DEVELOPMENT OF PREMIUM MUTTON NUGGETS ENRICHED WITH NUT BASED FUNCTIONAL COMPONENTS

Thesis

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पशुधन उत्पाद प्रौद्योगिकी विभाग

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CERTIFICATE-I

This is to be certified that the research work embodied in this thesis entitled ***"Development of premium mutton nuggets enriched with nut based functional components"*** *submitted by* ***R.R.Kumar,*** *Roll No.1440 for the award of Doctor of Philosophy Degree in Livestock Products Technology, at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

It is further certified that ***R.R.Kumar,*** *Roll No.1440 has worked for more than 30 months in the Institute and has put in more than 300 days of attendance under me from the date of registration for the Doctor of Philosophy degree in this Deemed University, as required under the relevant ordinance.*

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CERTIFICATE-II

We, the undersigned members of Advisory Committee of ***R.R.Kumar****, Roll No.****1440,*** *a candidate for the degree of Doctor of Philosophy with the major discipline in Livestock Products Technology agree that the thesis entitled* ***"Development of premium mutton nuggets enriched with nut based functional components"*** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of Doctor of Philosophy Degree of this Institute.

It is further certified that the candidate has completed all the prescribed requirements governing the award of Doctor of Philosophy Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.

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Date. 12th May 2014
Place

Rajiv Ranjan Kumar

(R.R.Kumar)
Author

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ABBREVIATION

%	:	Percent
@	:	at the rate
°C	:	Degree Celsius
µ	:	Micron
µl	:	Microlitre
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
APHA	:	American Public Health Association
cfu	:	Colony forming unit
cm	:	Centimetre
DAHD & F	:	Department of Animal Husbandry, Dairying and Fisheries
df	:	Degree of freedom
FFA	:	Free fatty acids
Fig.	:	Figure
g	:	Gram
GRAS	:	Generally recognized as safe
h	:	Hour
HDPE	:	High density polyethylene
i.e	:	that is
kg	:	Kilogram
KWh	:	Kilowatt hour
L	:	Litre
LDPE	:	Low density polyethylene
m	:	Meter
M	:	Molar
MDA	:	Malonaldehyde
Meq	:	Milliequivalent
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre

mm	:	Millimetre
MSS	:	Mean sum of square
MUFA	:	Monounsaturated fatty acids
N	:	Newton
nm	:	Nanometre
OD	:	Optical density
PCA	:	Plate count agar
ppm	:	Parts per million
psi	:	Pounds per square inch
PUFA	:	Polyunsaturated fatty acids
PV	:	Peroxide value
Rs.	:	Rupees
SE	:	Standard error
sec	:	Seconds
SPSS	:	Statistical package for the social sciences
TBA	:	Thiobarbituric acid
TBARS	:	Thiobarbituric acid reactive substances
TPA	:	Texture profile analysis
TPC	:	Total plate count

INTRODUCTION

Mutton is the meat from mature domestic sheep older than 1 year. India with 74.0 million stocks of sheep shares 6.8% of population, and occupies 2nd position in world (FAO 2010). Sheep with its multi-facet utility for wool, meat, milk, skins and manure, form an important component of rural economy particularly in the arid, semi-arid and mountainous areas of the country. Mutton earlier a by-product from wool industry, is occupying primary position to support a recovery in the sheep industry in light of declining wool demand across the world. With a production figure of 0.29 MT it contributes to 3.39% of world mutton production (FAO, 2011). The share of sheep meat in India's total meat production is 4.67%. The country nurtures 42 different breeds, majority of them better suited for mutton rather than prime quality wool. The sustainability of sheep industry in India like other parts of world depends on mutton.

Like all other meats, mutton is a rich source of nutrients and micronutrients that are needed for good health throughout life. Raw mutton contains about 22% of high biological value protein with around 94% digestibility (William, 2007). Besides an excellent diet source of essential amino acids, it also plays an important role in supplying our diet with minerals and vitamins such as iron, zinc, selenium, copper and B vitamins (Mulvihill 2004; Biesalski 2005). Just as many people eat beef on a daily basis in different parts of the world, people tend to incorporate mutton extensively in their cuisine. As compared to beef, mutton is comparatively rich in vitamin A and vitamin B components such as thiamine, riboflavin,niacin. $B_{6,}$ $B_{12,}$ pantothenic acid, iron, phosphorus, calcium and copper but poor in beta carotene, alpha tocopherol, zinc and selenium. Mutton is one kind of meat towards which there is no prejudice by any community in India. The meat is comparatively tough because the animal is older, yet it has a more prominent flavor relished by meat eaters.

In recent days, consumers often associate meat and meat products with a negative health image mainly due to its content of fat, saturated fatty acids, cholesterol and their association with chronic diseases such as cardiovascular diseases, some types of cancer, and obesity (Chan 2004 ; Ovesen 2004a, 2004b ; Fernandez - Gines *et al*., 2005; Valsta *et al.,* 2005). Mutton categorized under red meat attract objection because of intramuscular fat in lean cuts which accounts for about 4%, a comparatively higher figure than the beef. It also has a higher proportion of cholesterol (0.066%) and saturated fatty acids (1.46%) in their lean as compared to other meat in same category. The P/S quotient, the ratio of

polyunsaturated fatty acids to saturated fatty acids is generally unfavorable, as the saturated fatty acids prevail over the polyunsaturated FA. In a report South African mutton was reported to have good amount (2.37%) of cholesterol raising fatty acids (ARC, 2008). As per recommendation of medical authorities world-wide energy intake from fat should not exceed 30–35%, and more specifically energy intake from saturated fatty acid should not exceed 10% of total energy intake and energy intake from monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) should be approximately 16% and 7%, respectively, of energy intake. In order to produce ‘‘healthier’’ meat products, the positive as well as negative effects of meat constituents on health must be fully understood and it is necessary to avoid undesired substances (natural or otherwise) or reduce them to appropriate limits and to increase the levels (naturally or by programmed additions) of other substances with beneficial properties (functional ingredients) (Colmenero *et al*., 2001). Depending on the product type (composed of identifiable pieces of meat, coarsely or finely ground, emulsions, cooked, cured, etc.), one of the best moments at which to alter the composition of meat products is perhaps during one of the preparation stages. Replacing part of the animal fat normally present in the product with another more suited to human needs, i.e. with less saturated fatty acids and more monounsaturated (oleic) or polyunsaturated acids, and with no cholesterol is one of the established procedures by which meat fatty acid composition can be altered. Substitution with plant based bio-active enriched elements is an emerging concept in designing and development of healthier meat products.

Nuts have been part of the human diet for a long time and remains have been found in archaeological sites dating back to before 10,000 BC. It constitutes a good source of certain vital bioactive/functional compounds that could elicit many health benefits in human beings beyond those ascribed to the macro- and micronutrients. Nuts have favorable fatty acid and nutrient profiles, low in saturated fatty acids and higher in monounsaturated and polyunsaturated fatty acids accounting more than 75% (Damirchi *et al*., 2011). There are also other bioactive molecules in nuts that elicit cardioprotective effects which include plant protein, dietary fiber, micronutrients such as copper and magnesium, plant sterols, and phytochemicals. (Etherton *et al*., 1999). Nuts also contain significant amounts of squalene and tochopherols. Squalene has important beneficial effects on health and tochopherols are powerful antioxidants, which in high doses may

reduce the risk of chronic heart diseases (Ryan *et al*., 2006). Epidemiological studies show that regular consumption of nuts in general (Fraser *et al*., 1992; Sabaté *et al*., 1993; Feldman, 2002; Iwamoto *et al*., 2002; García-Arellano *et al*., 2003; Nus & Muniz, 2004; Fitó *et al*., 2007; Salvadó and Ballart, 2008; Banel and Hu, 2009; Tyrovolas and Panagiotakos, 2009) correlates inversely with myocardial infarction and CHD regardless of other factors associated with risk such as age, sex, smoking, hypertension, weight and exercise. Serrano *et al*., (2005) and Ayo *et al*., (2007) prepared the restructured beef steak and frankfurters with added walnuts. Delgado *et al*., (2010) concluded that some nuts might be a natural source of bioactive compounds that can be incorporated into new health-related products or be substitutes of synthetic compounds of questionable safety, promoting human health and reducing disease risks.

Almonds (*Prunus amygdalus*) are healthful nuts appealing to busy and health conscious consumers. One hundred grams of whole natural almonds provide high quality protein (21 g), dietary fibre (12 g), and out of its total fat content (50 g), 68 percent is present as monounsaturated fatty acids. In addition, few foods match the amount of vitamin E found in almonds (26 mg) with 96 percent as α-tocopherol, 2.3 percent as γ-tocopherol, 1 percent as β-tocopherol, and 0.2 percent as σ-tocopherol. α-Tocopherol is the most bioavailable form of vitamin E and is preferentially secreted from the liver into the circulation. A daily serving of almonds increases the dietary intake and plasma concentration of α-tocopherol (Jambazian *et al*., 2005). Importantly, many Europeans fall short of meeting their requirement of vitamin E and yet a handful of almonds would provide 50 percent of their daily allowance (Brigelius and Kelly, 2002). Almond skins are naturally rich in flavonoids and phenolic acids (Millbury *et al*., 2006). Research studies have shown that these compounds were bioavailable and acted as antioxidants in a synergistic manner with vitamins C and E (Chen *et al*., 2005). Almond skins (also referred to as almond bran) contribute 6.0 to 8.4 percent of the almond seed. Almond by-products such as almond skins may prove to be a high fiber ingredient useful both for the control of oxidative processes in food products and as a functional food ingredient (Ning *et al*., 2007; Garrido *et al*., 2008). Spiller *et al*., (1998) found that almond-based diet lowers LDL-c while preserving HDL-c and concluded that more favorable lipid-altering effects induced by the almond group may be due to interactive or additive effects of the numerous bioactive constituents found in almonds.

Peanuts (*Arachis hypogaea*) are legumes but are generally considered as nuts. They have a desirable fatty acid profile and are rich in vitamins, minerals and bioactive materials. They contain several known heart healthy nutrients including monounsaturated and polyunsaturated fatty acids, potassium, magnesium, copper niacin, arginine, fiber, α-tocopherol, folates, phytosterols and flavonoids. Peanut consumption has been associated with improved overall diet quality and nutrient profile (Singh and Singh, 1991; Etherton *et al*., 1999b; Kerckhoffs *et al*., 2002; Griel *et al*., 2004) and contains about 25% protein (a higher proportion than in any true nut (Weightlossforall.com). The total amount of carbohydrate in peanuts is low with over half consisting of healthy fiber. It has been identified that numerous compounds in peanuts and in their skins have added health benefits beyond basic nutrition. For example, arginine is a bioactive compound found in peanuts. It is an amino acid that is a precursor to nitric oxide, which helps to keep arteries relaxed, improving blood flow. Aside from the macro- and micronutrients, the functional compounds in peanuts can be clustered into four main categories: 1) flavonoids, 2) phenolic acids, 3) phytosterols, and 4) stilbenes. The compounds that fall under these categories function in various ways to promote health and some have antioxidant capacity (Francisco and Resurreccion, 2008). Resveratrol, for example, is a stilbene known for its role in various mechanisms that reduce cardiovascular disease (CVD) (Sanders *et al*., 2000) and cancer risk. It is said to have life promoting capabilities and to increase endurance. Percent Digestibility and protein digestibility corrected amino acid score values for Peanuts are 94 and 0.70 (Sarwar 1987). India the 2nd largest producer in the world also has no reported case of pea nut allergy. (Fraser, 2011)

Pine nuts are the edible seeds of pines (*Pinus gerardiana*) popularly called as *Chilgonga* in India. They are rich in calories, Vit B such as thiamin, riboflavin, niacin, pantothenic acid, vitamin B-6 (pyridoxine) and folates, Vit E, antioxidants, minerals like manganese, potassium, calcium, iron, magnesium, zinc as well as selenium and packed with numerous health promoting phyto-chemicals. The high caloric content of pines comes from their fats. However, the nuts are especially rich in mono-unsaturated fatty acids like *oleic acid* (18:1 undifferentiated fat) that helps to lower LDL or "bad cholesterol" and increase HDL or "good cholesterol" in the blood. Pine or cedar nuts contain essential fatty acid (ω-6 fat) pinolenic acid. Recent research has shown its potential use in weight loss by curbing the appetite. Pinolenic acid causes the triggering of hunger suppressant

enzymes *cholecystokinin* and *glucagon-like peptide-1* (GLP-1) in the gut. In addition, pinolenic acid may have LDL-lowering properties by enhancing hepatic LDL uptake. Pines are an excellent source of vitamin E; contain about 9.33 mg per 100 g (about 62% of RDA). Vitamin E is a powerful lipid soluble antioxidant, required for maintaining the integrity of cell membrane of mucus membranes and skin by protecting it from harmful oxygen free radicals. Further, like almonds and peanuts, pines are free from *gluten* and therefore are a popular ingredient in the preparation of gluten free food formulation. Pine nuts contain healthy amounts of essential minerals like manganese, potassium, calcium, iron, magnesium, zinc and selenium. At 8.802 mg per 100 g (about 383% of daily recommended intake), pines are one of the richest sources of manganese. (nutrition-and-you.com, 2012)

Since the phytochemicals are bioactive compounds in nuts and their usefulness is associated with promoting health and reducing the risk for chronic diseases, it seems appropriate to harness their functionality in meat products which is invariably alleged for health intricacies on ground of higher SFA, cholesterol, low dietary fibers and oxidation prone status. Comminuted, emulsion based meat products are not only ideally suited for reformulation strategies, using various non-meat ingredients to promote the presence of a wide variety of bioactive/functional compounds but also for the most judicious utilization of culled stock accounting for 60% for sheep and goats in India. Accordingly, the present study was envisaged with the following objectives:

1. **To optimize the level of incorporation of functionally rich nut pastes viz; almond, pea nuts and pine nuts for the preparation of premium mutton nuggets**
2. **To assess the efficacy of nut pastes viz: almond, pea nuts and pine nuts at their optimum level of incorporation for their functional components by determining the detailed product profile of premium mutton nuggets.**
3. **To determine the storage stability of developed premium mutton nuggets at refrigerated storage (4±1°C)**

REVIEW OF LITERATURE

2.1. Meat in Human Diet

Growing numbers of people world-wide are adopting energy-dense diets, high in animal protein and fat (Smil V., 2002). Improving access to nutrient-rich animal source foods is an easy way to improve nutritional status of the millions of people who are threatened with malnutrition. Meat consumption has increased by, on average, more than 10% worldwide since the beginning of 1960s (Valsta *et al*.,2005). Meat and meat products, essential components in the diets specially in developed countries, are important sources of proteins, vitamins and minerals. It is also very important in the nutrition of the most sensitive groups of population: pregnant women, lactating mothers, children and elderly (Campbell *et al*.,1999) because of its rich composition and therefore its great value as supplement. In addition to an excellent diet source of essential amino acids, minerals, vitamins such as iron, zinc, selenium, copper and B vitamins (Mulvihill, 2004; Biesalski, 2005), is also a significant source of ω-3 polyunsaturated fatty acids, especially long-chain fatty acids (C20 and longer important in the process of growth and development. Waylett *et al*.,(1999) reported that eliminating meat from the diet increases risks for deficiencies of vitamin B_{12}, iron and zinc. The growing number of studies show that meat and meat products could be the source of not only customary vital nutrients but also additional physiological active components that can promote human health (Williams, 2007). Meat is an important source for some micronutrients, due to the fact that meat is either the only source or provides a substantially higher bioavailability of some micronutrients. Being protein-rich and carbohydrate-low product, meat contributes to a low glycemic index, which is assumed to be beneficial with respect to diabetes development (Biesalski, 2005). Proteins have been found to suppress food intake as they contribute to satiety, promoting a feeling of fullness. The inclusion of lean red meat as part of a balanced diet, therefore, may help weight loss as part of a reduced-energy diet. Meat is likely to become as a central element in our eating, and the role of meat will remain prominent under discussion while talking about food trends instead of rising prices of meat, growing population and availability, negative image with respect to human health, animal welfare and environmental issues etc.

2.2. Red Meat Consumption and Health Concern

Meat of adult mammals such as cows, sheep, buffaloes, goats and horses is invariably considered red, while chicken and rabbit is invariably considered white. The meat of young mammals such as milk-fed veal calves, sheep and pigs is traditionally considered white; while the meat of duck and goose is considered red. In recent years, meat has been exposed to great criticism because it contains fats, saturated fatty acids, cholesterol, purine and also carcinogenic substances. Due to the mentioned points of view, nowadays, meat is often related to diseases of "civilisation" such as: overweight, increased blood pressure, diabetes, gout, cardiovascular diseases and cancerous diseases.

A number of epidemiological studies have associated red and processed meat consumption with the development of two of the major chronic diseases in the Western world; CVD and colon cancer (Giovannucci *et al*., 1994; Kelemen, *et al*., 2005; Cross *et al*., 2007; Kontogianni *et al*., 2007). High consumers of meat (P285 g/d) have been found to possess both higher intakes of cholesterol and higher plasma concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol and triglycerides (TG) than vegetarians, vegans or moderate and low consumers of meat (Li *et al*., 1999). Constituents of red meat that have been proposed to be responsible for these associations include the fat content, fatty acid composition and the possible formation of carcinogenic compounds, such as heterocyclic amines (HCAs), by cooking meat at high temperatures (Bingham *et al*.,2002). A major Harvard University meta-study in by Micha *et al*., 2010 involving over one million people who ate meat revealed that eating even 50g (less than 2oz) of processed meat per day increases risk of coronary heart disease by 42%, and diabetes by 19%, leading the researchers to suggest that differences in salt and preservatives, rather than fats, might explain the higher risk of heart disease and diabetes seen with processed meats

One of the 10 universal guidelines for healthy nutrition in a report of the World Cancer Research Fund released at the end of 2007 is to ''limit intake of red meat and avoid processed meat", as a result of the ''convincing evidence" for an association with an increased risk of colorectal cancer development (Willet *et al*.,1990;Giovannucci *et al*.,1994; Recommendations for Cancer Prevention, Tavani *et al*.,2000; Sinha, *et al*.,2005; Chao *et al*.,2005; American Cancer Society, BBC News. 2006, Kune, 2010). There is suggestive evidence that red meat intake increases the risk of oesophageal, lung, pancreatic and endometrial cancer (Food, Nutrition, Physical Activity, and the Prevention

of Cancer: a Global Perspective). Some studies have linked consumption of large amounts of red meat with breast cancer (Stein, 2006; Cho *et al*., 2006), stomach cancer,http://en.wikipedia.org/wiki/Red_meat_-_cite_note-30 lymphoma, bladder cancer (Fraser, 1999), lung cancer (Alavanja *et al*., 2001) and prostate cancer (Giovannucci *et al*., 1993, Agalliu *et al*., 2011). A 2011 study of almost 500,000 participants found that those in the highest quintile of red meat consumption had a 19% increased risk of kidney cancer (Daniel *et al*.,2011).Carcinogenic nature of red meat has been attributed to haemoglobin and myoglobin molecules which are found in red meat, and when ingested trigger a process called nitrosation in the gut which leads to the formation of carcinogens(Sesink *et al*., 2001, BBC News, 2006; Tappel, 2007;). Presence of carcinogenic compounds called heterocyclic amines, which are created in the cooking process is another important factor (Augustsson, K. *et al*.,1999; Sinha, and Rothman, 1999).

Many of the studies have associated red meat (processed and unprocessed) consumption with cardiovascular diseases, possibly because of its high content of saturated fat (Fraser, 1999, WHO 2003and NCI 2010). Specifically, increased beef intake is associated with ischemic heart disease. Some mechanisms that have been suggested for why red meat consumption is a risk factor for cardiovascular disease include: its impact on serum cholesterol (Gotto *et al*.,1990), that red meat contains arachidonic acid (Leaf, 1988), heme iron (Malaviarachchi *et al*., 2002), and homocysteine (Verhoef *et al*., 1996). The major SFA within beef (myristic acid C14:0, palmitic acid C16:0 and stearic acid C18:0) have each been found to be significantly associated with CHD risk in the Nurses Health Study (Hu *et al*., 1999a). Red meat consumption is also associated with acute coronary syndrome (Kontogianni *et al*., 2007), as well as stroke (Fung *et al*., 2004). It has also been associated with greater intima-media thickness, an indicator of atherosclerosis (Oh *et al*., 2010). A 2008 article published in Nature found that red meat consumption was "strongly associated" with increased odds of acute coronary syndrome, with those eating more than 8 servings of red meat per month being 4.9 times more likely to have cardiac events than those eating less than four servings per month (Kontogianni *et al*.,2007). A 21 year follow up of about thirty thousand Seventh-day Adventists (adventists are known for presenting a "health message" that recommends vegetarianism) found that people who ate red meat daily were 60% more likely to die of heart disease than those who ate red meat less than once per week (Kahn *et al*.,1984). The Seven Countries Study found a significant

correlation between red meat consumption and risk of CHD (Menotti *et al*., 1999). A significant relationship between red meat and CHD has been found specifically for women (Zyriax *et al*., 2005), most strongly with regards to processed red meat (Gramenzi *et al*.,1990) A 2009 study by the National Cancer Institute revealed a correlation between the consumption of red meat and increased mortality from cardiovascular diseases as well as increased mortality from all causes (Sinha *et al*., 2009)

Red meat intake has been associated with an increased risk of type II diabetes (Van *et al*., 2002; Song *et al*., 2004; Fung *et al*., 2004). Interventions in which red meat is removed from the diet can lower albuminuria levels (de Mello *et al*., 2006). Replacing red meat with a low protein or chicken diet can improve glomerular filtration rate (Gross *et al*., 2002).Other findings have suggested that the association might be due to saturated fat, trans fat and dietary cholesterol, rather than red meat per se (Feskens *et al*., 1990; Hu *et al*., 2001; Van *et al*., 2002). An additional confound is that diets high in processed meat could increase the risk for developing Type 2 diabetes (Schulze *et al*., 2003). Halkjær (2010) reported an association between increased waist circumference and red meat consumption

Seifert *et al*., (1992) in a ten-year follow up of 80,000 men and women found that "ten-year changes in body mass index were associated positively with meat consumption" as well as with weight gain at the waist. In a Mediterranean population of 8,000 men and women, meat consumption was significantly associated with weight gain Data from the National Health and Nutrition Examination Survey showed "consistent positive associations between meat consumption and BMI, waist circumference, obesity and central obesity (Wang *et al*., 2009). Western diets, which include higher consumption of red meats, are often associated with obesity (Song *et al*., 2009; Paradis *et al*., 2009). Regular consumption of red meat has also been linked to hypertension, and arthritis (Fraser 1999).

2.3. Nuts as Source of Functional Components and their Uses in Meat Products

Nuts are a good source of dietary fibre, essential fatty acids and protein. They also provide vitamins including folate, niacin, vitamins E and B6 and minerals, such as iron, magnesium, zinc, selenium, phosphorus and potassium. In addition, they are a source of plant bioactives that may have important health benefits. Although they are high in fat and thus energy, the fatty acids are predominantly unsaturated. There is a substantial body of evidence suggesting that frequent consumption of nuts is associated with lower risk of

cardiovascular disease (Aisbitt, 2007). Clinical trials have consistently shown that including nuts as part of a cholesterol-lowering diet improves lipid and lipoprotein profiles (Spiller *et al.,* 1992; Sabate *et al.,* 1993; Abbey *et al.,* 1994; O'Byrne *et al.,* 1997; Chisholm *et al.,* 1998; Edwards *et al.,* 1999; Kris-Etherton *et al.,* 1999; Zambon *et al.,* 2000; Curb *et al.,* 2000; Morgan and Clayshulte, 2000).

Trox *et al.,* (2010) studied the effects of various conventional shelling methods (oil-bath roasting, direct steam roasting, drying, and open pan roasting) as well as a novel "Flores" hand-cracking method on the levels of bioactive compounds of cashew nut kernels. The raw cashew nut kernels were found to possess appreciable levels of certain bioactive compounds such as β-carotene (9.57 μg/100 g of DM), lutein (30.29 μg/100 g of DM), zeaxanthin (0.56 μg/100 g of DM), R-tocopherol (0.29 mg/100 g of DM), γ-tocopherol (1.10 mg/100 g of DM), thiamin (1.08 mg/100 g of DM), stearic acid (4.96 g/100 g of DM), oleic acid (21.87 g/100 g of DM), and linoleic acid (5.55 g/100 g of DM). All of the conventional shelling methods including oil-bath roasting, steam roasting, drying, and open pan roasting revealed a significant reduction, whereas the Flores hand-cracking method exhibited similar levels of carotenoids, thiamin, and unsaturated fatty acids in cashew nuts when compared to raw unprocessed samples.

McKay and Sibley (2011) substantiated the walnuts as good source of Omega-3 Fatty Acids. They further added that nuts contain dietary fiber, protein, essential micronutrients, plant sterols, and other potentially beneficial phytochemical compounds and cited number of research findings on the same.

Serrano *et al.,* (2005) assessed amino acid, fatty acid profile, cholesterol, vitamin E and mineral contents in restructured beef steak with 20% added walnut (20W). Compared with control restructured beef steak (0% added walnut), the product with added walnut presented a lower ($P< 0.05$) lysine/arginine ratio, larger ($P <0.05$) quantities (mg/100 g product) of monounsaturated (MUFA) and *n*3 polyunsaturated (PUFA) fatty acids (mainly linolenic acid), a lower ($P<0.05$) *n*6/*n*3 PUFA ratio and a higher ($P <0.05$) polyunsaturated/saturated fatty acid ratio. The replacement of raw meat material by walnut reduced ($P< 0.05$) the cholesterol content and increased (more than 400 times) the amount of α-tocopherol. Iron, calcium, magnesium and manganese contents of 20W sample were greater ($P <0.05$) than in the control. They concluded that some changes induced by added walnut in the nutritional quality of the restructured product may present health benefits.

Ercoskun and Ercoskun (2010) studied the effects of the substitution of beef fat with walnut paste on chemical, physical and sensory quality attributes in sucuk. Four sucuk formulations were prepared as follows; one control using 90% beef and 10% beef fat and three treatments by replacing 15, 30 and 45% of beef fat with walnut paste. The addition of walnut paste increased the dry matter and decreased the fat contents of the products. The cholesterol content of control samples was significantly higher than 15, 30 and 45% walnut-substituted samples. The addition of walnut to sucuk improved the fatty acid profile compared with control sucuks. Sucuks with walnut had a healthier polyunsaturated fatty acid profile, a lower ω-6/ω-3 ratio, and a lower atherogenicity index and thrombogenicity index due to the walnut ratio, but increased thiobarbituric acid reactive substances values. Substitution of beef fat of sucuk with walnut did not affect the sensory properties of sucuk, including consistency, appearance and taste–flavor, except color.

Colmenero *et al*., (2010) presented a review on comprehensive model for the development of meat-based functional foods based on a presentation of the research achieved in terms of the design and development of qualitatively and quantitatively modified meat products (through reformulation) in nutrients associated with cardiovascular risk (walnut as a source of bioactive substances). They also discussed their bioavailability and the effect of their consumption on intermediate cardiovascular risk markers in humans.

Delgado *et al*., (2010) evaluated the potential of hazelnut kernels as a source of antioxidants to be incorporated into new products. First, the effects of extraction conditions on the isolation of hazelnut kernels' total phenols and antioxidants were evaluated. Six conditions, involving different solvents (water, methanol and aqueous acetone) and contact times, were studied. The highest total phenol contents were obtained with boiling water for 30 min, 44.3mgGAE/g extract, and 80% (v/v) aqueous acetone solution for 24 h, 36.2mgGAE/g extract. Increasing the contact time for the acetonic extractions did not improve the total phenols content. Regarding antioxidant activity, the highest DPPH-scavenging effect value was obtained with 80% (v/v) aqueous acetone for 24 h with an effective concentration (EC50) equal to1.12mg/mL. When other nuts walnuts, almonds, pine nuts and peanuts were extracted under this condition, only walnut extract exhibited higher phenol content (268±32mgGAE/gextract), antioxidant activity as measured by reducing power (EC50 = 0.091mg/mL) and free radical scavenging capacity (DPPH assay) (EC50 = 0.060±0.010 mg/mL) than hazelnut extract. The work demonstrated that some

nuts might be a natural source of bioactive compounds that can be incorporated into new health-related products or be substitutes of synthetic compounds of questionable safety, promoting human health and reducing disease risks.

Djarkasi *et al*., (2011) conducted a research project on the bio-active compounds from canarium nut. The observed parameters were proximate composition of protein, lipid, water, ash, carbohydrate contents, bioactive compounds and antioxidant activity expressed by DPHH value. The result from proximate analysis showed that the highest chemical component of canarium nut was lipid. Furthermore, the dominant fatty acid of triacylglycerol in three kind of canarium nut was oleic acid. Protein was the second highest compound of canarium nut, with glutamic acid as the most predominant amino acid. Canarium nut contained high concentration of phenolic and flavanoid as alpha tochopherol. Antioxidant activity of canarium nut expressed by Radical scavenging activity DPHH was in mutual accord with phenolic compound. Compared to BHT, Canarium nut had higher antioxidant capacity than BHT. The best solvent for extraction of phenolic and flavonoids compounds was ethanol. Indicated by phenolic content, flavanoid anti antioxidant capacity was as high as 0.89 mg/g, 1.58 mg/g and 69.71% respectively. On the other hand, the highest alpha tocopherol was found in hexane extracts. It was concluded that, canarium nut may be used as functional food due to its high antioxidant activity.

Damirchi (2011) reported nuts to be part of a healthy diet such as Mediterranean diet. Benefits of nuts in reducing the risk of heart disease have been reasonably attributed to their composition of vitamins, minerals, unsaturated fatty acids, fiber and phytochemicals such as polyphenols, tocopherols, squalene and phytosterols. More than 75% of total fatty acids of nuts are unsaturated. α- tocopherol is the main tocopherol isomer present in most of the nuts. While walnuts, Brazil nut, cashew nut, peanut, pecan and pistachio nuts are rich in γ- tocopherol. β- sitosterol is dominant sterol in nuts. Pistachio and pine nut have the highest total phytosterol and Brazil nut and English walnut the lowest. Walnuts also contain large amount of phenolic compounds compared with other nuts. Nuts are rich in compounds with antioxidant properties and their consumption can offer prevention from incidence of many diseases including cardiovascular

Simopoulos (2002) in his article on "Omega-3 fatty acids in wild plants, nuts and seeds: Efforts made in the direction of Health Meat Products" identified multiple sources of ALA from plants, legumes, nuts and seeds and emphasized the importance of the ratio

of omega-6 to omega-3 fatty acids for proper desaturation and elongation of ALA into EPA and DHA. He stated that α-linolenic acid was not equivalent in its biological effects to the long-chain omega-3 fatty acids found in marine oils. Eicosapentaenoic acid and DHA were more rapidly incorporated into plasma and membrane lipids and produce more rapid effects than does ALA. Relatively large reserves of linoleic acid in body fat, as are found in vegans or in the diet of omnivores in Western societies, would tend to slow down the formation of long-chain omega-3 fatty acids from ALA. Therefore, the role of ALA in human nutrition becomes important in terms of long-term dietary intake. One advantage of the consumption of ALA over omega-3 fatty acids from fish is that the problem of insufficient vitamin E intake does not exist with high intake of ALA from plant sources.

Ros and Mataix (2006) stated that due to their high content of saturated fatty acids (SFA), the intake of meat and meat products was strongly associated with elevated blood cholesterol concentrations and an increased risk of hypertension, diabetes and cardiovascular diseases. Conversely, the intake of foods rich in unsaturated fatty acids, such as those contained in most vegetable fats and oils and oily fish, was associated with improved lipid profiles, a lower potency of intermediate biomarkers of atherosclerosis and lesser incidence of cardiovascular diseases. There are persuasive evidences that dietary substitution of monounsaturated fatty acids (MUFA) or n-6 polyunsaturated fatty acids (PUFA) for SFA lower blood cholesterol and may have beneficial effects on inflammation, thrombosis, and vascular reactivity. MUFA may have an advantage over PUFA because enrichment of lipoprotein lipids with MUFA increases their resistance to oxidation. Marine n-3 PUFA has a number of anti-atherosclerotic effects, including anti-arrhythmic properties and, at relatively high doses, reduces serum triglycerides. These effects appear to be shared in part by vegetable n-3 PUFA. Nuts are natural foods rich in unsaturated fatty acids; most nuts contain substantial amounts of MUFA, while walnuts are especially rich in both n-6 and n-3 PUFA. Healthy fats in nuts contribute to the beneficial effects of frequent nut intake observed in epidemiological studies (prevention of coronary heart disease, diabetes, and sudden death) and in short-term feeding trials (cholesterol lowering, LDL resistance to oxidation, and improved endothelial function).

Etherton *et al.*, (1999) reported that, nuts had favorable fatty acid and nutrient profiles, there was growing interest in evaluating their role in a heart-healthy diet. They stated that nuts were low in saturated fatty acids and high in monounsaturated and

polyunsaturated fatty acids. In addition, emerging evidence indicates that there are other bioactive molecules in nuts that elicit cardioprotective effects. These include plant protein, dietary fiber, micronutrients such as copper and magnesium, plant sterols, and phytochemicals. Few feeding studies conducted by incorporating different nuts into the test diets to determine the effects on plasma lipids and lipoproteins revealed the total- and lipoprotein-cholesterol responses to these diets. Moreover, the actual cholesterol response was also compared with the predicted response derived from the most current predictive equations for blood cholesterol. Results from this comparison showed that when subjects consumed test diets including nuts, there was a 25% greater cholesterol-lowering response than that predicted by the equations. These results suggest that there are non–fatty acid constituents in nuts that have additional cholesterol-lowering effects. They concluded that further studies were needed to identify these constituents and establish their relative cholesterol lowering potency.

Venkatachalam and Sathe (2006) analyzed commercially important edible nut seeds for chemical composition and moisture sorption. Moisture (1.47-9.51%), protein (7.50- 21.56%), lipid (42.88-66.71%), ash (1.16-3.28%), total soluble sugars (0.55-3.96%), tannins (0.01-0.88%), and phytate (0.15-0.35%) contents varied considerably. They reported that regardless of the seed type, lipids were mainly composed of mono- and polyunsaturated fatty acids (>75% of the total lipids). Fatty acid composition analysis indicated that oleic acid C18:1) was the main constituent of monounsaturated lipids in all seed samples. With the exception of macadamia, linoleic acid (C18:2) was the major polyunsaturated fatty acid. In the case of walnuts, in addition to linoleic acid (59.79%) linolenic acid (C18:3) also significantly contributed toward the total polyunsaturated lipids. Amino acid composition analysis indicated lysine (Brazil nut, cashew nut, hazelnut, pine nut, and walnut), sulfur amino acids methionine and cysteine (almond), tryptophan (macadamia, pecan), and threonine (peanut) to be the first limiting amino acid as compared to human (2-5 year old) amino acid requirements. The amino acid composition of the seeds was characterized by the dominance of hydrophobic (range) 37.16-44.54%) and acidic (27.95-33.17%) amino acids followed by basic (16.16-21.17%) and hydrophilic (8.48-11.74%) amino acids. Trypsin inhibitory activity, hemagglutinating activity, and proteolytic activity were not detected in the nut seed samples analyzed. Sorption isotherms (a_w range) 0.08-0.97) indicated a narrow range for monolayer water content (11-29 mg/g

of dry matter). No visible mold growth was evident on any of the samples stored at a_w < 0.53 and 25 ℃ for 6 months.

Pasupathy *et al*., (2011) stated that walnuts were an important source of monounsaturated fats-approximately 15% of the fat found in walnuts was healthful monounsaturated fat. In addition to their heart-protective monounsaturated fats, walnuts' concentration of omega-3 essential fatty acids is also responsible for the favorable effects walnut consumption produces on cardiovascular risk factors. Omega-3s benefit the cardiovascular system by helping to prevent erratic heart rhythms, making blood less likely to clot inside arteries (which is the proximate cause of most heart attacks), and improving the ratio of good (HDL) cholesterol to potentially harmful (LDL) cholesterol. Omega-3s also reduce inflammation, which is a key component in the processes that turn cholesterol into artery-clogging plaques.

