EFFICACY OF DIFFERENT FLOURS AND NATURAL PRESERVATIVES ON THE PROCESSING AND STORAGE QUALITY OF CHICKEN MEAT CARUNCLES

Thesis

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

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LIVESTOCK PRODUCTS TECHNOLOGY

(Minor Subject: Veterinary Public Health)

By

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(L-2009-V-11-M)

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2011

CERTIFICATE – I

This is to certify that the thesis entitled, **"EFFICACY OF DIFFERENT FLOURS AND NATURAL PRESERVATIVES ON THE PROCESSING AND STORAGE QUALITY OF CHICKEN MEAT CARUNCLES"** submitted for the degree of M.V.Sc. in the subject of **Livestock Products Technology** (Minor Subject: **Veterinary Public Health**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Mr. Parminder Singh (L-2009-V-11-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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CERTIFICATE - II

This is to certify that the thesis entitled, **"EFFICACY OF DIFFERENT FLOURS AND NATURAL PRESERVATIVES ON THE PROCESSING AND STORAGE QUALITY OF CHICKEN MEAT CARUNCLES"** submitted by **Mr. Parminder Singh (L-2009-V-11-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M. V. Sc.** in the subject of **Livestock Products Technology** (Minor Subject: **Veterinary Public Health**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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Gur Parasadhee Vidhia Veecharai Parr Parr Pavai Man ||

Vidhiaa Veechaaree Thaan Paroupakaaree ||

Gur Giaan Prachandd Balaaeiaa Agiaan Andhhaeraa Jaae ||2||

Dandaa Giaan Vichaaru Doot Sadhaaiaa ||

-Shri Guru Granth Sahib Ji.

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ABSTRACT

Studies were conducted to develop shelf stable chicken meat caruncles using spent hen meat, starches, flours, natural preservatives along with modified atmosphere packaging for safety and benefit of the consumers. The process of development of chicken meat caruncles was optimized using three factor three level Box-Behnken design of response surface methodology. Three levels of meat (60%, 65% and 70%), oil level (2.5%, 5% and 7%) and cooking time (3, 4 and 5 mins) were considered for which 17 different runs were conducted. The process was standardized at 65% meat level, 5% oil level and at 4min cooking time. The flours and starches were used successfully to increase the cooking yield and to improve the sensory attributes. Developed chicken meat emulsion with 60% tapioca starch along with 0.2% clove powder produced better results in terms of physico-chemical characteristics, oxidative stability and microbiological parameters than 3% ginger and 2% garlic paste during the period of refrigeration storage at 4±1°C for 9 days. Chicken meat caruncles prepared by using 0.2% clove powder along with 50% CO_2:50% N_2 modified atmosphere packaging produced better acceptability of the product by improving the sensory attributes, decreasing the microbial load and inhibiting the lipid peroxidation (FFA, PV, TBARS number, DPPH % inhibition and ABTS % inhibition) and thus maintained freshness of quality better than their control counterparts up to a storage period of 60 days at room temperature (35±2°C and 70% R.H).

Key words: Shelf stable, clove powder, tapioca starch, chicken meat caruncles.

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Signature of Major Advisor Signature of the Student

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ABBREVIATIONS USED

APHA	:	American Public Health Association
AOAC	:	Association of Official Analytical Chemists
cfu	:	Colony forming unit
CP	:	Clove powder
ERV	:	Extract release volume
ES	:	Emulsion stability
FAO	:	Food and Agricultural Organisation
FFA	:	Free fatty acids
Fig	:	Figure
g	:	Gram
GaP	:	Garlic paste
GiP	:	Ginger paste
hrs	:	Hours
i.e.	:	That is
Kg	:	Kilogram
MDA	:	Malonaldehyde
Meq	:	Milliequivalent
MMb	:	Metmyoglobin
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre
mM	:	Millimolar
nm	:	Nanometre

°C	:	Degree celcius
PV	:	Peroxide value
SE	:	Standard error
Sec	:	Seconds
SPC	:	Standard plate count
TBARS	:	Thiobarbituric acid reactive substances
Tmts	:	Treatments
viz	:	namely
Wt	:	Weight
YMC	:	Yeast and mold count
%	:	Percentage
<	:	Less than
>	:	More than

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Chapter-I

INTRODUCTION

Meat plays an important role in human diet by providing essential amino acids, minerals, trace elements and B-complex vitamins. Besides it is well known for its palatability due to the inherent sensory attributes. At present total meat production of India is about 7.2 million tonnes of meat which accounts for 33.8% share of total export from the livestock industry (DGCIS 2009). So, Indian meat industry especially poultry meat segment is growing at a very fast pace from the last one decade. Chicken meat and its products have experienced increasing popularity and become widely spread all over the world. The processing of meat into different ready-to-eat value added products is also increasing due to urbanization and the growth of fast food sector. However, our traditional meat products due to their limited shelf life at room temperature and bulkiness find very limited role in our diets. So there is strong need to develop nutritionally superior meat products having longer shelf life at room temperature conditions. Moreover, due to increasing problems of hypertension and heart attacks, there is great concern for consumption of foods containing higher levels of unsaturated fatty acids. To resolve all these hurdles there is demand for meat based snack products. So modern trends towards convenience foods have resulted in consumer demand for pre-cooked and shelf-stable meat based snack products.

Snack foods are convenient food items typically designed to be portable, quick and satisfying for one person, house hold, working women, school age children and during travelling or to satisfy short term hunger (Lusas and Rhee 1987). Such foods are less perishable, more durable, more appealing and shelf stable in nature. Various snack foods such as cookies, pretzels, corn chips, tortilla chips, snack nuts, meat snacks etc. are prepared by the use of extrusion technology. Snacks are mainly prepared from cereal grains which are deficient in some amino acids like threonine, lysine and tryptophan (Jean *et al* 1993). So incorporation of meat greatly enhances its nutritional value

especially with respect to essential amino acids content and high biological value protein along with some sensory attributes like flavour and taste. Hence, meat based snack products are superior to other meat products by virtue of their good nutritional profile, less fat, sodium and calorie content. Moreover, it will also extend the market of meat based value added products.

With the rising poultry industry and egg production in our country, the number of spent hens has also increased manifold. Spent hens are 80 to 100 week old birds that are characterized by an objectionable toughness of meat because of high amounts of collagen. This largely reduces its use in meat products thereby causing huge economic losses. Therefore, there is strong need to develop additional approaches for increasing the value of spent hen meat as well as for the development of health oriented further processed poultry products.

In meat based snack products, crispiness, lipid oxidation and growth of yeast and mold are the bottle necks which should be combated to improve its shelf life and its acceptability amongst the masses. Various cereal flours and starches are being used as non-meat ingredients in the processed meat products (including snacks) to improve their processing and nutritional quality such as tapioca starch in beef patties (Nissar 2009), potato starch and barley flour in pork patties (Kumar *et al* 2004 and Kumar and Sharma 2004), rice flour in chicken snacks (Singh *et al* 2002), oat and ragi flour in chicken snack stick (Kale 2009).

Tapioca starch is mainly employed as thickener and stabilizer in fruit pies, soups, puddings, breads, sauces, soy and meat products. The most striking feature of tapioca starch is that it can withstand long cooking times without breaking down and affecting the sensory attributes. Potato starch besides a rich source of carbohydrates provides crispiness to meat products and improves suitability to extrusion (www.shubhamstarch.com). Rice flour a rich source of carbohydrates and some essential amino acids like leucine, isoleucine and

henylalanine. These starches and cereal flours besides acting as functional ingredients have a significant influence on the marketable traits of meat products such as cooking yield, flavour, colour, taste, crispiness, reduced oil pick up and better overall eating quality of the product. Lipid oxidation is an important factor responsible for the deterioration of shelf life of meat based snacks affecting its colour, flavour and nutritional value. Snack meat products are dried/low- moisture meat products with lower water activity (a_W) level and commonly stored at room temperature conditions which makes them more prone to the fungal spoilage.

Various chemicals are used as antimicrobial and antioxidant agents to combat the above mentioned problems. However, the use of synthetic compounds is quite debatable because of their ill effects on human health. Therefore, now a day the use of natural compounds possessing both antioxidant and antimicrobial activities such as ginger (Gupta and Ravishankar 2005), garlic (Yin and Cheng 2003), clove and red chilli (Leuschner and Lelsch 2003) etc. is warranted for maintaining meat quality, extending shelf life and preventing economic loss.

Clove besides acting as antioxidant and antimicrobial (Shan *et al* 2009), anti-inflammatory and anti-carcinogenic, it is a rich source of vitamin C, calcium, magnesium, manganese and omega-3-fatty acids (www.whfoods.com). Ginger (*Zingiber officinale)* has been used as a spice for over 2000 years (Bartley and Jacobs 2000). The extract obtained from its roots being rich in polyphenolic compounds, has high antioxidant (Chen *et al* 1986, Herrmann 1994 and Stoilova *et al* 2007) and antimicrobial activity (Gupta and Ravishankar 2005). Besides it is also used for the treatment of a wide spectrum of affections including atherosclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression, and impotence (Liang 1992). Garlic *(Allium sativum)* is always appreciated for its flavour enhancing and medicinal properties. It has potent antioxidant (Prasad *et al* 1995 and Jackson *et al* 2002), antimicrobial (Naidu 2000, Unal *et al* 2001 and Leuschner and Lelsch 2003),

antiviral, antifungal, antiprotozoal and anti-carcinogenic activity. Also it has beneficial effects on cardiovascular and immune system (Harris *et al* 2001). Hence the use of natural preservatives in meat snacks to increase shelf life at ambient temperature is mandatory.

In the view of above discussion the present study is envisaged with the following objectives:

1. To optimize the processing conditions and the level of incorporation of rice flour, potato and tapioca starch for the development of chicken meat caruncles.
2. To extend the shelf life of chicken meat caruncles with the use of clove, ginger and garlic.
3. To study the effect of different packaging methodologies on the storage stability of developed chicken meat caruncles at room temperature conditions.

Chapter- II

REVIEW OF LITERATURE

Meat and meat products have provided a valuable contribution to our diets since the time of hunter-gatherer societies. The importance of meat is reflected not only in terms of a concentrated source of proteins with high biological value but also due to containment of almost all essential amino acids, vitamins, minerals, fatty acids etc. Recently the outburst of dietary fat related health problems such as obesity, hypertension, high blood cholesterol level, coronary heart diseases and cancer set the trigger for various recommendations to minimize the dietary fat content to the 30% of total energy intake, out of which not more than 10% from the saturated fatty acids (Department of Health 1994). The concept of functional foods includes exploiting the properties of certain foods to treat, mitigate or prevent diseases in addition to their nutritional value. For these reasons, various organizations have recommended a total fat intake to no more than 30% of total calories.

Today majority of world's population is enjoying snack foods which are prepared from natural ingredients or components to yield products with some specific functional properties. Snack is a "titbit" which is a small meal in broadest sense. Snacking can be defined as problem free consumption of easy to handle, miniature portioned, hot or cold products in solid or liquid form which need little or no preparation and are intended to satisfy the occasional pangs of hunger. These snack foods are available in the market in different forms and shapes (Tettweiler 1991). The world snack food market was valued at $66 billion USD in 2003 with baked goods, cookies and crackers, meat snacks, and popcorn accounting for about 22% of these sales (Hodgen 2004). Since cereal based snack products lack some essential amino acids (Jean *et al* 1996), so corporation of meat in snacks enhances the nutritive value especially with respect to

amino acids, flavour and taste (Park *et al* 1993). Many meat based snack products are now marketed with "fat free" slogans, (97- to 98-percent fat-free) and in fact, most have been low in fat since their early development (Kale 2009).

2.1 Utilization of spent hen meat

Today the poultry industry is passing through a period of great turbulence due to a very large number of spent hens. According to an estimate there are about 2.6 billion spent hens each year and thus there is prompt need for this major resource to be used more efficiently and profitably (Singh *et al* 2001). Spent hens (80 to 100 week old birds) are byproducts of the egg industry and the meat obtained from these birds has poor functional properties such as objectionable toughness as compared to broilers and roasters (Baker *et al* 1969) due to its high collagen content (Nakamura *et al* 1975) and cross linkages (Wenham *et al* 1973 and Bailey 1984). The toughness prevents its use in whole meat food and thereby reduces its market value. However, meat from spent hens besides a good source of myofibrillar proteins (Lin and Chen 1989, Rhee *et al* 1999 and Lee *et al* 2003), is highly enriched with omega-3 fatty acids and there is less cholesterol content especially in breast muscle (Ajuyah *et al* 1992) which is good for health.

Spent hen meat primarily utilized for preparation of chicken soups and emulsified chicken products such as frankfurter and bologna. Tough meat from spent hens can be effectively utilized for restructured chicken patties (Hollender *et al* 1987), comminuted surimi based products (Sams 1990 and Nowasd *et al* 2000), smoked chicken sausages (Kondaiah and Panda 1992 and Panda 1996), turkey sausages (Sahoo and Berwal 1993), in canned products as solid particles in liquid products such as soups, sauces, stews, and gravies, or as stewing hens (Voller *et al* 1996), chicken meat *papads* (Ahlawat *et al* 1997), chicken meat balls (Mahajan *et al* 2000), chicken chips (Sharma and Nanda 2002), Bone-in meat pickle (Khanna *et al* 2004),

chicken nuggets (Devatkal *et al* 2008 and Kumar *et al* 2011) and for various other products. Rhee *et al* 1999 studied quality properties of expanded extrudates from blends of corn starch and goat meat, lamb, mutton, spent fowl meat or beef using a single-screw extruder.

Meat consumers and processors can also benefit from the development of efficient and economic technology for processing undervalued meat such as spent hen meat into value-added meat products that are palatable and reasonable in cost (Jin *et al* 2009).

2.2 Tenderization of spent hen meat

Due to persistent increase in the consumption of poultry and its products, the number of spent hens has also increased manifold. Because of unacceptable toughness and brittle bones, the use of spent fowl meat has long been a problem for the poultry industry. Spent hen meat becomes objectionably tough with increasing age and this toughness limits its use in whole muscle foods, resulting in considerable economic losses to the poultry industry (Shimokomaki *et al* 1972 and Nakamura *et al* 1975). Hence we have to explore the methods for meat tenderization. Tenderness of meat is one of considered to be the most important organoleptic attribute of meat (Lawrie 1991). If somehow we achieve this tenderness by physical degradation of the collagen protein, it would be possible to expand the market for the spent hen meat and increase its value (Kondaiah and Panda 1992).

