorporated with chicken gizzard powder was significantly (P<0.05) lower than the liver incorporated dog biscuits. The yellowness (b*) of T3 and T6 dog biscuits was significantly (P<0.05) lower than the other treatment products. Lightness (*L** value) of chicken liver powder incorporated dog biscuits was significantly (P<0.05) higher than the chicken gizzard powder incorporated dog biscuits. In the Single bowl test 12 dogs were selected having body weight of 4±2 Kg. 100 g of dog biscuits was served do the dogs for 5 days after their regular meals. Food intake is determined by difference from initial food on offer and orts (or leftovers). The same procedure was repeated for the other treatment dog biscuits. Under this test 500 g dog biscuits from each treatment was served to all the dogs. Analysis of data showed that intake of T_2 and T_3i.e 20% liver and 30% liver powder incorporated dog biscuits was highest in all the dogs. Among these treatment products Consumption of T3 was highest which might be due to the more meat flavour intensity as shown in the sensory analysis of dog biscuits. Texture profile analysis revealed that values for both fracturability and shear force showed declining trend with increase in the incorporation level of liver and gizzard powder, which might be due to the replacement of refined wheat flour with by-products powder having low starch content and lower gelatinization on cooking of the developed biscuits.

On the basis of results of sensory evaluation, dog palatability testinstrumental colour profile and texture profile analysis, the incorporation of chicken liver powder at 30% level (T3) was adjudged best for the development of dog biscuits. T_3 product was selected for further studies.

The developed dog biscuits incorporated with most suitable by-product i.e. chicken liver powder at optimum level i.e. 30% were fortified with dicalcium phosphate on the basis of recommended dietary allowance of dogs. The dicalcium phosphate was incorporated at 2% level after replacing the refined wheat flour in the standardized formulation. The pH values of liver powder incorporated and calcium fortified dog biscuits increased significantly ($P<0.05$) upon incorporation of 2% dicalcium phosphate. The addition of dicalcium phosphate showed a significant effect ($P<0.05$) on the cooking yield of liver incorporated dog biscuits. Cooking yield of dog biscuits decreased significantly ($P<0.05$) with the incorporation of 2% level of dicalcium phosphate in the formulation The developed dog biscuits had very low moisture content (4.75±0.26 %) and the values for moisture, protein, fat content in T-1 and T-2 dog biscuits were comparable to each other but the incorporation of 2% dicalcium phosphate significantly ($P<0.05$) increased the ash content of T-2.

Experiment 2: Evaluation of the storage stability of developed dog biscuits at ambient temperature (25 °C).

Dog biscuits selected from experiment No. 1 i.e. 2% dicalcium phosphate and 30% chicken liver powder incorporated dog biscuits were packed under aerobic and MAP conditions and stored at ambient temperature (25 °C). All the packaged samples were stored at ambient temperature for 80 days. The samples were drawn at 20 days intervals and analyzed on 1, 20, 40, 60 and 80 days. The storage quality was evaluated on the basis of various physico-chemical (pH, water activity, moisture, TBARS, PV, FFA) microbiological (TPC, PC, coliforms count, yeast and mold counts), water activity and sensory analysis.

The pH value followed a significant ($P<0.05$) decreasing trend with the advancement of storage period for both the treatment products under different packaging conditions. The linear decreasing trend of pH value might be due to production of acid by fermentation of sugar. pH value of MAP packaged product has significant ($P<0.05$) lower values than the aerobically packaged products during the entire storage period. The increase in the pH of MAP packaged products was at slower rate than products of Aerobic packaging, which might be due to buffering by carbonic acid produced due to the influx of CO_2 gas in the package. Water activity (aw) and Moisture content followed significantly ($P<0.05$) increasing trend in both the

dog biscuits of both packaging methods during gradual advancement of storage study, but the rate of increase of a_w was comparatively lower in MAP. TBARS, FFA and peroxide values followed a significantly ($P<0.05$) increasing trend throughout the storage period for both aerobic and MAP dog biscuits incorporated with liver powder and dicalcium phosphate. From start of storage study the highest TBARS, FFA and Peroxide values were found in aerobically packaged products as compared to MAP packaged products indicating a low degree of lipid oxidation in MAP products.

Incorporation of liver powder affected the lightness (L^* value) redness value (a^* value) and yellowness (b^* value) of dog biscuits, where the decreasing trend was observed in all these colour values. The rate of decrease in the lightness scores was lower for the MAP packaged products Incorporation of 30% liver powder and 2.0% dicalcium phosphate affected the yellowness (b^* values) and the rate of decrease was significantly ($P<0.05$) lower for all the treated samples throughout the storage period. The mean values for Total plate count (TPC) and Yeast and mold count for aerobically and MAP packaged dog biscuits increased significantly ($P<0.05$) at each subsequently storage interval. Though TPC increased significantly ($P<0.05$) during storage, it was significantly ($P<0.05$) lower in MAP than aerobic packaged products except on day one of storage. Coliforms and Psychrophills were not detected in all the control and treatment products during entire storage period.

The appearance scores showed significant ($P<0.05$) decline in products of both aerobic and MAP with the progress of storage period but decline in the scores for MAP products was significantly ($P<0.05$) slower than aerobic packaging The Meat flavour flavor intensity scores also showed a significant ($P<0.05$) decreasing trend as the days of storage advanced for both aerobic and MAP products. The flavor scores of control and treatment products of both packaging methods were comparable on 1^{st}, 20^{th} and 40^{th} day of storage, however on $60^{th}$and 80^{th}day of storage the appearance scores for aerobic packaged products was significantly ($P<0.05$) lower than MAP packaged products. Decline in all the sensory scores were significantly lower in MAP products than aerobic products. The developed dog biscuits can be successfully stored at ambient temperature under both aerobic and modified atmosphere packaging up to 80th day without any marked loss in physico-chemical, microbiological and sensory qualities.

Cost of production of dog biscuits was worked out taking the assumption that per day production of dog biscuits was 50 kg. To estimate accurate cost of production of dog biscuits under commercial conditions, the expenditure incurred in terms of recurring items, labour charges, water and electricity charges, depreciation on machineries, rent paid and maintenance cost was taken under consideration. The cost of 1 kg product was calculated Rs 146.00 for control and Rs 209.42 for treatment dog biscuits incorporated with 30% liver powder and 2% dicalcium phosphate.

CONCLUSIONS

1. Poultry slaughter byproducts namely liver and gizzard may be effectively utilized for the development of dog biscuits.
2. Dog biscuits with high nutritive value and sensory quality may be developed by incorporation of chicken liver powder at 30% level.
3. Incorporation of chicken liver powder at 30% level will lead to selenium fortification in dog biscuits.
4. Incorporation of Dicalcium phosphate at 2% level will lead to calcium and phosphorus fortification in dog biscuits.
5. The developed dog biscuits can be successfully stored at ambient temperature under both aerobic and modified atmosphere packaging up to 80th day without any marked loss in physico-chemical, microbiological and sensory qualities.
6. The cost of production of developed dog biscuits is Rs. 210/Kg

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