Spiller *et al*., (1998) compared the lipid-altering effects of an almond-based diet with an olive oil-based diet, against a cheese and butter-based control diet. Forty-five free-living hyperlipidemic men (n512) and women (n533) with a mean plasma total cholesterol (TC) of 251630 mg/dL followed one of three diets; almond-based, olive oil-based, or dairy-based for 4 weeks. Total fat in each diet was matched, and the study-provided sources of fat comprised the major portion of fat intake. Reductions in TC and low-density lipoprotein-cholesterol (LDL-C) between the three groups were significantly different from the almond group (both p,0.001). Within group analysis revealed that the almond-based diet induced significant reductions in TC (p,0.05), LDL-C (p,0.001), and the TC:HDL ratio (p,0.001), while HDL-C levels were preserved. TC and HDL-C in the control diet were significantly increased from baseline (both p,0.05), while the olive oil-based diet resulted in no significant changes over the study period. Weight did not change significantly. Results suggested that the more favorable lipid-altering effects induced by the almond group might be due to interactive or additive effects of the numerous bioactive constituents found in almonds.

Joice *et al*., (2008) stated that increased consumption of plant foods high in dietary fibre and phytochemicals was associated with a reduced risk of obesity, heart disease, diabetes, and some forms of cancer. As part of a healthful diet and lifestyle, almond consumption promotes satiety, serum cholesterol reduction, and blood sugar control by serving as a good source of monounsaturated fat, dietary fibre, phytochemicals, and

vitamin E. Awareness of the health benefits of whole almonds and their components could provide additional appeal for consumers and add value to food manufacturers reformulating products to provide higher levels of fibre and other important nutrients.

Etherton *et al*., (2007) cited epidemiologic and clinical trial evidence that demonstrated consistent benefits of nut and peanut consumption on coronary heart disease (CHD) risk and associated risk factors. They also stated that the epidemiologic studies had reported various endpoints, including fatal CHD, total CHD death, total CHD, and nonfatal myocardial infarct. A pooled analysis of 4 U.S. epidemiologic studies showed that subjects in the highest intake group for nut consumption had 35% reduced risk of CHD incidence. The reduction in total CHD death was due primarily to a decrease in sudden cardiac death. Clinical studies have evaluated the effects of many different nuts and peanuts on lipids, lipoproteins, and various CHD risk factors, including oxidation, inflammation, and vascular reactivity. Evidence from these studies consistently shows a beneficial effect on these CHD risk factors. The LDL cholesterol-lowering response of nut and peanut studies is greater than expected on the basis of blood cholesterol-lowering equations that are derived from changes in the fatty acid profile of the diet. Thus, in addition to a favorable fatty acid profile, nuts and peanuts contain other bioactive compounds that explain their multiple cardiovascular benefits. Other macronutrients include plant protein and fiber; micronutrients including potassium, calcium, magnesium, and tocopherols; and phytochemicals such as phytosterols, phenolic compounds, resveratrol, and arginine. Nuts and peanuts are food sources that are a composite of numerous cardioprotective nutrients and if routinely incorporated in a healthy diet, population risk of CHD would therefore be expected to decrease markedly.

Stephens *et al*., (2010) evaluated the effects of fat free peanut flour (FFPF), peanuts, and peanut oil on cardiovascular disease (CVD) risk factors and the development of atherosclerosis in male Syrian golden hamsters. Each experimental diet group was fed a high fat, high cholesterol diet with various peanut components (FFPF, peanut oil, or peanuts) substituted for similar metabolic components in the control diet. Tissues were collected atweek 0, 12, 18, and 24. Total plasma cholesterol (TPC), LDL-C, andHDL-C distributionswere determined by high-performance gel filtration chromatography, while aortic total cholesterol (TC) and cholesteryl ester (CE) were determined by gas liquid chromatography. Peanuts, peanut oil, and FFPF diet groups had significantly lower ($P <$

0.05) TPC, non-HDL-C than the control group beginning at about 12 wk and continuing through the 24-wk study. HDL-C was not significantly different among the diet groups. Peanut and peanut component diets retarded an increase in TC and CE. Because CE is an indicator of the development of atherosclerosis, this study demonstrated that peanuts, peanut oil, and FFPF retarded the development of atherosclerosis in animals consuming an atherosclerosis inducing diet.

MATERIALS AND METHODS

3.1. Source of Materials

3.1.1. Mutton

Mutton was procured from experimental abattoir of Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar. It was packed in clean polyethylene bags and brought to the laboratory. The meat cuts were deboned manually, packed in clean polyethylene bags and frozen at -18 ± 1^0C until use.

3.1.2. Condiments

Onion and garlic were procured from local market of Bareilly. To prepare condiment mix, onion and garlic were peeled off, cut into small pieces and homogenized separately in a grinder to obtain a fine paste. For preparation of mutton nuggets onion and garlic were used in the ratio 3:1.

3.1.3. Chemicals and other ingredients

All the chemicals used were of analytical grade and obtained from standard firms (Qualigen, Hi-Media, Sdefine etc.). Refined salt (Tata Chemicals Ltd., Mumbai), refined wheat flour (maida), cabbage, peanuts, almond and pine nut were purchased from local market.

3.1.4. Packaging materials

Low density polyethylene (LDPE) pouches (200 gauge) were purchased from local market and nylon barrier pouches (150 gauge) in natural colour were procured from M/s Hitkari Industries Ltd., New Delhi-14.

3.1.5. Spices mix

All the spices were purchased from local market of Bareilly. After removal of extraneous materials, the spices were oven dried at 60°C for overnight and were ground mechanically to powder. The coarse particles were strained out by using a sieve (100 mesh). The powder so obtained was mixed in required proportion to obtain spice mix. The spices mix was stored in plastic container for subsequent use. The spice mix was prepared for mutton nuggets as per given formulation:

Table- 1 Composition of spices mix

Ingredients	Percentage (w/w)
Cumin (Zeera)	15
Coriander (Dhania)	20
Aniseed (Soanf)	10
Black pepper (Kalimirch)	7
Caraway (Ajwain)	10
Capsicum (Mirch powder)	12
Cardamom (badi Elaichi)	5
Dried ginger (Saundh)	5
Cinnamon (Dalchini)	5
Clove (Laung)	2
Bay leave (Tej pat)	3
Nutmeg (Jaifal)	3
Mace (Javitri)	3
Total	**100**

3.1.6. Food ingredients: Peanut, almonds and pine nuts were purchased from local market. Nuts were subjected to drying at 60^0C in hot air oven for 4-6 hrs to rule out the seasonal moisture variation.

3.2. Preparation of Premium Mutton Nuggets

Suitable transformation technique for nut paste viz: peanut, almond and pine nuts were standardized based on sensory evaluation under preliminary trials. Proximate composition of various nut paste viz: peanut, almond and pine nuts were determined and with the aim to substitute the additionally incorporated animal fat in traditional emulsion preparation by 50, 75 and 100%, efficacy of each nut paste for their functionality were evaluated at three levels. After rounding off the figure for fat content of various nut pastes and keeping in view the aim of substitution as cited, the levels of incorporation for peanut and almond were set as 10, 15 and 20%, while for pine nut paste these were 8,12 and 16%. The cited levels were used in further experiments to determine the optimum level of incorporation of functionally rich nut paste viz;, peanut, almond and pine nuts for the preparation of premium mutton nuggets.

Table 2: Formulation of premium mutton nuggets enriched with nut based functional components

Ingredients (%)	**Control**	**Peanut paste (Fat≈50% Exp-2a)**			**Almond paste (Fat≈50% Exp-2b)**			**Pine nut paste (Fat≈62.5% Exp-2c)**		
		T_1	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**
Lean meat	70	65	62.5	60	65	62.5	60	67	65.5	64
Nut Paste	0	10	15	20	10	15	20	8	12	16
Ice flakes	10	10	10	10	10	10	10	10	10	10
Sodium chloride	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Sodium nitrite (ppm)	150	150	150	150	150	150	150	150	150	150
STPP	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Animal Fat	10	5	2.5	0	5	2.5	0	10	5	2.5
Refined wheat flour	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3
Condiment mix	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Spice mix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

(Substitution of animal fat at the level of 50%, 75% and 100% in T_1, T_2 and T_3 respectively on the inherent fat% in standardized nut paste, the extra lipid content of nut paste was adjusted by replacing lean on constant weight basis)

3.2.1. Formulation of mutton nuggets

3.2.2. Processing of mutton nuggets

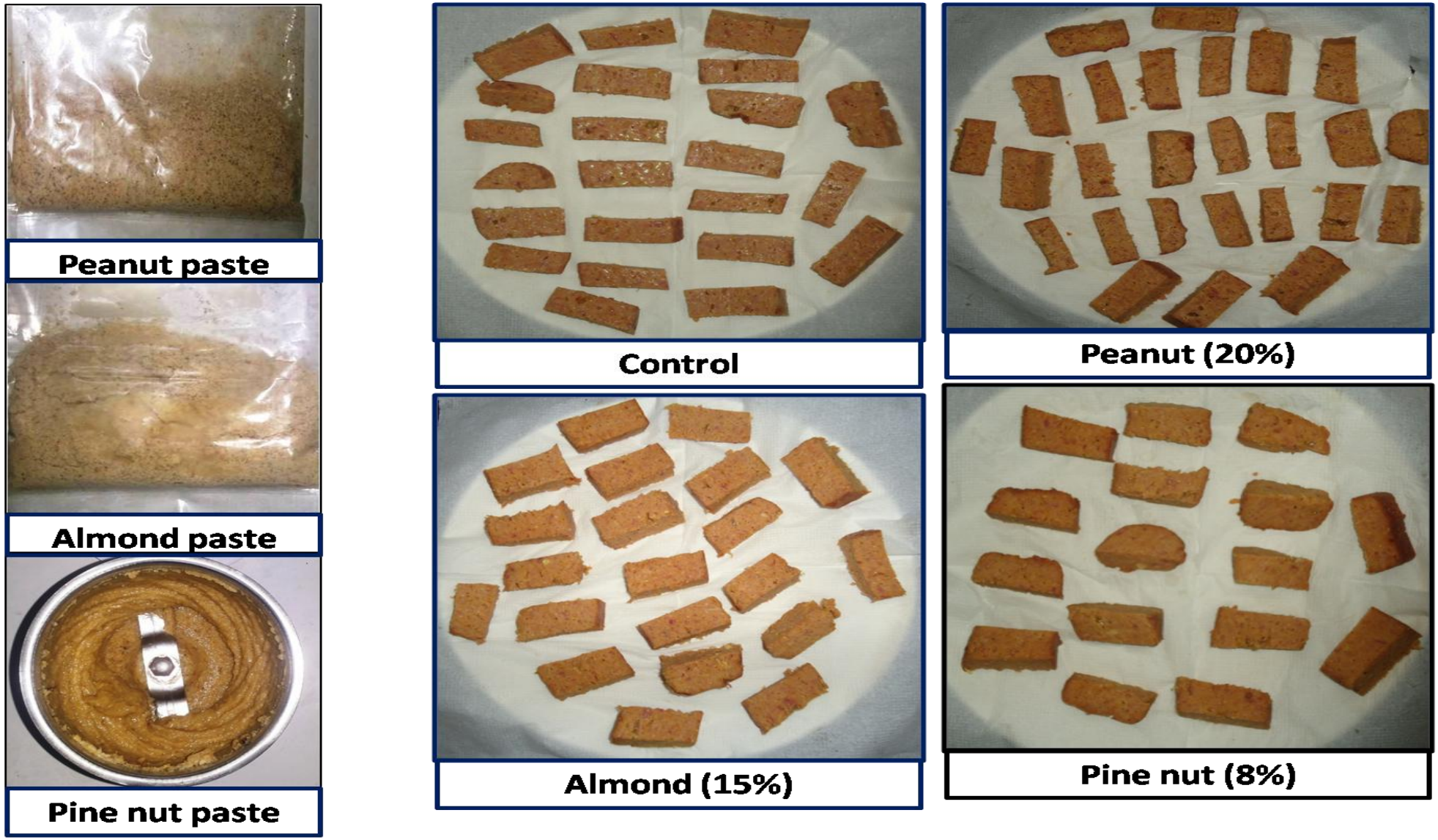
Mutton was partially thawed overnight, cut into small cubes and double minced with Electrolux meat mincer. Meat emulsion was prepared in a bowl chopper (Seydelmann K20, Ras, Germany). Pre-weighed quantity of minced mutton, salt, sodium tripolyphosphate, and sodium nitrite were added and chopped for about 2-3 minutes. It was chopped again for 2 minutes after the addition of ice flakes. Animal fat was slowly incorporated while chopping till it was completely dispersed in the batter. Refined wheat flour, condiment paste, dry spice mix, and other ingredients viz: peanut/almond/pine nut paste were added. Chopping was continued till uniform dispersion of all the ingredients and desired consistency of the emulsion was achieved. Weighed quantity of emulsion was taken and filled in stainless steel mould. Mould was covered with lid and tied with thread and steam cooked for 35 minutes. Core temperature of cooked blocks was recorded by using probe thermometer that should reach to 72° C. Mutton meat block so obtained was sliced and cut into pieces to get nuggets.

3.3. Experimental details

Experiment 1: Standardization of the suitable transformation technique for nuts paste and determination of proximate composition of various nut pastes viz; almond, pea nuts and pine nuts.

In the preliminary trials number of transformation techniques viz: soaking of dried nuts in water overnight and grinding, direct pan frying and then grinding, microwave treatment then grinding, sand heating and then grinding, direct grinding and their various combination-permutation were attempted. The resultant pastes were incorporated to the control mutton nuggets formulation at uniform levels and sensory evaluation of the products were made to determine the most acceptable transformation techniques for various nut pastes.

The nut pastes which were found most preferred by sensory panelists in preliminary trials were subjected to determination of their crude fat and protein content (AOAC, 1995). On the basis of crude fat content of nut pastes, levels of substitution with aim to replace added animal fat in traditional emulsion by 50, 75 and 100% were set for evaluation in further experiments.



Fig–1– Nut pastes and Premium mutton nuggets enriched with nut based functional components

3.2.3. Flow diagram for preparation of premium mutton nuggets

Frozen mutton at -18±1°C

↓

Thawing the mutton at 4±1°C for 24 hrs

↓

Mincing through 8 mm sieve plate

↓

Chopping in bowl chopper with salt, STPP and sodium nitrite with ice flakes

↓

Addition of animal fat

↓

Chopping again (2-3 minutes)

↓

Addition of refined wheat flour, spices, condiments and other ingredients *viz:* peanut/almond/pine nut paste

↓

Chopping till uniform dough formation

↓

Filling into stainless steel mould

↓

Steam cooking for 35 min

↓

Cooling to ambient temperature, slicing, cutting, packaging and storage at 4±1°C

↓

Sensory evaluation and sample analysis

Experiment 2: Optimization of the level of incorporation of functionally rich nut paste viz; peanut, almond and pine nuts for the preparation of premium mutton nuggets

Based on the proximate composition of each paste, mainly the fat content, the three levels of incorporation were determined with the aim to substitute the additionally incorporated animal fat in traditional emulsion preparation approximately by 50, 75 and 100%. Constituents of nuts paste also replaced partial amount of meat in contrast to control group on constant weight basis. Detail formulations of various categories of products are given in Table-1. After making the substitution with nut paste, the treatment products were subjected to evaluation for the physico-chemical parameters viz: pH, emulsion stability, cooking yield (%), shear force value, proximate parameters such as moisture content, protein, crude fat content and ash, and detailed sensory analysis for various sensory parameters namely general appearance, flavour, juiciness, texture and overall acceptability on 8-point descriptive scale where, 8 = extremely liked and 1 = extremely disliked to determine their optimum level of incorporation. On the basis of sensory scores and product yield optimal level of incorporation were adjudged for each nut paste.

Experiment 3: Assessment of the efficacy of nut's paste viz: peanut, almond and pine nuts at their optimum level of incorporation for their functional components by determining the detailed product profile of premium mutton nuggets

Nut based functionally enriched premium mutton nuggets with respective level of optimum incorporation, evolved from the earlier experiments, were analyzed for detailed profile viz: Lipid profile parameters such as total lipids, total cholesterol, total phospholipid, total glycolipids, total free fatty acids and total glycerides, calorific value, anti-oxidant capacity such as total phenolic content, DPPH radical-scavenging activity, and reducing power assay, total dietary fiber and detailed texture profile.

Experiment 4: Determination of the storage stability of premium mutton nuggets at refrigerated storage (4 ± 1^{o}C).

The storage studies were conducted on the nut based functionally rich premium mutton nuggets with selected levels of incorporation at refrigeration (4 ± 1^{o}C).The product were

1. Aerobically packaged in low-density polyethylene film (200 gauge) and stored at refrigerated temperature (4±1°C) for 15 days. The stored samples were analyzed at an interval of 5 days.

2. Vacuum packaged in nylon barrier films and stored at refrigerated temperature (4±1°C) for 60 days. The stored samples were analyzed at regular interval of 15 days.

The product were analysed for physico-chemical parameters such as pH, thiobarbituric acid reacting substance value, free fatty acid and peroxide value, instrumental colour analysis parameters for redness, yellowness, hue and chroma and microbiological quality viz: standard plate count, psychrophilic count and coliform count and sensory analysis (general appearance, flavour, texture, binding, juiciness and overall acceptability). In case of vacuum packaged products anaerobic count, lactic acid bacterial counts were also done.

Experiment 5: Estimation of production cost of the developed premium mutton nuggets

Production cost of nut based functionally rich premium mutton nuggets with selected levels of incorporation were determined and compared with control.

3.4 Analytical Procedure:

3.4.1 pH

The pH of emulsion as well as mutton nuggets was determined (Trout *et al*., 1992) by combination electrode digital pH meter (model CP 901, century Instrument Ltd. India), 10 gm of sample was homogenized with the help of ultra turrax tissue homogenizer (T-25 Germany) for about a minute in 50 ml of distilled water. pH was recorded by immersing the electrode directly into the meat suspension.

3.4.2 Emulsion stability

The emulsion stability was determined as per the method of Townsend *et al*., (1968) with some modifications. About 25 gm each of emulsion was placed in polyethylene bags and heated at 80°C in a thermostatically controlled water bath for 20 minutes. After cooling and draining the exudate, the cooked emulsion mass was weighed and yield was expressed as emulsion stability in percentage.

3.4.3 Cooking yield

Weights of raw and cooked mutton nuggets were recorded. The percent cooking yield for each mutton nuggets was calculated as follow.

$$\textbf{Cooking yield (\%)} = \frac{\text{Wt. of cooked mutton nuggets}}{\text{Wt. of raw dough}} \times 100$$

3.4.4 Proximate composition

The moisture, protein, fat and ash content of mutton nuggets were determined by standard methods using hot air oven, Kjeldahl assembly, Soxhlet extraction apparatus, Muffle furnace respectively as per AOAC (1995).

3.4.4.1 Moisture content

10 gm of mashed sample was transferred in pre-weighed flat bottom aluminum moisture cup, which was transferred to hot air oven at 101±1ºC and kept for 16-18 hrs. Dried sample was then placed in desiccator having silica gel as desiccant. After 1 hr, the cup containing dried sample was weighed. Moisture content was calculated by applying the following formula:

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1 = weight of empty cup

W_2 = weight of cup + sample

W_3 = weight of cup + dried sample

3.4.4.2 Protein content

The sample (2-2.2 gm) was digested using Micro- Kjeldahl digester in presence of digestion mixture which acts as catalyst (sodium sulfate/potassium sulfate: copper sulfate = 5:1) and 40 ml sulfuric acid. Flask was placed in an inclined position and heated gently until frothing ceases, then boiled rapidly until solution became clear. The sample was then cooled and distilled water was added to make the volume upto 250 ml. The diluted sample (10 ml) was distilled with 10 ml of 40% NaOH using Micro- Kjeldahl distillation unit. Steam was distilled over 2% boric acid (25 ml) containing mixed indicator (1 part 0.2% methyl red + 2 part 0.2% bromocresol green dye) for 30 min. The ammonia trapped in boric acid was determined by titrating with 0.1 N sulfuric acid. The nitrogen percentage was calculated using the following formula:

$$\textbf{Nitrogen (\%)} = \frac{(A - B) \times 0.0014 \times \text{Total volume made}}{\text{Weight of sample taken} \times \text{Volume of distillate}} \times 100$$

Where, A = Titrated value for sample

B = Titrated value for blank

Protein percentage was determined by conversion of nitrogen percentage to protein by using conversion factor (6.25) assuming that all the nitrogen in meat was present as protein i.e**. protein percentage = N% x 6.25.**

3.4.4.3 Fat content

Fat Content in the sample was extracted in Soxhlet extraction unit. Soxhlet Extractor was set with reflux condenser and oil flask which was previously dried and weighed. Meat sample (3-4 gm) was taken into fat free extraction thimble, dried in oven for 6 hr at 100-102 ºC and placed in Soxhlet extraction apparatus. 150 ml of petroleum ether (BP: 60-80ºC) was then poured into extraction flask and condenser was joined and placed on electric heater in order to boil the solvent gently. Extraction was carried out for 16 hrs. Fat content was calculated by using the following formula:

$$\textbf{Fat (\%)} = \frac{W2 - W1}{W3} \times 100$$

Where, W1 = weight of empty oil flask

W2 = weight of oil flask + Fat

W3 = weight of sample taken

3.4.4.4 Ash percentage:

The fresh minced sample (5-10 gm) was transferred in a pre-weighed crucible and transferred to Muffle furnace at (550°C) for 4-5 hr. ashed sample was transferred to desiccator having silica gel as desiccant. After 1 hr, the crucible was weighed. The ash content was calculated by the following formula:

$$\textbf{Ash (\%)} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

3.4.4.5. Moisture protein ratio

It was a derived value and calculated by simply dividing the moisture content by protein content.

3.4.4.6. Carbohydrate percentage

It was a derived value and was calculated by subtracting the sum of moisture, protein, fat and ash from 100.

Carbohydrate % = 100-(Moisture % + Protein% + Fat% +Ash%)

3.4. 5 Shear force value

Shear force value was determined as per the method described by Berry and Stiffler (1981). It is measured as force required for shearing 1 cm square block on Warner-Bratzler Shear Press (81031307 GR Elec. MFG. Co. USA) and expressed in kg/cm^2.

3.4.6 Total dietary fibre

Total Dietary Fibre (TDF), Soluble Dietary Fibre (SDF) and Insoluble Dietary Fibre (IDF) were determined by slight modification of an enzymatic method given by Furda (1981).

3.4.6.1 Extraction of water soluble material

Defatted product sample (2 gm) was dispersed in 200 ml of 0.005 N HCl and boiled for 20 minutes. The suspension was cooled to 60^0C, 0.3 gm disodium EDTA was added and pH was adjusted to 6.0-6.3 with 0.005 N NaOH. To this solution, phosphate buffer (12 ml, pH 6.0-6.5) was added and the interaction was continued for 40 minutes at 60^0C to ensure interaction of pectin with minimum degradation.

3.4.6.2 Starch and protein hydrolysis

pH of solution was adjusted to 6.0-6.5 for optimum amylase and protease activity. The suspension was then cooled to 20-30^0C before incubating over night with 10 mg of bacterial-amylase and 10 mg of bacterial protease. The incubation was accompanied by slow stirring with magnetic bar.

3.6.3 Isolation of IDF

The suspension was filtered through a coarse tarred gooch filtering crucible containing glass wool and the residue was washed with small amount of water. The filtrate was saved for next step. This residue was then washed with water, alcohol and acetone before being dried at 70^0C in vacuum, overnight. This dried residue constituted IDF.

3.6.4 Precipitation and isolation of SDF

The saved filtrate was acidified with a few drops of conc. HCl to bring the pH to 2-3. This pH tends to facilitate the rapid precipitation of polysaccharides. To this, four volumes of ethanol were added slowly and the suspension was kept as such for about one hour. The precipitate was filtered through a tared, coarse Gooch crucible containing glass wool and then washed with 75% ethanol, absolute ethanol and acetone before drying at 70^0C in a vacuum oven overnight. The residue was weighed to give the SDF content.

3.4.6.5 TDF

TDF was calculated by adding IDF and SDF contents.

3.4.7 Calorific value

2 gm sample was ignited electrically burnt in excess of oxygen in the bomb calorimeter. The maximum temperature rise of bomb calorimeter was measured with the thermocouple and the galvanometer system. By comparing this rise with that obtained when a sample of known calorific value is burnt, the calorific value of the sample material could be determined.

Gross energy of samples was determined by Gallenkamp and Ballistic Bomb Calorimeter (Haque and Murali Lal, 1999). Approximately 1-2 single piece of meat nuggets sample was taken and weighed along with pre-weighed steel crucible. This crucible was placed on the support pillar in the base of the bomb. The firing wire and the sample were connected with the help of cotton thread. The bomb was fired under an oxygen pressure of 25 atmosphere. The initial and final temperature readings on the galvanometer were noted. The deflection on the galvanometer was compared with 1 gram standard Benzoic acid of known calorific value (6.318 kcal/g). The calorific value of the sample was calculated and expressed as kcal/g.

3.4.8. Antioxidant capacity

3.4.8.1 Determination of Total Phenolics by F-C Method:

3.4.8.1.1 Principle

Folin-Ciocalteau (F-C) colorimetry is based on a chemical reduction by transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid of the F-C reagent; the products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 760 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols.

3.4.8.1.2.Extraction of phenolics from mutton nuggets

5 gm sample of mutton nuggets was taken in a beaker of 100 ml capacity. 25 ml of aqueous ice cold 70% acetone was added and subjected to homogenization for 60s in Ultra Turrax T25 tissue homogenizer (Janke and Kenkel IKA Labortechnik, Germany) and then the beaker was wrapped with aluminium foil and kept overnight for extraction at refrigeration temperature 4±1°C (Naveena *et al*., 2008).

Table-3. Preparation of Calibration Curve Using Standard Tannic Acid (TA)

Stock standard (Tannic acid) concentration - 0.1mg/ml

Test tube	Final Conc. of TA (µg/ml)	Stock TA 0.1mg/ml (µl)	Distilled water (ml)	F-C reagent (ml)	Sodium carbonate solution (ml)	Final volume (ml)	Vortexed and kept at room temp for 40 mints then recorded at 760 nm
Blank	0	0	0.50	0.25	1.25	2.0	
T1	2	20	0.48	0.25	1.25	2.0	
T2	4	40	0.46	0.25	1.25	2.0	
T3	6	60	0.44	0.25	1.25	2.0	
T4	8	80	0.42	0.25	1.25	2.0	
T5	10	100	0.40	0.25	1.25	2.0	

Calibration curve was drawn and the equation was calculated in Microsoft Excel 2007 spread sheet. The linear correlation between standard concentration and absorbance was expressed with the equation y=f(x) and r^2 value. Where, y = absorbance, x = standard concentration (µg/ml) and r^2 = correlation coefficient.

3.4.8.1.3. Total Phenolics

Total phenolic content in phyto-ingredients powder and mutton nuggets were quantified using the Folin-Ciocalteu colorimetric method as described by Makkar (2000). Suitable aliquots of the extracts were taken in test tubes, and the volume was made upto 0.5 ml with distilled water and 0.25 ml Folin-Ciocalteu (1N) reagent was added and then the reaction was neutralized by addition with 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance of the resulting blue colour was measured using *BECKMAN DU-640 UV/Vis* Spectrophotometer at 760 nm against blank after incubation for 40 min at room temperature. From the standard calibration curve equation y=f(x), quantification of phenolics was done and expressed as mg tannic acid equivalents per gm of sample.

3.4.8.2 Reducing power assay

3.4.8.2.1 Principle

The reducing power was determined by the method of Oyaizu (1986)**.** Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride in acidic medium to form ferric

ferrous complex. Therefore, depending on the reducing power of the test compounds, the yellow colour of the test solution changes to various shades of green or blue (Amarowicz *et.al*., 2004) that has an absorption maximum at 700 nm. The reducing power of the extract is linearly proportional to the concentration of the sample.

3.4.8.2.2 Procedure

Suitable aliquots of the extracts containing 50–100 µg phenolics from phyto-ingredients and mutton nuggets were taken in test tubes, and the volume was made equal with acetone (70%) and mixed with 2.5 ml phosphate buffer (200 mM, pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v).

This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 5000 rpm for 10 min in REMI research centrifuge. The upper layer of the solution (2.5 ml) was mixed with (2.5 ml) distilled water and 0.5 ml of freshly prepared ferric chloride (0.1% w/v) solution. The absorbance was measured using BECKMAN DU-640 UV/Vis Spectrophotometer at 700 nm against blank without any extracts and 0.1% ferric chloride. Increase in absorbance of the reaction mixture indicated the reducing power of the sample.

3.4.8.3 DPPH radical scavenging activity

The ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by phyto-ingredients and mutton nuggets was estimated by the method of Singh *et al*., (2002). Different concentrations (50 and 100 µL equivalent to 50 and 100 ppm) of phyto-ingredients and mutton nuggets were taken in different test tubes. The volume was adjusted to 100 µL by adding MeOH. 5 ml of a 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm using a BECKMAN DU-640 *UV-VIS* spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\textbf{\% radical scavenging activity} = \frac{\text{control OD - sample OD}}{\text{Control OD.}} \times 100$$

3.4.9. Lipid profile

3.4.9.1. Extraction of lipids

The method of Folch *et al.,* (1957) was used to extract lipids from the mutton nuggets samples. 5 g of sample was taken and ground using mortar and pestle with 20 volumes (ml) of solvent mixture comprising of chloroform : methanol (2:1 v/v). The contents were allowed to stand at room temperature with occasional stirring for 6 to 8 hours. The extract was filtered through Whatman No. 1 filter paper and residue was re-extracted with 10 more volumes of the same solvent mixture for two hours and filtered. The filtrate were combined and evaporated to dryness *in vacuo* at 55-60°C in rotary evaporator and this process was repeated three times. For breaking the proteolipids, dried lipid residue was dissolved in one tenth volume of the original lipid extract in chloroform: methanol: water (64: 32: 4 v/v/v) and evaporated to dryness *in vacuo* at 55°C. This step was repeated twice and dried lipid residue was dissolved and filtered quantitatively into a separatory funnel with 100 ml of chloroform: methanol (2:1 v/v). The lipid extract was then washed with one fifth volume of 0.9 per cent sodium chloride solution (mix 20 ml of normal saline and shake vigorously) so as to remove non-lipid impurities from the lipid sample. It was then allowed to stand overnight at room temperature. The chloroform layer was collected and evaporated to dryness *in vacuo* at 55-60°C and repeat the process 3-4 times and finally volume was made to 5 ml with chloroform. After adding a drop of 0.5 per cent butylated hydroxy toluene (BHT in chloroform), the lipid samples were stored in glass stoppered test tubes at -18°C till further analysis.

3.4.9.2. Assay of total lipids

The total lipids of the samples were determined gravimetrically by the method described by Bligh and Dyer (1959). One ml of aliquot of the lipid extract was pipetted into dried stainless steel planchets with constant predermined weights. The samples were then dried at 60°C in a hot air oven to a constant weight. Total lipids were expressed as mg/g of sample.

3.4.9.3. Assay of total phospholipids

Total phospholipids were estimated by determining the phosphorus content of the lipid extract by converting into inorganic phosphorus. Method described by Barlett (1959) and modified by Marinetti (1962) was used for the estimation of phospholipids in the present study.

Twenty five micro litre lipid extract and standard phosphate solution (25, 50, 75 and 100 µl) containing 0.4 mg/5 ml potassium dihydrogen phosphate (the standard was prepared by dissolving 0.351 g potassium dihydrogen phosphate in 10 ml 10N H_2SO_4 and made the volume to 1 litre) were digested with 1 ml of 60 per cent perchloric acid (for digestion heat sample at 60-65°C and colour changes gradually from black, brick and lastly colourless). Blank was prepared with perchloric acid (1 ml) only and treated in similar fashion. After digestion, colour was developed by heating in boiling water bath after the addition of 7 ml of distilled water, 0.5 ml of 2.5 per cent ammonium molybdate and 0.2 ml of ANS reagent (the ANS reagent was prepared by dissolving 0.5 aminonaphthal-sulphonic acid in 200 ml of 15 per cent anhydrous sodium sulphite. The reagent is stable for one week when stored at 4ºC in brown bottle). The molybdenum blue formed was measured by reading the optical density at 830 nm in a spectrophotometer (Elico, Scanning minispec SL 117). The phospholipid content was arrived at by multiplying the inorganic phosphorus content with the factor of 25 and expressed as mg per g of tissue.

3.4.9.4. Assay of total cholesterol

Total cholesterol in the lipid extracts was determined by adopting the Tschugaeff reaction as modified by Hanel and Dam (1955).Fifty micro-litre of lipid extract and standard cholesterol solution (1 mg in 1 ml Chloroform were evaporated to dryness and dissolved in 2 ml of chloroform to which 1 ml of $ZnCl_2$ reagent (reagent was prepared by dissolving 40 g anhydrous zinc chloride in 153 ml glacial acetic acid at 80 ºC for two hours and filtered through Whatman No.1 filter paper) and 1 ml of acetyl chloride were added and heated in a water bath at 60ºC for 10 minutes. Blank containing 2 ml of chloroform and 1 ml of each zinc chloride and acetyl chloride was run at the same time. The colour complex developed was measured by reading the optical density at 528 nm in a spectrophotometer (Elico, Scanning minispec SL 117) and expressed as mg per g of tissue.

3.4.9.5. Assay of glycolipids

Total glycolipids were estimated by determining the hexose (galactose) content of the lipid extract. In the present study a method of Roughan and Batt (1968) was followed for the estimation of galactose in lipid extracts.

The galactose standard solutions of 20, 40, 60, 80 µl of concentration 1 mg/1ml of distilled water was taken in test tubes. One ml of 2 per cent phenol along with 4 ml of concentrated H_2SO_4 was added. Orange colour developed was measured at 480 nm after

cooling to room temperature for 15 minutes. The standard curve was plotted, taking concentration on X-axis and O. D. on Y-axis.

One ml of lipid extract was evaporated to dryness and hydrolyzed with 2 ml of 2N H_2SO_4 for 2 hours at room temperature. After hydrolysis, 4 ml of chloroform was added and mixture was centrifuged. To 1 ml of aqueous layer, 1 ml of 2% phenol was added followed by 4 ml of concentrated H_2SO_4. The orange colour developed was measured at 480 nm in a spectrophotometer (Elico, Scanning minispec SL 117). The concentration of galactose of the lipid was calculated from the standard curve. This was multiplied by 4.45 to estimate the glycolipid content and expressed as mg/g of sample.

3.4.9.6. Assay of free fatty acids

For the determination of free fatty acids, the method described by Koniecko (1979) was followed. Exactly 5 g of mutton nuggets was blended for 2 minutes with 30 ml of chloroform in the presence of about 5 g of anhydrous sodium sulphate. Then it was filtered through Whatman No.1 filter paper into a 150 ml conical flask. About 2 to 3 drops of 0.2% phenolphthalein indicator were added to the chloroform extract, which was titrated against 0.1N alcoholic potassium hydroxide to get the pink colour end point. The quantity of potassium hydroxide consumed during titration was recorded. Per cent free fatty acid was calculated as follows and was also taken as absolute values in lipid profile (Same sample wt).

Free fatty acids (% oleic acid) = $\frac{(0.1 \times \text{ml 0.1N 90\% alcoholic KOH} \times 0.282) \times 100}{\text{Wt. of sample}}$

3.4.9.7. Total glycerides

The total glycerides were indirectly calculated by subtracting the sum of total lipids, total cholesterol, total glycolipids and total free fatty acids from the total lipid values.

3.4.10. Texture profile analysis

The texture profile of mutton nuggets was measured with the help of instrumental texture profile analyser (TA HD Plus Texture analyser). The procedure used for instrumental texture profile analysis was similar to those described by Bourne (1978). Chilled samples were tempered to bring to room temperature and were cut into 1cm squares. The samples were placed on a platform in a fixture and compressed twice to 85% of their original height by a compression probe (P75) at a cross head speed of 10 mm/s through a two cycle sequence, using a 50 kg load cell.