Practically, there is no economically feasible way to increase meat tenderness by decreasing collagen content in spent hen meat (Kondaiah and Panda 1992 and Woods *et al* 1997). In order to achieve efficient utilization of spent hen meat, many workers have attempted to improve meat tenderness by using phosphates (Baker and Darfler 1968), enzymes (Devitre and Cunningham 1985), electrolytes (Lyon and Hamm 1986), pressure treatment (Mendiratta and Panda 1995), using cross linking inhibitor aminoguanidine (Brownlee *et al* 1986, Khatan *et al* 1988, Oxlund and Andreassen 1992, Wu 1995 and Klandorf *et al* 1996) etc. However the

basic idea is that applied treatment should not pose adverse effect on the sensory attributes of the product. Kijowski and Mast (1993) observed that drumsticks from spent layer hens marinated in 2% NaCl brine for 72 h to improve 'tenderness' as measured by shear-force, showed reduction of 40% of shear force of the meat. Marinating in 1.5% lactic or acetic acid for 72h reduced shear-force of the *M. gastrocnemius* by 21.7-fold and 8.1-fold, respectively to values comparable to those observed for broiler chicken drumstick meat. Nurmahmudi *et al* (1997) used 0.6M NaCl and 0.3M $CaCl_2$ for spent hen meat tenderization. The results indicated that 10% level of 0.3M calcium solution into spent hen fillets was sufficient to cause maximum tenderization. Naveena and Mendiratta (2001) studied the tenderization of spent hen meat at pre-or post-chilled stage using different concentrations (0%, 1%, 3% and 5% v/w) of ginger extract (GE). The chunks were allowed to marinate at 4±1°C, cooked in a gas tandoor oven to an internal temperature of 70°C. They observed that treatment of post-chilled spent hen meat with 3% GE for 24 h was found optimum for tenderization. Kanimozhi and Mendiratta (2002) studied the effect of calcium chloride (0.3M $CaCl_2$), Lactic acid (0.5% v/v LA) and combination of $CaCl_2$ and LA. Treated and control cuts were evaluated after 4 h and 24 h of storage at 4±1°C. They observed that combination of 0.3M $CaCl_2$ + 0.5% (v/v) LA was found to be very effective in improving texture and tenderness. Tough turkey meat chunks were tenderized by using calcium chloride, lactic acid and papain by Biswas *et al* (2009). They observed that marination of turkey meat chunks with 0.15M lactic acid and 0.15M calcium chloride is useful to improve the acceptability of turkey meat as it significantly improved the juiciness, texture, tenderness and overall acceptability scores with lower cooked release volume (CRV), higher cooking yield and lower pH.

2.3 Extrusion Technology

Food extrusion is a technique which is used to form snacks of different shapes by forcing the raw food material through a special die which is designed to form and/or puff dry extrudates (Rhee *et al* 1999a). In snack food industry, the use of

extruders was started in 1930, when corn curls were first extruded. Later on, their use for production of second and third generation snacks was increased (Moore 1993). Today a large number of single screw, multiple and twin screw extruders are available for preparation of a wide variety of snacks such as pastas, multigrain snacks, jelly beans, breakfast cereals, cookies, pretzels, French Fries, Idiappam, corn chips, tortilla chips, snack nuts, fish crackers, turkey meat papads, extruded snacks, meat snacks etc.

Extrusion cooking is a highly versatile and efficient technology for food processing as it denatures antinutritional factors and improves the quality and digestibility of proteins (Harper 1978). During extrusion, raw materials undergo various structural and chemical changes such as starch gelatinization, protein denaturation, complex formation between amylase and lipids, inactivation of raw food enzymes, destruction of naturally occurring toxins and reduction of microbial load in the final product (www.industrialextrusionmachinery.com). Some of the scientific studies related to extrusion cooking have been reported for starch conversion (Bhattacharya and Hanna 1987, Cai and Diosady 1993 and Zheng and Wang 1994), degradation of starch (Davidson *et al* 1984, Diosady *et al* 1985 and Cai *et al* 1995) and destruction of some vitamins or pigments (Guzman-Tello and Cheftel 1987, Guzman-Tello and Cheftel 1990 and Ilo and Berghofer 1998). The final quality of the product depends upon structural and chemical alterations occurring due to extensive shear forces during extrusion (Ilo and Berghofer 1999).

2.4 Extruded type meat based snacks

Although most snack products are based on cereals, there are a few that are composed primarily of animal derived raw materials. Traditional meat based snacks mainly include expanded pork rinds (bacon skins or skeens), jerky, chimni etc. However, in many countries, meat snacks such as beef jerky and fermented/cured low-moisture meat sticks are popular (Park *et al* 1993). Similarly popped pork rinds have been popular as a between meal snack in south for

many years. Extruded meat based snack products are convenient, easy to carry, highly crispy and attractive (Zeuthen *et al* 1984), nutritionally sound (Battacharya *et al* 1988) and shelf stable in nature. Extruded snack foods were produced with meats in combination with other ingredients (Mittal and Lawrie 1984, Megard *et al* 1985, Alvarez *et al* 1990 and Alvarez *et al* 1992). For their preparation, Extrusion technology is commonly used and is becoming more and more popular due to their technological advantages over the traditional food processing techniques.

Park *et al* (1993) prepared good quality snacks by using three different levels of beef and fat along with defatted soy flour and corn starch in a single-screw extruder. Berwal *et al* (1996) developed turkey meat *papads* using raw and heat treated meat along with rice flour (50:50) and observed that heat treated turkey meat *papads* had significantly higher sensory scores and better acceptability. Rhee *et al* (1999b) prepared the expanded extrudates from blends of corn starch (81.72-84.86%) and goat meat, lamb, mutton, spent fowl meat, or beef (15.14-18.28%) by using a single screw extruder. They observed that all extrudates were well expanded and low in fat (<1.5%), water activity (<0.12%), bulk density and shear force. Sharma and Nanda (2002) developed and studied the shelf life of chicken chips and reported that sensory properties remained stable up to 12 weeks of aerobic storage. Cosenza *et al* (2003) prepared the fermented cabrito snack stick by using goat meat with different levels of soy protein concentrate and stored at 2±1°C. Anna Anandh *et al* (2005) prepared extruded tripe snack food from buffalo rumen meat (50%) and corn flour (at 40%, 50% and 60%) and control with 100% flour. They reported that 50% corn flour incorporation was optimal with highest score in different sensory attributes like flavour, texture, after taste and overall acceptability. Defreitas and Molins (2006) formulated snack dips with the combination of 50% ham, 26% bacon or 28% pepperoni with added sour cream, unflavored yogurt and tofu and concluded that snack dips were stable and microbiologically safe under simulated wholesale (3 weeks, 2-4°C), retail (10

days, 5°C) and household (10 days, 8-10°C) storage conditions. Ray *et al* (1996) prepared highly nutritious

jerky-type extruded products by using potato flour with beef / chicken and chile powder and proved that extrusion processing resulted in lower microbial counts in the finished product. Kong *et al* (2008) developed value-added jerky-style fish meat snacks from salmon flesh and observed that extrusion cooking did not adversely affect content of omega-3 fatty acids. Kale (2009) developed chicken snack sticks incorporated with 4.2% oat meal and 8.4% ragi flour and extended its shelf life with nisin and potassium sorbate.

2.5 Microwave Cooking

Cooking is the process of preparing food by application of heat. The ultimate satiety or enjoyment that comes from eating meat is largely dependent on cooking method. Cooking not only ensures palatability and microbial safety (Tornberg 2005) but also increases the shelf life of product and enhances flavor and taste (Bognar 1998), improves digestibility (Rodriguez-Estrada *et al* 1997), softens the connective tissue and thus improves texture, coagulates and denatures meat proteins thereby changing their solubility and color, inactivates proteolytic enzymes and thus preventing the development of off flavors, lowers the water activity of meat and improve the peelability of frankfurters and lastly stabilizes the red color of cured meats (Pearson and Gillett 1997).

Microwave treatment is a convenient way for thawing, heating, cooking, drying, frying, pasteurization and blanching of foods (Giese 1992 and Datta and Davidson 2001). Microwave ovens make use of a portion of the electromagnetic spectrum. The two commonly used frequencies for microwave heating are 915 and 2450 MHz with wavelengths of 32.8 and 12.25 cm respectively. Once microwave energy is absorbed, polar molecules (such as water molecules) and ions (dissolved salts) inside the food will rotate or collide according to the alternating electromagnetic field and heat is subsequently generated for cooking. Microwave cooking has many advantages over conventional cooking such as rapid and thorough cooking of product, ease

of control, wide degree of selectivity, reduction in cooking losses, less energy usage and retaining the initial characteristics of the product. Microwave ovens are about 40% efficient as compared to 14% for standard electric ovens and 7% for gas ovens (Traub 1979). The use of microwave oven in the development of snacks, fried without oil, envisages a very interesting field in product development and innovation.

Echarte *et al* (2003) prepared chicken and beef patties and he observed that microwave heating hardly modified the fatty acid profiles of both chicken and beef patties. Total cholesterol oxidation product (COP) increments were 5.3-6.1-fold with microwave heating and 1.5-2.6-fold with frying. **Rababah *et al* (2006)** observed that meats cooked by microwave had higher redness and lower lightness values than those cooked by conventional electric oven. Also they found that meats cooked by microwave had higher maximum shear force, working of shear, hardness, springiness, cohesiveness, and chewiness values than meats cooked by conventional electric oven. Mohammad Nisar *et al* (2010) observed that moisture, fat retention and cooking yield were better in microwave cooked meat patties than those cooked by hot air oven. Some workers believe that microwave oven cooked meat products had lower moisture than conventional oven cooking (Salama 1993 and Hoda *et al* 2002); but Nath *et al* (1996) and Mendiratta *et al* (1998) reported no moisture difference in microwave oven and conventional oven cooked chicken patties.

2.6 Tapioca starch

Tapioca starch is a white fluffy powder which is extracted from the roots of *Manihot esculenta*. It is used as a thickening and stabilizing agent in soups, puddings, breads, sauces, soy and meat products. It becomes clear and gel-like when cooked and dissolves when used as a thickener. Also it can withstand prolonged cooking times without breaking down affecting the sensory attributes. It is also used in bakery food items like chocolates, biscuits, cakes, ice creams

etc. for providing characteristic gelling and bodying properties. Similarly a variety of other products like Instant noodles, Noodles, Vermicelli, Sago etc are based on tapioca starch. Tapioca starch does not mask the flavor of food item in which it is present. Sometimes it is also used as a quick thickener in sauces before serving. As far as appearance is concerned, it provides transparent and glistening sheen to product (www.starch.dk). Tapioca starch can improve flavour and flavour release, increase moisture retention as well as reduce cooking losses (Knight and Perkin 1991 and McAuley and Mawson 1994).

Knight and Perkin (1991) prepared restructured meat products and observed decreased cooking losses with dry addition of tapioca starch. They revealed that combination of preformed whey protein/ carrageenan gel and 3% tapioca starch resulted in low fat sausage with similar mechanical and organoleptic attributes as those of full fat controls. Berry (1997) evaluated the combination of sodium alginate and tapioca starch in low fat beef patties cooked by broiling or grilling to 68 or 74°C and found out that the combination provided improvement in tenderness, juiciness and cooking yields without increasing fat retention or affecting beef flavour.

Hughes *et al* (1998) prepared low-fat frankfurters from lean pork and beef by addition of 3% tapioca starch and they revealed that addition of starch significantly reduced the cooking losses, increased emulsion stability and altered the fat to water ratio of expressible fluid in product. They reported that decreasing the fat content decreased the cooking yield, emulsion stability and product lightness. Fat reduction increased smoke, spice and salt intensities and increased overall flavour intensity and juiciness. Lyons *et al* (1999) reported that dry addition of tapioca starch had a positive effect on the physical and organoleptic parameters of low fat pork sausages when used alone, and in combination with the preformed whey protein/ carrageenan gel blends.

Kong *et al* (2008) developed value-added jerky-style fish meat snacks from salmon flesh using modified tapioca starch (3%), salt (2%), and teriyaki flavoring (2%). Three oil binding agents (tapioca starch, high-amylose cornstarch, oat fiber) were each studied at the 4% level. For the tapioca starch formulation, total fat content was the lowest before extrusion and after drying and, no fatty acid changes were found post extrusion. There were no significant differences between two starch formulations for acceptability of appearance, aroma, taste, texture, and overall acceptability. Mohammad Nisar *et al* (2009) revealed that tapioca starch as a fat replacer has better moisture and fat retention properties in buffalo meat patties. Ponsingh *et al* (2011) prepared buffalo meat sausages with three different levels of tapioca starch (3%, 7% and 10% TS) with 30% less value meat. They revealed that sausages prepared with 7% TS had superior scores for appearance, texture, flavor, juiciness and overall palatability ($P<0.01$) compared to 3 and 10%.

2.7 Potato Starch

Potato starch is fine grounded white powder obtained from the root tubers of *Solanum tuberosum*. Potato starch is used as filler/ thickener in various foods including fast foods, processed meats (cooked sausages, frankfurters, forcemeat and forcemeat products, ham, meat stuffing), fish products (imitation crabmeat, fish semi-finished products–cutlets etc.), baked foods, noodles, soups, sweets, food concentrates. Besides a rich source of carbohydrates, it provides crispiness to meat products and improves suitability to extrusion. Potato starches are recommended as water binders in comminuted meat products to increase yields, reduce losses from cooking, improve texture and extend shelf life (Murphy 2000). Amylopectin potato starch can be used as a thickener and or stabilizer in a wide range of sectors of the food industry (Vries 1995). The pregelatinized waxy starch and potato

starch are hydrated prior to cooking which allows bubbles to be produced and retained and thus controls the crisp and crunchy texture of the

snack foods (Carey *et al* 1998). Bushway *et al* (1982) reported that 1.5% potato starch (PS) plus 1.5% potato flour (PF) or 3% PS may be used as an extender in the formulation of frankfurters without changing the chemical and sensory properties of the finished product. Also the frankfurters prepared from lean beef using 1.5% each of PS and PF were rated more tender and juicy than those made with 3% PS. Dexter *et al* (1993) found that starch added in the chopping process of turkey bologna was very effective in reducing cooking loss as well as decreasing purge loss during storage while not increasing product hardness. Yang *et al* (1995) observed that potato starch was very beneficial in improving the texture of low-fat frankfurters. Bloukas *et al* (1997) prepared low-fat frankfurters (9% fat and 13% protein) with 3.5% potato starch. They observed a significant improvement in the lightness, hardness and skin strength of product. Shand (2000) prepared low fat (<1%) pork bolognas and he reported no significant differences in cooking yield percentage between a control, potato starch (4%) and κ-carrageenan (0.25%) groups. Garcia-Garcia and Totosaus (2008) observed that inclusion of 10% potato starch in low-fat sodium-reduced sausages (prepared from combination of lean beef and pork) had a notable affect on cooking yield and texture, and produced a harder and more resilient product. Potato starch can be used as an extender in low-fat cooked meat products.