Other conditions (test descriptions) set for analyses were:

1. Pretest speed : 1 mm/sec
2. Test speed : 5 mm/sec
3. Posttest speed : 5 mm/sec
4. Target mode-strain : 75%
5. Time : 5secs
6. Trigger type : Auto (Force)
7. Trigger force : 0.04903 N
8. Tare mode : Auto
9. Probe : P /75 75mm compression plating

The calculation of TPA values was obtained by graphing a curve using force and time plots. Results obtained were interpreted as follows:

Table-4: Interpretation for texture profile parameters

Parameters	Method of curve interpretation (Bourne, 1978)	Sensory description (Chen and Trout,1991)
Hardness (N/cm^2 or gm/mm^2)	Peak force of the first compression cycle	Amount of force required to bite through sample
Adhesiveness (Ns/gms)	Negative area under the baseline between the compression cycles	Work necessary to pull the compressing plunger away from sample
Springiness (cm/mm)	Ratio of the time duration of the second compression to that of the first compression	The degree to which sample deforms before shearing
Cohesiveness	Ratio of the positive force area during the second compression to that of the first compression	The previewed degree with which the sample returned to the original height and thickness after pressing five times
Gumminess (N/cm^2 or gm/mm^2)	Breaking force multiplied by cohesiveness	Degree to which sample sticks to mouth/cheek
Chewiness (N/cm or gm/mm)	Hardness multiplied by cohesiveness multiplied by springiness	Number of chews required to prepare sample for swallowing

3.4.11. TBARS value

The TBARS value of mutton nuggets was determined by using the distillation method described by Tarladgis *et al.,* (1960). 10 gm of sample was homogenized with 50 ml distilled water using homogenizer for 2 minutes. The slurry was quantitatively transferred to a 500 ml Kjeldahl flask which was then rinsed with 45ml of distilled water and washings were transferred to the flask to which 5ml of 6N HCl was added. Few drops of liquid paraffin and glass beads were added to prevent frothing and bumping respectively, during heating. The flask was heated to high temperature and 50 ml distillate was collected in a graduated stopper glass cylinder.

The distillate was thoroughly mixed and 5 ml of distillate was pipetted in duplicate into 20 ml glass stopper test tubes. 5 ml of TBA (0.02M 2-thiobarburic acid in 90% glacial acetic acid) was added to each test tube. The contents were mixed well and immersed in boiling water bath for 30 minute. A blank consisting of 5 ml of distilled water and 5 ml of TBA reagent was similarly prepared. The tubes were cooled for 10 minute under tap water and optical density was recorded using spectrophotometer (Model: Beckman DU 640, USA) at 538 nm. The O.D. was multiplied by the factor 7.8 and TBARS value was expressed as mg malonaldehyde/kg of sample.

3.4.12 Peroxide value

Peroxide value (POV) of mutton nuggets was determined according to the AOAC International (1999). The sample (5 g) was weighed in a 250 ml glass stoppered Erlenmeyer flask and heated in a water bath at 6°C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered using filter paper to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into the burette. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). POV was calculated and expressed as milliequivalent peroxide per kg of sample

POV (meq/kg) =S x N/W x 1000

where S is the volume of sodium thiosulfate solution used for titration (ml), N the normality of sodium thiosulfate solution (N = 0.01), and W the sample weight (kg).

3.4.13. Instrumental colour analysis

The colour of mutton nuggets was measured using a Lovibond Tintometer (Model F, Greenwich, UK). Samples were cut with the help of scissors to the inner diameter of sample holder and secured against the viewing aperture. The sample colour was matched by adjusting the red (a*) and yellow (b*) units, while keeping the blue unit fixed at 0.1. The corresponding colour units were recorded. The hue and chroma values were determined by using the formulae, $\tan^{-1}(b/a)$ (Little, 1975) and $(a^2+b^2)^{1/2}$ (Froehlich *et al.*, 1983), respectively where a = red unit, b = yellow unit.

3.4.14. Microbiological quality

All the microbiological parameters were determined following the methods as described by APHA (1984). Readymade media (Hi-Media, India) were used for the analysis.

3.4.14.1. Preparation of serial dilutions

The stored samples of mutton nuggets were opened in laminar flow chamber sterilized by ultra-violet irradiation. About 10 gm of sample was aseptically weighed and transferred to a sterile mortar containing 90 ml of sterile 0.1% peptone water (Code No. M028S). The sample was homogenized for 2 minutes using a sterile pestle to make 10^{-1} dilution. Further dilutions were made using sterile 0.1% peptone water as diluent. To prepare 10^{-2} dilution 1 ml from 10^{-1} dilution was mixed with 9 ml of 0.1% peptone water and so on. Proper mixing in serial dilutions was ensured by vortexing the test tubes. Preparation of sample and serial dilutions were made near flame in a horizontal laminar flow apparatus (Model: YS1-188, Varco Sales, Pvt. Ltd., New Delhi) observing all possible aseptic conditions.

3.4.14.2. Total plate count (TPC)

The Plate Count Agar of amount 23.5 gm (Hi-Media Laboratories Pvt. Ltd., Mumbai) was suspended in 1000 ml of distilled water and boiled to dissolve completely and sterilized by autoclaving at 121°C and 15 lbs pressure for 15 min. The final pH of the media was adjusted to 7.0±0.2 at 25°C. Duplicate sets of sterilized petridishes were inoculated aseptically with 1 ml of aliquots from appropriate dilution. About 10-15 ml of plate count agar melted and maintained at 44-46°C was poured gently and rotated the disc clockwise and anticlockwise to mix the media uniformly. The plates were incubated at

35±2°C for 48 hrs. Plates' showing 30-300 colonies were counted. The number of colonies was multiplied by the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

3.4.14.3. Psychrophilic Count

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

3.4.14.4. Total coliforms

41.5 gm of violet red bile agar obtained from Hi-media Laboratories Pvt Ltd., Mumbai (Code:M049S) was suspended in 1000 ml of distilled water, boiled to dissolve the medium completely and cooled to 45^0C. Final pH of the medium was 7.4±0.2 at 25^0C. Precautions were taken not to autoclave the medium. One ml of suitable dilutions in duplicates was introduced into the sterile petridishes and about 20 ml molten medium was poured into the petridishes. The petridishes were incubated at 35^0C for 24 hrs. The numbers of red or purple colonies with about 0.5mm diameter surrounded by a zone of precipitated bile were counted. The average numbers of colonies were multiplied with reciprocal of the respective dilutions and expressed as $\log_{10}$ cfu/g.

3.4.14.5. Anaerobic plate count

58 gm of anaerobic agar media (hi-Media laboratories Pvt. Ltd., Mumbai) was dissolved in 1000 ml of distilled water and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Final pH of the medium was adjusted to 7.2±0.2. One ml of suitable dilutions in duplicates was introduced into the sterile petridishes and about 20 ml molten medium was poured into the petridishes. The Petridishes were put into anaerobic jar and incubated at 35±2ºC for 48 hours. The colonies counted and expressed as $\log_{10}$ cfu/g of sample.

3.4.14.6. Lactic acid bacteria count

67.2 gm of lactobacillus MRS agar media (M641) was dissolved in 1000 ml of distilled water and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Final pH of the medium was adjusted to 6.5±0.2. One ml of suitable dilutions in duplicates was introduced into the sterile petridishes and about 20 ml molten medium was poured into the petridishes. The Petridishes were put into anaerobic jar and incubated at 35±2ºC for 24 hours. The colonies counted and expressed as $\log_{10}$ cfu/g of sample.

3.4.15. Sensory evaluation

Semi-trained experienced sensory panel consisting of scientists and post graduate students of the LPT Division obliged in conducting the sensory evaluation of the product. Sensory evaluation was organized around 3.30-4.00 PM every time. The products were evaluated for appearance, flavour, texture, binding, juiciness and overall acceptability using 8-point descriptive scale (Keeton, 1983), where 8 is extremely desirable and 1 is extremely undesirable. Plain water was provided to rinse the mouth between the samples.

3.5. Statistical Analysis

Duplicate samples were taken for each parameter and three trials were conducted for each experiment, total being six observation (n=6) for consistency of the results. The results were analysed statistically for variance and least significance difference test as per Snedecor and Cochran (1989) and means were compared by using Dunkan's multiple range test (Dunkan, 1995). Statistically analysed data using SPSS software were tabulated and interpreted.

RESULTS AND DISCUSSION

The present chapter describes the results obtained from the different studies (1-5 experiments) carried out during the present investigation. The results have been presented through narration and supported with Tables (5-45). A critical analysis of the results and discussion as well as comparison has also been attempted.

4.1. Standardization of the Suitable Transformation Technique for Nuts Paste and Determination of Proximate Composition of Various Nut Pastes Viz; Almond, Pea Nuts and Pine Nuts.

Based on sensory evaluation under preliminary trials, paste from microwave sand heating (2 ½ minutes) followed by grinding was rated high for peanut, whereas for almond and pine nut pastes, direct grinding was found better. The probable reason for difference with peanut might be because of unique amino acid profile. Some amino acids such as aspartic acid, asparagine, glutamic acid, phenylalanine and histidine were shown to be the precursors of typical peanut flavors and amino acids like threonine, tyrosine, and lysine were considered to contribute to atypical peanut flavors (Newell *et al*., 1967). The microwave sand heating might have led to changes in these amino acids as well as removal of volatile flavour components. The usual consumption pattern viz: roasted, soaked or direct use, for various nuts by the members of sensory panel might also have influenced the sensory scores.

The fat and protein percentage of various nut pastes which were found most acceptable based on sensory ratings is presented in Table-5.

Table-5. Proximate composition of various nut paste used in preparation of premium mutton nuggets

Nut Pastes	Moisture (%)	Protein (%)	Fat (%)
Peanut	4.54+0.19	31.00+0.75	50.46+0.97
Almond	4.29+0.32	22.82+0.67	50.38+1.22
Pine nut	2.24+0.14	12.76+0.41	62.6+1.25

The peanut and almond paste had almost similar fat content with approx figure of 50% whereas pine nut paste had slightly higher i.e. 62.5% fat. Peanut seeds were reported

to contain 36-54% oil (Worthington and Hammons, 1971; Dwivedi *et al*., 1990; Hashim *et al*., 1993; Isleib *et al*., 2004; Yaw *et al*., 2008; Atasie *et al*., 2009) and 16-34% protein (Young and Hammons 1973; Pancholy *et al*., 1978; Jambunathan *et al*., 1985; Dwivedi *et al*., 1990; Ory *et al*., 1992; Grosso *et al*., 2000). Similarly, the fat content of almond has been reported in the range of 43.37–47.50% by Ahrens *et al*., (2005), 49.42% by USDA data base, 47.48-56.70% by Aslantas *et al*., (2001) and 48% to 67% of the total kernel dry weight by Kodad and Company, (2008). The range for protein content of almonds has been reported to be 16.42–22.17% by Sathe, (1992), 19.04-24.51% by Aslantas *et al*., (2001), 20.68–23.30% by Ahrens *et al*.,(2005) and 21.22 by USDA data base.

The fat and protein percentage of pine nuts as per USDA data base are 68.37% and 13.69% respectively, whereas, Venkatchallam and Sathe, (2006) reported the fat and protein percentage of pine nuts (*Pinus pinea*) as 61.73% and 13.08% respectively. The proximate composition of various nut pastes in our study was almost similar or in the range of the reported values.

4.2 Optimization of the Level of Incorporation of Functionally Rich Nut Paste Viz; Almond, Pea Nuts and Pine Nuts for the Preparation of Premium Mutton Nuggets

In this experiment, three different levels of each functionally enriched nut pastes were incorporated by substituting the added animal fat in traditional emulsion preparation and lean meat into the prestandardized formulation of mutton nuggets as indicated below.(Detailed formulation have been given in Table-2.

Table-6: Levels of substitution evaluated for optimization of nut pastes in premium mutton nuggets

Functionally rich nut paste (%)	Control	Treatment-I	Treatment-II	Treatment-III
Peanut	0	10	15	20
Almond	0	10	15	20
Pine nut	0	8	12	16

The control and treatment mutton nuggets were assessed for quality and acceptability on the basis of physico-chemical and sensory parameters. The results are presented through statistically analysed tables (3-14) and critically discussed and reviewed in the light of objectives.

4.2.1: Optimization of peanut paste incorporation level for the preparation of premium mutton nuggets.

In this study, peanut paste was incorporated at the level of 10, 15 and 20% by substituting the added animal fat in traditional emulsion by 50%, 75% and 100% and also replacing the lean meat (wt basis) in prestandardized mutton nuggets formulation (Table-2). Physico-chemical properties such as emulsion stability, pH of emulsion and product, cooking yield, moisture, protein, moisture protein ratio, fat, ash, calculated carbohydrates as well as shear force value and sensory attributes viz. general appearance, flavour, texture, binding, juiciness and overall acceptability were evaluated to find the optimum level of incorporation.

The physico-chemical properties of premium mutton nuggets incorporated with different levels of peanut paste are presented in Table -7 and their corresponding ANOVA is given in Table-8. The pH values of raw emulsion increased with increase in incorporation level of peanut paste and were significantly higher ($P<0.05$) at subsequent level of incorporation. This increase in pH might be attributed to replacement of lean meat (approx pH of 5.4) with comparatively high pH peanut paste (pH 6.28 -7.50; http://www.foodscience.caes.uga.edu). The emulsion stability of premium mutton nuggets also increased with increase in incorporation level and at 20% incorporation, it was significantly higher ($P< 0.05$) than control and 10% incorporated premium mutton nuggets. Premium mutton nuggets prepared with 15% peanut paste had comparable emulsion stability to both 10 and 20% levels. The emulsion stability at 10% incorporation level was comparable to control as well as 15% incorporation level. The increase in emulsion stability might be due to increase in total emulsifying protein content in the formulation after substitution. Peanut proteins have been reported to posses good emulsifying property by Khan *et al*., (1975); Beuchat., (1977); McWatters *et al*., (1976 and 1977); Xiao *et al*., (2011 and 2013).

The cooking yield of the product improved with increase in incorporation level of peanut paste and was significantly higher ($P< 0.05$) at 15% and 20% levels as compared to both control and 10% incorporation level. Product with 10% peanut paste had comparable cooking yield to control. Similarly, 15% incorporation level had comparable cooking yield to that of product with 20% peanut paste. The improvement in the cooking yield with increasing level of peanut paste, coincided with the emulsion stability of respective

products and might be due to characteristic property of non-meat additives (Reitmer and Prusa, 1991) that too rich with protein. Prinyawiwatkul *et al*., (1997) reported that the fat and water binding and heat-induced gelation properties of fermented partially defatted peanuts (FPDPF) were quiet advantageous in comminuted meat systems. The pH of the product with 10% peanut paste was significantly lower ($P< 0.05$) to product with 20% incorporation level, but comparable to other two i.e. control as well as 15% incorporation level. At 15% incorporation level, the product pH was comparable to both 10 and 20% incorporation levels but significantly higher than control. The pH of the product increased with increase in incorporation level similar to pH of emulsion. Increase in pH of cooked product over emulsion could be due to the changes in net charge of proteins due to denaturation (Babu *et al.,* 1994).

The moisture percentages of the treatment products were significantly lower ($P< 0.05$) than control. The moisture content significantly decreased at subsequent level of incorporation and it was lowest at 20% incorporation. Premium mutton nuggets with 10% peanut paste had significantly lower moisture percentage than control but higher than product with 15% peanut paste. There was significant increase ($P< 0.05$) in protein percentage at each subsequent higher level of incorporation. Decrease in moisture percentage and increase in protein percentage in the product was due to replacement of lean meat with peanut paste which had comparatively low moisture but high protein content. The moisture:protein ratio being a derived value, showed declining trend with increase in level of incorporation.

The fat percentages of treatment and control product were comparable. A slight increase in fat percentage with increase in incorporation level might be because of improved fat retention and gelling effects. Increase in fat percentage of chicken burgers prepared by incorporation of 20% roasted defatted peanut flour was reported by Soher *et al*., (2013). Ash content of the treatment products were marginally higher ($P > 0.05$) than control product and gradual increase in ash percentage with increase of incorporation level of peanut paste might be due to replacement of lean meat which had lower ash content than peanut. Calculated carbohydrate percentage of the product increased marginally ($P >0.05$) with increase in incorporation level, obviously due to incorporation of peanut paste having substantial carbohydrate as compared to meat. The shear force values of treated products and control products were comparable among themselves. A slight decrease in shear force

value with increase in the incorporation of non- meat additives was in accordance with Das *et al*., (2006) and Atughonu *et al*., (1998). These results are in contrast to those obtained by Soher *et al*., (2013), who found an increase in shear force value with increase in defatted peanut flour (DPF) supplementation in chicken burger.

Mean sensory scores of premium mutton nuggets incorporated with different levels of peanut paste are presented in Table -9 and their corresponding ANOVA is given in Table -10. Sensory scores for general appearance, flavour, texture, binding, juiciness and overall acceptability showed a declining trend with increase in level of peanut paste incorporation. Score for general appearance of product with peanut paste was lower than control but even at 20% incorporation level, the difference was not significant ($P>0.05$). A gradual decrease in appearance score might be attributed to decrease in the intensity of red meat colour with increase in peanut paste. The flavour scores gradually decreased with increase in incorporation level but even at 20% incorporation, the score was comparable to control. The lowering of flavour scores might be attributed to the dilution of meaty flavour and dominance of flavour of peanut paste itself. There was gradual decrease in texture scores also, which were concurrent to shear force values too. The juiciness scores of treated products were slightly less than control and showed declining trend with increase in the level of incorporation. The overall acceptability of treated products and control were comparable among themselves. The scores for overall acceptability decreased with increase in incorporation level but remained comparable to control even at highest level i.e. 20% . Prinyawiwatkul *et al*., (1997) also reported a similar decrease in the scores for all the sensory attributes with increase in incorporation level of fermented defatted peanut flour in chicken nuggets.

The sensory scores for most attributes in 20% peanut paste incorporated treatment product were comparable to control and had almost very good sensory rating. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of peanut paste was adjudged as 20% for preparation of premium mutton nuggets.

Table 7. Physico-chemical properties of premium mutton nuggets enriched with peanut based functional components. (Mean±S.E.)

Parameters	**Control**	**peanut paste** (containing approx 50% fat)		
		10	**15**	**20**
Emulsion pH	6.23 ± 0.006^{a}	6.26 ± 0.017^{b}	6.29 ± 0.009^{b}	6.34 ± 0.008^{c}
Emulsion stability	95.12 ± 0.61^{a}	96.20 ± 0.93^{ab}	97.38 ± 0.68^{bc}	98.79 ± 0.36^{c}
Cooking yield (%)	95.57 ± 0.35^{a}	96.15 ± 0.12^{a}	97.03 ± 0.05^{b}	97.59 ± 0.02^{b}
Product pH	6.28 ± 0.030^{a}	6.32 ± 0.013^{ab}	6.35 ± 0.012^{bc}	6.39 ± 0.018^{c}
Moisture (%)	61.00 ± 0.62^{a}	58.57 ± 0.42^{b}	56.60 ± 0.45^{c}	53.72 ± 0.48^{d}
Protein (%)	17.60 ± 0.49^{a}	19.54 ± 0.45^{b}	20.85 ± 0.27^{b}	23.12 ± 0.65^{c}
Moisture Protein ratio	3.42 ± 0.083^{a}	3.28 ± 0.097^{ab}	3.07 ± 0.088^{bc}	2.90 ± 0.11^{c}
Fat (%)	14.64 ± 0.71^{a}	14.79 ± 0.68^{a}	15.31 ± 0.43^{a}	15.73 ± 0.25^{a}
Ash (%)	2.45 ± 0.081^{a}	2.55 ± 0.089^{a}	2.57 ± 0.092^{a}	2.65 ± 0.076^{a}
Carbohydrates	4.30 ± 0.14^{a}	4.54 ± 0.24^{a}	4.65 ± 0.15^{a}	4.77 ± 0.43^{a}
Shear force value (Kg/cm^2)	0.32 ± 0.024^{a}	0.29 ± 0.027^{a}	0.29 ± 0.027^{a}	0.28 ± 0.018^{a}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).

n_1 (Cooking yield) =3, n_2 (Physico-chemical parameters) =6, n_3 (Shear force value) =15 for each treatment.

Table 8: ANOVA for physico-chemical properties of premium mutton nuggets enriched with peanut based functional components.

Parameters	**Source of variation**				
	Treatment			**Error**	
	d.f.	**MSS**	**F value**	**d.f.**	**MSS**
Emulsion pH	3	0.015	21.152**	20	0.001
Emulsion stability (%)	3	0.015	6.195**	20	0.002
Cooking yield (%)	3	2.440	22.853**	8	0.107
Product pH	3	14.966	5.429**	20	2.757
Moisture (%)	3	56.919	38.123**	20	1.493
Protein (%)	3	32.182	22.862**	20	1.408
Moisture Protein ratio	3	1.392	39.881	20	0.035
Fat (%)	3	1.493	0.824	20	1.812
Ash (%)	3	0.041	0.942	20	0.043
Carbohydrates	3	0.242	0.574	20	0.422
Shear force value(Kg/cm^2)	3	0.004	0.438	56	0.009

* Significant (P<0.05); ** Highly significant (P<0.01).

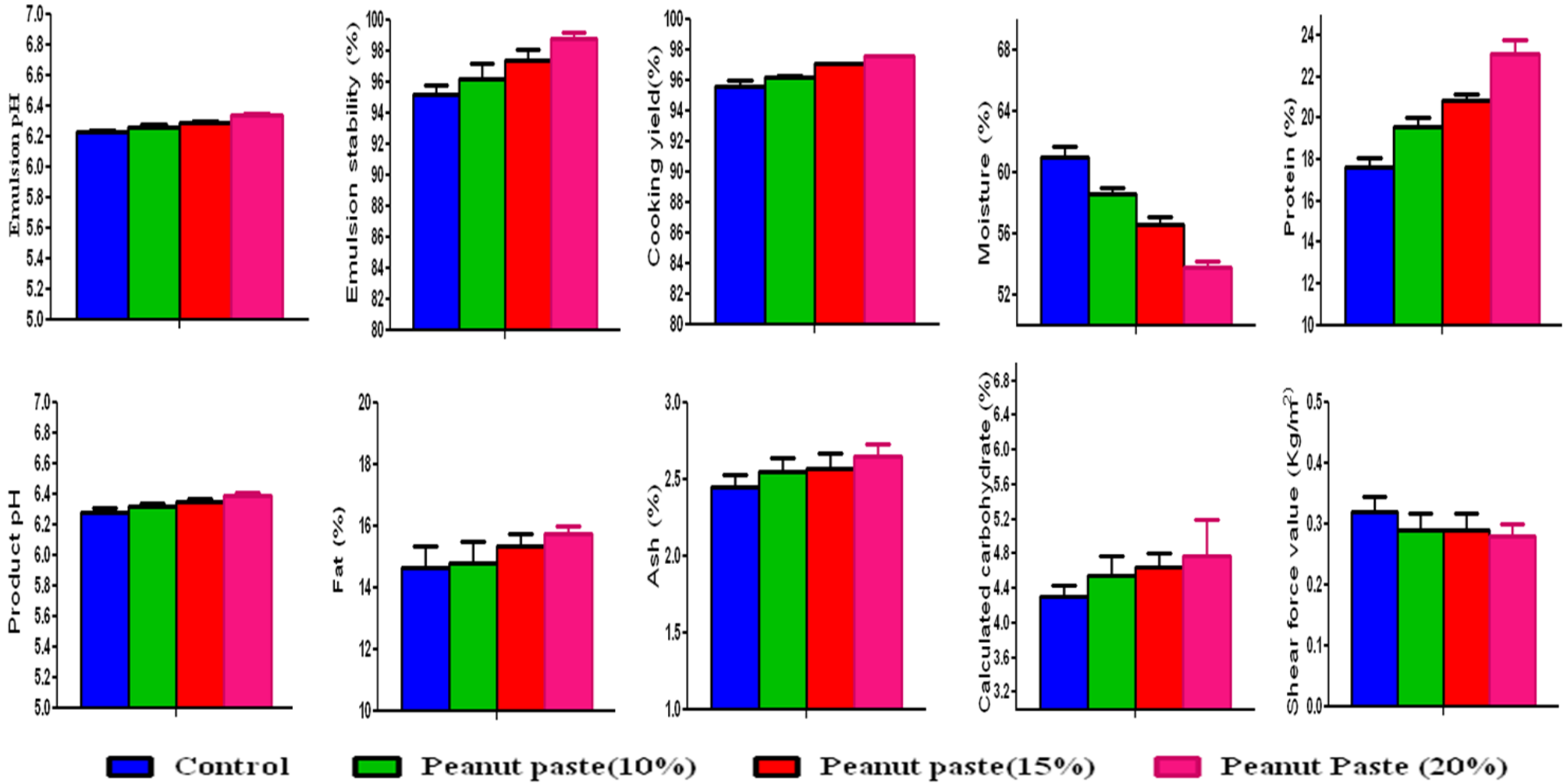


Fig-2- Physico-chemical properties of premium mutton nuggets enriched with peanut based functional components

Table 9. Sensory characteristics of premium mutton nuggets enriched with peanut based functional components. (Mean±S.E.)

Attributes	Control	Peanut paste(containing approx 50% fat)		
		10	15	20
General appearance	7.01 ± 0.080	6.98 ± 0.059	6.94 ± 0.079	6.91 ± 0.063
Flavour	7.02 ± 0.082	6.91 ± 0.080	6.84 ± 0.096	6.79 ± 0.103
Texture	7.07 ± 0.081	7.03 ± 0.045	6.93 ± 0.056	6.91 ± 0.086
Juiciness	7.06 ± 0.055	6.96 ± 0.090	6.94 ± 0.099	6.90 ± 0.074
Overall acceptability	7.01 ± 0.058	6.91 ± 0.068	6.87 ± 0.090	6.79 ± 0.103

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).
n=21 for each treatment.

Table 10: ANOVA for Sensory attributes of premium mutton nuggets enriched with peanut based functional components.

Attributes	Source of variation				
	Treatment			Error	
	d.f.	MSS	F value	d.f.	MSS
General appearance	3	0.040	0.376	80	0.106
Flavour	3	0.208	1.199	80	0.174
Texture	3	0.131	1.311	80	0.100
Juiciness	3	0.093	0.673	80	0.139
Overall acceptability	3	0.164	1.170	80	0.140

** Highly significant (P<0.01).

4.2.2: Optimization of almond paste incorporation level for the preparation of premium mutton nuggets

In this study, almond paste was incorporated at the level of 10, 15 and 20% by substituting the added fat in traditional emulsion by 50%, 75% and 100% and also replacing the lean meat (wt basis) in pre-standardized mutton nuggets formulation (Table-2). Physico-chemical properties such as emulsion stability, pH of emulsion and product, cooking yield, moisture, protein, moisture protein ratio, fat, ash, calculated carbohydrates as well as shear force value and sensory attributes viz. general appearance, flavour, texture, binding, juiciness and overall acceptability were evaluated to find the optimum level of incorporation. The physico-chemical properties of premium mutton nuggets incorporated with different levels of almond paste are presented in Table-11 and their corresponding ANOVA in Table-12. The pH values of raw emulsion increased with increase in

incorporation level of almond paste and were significantly higher in treatment products as compared to control. The pH value of raw emulsion with 10 and 15% levels of almond paste were comparable to each other but significantly lower ($P<0.05$) than treatment emulsion with 20% almond paste. This increase in pH might be attributed to replacement of lean meat (approx pH of 5.4) with slightly alkaline almond paste. Faid *et al*., (1995) evaluated the physico-chemical characteristics of almond paste and categorized it under slightly basic. The emulsion stability of premium mutton nuggets also increased with increase in incorporation level and at 20% incorporation, it was significantly higher ($P <0.05$) than others. Premium mutton nuggets prepared with 15% almond paste had comparable emulsion stability to both 10 and 20% levels but significantly higher ($P<0.05$) than control. A significantly high ($P<0.05$) emulsion capacity of almond was reported by Mostafa and Awadh (2013) and they suggested that the high emulsion capacity of nut meal protein might be useful in the food applications. Sze-Tao and Sathe (2000b) cited that emulsion activity of almond was even higher than that of soybean. Improvement in emulsion stability of goat meat nuggets by addition of almond was also reported by Rajkumar *et. al.,* (2012).

The cooking yield of the product increased with increase in incorporation level of almond paste and was significantly ($P< 0.05$) higher at 15% and 20% levels as compared to control. The premium nuggets with 15% almond paste had comparable cooking yield to both 10 and 20% levels incorporated products. Product with 10% almond paste was having comparable cooking yield with that of control. The improvement in the cooking yield with increasing level of almond paste coincided to the emulsion stability at respective levels and might be due to superior functional property of almond protein with respect to its use in food products (Mostafa and Awadh, 2013).

The moisture percentages in almond paste incorporated products decreased with increase in incorporation level. Premium mutton nuggets with 10% almond paste had significantly higher ($P<0.05$) moisture percentage than 20% paste incorporated product but it was comparable to that of control and 15% incorporated product. Likewise, at 15% incorporation level, the moisture percentage of the product was significantly lower ($P<0.05$) than control but comparable to products with 10% or 20% levels of almond paste. Moisture percentage was the least for the product with 20% almond paste. There was gradual increase in protein percentage with increase in the level of incorporation and at

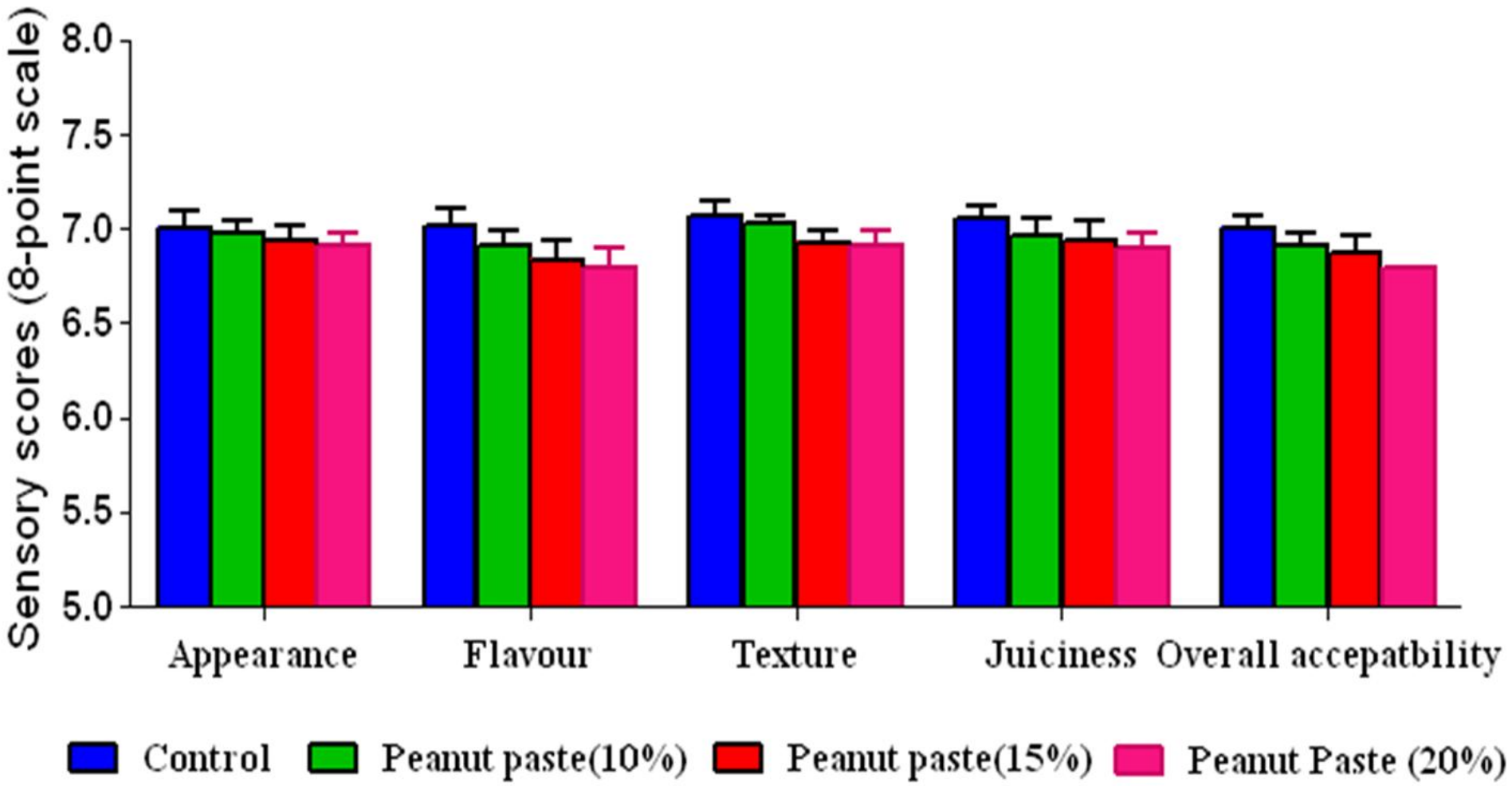


Fig-3-Sensory characteristics of premium mutton nuggets enriched with peanut based functional components

20% incorporation, it was significantly higher than either control or product with 10% almond paste. Decrease in moisture percentage and increase in protein percentage in the product was because of replacement of high moisture lean with almond paste which had comparatively low moisture. The moisture:protein ratio being the derived value, showed a significant decline at each increase in almond paste incorporation.

The fat percentage of treatments and control products were comparable. A slight increase in fat percentage with increase in the incorporation level might be due to improved fat retention and gelling effects. Ash content of the treatment products were marginally higher ($P > 0.05$) than control product and progressive increase in ash percentage with increase in the incorporation level of almond paste might be due to replacement of lean meat which had lower ash content than almond. The ash percentage at 15% and 20% levels of incorporation were comparable to each other but significantly higher than either control or 10% almond paste incorporated products. Calculated carbohydrate content of the product increased insignificantly ($P >0.05$) with increase in incorporation level which was obviously due to incorporation of carbohydrate rich almond paste. Rajkumar *et al.,* (2012) also reported a similar increase in all the compositional parameters except moisture with increase in almond paste incorporation in goat meat nuggets.

The shear force values decreased gradually with increase in the level of incorporation of almond paste. At 20% level it was significantly lower than others. Upto 15% incorporation the shear force values of the treatment products remained comparable to control but at 20% level, it was significantly lower ($P<0.05$) than others. Almonds have been reported to possess good amount of dietary fibers. McDonagh *et al.,* (2005) have cited a decrease in shear force value of meat products with increase in the incorporation of dietary fiber rich components.

Mean sensory scores of premium mutton nuggets incorporated with different levels of almond paste are presented in Table-13 and their corresponding ANOVA is given in Table -14. Sensory scores for general appearance, flavour, texture, binding, juiciness and overall acceptability showed a declining trend with increase in the level of almond paste incorporation. At 10% incorporation level, general appearance score of the product was comparable to both control and 15% incorporation level but score for general appearance of product with 20% incorporation was significantly ($P<0.05$) lower than control.

Table 11. Physico-chemical properties of premium mutton nuggets enriched with almond based functional components. (Mean±S.E.)

Parameters	Control	Almond paste(containing approx 50% fat)		
		10	15	20
Emulsion pH	6.23 ± 0.006^{a}	6.27 ± 0.013^{b}	6.29 ± 0.007^{b}	6.34 ± 0.007^{c}
Emulsion stability	94.74 ± 0.59^{a}	95.74 ± 0.51^{a}	96.47 ± 0.59^{ab}	97.68 ± 0.55^{b}
Cooking yield (%)	94.95 ± 0.44^{a}	95.62 ± 0.31^{ab}	96.34 ± 0.37^{bc}	97.04 ± 0.29^{c}
Product pH	6.30 ± 0.011^{a}	6.33 ± 0.011^{ab}	6.33 ± 0.012^{bc}	6.39 ± 0.006^{c}
Moisture (%)	60.52 ± 0.58^{a}	59.29 ± 0.34^{ab}	58.03 ± 0.50^{bc}	56.99 ± 0.50^{c}
Protein (%)	17.72 ± 0.29^{a}	18.16 ± 0.43^{a}	18.97 ± 0.40^{ab}	19.69 ± 0.59^{b}
Moisture Protein ratio	3.48 ± 0.099^{a}	3.00 ± 0.063^{b}	2.71 ± 0.046^{c}	2.34 ± 0.086^{d}
Fat (%)	14.69 ± 0.27	14.76 ± 0.31	14.79 ± 0.24	14.88 ± 0.26
Ash (%)	2.45 ± 0.032^{a}	2.51 ± 0.032^{a}	2.63 ± 0.037^{b}	2.72 ± 0.043^{b}
Carbohydrates	4.62 ± 0.24^{a}	5.28 ± 0.34^{ab}	5.59 ± 0.23^{b}	5.71 ± 0.36^{b}
Shear force value (Kg/cm^2)	0.31 ± 0.021^{a}	0.30 ± 0.018^{a}	0.29 ± 0.018^{a}	0.23 ± 0.017^{b}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).

n_1 (Cooking yield) =3, n_2 (Physico-chemical parameter) =6, n_3 (Shear force value) =15 for each treatment.

Table 12: ANOVA for physico-chemical properties of premium mutton nuggets enriched with almond based functional components.

Parameters	Source of variation				
	Treatment			Error	
	d.f.	MSS	F value	d.f.	MSS
Emulsion pH	3	0.013	26.820**	20	0.000
Emulsion stability (%)	3	9.171	4.822**	20	1.902
Cooking yield (%)	3	2.443	6.358*	8	0.384
Product pH	3	0.004	6.682**	20	0.001
Moisture (%)	3	14.021	9.784**	20	1.433
Protein (%)	3	4.590	3.922*	20	1.171
Moisture Protein ratio	3	0.306	5.734**	20	0.53
Fat (%)	3	0.036	0.083	20	0.439
Ash (%)	3	0.089	11.353**	20	0.008
Carbohydrates	3	1.419	2.674	20	0.531
Shear force value(Kg/cm^2)	3	0.021	4.000*	56	0.005

* Significant (P<0.05); ** Highly significant (P<0.01).

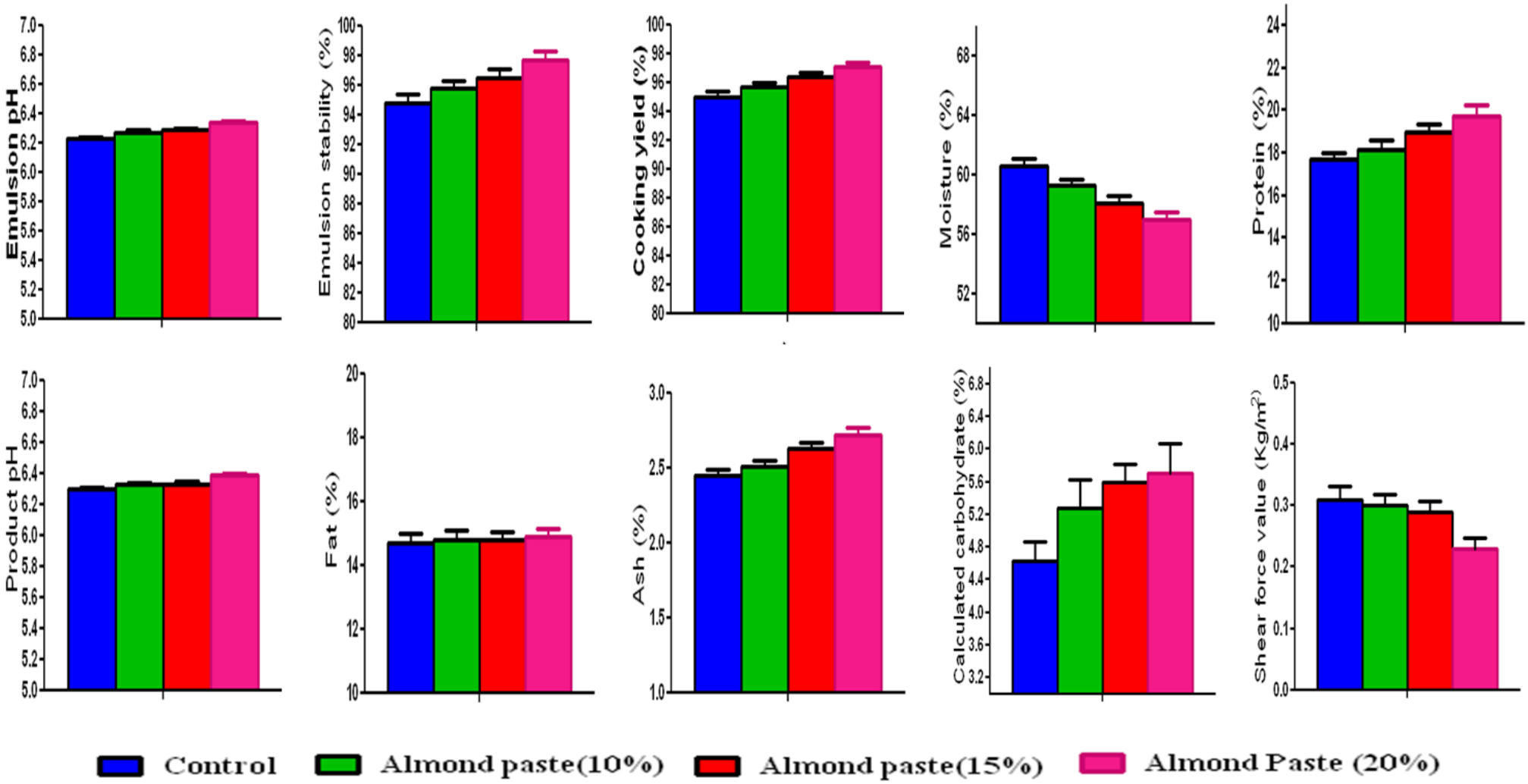


Fig-4- Physico-chemical properties of premium mutton nuggets enriched with Almond based functional components

Table 13. Sensory characteristics of premium mutton nuggets enriched with almond based functional components. (Mean±S.E.)

Attributes	Control	Almond paste(containing approx 50% fat)		
		10	15	20
General appearance	7.09 ± 0.069^{a}	6.97 ± 0.077^{ab}	6.83 ± 0.062^{b}	6.80 ± 0.071^{b}
Flavour	7.05 ± 0.061^{a}	6.95 ± 0.079^{ab}	6.87 ± 0.079^{ab}	6.75 ± 0.092^{b}
Texture	7.08 ± 0.064^{a}	7.01 ± 0.058^{a}	6.88 ± 0.086^{ab}	6.72 ± 0.090^{b}
Juiciness	7.14 ± 0.048^{a}	7.09 ± 0.070^{a}	6.98 ± 0.076^{ab}	6.79 ± 0.094^{b}
Overall acceptability	7.05 ± 0.055^{a}	7.01 ± 0.076^{a}	6.90 ± 0.080^{ab}	6.75 ± 0.071^{b}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).
n=21 for each treatment**.**

Table 14: ANOVA for Sensory attributes of premium mutton nuggets enriched with almond based functional components.

Attributes	Source of variation				
	Treatment			Error	
	d.f.	MSS	F value	d.f.	MSS
General appearance	3	0.387	3.742*	80	0.103
Flavour	3	0.320	2.456	80	0.130
Texture	3	0.524	4.321**	80	0.121
Juiciness	3	0.510	4.399**	80	0.116
Overall acceptability	3	0.360	3.379*	80	0.107

* Significant (P<0.05); ** Highly significant (P<0.01).

A decline in general appearance scores of premium mutton nuggets with increase of incorporation level might be due to inclusion of white coloured almond paste which diluted normal colour of mutton nuggets. The flavour scores gradually decreased with increase in incorporation level but remained comparable to control upto 15% incorporation level. The lowering of flavour scores might be attributed to dilution of meaty flavour. There was decrease in texture scores also with increase in incorporation level. The texture score even at 15% incorporation level was comparable to control but after that it was significantly lower (P<0.05) than control and 10% incorporation. Increase in dietary fiber content and changed firmness of substituted fat could have been the reason for lowering of textural scores.

The juiciness scores of treatment products were less and showed a declining trend with increase in incorporation level. The juiciness scores of the products were comparable to control upto 15% incorporation level but at 20% incorporation level, these were significantly lower than both control and 10% paste incorporated products. The overall acceptability scores were reflectance of the scores for other sensory attributes. The scores for overall acceptability decreased with increase in incorporation level but remained comparable to control upto 15% incorporation. A non-significant decrease in scores for various attributes in almond incorporated goat meat nuggets was reported by Rajkumar *et al*., (2012) but they had evaluated the effect only upto 5% incorporation.

The sensory scores for most of the attributes at 15% almond paste incorporation were comparable to control and had very good rating. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of almond paste was adjudged as 15% for preparation of premium mutton nuggets.

4.2.3: Optimization of pine nut paste incorporation level for the preparation of premium mutton nuggets

In this study, pine nut paste was incorporated at the level of 8, 12 and 16% by substituting the added fat in traditional emulsion by 50%, 75% and 100% and also replacing the lean meat (wt basis) in prestandardized mutton nuggets formulation. Physico-chemical properties such as emulsion stability, pH of emulsion and product, cooking yield, moisture, protein, moisture protein ratio, fat, ash, calculated carbohydrates as well as shear force value and sensory attributes viz. general appearance, flavour, texture, binding, juiciness and overall acceptability were evaluated to find the optimum level of incorporation.

The physico-chemical properties of premium mutton nuggets incorporated with different levels of pine nut paste are presented in Table-15 and their corresponding ANOVA is given in Table-16. The pH value of raw emulsion decreased slightly with increase in the level of incorporation level of pine nut paste but it was comparable to control even at 12% incorporation. The pine nut has been categorized under low acid food (http://www.erichsenwellness.com/wp-content/uploads/2012/01/Balance-Ph-with-Foods-Chart.pdf) and as per Nunes *et al*., (1999), the pH of the bark of the tree was also slightly acidic. The emulsion stability of premium mutton nuggets increased with increase in incorporation level and 16% incorporation, it was significantly higher ($P < 0.05$) than control and 8% incorporation. Premium mutton nuggets prepared with 12% pine nut paste

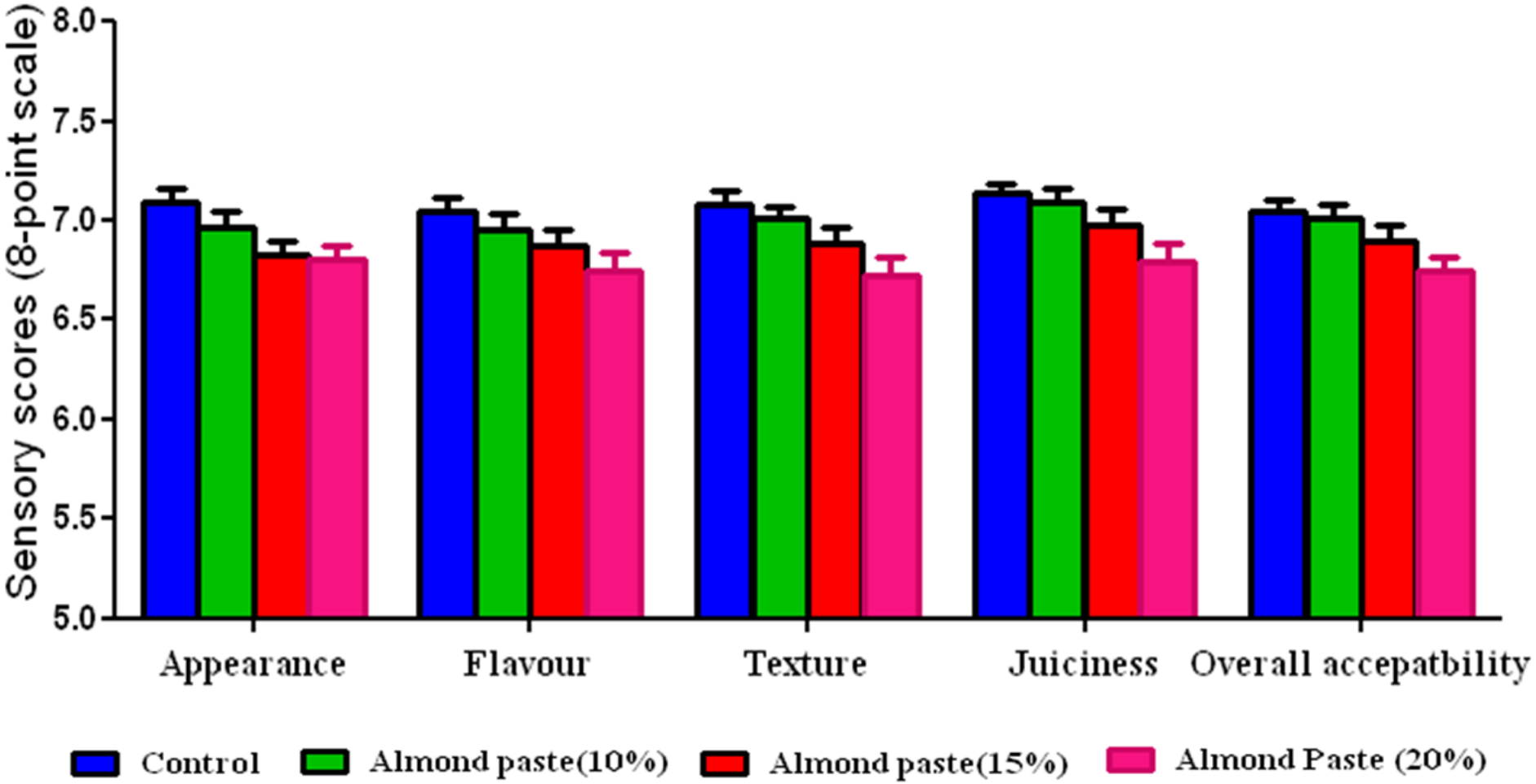


Fig-5-Sensory characteristics of premium mutton nuggets enriched with Almond based functional components

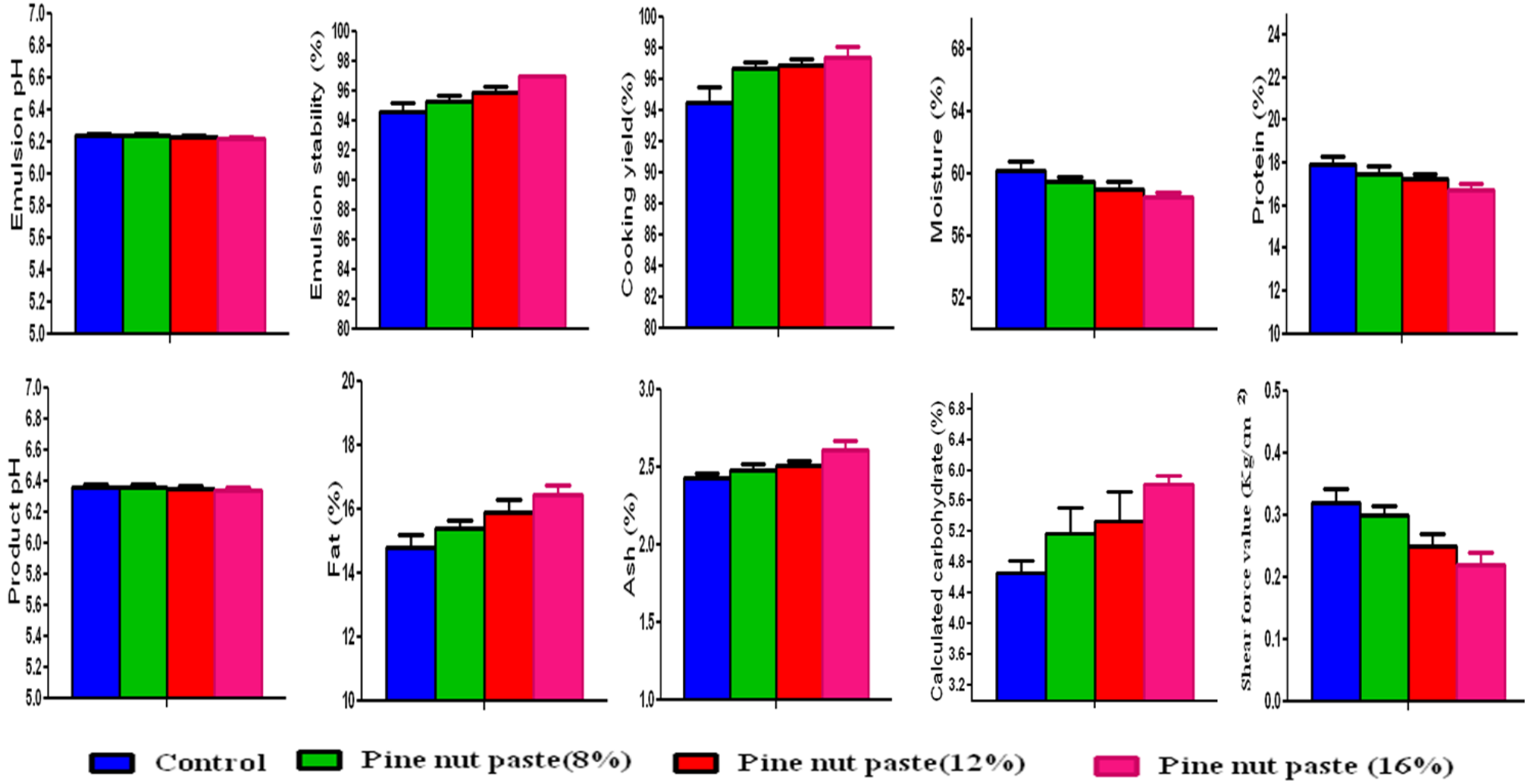


Fig-6- Physico-chemical properties of premium mutton nuggets enriched with Pine nut based functional components

had comparable emulsion stability to all others. The emulsion stability at 8% incorporation was comparable to control as well as 12% incorporation. Pine nut has been reported to possess very good amount of emulsifying protein by Ming (2012) and Mostafa and Awadh (2013) which could have been the reason behind increased emulsion stability in treatment products.

The cooking yield of the product increased with increase in the level of incorporation of pine nut paste and was significantly higher ($P< 0.05$) at 12% and 16% levels as compared to control. The premium nuggets with 12% pine nut paste had comparable cooking yield to 8 and 16% incorporated products. Product with 8% pine nut paste had comparable cooking yield to that of control. The improvement in the cooking yield with increasing level of pine nut paste coincided with the emulsion stability of respective products and might be due to superior functional property of pine nut protein with respect to its use in food products (Mostafa and Awadh, 2013). Use of various nuts viz: malva nut in chicken meat batter, tiger nuts in pork burger have been reported to decrease the cooking loss by Barbut and Somboonpanyakul (2007) and Sánchez-Zapata *et al*., (2010) respectively.

The moisture percentage in pine nut paste incorporated products decreased with increase in level of incorporation. Premium mutton nuggets with 16% pine nut paste had significantly lower ($p<0.05$) moisture percentage than control product but moisture percentage was comparable to that of control upto 12% incorporation. There was gradual decrease in protein percentage with increase in the level of incorporation and it was significantly ($P<0.05$) lower than control at 16% incorporation level. The protein percentage in the treatment products remained comparable to control upto the level of 12%. Decrease in moisture and protein percentage in the treatment product could be due to replacement of lean meat with pine nut paste which was comparatively low in moisture and protein. The moisture:protein ratio being the derived value, showed marginal increase with increase in the level of pine nut paste incorporation. The fat percentage in the treatment products increased progressively with increase in the level of incorporation and was significantly higher at 12% and 16% levels as compared to control. Product with 8% pine nut paste showed comparable fat percentage to all others including control. A slight increase in fat percentage with increase in incorporation level could be due to improved fat retention and gelling effects. Ash content of the 16% pine nut paste incorporated product was

significantly higher ($P<0.05$) than control product and gradual increase in ash percentage with increase in the incorporation level of pine nut paste might be due to replacement of lean meat which had a lower ash content than pine nut. The ash percentage at 8% level of incorporation was comparable to other all other treatment products including control. Calculated carbohydrate content of the product increased with increase in incorporation level which was obvious because of incorporation carbohydrate rich pine nut paste. The change in meat batter composition because of nut incorporation was also studied by Ayo *et al.,* (2005) and they reported a proportionate increases of fat and decrease in moisture by addition of walnut.

The shear force values decreased gradually with increase in incorporation level of pine nut paste. Values were significantly lower at 12 and 16% level of incorporation as compared to control and at 16% level it was even significantly lower than 8% incorporation level. Products with 8% pine nut paste showed shear force value comparable to control as well as 12% pine nut incorporated product. A decrease in shear force values might be due to reduction in binding of myofibrillar protein and increase in binding of dietary fiber content of pine nut paste with water and fat.

Mean sensory scores of premium mutton nuggets extended with different levels of pine nut paste are presented in Table-17 and their corresponding ANOVA in Table-18. Sensory scores for general appearance, flavour, texture, binding, juiciness and overall acceptability showed a declining trend with increase in level of pine nut paste incorporation. At 8% and 12% incorporation level the scores for general appearance were comparable to each other, control as well as 16% incorporation level, whereas score for general appearance of product with 16% pine nut paste was significantly lower than control but comparable to other two levels of incorporation evaluated. A decrease in general appearance scores with increase in incorporation level of pine nut paste might be attributed to cream colour of paste which diluted the colour intensity of control products. The flavour scores of treatment products were significantly lower ($P<0.05$) than control at 12 and 16% levels of incorporation. However, at 8% incorporation level, the flavour score was comparable to control as well as higher levels evaluated. Decrease in flavour scores might be attributed to the predominance of nut flavour which increased proportionately. Sharashkin and Gold (2004) reported about the creamy colour and strong nutty flavour of pine nuts . There was decrease in texture scores also with increase in incorporation level.

The texture score at 8% incorporation was comparable to control but it was significantly lower (P<0.05) than control thereafter. The juiciness score decreased gradually with increase in incorporation level and even at 12% incorporation level it was comparable to control. Product with 16% pine nut paste had significantly lower (P<0.05) juiciness score than control and 8% pine nut incorporated product. A decrease in texture and juiciness scores matched with changes in shear force values and moisture percentage. Rocha-Garza and Zayas (1995) reported that the ability of protein to retain water and bind fat governs the texture, juiciness and structural binding characteristics of extended meat products. The overall acceptability scores were a reflectance of the ratings for other attributes. The overall acceptability scores were comparable to control at 8% incorporation. At 12% incorporation, the score was comparable to the product with 16% pine nut paste.The adverse nut mouth taste has been reported at higher level by Middleton, (2009); Hutchison, (2010); Jamieson, (2011); Daily Mail Reporter, (2011) and this mouth taste disturbance could have significantly lowered the overall acceptability at higher level of incorporation.

The sensory scores for most of the attributes at 8% pine nut paste incorporation were comparable to control and had very good rating. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of pine nut paste for preparation of premium mutton nuggets was adjudged as 8%.

Table 15. Physico-chemical properties of premium mutton nuggets enriched with pine nut based functional components. (Mean±S.E.)

Parameters	**Control**	**Pine nut paste** (containing approx 62.5% fat)		
		8	**12**	**16**
Emulsion pH	6.24 ± 0.006^{a}	6.24 ± 0.005^{ab}	6.23 ± 0.008^{ab}	6.22 ± 0.002^{b}
Emulsion stability	94.54 ± 0.59^{a}	95.22 ± 0.44^{a}	95.89 ± 0.37^{ab}	97.01 ± 0.66^{b}
Cooking yield (%)	94.43 ± 1.04^{a}	96.64 ± 0.46^{ab}	96.89 ± 0.46^{b}	97.38 ± 0.68^{b}
Product pH	6.36 ± 0.020	6.36 ± 0.020	6.35 ± 0.013	6.34 ± 0.012
Moisture (%)	60.18 ± 0.57^{a}	59.49 ± 0.29^{ab}	58.98 ± 0.50^{ab}	58.44 ± 0.34^{b}
Protein (%)	17.94 ± 0.39^{a}	17.50 ± 0.37^{ab}	17.28 ± 0.19^{ab}	16.70 ± 0.31^{b}
Moisture Protein ratio	3.36 ± 0.093	3.41 ± 0.085	3.41 ± 0.035	3.50 ± 0.070
Fat (%)	14.77 ± 0.43^{a}	15.36 ± 0.26^{ab}	15.88 ± 0.40^{b}	16.42 ± 0.30^{b}
Ash (%)	2.43 ± 0.030^{a}	2.48 ± 0.033^{a}	2.51 ± 0.024^{ab}	2.61 ± 0.053^{b}
Carbohydrates	4.67 ± 0.15^{a}	5.17 ± 0.34^{a}	5.34 ± 0.38^{ab}	5.82 ± 0.11^{b}
Shear force value (Kg/cm^{2})	0.32 ± 0.021^{a}	0.30 ± 0.015^{ab}	0.25 ± 0.018^{bc}	0.22 ± 0.019^{c}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).

n_1 (Cooking yield) =3, n_2 (Physico-chemical parameter) =6, n_3 (Shear force value) =15 for each treatment.

Table 16: ANOVA for physico-chemical properties of premium mutton nuggets enriched with pine nut based functional components

Parameters	Source of variation				
	Treatment			Error	
	d.f.	MSS	F value	d.f.	MSS
Emulsion pH	3	0.000	2.046	20	0.000
Emulsion stability (%)	3	6.668	4.007*	20	1.664
Cooking yield (%)	3	5.113	3.448	8	1.483
Product pH	3	0.001	0.569	20	0.002
Moisture (%)	3	3.308	2.879	20	1.149
Protein (%)	3	1.597	2.515	20	0.635
Moisture Protein ratio	3	0.021	0.641	20	0.033
Fat (%)	3	2.978	3.979*	20	0.748
Ash (%)	3	0.036	4.410*	20	0.008
Carbohydrates	3	1.370	3.123*	20	0.59
Shear force value(Kg/cm^2)	3	0.030	5.917**	56	0.005

* Significant (P<0.05); ** Highly significant (P<0.01).

Table 17. Sensory characteristics of premium mutton nuggets enriched with pine nut based functional components. (Mean±S.E.)

Attributes	Control	Pine nut paste(containing approx 62.5% fat)		
		8	12	16
General appearance	7.10 ± 0.043^{a}	6.99 ± 0.052^{ab}	6.89 ± 0.057^{ab}	6.76 ± 0.082^{b}
Flavour	7.08 ± 0.045^{a}	6.90 ± 0.066^{ab}	6.87 ± 0.059^{b}	6.77 ± 0.084^{b}
Texture	7.18 ± 0.041^{a}	7.04 ± 0.047^{ab}	6.95 ± 0.050^{b}	6.79 ± 0.069^{c}
Juiciness	7.18 ± 0.045^{a}	7.12 ± 0.035^{a}	7.03 ± 0.050^{ab}	6.92 ± 0.073^{b}
Overall acceptability	7.13 ± 0.045^{a}	7.02 ± 0.053^{ab}	6.90 ± 0.070^{bc}	6.71 ± 0.099^{c}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).
n=21 for each treatment.

Table 18: ANOVA for Sensory attributes of premium mutton nuggets enriched with pine nut based functional components.

Attributes	Source of variation				
	Treatment			Error	
	d.f.	MSS	F value	d.f.	MSS
General appearance	3	0.457	5.978**	80	0.077
Flavour	3	0.343	3.844*	80	0.089
Texture	3	0.551	9.281**	80	0.059
Juiciness	3	0.267	4.614**	80	0.058
Overall acceptability	3	0.692	6.727**	80	0.103

* Significant (P<0.05); ** Highly significant (P<0.01)

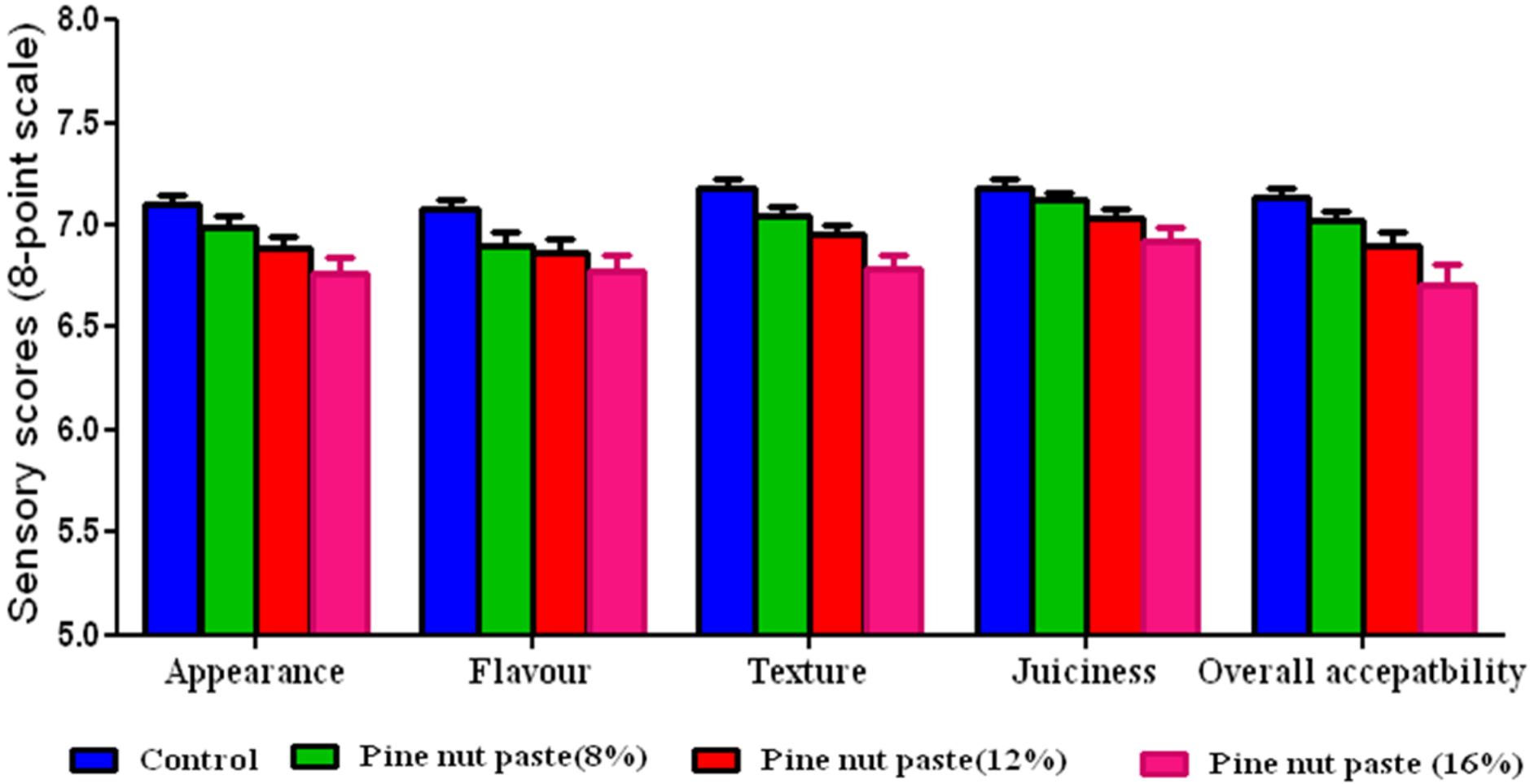


Fig-7-Sensory characteristics of premium mutton nuggets enriched with Pine nut based functional components

4.3. Assessment of the Efficacy of Nut Pastes viz: Almond, Peanuts and Pine Nuts at their Optimum Level of Incorporation for their Functional Components by Determining the Detailed Product Profile of Premium Mutton Nuggets

Nuts based functionally enriched premium mutton nuggets with respective level of optimum incorporation, evolved from the earlier experiments were analyzed for detailed profile viz: lipid profile parameters, calorific value, dietary fiber, total phenolics, DPPH radical scavenging activity, reducing power assay and texture profile parameters.

4.3.1. Lipid profile of premium mutton nuggets enriched with nut based functional components

In this part of study the premium mutton nuggets developed by incorporation of optimum level of nut pastes viz: 20% of peanut, 15% of almond and 8% of pine nut were evaluated for detailed lipid parameters such as total lipids, total cholesterol, total phospholipids, total glycolipids, total free fatty acids and total glycerides. The detailed lipid profile of developed premium nuggets and control is presented in Table-19 and corresponding ANOVA is given in Table-20.

The total lipids content of treatment product was comparatively higher than control. Product with pine nut paste showed significantly higher ($P<0.05$) total lipids content than control and 20% peanut and 15% almond paste incorporated premium mutton nuggets. The products containing peanut paste or almond paste were having total lipids content comparable to each other as well as control. The difference in total lipid content might be due to different protein fat matrix and moisture percentages in different classes of products which affected the lipid extraction. The factors affecting the extractability of lipids by solvents have been reviewed extensively by Zahler and Niggli (1977). Further, Christie (1993) reported that the two main structural features of lipids controlling their solubility in organic solvents are hydrophobic hydrocarbon chains of the fatty acid or other aliphatic moieties and other polar functional groups such as phosphate or sugar residues, which are markedly hydrophilic. The total cholesterol content in treatment products was significantly less than control product. Among the treatments, the cholesterol contents were comparable and varied as per the replacement of lean meat in the formulation. A decrease in cholesterol content in treatment products was obvious because of substitution cholesterol rich animal fat (William, 2007, Bronislaw *et al*., 2012) with nut based lipids which are devoid of cholesterol.

The total phospholipids and total glycolipids of treatment products were significantly higher ($p<0.05$) than control. The difference in the values for phospholipids and glycolipids among the treatment groups was not significant. Phospholipid content was highest for almond incorporated product followed by pine nut and peanut, whereas total glycolipid was highest in peanut followed by almond and pine nut respectively. Phospho-lipids and glycolipids, together with proteins, are the building blocks of biological membranes. Glycolipids are lipids containing covalently linked carbohydrates and do not contain phosphorus. Channon *et al*., (2003) reported that polyunsaturated fatty acids are generally associated with the phospholipid fraction in sheep meat that has been cited to possess lowest phospholipid content among the food animal species (Mottram, 1991). On the contrary, nuts are rich with PUFA and considered as good source of phospholipids which also act as natural anti oxidant for their lipids. The reported phospholipid content in peanut is 0.4-1.6 % of total fat (Federica *et al*., 2013), 78% of total polar lipids in almond (Malisiova *et al*., 2004; Federica *et al*., 2013), 43-72% of total polar lipids in pine nut (Miraliakbari and Shahidi, 2007 and 2008). Federica *et al*., (2013) reported a comparatively lower concentration of phospholipids which was also susceptible to microwave treatment in case of peanuts (Yoshida *et al*., 2005). This could account for slightly lower phospholipid content in 20% peanut incorporated product among the treatment products. Nuts rich with carbohydrate moieties and therefore glycolipids, resulted in premium mutton nuggets with higher glycolipid content as compared to control. Higher glycolipid content has been reported in nuts viz: peanut (Gopala-Krishna and Prabhakar, 1994), almond (Pacettia *et al*., 2007) and pine nuts (Lee and Rhee, 2003). Slightly higher glycolipids in peanut paste incorporated premium mutton nuggets might be due to higher level of incorporation.

The total free fatty acids content in peanut incorporated product was significantly lower than control and pine nut incorporated product. Premium mutton nuggets with almond paste also showed significantly lower ($P<0.05$) total free fatty acid content than control but comparable to pine nut as well as peanut incorporated products. Pine nut paste incorporated product was having total free fatty acid content comparable to control and almond incorporated products but significantly higher ($P<0.05$) than peanut incorporated product. Nuts are rich in phospholipids, polyphenols, tocopherols and dietary fibers (Ros, 2010, Mandalari *et al*., 2010 and Wongama *et al*., 2014). The role of these ingredients are

well known in stabilizing the lipids resulting in lower total free fatty acid contents in nut incorporated products. The total glycerides content was significantly higher (P<0.05) than control in pine nut incorporated product. Premium mutton nuggets with peanut paste as well as almond paste, showed total glyceride contents comparable to control and pine nut incorporated product. The difference in total glyceride contents, a derived value was due to differences in the values of other lipid parameters among the treatments.

Table 19. Lipid profile of premium mutton nuggets enriched with nut based functional components. (Mean±S.E.)

Parameters	Control	Nut paste		
		Pea nut (20%)	Almond (15%)	Pine nut (8%)
Total lipids (mg/gm)	126.00 ± 5.58^{a}	135.50 ± 5.54^{ab}	139.83 ± 5.8^{ab}	146.00 ± 4.22^{b}
Total cholesterol (mg/gm)	1.13 ± 0.079^{a}	0.86 ± 0.049^{b}	0.88 ± 0.053^{b}	0.92 ± 0.049^{b}
Total phospholipids (mg/gm)	6.71 ± 0.60^{a}	8.58 ± 0.61^{b}	10.29 ± 0.56^{b}	9.00± 0.43^{b}
Total glycolipids (mg/gm)	0.34 ± 0.017^{a}	0.47 ± 0.029^{b}	0.42 ± 0.028^{b}	0.41 ± 0.019^{b}
Total free fatty acids (mg/gm)	0.36 ± 0.040^{a}	0.19 ± 0.025^{c}	0.25 ± 0.029^{bc}	0.31 ± 0.040^{ab}
Total glycerides (mg/gm)	117.47 ± 5.85^{a}	125.39 ±5.25^{ab}	127.99 ± 5.84^{ab}	135.35 ±3.90^{b}

*Mean±S.E. with different superscripts in a row differ significantly (P<0.05).

n =6 for each treatment

Table 20: ANOVA for Lipid profile of premium mutton nuggets enriched with nut based functional components

Parameters	Source of variation				
	Treatment		Error		
	d.f.	MSS	d.f.	MSS	F value
Total lipids	3	424.33	20	169.82	2.499
Total cholesterol	3	0.09	20	0.02	4.267*
Total phospholipids	3	13.18	20	1.85	7.139**
Total glycolipids	3	0.02	20	0.003	5.65**
Total free fatty acids	3	0.03	20	0.007	4.774*
Total glycerides	3	326.98	20	166.65	1.962

* Significant (P<0.05); ** Highly significant (P<0.01)

4.3.2. Calorific value, dietary fiber, total phenolic content and antioxidant activity of premium mutton nuggets enriched with nut based functional components

In this part of study, the premium mutton nuggets developed by incorporation of optimum level of nut pastes viz: 20% of peanut, 15% of almond and 8% of pine nut were evaluated for calorific value, total dietary fiber, total phenolic content and antioxidant

properties such as DPPH radical scavenging activity and reducing power assays. The mean values of these parameters for premium mutton nuggets and control are presented in Table-21 and their corresponding ANOVA is given in Table-22.