2.8 Rice Flour

Rice flour is fine powder obtained from milling of *Oryza sativa.* Although it is a good source of carbohydrates, proteins and a few amino acids like leucine, isoleucine, phenylalanine etc (Traitilevich 1984), yet it lacks some other essential amino acids which are required for good health. So incorporation of essential amino acids can be done by addition of nuts, fish, meat etc to rice flour (Wu *et al* 2002). Since rice like other cereals can be puffed or popped easily, so it is commonly used in snack foods to increase cooking yield, flavor, colour, taste, crispiness etc.

Maga and Reddy (1985) prepared extrudates of varying amounts (10–35%) of deboned minced carp. They blended these extrudates with non-extruded rice flour and prepared *pakodas* (a fried Indian snack food). Clayton and Miscourides (1992) prepared shelf stable, texturised and puffed snack food products from underutilized fish tissue alone and in combination with rice flour blends using a single screw extruder. Singh *et al* (2002) developed chicken snacks using spent hen meat, rice flour, sodium caseinate, spice mix, condiments, salt, phosphate and baking powder. They observed that product with 50% spent hen meat got highest score for colour, appearance, texture, crispiness and overall acceptability. Kumar and Sharma (2005) prepared chicken patties using pressed rice flour as extender at three different levels (5%, 10% and 15% in 1:1 hydration w/w). They observed that at 5 per cent extension, the scores for all the sensory attributes were comparable to control.

2.9 Clove

Clove is a dried floral bud of *Syzygium aromaticum* and it is known to have antimicrobial activity for long time due to its active ingredient - eugenol (Cort 1974). Menon and Garg (2001) reported that clove oil at 0.5% and 1% level inhibited the growth of *L. monocytogenes* in minced mutton. At 1% level, the number of *L. monocytogenes* decreased by 1–3 log cfu/g in the mutton. Leuschner and Lelsch (2003) observed antimicrobial effects of fresh garlic, ground clove and red dried chilli on *Listeria monocytogenes* in broth systems at 37°C and at 4°C for 7 h. Raj *et al* (2005) obseved the effect of ginger extract (GE) and clove powder (CP) on preserving the quality of microwave cooked chevon patties. They blended minced chevon meat with no additives (control), 2% GE, 0.2% CP or a combination of 2% GE + 0.2% CP and prepared four different groups. Results indicated that the GE treated patties were superior (in terms of cooking yield %, moisture %, fat %, Warner-Bratzler shear force, colour / appearance and flavour scores) to

the control and other treated groups. Mytle *et al* (2006) observed that in ready-to-eat chicken

frankfurters, clove oil at 1% and 2% level inhibited the growth of *L. monocytogenes* during storage at 5°C and 15°C. Sofia *et al* (2007) evaluated the antimicrobial activity of six indian spice extracts including clove, cinnamon, mustard, garlic, ginger and mint; and found that the extracts of clove had good inhibitory action at 1% concentration and at 3% concentration, complete bactericidal effect was achieved. Shan *et al* (2009) studied antibacterial and antioxidant effects of clove, oregano, cinnamon stick, pomegranate peel and grape seed extracts on *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella enterica* in raw pork at room temperature (20°C). They observed that all five natural extracts, especially clove, were effective against the bacteria. Also, clove was most effective for retarding lipid oxidation and presented the highest antioxidant activity.

2.10 Ginger

Ginger *(Zingiber officinale)* as a natural preservative finds its potential use in food and pharmaceutical industries and is one of the most popular spices in oriental cuisine. Its antioxidant (Lee *et al* 1986 and Stoilova *et al* 2007) and antimicrobial (Gupta and Ravishankar 2005) activities have been well documented. Ginger bears enormous pharmacological activities such as cardio protective activity, anti-inflammatory activity, anti-microbial activity, anti-proliferative activity, neuro-protective activity, hepato-protective activity etc.

Lee *et al* (1986) studied the antioxidant effect of ginger rhizome in pork patties. They observed that the antioxidant effectiveness was dependent on the kind of preparation, pH and concentration. The storage stability of the product as determined by TBARS value was improved by inclusion of ginger extract. El-Alim *et al* (1999) compared the antioxidant potential of ethanolic extract of ginger with that of ethanolic extracts of sage, basil and thyme in refrigerated and chilled pork patties pretreated with NaCl. They observed that these spices significantly inhibited lipid peroxidation (both peroxide value and TBARS) in

heat-treated meat products during frozen storage. Out of all these, the highest antioxidant activity was observed in the case of

ginger. Naveena *et al* (2001) revealed that incorporation of 2.5% v/v ginger extract (GE) in curing solution significantly ($P<0.05$) reduced the shear force value and improved the sensory attributes of smoked spent hen meat. They demonstrated the tenderizing, antioxidant and antimicrobial properties of GE. Hence GE can be used to improve the qualities of tough meat to produce highly palatable smoked product with improved shelf life.

Gupta and Ravishankar (2005) studied the antimicrobial effects of garlic, ginger, carrot and turmeric pastes against *Escherichia coli* O157:H7. Turmeric paste, fresh carrot, ginger and garlic pastes, and commercial ginger and garlic paste were heated alone or with buffered peptone water (BPW) or ground beef at 70°C for 7 min. Commercial ginger paste and fresh garlic paste showed the strongest antimicrobial activity with complete inactivation of *E. coli* O157:H7 in the paste at 3 days at 4°C and 8°C. Fresh ginger paste showed antimicrobial activity only at 8°C. Only commercial ginger paste had antimicrobial activity in BPW at 4°C for 2 weeks. However, commercial ginger paste showed antimicrobial activity in ground beef at 3 days and after (about 1–2 log CFU/g) compared to control samples at 8°C for 2 weeks.

Rongsensusang *et al* (2005) developed spent hen meat balls using three different levels of fresh ginger paste (2%, 4% and 6%) as an antioxidant and stored at 20±2°C. They revealed that 6% ginger paste can be used in chicken meat balls preparation with optimum antioxidant and desired sensory attributes.

Pawar *et al* (2007) marinated five groups of goat meat ginger rhizome extract (GRE) at 1, 3, 5, and 7% along with 600 ppm of ascorbic acid, 2% sodium chloride and 0.5% sodium tripolyphosphate. The samples were packed in low-density polyethylene bags and stored at

a refrigerated condition of 4±1°C for 1, 3, 5 and 7 days. The GRE-treated chevon patties received a higher score for colour, tenderness, flavour, juiciness, springiness and overall acceptability.

The study revealed that GRE can be used as a potential source of additive in ground, comminuted chevon products because of its antioxidant, proteolytic and antimicrobial properties, and may be used as an effective alternative to many other plant enzymes.

2.11 Garlic

Garlic *(Allium sativum)* is an important spice used in various foods for flavour, antioxidant and antimicrobial activity. Several components of garlic and garlic extracts possess antioxidant activity, which is concentration dependent (Yang *et al* 1993). Many studies have shown the antimicrobial effect of garlic on *S*. *aureus*, *S*. *albus*, *S*. *typhi*, *E*. *coli*, *L*. *monocytogenes*, *A*. *niger*, *Acari parasitus*, *Pseudomonas aeruginosa* and *Proteus morganni* (Kumar and Berwal 1998 and Maidment *et al* 1999).

Though garlic contains various substances such as allicin, diallyl sulfide, allyl sulfide and propyl sulfide, which account for the antioxidant and antimicrobial activity, but allicin is the principal ingredient. In addition, it also contains ascorbic acid, nitrates and nitrites (Aguirrezabal *et al* 1998). Use of garlic is rising in health conscious population. It shows beneficial effects in diseases such as ischemic-reperfusion arrhythmias, infarction, ischemic heart disease, hypertension, hyperlipidemia, peripheral arterial occlusive disease (Prasad *et al* 1995). Sallam *et al* (2004) studied the antioxidant and antimicrobial effects of fresh garlic, garlic powder and garlic oil in raw chicken sausage during cold storage (3°C) and these results showed that the effects are concentration dependent. They observed that fresh garlic (at a concentration of 50 g/kg sausage) demonstrated the most potent effect, but such a high concentration was not acceptable because of its strong flavor. However, addition of fresh garlic (30 g/kg) or garlic powder (9 g/kg) did not result in a strong flavor and, at the same time, they produced significant antioxidant and antimicrobial effects and extended the shelf-life of the product up to 21 days. Hence they suggested that garlic, as a natural herb,

could be used to extend the shelf-life of meat roducts, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin. Oliveira *et al* (2005) reported that in

refrigerated poultry meat, aqueous garlic extract inhibited the growth of microbial contaminants including facultative aerobic, mesophilic, and faecal coliforms on the surface of poultry carcasses. Park and Kim (2009) demonstrated that addition of 3% garlic juice was most effective in decreasing peroxide value, TBARS, residual nitrite and total microbiological counts than those of control samples in emulsified sausage during cold storage.

2.12 Response Surface Methodology

Response surface methodology (RSM) is an important branch of experimental design. It is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. Montgomery (2005) defines RSM as a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The most extensive applications of RSM are in the particular situations where several input variable potentially influence some performance measure or quality characteristic of the process. This performance measure or quality characteristic is called the response. The input variables are called independent variables.

The field of response surface methodology consists of the experimental strategy for exploring the space of the process or independent variables, empirical statistical modeling to develop an approximated relationship between the yield and the process variables. Also, with the help of response surface methodology, optimization can be done for finding the values of the process variables that produce desirable values of the response. The basic idea of RSM is that for any given system, there must be a functional relationship ϕ that correlates the factor x_1 (decision values) to the response y (performance function).

$$y = \phi\,(X_1, X_2, \ldots\ldots X_n)$$

If the function is unknown or extremely complicated, another mathematically simpler function ƒ must be found to approximate ϕ and describe the system. The new function – y' = (x_1, x_2, ……$x_{n)}$ estimates y' rather than the true value of y (Cox and Cochran 1964). It is called approximate or graduating function and may take the form of practically any mathematical expression. The most commonly used expressions are polynomials of first or second degree given as:

$$y' = \beta_o + \sum_{i=1}^{n} \beta_i\ x_i + \varepsilon$$

$$y' = \beta_o + \sum_{i=1}^{n} \beta_i\ x_i + \sum_{i=1}^{n} \beta_{ii}\ x_i^2 + \sum_{i=1}^{n-1} \sum_{j=1}^{n-1} \beta_{ij}\ x_i x_j + \varepsilon$$

Where $\beta_{0,}$ β_{ii} and β_{ij} are constant coefficients and x_i, x_j are coded independent variables usually determined by least squares method and ε is the error involved in estimating the coefficients β from the experimental data. After the coefficients β's have been determined, the stationary points (s) may be determined by taking the first partial derivatives of above equations(s) ,equating them to zero and solving the system of n equations.

$$\partial y'/\partial x_i = 0 (i = 1,2, \ldots\ldots n)$$

It is a commonly employed tool for optimization of processes or products.

RSM has several advantages such as it helps to determine the factor levels that will simultaneously satisfy a set of desired specifications, to determine the optimum combination of factors that yield a desired response and describes the response near the optimum, to achieve a quantitative understanding of the system behavior over the region tested, to determine how a specific response is affected by changes in the level of the factors over the specified levels of interest, to find conditions for process stability etc. However, the main advantage of RSM is the

reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. The application of RSM in novel areas has opened up new

avenues of research towards accurate prediction of responses in the experimental region. In the present scenario RSM can be envisaged to be a still better tool if integrated with an efficient simulation system for prediction and optimization of process parameters to meet the future requirement of product and process specificity.

2.13 Product optimization using RSM

RSM applications are from areas such as chemical or engineering processes, industrial research and biological investigations with emphasis on optimizing a process or system. It is a faster and less expensive method for performing scientific research compared to the classical one-at-a-time or full factorial experiment (Floros and Chinnan 1987).

Park *et al* (1993) studied the effects of feed moisture, fat and corn starch levels and process temperature on physical properties of extrudates of defatted soy flour-amylose corn starch raw beef blends using central composite design of RSM. They observed that fat decreased expansion ratio (ER) and increased bulk density (BD), whereas corn starch increased ER. Products with high ER and low BD and shear-force (SF) tended to have prominent air cells. According to the above said design, the optimum extrusion conditions for minimal SF values, with 20% non-dehydrated beef muscle and varied amounts of defatted soy flour, were: 29.1% feed moisture; 2.96% feed fat; 22% feed corn starch; and 162°C process temperature. Rhee *et al* (1999a) studied the effects of raw material moisture, process temperature and screw speed on expansion ratio (ER) and shear force (SF) of extrudates from blends of corn starch and lean lamb. The process was optimized at 26.5% raw material moisture, 148°C process temperature and 134 rpm screw speed by using central composite second-order design of RSM. Woelfel *et al* (2002) focused on the characterization and incidence of Pale, Soft, and Exudative broiler meat in

a commercial processing plant using RSM. They observed a large portion of commercially processed broiler meat as pale in color and with lower water-holding capacity. *L* value seems to be of more predictive value than pH, is easier and faster than pH, and could therefore be used to sort chicken meat in commercial processing plants. *Rhee et al (2004) prepared snack extrudates of a maximal expansion ratio from blends of catfish flesh (20%), corn flour and defatted soy flour (DSF) using a single-screw extruder. The process was optimized at 26.9% feed moisture, 160.1°C and 4.95% DSF using central composite design of RSM.* Modi *et al* (2007) optimized the level of binders (starch, refined wheat flour and milk powder) with respect to sensory quality of ready-to-eat chicken kebab made from a dehydrated mix using Box–Behnken design of RSM. The optimised levels (as % of cooked meat) were 4.5% starch, 9.5% refined wheat flour and 2% milk powder. They observed that chicken kebab mix was microbiologically safe as indicated by low bacterial counts and absence of coliforms throughout the storage period of 6 months. Fried chicken kebabs prepared from mix stored for up to 6 months were acceptable.

2.14 Physico-chemical and rheological characteristics of fresh meat based snack food

The acceptability of meat based snacks depends on certain physical characteristics of the product along with sensory attributes. It has been observed that some properties especially expansion, density, hardness, crispiness etc are more important for such types of products (Peri *et al* 1983). Shear force value is considered as the most critical factor in determining the texture of extruded meat based snack products and generally lower shear force value indicates lower bulk density, higher expansion ratio and higher water absorption by products (Park *et al* 1993). Also it was observed that >5% fat level interfered with extrusion process and produced irregular extrudates and die head blockage. They also observed that high corn starch and low fat level resulted in higher water absorption index of extrudates. Siriburi and Hill (2000) reported that

water absorption index and water solubility index can be used to characterize extruded products. They also concluded that increasing WSI was due to increasing starch conversion.

When starch was used in large quantity, water absorption index of extrudates increased due to increased starch gelatinization (Davidson *et al* 1984 and Cheftel 1986). Expansion in extrudates with 20% chicken thigh meat was more due to hydrophilic nature of chicken proteins which facilitated starch gelatinization as compared to 40% chicken meat (Chandrashekhar and Kirleis 1998). Lower value of pH in snacks due to increase in level of meat and decrease in starch content in the mix was reported by Mittal and Usborne (1986).