The calorific values of treatment products were higher than the control. Product with 20% peanut had significantly higher ($P<0.05$) calorific value than control, pine nut incorporated product as well as almond based premium mutton nuggets. Likewise, 15% almond incorporated product was having significantly higher calorific value than either control or pine nut paste incorporated products but significantly lower ($P<0.05$) than peanut based premium mutton nuggets. 8% pine nut paste incorporated premium mutton nuggets showed calorific value comparable to control, but significantly less than other two viz: peanut and almond based premium mutton nuggets. Higher calorific values of treatment products might be due to increased dry matter in the products. Nuts have been categorized as energy dense foods (Tey, 2011). Onyeike and Oguike (2003) and Eshun *et al*., (2013) reported the calorific values of differently processed peanuts and varieties of peanuts respectively and the values were almost 4-5 times higher than the reported caloric value of 120-175kcal/ 100 gm for sheep meat (USDA data base, 2001, William, 2007). Likewise, Novonty *et al*., (2012) and Soliman (2012) reported the calorific values of nearly 610 Kcal/100 gm of almond. As per USDA data base pine nut seeds contain 673Kcla/100gm and in both the cases it is quiet high. Shakerardekani *et al*., (2013) reported the energy content in peanut, almond and pine nut as 567, 578 and 629 Kcal/100gm respectively.

The total dietary fiber in the premium mutton nuggets incorporated with peanut and almond pastes was significantly higher ($P<0.05$) than control and pine nut paste incorporated products which had comparable dietary fiber content. Spliller (1997) reported a dietary fiber range of 5-14 % in various nuts and it was highest in almond (10-14%). Among the three nuts evaluated, the reported dietary fiber content in almond, peanut and pine nuts were approx 12%, 8% and 3.2% respectively (USDA data base). The observed dietary fiber content in premium mutton nuggets varied according to the proportion of nut paste added.

The total phenolic content of control nuggets was less as compared to nut paste incorporated premium mutton nuggets. It was significantly higher ($P<0.05$) in 20% peanut paste incorporated products as compared to others, followed by 15% almond incorporated

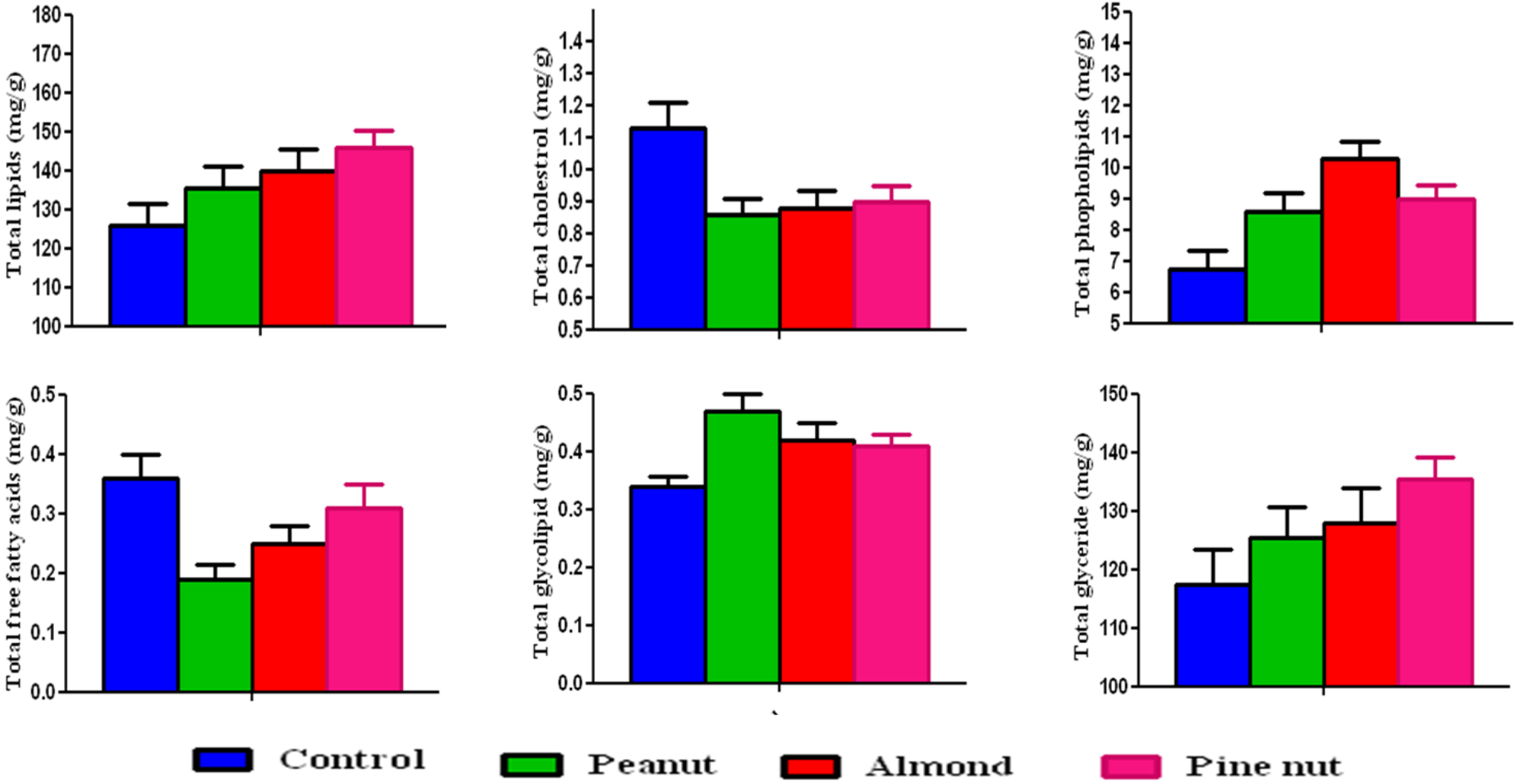


Fig-8- Lipid profile of premium mutton nuggets enriched with nut based functional components.

product which was comparable with pine nut paste incorporated product but still significantly higher than control. Nuggets incorporated with 8% pine nut showed lowest total phenolic content among the treatment products. The total phenolic content in peanut incorporated product was comparatively more because of higher level of substitution. The sheep meat with meager natural antioxidant (Tarzi *et al.,* 2012) is considered poor in total phenolics whereas, nuts are rich in total phenolics. Song *et al*., (2013) reported the total phenol contents of beef, chicken, pork by the Folin-Denis method as 5.1±0.7, 4.5±0.9 and 4.8±1.2 µg/g of gallic acid equivalent whereas, Wu *et al*., (2004) reported the total phenolic content in peanut, almond and pine nut as 3.96+0.54, 4.18 ± 0.84 and 0.68 ± 0.25 mg of GAE/g respectively, indicating almond with highest total phenolic content among the nuts included in our study. Delgado (2010) reported that total phenols in pine nuts as 6.9mgGAE/gextract or 37mgGAE/100 g fruit weight, almost equal to the range of 32mgGAE/100 g fruit weight as reported by Kornsteiner *et al*., (2006). As per Delgado *et al*., (2010), peanuts showed a significantly higher ($P<0.05$) total phenol content (33.6mgGAE/g extract or 248mgGAE/100 g fruit weight) than pine nuts; slightly lower than those reported by Kornsteiner *et al*. (2006) for peanuts with skin i.e. 326–552mgGAE/100. There are few reports of total phenolic contents in differently cooked meat products like cooked goat meat patties as 152µg/gm (Naveena *et al*., 2008), cooked goat meat patties about 580µg/gm (Devatkal *et al*., 2010) and cooked beef patties as 300µg/gm (Hayam *et al*., 2012).

The DPPH radical scavenging activity was significantly higher ($p<0.05$) in nut paste incorporated products as compared to control. It was significantly higher in almond paste incorporated products, followed by peanut based product and then pine nut based product. Pine nut paste incorporated products was having DPPH radical scavenging activity lowest among the treatments but significantly higher ($P<0.05$) than control product. DPPH is more stable than hydroxyl and superoxide radicals, which enhances its utility in evaluation of antioxidant activities (Siriwardhana & Shahidi, 2002).The higher DPPH radical scavenging activity in almond paste incorporated product as compared to peanut and pine nut based products was in accordance with the findings of Delgado *et al*., (2010) who reported significantly higher DPPH radical scavenging activity in almond extract as compared to peanut followed by pine nut and walnut. Win *et al*., (2011) evaluated the DPPH scavenging activity in different parts of peanuts which ranged from 11.795 in raw

kernel to 89.97% in its skin. Sarwar *et al*., (2012) reported the DPPH radical scavenging capacity in almond shell as 12.15 to 57.90% depending upon the method of extraction. Almost similar antioxidant activity against DPPH radicals in oils of peanut and pine nut was reported by Orhan *et al*., (2011). In a related study, Hayam *et al*., (2012) reported the DPPH radical scavenging activity of 16.51± 0.09 % in cooked beef sausages containing 49% of beef meat.

Results from reducing power assay showed that incorporation of nut paste improved the reducing power (i.e., higher absorbance) which was significantly higher ($P<0.05$) in peanut and almond incorporated products as compared to control. The pine nut incorporated product had a reducing power comparable to control but significantly less than peanut and almond based premium mutton nuggets. Reducing properties are generally associated with the presence of reductones (Duh, 1998). Gordon (1990) reported that the antioxidative action of reductones is based on the breaking of free radical chains by the donation of hydrogen atom. Huang *et al*., (2011) compared the reducing power assay in raw and cooked samples which were almost same and in porcine and bovine meats as 0.18 and 0.26 respectively. Chen *et al*., (2008) and Delgado *et al*., (2010) reported a higher reducing power in almond extract as compared to peanut and pine nut. Ying *et al*., (2013) reported reducing ferric ion antioxidant potential (FRAP) in extracts of peanuts as 47966.67 µM Fe equivalent/100g dry weight. In another study, Isfahlan *et al*., (2010) reported the reducing power in hull and shell of almond as 0.667-0.343, 0.267-0.114 AU at 700 nm respectively. Boiling *et al*., (2010) reported a much lower ferric reducing antioxidant power (FRAP) in pine nut (13.4 µmol Fe^{2+}/g) as compared to almond (41.3 µmol Fe^{2+}/g).

Table 21. Calorific value, dietary fiber, total phenolic content and antioxidant activity of premium mutton nuggets enriched with nut based functional components. (Mean±S.E.)

Parameters	**Control**	**Nut paste**		
		Pea nut (20%)	**Almond (15%)**	**Pine nut (8%)**
Calorific value (Kcal/100gm)	242.50 ± 5.25^{a}	282.63 ± 3.11^{b}	256.23 ± 3.15^{c}	252.31 ± 2.30^{a}
Total Dietary fiber (%)	0.48 ± 0.023^{a}	0.68 ± 0.034^{b}	0.71 ± 0.041^{b}	0.51 ± 0.034^{a}
Total phenolic content (µg TA eq./ gm)	367.0 ± 26.52^{a}	635.0 ± 35.15^{c}	522.0 ± 30.17^{b}	439.0 ± 27.19^{ab}
DPPH radical-scavenging activity (%)	13.33 ± 1.06^{a}	26.40 ± 1.86^{c}	32.48 ± 1.40^{d}	18.85 ± 1.14^{b}
Reducing power (absorbance at 700nm)	0.26 ± 0.027^{a}	0.38 ± 0.026^{b}	0.40 ± 0.024^{b}	0.30 ± 0.018^{a}

*Mean±S.E. with different superscripts in a row differ significantly ($P<0.05$).
n =6 for each treatment

Table 22: ANOVA for calorific value, dietary fiber, total phenolic content and antioxidant activity of premium mutton nuggets enriched with nut based functional components

Parameters	Source of variation				
	Treatment		Error		F value
	d.f.	MSS	d.f.	MSS	
Calorific Values	3	1763.10	20	78.68	22.419**
Total Dietary fiber	3	0.08	20	0.007	12.127**
Total phenolic content	3	79595.33	20	5383.067	14.786**
DPPH radical-scavenging activity	3	424.21	20	11.73	36.152**
Reducing power	3	0.03	20	0.003	7.665**

* Significant (P<0.05); ** Highly significant (P<0.01)

4.3.3. Texture profile of premium mutton nuggets enriched with nut based functional components

In this part of study, the premium mutton nuggets developed by incorporation of optimum level of nut pastes viz: 20% of peanut, 15% of almond and 8% of pine nut were evaluated for textural properties viz: hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness. The mean values of these parameters for premium nuggets and control are presented in Table-23 and their corresponding ANOVA is given in Table-24.

The hardness scores were significantly higher, for treatment products except for peanut paste incorporated product which were comparable among each other. A higher score for hardness might be due to transformation technique adopted for peanut paste i.e. microwave treatment followed by grinding. It could have imparted grittiness in paste form which led to somewhat high hardness scores. Another probable reason might be surface hardening in comparatively less moistened product. Adhesiveness values for treatment products were comparable to control and also among themselves. Springiness and cohesiveness scores in treatment products were lower as compared to control product. Premium mutton nuggets with almond paste had significantly lower (P<0.05) springiness and cohesiveness values than others. Peanut paste incorporated product was having intermediate values for springiness and cohesiveness, whereas value for pine nut incorporated mutton nuggets were comparable to control. Decrease in springiness and cohesiveness values was indicative of poor binding and fragility of product. Farouk *et al*.,

(2000) reported that addition of ingredients reduces the proportion of water available to form a gel matrix between meat pieces, which could again limit the binding process. This behavior was attributed primarily to the dilution effect of non-meat ingredients in meat protein systems (Rocha-Garza & Zayas, 1996 and Tsai *et al*., 1998) or to their ability to reduce friction and/or binding among meat particles (Saleh & Ahmed, 1998); or again, in relation to cooking yield, it was suggested that less cooking loss made for products that were less rigid and more easily broken apart during binding valuations (Shao *et al*., 1999). The gumminess values for treatment products were comparable to control and also among themselves. The chewiness scores for treatment products were higher than control product. In peanut incorporated premium mutton nuggets, chewiness value was significantly higher ($P<0.05$) than control but comparable to almond and pine nut based premium mutton nuggets. In this aspect, almond incorporated product it was significantly higher ($P<0.05$) than control but comparable to peanut and pine nut based products. Presence of dietary fiber might have contributed to higher chewiness value. Adverse effect on textural properties of restructured beef steak incorporated with walnut has been reported by Colmenero *et al*., (2003).

On the basis of analysis of detailed product profile, premium mutton nuggets incorporated with optimum levels of nut pastes had improved lipid profile viz: low in cholesterol and FFA, high total dietary fiber and antioxidant capacity in terms of total phenolics, DPPH radical scavenging activity and reducing power assay than control.

Table 23. Texture profile of premium mutton nuggets enriched with nut based functional components. (Mean±S.E.)

Textural Properties	**Control**	**Nut paste**		
		Peanut (20%)	**Almond (15%)**	**Pine nut (8%)**
Hardness (N/cm^2)	51.82 ± 3.49^{ab}	57.50 ± 1.51^{b}	48.87 ± 2.00^{a}	50.11 ± 1.25^{a}
Adhesiveness (Ns/gms)	-0.08 ± 0.011	-0.11 ± 0.013	-0.12 ± 0.012	-0.11 ± 0.011
Springiness (cm/mm)	0.82 ± 0.037^{a}	0.67 ± 0.036^{bc}	0.56 ± 0.040^{c}	0.75 ± 0.048^{ab}
Cohesiveness	0.39 ± 0.036^{a}	0.33 ± 0.029^{ab}	0.28 ± 0.024^{b}	0.36 ± 0.025^{ab}
Gumminess (N/cm^2)	21.40 ± 1.47	21.02 ± 0.98	20.94 ± 1.14	22.21 ± 1.13
Chewiness	13.77 ± 1.00^{a}	18.12 ± 1.20^{b}	18.31 ± 1.05^{b}	16.81 ± 1.17^{ab}

*Mean±S.E. with different superscripts in a row differ significantly ($P<0.05$).

n =6 for each treatment

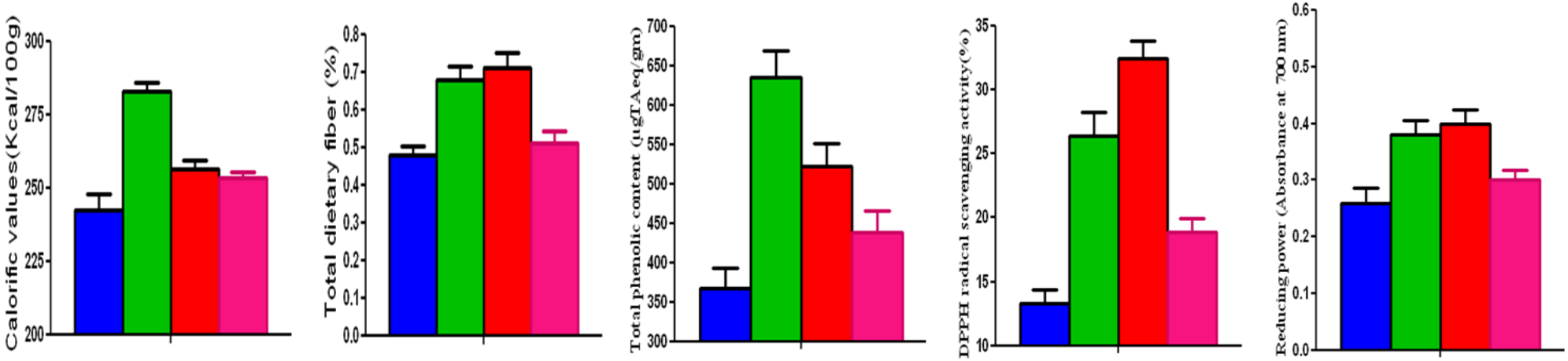


Fig-9- Calorific value, dietary fiber, total phenolic content and antioxidant activity of premium mutton nuggets enriched with nut based functional components

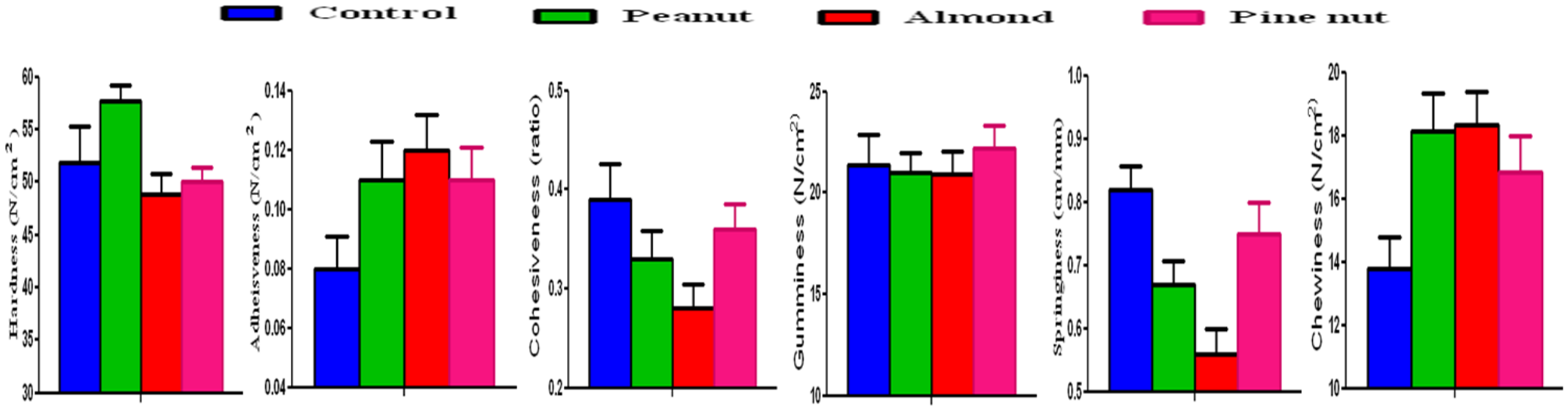


Fig-10- Texture profile of premium mutton nuggets enriched with nut based functional components

Table 24: ANOVA for texture profile of premium mutton nuggets enriched with nut based functional components.

Parameters	Source of variation				
	Treatment		Error		F value
	d.f.	MSS	d.f.	MSS	
Hardness (N/cm^2)	3	87.23	20	30.05	2.903
Adhesiveness (Ns/gms)	3	0.001	20	0.001	1.522
Springiness (cm/mm)	3	0.08	20	0.01	7.609**
Cohesiveness	3	0.01	20	0.005	2.786
Gumminess (N/cm^2)	3	1.10	20	8.55	0.233
Chewiness	3	26.46	20	7.396	3.577

* Significant (P<0.05); ** Highly significant (P<0.01)

4.4. Determination of the Storage Stability of Premium Mutton Nuggets at Refrigerated Storage (4±1°c).

Premium mutton nuggets incorporated with optimum levels (Experiment 2) of nut pastes viz. 20% peanut, 15% almond and 8% pine nut were stored as follows:

1. Aerobically packaged in low density polyethylene pouches (200 gauge) and stored at refrigerated temperature (4±1°C) for 15 days. The stored samples were analyzed at an interval of 5 days.
2. Vacuum packaged in impermeable nylon pouches (150 gauge) and stored at refrigerated temperature (4±1°C) for 60 days. The stored samples were analyzed at regular interval of 15 days.

The products were analyzed for physico-chemical parameters viz: pH, TBARS value, free fatty acids (FFA), peroxide value; instrumental colour analysis viz: redness, yellowness, hue and chroma; microbiological quality viz: standard plate count, psychrophilic count, coliform count and additionally in case of vacuum packaged product anaerobic plate count, lactic acid bacteria count and sensory characteristics for attributes such as general appearance, flavour, texture, juiciness and overall acceptability. The results are presented through statistically analysed tables (25-32) and critically discussed and reviewed.

4.4.1. Storage quality of aerobically packaged products

4.4.1.1 Physico-chemical parameters

The physico-chemical parameters (pH, TBARS, FFA and peroxide value) of aerobically packaged functional mutton patties during refrigerated storage are presented in Table -25 and their corresponding ANOVA is given in Table -26.

4.4.1.1.1 pH

There was increasing trend in the pH values of control as well as treatment premium mutton nuggets with the progressive increase in storage period. The pH of day 0 was comparable upto day 5 of storage and then significantly higher ($P<0.05$) on subsequent day 10 and 15 of storage. Among the treatments, peanut and pine nut paste incorporated products had significantly higher pH values than control and almond incorporated product throughout the storage period. The almond paste incorporated product had higher pH than control on day 0 and 5 but subsequent values were comparable. On day 0 and 5 , pH values of peanut and pine nut paste incorporated products were comparable but, on day 10, pH was significantly higher ($P<0.05$) for peanut product, whereas it was significantly higher for pine nut based products on day 15.

The increase in pH during storage could be due to protein breakdown and liberation of protein metabolites, mainly amines due to bacterial activity during storage (Jay, 1996). The pH of peanut and pine nut incorporated products were higher throughout the period of storage. The increased pH from day 5 onwards in all categories of products was indicative of progressive microbial proteolysis. Webster *et al*., (1982) reported that release of amino groups because of hydrolysis of collagen molecules in meat system raised its pH. An increase in pH of meat products during refrigerated storage was reported by McCarthy *et al*., (2001) in pork patties, Kumar and Sharma (2004a, b) in chicken patties, Rajkumar *et al*., (2004) in goat meat patties, Chidanandaiah *et al*., (2009) in buffalo meat patties and Kumar and Tanwar (2011) in chicken nuggets.

4.4.1.1.2. Thiobarbituric acid reacting substances (TBARS) values

The TBARS values of control as well as treatment premium mutton nuggets increased with storage period. The values were significantly higher ($P<0.05$) even on day 5, as compared day 0 values in case of control and pine nut incorporated products. However, in case of peanut and almond incorporated products the values on day 0 and 5 were comparable to each other. On day 10 and 15, the TBARS values increased significantly ($P<0.05$) as compared to earlier recorded values in all categories of products.

Among the treatments, TBARS values for peanut and pine nut incorporated products were comparable whereas control and pine nut incorporated products showed comparable values throughout storage period. The values for peanut and almond incorporated products were significantly less ($P<0.05$) as compared to control or pine nut incorporated products during entire storage period. The mean TBARS values were below the minimum threshold value of 1-2 mg malonaldehyde/kg meat (Watts, 1962) during the storage period. The increase in TBARS values was much higher at latter stage which was in accordance with findings of Gutiérrez *et al*., (2011) in lamb meat. General increase in TBARS values might be attributed to the lipid oxidation and the production of volatile metabolites. This was in agreement with the findings of Chidanandaiah *et al*., (2009), Reddy *et al*., (2009), Sudheer *et al*., (2011), Devatkal (2011) and Gadekar (2014) who also found a similar increase in TBARS values during storage of different meat products.

4.4.1.1.3. Free fatty acids

The free fatty acid values of control as well as treatment products also increased with progressive storage period. The FFA values on each subsequent storage period were found significantly higher ($P<0.05$) than previous ones in all categories of products. Control product had significantly higher FFA value than peanut and almond incorporated products throughout the storage period, but the values were comparable with that of pine nut incorporated product except on day 0. An increase in FFA values in meat products during storage because of lipase activity has been reported by many authors such as Fernandez and Rodriguez (1991) and Zalacain *et al*., (1995) in pork sausages, Yashoda *et al*., (2004) in fried chicken and Modi *et al*., (2004) in chicken nuggets

4.4.1.1.4.Peroxide value

The peroxide values of control as well as treatment products increased with progress of storage period. The peroxide values on each subsequent day were significantly higher ($P<0.05$) than previous readings in almost all categories of products throughout storage period except those of day 5 values of peanut and almond incorporated products, which were comparable to their respective day 0 readings. Peanut incorporated product had significantly less peroxide values than control and pine nut incorporated products throughout storage period. Almond incorporated product had shown slight inconsistency, and on day 0 and 15 of storage period, the peroxide values were comparable to all categories of products. Control and pine nut incorporated products showed comparable peroxide values throughout the storage period.

The significantly lower TBARS, free fatty acids and peroxides values for products with peanut and almond incorporated products might be due to associated antioxidant activity in their paste. Naturally-occurring phytochemicals in peanuts (Arachis hypogaea L.) such as tocopherols, carotenoids and polyphenolics, could have a role in slowing or preventing lipid oxidation due to their antioxidative nature (Angelo, 1996; Talcott *et al*., 2005 and Maestri *et al*., 2006). Similar, antioxidant activity of almond has been reported in various studies (Wu *et al*., 2004; Amarowicz *et al*., 2005 and Aiello *et al*., 2010). Incorporation of tocopherol and vitamin E in meat system has been associated with reduced lipid oxidation (Sante and Lacourt 1994, Pfalzgraf *et al*., 1995, Aksu and Kaya 2005, Rodríguez-Carpena *et al*., 2012 and Hygreeva *et al*., 2013). Maguire *et al*., (2004) reported higher levels of total tocopherols in almond, hazelnut, walnut, peanut and macadamia nuts (122.3-452.0 mg/g) and α tocopherol was the principal vitamin E analogue present in these nuts with the exception of walnut (Ryan *et al*., 2006).

Table 25: Effect of refrigerated storage on physico-chemical characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)			
	0	5	10	15
pH				
Control	6.28 ± 0.009^{a1}	6.30 ± 0.012^{a1}	6.39 ± 0.018^{b1}	6.44 ± 0.014^{c1}
Peanut (20%)	6.38 ± 0.009^{a3}	6.40 ± 0.011^{a3}	6.47 ± 0.015^{b3}	6.52 ± 0.012^{c2}
Almond (15%)	6.33 ± 0.011^{a2}	6.34 ± 0.012^{a2}	6.39 ± 0.010^{b1}	6.42 ± 0.012^{c1}
Pine nut (8%)	6.36 ± 0.012^{a3}	6.39 ± 0.011^{a3}	6.43 ± 0.011^{b2}	6.55 ± 0.014^{c3}
TBARS values (mg malonaldehyde/Kg)				
Control	0.19 ± 0.014^{a3}	0.26 ± 0.012^{b2}	0.31 ± 0.011^{c2}	0.40 ± 0.012^{d2}
Peanut (20%)	0.14 ± 0.011^{a1}	0.16 ± 0.010^{a1}	0.23 ± 0.010^{b1}	0.33 ± 0.015^{c1}
Almond (15%)	0.14 ± 0.010^{a12}	0.18 ± 0.010^{a1}	0.26 ± 0.012^{b1}	0.34 ± 0.010^{c1}
Pine nut (8%)	0.18 ± 0.012^{a23}	0.23 ± 0.010^{b2}	0.31 ± 0.016^{c2}	0.41 ± 0.010^{d2}
Free fatty acid values (% oleic acid)				
Control	0.36 ± 0.023^{a3}	0.43 ± 0.014^{b2}	0.50 ± 0.015^{c3}	0.61 ± 0.020^{d2}
Peanut (20%)	0.22 ± 0.017^{a1}	0.30 ± 0.015^{b1}	0.39 ± 0.016^{c1}	0.49 ± 0.016^{d1}
Almond (15%)	0.26 ± 0.021^{a1}	0.34 ± 0.018^{b1}	0.42 ± 0.012^{c2}	0.47 ± 0.018^{d1}
Pine nut (8%)	0.32 ± 0.015^{a2}	0.42 ± 0.016^{b2}	0.48 ± 0.019^{c3}	0.57 ± 0.013^{d2}
Peroxide values (meq oxygen/kg lipid)				
Control	0.62 ± 0.050^{a2}	0.81 ± 0.029^{b3}	1.01 ± 0.027^{c2}	1.25 ± 0.045^{d2}
Peanut (20%)	0.46 ± 0.020^{a1}	0.52 ± 0.030^{a1}	0.73 ± 0.029^{b1}	1.03 ± 0.057^{c1}
Almond (15%)	0.57 ± 0.045^{a12}	0.67 ± 0.040^{a2}	0.96 ± 0.049^{b2}	1.12 ± 0.032^{c12}
Pine nut (8%)	0.58 ± 0.031^{a2}	0.71 ± 0.035^{b23}	0.94 ± 0.047^{c2}	1.23 ± 0.032^{d2}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numeral) differ significantly ($P<0.05$). n =6 for each treatment.

Table 26: ANOVA for effect of refrigerated storage on physico-chemical characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters							
		pH		TBA		FFA		Peroxide Value	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	3	0.108	120.880**	0.197	242.809**	0.268	156.113**	1678	186.90**
Between treatments	3	0.047	52.213**	0.033	40.306**	0.083	48.523**	0.243	27.103**
Treatment × storage days	9	0.003	3.04**	0.001	1.122	0.001	0.735	0.009	0.965
Error (days of storage)	80	0.001	-	0.001	-	0.002	-	0.009	

* Significant (P<0.05); ** Highly significant (P<0.01).

4.4.1.2. Instrumental colour analysis

The results of instrumental colour analysis (redness, yellowness, hue and chroma) of aerobically packaged premium mutton nuggets observed during refrigerated storage on days 0, 5, 10 and 15 are presented in Table -27 and their corresponding ANOVA is given in Table -28.

4.4.1.2.1. Redness (a^* Value)

The redness (a* value) is the most important color parameter in evaluating meat oxidation, since a decrease in redness reduces the acceptability of the meat products to consumers (Renerre, 2000). There was a decreasing trend in the redness (a* value) as the storage time progressed in all categories of products. Redness scores (a values) for control and peanut incorporated products were comparable to their respective day 0 values, upto day 5 of storage but decreased significantly at each subsequent storage period. The values of almond incorporated product were comparable upto day 10 whereas those in pine nut were comparable throughout storage period. Among the treatments, peanut incorporated product had significantly higher redness (a^* value) than all other categories of products almost during entire storage period except on day 15, when it was comparable to almond based product. On the other hand, pine nut incorporated product had significantly lower redness (a^* value) than all other categories of products. Control and almond incorporated products had comparable redness (a^* values) throughout the storage. A decrease in a* values corresponding to decreased redness of lamb meat due to oxidation of myoglobin

and formation of metmyoglobin was reported by Kerry *et al*., (2000), Vergara and Gallego (2001) and Kennedy *et al*., (2004). The peanut paste incorporated product showed higher redness value because of i) characteristically brownish color of peanut hulls which were included while forming paste ii) 20% peanut incorporated product had comparatively less moisture which might have further concentrated the color pigment. Further, heat treatment before making paste could have induced Maillard reaction. In pine nut incorporated product, the paste itself was creamish resulting in dilution of redness value to such an extent that the product scored significantly lower redness than control. Rocha-Garza & Zayas (1996) have also reported the dilution of meat pigments by non-meat ingredients.

4.4.1.2.2. Yellowness (b^* Value)

The yellowness (b* value) also decreased in all categories of products with progressive increase in the period of storage. In all categories of products, except pine nut incorporated products yellowness on day 5 was comparable to day 0, but decreased thereafter. Peanut incorporated product had yellowness value comparable to control upto day 5 of storage and latter on significantly lower ($P<0.05$) yellowness than all other categories of products. Almond incorporated product had significantly higher ($P<0.05$) yellowness than control throughout the storage. Pine nut incorporated product maintained significantly higher ($P<0.05$) yellowness than all other categories of products throughout the storage period. The lower yellowness score in case of peanut incorporated product might be associated with the color of paste and antioxidant activity. Georgantelis *et al*., (2007) reported a decreasing trend of redness and yellowness values of beef burgers with the addition of rosemary extract during frozen storage and the decrease was negatively correlated with lipid oxidation. This report was in agreement with our results where clear correlation was observed between instrumental color and lipid oxidation (TBARS). Mancini and Hunt (2005) noted that the decrease of redness and yellowness is attributed to the gradual oxidation of myoglobin and accumulation of metmyoglobin.

4.4.1.2.3. Hue

The changes in Hue angle were quiet inconsistent. Hue angles remained almost comparable during the entire storage period in all categories of products except on day 15 in case of control and peanut incorporated products which showed significantly higher values than day 0 value. Among the treatments pine nut incorporated product had significantly higher ($P<0.05$) whereas peanut incorporated product had significantly lower

hue angle than other categories of products throughout storage. Hue angles for control and almond incorporated products were comparable in later stage of storage period i.e. day 10 and 15 of storage. Hue angle is a good descriptor of meat browning (Young Priolo, Simmons, & West, 1999; Lee, Decker, Faustman, & Mancini, 2005). Increase in hue angle is indicative of shift from redness to yellowness because of metmyoglobin formation. A hue angle of 0/360° is red, 90° is yellow, 180° is green and 270° is blue (Lindahl 2003). However, yellowness scores did not follow the usual pattern of increase in our results which might be due to characteristic colour of pastes and associated tocopherol, although redness scores declined shifting the derived hue angle towards an increase. Increase in hue angles with storage period was in agreement with findings of Luciano *et al*., (2009 a&b) and Greathousea *et al*., (2013).