Mohamed (1990) observed that by increasing the meat protein resulted in decreased expansion of the extruded corn starch. Extrudates containing meat with more moisture expanded more (Rhee *et al* 1999b). But moisture content of the meat extrudates decreased as the concentration of non-meat binders increased (Alvarez *et al* 1990). Extrudates should have a final moisture content of less than 5% to make the products brittle (Jean *et al* 1996).

Smith *et al* (1991) substituted 5 different levels (0, 5, 10, 15 and 20%) of partially defatted chopped beef (PDCB) for lean during the preparation of fermented beef snack sausages. It was observed that replacement of these levels of lean with PDCB did not ($P>0.05$) alter percentages of fat, moisture and protein, moisture: protein ratio or a_w of the final product. Cooked yields were similar for all the treatments, averaging about 75%. TBA values were low initially (0.22-0.24) and did not differ ($P>0.05$). Shear force values increased incrementally with increasing levels of PDCB. Overall, addition of PDCB up to 20% was found not to cause significant changes in flavor, texture, or oxidative rancidity.

Park *et al* (1993) studied the physical and sensory properties of high protein texturized extruded products. The selected mixes consisting of Bf [high-beef (29%) low-fat (2.96%)], bf

[low-beef (20%) low-fat] and BF [high-beef high-fat (5%)] which incorporated raw beef, defatted soy flour, and corn starch were extruded through a single screw extruder. The hardness scores for BF were higher than the Bf. Fracturability scores were higher for bf than for Bf or BF. No significant differences among the three were detected in other textural attributes, such as denseness and tooth picking. Off flavor scores were not significantly different among the products. Moreover BF had lower expansion ratio and water absorption values and higher bulk density and shear force values when compared to Bf and bf. The sample bf was higher in bulk density and shear force and lower in water absorption than Bf.

Ray *et al* (1996) developed extruded, nutritious, jerky-type products using potato flour along with either partially defatted chopped beef (PDCB), mechanically separated chicken (MSC) or chicken thigh and leg meat (C) and flavored with three levels (0.5%, 1.0% and 1.5%) of chile powder. They observed that energy required to shear extrudates from C increased with the addition of more chile powder while extrudates from PDCB required more energy for tensile strength at the lower levels of chile powder. Energy required to pull apart C jerky-type extrudates (tensile strength) increased with an added level of chile powder while the opposite was found for PDCB indicating that chile may enhance or decrease binding capability. Extrudates composed of PDCB were higher in protein, lower in fat, and contained more iron than C and MSC extrudates. Several raw ingredients were found to have high microbial counts. Extrusion processing resulted in low microbial counts in the finished products.

Berwal *et al* (1996) prepared turkey meat *papads* using mixed turkey raw meat and heat treated (50°C/20min.) turkey meat by blending with rice flour (50:50) and traditional rice *papads* were used as control. It was observed that there were significant ($P<0.05$) increases in protein and fat contents and decrease in ash content in turkey raw meat and heat treated turkey meat *papads*, compared to control *papads*. The % yield and % expansion on frying were higher

(P<0.05) for control *papads*. The acceptability scores were highest for control, followed by heat treated turkey meat and turkey raw meat *papads*. Moreover, heat treated turkey meat gave a better blend with rice flour, compared to turkey raw meat.

Rhee *et al* (1999b) studied the sensory properties of expanded extrudates prepared from corn starch, ground meat (goat meat, lamb, mutton, spent hen meat, beef). Trained panel sensory scores indicated all products were bland, with no differences found in flavour attributes among products. The dominant flavour notes were rice and dried grassy (mean scores of 2.23-2.29 and 1.81-2.15, respectively, on a 0-15 scale). Extrudates with goat meat had higher fracturability, hardness, and denseness scores than other extrudates. Total polyunsaturated fatty acid percentage was similar for extrudates with beef, lamb and mutton and highest for those with chicken. Water activity for all the extrudates was very low (0.11-0.12) and no notable differences were found among the five products with different meat sources. Total aerobic plate counts were <10 (log_{10}CFU/g) for all products, indicating that the extrusion process had destroyed microorganisms in ingredients and low water activity of finished extrudates prevented the microbial growth.

Sharma and Nanda (2002) developed chicken chips using four different levels of spent hen meat i.e 95, 85, 80 and 75% (WIW) in I, II, III and IV formulations respectively. Flours were incorporated in formulations II, III and IV replacing meat. After adding all the ingredients the chips were extruded in chip form and deep fat fried. They observed that cooking yield of chips in formulation I was significantly lower (P<0.05) than all other formulations whereas moisture and crude protein contents in formulation I were significantly higher (P<0.05) as compared to other formulations. Upon sensory evaluation, no significant differences (P>0.05) were observed in the colour and appearance as well as meat flavour intensity of chicken chips in all the formulations.

However, formulation IV was significantly (P>0.05) better with regard to crispiness and overall acceptability.

Singh *et al* (2002) developed chicken snacks using four different levels of spent hen meat i.e. 0% (control), 40% (I), 50% (II) and 60% (III) along with rice flour, sodium caseinate, spice mix, condiments, salt, phosphate and baking powder. Level of meat significantly (P<0.01) influenced the contents of fat, protein, ash and carbohydrates as well as colour and appearance, flavour, texture, crispness, after taste, meat flavour intensity (MFI) and overall acceptability of the products. Product with 50% chicken meat obtained highest score for colour and appearance, texture, crispness and overall acceptability while the product with 60% chicken meat scored highest for flavour, after taste and MFI. It was also documented that among all the products, pH and emulsion stability did not differ significantly but had an inverse and direct relationship respectively with the level of meat in the emulsion. Use of 50% of spent hen meat is recommended for making a good quality chicken snack.

Lee *et al* (2003) developed the popped cereal snacks with optimum ratio of spent hen meat to grain 1:2 and 1:3 to corn starch and potato starch, respectively. They documented that as the grain ratio of the snacks increased, bulk density decreased and air cells became lager and more at cross-sectional morphologies of the popped snacks. All popped snacks were significantly different (P<0.001) in bulk density, color, and breaking force. Lowest bulk density was observed in the snack with 1:2 ratio of meat and potato starch. The popping degree of snack with starch and spent hen meat was affected by the presence of meat. Also they observed that except for the popped snack with meat and rice flour, as the starch content increased, bulk density decreased gradually. Popped snacks with grains only were higher in *L* value than those with meat and grains. The *a* and *b* values increased with increasing meat content. The optimum ratios of meat to

grain for high expansion ratio were determined to be 1:2 and 1:3 of meat to corn starch and potato starch.

Anna Anandh *et al* (2005) prepared extruded tripe snack food from buffalo rumen meat (50%) and corn flour (at 40%, 50% and 60%) and control with 100% flour. They reported that 50% corn flour incorporation was optimal with highest score in different sensory attributes like flavour, texture, after taste and overall acceptability except for appearance. Linear and significant increasing trends were observed for the physicochemical properties like pH, moisture, protein, fat and ash from the control to 3 levels of corn flour incorporation; and a similar trend was observed for bulk density (BD). However, significant reverse trends were observed for hydratability, water absorption index (WAI) and water solubility index (WSI).

Nurul *et al* (2009) prepared fish meat crackers using different ratios of fish meat to tapioca flour: 1:1 (A), 1.5:1 (B), 2:1 (C) and 2.5:1 (D). They observed that protein and fat content increased with the increase in the ratio of the fish meat. But linear expansion and oil absorption decreased with an increase in the ratio of the fish. Hardness also increased with the increase in the ratio of the fish meat.

2.15 Storage studies of meat based snacks

Cereal based snacks and thermally processed meat products are shelf stable (De-Freitas and Molins 1988). Application of extrusion technology helps in nutrient retention and inactivation of both contaminating and disease producing micro-organisms (Harper 1981).

Smith *et al* (1991) reported that TBA value of fermented beef snack sausages after 0, 30, 60 and 90 days at 24°C, decreased with increase in storage time. Sensory profiles indicated that concentration of PDCB in the formulation had no effect on aroma, mouthfeel, taste, or texture of the product throughout the storage period.

Park *et al* (1993) studied the shelf life of texturized extruded products- Bf [high-beef (29%) low-fat (2.96%)], bf [low-beef (20%) low-fat] and BF [high-beef high-fat (5%)] stored at 37°C for 5 months under aerobic conditions. It was observed that at 0, 30 and 150 days, Total aerobic plate counts, yeast and mold count, coliforms, *E. coli* were all very low and within the normal microbiological profile of breakfast cereals and cereal snack products. *Salmonella* was not present in any sample. Also at day 0, TBA values were highest for BF and lowest for bf. However, TBA values of all products decreased rapidly in 15 days and changed little thereafter. No consistent or marked differences in TBA values were found among the products when stored for 15-210 days.

Rhee *et al* (1999b) stored the developed meat extrudates at 37°C under aerobic conditions for 4 months and it was observed that peroxide values (as measured in meq. peroxides/kg fat) for all products steadily increased ($P<0.05$) throughout the storage study. Moreover, these values were lower for products containing goat meat, lamb, or mutton than for those with beef or chicken. Sharma and Nanda (2002) assessed the shelf life of chicken chips packaged under nitrogen atmosphere in laminate pouches (Aluminium/LDPE) and kept at ambient temperature. During the storage, it was observed that total aerobic count and yeast and mould counts remained well below the permissible limits and TBA value was also far below the threshold value for sensory detection. Sensory scores for various attributes showed a progressive but slow decrease during the entire period of storage. However, sensory ratings of colour and appearance, meat flavour intensity and overall acceptability did not decrease significantly ($P>0.05$) from 2-week onwards and the product rating still remained between good to very good.

Singh *et al* (2002) also reported that there was no significant difference in sensory characteristics of chicken snack throughout storage at 30 ± 2°C for 30 days.

Anna Anandh *et al* (2005) studied the storage changes of aerobically packaged extruded buffalo meat snacks kept at ambient temperature (30±2°C). It was observed that during 7, 14, 21 and 30 days of storage period,there were no significant changes in physicochemical properties (moisture, TBA) except pH, which progressively and significantly

decreased with increasing periods of storage. Total plate, coliform and yeast and mould counts significantly increased on storage and all these counts were <2.5 cfu/g by 30 days. At the 30^{th} day of storage sensory panelists suggested that buffalo meat snacks were either equal (texture, crispness and overall palatability) or better (flavour) in sensory properties than the control, except for appearance. The product was rated "moderate to very acceptable" even after 30 days of storage.

Jin *et al* (2010) studied sensory attributes of dry cured pork stored at 4°C for a period of 90 days under VP: vacuum package, NP: 100% N_2 package, MP: 25% CO_2 + 75% N_2 package. They observed that sensory scores for all parameters except colour reduced significantly at day 60 and 90. The colour scores remained unaffected by the days of storage or by the packaging methods. The scores on aroma and flavour were in acceptable range up to 90 days of storage in all three packaging methods.

2.16 Packaging of processed meat products

Packaging is an important tool for preservation of meat as it avoids contamination, delays spoilage; permit some enzymatic activity to improve tenderness, reduce weight loss and sometimes to ensure an oxymyoglobin or cherry-red colour in red meats at retail or customer level (Brody 1989). The type of packaging i.e. aerobic, vacuum or modified atmosphere to be used for a particular product depends on the nature of product and their shelf life (Sahoo and Anjaneyulu 1995).

Edward *et al* (1987) reported that among vacuum packaging, aerobic packaging and modified atmosphere packaging, vacuum packaging is most widely used for extruded

products as this type of packaging can keep the product safe especially beef products for about 45 days at ambient temperature (32±2°C). Packaging can lower the weight loss, cost of transportation and increase the shelf life of food products (Rozbeh *et al* 1993). Singh *et al* (2002) also studied the changes in quality of vacuum packaged chicken snack during storage at ambient temperature (30±2°C). Extruded type products are mostly packaged in laminated pouches for convenience and to maintain their quality. Vacuum packaging can delay the growth of aerobic spoilage micro-organisms and retard oxidation of lipids in fresh meats (Genigeorgis 1985). MAP provides too many advantages of cost, shelf life, product uniformity, label information, and supply chain integration for most industrialized countries to return to in-store cutting and packaging to supply self-service meat cases.

Aksu *et al* (2005) studied the effect of modified atmosphere packaging, storage period, and storage temperature on the residual nitrate of sliced-pastrima, dry meat product, produced from fresh meat and frozen/thawed meat (stored at -18°C for 240 days and then thawed at 10°C for 24 hrs.). They observed that the storage temperature, storage period and the storage period x the storage temperature interaction had significant ($P<0.01$) effects on the amount of the residual nitrite.

Gok *et al* (2008) concluded that Pastrimas packaged by modified atmosphere packaging using CO_2 and N_2 in the ratio of 35%:65% were given higher sensory ratings than those packaged by Vacuum Packaging (VP) or Aerobic Packaging (AP) when stored at 4°C for 120 days. As storage time increased, color scores declined with the lowest scores observed on day 120. MAP preserved color better than VP or AP which was reflected in color scores. With increased storage time, taste scores decreased and became lowest on day 120. Texture, appearance and acceptability scores showed similar decreasing trends with increased storage time.

3.1 SOURCE OF RAW MATERIALS

3.1.1 Chicken meat

The white Leghorn layer spent hens were obtained from poultry farm of GADVASU, Ludhiana and slaughtered as per standard procedure in the experimental slaughterhouse of Department of Livestock Products Technology, College of Veterinary Science, GADVASU, Ludhiana, Punjab. The dressed layer carcasses were brought to the laboratory and hot deboned manually. After removal of all separable connective tissues, fat, fascia and blood vessels the deboned chicken meat (DCM) was packed in low density polyethylene (LDPE) bags and stored over night at 4±1°C in a refrigerator for conditioning and then frozen at -18±1°C for subsequent use. Frozen meat samples were taken out as per requirement and cut into smaller cubes after partial thawing in a refrigerator (4±1°C). These meat cubes were dipped in a solution containing 0.25% papain (w/w) and 0.15 M calcium chloride (w/v) for about 36-40 hrs at refrigeration temperature (4±1°C) for tenderization (Biswas *et al* 2009). Thereafter the meat was taken out from the solution, washed thoroughly 2-3 times with running water; extra moisture was drained out, then packed in low density polyethylene (LDPE) bags and kept at -18±1°C for subsequent use. Frozen tenderized meat sample were taken out as per requirement and cut into smaller cubes after partial thawing in a refrigerator (4±1°C). The meat chunks were then double minced using 6 mm and 4 mm grinder plates to get fine tenderized minced chicken meat (TMCM) (Plate 5) for experimental use.**3.1.2 Refined wheat flour**

Fresh refined wheat flour (RWF) *i.e*. maida used in the study was procured from local market of Ludhiana.

3.1.3 Salt, sugar and oil

The salt used in the study was food grade TATA Salt (NaCl), 904043901015 Tata chemicals limited, Mumbai; grounded cane sugar and refined soybean oil of FORTUNE brand, all were procured from local market Ludhiana.

3.1.4 Spice mix

All the spice ingredients were procured from local market, Ludhiana, Punjab. Thereafter, it was carefully cleaned and dried in hot air oven at 45±2°C for 2 hours. The ingredients were grounded mechanically in a grinder (Inalsa) and sieved through a fine mesh. The fine powder form of spice mix was obtained using a spice mix formulation as mentioned in Table 1. The spice mixture was stored in a PET (Polyethylene Terephthelate) jar for subsequent use.

3.1.5 Baking Powder

Food grade baking powder (Brand: Ajanta Baking Powder), Product no. 288668, manufactured by Ajanta Food Products Co., Solan, was procured from the local market.

3.1.6 Carboxymethyl cellulose

Carboxymethyl cellulose, sodium salt High Viscosity 500-800 CPs LR, product no. 56095, manufactured by S d fine-CHEM Limited, Mumbai was used.

3.1.7 Rice flour

The excellent quality rice (Brand - Dawat, Rozana Basmati rice) was purchased from local market of Ludhiana, Punjab then kept it for drying at 65°C in a hot air oven for about 2-3 hrs. After drying, it was grounded in a food mixer (Inalsa make) to obtain rice flour (RF, shown in Plate 3).

Table 1. Composition of spice mixture.

S.No.	Name of ingredient	Percentage(w/w)
1	Coriander (Dhania)	**15.00**
2	Cumin seeds (Zeera)	**15.00**
3	Caraway seeds (Ajwain)	**10.00**
4	Aniseed (Soanf)	**10.00**
5	Black pepper (Kalimirch)	**10.00**
6	Capsicum (Mirch powder)	**8.00**
7	Dry ginger powder (Soanth)	**8.00**
8	Cinnamon (Dalchini)	**5.00**
9	Cloves (Laung)	**5.00**
10	Cardamom large (Badi Elaichi)	**5.00**
11	Mace (Javitri)	**5.00**
12	Nutmeg (Jaifal)	**2.00**
13	Cardamom small (Chhoti Elaichi)	**2.00**
	Total	**100**

3.1.8 apioca starch

3.1.9 Tapioca starch (TS) was procured from Shubham Starch Chemical Pvt. Limited, Faridabad, Haryana. The technical specifications and composition details of the tapioca starch (shown in Plate 2) are given in Table 2.

3.1.9 Potato starch

Potato starch (PS) was procured from Shubham Starch Chemical Pvt. Limited, Faridabad, Haryana. The technical specifications and composition details of the potato starch are given in Table 3.

3.1.10 Clove powder

Good quality cloves were procured from the local market and thereafter these were grounded mechanically in a grinder (Inalsa) and sieved through a fine mesh. The fine powder form of clove powder (CP) was stored in a PET (Polyethylene Terephthelate) jar for subsequent use. CP (Plate 4) was used at the level of 0.2% in final product.

3.1.11 Ginger paste

Fresh ginger was purchased from the local supermarket. It was peeled, washed, and minced in a grinder (Inalsa made) in the form of a uniform paste. Ginger paste (GiP) was used at the level of 3% in final product.

3.1.12 Garlic paste

Fresh garlic was purchased from the local market. It was peeled, washed, and minced in a grinder (Inalsa made) in the form of a uniform paste. Garlic Paste (GaP) was used at the level of 2% in final product.

3.1.13 Packaging materials

Low density polyethylene (LDPE) bags of 200 Gauze were procured from the local market for aerobic packaging. For MAP packaging, two layered (Polyester / Polypropylene) laminated pouches were used.

Table 2: Technical and composition details of tapioca starch.

Appearance	White Powder
Moisture %	13 to 14
Ash %	0.3 to 0.4
Soluble	0.7
pH of 10% solution	5 to 7
Acidity for 5gms using NaOH	1.00 ml.
Mesh size-pass through 100 Mesh %	99.80
Pass through 200 Mesh	97.00%
Protein %	0.3
Fibres	0.05 to 0.10
Iron ppm	30 to 40

Table 3: Technical and composition details of potato starch.

Appearance	White fine powder
Odour and taste	Specific pure
Moisture	18.0-20.0%
Raw Protein	Max. 0.1% is.(in substance)
Raw fat	Max. 0.1%
Ash (800^{o}C)	Max. 0.25%
pH-value(20% solution)	6.0-8.0
Bulk Weight	600-700g/l
Gelatinization Temperature	60-65^{o}C

PLATES

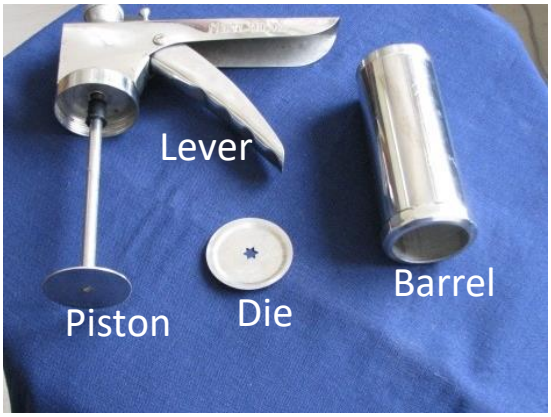


Plate 1: Different parts of manually operated stainless steel extruder

Plate 2: Tapioca starch

Plate 3: Rice flour

Plate 4: Clove powder

Plate 5: Meat Keema

Plate 6: Preparation of meat emulsion

3.2 Experiment No.1: Standardization of the formulation and processing conditions for the development of chicken meat caruncles.

3.2.1 Experimental design

Preliminary trials were conducted for standardization of development of chicken meat caruncles (CMC). Raw chicken caruncles were cooked by different cooking methods including baking, frying and microwave cooking. On the basis of physico-chemical and sensory properties, it was suggested that microwave cooking was the most suitable for development of chicken caruncles.

The experiments were conducted as designed by using Box-Behnken design of RSM including factors as meat level, oil level and microwave cooking time. The three levels under the design are presented in Table 4. The design was taken from the response surface designs and it fulfills most of the requirements needed for optimization of the product. In the above design, X_1, X_2 and X_3 are the coded variables, which are related to uncoded variables in actual units linearly by the relation:

$$X_i = 2 (\xi_i - \bar{\xi}_i) / d_i$$

Where:

ξ_i = Variable value in actual units of the i^{th} observation

$\bar{\xi}_i$ = Mean of the highest and lowest variable value of ξ_i.

d_i = Difference between the highest and lowest variable value of ξ_i.

Based on the above relation the independent variables and their levels in the form of coded variables are given in Table 5.

3.2.2 Preparation of meat emulsion

Tenderized minced chicken meat was blended with common salt, sugar (as mentioned in

Table 4: Experimental Design for Three Variable-Three levels.

X_1	X_2	X_3	Number of runs
±1	±1	0	
±1	0	±1	3×4(combination)=12
0	±1	±1	
0	0	0	1×5replication=5
			Total runs=17

Table 5: Process variables and their levels.

Independent variables	Symbol		Levels	
	Coded	Uncoded	Coded	Uncoded
Meat level (%)	X_1	M	1	70
			0	65
			-1	60
Oil level (%)	X_2	O	1	7.5
			0	5.0
			-1	2.5
Cooking time (mins.)	X_3	t	1	5.0
			0	4.0
			-1	3.0

Overall process schedule for preparation of chicken meat caruncles.

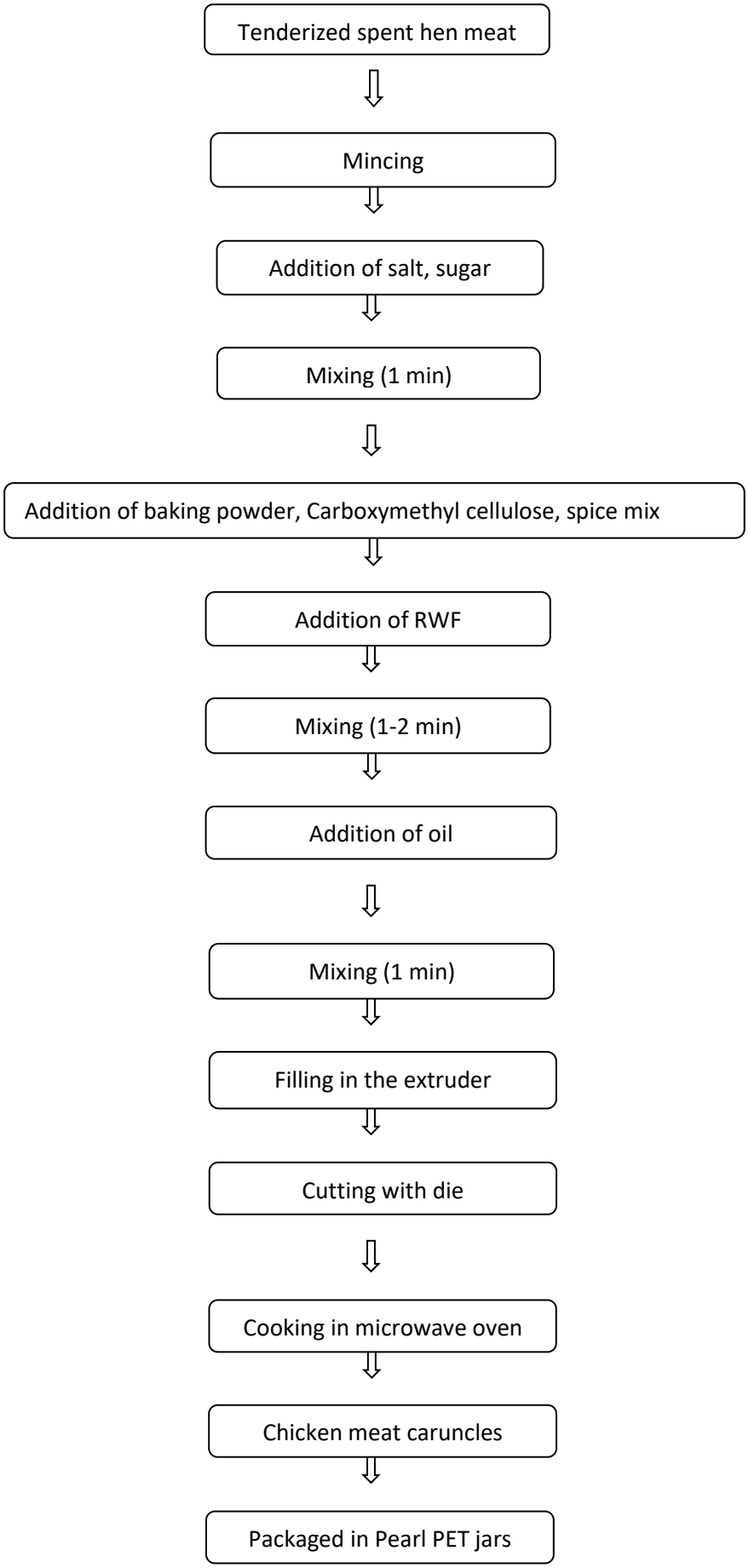


Table 6) and mixed with clean hand up to 1 min. The emulsion was mixed in mixer (Inalsa Maxie plus, 07120219, Inalsa Technologies, New Delhi) for 1 min, followed by mixing of baking powder, Carboxymethyl cellulose, spice mix, up to 30 sec in the mixer. Then RWF was added and again mixed for 1-2 min. At the last the refined oil was added slowly by the side of the dough mixer and mixing was done for another 1 min (Plate 6).

3.2.3 Preparation of chicken meat caruncles

The prepared emulsions were extruded through the manually operated stainless steel extruder (Plate 1) into a thin chip like shape (Plate 7) in a microwave plate. Cooking was done by putting this plate in a microwave oven (Inalsa make) for required time (3-5 min.). Then CMC (Plate 8) were kept in Pearl PET jars and thereafter analyzed for different physico-chemical parameters viz cooking yield, texture profile, colour profile and sensory attributes (colour and appearance, flavour, texture, crispiness, after taste, meat flavour intensity and overall palatability) to determine the optimum chicken meat level, oil level and cooking time using Response Surface Methodology (RSM) software.

3.3 Experiment No.2: Optimization of the level of rice flour, tapioca starch and potato starch in chicken meat caruncles.

3.3.1 Preparation of meat emulsion

Preliminary trials were conducted for selection of three suitable levels of each of RF, TS and PS replacing RWF. Final product obtained in Experiment No. 1 (Meat level 65%, oil level 5% and cooking time 4 mins) acted as control. On the basis of physico-chemical and sensory properties, it was suggested that three levels of RF (35%, 50% and 65% of RWF), TS (50%, 60% and 70% of RWF) and PS (60%, 80% and 100% of RWF) were most suitable for development of CMC.

able 6: Formulation used to prepare meat emulsion.

Ingredients	Percentage level
TMCM	X_1
Refined wheat flour	35
Oil	X_2
Spices	2
Salt	1
Sugar	1
Carboxymethyl cellulose	0.7
Baking powder	0.5

X_1 (TMCM) = 70% or 65% or 60%

X_2 (Oil) = 7.5% or 5.0% or 2.5%

X_3 (Cooking time) = 3 mins or 4 mins or 5 mins

Table 7: Formulation of emulsion of rice flour batch.

Ingredients (%)	Control	T_1	T_2	T_3
TMCM	65	65	65	65
RWF	35	22.75	17.50	12.25
RF	0	12.25	17.50	22.75
Oil	5	5	5	5
Spice	2	2	2	2
Salt	1	1	1	1
Sugar	1	1	1	1
Carboxymethyl cellulose	0.7	0.7	0.7	0.7
Baking powder	0.5	0.5	0.5	0.5

for RF batch, CMC were prepared using three different levels of RF i.e. 35% (T_1), 50% (T_2) and 65% (T_3) replacing RWF, along with control as mentioned in Table 7. For TS batch, CMC were prepared using three different levels of TS i.e. 50% (T_1), 60% (T_2) and 70% (T_3) replacing RWF, along with control as mentioned in Table 8. For PS batch, CMC were prepared using three different levels of PS i.e. 60% (T_1), 80% (T_2) and 100% (T_3) replacing RWF, along with control as mentioned in Table 9.

3.3.2 Preparation of chicken meat caruncles

Chicken meat caruncles of three different batches of each of RF, TS and PS were prepared following the same procedure as in Experiment No.1. CMC were packed in pearl PET jars and thereafter analyzed for different physico-chemical, quality and sensory attributes viz. pH, emulsion stability, cooking yield, compositional parameters (moisture, fat, protein, ash and crude fiber), sensory attributes, texture profile analysis, colour profile, hydratability, water absorption index, water solubility index, water activity etc.