Table 27: Instrumental colour analysis of aerobically packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)			
	0	5	10	15
Redness (a* value)				
Control	12.11 ± 0.99^{a2}	11.77 ± 0.84^{a2}	9.76 ± 0.49^{b12}	8.28 ± 0.46^{c12}
Peanut (20%)	15.02 ± 0.44^{a3}	14.04 ± 0.49^{a3}	12.37 ± 0.42^{b3}	10.39 ± 0.47^{c3}
Almond (15%)	12.73 ± 0.44^{a2}	11.82 ± 0.47^{a2}	10.41 ± 0.51^{ab2}	9.47 ± 0.52^{b23}
Pine nut (8%)	8.59 ± 0.49^{a1}	8.45 ± 0.50^{a1}	8.24 ± 0.43^{a1}	7.33 ± 0.27^{a1}
Yellowness (b* value)				
Control	17.85 ± 0.72^{a1}	16.60 ± 0.46^{ab1}	15.70 ± 0.23^{bc2}	14.83 ± 0.33^{c2}
Peanut (20%)	16.80 ± 0.33^{a1}	15.49 ± 0.39^{ab1}	14.20 ± 0.38^{bc1}	13.38 ± 0.32^{c1}
Almond (15%)	21.54 ± 0.68^{a2}	20.70 ± 0.46^{a2}	18.52 ± 0.36^{b3}	17.45 ± 0.30^{b3}
Pine nut (8%)	24.70 ± 0.49^{a3}	23.17 ± 0.36^{b3}	20.89 ± 0.60^{c4}	18.92 ± 0.33^{d4}
Hue angle				
Control	55.92 ± 2.68^{a2}	54.78 ± 2.31^{a2}	58.21 ± 1.18^{ab2}	60.96 ± 0.093^{b2}
Peanut (20%)	48.22 ± 0.49^{a1}	47.86 ± 0.47^{a1}	48.98 ± 0.69^{ab1}	52.27 ± 0.93 ^{b1}
Almond (15%)	59.41 ± 0.48^{a3}	60.28 ±1.12^{a3}	60.71 ± 1.21^{a2}	61.57 ±1.32^{a2}
Pine nut (8%)	70.78 ± 1.28^{a4}	70.01± 1.13^{a4}	68.48± 0.93^{a3}	68.81 ± 0.87^{a3}
Chroma				
Control	21.69 ± 0.70^{a1}	20.44 ± 0.46^{a1}	18.50 ± 0.39^{b1}	16.99 ± 0.50^{c1}
Peanut (20%)	22.54 ± 0.52^{a1}	20.91 ± 0.60^{b1}	18.84 ± 0.52^{c1}	16.95 ± 0.50^{d1}
Almond (15%)	25.02 ± 0.79^{a2}	23.86 ±0.46^{a2}	21.27 ± 0.44^{b2}	19.88 ±0.38^{b2}
Pine nut (8%)	26.18 ± 0.39^{a2}	24.61± 0.42^{a2}	22.47± 0.64^{b2}	20.30 ± 0.30^{c2}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numerals) differ significantly (P<0.05). n =6 for each treatment.

Table 28: ANOVA for instrumental colour analysis of aerobically packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters							
		Redness		Yellowness		Hue		Chroma	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	3	50.295	28.603**	77.511	65.702**	33.762	3.511*	133.117	83.125**
Between treatments	3	94.335	53.649**	239.501	203.012**	1668.756	173.549**	93.787	58.565*
Treatment × storage days	9	2.817	1.602	2.046	1.734	15.646	1.627	0.372	0.232
Error (days of storage)	80	1.758	-	1.180	-	9.615	-	1.601	-

* Significant (P<0.05); ** Highly significant (P<0.01).

4.4.1.2.4. Chroma

The chroma values invariably decreased with progress of storage period in all categories of products. At day 0 the chroma values were comparable to respective day 5 values, except peanut based product. On day 10 and 15 in each category of products, chroma values decreased and were significantly less (P<0.05) than day 0 or day 5 values. Chroma describes the colour saturation sometimes termed as vividness (Tapp *et al*., 2011). Greathousea *et al*., (2013) reported that it was a measure of the total color (larger value indicated more total color) and being a calculated value, the decrease was obvious with decrease in a^* and b^* values. **4.4.1.3. Microbiological quality**

The microbiological parameters (total plate count, psychrophilic count and coliform count) for the control as well as treatment premium mutton nuggets as detected on days 0, 5, 10 and 15 of refrigerated storage (4±1°C) in aerobic packaging are presented in Table-29 and their corresponding ANOVA is given in Table -30.

4.4.1.3.1 Total plate count

The mean values of the total plate count (TPC) for control as well as treatment products increased significantly (P< 0.05) at each subsequent storage interval. However, even after 15 days storage, the counts were well below the permissible limit of $\log_{10}7$ cfu/g for cooked meat products (Jay, 1996). Among the treatments, peanut and almond incorporated products had significantly higher count on day 0 but subsequently, all categories of products had comparable counts. Increase in TPC with storage period was obvious as meat products serve as good medium for microbial growth. Increase in total

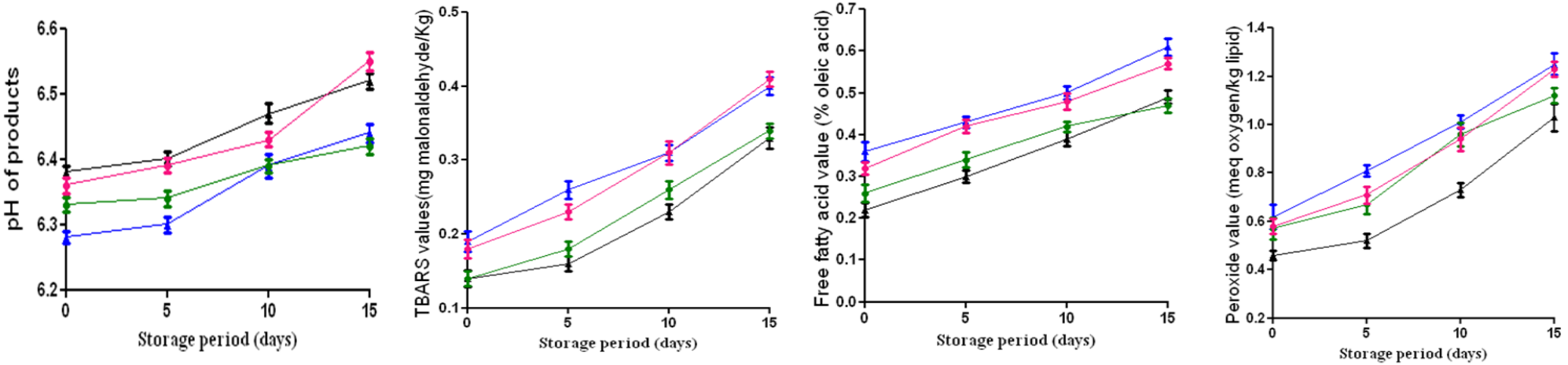


Fig-11- Effect of refrigerated storage on physico-chemical characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components

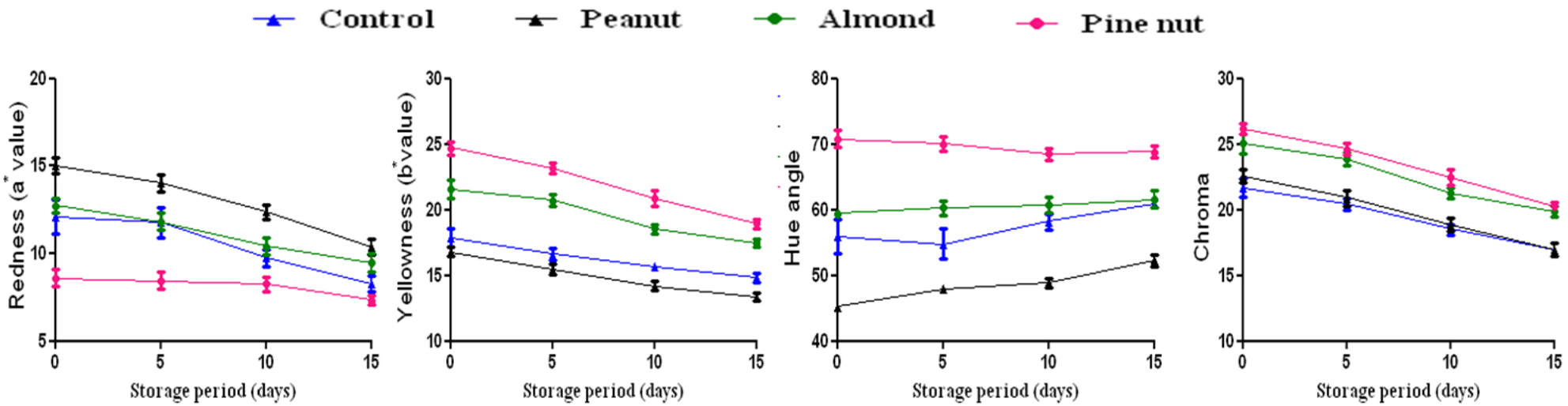


Fig-12- Instrumental colour analysis of aerobically packaged premium mutton nuggets enriched with nut based functional components

plate count of meat products has been reported by various workers like Sachindra *et al*., (2005), Kumar and Tanwar (2011), Syne *et al*., (2013), Bhat *et al*. (2010b), (2013a) and (2013b) who reported similar results in different meat products.

4.4.1.3.2 Psychrophilic count

The psychrophilic counts (PC) were not observed in control as well as treatment premium nuggets on day 0 and 5 of storage, although thereafter psychrophilic count increased significantly ($P<0.05$) at each storage interval. The psychrophilic count of almond and pine nut incorporated products were significantly higher on day 10 of their appearance as compared to control and peanut based products. On day 15 of storage period, the psychrophilic counts in all categories of products were comparable among themselves. In present study, PC remained below the threshold level for acceptability of cooked meat products that have been reported as $\log_{10}$ 4 cfu/gm (Jay, 1996) and $\log_{10}$ 4.6 cfu/gm (Cremer and Chipley, 1977). Absence of psychrophilic bacteria in the nuggets during initial periods of storage might be attributed to a retardation of log phase as a result of reduced metabolic rate due to sudden change in the physical environment. A detectable count on day 10 might be attributed to the fact that bacteria generally need some lag phase before active multiplication is initiated. . Similar results in early phase of aerobic refrigerated storage were reported by Kumar and Shrama (2004), Thomas *et al*., (2006), Gadekar *et al*., (2014) and various others authors.

4.4.1.3.3 Coliform count

The coliforms were not detected in control as well as treatment products throughout the storage period of 15 days. It could be due to the destruction of coliforms during cooking at high temperature, much above their death point of 57°C. Denis *et al*., (2006) found that coliform species were sensitive to heat treatment with a decimal reduction time under 2 min at 60^0C. In present study, the premium mutton nuggets were subjected to intense internal end point temperature of 75^0C. Further, good hygienic and sanitation practices followed during handling, processing and packaging of the cooked products could also account for the absence of coliforms. Similar results were reported by Sachdev and Gopal (2000) in cooked chicken rolls, Kumar and Sharma (2004a, b) in pork patties, Kandeepan *et al*., (2010) in buffalo meat keema, Bhat *et al*., (2012) in mutton harrisa and Zargar *et al*., (2014) in chicken sausages.

Table 29: Effect of refrigerated storage on microbiological characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)			
	0	5	10	15
Total plate count (log_{10} cfu/gm)				
Control	0.96 ± 0.208^{a1}	1.61 ± 0.154^{b1}	2.64 ± 0.052^{c1}	3.18 ± 0.040^{d1}
Peanut (20%)	1.23 ± 0.078^{a12}	1.83 ± 0.079^{b1}	2.73 ± 0.082^{c1}	3.20 ± 0.017^{d1}
Almond (15%)	1.31 ± 0.105^{a2}	1.88 ± 0.089^{b1}	2.81 ± 0.077^{c1}	3.25 ± 0.014^{d1}
Pine nut (8%)	1.15 ± 0.067^{a12}	1.74 ± 0.088^{b1}	2.76 ± 0.051^{c1}	3.26 ± 0.021^{d1}
Psychrophilic count (log_{10} cfu/gm)				
Control	Not detected	Not detected	0.77 ± 0.248^{a1}	1.62 ± 0.125^{b1}
Peanut (20%)	Not detected	Not detected	0.80 ± 0.263^{a1}	1.74 ± 0.090^{b1}
Almond (15%)	Not detected	Not detected	1.18 ± 0.085^{a2}	1.83 ± 0.080^{b1}
Pine nut (8%)	Not detected	Not detected	1.15 ± 0.067^{a2}	1.67 ± 0.111^{b1}
Coliform count (log_{10} cfu/gm)				
Control	Not detected	Not detected	Not detected	Not detected
Peanut (20%)	Not detected	Not detected	Not detected	Not detected
Almond (15%)	Not detected	Not detected	Not detected	Not detected
Pine nut (8%)	Not detected	Not detected	Not detected	Not detected

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

n =6 for each treatment.

Table 30: ANOVA for microbiological characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters			
		TPC		Psychrophilic count	
		MSS	F Value	MSS	F Value
Between storage days	3	21.719	419.470**	16.667	240.461**
Between treatments	3	0.193	3.897*	0.116	1.668
Treatment × storage days	9	0.020	0.409	0.076	1.095
Error (days of storage)	80	0.049	-	0.069	-

** Highly significant (P<0.01).

4.4.1.4. Sensory quality

Mean sensory scores for aerobically packaged premium mutton nuggets during storage at 4±1°C are presented in Table-31 and their corresponding ANOVA is given in Table-32. The sensory scores for control as well as nut paste incorporated premium mutton nuggets showed a decreasing trend with increase in storage period. The general appearance scores for all the treatment products and control showed a progressive non-significant decline ($P > 0.05$) with increase in the storage period. Among the treatments, 15% almond incorporated product showed significantly lower general appearance scores as compared to control throughout the storage period. Peanut and pine nut incorporated products maintained their general appearance scores comparable to control during entire period of storage. The decrease in appearance scores might be due to pigment and lipid oxidation. A comparable general appearance score for chevon nuggets during storage was reported by Das *et al*., (2006).

There was gradual decrease ($P > 0.05$) in flavour scores for control and treatment products upto day 10 but the scores declined significantly ($P < 0.05$) on day 15 of storage as compared to day 0 score, in all categories of products. The flavour scores of nut incorporated products were marginally lower than control but comparable among themselves throughout the period of storage. A gradual decline in flavour scores might be due to expected loss of volatile flavour components on storage of meat products. The progressive decrease in flavour scores could also be correlated to increase in TBARS values and free fatty acids in the meat products (Tarladgis *et al.*, 1960) under aerobic conditions. A decrease in flavour scores during storage was also reported by Sahoo and Anjeneyulu (1997), Kumar and Sharma (2004), Thomas *et al*., (2006) and Das *et al*., (2008).

Juiciness scores for control and treatment products declined gradually with increase in the period of storage and were significantly lower ($P < 0.05$) on day 15 of storage period as compared to respective initial score in all categories of products. The treatment products had marginally lower scores than control product and among treatment products, peanut incorporated product had least scores throughout the storage. The scores for juiciness in all categories of products were comparable among themselves. A gradual decrease in juiciness scores might be attributed to loss of moisture from the products during aerobic refrigerated

storage. Similar results were reported by Huffman *et al*., (1987), Sahoo and Anjaneyulu (1997), Kumar and Sharma (2004), Ahamad *et al*., (2007) and many others.

The texture scores of the control as well as treatment products decreased with progressive increase in storage period. Except peanut incorporated product which had comparable scores to its initial score throughout storage period, all other categories of products showed significantly lower ($P<0.05$) texture scores to their 0 day score on 15th day of storage. All categories of products had comparable texture scores throughout storage period. Decline in texture might be attributed to proteolytic and disulphide bonds changes (Santamaria *et al*., 1992) taking place with progress of storage period. Peanut incorporated product had additional protein from high level of substitution which might be held responsible for comparable texture scores throughout storage period. A decrease in texture scores of meat products with advancement of storage period was has been reported in sausages (Skrede 1989) and bologna (Colmenero *et al.* 1996) and others.

The overall acceptability scores decreased gradually as the storage period increased. Control and peanut incorporated products could maintain their overall acceptability scores comparable to day 0 scores throughout storage period. However, almond and pine nut incorporated products showed significantly lower ($P<0.05$) scores than their initial scores on day 15 of storage. In these products overall acceptability scores were comparable to their day 0 scores only upto day 10 of storage. Among the treatments, overall acceptability scores of peanut incorporated product was slightly lower than other categories while control product had a marginal edge in overall acceptability scores than others throughout storage. A decrease in overall acceptability scores was expected because of decrease in scores of other attributes with the advancement of storage period. Peanut incorporated product with maximum level of substitution had lower but comparable overall acceptability scores throughout storage period. The decrease in overall acceptability scores of the products during storage has been reported by various authors such as Radziejewska *et al*., (2008) in poultry meat products, Biswas *et al*., (2011) in duck patties, Malav *et al*., (2013) in restructured meat rolls and Zargar *et al*., (2014) in chicken sausages.

These observations from the present study indicated that premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond, and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and good to very good sensory ratings during aerobic storage in

LDPE pouches under refrigeration at 4±1°C for 15 days. Hence, premium mutton nuggets evolved in this study could be safely stored upto 15 days at 4±1°C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

Table 31: Effect of refrigerated storage on sensory attributes of aerobically packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)			
	0	5	10	15
General appearance				
Control	7.09 ± 0.069^{a1}	7.06 ± 0.042^{a1}	6.97 ± 0.044^{a1}	6.92 ± 0.076^{a1}
Peanut (20%)	6.91 ± 0.063^{a1}	6.88 ± 0.079^{a12}	6.83 ± 0.062^{a12}	6.79 ± 0.095^{a12}
Almond (15%)	6.83 ± 0.062^{a2}	6.81 ± 0.067^{a2}	6.73 ± 0.041^{a2}	6.67 ± 0.050^{a2}
Pine nut (8%)	6.99 ± 0.052^{a1}	6.94 ± 0.036^{a1}	6.87 ± 0.041^{a12}	6.83 ± 0.076^{a12}
Flavour				
Control	7.05 ± 0.061^{a1}	6.95 ± 0.057^{ab1}	6.82 ± 0.071^{ab1}	6.73 ± 0.082^{b1}
Peanut (20%)	6.81 ± 0.109^{a2}	6.76 ± 0.092^{ab1}	6.70 ± 0.085^{ab1}	6.65 ± 0.067^{b1}
Almond (15%)	6.88 ± 0.079^{a12}	6.82 ± 0.071^{ab1}	6.72 ± 0.045^{ab1}	6.63 ± 0.057^{b1}
Pine nut (8%)	6.90 ± 0.066^{a2}	6.82 ± 0.071^{ab1}	6.74 ± 0.053^{ab1}	6.60 ± 0.0075^{b1}
Juiciness				
Control	7.09 ± 0.064^{a1}	7.01 ± 0.063^{ab1}	6.90 ± 0.071^{ab1}	6.81 ± 0.075^{b1}
Peanut (20%)	6.91 ± 0.086^{a1}	6.83 ± 0.0062^{ab1}	6.76 ± 0.092^{ab1}	6.64 ± 0.107^{b1}
Almond (15%)	6.98 ± 0.076^{a1}	6.91 ± 0.080^{ab1}	6.84 ± 0.096^{ab1}	6.74 ± 0.071^{b1}
Pine nut (8%)	7.04 ± 0.048^{a1}	6.95 ± 0.045^{a1}	6.84 ± 0.064^{ab1}	6.75 ± 0.082^{b1}
Texture				
Control	7.10 ± 0.062^{a1}	6.96 ± 0.079^{ab1}	6.90 ± 0.080^{ab1}	6.80 ± 0.071^{b1}
Peanut (20%)	6.89 ± 0.086^{a1}	6.80± 0.071^{a1}	6.73 ± 0.090^{a1}	6.64 ± 0.056^{a1}
Almond (15%)	6.89 ± 0.086^{a1}	6.83 ± 0.094^{ab}	6.73 ± 0.090^{ab1}	6.62 ± 0.0085^{b1}
Pine nut (8%)	7.12 ± 0.035^{a1}	6.95 ± 0.050^{ab1}	6.87 ± 0.089^{b1}	6.80 ± 0.071^{b1}
Overall acceptability				
Control	7.01 ± 0.058^{a1}	6.94 ± 0.079^{a1}	6.84 ± 0.096^{a1}	6.84 ± 0.073^{a1}
Peanut (20%)	6.79 ± 0.114^{a1}	6.73 ± 0.076^{a1}	6.64 ± 0.107^{a1}	6.58 ± 0.100^{a1}
Almond (15%)	6.90 ± 0.080^{a1}	6.83 ± 0.062^{ab1}	6.73 ± 0.060^{ab1}	6.63 ± 0.053^{b1}
Pine nut (8%)	7.02 ± 0.079^{a1}	6.94 ± 0.062^{ab1}	6.83 ± 0.077^{ab1}	6.73 ± 0.021^{b1}

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

n =21 for each treatment.

Table 32: ANOVA for effect of refrigerated storage on sensory attributes of aerobically packaged premium mutton nuggets enriched with nut based functional components.

Source of variation	d.f.	Mean sum of square									
		General appearance		Flavour		Texture		Juiciness		Overall acceptability	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	3	0.395	4.908**	1.014	9.066**	1.098	9.127**	1.218	9.862*	0.916	6.972**
Between treatments	3	0.933	11.599**	0.406	3.630*	0.395	3.284*	0.824	6.671**	0.849	6.456**
Treatment × storage days	9	0.004	0.046	0.022	0.196	0.006	0.047	0.013	0.107	0.015	0.712
Error (days of storage)	320	0.080	-	0.112	-	0.120	-	0.123	-	0.131	-

* Significant (P<0.05); ** Highly significant (P<0.01).

4.4.2. Storage quality of vacuum packaged products

4.4.2.1. Physico-chemical parameters

The physico-chemical parameters (pH, TBARS and FFA) of vacuum packaged premium mutton nuggets observed on 0, 15, 30, 45 and 60 days during refrigerated storage are presented in Table-33 and their corresponding ANOVA is given in Table-34.

4.4.2.1.1 pH

The pH values of control and treatment products except pine nut incorporated product showed initial stability upto day15 of storage and had a declining trend thereafter. The pH values of control, peanut and almond incorporated products were significantly lower (P<0.05) at each subsequent interval compared to their preceding observation from day 30 onwards. The pine nut incorporated product showed a faster decline and pH values on day 15 onwards and were significantly lower (P<0.05) at each subsequent storage interval. Among the treatments, pH of peanut and pine nut incorporated products were comparable at day 0 and significantly higher than control and almond incorporated products. On day 15, peanut incorporated product still maintained significantly higher pH

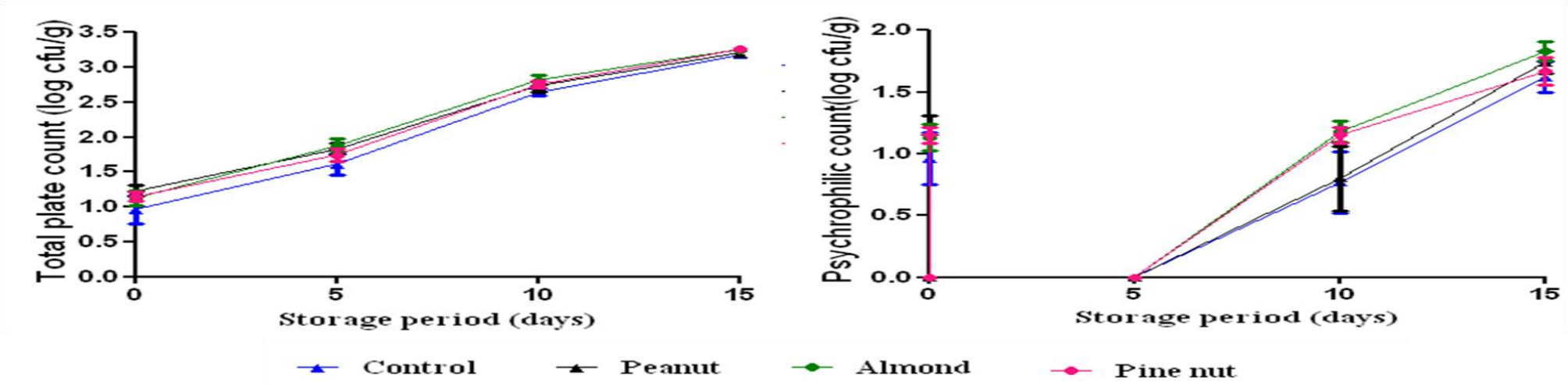


Fig- 13-Effect of refrigerated storage on microbiological characteristics of aerobically packaged premium mutton nuggets enriched with nut based functional components

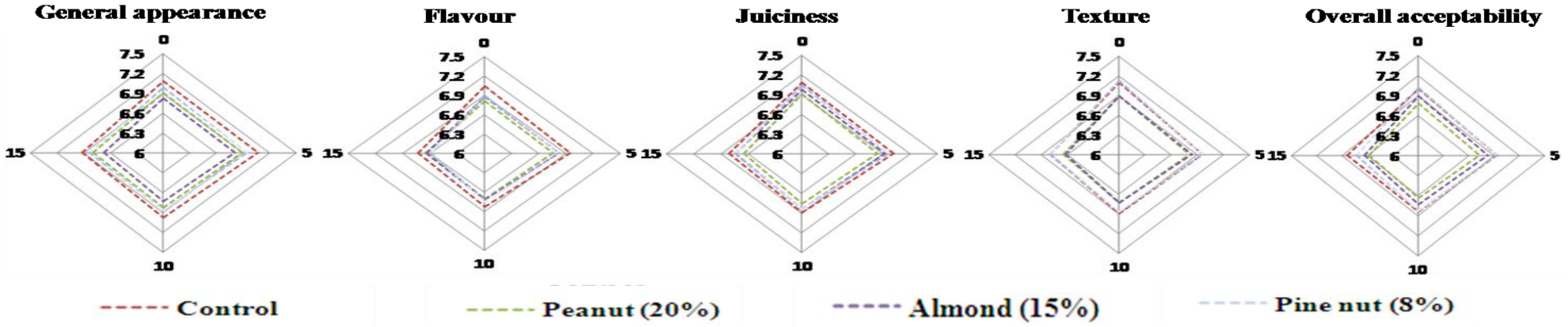


Fig- 14- Effect of refrigerated storage on sensory attributes of aerobically packaged premium mutton nuggets enriched with nut based functional components

than other products and on day 30 onwards, all categories of products except that of pine nut had comparable pH values.

A decline in pH values of vacuum packaged meat products might be attributed to the anaerobic cycle in later period of storage. Breakdown of carbohydrate components to organic acids might be the reason of comparatively faster pH decline in treatment products particularly pine nuts. Mahmoudzadeh *et al*., (2010) reported that constant or lower pH levels might be attributed to increasing solubility of CO_2 with storage time, effecting growth of aerobic microflora. A decline in pH of vacuum packaged product with storage has also been reported by Sallama and Samejimab (2004), Kumar *et al*., (2007), Taheri and Motallebi (2012) and Hur *et al*., (2013).

4.4.2.1.2.Thiobarbituric acid reacting substances (TBARS) valuess

The TBARS values gradually increased with the advancement of storage period and were comparable upto day 15 of storage in all categories of products. After day 15, there was significant increase at each subsequent interval. Among the treatment products TBARS values were quiet inconsistent. Peanut and almond incorporated products had TBARS values comparable to each other and lower than control and pine nut incorporated products throughout storage period. Control and pine nut incorporated products had comparable TBARS values during entire storage period except on day 45 of observation, when pine nut incorporated products showed higher TBARS values. At the end of day 60, all categories of products had comparable TBARS values which were still lower than threshold value of 1-2 mg malonaldehyde/kg meat (Watts, 1962).

An increase in TBARS values with the advancement of storage period might be attributed to lipid oxidation. The vacuum packaging could drastically reduce the rate of change in TBARS values, but lipolytic organism and presence of pro-oxidant such as salt in the product formulation might have played a role in increasing TBARS values of the products even in absence of oxygen. A similar increase in TBARS values in vacuum packaged meat products was also reported by Ahn *et al.*, (1993), Przysiężna (2007), Kumar *et al*., (2007), Popova *et al*., (2009) and Hur *et al*., (2013).

4.4.2.1.3.Free fatty acids

The free fatty acid (FFA) content in control and treatment products showed a gradual increase but showed only marginal increase upto day 15 of storage. Then day 30 onwards FFA values increased significantly ($P<0.05$) at each subsequent storage interval.

In general, peanut and almond incorporated products showed comparable FFA values and control and pine nut incorporated products showed comparable FFA values during the entire period of storage. Moreover, peanut and almond incorporated products showed significantly lower ($P<0.05$) FFA values than control or pine incorporated products.

The increase in free fatty acids values might be attributed to the hydrolysis of phospholipids and triglycerides by the action of lipases and phospholipases (Serdaroglu and Felekoglu, 2005) which might be of microbial origin. Monin *et al*., (2003) reported that increase in FFA took place because of hydrolysis of lipids viz; triglycerides, diglycerides and phospholipids by endogenous lipolytic enzymes or other factors. As FFAs are produced by hydrolysis, it was expected that the vacuum packaged premium mutton nuggets with the highest moisture levels would have higher levels of FFAs and in this study it could be the reason for the lowest FFA in peanut incorporated product. Increase in FFA values of meat products during the period of storage under vacuum packaging has also been reported by Rao *et al*., (1996), (Modi *et al*., 2004a & b), Kumar and Sharma (2007), Khaksar *et al*., (2010), Yilmaz, and Demirci, (2010) and Ježek and Buchtová (2011).

4.4.2.1.4. Peroxide values

The peroxides values of control and treatment premium mutton nuggets increased with the progress of storage period. The treatment mutton nuggets had comparable peroxide values upto day 15 reflecting initial stability. There was significant increase in peroxide values of treatment on day 30. Control and pine nut incorporated products had comparable peroxide values on days 45 and 60 of storage whereas there were inconsistencies in the rate of increase of peroxide values throughout storage among various treatments. Initial and day 60 of storage, peroxide values for all categories of products were comparable. Peanut incorporated product maintained least value among the treatments throughout storage period followed by almond incorporated products and then pine nut based products. Pine nut based products had highest values which were comparable to that of control product.

The peroxide value measures primary products of lipid oxidation and is used to determine the oxidative state of lipid containing foods (Istrati *et al*., 2011). Peroxides are intermediate reaction products, which react further to form the odorous aldehydes, ketones, and other materials that contribute to flavor deterioration of foods (Frankel, 1991) and

indicative of oxidative rancidity. Inconsistency in the rate of increase of peroxides values was expected as it measures transitory compounds. Further, induction period for generation of peroxides are dependent on nature of the fat and the presence of antioxidants (Gheisari, 2011). A positive correlation has been observed between different lipid oxidation parameters viz: TBARS values, FFA and peroxide values and increase with progress of storage period has been reported by various authors such as Przysiężna *et al*., (2007), Gheisari, (2011), Istrati *et al*., (2011) and Bilsaka *et al*.,(2012).

4.4.2.2. Instrumental colour

The results of instrumental colour analysis (redness, yellowness, hue and chroma) of vacuum packaged premium mutton nuggets observed during refrigerated storage on 0, 15, 30, 45 and 60 days are presented in Table 35 and their corresponding ANOVA is given in Table 36.

There was a decreasing trend in the redness (a* value) values with increase in storage period in control as well as treatment products. The decrease in redness (a^*) values in all the groups in early storage period was comparatively faster than the later part of storage. The redness values for almost all categories of products were significantly lower ($P<0.05$) at each subsequent interval of observation. Except for peanut incorporated product which continued to decrease significantly ($P<0.05$) upto day 45 of storage, all other products showed marginal decline in their redness values from day 30 onwards and redness values remained comparable thereafter. Among the treatment products, pine nut incorporated product maintained lowest redness values throughout storage period and was comparable with control at the end of day 60. Peanut incorporated product showed higher redness values than other groups during entire storage period except day 60, when almond incorporated product had the highest value. Almond incorporated product and control products had comparable redness values comparable throughout the storage.

The decrease in redness (a^*) value with increase in storage period might be attributed to oxidative changes in lipid and pigments. Hur *et al*., (2013) reported less influence of packaging method on myoglobin redox chemistry but cited that decrease in redness is associated with rancidity. Decrease in redness value with progress of storage period under vacuum has also been reported by Du *et al*., (2002), Kenawi *et al*. (2004), and Hur *et al*., (2013).

Table 33: Effect of refrigerated storage on physico-chemical characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)				
	0	15	30	45	60
pH					
Control	6.28 ± .009a^{a3}	6.28 0.007^{a2}	6.14± 0.012^{b1}	5.95 ± 0.018^{c1}	5.89 ± 0.018^{d1}
Peanut (20%)	6.38 ± 0.009^{a1}	6.39 ± 0.009^{a1}	6.11 ± 0.009^{b1}	5.88 ± 0.022^{c2}	5.72 ± 0.011^{d3}
Almond (15%)	6.32 ± 0.009^{a2}	6.32 ± 0.007^{a2}	6.13 ± 0.011^{b1}	5.93 ± 0.017^{c1}	5.78 ± 0.007^{d2}
Pine nut (8%)	6.36 ± 0.012^{a1}	6.29 ± 0.08^{b2}	6.14 ± 0.011^{c1}	5.94 ± 0.009^{d1}	5.86 ± 0.015^{e1}
TBARS values (mg malonaldehyde/Kg)					
Control	0.19 ± 0.014^{a1}	0.22 ± 0.008^{a1}	0.28 ± 0.009^{b1}	0.32 ± 0.007^{b1}	0.43± 0.074^{c1}
Peanut (20%)	0.14 ± 0.011^{a1}	0.15 ± 0.007^{a2}	0.22 ± 0.007^{b2}	0.32 ± 0.011^{c1}	0.40 ± 0.013^{d1}
Almond (15%)	0.14 ± 0.009^{a1}	0.17 ± 0.008^{a12}	0.24 ± 0.010^{b12}	0.32± 0.007^{c1}	0.38± 0.008^{c1}
Pine nut (8%)	0.18 ± 0.012^{a1}	0.21 ± 0.008^{a12}	0.28 ± 0.019^{b12}	0.39 ± 0.010^{c2}	0.42 ± 0.010 ^{c1}
Free fatty acids values (%)					
Control	0.36 ± 0.023^{a1}	0.39 ± 0.012^{a1}	0.45 ± 0.011^{b1}	0.51 ± 0.015^{c1}	0.57 ± 0.018^{d2}
Peanut (20%)	0.22 ± 0.017^{a2}	0.26 ± 0.014^{a2}	0.32 ± 0.016^{b2}	0.44 ± 0.015^{c2}	0.52 ± 0.017^{d1}
Almond (15%)	0.25 ± 0.015^{a2}	0.28 ± 0.016^{a2}	0.37 ± 0.011^{b3}	0.43 ± 0.015^{c2}	0.53 ± 0.015^{d1}
Pine nut (8%)	0.32 ± 0.015^{a1}	0.34± 0.011^{a3}	0.43 ± 0.018^{b1}	0.53 ± 0.011^{c1}	0.55 ± 0.014^{c12}
Peroxide values					
Control	0.62 ± 0.050^{a1}	0.66 ± 0.024^{b3}	0.79 ± 0.019^{b1}	1.05 ± 0.044^{c1}	1.08 ± 0.017^{c1}
Peanut (20%)	0.46 ± 0.020^{a2}	0.46 ± 0.021^{a1}	0.64 ± 0.021^{b2}	0.84 ± 0.040 ^{c2}	1.00 ± 0.025^{d1}
Almond (15%)	0.57 ± 0.045^{a12}	0.53 ± 0.021^{a2}	0.78 ± 0.044^{b1}	0.89 ± 0.035^{b2}	1.10 ± 0.100^{c1}
Pine nut (8%)	0.58 ± 0.031^{a1}	0.59 ± 0.015^{ab2}	0.74 ± 0.047 ^{b12}	1.03 ± 0.032^{c1}	1.09 ± 0.031^{c1}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numeral) differ significantly (P<0.05). n =6 for each treatment.

Table 34: ANOVA for effect of refrigerated storage on physico-chemical characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters							
		pH		TBA		FFA		Peroxide Value	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	4	1.297	1429.629**	0.255	113.732**	0.284	203.205**	1.316	140.285**
Between treatments	3	0.003	3.358*	0.020	9.042**	0.075	53.818**	0.134	14.297**
Treatment × storage days	12	0.017	18.260**	0.001	0.593	0.002	1.750	0.011	1.120
Error (days of storage)	100	0.001	-	0.002	-	0.001	-	0.009	-

* Significant (P<0.05); ** Highly significant (P<0.01).

The yellowness scores of control and treatment products decreased with progress of storage period. Yellowness values in all categories of products decreased significantly ($P<0.05$) at each subsequent storage interval upto day 30 of storage. In all categories of products, yellowness values on day 45 were comparable to their day 30 value. Among the treatment products, yellowness values of pine nut incorporated product were higher than all other categories of products throughout storage period. Almond incorporated product ranked second in yellowness values followed by control and peanut incorporated product.