3.4 Experiment No.3: Comparative study of natural preservatives on the quality characteristics of chicken meat emulsion.

3.4.1 Preparation of meat emulsion

From Experiment No. 2, on the basis of physico-chemical, quality and sensory attributes, it was known that RF (35% of RWF), TS (60% of RWF) and PS (100% of RWF) were considered most acceptable. From comparative study between control (100% RWF), RF (35% of RWF), TS (60% of RWF) and PS (100% of RWF), it was known that product with TS (60% of RWF) was most acceptable. TS (60% of RWF) acted as control in this experiment.

Four different batches of chicken meat emulsion i.e. control, T_1, T_2 and T_3 were prepared with all other ingredients except that T_1, T_2 and T_3 were having different

Table 8: Formulation of emulsion of tapioca starch batch.

Ingredients (%)	Control	T_1	T_2	T_3
TMCM	65	65	65	65
RWF	35	17.50	14.00	10.50
TS	0	17.50	21.00	24.50
Oil	5	5	5	5
Spice	2	2	2	2
Salt	1	1	1	1
Sugar	1	1	1	1
Carboxymethyl cellulose	0.7	0.7	0.7	0.7
Baking powder	0.5	0.5	0.5	0.5

Table 9: Formulation of emulsion of potato starch batch.

Ingredients (%)	Control	T_1	T_2	T_3
TMCM	65	65	65	65
RWF	35.00	14.00	7.00	0
PS	0	21.00	28.00	35.00
Oil	5	5	5	5
Spice	2	2	2	2
Salt	1	1	1	1
Sugar	1	1	1	1
Carboxymethyl cellulose	0.7	0.7	0.7	0.7
Baking powder	0.5	0.5	0.5	0.5

proportions of clove powder (CP), ginger paste (GiP) and garlic paste (GaP) respectively. The detailed formulation of four batches is given in Table 10.

3.4.2 Packaging and storage of meat emulsions

The different groups were packaged in Low Density Polyethylene (LDPE) bags and stored for 9 days in a refrigerator (4±1°C). The sample was drawn every alternate day i.e. 1, 3, 5, 7, 9 and analyzed for different physico-chemical quality and sensory attributes like visual colour and odor scores, pH, metmyoglobin percentage, titrable acidity, Extract release volume, Free fatty acid content, Thio Barbituric Acid Reactive Substances (TBARS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonicacid) ($ABTS^{+}$) activity, peroxide value, color profile, Standard Plate count, yeast and mold count, coliforms, *Staphylococcus aureus* etc.

3.5 Experiment No.4: Storage stability of chicken meat caruncles incorporated with clove powder as a natural preservative with different packaging conditions at room temperature (35±2°C, 70% R.H).

3.5.1 Preparation of meat emulsion

Two batches i.e. Control and Treated (0.2% CP) were prepared following procedure as mentioned in experiment No. 2 and formulation given in Table 11 was used.

3.5.2 Preparation and Storage studies of chicken meat caruncles

The above prepared meat emulsion was used to prepare CMC following procedure mentioned in experiment No. 2. Both control and treated batches were divided into two separate groups. The first group was packaged in Low density Polyethylene for aerobic packaging and second group was packaged in Roschermatic packaging machine, type 19/S/CL, Germany, using 50:50 CO_2/N_2 gas mixture in MAP laminated pouches. The latter batch was named as modified atmosphere packaged sample. Finally four different variants of

Table 10: Formulation used to prepare four different meat emulsions.

Ingredients (%)	Control	T_1	T_2	T_3
TMCM	65	65	65	65
RWF	14	14	14	14
TS	21	21	21	21
Oil	5	5	5	5
Salt	1	1	1	1
Sugar	1	1	1	1
Carboxymethyl cellulose	0.7	0.7	0.7	0.7
Baking powder	0.5	0.5	0.5	0.5
CP	0	0.2	0	0
GiP	0	0	3	0
GaP	0	0	0	2

Table 11: Formulation used to prepare chicken meat caruncles.

Ingredients (%)	Control	Treated
TMCM	65	65
RWF	14	14
TS	21	21
Oil	5	5
Salt	1	1
Sugar	1	1
Carboxymethyl cellulose	0.7	0.7
Baking powder	0.5	0.5
CP	0	0.2

CMC were prepared viz. Control aerobic (CA), Control modified (CMAP), treated aerobic (TA) and treated modified (TMAP) (Plate 11). All the samples were stored in Controlled temperature humidity cabinet (Sonar plus BOD 1062M, F-0031900610, Assocaited Scientific Technologies, Delhi, India) at 35±2°C and 70% RH for a storage period of 60 days. The samples were withdrawn at 10 days interval for evaluation of storage quality on the basis of physico-chemical characteristics viz. pH, Thio Barbituric Acid Reactive Substances (TBARS) number, Free Fatty acids % (FFA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonicacid) ($ABTS^{+}$) activity, peroxide value (PV), sensory attributes (appearance, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability) and microbiological quality (Standard plate count, yeast and mold count, coliforms count and *Staphylococcus aureus* count).

3.6 Analytical techniques

3.6.1 pH

The pH of chicken meat emulsion and cooked chicken caruncles (n=6) was determined as per the method given by Trout *et al* (1992) using digital pH tester equipped with a combined glass electrode. For this, 10 g of sample was homogenized with 50 ml of distilled water for 1 min using pestle and mortar. The pH meter was calibrated using standard buffer solution. Then electrode was dipped into the test sample suspension and the pH value of the sample was recorded.

3.6.2 Emulsion stability Twenty gram of meat emulsion was taken in low density polyethylene (LDPE) bags of 150 gauge (size 11 × 10 cm) and were placed in a thermostatically controlled water bath (Model: NSW 125) at 80±1°C for 20 min. After that the bags were removed from water bath, drained off cook out fluid (fat, water soluble solids) and weight of the cooked mass was recorded.

e cooked emulsion was weighed and expressed as percentage (Baliga and Madaiach 1970).

3.6.3 Cooking yield

The weight of raw and cooked CMC of each replicate was recorded before and after cooking and yield (n=3) was expressed as percentage by using following formula.

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

3.6.4 Extract release volume

The standard method of Jay (1964) was followed. Fifteen gram of CME was blended with 60 ml of 0.05M phosphate buffer solution (pH 5.8) for two minutes in a pestle and mortar. The homogenate was filtered through Whatman filter paper No. 1. Filtration was carried out for 15 min and filtrate was collected in a measuring cylinder. The volume of the extract was measured and expressed as ERV (ml).

3.6.5 Titarble acidity

The titrable acidity was measured as per method described by Shelf and Jay (1970) with suitable modifications. Ten gram of CME was blended with 200 ml of distilled water and made the volume 250 ml in a volumetric flask. The slurry was filtered through Whatman filter paper No.1. Then, 25 ml of filtrate was collected and added 75 ml distilled water with three drops of 1% phenolphathalein indicator solution and titrated against 0.1 N NaOH till the final end point was reached (pink colour). The amount of 0.1 N NaOH required was used to calculate the titrable acidity. The titrable acidity was expressed as percent lactic acid and was calculated as given below:

$$\text{Titrable acidity} = \frac{\text{ml of 0.1N NaOH} \times 0.1 \times \text{meq wt. of lactic acid}}{} \times 100$$

weight of sample (g)

3.6.6 Thiobarbituric acid reactive substances (TBARS) number

The extraction method described by Witte *et al* (1970) was used with suitable modifications for the determination of TBARS value in CME and CMC. 10g of sample was triturated with 25 ml of precooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was then quantitatively transferred into a beaker by rinsing with 25 ml of cold distilled water, mixed properly and filtered through ashless filter paper (Whatman filter paper No. 1 supplied by s. d. Fine Chemicals Ltd., Mumbai, India). Then 3 ml of TCA extract (filtrate) was mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a dark room for 16 hrs. A blank sample was made by mixing 1.5 ml of 20% TCA, 1.5 ml distilled water and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India). TBARS number was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

3.6.7 Hydratability

Hydratability of CMC was determined as per procedure of Mittal and Lawrie (1986). About 2.5 gm weighed sample of CMC were placed in a test tube with excess boiling water. The tubes were immersed in a boiling water bath for 5 min. to hydrate the chicken caruncles. The hydrated samples were drained out for 5 min., with an intermittent blotting and then weighed carefully. Hydratability of chicken caruncles was determined as weight of water absorbed by the chicken caruncles (gm)/ weight of dry sample of chicken caruncles.

3.6.8 Water Absorption Index The water absorption index (WAI) was determined as per the procedure given by Anderson *et al* (1969). 2.5 gm of finely ground sample of CMC was weighed into 100ml centrifuge tubes. Then 30 ml of distilled water was added and the sample was left to equilibrate for 30 min with occasional stirring. After centrifugation at

5000 rpm for 10 min, the supernatant was collected in a petridish and the remaining gel was weighed. The water absorption index was calculated as the ratio of weight of gel obtained to that of initial weight of the sample (g/g).

3.6.9 Water Solubility Index

The water solubility index (WSI) was measured according to procedure described by Machado *et al* (1998). The supernatant liquid obtained from WAI determination was used for determination of water solubility index. The supernatant liquid was kept in a hot air oven to evaporate to dryness. After drying, the petridishes were cooled and weighed. The water solubility index was determined as weight of solids to the initial weight of the sample (g/g).

3.6.10 Free fatty acids

The free fatty acids (FFA) in the sample were quantified using method as described by Koniecko (1979). 5 g of the CME or CMC was blended for 2 min. with 30 ml of chloroform in the presence of anhydrous sodium sulphate. Then, it was filtered through Whatman filter paper No. 1 into a 250 ml conical flask. About 2 or 3 drops of 0.2 percent phenolphthalein indicator solution were added to the chloroform extract and titrated against 0.1N alcoholic potassium hydroxide with regular shaking till the end point, permanent pink colour appeared. The quantity of potassium hydroxide consumed during titration was recorded. Percent free fatty acid content was calculated as follows.

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

3.6.11 Peroxide value

The procedure as described by Koniecko (1979) was used with slight modifications. Five gram of CME or CMC was blended for 2 min. with 30 ml chloroform in the presence of

anhydrous sodium sulphate. The mixture was filtered through Whatman filter paper No.1 and 25 ml aliquot of the filtered chloroform extract was transferred to 250 ml conical flask to which 30 ml of glacial acetic acid and 2 ml of saturated potassium iodide solution were added and allowed to stand for 2 min with occasional shaking (swirling) after which 100 ml of distilled water and 2 ml of fresh 1 percent starch solution were added. Flask contents were titrated immediately against 0.1N sodium thiosulphate till the end point was reached (non-aqueous layer turned to colourless). The peroxide value (PV) was calculated in meq/kg of the meat as per the following formula.

$$PV(\text{meq/kg sample}) = \frac{0.1\times \text{ml 0.1N sodium thiosulphate}}{\text{Sample weight (g)}} \times 1000$$

3.6.12 Percentage metmyoglobin

The percent metmyoglobin was measured as per the method described by Trout (1989) with slight modifications. Three grams of CME was taken to which 30 ml of cold phosphate buffer 0.04 M (pH 6.8) was added. The meat sample was homogenized with the help of pestle and mortar for 20 sec and kept at refrigerated temperature (4°C) for 1 hour. Then, it was centrifuged at 10,000 rpm for 5 minutes in a refrigerated centrifuge (Eltek MP-400-R Eltek India, Delhi) at 4°C. The supernatant was collected and filtered through a Whatman filter paper No. 42. The optical density was measured in a UV-VIS spectrophotometer (Elico India Limited, Mumbai) at 525, 572 and 700 nm. Metmyoglobin percent was calculated using the formula of Krzywicki (1979).

$$MMb\% = [1.395-(OD_{572}- OD_{700})/ (OD_{525}- OD_{700})] \times 100$$

3.6.13 Water activity

Water activity (a_w) is determined using hand held potable digital water activity meter (Rotonix HYGRO Palm AW1 Set/40, Serial no. 60146499). Finely ground CMC is filled up (80%) in a moisture free sample cup provided along with a_w meter. The sample cup is

placed into the sample holder, and then sensor is placed on it for five min for getting a_w value. Duplicate reading was performed for each sample.

3.6.14 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The ability to scavenge 1, 1 diphenyl-2picrylhydrazyl (DPPH) radical by added antioxidants in CME or CMC was estimated following the method of Kato *et al* (1988) with slight modifications. DPPH can make stable free radicals in aqueous or ethanol solution, however, fresh DPPH solution was prepared before every measurement. Sample extract was prepared by blending 10g of raw meat emulsion or cooked chicken caruncles with 20 ml of ethanol for 2 min. The content was quantitatively transferred into a beaker and filtered through Whatman filter paper No 42 to get sample extract. Prior to use about 1 ml of DPPH stock solution was diluted with 9 ml of ethanol to make working solution. Then, 200µl of the sample extract was mixed with 1300µl of 0.1M Tris-HCl buffer previously adjusted to a pH of 7.4 and 1 ml of DPPH working solution (250 µM) in test tubes. Ethanol was used as blank sample. After properly mixing the samples, the absorbance (At_0) at time t=0 min, was measured at 517-518 nm using a UV-VIS Spectrophotometer (Elico India Limited, Mumbai) and then incubated at room temperature in dark for 20 mins. After 20 mins, the absorbance (At_{20}) at time t=20 min was measured at the same wavelength. The free radical scavenging activity was calculated as a decrease of absorbance from the equation: Scavenging activity (% inhibition) = $100 - [(At_{20}/At_0) \times 100]$.

3.6.15 ABTS (2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid) radical cation

The spectrophotometric analysis of $ABTS^+$ radical scavenging activity was determined according to method of ABTS, also a relatively stable free radical (Shirwaikar *et al* 2006). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of standard antioxidants. $ABTS^+$ was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting $ABTS^+$ stock solution with 2.45 mM potassium persulphate ($(K_2S_2O_8)$ and allowing the mixture to stand in the dark

at room temperature for 16 hrs before use. Because $ABTS^+$ and potassium persulphate react stoichiometrically at a ratio of 1:0.5 (mol/mol), this will result in complete oxidation of $ABTS^+$. Oxidation of $ABTS^+$ commenced immediately, but the absorbance was not maximal and stable until 6 hrs had elapsed. The radical was stable in this form more than two days, when stored in dark at room temperature. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 at t_0 (t=0 min) and equilibrated at 30°C exactly 6 min after initial mixing. About 2 ml of $ABTS^+$ working standard solution was mixed with 1ml of sample extract (sample extract was prepared similar to the procedure as mentioned for DPPH) and absorbance was measured after 20 min (t_{20}) at 734 nm. The $ABTS^+$ activity was calculated by using formula:

$ABTS^+$ activity (% inhibition) = $[(0.7 - At_{20})/ 0.7] \times 100$.