The yellowness value is dependent up on lipid oxidation and generally increases with storage period. The observations in this study were contrary to this perception which could be due to the presence of nitrite in the formulation. Harms *et al*., (2003) reported that nitrite could bind to myoglobin to form a nitrosyle myoglobin which might not act as a catalyst of lipid peroxidation. The decrease in yellowness scores in meat products during storage was also reported by Mancini and Hunt (2005); Georgantelis *et al*., (2007) and Cachaldora *et al*., (2013) in beef burger, meat sausages and blood sausages respectively.

In general, hue values increased gradually with progress of storage period. Hue values of pine incorporated product remained comparable throughout storage period. In peanut incorporated product, hue values were comparable upto day 30 of storage and then increased significantly ($P<0.05$) on day 45 to remain comparable on day 60 of storage as well. Control and almond incorporated products showed comparable yellowness value upto day 15, then increased and remained comparable upto day 60 of storage. The increase in hue index was explained by the gradual oxidation of myoglobin and accumulation of metmyoglobin with time (Ruiz *et al*., 2003; Mancini and Hunt, 2005). The hue being a derived value depended on redness and yellowness values. An increase in hue with progressive storage period under vacuum for meat products was also reported by Rodas-González *et al*., (2011) in beef steak.

The chroma values of control and treatment products decreased with advancement of storage period. In general, chroma values for all categories of products decreased significantly at each subsequent storage interval. In peanut incorporated product, chroma value on day 45 was also significantly lower ($P<0.05$) than day 30 value. Control and pine nut incorporated product behaved similarly and their chroma values on day 45 were comparable to respective day 30 values, but values on day 60 were significantly lower

Table 35: Instrumental colour analysis of vacuum packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)				
	0	15	30	45	60
Redness					
Control	12.11± 0.99^{a1}	9.56 ± 0.70 ^{b1}	6.91 ± 0.36^{c1}	5.94 ± 0.25^{c1}	5.59 ± 0.26^{c12}
Peanut (20%)	15.02 ±0.44^{a2}	11.56 ± 0.30^{b2}	9.70 ± 0.32^{c2}	7.72 ± 0.38^{d2}	6.39± 0.23^{d12}
Almond (15%)	12.73 ± 0.44^{a1}	9.16 ± 0.46^{b1}	7.58 ± 0.42^{c1}	7.31 ± 0.49^{c2}	6.95± 0.42^{c2}
Pine nut (8%)	8.59± 0.49^{a3}	6.78± 0.42^{b3}	6.41± 0.29^{bc1}	5.83± 0.24^{bc1}	5.28± 0.20^{c1}
Yellowness					
Control	17.68 ± 0.71^{a1}	15.10 ± 0.35^{b1}	13.70 ± 0.23^{c1}	12.10 ± 0.43^{c1}	10.63 ± 0.54^{d1}
Peanut (20%)	16.80 ± 0.33^{a1}	13.69 ± 0.39^{b2}	11.70 ± 0.22^{c2}	11.22 ± 0.29^{c2}	10.63 ± 0.39^{c1}
Almond (15%)	21.54 ± 0.68^{a2}	17.54 ± 0.35^{b3}	16.11 ± 0.27^{c3}	15.28 ± 0.29^{cd3}	14.05 ± 0.35^{d2}
Pine nut (8%)	24.70 ± 0.49^{a3}	20.49 ± 0.53^{b4}	18.22 ± 0.50^{c4}	17.09 ± 0.24^{c4}	15.54 ± 0.34^{d3}
Hue					
Control	55.68 ± 2.67^{a1}	57.79 ± 2.13^{a1}	63.30 ± 0.98^{b1}	65.44 ± 0.51^{b1}	61.99 ± 2.11^{b12}
Peanut (20%)	48.22 ± 0.49^{a2}	49.82 ± 0.72^{a2}	50.38 ± 0.86^{a2}	55.56 ± 0.72^{b2}	58.87 ± 1.62^{b1}
Almond (15%)	59.41± 0.48^{a1}	62.49 ± 1.05^{ab3}	64.87 ± 1.13^{b1}	64.52 ± 1.43^{b1}	63.73 ± 1.40^{b2}
Pine nut (8%)	70.78 ± 1.24^{a3}	71.72 ± 0.98^{a4}	70.63 ± 0.71^{a3}	71.18 ± 0.71^{a3}	71.20 ± 0.87^{a3}
Chroma					
Control	21.55 ± 0.69^{a1}	17.93 ± 0.41^{b1}	15.35 ± 0.33^{c1}	14.29 ± 0.48^{c1}	12.05 ± 0.40^{d1}
Peanut (20%)	22.54 ± 0.52^{a1}	17.93 ± 0.44^{b1}	15.21 ± 0.32^{c1}	13.62 ± 0.45^{d1}	12.42 ± 0.28^{d1}
Almond (15%)	25.02 ± 0.79^{a2}	19.80 ± 0.45^{b2}	17.82 ± 0.36^{c2}	16.97 ± 0.37^{cd2}	15.69 ± 0.40^{d2}
Pine nut (8%)	26.18 ± 0.39^{a2}	21.60± 0.56^{b3}	19.32 ± 0.53^{c3}	18.06 ± 0.25^{c3}	16.42 ± 0.30^{d2}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numeral) differ significantly (P<0.05). n =6 for each treatment.

Table 36: ANOVA for Instrumental colour analysis values of vacuum packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters							
		Redness		Yellowness		Hue		Chroma	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	4	140.987	120.181**	198.039	188.004**	137.968	14.011*	340.554	273.561**
Between treatments	3	63.930	54.496**	249.300	236.667**	1740.646	176.763**	123.450	99.165**
Treatment × storage days	12	5.807	4.950**	2.214	2.102*	36.214	3.678**	0.867	0.696
Error (days of storage)	100	1.173	-	1.053	-	9.847	-	1.245	-

* Significant (P<0.05); ** Highly significant (P<0.01).

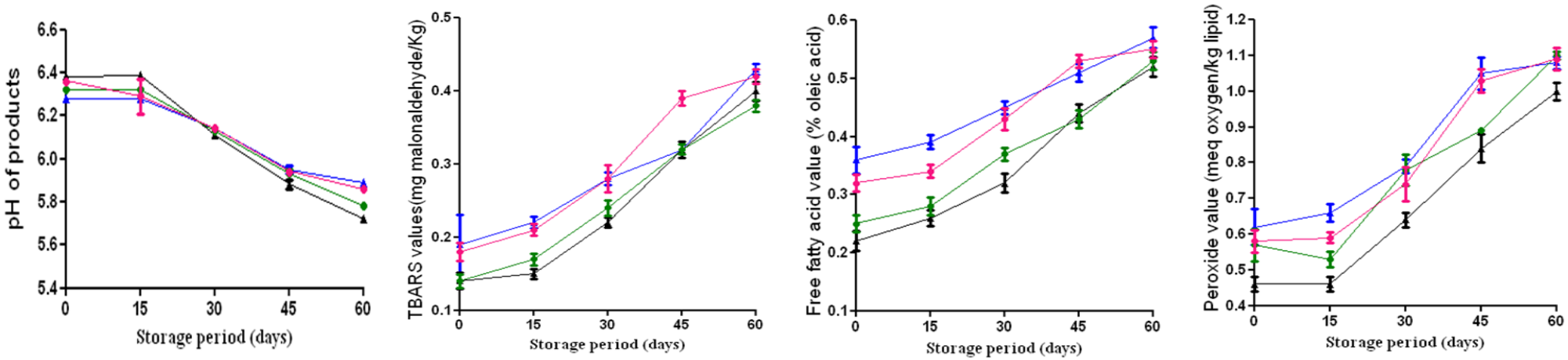


Fig-15- Effect of refrigerated storage on physico-chemical characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components

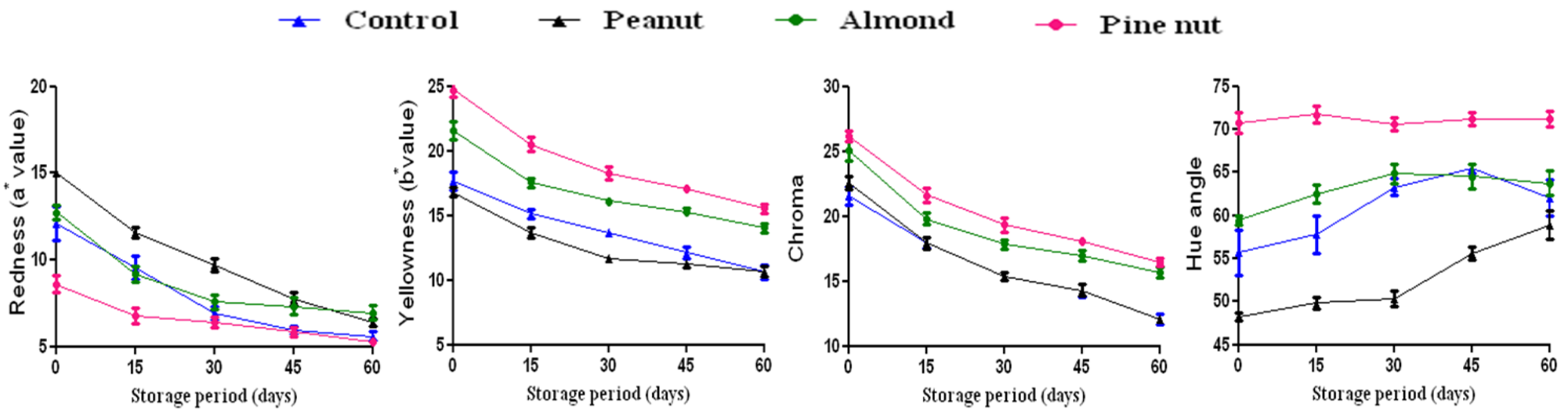


Fig-16- Instrumental colour analysis of vacuum packaged premium mutton nuggets enriched with nut based functional components

than day 45. Almond incorporated product showed significantly lower chroma value on day 60 of storage as compared to its day 30 value. Among the treatments pine nut incorporated products showed almost significantly higher ($P<0.05$) chroma values than other categories of products throughout storage period. During storage of meat, the decrease in chroma is a measure of the variation in color intensity or colorfulness, and the increase in hue angle indicates the degree of change from redness to yellowness (Lanari *et al*., 1994). A decrease in chroma value with storage period was also reported by Haile *et al*., (2011) in cooked ham.

4.4.2.3. Microbiological quality

The microbiological parameters (total plate count, psychrophilic count, coliform count, anaerobic count and lactic acid bacteria count) for the control as well as treatment premium mutton nuggets as detected on days 0, 15, 30, 45 and 60 of refrigerated storage (4±°C) in vacuum packaging are presented in Table 37 and their corresponding ANOVA is given in Table 38.

4.4.2.3.1. Total plate count

The mean values of total plate count in control as well as treatment products increased with advancement of storage period. The total plate count in all categories of products increased significantly ($P<0.05$) at each subsequent storage interval upto day 45 but on day 60 the count were comparable to respective day 45 count. Among the treatments, the total plate counts for all categories of products were comparable among themselves on corresponding day of observation. Even after 60 days of storage, TPC were well below the permissible limit of $\log_{10}7$ cfu/g for cooked meat products (Jay, 1996) and also below the limit set by Cremer and Chipley (1977) ($\log_{10}$ 5.33 cfu/gm) as indicative of unacceptability of cooked meat products. Meat products are very good medium for microbial growth and vacuum and refrigeration only retarded the growth. Increase in total plate count of meat products packaged in vacuum and stored at refrigeration temperature with progressive storage period was also reported by Babji *et al*., (2000), Kenawi *et al*., (2007), Mantis *et al*., (2007) and many others.

4.4.2.3.2. Psychrophilic count

The psychrophiles were not detectable upto day 15 of observation in all categories of products, but after their appearance on day 30, count increased significantly at each subsequent interval. Among the treatment products, psychrophilic counts were comparable

among themselves at each subsequent interval of observation throughout storage period. In present study, PC always remained below the threshold level of acceptability for cooked meat products that have been reported as $\log_{10}$ 4 cfu/gm (Jay, 1996) and $\log_{10}$ 4.6 cfu/gm (Cremer and Chipley, 1977). Nottingham (1982) reported an increase in total plate count and psychrophilic count as the condition inside vacuum packages are not completely anoxic. A detectable count on day 30 while nil on preceding observation might be attributed to the fact that bacteria generally need some lag phase before active multiplication is initiated. Absence of psychrotrophic bacteria in the nuggets during initial periods of storage might be attributed to a retardation of log phase as a result of reduced metabolic rate due to sudden change in the physical environment. It might also be due to thorough cooking of the products during processing. Similar results, where psychrophiles were absent in early phase of vacuum refrigerated storage and then increase in their count after appearance were reported by Kumar *et al*., (2005) and Kumar *et al*., (2007). Increase in psychrophilic counts in vacuum packaged meat products stored at refrigerator temperature was also reported by Babji *et al*., (2000) and Kenawi *et al*., (2005) in minced goat meat and chicken breast respectively.

4.4.2.3.3. Coliform count

The coliforms were not detected in control as well as treatment products throughout the storage period of 60 days. It could be due to the destruction of coliforms during cooking at high temperature, much above their death point of 57°C. Denis *et al*., (2006) found that coliform species were sensitive to heat treatment with a decimal reduction time under 2 min at 60^0C. In present study, the premium mutton nuggets were subjected to much intense internal end point temperature of 75^0C. Further, good hygienic and sanitation practices followed during handling, processing and packaging of premium mutton nuggets could also be one of the reasons for the absence of coliforms. A coliform count of 10^1–10^3per gram was observed in cooked frankfurters and there was no significant change in the number of coliforms during storage at 3^0C (Simard *et al*., 1983). Similarly, coliforms 0.2 (MPN/g) were reported by Sachindra *et al*., (2005) who predicted their presence because of cross contamination only. A total absence of coliforms throughout storage period under vacuum packaging and refrigerated storage was reported by Kumar *et al*., (2007) and Afshin *et al*., (2011) in chicken patties and coktail sausages respectively.

4.4.2.3.4. Anaerobic count

Anaerobes were not detectable upto day 15 of observation in all categories of products but after their appearance on day 30, the counts increased significantly ($P<0.05$) at each subsequent storage interval. Among the treatments, anaerobic counts were comparable among themselves on corresponding day during entire period of storage. The non detection of anaerobes in early storage period (upto day 30) might be attributed to sufficient heat treatment, use of nitrite in formulation which diminished with time and residual oxygen in early phase. Wang *et al*., (2004) found no colonies from the anaerobic plates of chicken wings during the first week of vacuum packaged refrigerated storage and after 2 weeks of storage, most of the anaerobic count increased as the storage time progressed. Similarly, Kumar *et al*., (2007) reported non detection of anerobes upto days 21 of vacuum packaged refrigerated storage of chicken patties and then progressive increase with extended storage. Djeri Noufoh (2007) also reported non detection of anaerobes in early phase but afterwards an increase in anaerobic count during vacuum storage of goat meat products. Increase in anaerobic count under vacuum packaged refrigerated storage was also reported by Schindra *et al*., (2005) and Diaz *et al*., (2008) in buffalo sausages and cooked pork loin respectively.

4.4.2.3.5. Lactic acid bacteria count

Lactic acid bacteria were not detectable upto day 30 of observation in all categories of products but after their appearance on day 45, counts increased significantly ($P<0.05$) at each subsequent storage interval. Among the treatments, lactic acid counts for peanut and almond incorporated products were comparable to each other, but their counts were significantly higher than both control and pine nut incorporated products on similar day of observation. Peanut incorporated product showed lactic acid bacterial count comparable to control on day 45, but on day 60 count were significantly higher than control product. The lactic acid bacterial count remained the least in control product throughout storage period. In all categories of products, the lactic acid bacterial counts were low enough to cause any fermentative repugnant odour and flavour in the products. Rodriguez *et al*. (2003) reported a critical limit of 10^7-10^8 for lactic acid bacteria. The initial absence and then increase in lactic acid bacteria with progressive storage period might be attributed to the presence of non conducive factors in early phase of storage such as residual oxygen, nitrite and high pH. Similar findings were also reported in vacuum packaged diverse cooked meat products by Waites (1988), Zurera Cosano *et al*., (1988), Yoshikatsu *et al*., (2003), Rodriguez *et al*. (2003) and Kumar *et al*., (2007).

Table 37: Effect of refrigerated storage on microbiological characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)				
	0	**15**	**30**	**45**	**60**
Total plate count (log_{10} cfu/gm)					
Control	0.96 ± 0.21^{a1}	1.41 ± 0.09^{b1}	2.29 ± 0.06^{c1}	2.93 ± 0.04^{d1}	3.14 ± 0.02^{d1}
Peanut (20%)	1.23 ± 0.08^{a2}	1.62 ± 0.07^{b1}	2.41 ± 0.09^{c1}	2.95 ± 0.02^{d1}	3.18 ± 0.02^{d1}
Almond (15%)	1.31 ± 0.11^{a2}	1.64 ± 0.05^{b1}	2.49 ± 0.11^{c1}	2.97 ± 0.01^{d1}	3.17 ± 0.02^{d1}
Pine nut (8%)	1.15 ± 0.07^{a12}	1.63 ± 0.04^{b1}	2.50 ± 0.04^{c1}	2.99 ± 0.02^{d1}	3.15 ± 0.01^{d1}
Psychrophilic count (log_{10} cfu/gm)					
Control	Not detected	Not detected	0.72 ± 0.23^{a1}	1.39 ± 0.12^{b1}	2.10 ± 0.07^{c1}
Peanut (20%)	Not detected	Not detected	0.98 ± 0.21^{a1}	1.60 ± 0.10^{b1}	2.15 ± 0.09^{c1}
Almond (15%)	Not detected	Not detected	1.15 ± 0.07^{a1}	1.62 ± 0.09^{b1}	2.18 ± 0.09^{c1}
Pine nut (8%)	Not detected	Not detected	0.93 ± 0.20^{a1}	1.52 ± 0.09^{b1}	2.11 ± 0.06^{c1}
Coliform count (log_{10} cfu/gm)					
Control	Not detected	Not detected	Not detected	Not detected	Not detected
Peanut (20%)	Not detected	Not detected	Not detected	Not detected	Not detected
Almond (15%)	Not detected	Not detected	Not detected	Not detected	Not detected
Pine nut (8%)	Not detected	Not detected	Not detected	Not detected	Not detected
Anaerobic count (log_{10} cfu/gm)					
Control	Not detected	Not detected	1.80 ± 0.05^{a1}	2.43 ± 0.06^{b1}	2.86 ± 0.03^{c1}
Peanut (20%)	Not detected	Not detected	1.90 ± 0.03^{a12}	2.58 ± 0.06^{b2}	2.95 ± 0.03^{c12}
Almond (15%)	Not detected	Not detected	1.93 ± 0.04^{a2}	2.74 ± 0.05^{b3}	3.01 ± 0.02^{c2}
Pine nut (8%)	Not detected	Not detected	1.81 ± 0.05^{a1}	2.47 ± 0.07^{b1}	2.90 ± 0.04^{c1}
Lactic acid bacterial count (log_{10} cfu/gm)					
Control	Not detected	Not detected	Not detected	1.62 ± 0.05^{a1}	2.03 ± 0.04^{b1}
Peanut (20%)	Not detected	Not detected	Not detected	1.84 ± 0.04^{a2}	2.18 ± 0.04^{b2}
Almond (15%)	Not detected	Not detected	Not detected	1.86 ± 0.04^{a2}	2.21 ± 0.04^{b2}
Pine nut (8%)	Not detected	Not detected	Not detected	1.78 ± 0.05^{a1}	2.11 ± 0.03^{b3}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numeral) differ significantly ($P<0.05$). n =6 for each treatment.

Table 38: ANOVA for microbiological characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components

Source of variation	d.f.	Parameters							
		SPC		Psychrophiles		Anaerobic		LAB	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between storage days	4	0.168	4.988**	0.118	1.931	0.087	10.930**	0.040	10.462**
Between treatments	3	17.972	532.905**	21.281	347.021**	46.698	5883.934**	27.866	7202.013**
Treatment × storage days	12	0.024	0.726*	0.037	0.597	0.021	2.617**	0.016	4.174**
Error (days of storage)	100	0.034	-	0.61	-	0.008	-	0.004	-

* Significant (P<0.05); ** Highly significant (P<0.01).

4.4.2.4. Sensory quality

Mean sensory scores of vacuum packaged premium mutton nuggets during storage at 4±1°C are presented in Table 39 and their corresponding ANOVA is given in Table -40. The scores for all the sensory attributes for control as well as nut paste incorporated premium mutton nuggets showed a decreasing trend with increase in storage period. The general appearance scores for all the treatment products and control showed a gradual decline with progress in storage period. The scores for control products were comparable to 0 day score only upto day 15 of storage. On day 30 scores for control product were comparable to day 15 and day 45 score. The general appearance scores after day 45 had significant decrease (P<0.05) only in case of control products. The general appearance scores of control products were significantly higher (P<0.05) than nut incorporated products upto day 15 of storage. However, these scores were comparable for all categories of products on day 30 and day 45. On day 60, the general appearance score of control product was significantly lower (P<0.05) than peanut and pine nut incorporated products but comparable to almond incorporated product.

A decrease in general appearance score with storage was concurrent with decrease in color value parameters such as redness and yellowness. Besides the changes due to oxidation in lipid as well as pigment, the evaporative moisture condensation on the surface

of product was also one of the main factors affecting the general appearance scores. Higher general appearance score for control product over nut incorporated products in early phase of storage might be due to preference of panelists for original meat product colour which was diluted by nut pastes in treatment products. Later on, changes in colour values were much pronounced in control product whereas nut incorporated products got some protection due to associated antioxidants. A decrease in general appearance score with progress of storage period was also reported by Kumar *et al*., (2007) and Das *et al*., (2008).

The flavour scores of control and treatment products decreased with progressive increase in storage period. The flavour scores of control product were comparable upto day 30 of storage. Later on, the scores decreased significantly ($P<0.05$) on day 45 and 60 of storage respectively. In peanut incorporated product, changes in flavour scores were marginal and the scores remained comparable throughout storage period. Product incorporated with almond maintained comparable falvour scores upto day 45 of storage but recorded significantly lower score on day 60 of storage. Pine nut incorporated product maintained similar trend upto day 30 of storage and then scores decreased significantly as on day 45 and 60 of storage respectively. At the end of 60 days storage period, nut incorporated products showed comparable flavor scores among themselves as well as control with sensory ratings between good to very good. Das *et al*., (2008) cited the expected loss of volatile flavour components from spices and condiments on storage of meat products as a reason of gradual decline in flavour score. Sun and Holley (2012) reported that growth of microbes could lead to formation of objectionable compounds including those causing off-odors. Further, oxidation of lipids and pigments also cause undesirable flavors and discoloration in meat products. The comparative flavour stability in nut incorporated products particularly comparatively high level of peanut and almond indicated the role of associated antioxidants against flavour diminishing lipid oxidation. Decline in flavour scores of meat products during storage was reported by Sahoo & Anjaneyulu (1997), Thomae *et al*., (2006) and Kumar *et al*., (2007).

The juiciness score of control as well as nut incorporated products decreased with progression of storage period. In control and peanut incorporated products juiciness scores were not significantly affected ($P>0.05$) upto day 45, although these were significantly lower on day 60 of storage. In almond and pine nut incorporated products, juiciness scores were not affected upto day 30 of storage, then decreased significantly ($P<0.05$) day 45 and

day 60 of storage. Among the treatment products, juiciness scores were comparable at each storage interval during the period of storage.

A gradual decrease in juiciness scores might be attributed to evaporative moisture loss from product which remained as film on the surface as it was opened. Lund *et al*., (2007) reported that in meat, protein oxidation may decrease the eating quality by reducing tenderness as well as juiciness and increasing flavor deterioration as well as discoloration. In fact the proteolytic changes taking place during course of storage affects the water holding capacity and therefore associated juiciness. Decrease in juiciness scores of meat products with progression of storage period were in agreement with the findings of Kumar *et al*., (2007), Diaz *et al*. (2008) and Hur *et al*. (2013).

The texture of control and nut incorporated premium mutton nuggets decreased gradually with increase in storage period. In control and pine nut incorporated products texture scores were were not affected upto day 30 of storage and after that, these were significantly lower ($P<0.05$) as compared to day 0 score on day 45, and further on day 60 of storage. In peanut and almond incorporated products, texture scores were not affected upto day 30 of storage, but on day 45 and 60, the flavour scores were significantly lower than day 0 and day 15 scores. Control and pine nut incorporated products had consistently higher texture scores than peanut and almond incorporated products. A decrease in texture scores of the products were expected because of proteolytic and lipolytic changes during storage. As per Diaz *et al*., (2008) this would mainly correspond to protein degradation due to chemical and enzymatic activity. They opined that although heating at 70^0C inactivate part of the muscle proteases, residual protease activity continued in the refrigerated products. During storage of meat products, texture defect in the form of associated softness and loss of elasticity could be felt while masticating as reported by García-Garrido *et al*., (1999) and Arnau *et al*., (2007). A decrease in textural scores of fermented sausages was reported by Ahmad and Amer (2013) with advancement of storage period and they concluded that decline in pH which coagulated the protein adversely affected the texture.

The overall acceptability scores of control and premium muuton nuggets decreased with the progression of storage period. The scores were not affected ($P>0.05$) upto day 45 of sorage in all categories of products. Overall acceptability scores on day 60 of storage was significantly lower than respective day 0 scores in almost all categories of products except peanut incorporated product which had overall acceptability scores comparable to

day 0 score even on day 60 of storage. The decrease in overall acceptability scores during refrigerated storage might be a reflection of decline in the scores of attributes like general appearance, flavour, juiciness and texture. A decrease in overall acceptability scores during storage was in agreement with findings of Kumar *et al*., (2007), Diaz *et al*., (2008) and Hur *et al*., (2013).

Table 39: Effect of refrigerated storage on sensory attributes of vacuum packaged premium mutton nuggets enriched with nut based functional components (Mean±S.E.)*.

Treatments	Refrigerated storage period (days)				
	0	15	30	45	60
General appearance					
Control	7.10 ± 0.069^{a1}	7.05 ± 0.040^{ab1}	6.90 ± 0.037^{bc1}	6.77 ± 0.061^{c1}	6.54 ± 0.048^{d1}
Peanut (20%)	6.91 ± 0.063^{a2}	6.89 ± 0.043^{a12}	6.83 ± 0.041^{a1}	6.78 ± 0.058^{a1}	6.74 ± 0.061^{a2}
Almond (15%)	6.83 ± 0.062^{a2}	6.79 ± 0.048^{ab2}	6.73 ± 0.041^{abc1}	6.65 ± 0.062^{bc1}	6.60 ± 0.068^{c12}
Pine nut (8%)	6.99 ± 0.052^{a12}	6.88 ± 0.044^{ab12}	6.84 ± 0.044^{ab1}	6.78 ± 0.061^{b1}	6.73 ± 0.057^{b2}
Flavour					
Control	7.05 ± 0.061^{a1}	6.97 ± 0.051^{ab1}	6.87 ± 0.047^{abc1}	6.80 ± 0.061^{bc1}	6.69 ± 0.070^{c1}
Peanut (20%)	6.81 ± 0.108^{a2}	6.77 ± 0.088^{a1}	6.70 ± 0.085^{a1}	6.65 ± 0.059^{a1}	6.60 ± 0.059^{a1}
Almond (15%)	6.88 ± 0.079^{a12}	6.84 ± 0.058^{ab1}	6.77 ± 0.046^{ab1}	6.69 ± 0.049^{ab1}	6.62 ± 0.057^{b1}
Pine nut (8%)	6.90 ± 0.066^{a12}	6.87 ± 0.047^{ab1}	6.78 ± 0.048^{abc1}	6.65 ± 0.065^{bc1}	6.55 ± 0.067^{c1}
Juiciness					
Control	7.09 ± 0.064^{a1}	7.05 ± 0.047^{ab1}	6.93 ± 0.058^{ab1}	6.90 ± 0.055^{ab1}	6.87 ± 0.062^{b1}
Peanut (20%)	6.91 ± 0.086^{a1}	6.87 ± 0.055^{ab1}	6.83 ± 0.059^{ab1}	6.77 ± 0.052^{ab1}	6.70 ± 0.068^{b1}
Almond (15%)	6.98 ± 0.076^{a1}	6.95 ± 0.065^{ab1}	6.89 ± 0.078^{abc1}	6.81 ± 0.061^{bc1}	6.74 ± 0.068^{c1}
Pine nut (8%)	7.04 ± 0.048^{a1}	6.96 ± 0.043^{ab1}	6.84 ± 0.040^{abc1}	6.81 ± 0.087^{bc1}	6.72 ± 0.077^{c1}
Texture					
Control	7.10 ± 0.062^{a1}	7.02 ± 0.057^{ab1}	6.95 ± 0.068^{abc1}	6.83 ± 0.056^{bc1}	6.79 ± 0.057^{c1}
Peanut (20%)	6.89 ± 0.086^{a2}	6.85 ± 0.053^{a1}	6.79 ± 0.079^{ab1}	6.69 ± 0.058^{b1}	6.61 ± 0.054^{b1}
Almond (15%)	6.89 ± 0.086^{a2}	6.85 ± 0.070^{a1}	6.78 ± 0.078^{ab1}	6.68 ± 0.073^{b1}	6.60 ± 0.082^{b1}
Pine nut (8%)	7.12 ± 0.035^{a1}	6.97 ± 0.046^{ab1}	6.85 ± 0.041^{abc1}	6.80 ± 0.055^{bc1}	6.74 ± 0.067^{c1}
Overall acceptability					
Control	7.01 ± 0.058^{a1}	6.94 ± 0.079^{ab1}	6.91 ± 0.057^{ab1}	6.85 ± 0.07^{ab1}	6.78 ± 0.062^{b1}
Peanut (20%)	6.79 ± 0.114^{a1}	6.76 ± 0.054^{a1}	6.69 ± 0.069^{a1}	6.64 ± 0.077^{a1}	6.59 ± 0.083^{a1}
Almond (15%)	6.90 ± 0.080^{a1}	6.87 ± 0.055^{ab1}	6.78 ± 0.050^{ab1}	6.67 ± 0.051^{ab1}	6.59 ± 0.049^{b1}
Pine nut (8%)	7.01 ± 0.053^{a1}	6.95 ± 0.054^{ab1}	6.82 ± 0.046^{ab1}	6.75 ± 0.059^{ab1}	6.66 ± 0.071^{b1}

*Mean±S.E. with different superscripts row wise (small alphabet) and column wise (numeral) differ significantly ($P<0.05$). n =6 for each treatment.

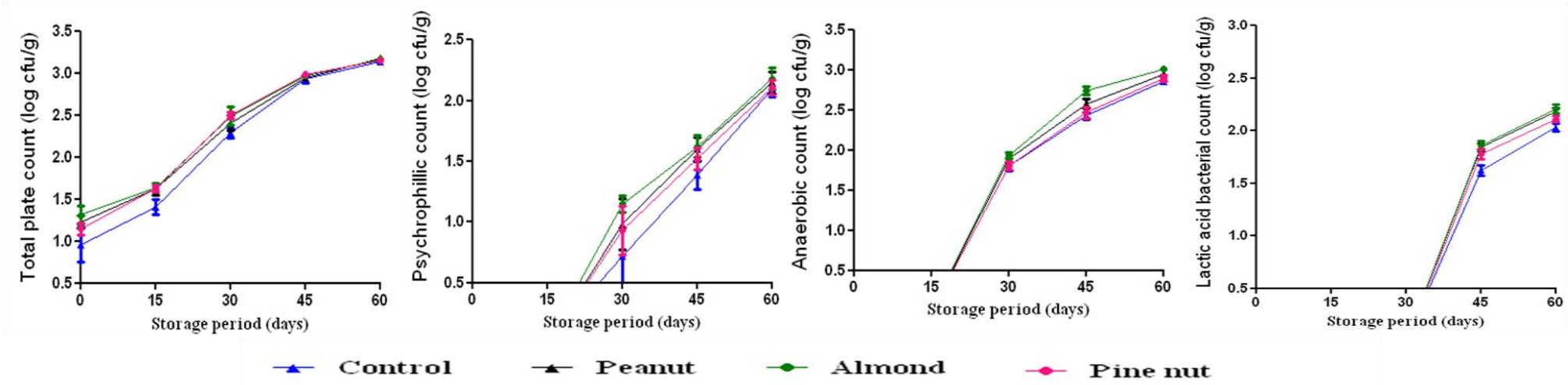


Fig- 17-Effect of refrigerated storage on microbiological characteristics of vacuum packaged premium mutton nuggets enriched with nut based functional components

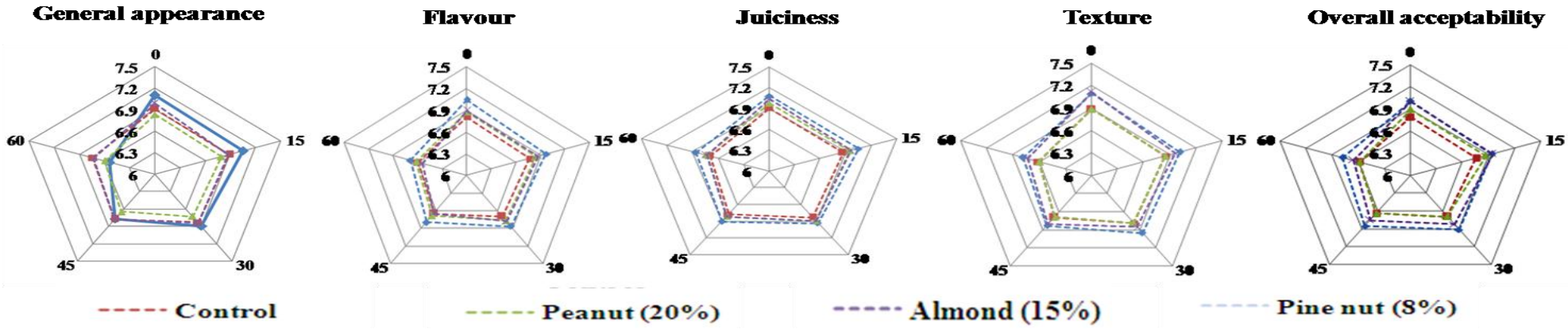


Fig- 18- Effect of refrigerated storage on sensory attributes of vacuum packaged premium mutton nuggets enriched with nut based functional components

Table 40: ANOVA for effect of refrigerated storage on sensory attributes of vacuum packaged premium mutton nuggets enriched with nut based functional components.

Source of variation	d.f.	Mean sum of square									
		General appearance		Flavour		Texture		Juiciness		Overall acceptability	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Between treatments	3	0.470	7.387**	0.545	6.038**	0.861	9.798**	0.432	5.326**	0.798	8.182**
Between storage days	4	1.251	19.670**	1.182	13.099**	1.355	15.425**	0.853	10.527**	1.038	10.643**
Treatment × storage days	12	0.111	1.747	0.021	0.232	0.018	0.207	0.015	0.184	0.025	0.253
Error (days of storage)	400	0.064	-	0.090	-	0.088	-	0.081	-	0.098	-

* Significant (P<0.05); ** Highly significant (P<0.01).

These observations indicated that premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and good to very good sensory ratings when stored in vacuum pouches under refrigeration at 4±1°C for 60 days. Hence, it was concluded that vacuum packaged premium mutton nuggets developed in this study could be safely stored in multilayered nylon pouches under refrigeration upto day 60 at 4±1°C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

4.4. Estimation of Production Cost of the Developed Premium Mutton Nuggets.

Along with nutritive value and sensory acceptability of meat product, economics is also very important criteria that determine the marketability of any product. In the present study, production cost of premium mutton nuggets incorporated with functionally enriched nut pastes viz: 20% peanut paste, 15% almond paste and 8% pine nut paste were determined and compared with control.

4.5.1 Raw material cost

Raw materials are the basic ingredients in the manufacture of meat products. The raw materials required for preparation of premium mutton nuggets were mutton, sheep fat, table salt, spices mixture, condiments, refined wheat flour, STPP and sodium nitrite. In addition, peanut, almond and pine nut paste 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. The cost of raw materials is presented in Table-41.

4.5.2. Cost of processing equipments

The essential equipments and accessories required for processing of premium mutton nuggets and approximate cost of processing and other machineries required for the preparation of 50 kg premium mutton nuggets are presented in Table 42.