3.6.16 Texture Profile Analysis

Texture profile analysis (TPA) was conducted using Texture Analyzer (TMS-PRO, Food Technology Corporation, USA). Each CMC was subjected to pretest speed (30mm/sec), post test speed (100mm/sec) and test speed (100mm/sec) to a single Warner-Bratzler shear blade with a load cell of 2500 N. The texture TPA was performed as per the procedure outlined by Bourne (1978). Parameters like hardness [peak force (N) required to cut the sample (V_1)], adhesive force [displacement (N) between initial minute and final minutes (V_3)], adhesiveness [displacement (milli Joules; mJ) from V_5 (value of displacement when load is zero) to final mm (end of the test)] and stringiness [value of displacement at trough (V_4) - value of displacement when load is zero (V_5) and expressed as mm] were calculated automatically by the preloaded Texture Pro software in the equipment from the force-time plot.

3.6.17 Colour Profile Analysis

Colour profile was measured using Lovibond Tintometer (Lovibond RT-300, Reflactance Tintometer, United Kingdom) set at 2° of cool white light (D_{65}) and known as '*L*', *a*, and *b* values. '*L*' value denotes (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/-blueness) values were recorded on CMC kept in a plate or on the surface of petriplate uniformly filled with CME. The instrument was calibrated using light trap (black hole) and white tile provided with the instrument. Then the above colour parameters were selected. The instrument was directly put on the surface of 3 individual CMC. Mean and standard error for each parameter were calculated. The Hue (relative position of colour between redness and yellowness) and chroma (Intensity, brightness or vividness of colour) was determined by using formula (Little 1975).

$$\text{Hue} = (\tan^{-1}) \, b/a$$

$$\text{Chroma} = [a^2 + b^2]^{0.5}$$

Where a = red unit, b= yellow unit

3.6.18 Moisture Estimation

The moisture content in CMC (n=6) was determined using automatic moisture analyzer (Essae, AND MX-50). Finely ground CMC (<5gm) were kept in sample plate and wait for 10-12 min for final reading. All samples were analyzed in duplicate.

3.6.19 Fat Estimation

Fat content in CMC was estimated by ether extraction following AOAC, 1995 method using Socs Plus (SCS-6-AS, Pelican Industries, Chennai). 2g of finely grounded, moisture free meat sample was taken in an extraction thimble fitted in a specially designed beaker. The initial weight of the empty beakers was noted (W_1). The thimbles with the samples were placed in the beakers containing around 80 ml of petroleum ether. The extraction

was carried out automatically using 5 segments programme. After the process was over the beakers containing the fat residue were placed in hot air oven (100°C) for 20-30 minutes. Thereafter, beakers were removed and cooled in a dessicator. The final weight of the beakers was noted as W_2. The fat percentage in the sample was calculated using the following formula.

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

3.6.20 Protein Estimation

The protein content of CMC in the study was estimated as per method described in AOAC, 1995 with suitable modifications using automatic digestion and distillation unit (Kel Plus-KES 12L, Pelican Industries, Chennai). Pre-weighed moisture free meat sample of approximately 0.2-0.3 g was digested in a Kjeldahl's digestion tubes after adding 10 ml of concentrated sulphuric acid and a pinch of digestion mixture (Potassium sulphate and Copper sulphate in 5:1 ratio) at 420°C in the digestion unit. The appearance of clear green colored liquid indicated the completion of digestion. Distillation was carried out automatically in the distillation unit. The ammonia liberated during the process gets collected in boric acid containing indicator (Toshiro's reagent) placed at the receiver end of the distillation unit. The distillate obtained was titrated against standard N/10 hydrochloric acid to light pink end point. The percentage Crude protein was calculated using the following formula. A parallel blank was run to eliminate the error.

$$\text{Nitrogen (\%)} = \frac{14.01 \times 0.1\ N \times (TV-BV)}{W \times 1000} \times 10$$

$$\text{Protein (\%)} = \text{\% Nitrogen} \times 6.25$$

Where:

14.01	=	Molecular weight of ammonia
0.1N	=	Titration solution's normality
TV	=	Titer value
BV	=	Blank value
W	=	Sample weight in g

3.6.21 Crude Fiber Estimation

Crude fiber of CMC was estimated as per AOAC, 1995 method using Fibra Plus, automatic unit, (FES-6, F-09014, Pelican Industries, Chennai). Fat free sample was weighed (W) and transferred to the crucibles and loaded in the Fibra plus unit. Acid digestion was carried out using 150 ml of 1.25% H_2SO_4. Digestion was carried out at 450°C for 45 min. The acid was drained out and rinsed thoroughly with distilled water. In second phase, alkali digestion was done using 150 ml of 1.25% NaOH in the same manner. After draining out the alkali rinsing was done using distilled water. The crucibles were dried in a hot air oven at 100°C for 20-30 min. Then, the crucibles were cooled in desiccators. The weights of the crucibles (before ashing) were noted (W_1). Then, crucibles were transferred to the muffle furnace and heated to 400°C for 2 h for ashing. After cooling the crucibles in a desiccator, weighed (after ashing) as W_2 and crude fiber content in the sample was calculated using the following formula.

$$\text{Crude Fiber \%} = \frac{W_1 - W_2}{W} \times 100$$

3.6.22 Ash Estimation

The ash content in the CMC was estimated as per AOAC, 1995 method using muffle furnace. About 2 g of moisture free sample was taken in pre-weighed moisture free silica crucibles.

The crucibles were then placed on a hot plate for charring. Thereafter silica crucibles with charred mass were placed in muffle furnace at 550°C for about 7-8 hours to obtain white ash. After cooling of the furnace the crucibles were taken out in desiccators and final weight is recorded. The % ash content was calculated using the following formula.

$$\text{Total ash \%} = \frac{\text{Weight of ash (g)}}{\text{Sample weight (g)}} \times 100$$

3.6.23 Carbohydrate Estimation

An analysis of the percentage of carbohydrate in the CMC was not performed. It was simply calculated by subtracting % moisture, fat, protein, fiber and ash from 100.

3.6.24 Energy/Calorie value

Estimates of total calories in CMC were calculated on the basis of 100 g portion using Atwater values for fat (9 kcal/g), protein (4.02 kcal/g) and carbohydrate (4 kcal/g). Therefore, the calorie values were estimates and not actual values.

3.6.25 Moisture: Protein ratio

Moisture: Protein ratio of CMC was calculated simply by dividing % moisture with % protein.

3.6.26 Sensory evaluation

A seven member experienced panel of judges from the pool of teachers and postgraduate students of College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University evaluated the CMC for sensory attributes viz. colour and appearance, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability following 8- point hedonic scale (Keeton, 1983 with slight modification) where 8=extremely desirable and 1=extremely undesirable. Three replicates (n=21) were conducted.

3.6.27 Colour and Odour evaluation

The visual colour score card 5- point descriptive scale (where 1- Very undesirable, 2- Moderately undesirable, 3- Moderately desirable, 4- Desirable and 5- Very desirable) and odour score card 5- point scale (where 1- Very unpleasant, 2-Moderately unpleasant, 3- Moderately pleasant, 4- Pleasant and 5- Very pleasant.) were used by a panel of seven judges consisting of teachers and postgraduate students of College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University to evaluate the visual colour and odour scores of CME.

3.6.28 Microbiological quality parameters

Standard plate counts, *Staphylococcus aureus* count, Total Coliforms counts and Yeast and mold counts of the samples were enumerated following the methods as described by American Public Health Association (APHA 1984).

3.6.28.1 Preparation of sample and serial dilutions

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

3.6.28.2 Standard plate count (SPC)

23.5 g of plate count agar (obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India; Code No.M091) was suspended in 1000 ml of distilled water followed by boiling to dissolve the medium completely, distributed into 250 ml conical flasks and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. Final pH of the medium was 7.0±0.2 at 25ºC. One ml in duplicate of suitable dilution was pipetted into the sterilized petriplates. About 20 ml of the sterilized and melted medium was poured over it, mixed slowly with rotating actions. Pour plate technique was used. The plates were incubated at 35ºC for 48-72 hrs in an inverted position. Plates showing 30 to 300 Colonies were counted manually. The average number of colonies was multiplied by reciprocal of the dilution and expressed as $\log_{10}$ cfu /g of sample.

3.6.28.3 Total coliform counts

40.62 g of violet red bile glucose agar (VRBGA) procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India (Code No. ME581) was suspended in 1000 ml of distilled water, boiled to dissolve the medium completely and cooled to 45°C. The final pH of the medium was adjusted to 7.4±0.2 at 25°C. One ml in duplicate of suitable dilution was pipetted into the sterilized petriplates. About 20 ml of the melted medium was poured over it, mixed slowly with rotating actions. The plates were allowed to stand for some time till the agar media got solidified. Then, an anaerobic layer was created by pouring 4-5 ml of the agar media. The plates were incubated at 35°C for 24 hrs. The numbers of red purple colonies with about 0.5 mm diameter surrounded by a zone of precipitated bile were counted. Colonies judged to be borderline cases were also counted. The average number of colonies was multiplied by the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

3.6.28.4 Yeast and mold counts

39g of potato dextrose agar (obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India; Code No. M096) was suspended in 1 litre distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The pH of sterilized medium was set to pH 3.5 by acidifying with 10 ml of 10% tartaric acid. Precaution was taken not to heat the medium after addition of the acid. Pour plate technique was followed for inoculation of suitable sample dilution and plates were incubated at 25°C for 5 days. Black, white, red, greenish black coloured colonies on the plates were counted and expressed as $\log_{10}$cfu/g.

3.6.28.5 *Staphylococcus aureus* count

63g of baird parker agar (BPA) procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India (Code No.MM043) was suspended in 950 ml of distilled water, boiled to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 min. and cooled to 50°C. Add aseptically 50 ml concentrated egg yolk emulsion and 3 ml sterile 3.5% potassium tellurite(obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India; Code No.RM090) solution and mix well before pouring. Final pH of the medium was 7.0±0.2. One ml in duplicate of suitable dilution was pipetted into the sterilized petriplates. About 20 ml of the sterilized and melted medium was poured over it, mixed slowly with rotating actions. Pour plate technique was used. The plates were incubated at 35ºC for 48 hrs in an inverted position. The number of the intensely dark, shiny, regularly shaped colonies surrounded by clear halos were counted and expressed as $\log_{10}$ cfu /g of sample.

3.6.29 Statistical analysis

In first experiment, Box-Behnken Design (Design-Expert 8.0.4.1 Trial version, 2010) of RSM was used for process optimization of development of CMC. In rest of the

experiments,

Duplicate Samples were taken for each parameter and the experiment was repeated three times, total being six observations (n=6) were taken for consistency of the results except sensory attributes in which 21 observations (n=21) were taken. The data were statistically analyzed on 'SPSS-16.0' software package (Trial version) as per standard methods (Snedecor and Cochran 1994) for one-way and two-way analysis of variance using Duncan's Multiple Range Tests and Homogeneity tests to test the significance of difference between means.

PLATES

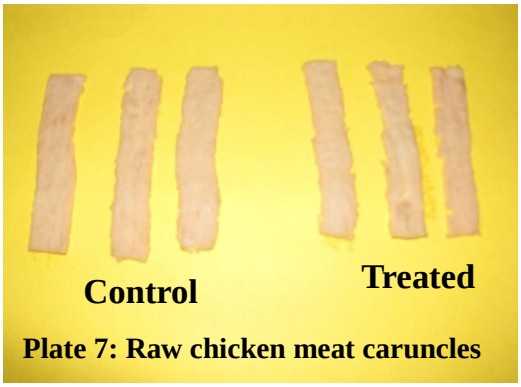


Plate 7: Raw chicken meat caruncles

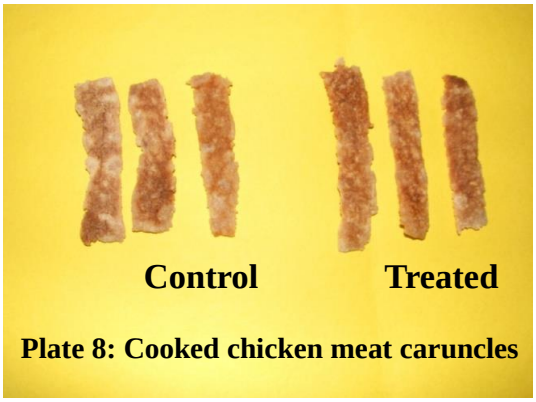


Plate 8: Cooked chicken meat caruncles

Control : without natural preservative and Treated : with 0.2% clove powder.

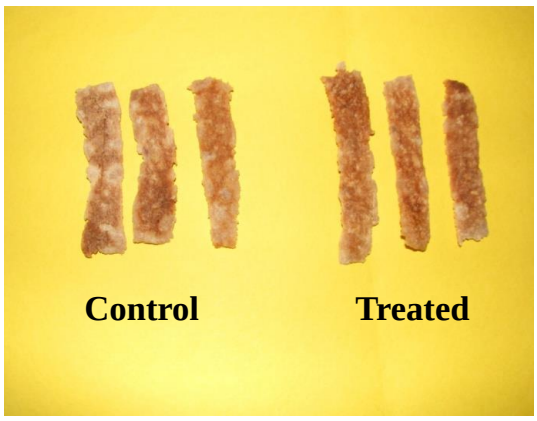


Plate 9: Before storage chicken meat caruncles

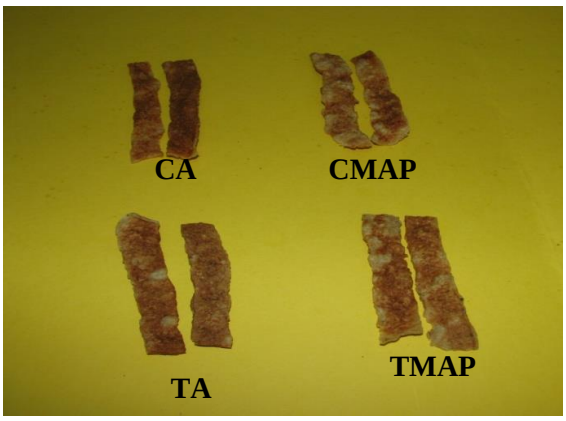


Plate 10: After storage chicken meat caruncles

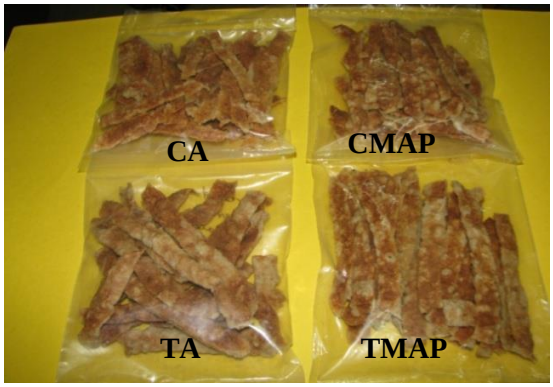


Plate 11: Before storage chicken meat caruncles

Plate 12: After storage chicken meat caruncles

CA: Control Aerobic, CMAP: Control modified,

TA: Treated Aerobic and TMAP: Treated modified

4.1 Experiment No.1: Standardization of the formulation and processing conditions for the development of chicken meat caruncles.