Depreciation rate = 10% per annum **4,18,200**
i.e. = ₹ 41,820/annum
i.e. = ₹ 139.4 ≈ **140/day** (300 working days per annum)

4.5.3. Cost of electricity

A processing plant requires electricity for the operation of various equipments and adequate illumination of the working space. Presently, the electricity charges are approximately Rs. 6/KWh under industry category use. The cost of electricity incurred for processing of 50 kg of premium mutton nuggets can be calculated as shown in Table 41.

Therefore the cost of electricity = 78.5 KWh × Rs. 6/ KWh = ₹ **471 /day**

Table 41: Comparative cost of raw materials for preparation of 100 Kg emulsion of control and premium mutton nuggets

Ingredients	Rate ₹/kg	Control		Peanut (20%)		Almond (15%)		Pine nut (8%)	
		Qt. (Kg)	₹	Qt (Kg)	₹	Qt. (Kg)	₹	Qt. (Kg)	₹
Lean mutton (Deboned)	500	70	35000	60	30000	62.5	31250	67	33500
Peanut paste	140	-	-	20	2800	-	-	-	
Almond paste	540	-	-	-	-	15	8100	-	
Pine nut paste	1600	-	-	-	-	-	-	8	12800
Ice water	2	10	20	10	20	10	20	10	20
Animal fat	100	10	1000	-	-	2.5	250	5	500
Spice mix	400	1.5	600	1.5	600	1.5	600	1.5	600
Condiment mix. (Onion: garlic -3:1)	40	3.2	128	3.2	128	3.2	128	3.2	128
Refined wheat flour	20	3.0	60	3.0	60	3.0	60	3.0	60
Salt	20	1.7	34	1.7	34	1.7	34	1.7	34
STPP	700	0.3	210	0.3	210	0.3	210	0.3	210
Nitrite	200	0.015	3	0.015	3	0.015	3	0.015	3
Sugar	40	0.3	12	0.3	12	0.3	12	0.3	12
Transportation cost	-		100		100		100		100
Total (₹)	-	-	**37167**	-	**33967**		**40767**		**47967**

Table 42: Cost of processing equipments for preparation of premium mutton nuggets

Equipments	**No. required**	**Cost (₹)**
Bowl chopper	1	100000
Meat mincer	1	50000
Cooking drum	4	20000
Refrigerator (500 L)	2	40000
Impulse sealer	1	3000
Geyser (50 L)	1	5000
Air conditioners (2 ton)	1	25000
Deep freezer (360L)	1	40000
Weighing balances	2	5000
Thermometer	1	200
Meat Slicer	1	30000
Furniture and utensils (steel table, knives, vessels etc.)	-	50000
Cost of bore well, water storage tanks and one pump set	1	50000
Total		**418200**

Table 43: Cost of electricity for preparation of premium mutton nuggets

Equipments	**Watt × hr**	**KWh**
Bowl chopper	1000 × 1	1.0
Meat mincer	1000 × 1	1.0
Geyser	500 × 3	1.5
Cooking drum	2000 × 4 × 2	16
Refrigerator	250 × 2 × 20	10
Deep freezer	1000 × 1 × 20	20.0
Air conditioners	1500 × 2 × 8	24.0
Lights, fans, weighing balance etc.	500 × 10	5.0
Total unit		**78.5**

4.5.4 Packaging cost

LDPE film (about 4 gm each pouch and dispensing size for nuggets is around 200gm per packets). So total number of packets required will be around 500 which will weight about 2Kg. LDPE cost is Rs 240/Kg. Cartons would be also required for bulk packaging, storage, transportation and distribution. So packaging material cost would be **Rs 500/day.**

4.5.5. Labour cost

The labour cost of skilled person and unskilled person would be ₹ 300 per day and ₹ 200 per day respectively. For preparation of premium mutton nuggets from 100 kg emulsion, one skilled and two unskilled labours would be required per day.

So, the labour cost can be calculated as

Skilled staff = 300 × 1 = ₹ 300 /day
Unskilled staff = 200 × 2 = ₹ 400 /day
Total labour cost = ₹ **700 /day**

4.5.6. 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 ₹ 15,000 per month. Therefore, rent per day ₹ **500 /day.**

4.5.7. 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 ₹**300 per day.**

4.5.8. Financing

Project Cost (Approx)	= 5 Lakhs
Highest amount of loan eligibility @ 85%	= 4.25 lakhs
Rate of interest	= 10.25% per annum
Therefore total interest per month	= 3630 per month
Installment per day (25 working days)	**= Rs 145/day**

4.5.9. Total expenditure

The sum of all the above costs **(4.1-4.8)** account for total cost for the production of premium mutton nuggets from 100 kg meat batter are shown in the Table 42.

4.5.10. Product yield

The product yield was around 94.4%, 97.6%, 96.3% and 96.6% for control and treatments viz; peanut, almond and pine nut incorporated products. However, a safety margin of 1 to 2 % should be considered to compensate the losses that might occur during various steps of processing, handling, cutting, weighing, packaging and marketing, The cost of product was calculated considering a final yield at 92%, 95%, 94% and 94% for control and treatments viz peanut, almond and pine nut incorporated premium mutton nuggets respectively.

Table 44: Total Input for preparation of premium mutton nuggets

Parameter	**Control**	**Peanut (20%)**	**Almond (15%)**	**Pine nut (8%)**
Raw materials cost	37167	33967	40767	47967
Cost of machineries (depreciation cost)	140	140	140	140
Cost of electricity	471	471	471	471
Packaging cost	500	500	500	500
Labour cost	700	700	700	700
Premises rent	500	500	500	500
Maintenance cost	300	300	300	300
Bank interest on finance	145	145	145	145
Total expenditure	**39923**	**36723**	**43523**	**50723**

Table-45: Retail cost calculation of premium mutton nuggets

Parameter	**Control**	**Peanut (20%)**	**Almond (15%)**	**Pine nut (8%)**
Cost of formulation (₹)	37167	33967	40767	47967
Overhead production cost (₹)	2756	2756	2756	2756
Total Input (₹)	39923	36723	43523	50723
Pragmatic Product yield (Kg)	92	95	94	94
Actual production cost per Kg product (₹)	**433.95**	**386.55**	**463.01**	**539.61**
Average gross Profit of the Producer (A gross profit of 12% is considered reasonable for the product) (₹)	52.07	46.39	55.56	64.75
Actual Retail cost of the products per kg(₹)	486.024	432.936	518.5712	604.3632
Retail cost of Nuggets per 200gm packet. (₹)	97.2048	86.5872	103.7142	120.8726
Approx cost per packet of 200gm(₹)	**97**	**87**	**104**	**121**

4.5.11. Production cost of premium mutton nuggets

Total input cost for the preparation of premium mutton nuggets from 100 kg meat batter was ₹39923, 36723, 43523 and 50723 and the product yield (Kg) was 92, 95, 94, and

94 for control and treatments viz peanut, almond and pine nut incorporated premium mutton nuggets respectively.

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

Therefore, the calculated production cost of 1 kg product was ₹ 433.95, 386.55, 463.01 and 539.61 for control peanut, almond and pine nut incorporated premium mutton nuggets respectively

Thus, the studies indicated that incorporation of functionally enriched nut paste in premium mutton nuggets at their optimum level viz. peanut, almond and pine nut resulted fluctuation of the cost of premium mutton nuggets by ₹ **10, 7** and **24** per 200gm packet respectively as compared to control product. The peanut incorporated product was 10.30% cheaper whereas, almond and pine nut based premium mutton nuggets were also in the affordable range with price fluctuation of 7.2% and 24.72% respectively.

SUMMARY AND CONCLUSION

The present research work was carried out to develop premium mutton nuggets enriched with nut based functional components. Based on the lipid profile of nuts suited to healthy diets, their availability and cost, three types of nuts viz: peanut, almonds and pine nuts were included in the present study. Study was made under different experimental heads which were interrelated to each other.

In very first experiment suitable transformation technique for nuts paste preparation was evolved and standardized. Preliminary trials were carried out to explore a number of transformation techniques viz: soaking of dried nuts in water overnight and grinding, direct pan frying and then grinding, microwave treatment followed by grinding, sand heating and then grinding, direct grinding and their various permutation combination were attempted. The resultant pastes were incorporated in the control formulation of mutton nuggets at uniform levels and sensory evaluation of the products were conducted to determine the most acceptable transformation technique for each nut paste. The selected nut pastes were subjected to determination of their crude fat and protein content. The nut pastes prepared by adopting respective most suitable transformation techniques had approx 50% fat in peanut and almond paste and 62.5% fat in pine nut paste. On the basis of crude fat content of nut pastes, level of their incorporation with the aim to replace added animal fat in traditional emulsion by 50, 75 and 100% were decided. Accordingly, the levels of substitution were fixed as 10,15 and 20% for peanut and almond whereas 8,12, and 16% for pine nut based premium mutton nuggets. Nuts pastes also replaced partial amount of lean meat in contrast to control group on constant weight basis.

In second experiment conducted in three parts, the levels of nut paste incorporation in premium mutton nuggets were optimized based on the physico-chemical and sensory characteristics of developed products. For preparation of mutton nuggets lean meat, ice flakes, nitrite, STPP, salt, animal fat, refined wheat flour, condiment mix (onion:garlic-3:1), dry spice mix, sugar and nut paste were used for batter preparation, followed by stuffing in mould box, cooking in steam for 35 minutes, cooling, slicing, cutting and finally packaging and evaluation. For development of peanut based premium mutton nuggets, 10, 15 and 20% of peanut paste prepared by microwave sand heating of peanut (2 ½ minutes) and then grinding, was added to control formulation replacing added animal fat by 50%, 75% and 100%. The extra fat constituent of nut paste replaced the lean meat also

in control formulation by 5, 7.5 and 10% respectively. The products were subjected to detailed physico-chemical and sensory analysis. There was gradual increase in emulsion and product pH, emulsion stability, cooking yield, and protein content with the increase in incorporation level of peanut paste and these treatments were significantly higher ($P<0.05$) than control at 15 and 20% levels, while at 10% level emulsion stability, cooking yield and product pH remained comparable with control values. Increase in fat, ash and calculated carbohydrate content was marginal and values were comparable among treatment groups. Moisture percent and moisture: protein ratio decreased with increase in the incorporation level and moisture percentage was significantly lower ($P<0.05$) at subsequent increased level of incorporation. Shear force values, with marginal decrease, remained comparable with control even at highest level of incorporation. Sensory evaluation of the premium products showed a general gradual decline in scores for all sensory attributes but even at 20% incorporation level the scores for all the attributes were comparable with control product. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of peanut paste for the preparation of premium mutton nuggets was adjudged as 20%.

For development of almond based premium mutton nuggets, 10, 15 and 20% of almond paste prepared by direct grinding, was added to control formulation replacing added animal fat by 50%, 75% and 100%. The extra fat constituent of nut paste replaced the lean meat also in control formulation by 5, 7.5 and 10% respectively. The products were subjected to detailed physico-chemical and sensory analysis. There was gradual increase in emulsion and product pH, emulsion stability, cooking yield, protein content, ash and calculated carbohydrate content with the increase in level of incorporation of almond paste and these parameters were significantly higher ($P<0.05$) than control at 20% level. At 15% level the emulsion stability and protein percentage remained comparable with control but parameters were significantly higher ($P<0.05$) than control group. Increase in fat was marginal and values were comparable among treatment products. Moisture content and moisture: protein ratio decreased significantly ($P<0.05$) almost with each subsequent increase in incorporation level. Shear force values, with marginal decrease, remained comparable with control upto 15% level of incorporation, but it was significantly lower than control group at 20% level. Sensory evaluation of the treatment products showed a general gradual decline in scores for all sensory attributes but at 20%

incorporation level, scores for all the attributes were significantly lower than that of control product. Except for significantly lower ($P<0.05$) score for general appearance, the scores for all other attributes including overall acceptability at 15% incorporation level were comparable with score of control product. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of almond paste for the preparation of premium mutton nuggets was adjudged as 15%.

For development of pine nut based premium mutton nuggets 8, 12 and 16% of pine nut paste prepared by direct grinding, was added to control formulation replacing added animal fat by 50%, 75% and 100%. The extra fat constituent of nut paste replaced the lean meat also in control formulation by 3, 4.5 and 6% respectively. The products were subjected to detailed physico-chemical and sensory analysis. There was gradual increase in emulsion stability, cooking yield, moisture: protein ratio, fat content, ash and calculated carbohydrate content with the increase in incorporation level of pine nut paste and these parameters were significantly higher ($P<0.05$) than control at 16% level while at 12% level, only cooking yield and fat percentage were higher and other parameters remained comparable to control. Increase in moisture: protein ratio was marginal and values were comparable among treatment products. Emulsion and product pH, moisture content and protein percentage decreased gradually and were almost significantly lower ($P<0.05$) as compared to control only at the 16% incorporation. Shear force values decreased with increase in incorporation level and was significantly lower ($P<0.05$) at 12 and 16% levels of incorporation than control. All the physico-chemical parameters at 8% incorporation were comparable with respective values for control product. Sensory evaluation of the treatment products showed a general gradual decline in scores for all sensory attributes and at 16% incorporation level, scores for all the attributes were significantly lower than control products. Except for comparable scores for general appearance and juiciness, scores for all other attributes including overall acceptability at 12% incorporation level were significantly lower ($P<0.05$) as compared to control product. However, at 8% incorporation level, the sensory scores for all attributes were comparable with control product. Hence, on the basis of sensory scores and physico-chemical properties, the optimum incorporation level of pine nut paste for the preparation of premium mutton nuggets was adjudged as 8%.

In third experiment premium mutton nuggets by incorporated with optimum level of nut paste viz: 20% of peanut, 15% of almond and 8% of pine nut paste, evolved from the earlier experiments were analyzed for detailed profile viz: lipid profile parameters, calorific value, dietary fiber, total phenolics, DPPH radical scavenging activity, reducing power assay and texture profile parameters.

The detailed lipid profile which included parameters such as total lipids, total cholesterol, total phospholipids, total glycolipids, total free fatty acids and total glycerides were evaluated for developed products and compared with control. The total lipids, total phospholipids, total glycolipids and total glycerides were comparatively lower in control product as compared to nut incorporated products, while cholesterol and total free fatty acids were higher than nut based premium mutton nuggets. Peanut and almond incorporated products had comparable values for selected lipid parameters measured. These products also had significantly lower ($P<0.05$) cholesterol as well as total free fatty acids and significantly higher phospholipids as well as total glycolipids but comparable total lipids as well as total glycerides in comparision to control. Pine nut incorporated product showed significantly higher ($P<0.05$) total lipids, total phospholipids, total glycolipids and total glycerides, comparable total free fatty acids and significantly lower ($P<0.05$) cholesterol than control products. Except for total free fatty acids, the values for all other lipid parameters for nut based premium mutton nuggets were comparable among themselves.

The calorific values for nut based premium mutton nuggets were higher than control products. Peanut incorporated product showed significantly higher ($P<0.05$) calorific value than almond incorporated product, which in turn was significantly higher than comparable calorific values for control and pine nut incorporated products. Total dietary fiber was highest in almond incorporated product which was comparable to the dietary fiber content in peanut based premium mutton nuggets but significantly higher than comparable dietary fiber contents for control and pine nut incorporated products.

Nut based premium mutton nuggets showed much improved antioxidant activity as compared to control product. Peanut incorporated product showed significantly higher ($P<0.05$) total phenolic content than almond incorporated product which in turn was significantly higher than control product. DDPH radical scavenging activity was highest in almond incorporated products significantly higher than peanut incorporated product, which

in turn was significantly higher than pine incorporated premium mutton nuggets. Reducing power activity for almond and peanut incorporated products were comparable and significantly higher ($P<0.05$) than comparable activity values of control and pine nut incorporated products. In pine nut based premium mutton nuggets, total phenolic content and reducing power activity were comparable and DPPH radical scavenging activity was significantly higher ($P<0.05$) as compared to control product. Among the textural profile parameters, hardness of peanut incorporated product was significantly higher ($P<0.05$) than comparable hardness values of almond and pine nut incorporated products but was comparable with control product. Adhesiveness and gumminess values for all categories of products were comparable. Springiness in almond based product was significantly lower ($P<0.05$) than control and pine nut incorporated products. Springiness value for peanut based premium mutton nuggets were comparable to other two nut based products but significantly lower than control. Cohesiveness value for almond incorporated product was significantly lower than control product, but comparable to other two nut based premium mutton nuggets. Both peanut and pine nut based products had springiness values comparable to control as well as almond incorporated product. Chewiness values for peanut and almond based products were comparable to each other but significantly higher ($P<0.05$) than control product. Chewiness value for pine nut incorporated product was comparable to control as well as other two nut based products.

The results of detailed product profile confirmed that premium mutton nuggets incorporated with optimum levels of nut pastes had improved lipid profile viz: lower in cholesterol and FFA, higher total dietary fiber and antioxidant capacity in terms of total phenol, DPPH radical scavenging activity and reducing power assay as compared to control.

Premium mutton nuggets incorporated with optimum levels of nut pastes viz. 20% peanut, 15% almond and 8% pine nut, were packaged aerobically in low density polyethylene pouches (200 gauge) and vacuum in impermeable nylon pouches (150 gauge) and then stored at refrigerated temperature (4±1°C) for 15 days and 60 days respectively. The stored samples were analyzed for physico-chemical parameters viz: pH, TBARS value, free fatty acids (FFA), peroxide value; instrumental colour analysis viz: redness, yellowness, hue and chroma; microbiological quality viz: standard plate count, psychrophilic count, coliform count and in case of vacuum packaged product, additionally

for anaerobic plate and lactic acid bacteria count; and sensory characteristics for the attributes such as general appearance, flavour, texture, juiciness and overall acceptability at regular interval of 5 days and 15 days respectively.

In aerobic packaged products there were gradual increases in pH, TBARS, FFA and peroxide values with advancement of storage period. The pH values of all categories of products behaved almost similar during storage period and were comparable upto day 5 of storage to their respective day 0 values. On day 15 of storage the pH values were significantly higher ($P<0.05$) than respective day 10 values, which were significantly higher ($P<0.05$) than values on day 5 of storage period. Inconsistency in pH values of peanut and pine nut incorporated products were observed, which were higher than almond incorporated product followed by control product. TBARS and peroxide values of the products followed similar increasing trend with the progress of storage period. Values for both the parameters in control and pine nut incorporated products increased significantly ($P<0.05$) on subsequent interval of observation. The peanut and almond incorporated products, had comparable values upto day 5 of storage, after which these also increased significantly ($P<0.05$) on 10^{th} and day 15 as compared to preceding interval values. TBARS values for control and pine nut incorporated products were comparable to each other, while TBARS values of peanut incorporated product were comparable to almond incorporated product on corresponding day of observation. Likewise, peroxide values for control and pine nut incorporated products were comparable to each other and almost comparable, even to almond incorporated products except on day 5 when peroxide values of control product was significantly higher ($P<0.05$) than almond based product. Peanut incorporated product had significantly lower peroxide value than all other categories of products, with exception on day 0 and day 15 when the values were comparable with almond incorporated product.

FFA values of all categories of products increased significantly ($P<0.05$) at each subsequent interval of storage period. FFA values for control and pine nut incorporated products were comparable to each other, while values of peanut incorporated product remained comparable to almond incorporated product throughout storage period. FFA values for control and pine nut incorporated products were significantly higher ($P<0.05$) than respective values of peanut and almond incorporated products throughout storage period. There was a general decrease in the values of colour attributes viz: redness values,

yellowness values and chroma with the progress of storage period in all categories of products, but hue values decreased simultaneously. Redness values remained almost same upto day 5 in case of control as well as peanut based product and , upto day 10 in almond incorporated product and throughout storage period in pine nut incorporated product. In control and peanut incorporated products there was a significant decrease on subsequent interval of observation after day 10 onwards, while these values were comparable at day 10 and day 15 of storage in almond incorporated product. Redness values of products were in order of peanut > almond > control > pine nut. Yellowness values for control and peanut products behaved similarly and were comparable to respective day 0 values upto day 5 and decreased significantly on day 10 as compared to day 0 and day 15 as compared to day 5 of storage. Almond incorporated product also showed comparable yellowness value upto day 5 but day 10 and day 15 values although comparable to each other, were significantly lower ($P<0.05$) to both 0 and day 5 yellowness values. Yellowness values of pine incorporated product decreased significantly at each subsequent storage interval throughout storage.

Hue angle of control and pea nut incorporated products were comparable to day 0 values upto day 10 of storage but significantly higher on day 15 of storage. Almond and pine incorporated products showed comparable hue values throughout the storage period. Chroma values for control and pine incorporated products were comparable upto day 5 of storage, then decreased significantly at each subsequent storage interval. Peanut incorporated product showed significant decline ($P<0.05$) at each storage interval while almond incorporated product had comparable value for chroma on day 0 and 5 and also on day 10 and 15. Yellowness and hue values of these products were in the order of pine nut > almond > control > peanut, whereas, chroma values also behaved almost similarly except for marginally higher value for peanut based product than control.

Total plate counts in all categories of products increased significantly ($P<0.05$) at each subsequent interval. Except for a significantly lower total plate count in control product as compared to almond based product, counts were comparable within treatment at corresponding interval of observation. Psychrophiles were not detectable upto day 5 of storage but afterwards detection, increased significantly at subsequent storage interval. Comparable psychrophiles count for control and peanut incorporated product were significantly lower ($P<0.05$) than comparable counts of almond and pine nut based

products on day 10 of observation. On day 15 of storage, psychrophil counts were comparable among the treatment groups. Coliforms were not detected in the products throughout storage period.

Sensory scores for various attributes in all categories of products showed a gradual decline with progressive increase in the storage period. General appearance scores were least affected and remained comparable to their day 0 scores in all categories of products throughout storage period. General appearance scores of control product were highest followed by pine incorporated product, peanut based product and then least for almond based product. Flavour and juiciness scores in all categories of products remained comparable upto day 10 of storage but on day 15 of storage, these were significantly lower than day 0 scores. Except significantly higher flavor score for control product on day 0 than peanut and pine nut based products, the flavor scores among the treatment groups at each interval, were comparable among themselves throughout storage period. Texture scores for control and almond based product were comparable upto day 10, peanut based products upto day 15 and pine nut incorporated products upto day 5 of storage as compared to their respective day 0 scores. Except for peanut incorporated product on day 15, texture scores for all other categories of products were significantly lower ($P<0.05$) than their day 0 scores. Overall acceptability scores for control and peanut incorporated product remained comparable throughout the storage but for almond and pine nut incorporated products it remained comparable only upto day 10 of storage period. Juiciness, texture and overall acceptability scores among the treatment groups were comparable at corresponding interval during entire period of storage.

The study indicated that premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and had good to very good sensory ratings when stored aerobically in LDPE pouches under refrigeration at 4±1°C for 15 days. Hence, premium mutton nuggets evolved in this study could be safely stored upto 15 days at 4±1°C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

Vacuum packaged premium mutton nuggets showed general increase in TBARS, FFA and peroxide values but decrease in pH values with the advancement of storage period. The pH values in all categories of except pine nut based product remained

comparable upto day 15 of storage and decreased significantly at subsequent interval. The pH values of nut incorporated products were in general higher than control in the initial stage but declined comparatively much faster. TBARS values for all categories of products showed stability upto day 15 of storage but then decreased significantly at each successive storage interval. TBARS values among the treatment groups were comparable at day 0 and day 60 of storage with inconsistencies in the intervening period. Peroxide values for nut based nuggets were comparable upto day 15 of storage. With few variations peroxide values of control product remained highest followed by pine nut based product, almond based product and then least for peanut incorporated product during most part of storage period.

There was general decrease in redness, yellowness and chroma values and increase in hue values of all categories of products as the storage period advanced. The values for redness, yellowness and chroma varied in early part of storage and were then significantly lower in all categories as compared to their preceding scores upto day 30 of storage. Contrary to other attributes, hue values showed stability during storage and values were comparable upto day 15 in each categories and even upto day 30 in peanut and day 60 in pine nut incorporated products. In later part also hue, values in most of the categories of products showed comparable values on day 30 onwards or day 45 onwards.

The total plate counts showed consistently significant increase ($P<0.05$) as compared to their previous count upto day 45 but on day 60 in all categories of products it remained comparable to their day 45 counts. Except for differences in counts on day 0, the total plate counts remained comparable after day 15 among the treatment groups throughout storage period. Psychrophiles and anaerobes were not detected in different categories of products upto day 15 of storage but after their appearance on day 30, there was significant increase ($P<0.05$) at each subsequent storage interval. Psychrophil count among the treatment groups were comparable among themselves but anaerobic count were comparatively higher in almond based products followed by peanut, pine nut then control product. Coliforms were not detected in any products throughout the storage period. Lactic acid bacteria also appeared in all categories of products only on day 45 and their counts were significantly higher ($P<0.05$) on day 60. The lactic acid bacterial count in the products followed almond > peanut > pine nut > control pattern.

The sensory scores for all the attributes showed general decline with the advancement of storage period in all categories of products. Except control product, the general appearance scores in other categories of products were comparable upto day 45 of storage and even upto day 60 in peanut incorporated product. Decline in general appearance scores of control was much faster followed by almond based product, pine nut based product and then peanut incorporated product. Flavour scores of control, peanut, almond and pine nut based products were comparable to their respective day 0 scores upto day 30, 60, 45 and 30 of storage respectively, followed by significant decrease afterwards. Flavour scores of control product remained highest followed by inconsistent pine nut and almond based products and then least for peanut incorporated product. Juiciness scores for control and peanut incorporated products were comparable upto day 45 of storage, whereas these were comparable only upto day 30 in case of almond and pine nut incorporated products. There was significant decrease in juiciness scores afterwards. Juiciness scores were comparable among treatment groups on corresponding day throughout storage period. Scores for textural attributes in all categories of products were comparable upto day 30 of storage but decreased significantly ($P<0.05$) on day 45 and even further on day 60 in control and pine nut based products. Except for the differences on day 0, the texture scores among the treatment groups remained comparable to each other throughout storage period. Overall acceptability scores in all categories of products were comparable to respective day 0 scores upto day 45 of storage and even upto day 60 of storage in peanut incorporated product. Overall acceptability scores among the treatment groups remained comparable to each other throughout storage period.

These observations indicated that premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and had good to very good sensory ratings when stored vacuum pouches under refrigeration at 4±1°C for 60 days. Hence, it was concluded that vacuum packaged premium mutton nuggets developed in this study could be safely stored in multilayered nylon pouches under refrigeration upto day 60 at 4±1°C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

The detailed production cost of the developed products worked out. Studies indicated that incorporation of functionally enriched nut paste in premium mutton nuggets at their optimum level viz. peanut, almond and pine nut resulted in fluctuation of the cost of premium mutton nuggets by ₹ **10,** ₹ **7** and ₹ **24** per 200gm pack respectively as compared to control product. The peanut incorporated product was 10.30% cheaper whereas, almond and pine nut based premium mutton nuggets were also in the affordable range with price fluctuation of 7.2% and 24.72% respectively.

Salient findings:

1. The transformation techniques for various nuts pastes were evolved based on the sensory evaluation of incorporated products.
2. Peanut paste prepared by microwave heating of peanuts in sand for 2 ½ minutes followed by grinding, whereas almond and pine nut paste prepared by direct grinding were found most acceptable when incorporated to the mutton nuggets formulation.
3. The approx fat percentage in peanut paste, almond paste and pine nut paste were 50, 50 and 62.5% and on this basis, levels fixed for evaluation were 10,15 and 20% for peanut and almond pastes and 8,12 and 16% for pine nut paste.
4. On the basis of sensory scores and physico-chemical properties, the optimum incorporation level of peanut paste, almond paste and pine nut paste for preparation of premium mutton nuggets were adjudged as 20%., 15% and 8% respectively.
5. On the basis of analysis of detailed product profile, premium mutton nuggets incorporated with optimum levels of nut pastes had improved lipid profile viz: low in cholesterol and FFA, high total dietary fiber and antioxidant activity in terms of total phenol, DPPH radical scavenging activity and reducing power assay as compared to control.
6. Premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and had good to very good sensory ratings when stored aerobically in LDPE pouches under refrigeration at 4±1°C for 15 days.

7. Premium mutton nuggets enriched with nut based functional components viz. 20% peanut, 15% almond and 8% pine nuts in paste form retained acceptable physico-chemical characteristics, color values, microbiological counts and had good to very good sensory ratings when stored vacuum pouches under refrigeration at 4±1°C for 60 days.
8. The peanut incorporated product was 10.30% cheaper, whereas almond and pine nut based premium mutton nuggets were also in the affordable range with price higher only by of 7.2% and 24.72% respectively.

Conclusion

Premium mutton nuggets with improved lipid profile, antioxidant activity, total dietary fiber content could be developed by incorporation of nuts viz; peanut, almond and pine nuts at their optimum level, without much effect on physico-chemical, colour, texture and sensory characteristics, which in aerobic and vacuum package at refrigeration temperature had shelf life of 15 and 60 days respectively.

MINI ABSTRACT

Nuts besides favourable fatty acid and nutrient profile posses certain bioactive/functional compounds which elicit numerous health benefits. Premium mutton nuggets with improved lipid profile were developed by substituting the traditionally added animal fat in emulsion by incorporation of nuts. Nuts included in the study were peanut, almond and pine nut and their transformation to paste forms was standardized based on preliminary sensory trials. With the aim to substitute added fat by 50, 75 and 100%, three levels of each nut paste viz 10, 15 and 20% for peanut and almond and 8, 12 and 16% for pine nut paste were explored to determine the optimum level of their incorporation. The premier products were further analyzed and compared for detailed product profile which included lipid profile viz: total lipids, total cholesterol, total phospholipids, total glycolipids, total FFA and total glycerides; calorific value, dietary fiber, total phenolic, DPPH radical scavenging activity reducing power assay; and texture profile parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness. The products were also evaluated for their storage stability in aerobic and vacuum packaging at refrigeration temperature for which physico-chemical parameters viz; pH, TBARS, FFA, peroxide value; microbiological characteristics such as TPC, Psychrophilic count, coliform count and additionally anaerobic and lactic acid bacterial count under vacuum packaging; and sensory analysis were made. Based on physico-chemical viz: pH of raw and cooked, emulsion stability, cooking yield, proximate composition, shear force values and sensory scores for attributes such as general appearance, flavour, texture, juiciness and overall acceptability, the optimum levels of incorporation for peanut, almond and pine nut paste were adjudged as 20%, 15% and 8% respectively. Developed premium mutton nuggets had significantly less cholesterol content, increased dietary fiber, enhanced antioxidant activity and acceptable texture profile. It was found that the developed premium mutton nuggets enriched with nut based functional components were stable for 15 days in aerobic packaging and 60 days in vacuum packaging at refrigeration temperature. Thus, it was concluded that functionality components of nuts can be well incorporated to conventional processing to develop superior products in terms of improved lipid profile, enriched dietary fiber and antioxidant activity.

HINDI ABSTRACT

काष्ठ फल आधारित, कार्यात्मक संघटकों से समृद्ध उत्कृष्ट भेड़ मांस नगेट्स का विकास

काष्ठ फलो में अनुकूल वसीय अम्ल एवं पोषक वर्णन के अतिरिक्त अनेक स्वास्थ्य लाभ परिलक्षित करने वाले कुछ जैव–सक्रिय/ कार्यात्मक संघटक भी होते हैं। मांस इमल्शन में पारम्परिक रूप से मिलाये जाने वाले पशु वसा को विस्थापित कर उन्नत वसीय वर्णन वाले उत्कृष्ट भेड़ मांस नगेट्स तैयार किए गए। अध्ययन में मूँगफली, बादाम एवं चिलगोजा जैसे काष्ठ फल सम्मिलित किए गए एवं प्रारंभिक संवेदी परीक्षणों के आधार पर इनके पेस्ट रूप में रूपान्तरण को मानकीकृत किया गया। मिलाये गए वसा को 50, 75 एवं 100 प्रतिशत स्तर पर विस्थापन के ध्येय से प्रत्येक काष्ठ फल के तीन स्तर जैसे कि मूँगफली एवं बादाम के लिए 10, 15 एवं 20 प्रतिशत तथा चिलगोंजा के लिए 8, 12 एवं 16 प्रतिशत पर, पेस्ट को उनके समावेश के उचित स्तर निर्धारण हेतु परखा गया।

विकसित उत्पादों को आगे विस्तृत उत्पाद वर्णन जिसमें कि वसा वर्णन, जैसे कुल वसा, कोलेस्ट्रॉल, कुल फास्फोलिपिड, कुल गलाइकोलिपिडस, कुल ग्लीसराइड, कैलोरी मान, आहार रेशा, कुल फिनॉलिक्स, डी.पी.पी.एच. तत्वरूप ग्राह्यता गतिविधि, अनॉक्सीकारक क्षमता परख तथा बनावट वर्णन, प्राचल जैसे कि कठोरता, चिपचिपाहट, लचक, सामंजस्य, सूजन और चब्यता के लिए विश्लेषित किया गया। उत्पादों को वायुजीवी एवं निर्वात थैलियों में प्रशीतन तापमान पर (4 ± 1^0 से0) पर रखकर भौतिक रसायनिक मापदंडक जैसे पी.एच., टी.बी.ए.आर.एस., एफ.एफ.ए., पेराक्साइड मान, जीवाणुतत्वीय मापदंडक जैसे कि टी.पी.सी., साइक्राफिलिक गिनती, कालीफॉर्म गिनती तथा निर्वात थैलियों, इनके अतिरिक्त एनॉराबिक एवं लैक्टिक एसिड बैक्टीरिया गिनती के आधार पर भंडारण स्थिरता के लिए आंका गया। भौतिक रसायनिक मापदंड जैसे कच्चे एवं पके उत्पाद का पी.एच., पायस स्थिरता, पकवान उपज, आसन्न संघटक, कतरनी बल मूल्य, संवेदी विशेषतायें जैसे सामान्य प्रकटन, सुगंध, सरसता, बनावट एवं समग्र स्वीकार्यता के सूचकांकों के आधार पर मूँगफली, बादाम एवं चिलगोंजा पेस्ट के समावेश का उचित स्तर क्रमशः 20 प्रतिशत, 15 प्रतिशत एवं 8 प्रतिशत विनिर्णीत किया गया। उत्कृष्ट भेड़ मांस नगेट्स में महत्वपूर्ण रूप से कम कोलेस्ट्रॉल, अधिक आहार रेशा, बढ़ी हुई अनॉक्सीकारक गतिविध तथा स्वीकार्य बनावट वर्णन पाया गया।

विकसित काष्ठफल आधारित कार्यात्मक संघटकों से समृद्ध उत्कृष्ट भेड़ मांस नगेट्स वायुजीवी एवं निर्वात संवेष्ठन में प्रशीतन तापमान पर क्रमशः 15 एवं 60 दिन तक स्वतः स्थाई पाये गए। इस प्रकार, यह निष्कर्ष निकाला गया कि काष्ठफल के कार्यात्मक संघटकों को पारम्परिक प्रसंस्करण में समावेशित कर उन्नत वसीय वर्णन, आहार रेशा एवं अनॉक्सीकारक गतिविधियों वाले बेहतर उत्पाद का विकास किया जा सकता है।

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ANNEXURE

Annexure-I

PERFORMA USED FOR SENSORY EVALUATION OF PREMIUM MUTTON NUGGETS

Name of the Panelist_____, Exp. No._____, Trial No._____, Date_____.

Scoring Guide

Attributes	Scale of descriptive attribute of product							
	8	7	6	5	4	3	2	1
General Appearance	Excellent	Very Good	Good	Fair	Slightly poor	Moderately Poor	Very Poor	Extremely Poor
Flavour	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
Texture	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Sample	General Appearance	Flavour	Juiciness	Texture	Overall acceptability
1					
2					
3					
4					

Remarks:

Signature

VITAE

The author of this manuscript was born on 15th Sep, 1978 at Sikta, Distt- West Champaran, Bihar. He cleared his 10th from Janta High School Sikta, in 1993 with 67% marks and 12th from B.D. Evening College in 1995 with 77% marks. Then he obtained the degree of B.V.Sc & A.H. from College of Veterinary Sciences, GBPUA&T, Pantnagar, 2002 with an OGPA of 4.706/5.00. In the year 2004, he completed his M.V.Sc degree from IVRI, Deemed University, Izatnagar, with major in Livestock Products Technology and minor in Veterinary Public Health and secured an OGPA of 8.42/10. He joined PhD as in-service candidate at IVRI in Sep 2011.He had been working in various organizations BARC, Trombay, SOA, IGNOU, FVSc and A.H. SKUAST-Jammu, NAARM Hyderabad and presently serving as Scientist (SS), Div of LPT, IVRI, Izatnagar, Bareilly

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