4.1.1 Selection of the product parameters for optimization

For selection of product with respect to meat level, oil level and cooking time, a statistical design of RSM was used namely, Box-Behnken Design. Under this experiment 17 runs were conducted using three meat levels (60, 65 and 70%), three oil levels (2.5%, 5.0% and 7.5%) and three cooking times (3, 4 and 5 mins) as variables. Data pertaining to each run along with different physico-chemical parameters (texture profiles, colour profiles, moisture, cooking yield and water activity) and sensory characteristics (appearance, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability) are given in Table 12. After conducting the runs, second order polynomial was fitted to each response by using RSM software i.e. Design Expert, version 8.0.4 (Design Expert 8.0.4.1, Trial version, 2010). After fitting the equation several targets of response were given through the software for achieving the best combination of variables which would result in required product. Target values were selected based upon preliminary trials conducted, sensory evaluation and available literature. For selection of the product, ANOVA tables were prepared for each response and 3-D graphs were made which showed effect of interaction of two variables on each response.

4.1.2 Model fitting

Three meat levels (60, 65 and 70%), three oil levels (2.5%, 5% and 7.5%) and three cooking times were taken for optimization and 17 runs were conducted. The multiple regression analysis was used to fit the second order polynomial by least square method to the experimental results as shown in Table 12 and the experimental result in uncoded values are given in Table 13. There is a functional relationship ϕ that correlates the decision variables

Table 12: Experimental data for the three factors (coded) three level response surface analyses.

Run	Factors			Responses															
	% Meat level (X_1)	% Oil level (X_2)	Cooking time in mins. (X_3)	CY (%)	Hardness (N)	Adhesiveness (mJ)	Adhesive force (N)	Stringiness (mm)	*L*	*a*	*b*	a_w	M (%)	CA	Flv	Csp	AT	MFI	OA
1	1	-1	0	54.40	100.30	61.04	8.20	0.41	22.59	8.27	19.17	0.56	6.91	6.57	6.21	6.64	6.64	6.79	6.64
2	0	1	1	50.90	119.65	73.90	8.80	0.54	32.61	8.99	21.37	0.60	8.23	6.64	6.50	6.64	6.64	6.36	6.64
3	-1	0	-1	51.79	102.82	72.85	8.35	0.54	30.11	8.08	20.45	0.58	8.56	6.50	6.64	6.71	6.71	6.93	6.93
4	1	1	0	54.40	107.83	70.23	9.13	0.66	28.44	9.54	20.42	0.58	8.64	6.57	6.57	6.57	6.64	6.64	6.93
5	0	1	-1	54.93	105.68	78.68	8.48	0.29	30.42	9.21	19.98	0.56	6.27	6.64	6.36	6.64	6.64	6.64	6.64
6	-1	-1	0	59.00	102.35	75.12	8.72	0.85	21.57	8.95	18.88	0.48	8.66	6.57	6.07	6.71	6.64	6.14	6.07
7	-1	0	1	50.88	102.12	74.98	8.40	0.53	28.89	8.09	18.49	0.59	8.41	7.00	6.29	6.64	6.36	7.00	6.64
8	-1	1	0	53.70	101.69	71.63	8.55	0.43	30.26	8.42	18.86	0.65	8.55	6.50	6.14	6.57	6.29	6.79	6.64
9	0	-1	1	54.40	103.13	72.69	9.85	0.24	29.92	8.67	20.83	0.70	8.68	6.50	7.00	6.87	6.57	6.64	6.36
10	1	0	-1	54.37	102.58	72.79	8.35	0.24	29.90	9.04	19.06	0.56	7.21	6.64	6.36	6.64	6.57	6.50	6.64
11	0	-1	-1	52.8	101.2	76.0	8.92	0.43	28.3	9.8	19.6	0.65	7.2	6.5	6.2	6.79	6.86	7.00	6.29

				0	7	2			3	9	0		2	0	1				
12	1	0	1	56.90	102.66	82.86	8.90	0.12	29.94	8.49	20.85	0.48	7.27	6.29	6.36	6.79	6.50	6.14	6.21
13	0	0	0	51.85	104.55	78.74	8.92	0.62	29.29	8.58	18.80	0.56	8.66	6.57	6.64	6.79	6.86	6.93	6.93
14	0	0	0	54.00	100.25	79.67	9.93	0.23	27.29	9.69	19.60	0.44	7.64	6.29	6.14	6.86	6.29	6.14	6.21
15	0	0	0	52.92	101.27	78.16	8.88	0.37	31.85	9.73	19.00	0.56	7.22	6.36	6.07	6.71	6.50	6.36	6.36
16	0	0	0	54.00	101.35	81.37	9.48	0.44	30.96	8.87	20.59	0.62	8.41	6.64	6.14	6.64	6.00	6.00	6.14
17	0	0	0	53.30	101.74	74.88	8.80	0.23	33.31	9.95	19.53	0.48	7.24	6.43	6.00	6.64	6.50	6.36	6.36

CY= Cooking yield (%), *L*=Lightness, *a*= redness, *b*= yellowness, a_w= water activity, M= moisture %, CA = colour/appearance, Flv= flavour, Csp= crispiness, AT=After-taste, MFI=Meat flavour intensity and OA=overall acceptability.

Table 13: Experimental data for the three factors (uncoded) three level response surface analyses.

Run	Factors			Responses															
	% Meat level	% Oil level	Cooking time (mins)	CY (%)	Hardness (N)	Adhesiveness (mJ)	Adhesive force (N)	Stringiness (mm)	*L*	*a*	*b*	a_w	M (%)	CA	Flv	Csp	AT	MFI	OA
1	70	2.5	4	54.40	100.30	61.04	8.20	0.41	22.59	8.27	19.17	0.56	6.91	6.57	6.21	6.64	6.64	6.79	6.64
2	65	7.5	5	50.90	119.65	73.90	8.80	0.54	32.61	8.99	21.37	0.60	8.23	6.64	6.50	6.64	6.64	6.36	6.64
3	60	5.0	3	51.79	102.82	72.85	8.35	0.54	30.11	8.08	20.45	0.58	8.56	6.50	6.64	6.71	6.71	6.93	6.93
4	70	7.5	4	54.40	107.83	70.23	9.13	0.66	28.44	9.54	20.42	0.58	8.64	6.57	6.57	6.57	6.64	6.64	6.93
5	65	7.5	3	54.93	105.68	78.68	8.48	0.29	30.42	9.21	19.98	0.56	6.27	6.64	6.36	6.64	6.64	6.64	6.64
6	60	2.5	4	59.00	102.35	75.12	8.72	0.85	21.57	8.95	18.88	0.48	8.66	6.57	6.07	6.71	6.64	6.14	6.07
7	60	5.0	5	50.88	102.12	74.98	8.40	0.53	28.89	8.09	18.49	0.59	8.41	7.00	6.29	6.64	6.36	7.00	6.64
8	60	7.5	4	53.70	101.69	71.63	8.55	0.43	30.26	8.42	18.86	0.65	8.55	6.50	6.14	6.57	6.29	6.79	6.64
9	65	2.5	5	54.40	103.13	72.69	9.85	0.24	29.92	8.67	20.83	0.70	8.68	6.50	7.00	6.87	6.57	6.64	6.36
10	70	5.0	3	54.37	102.58	72.79	8.35	0.24	29.90	9.04	19.06	0.56	7.21	6.64	6.36	6.64	6.57	6.50	6.64
11	65	2.5	3	52.80	101.27	76.02	8.92	0.43	28.33	9.89	19.60	0.65	7.22	6.50	6.21	6.79	6.86	7.00	6.29
12	70	5.0	5	56.90	102.66	82.86	8.90	0.12	29.94	8.49	20.85	0.48	7.27	6.29	6.36	6.79	6.50	6.14	6.21
13	65	5.0	4	53.30	101.74	74.88	8.80	0.23	33.31	9.95	19.53	0.48	7.24	6.43	6.00	6.64	6.50	6.36	6.36
14	65	5.0	4	51.85	104.55	78.74	8.92	0.62	29.29	8.58	18.80	0.56	8.66	6.57	6.64	6.79	6.86	6.93	6.93
15	65	5.0	4	52.92	101.27	78.16	8.88	0.37	31.85	9.73	19.00	0.56	7.22	6.36	6.07	6.71	6.50	6.36	6.36
16	65	5.0	4	54.00	101.35	81.37	9.48	0.44	30.96	8.87	20.59	0.62	8.41	6.64	6.14	6.64	6.00	6.00	6.14
17	65	5.0	4	54.00	100.25	79.67	9.93	0.23	27.29	9.69	19.60	0.44	7.64	6.29	6.14	6.86	6.29	6.14	6.21

CY= Cooking yield (%), *L*=Lightness, *a*= redness, *b*= yellowness, a_w= water activity, M= moisture %, CA = colour/appearance, Flv= flavour, Csp= crispiness, AT=After-taste, MFI=Meat flavour intensity and OA=overall acceptability.

(X_1, X_2…..X_n) to the response y (performance function). The exact mathematical representation of the function (*y'*) is either unknown or extremely complex. However second order polynomial equation can be assumed to approximate the true functions. Hence the fitting of second order polynomial resulted in the following models/equations for each response optimized.

Hardness = $102.51 + 0.75X_1 - 1.91X_2 + 1.04X_3 - 0.36X_1X_2 + 0.023X_1X_3 - 6.37X_2X_3 - 2.54X_1^2 + 3.12X_2^2 + 1.74X_3^2$

Adhesiveness = $76.11 - 2.47X_1 - 3.48X_2 - 0.79X_3 + 0.46X_1X_2 + 1.00X_1X_3 - 2.87X_2X_3 + 4.63X_1^2 - 3.35X_2^2 - 3.56X_3^2$

Adhesive force = $8.69 – 0.19X_1 – 0.096X_2 – 0.21X_3 – 0.096X_1X_2 - 0.47X_1X_3 - 0.30X_2X_3 + 0.39X_1^2 - 0.36X_2^2 + 0.34X_3^2$

Stringiness = $0.31 + 0.035X_1 + 0.095X_2 – (8.333E\text{-}003)X_3 – 0.20X_1X_2 + 0.13X_1X_3 - 0.089X_2X_3 + 0.032X_1^2 + 0.19X_2^2 + 0.013X_3^2$

$L = 29.16 + 1.06X_1 – 2.42X_2 – 1.02X_3 + 2.30X_1X_2 + 0.66X_1X_3 – 2.05X_2X_3$

$a = 9.56 - 0.32X_1 + 0.045X_2 – 0.057X_3 – 0.24X_1X_2 - 0.35X_1X_3 - 0.46X_2X_3 - 0.51X_1^2 - 0.53X_2^2 - 0.23X_3^2$

$b = 19.73 - 0.17X_1 - 0.12X_2 – 0.26X_3 + 0.90X_1X_2 - 0.34X_1X_3 - 0.94X_2X_3$

Water activity = $0.57 + 0.052X_1 - 0.012X_2 – 0.045X_3$

Moisture = $7.03 + 0.29X_1 + (7.500E\text{-}003)X_2 – 0.39X_3 – 0.37X_1X_2 + 0.13X_1X_3 - 0.35X_2X_3 + 0.73X_1^2 + 0.53X_2^2 + 0.52X_3^2$

Cooking yield = $53.80 – 1.87X_1 + 0.78X_2 – 0.79X_3$

Colour / Appearance = $6.54 + 0.098X_1 + 0.027X_2 + 0.036X_3 – 0.089X_1X_2 + 0.21X_1X_3 – 0.036X_2X_3$

Flavour = $6.34 + 0.23X_1 - 0.018X_2 - 0.089X_3 + 0.071X_1X_2 - 0.18X_1X_3 – 0.18X_2X_3$

Crispiness = $6.69 + (1.786E\text{-}003)X_1 - 0.018X_2 + 0.016X_3 – (1.429E\text{-}007)X_1X_2 - 0.11X_1X_3 + 0.000X_2X_3 + 0.11X_1^2 - 0.041X_2^2 - 0.037X_3^2$

After-taste = $6.61 + 0.13X_1 + 0.045X_2 + 0.054X_3 – 0.071X_1X_2 - 0.12X_1X_3 - 0.089X_2X_3 - 0.093X_1^2 + 0.16X_2^2 - 0.22X_3^2$

Meat flavour intensity = $6.55 + 0.38X_1 + 0.036X_2 + 0.027X_3$

Overall acceptability = $6.51 + 0.28X_1 + 0.018X_2 + (8.929E\text{-}003)X_3$

The above given equations followed quadratic model except for *b* value, Colour / Appearance and Flavour responses which followed 2FI model and water activity, cooking yield, meat flavour intensity and overall acceptability responses which followed Linear model.

4.1.3 Effect of variables on various responses

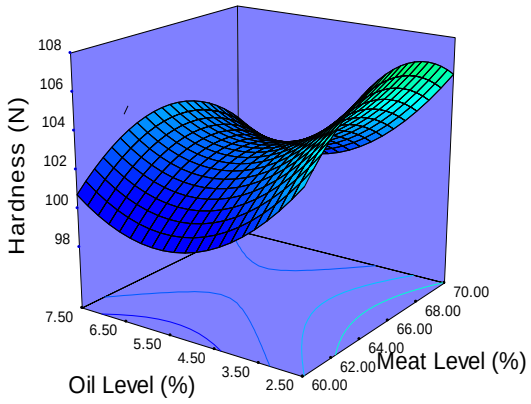
4.1.3.1 Hardness

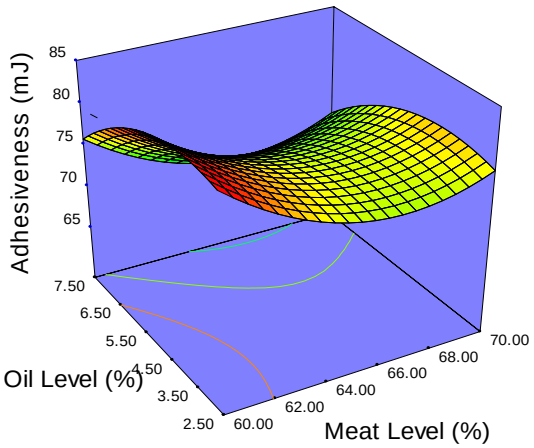
Hardness is the force required to compress a food between the molars. It is defined as force necessary to attain a given deformation and it is commonly expressed in Newton (N). Hardness of CMC was found to have quadratic relationship with the three variables. Two variables, % oil level * cooking time (BC) and % oil level (B^2) were found to have significant effect ($P<0.05$) on hardness at 5% level of significance (shown in Table 14). The Model F-value of 4.48 implied the model was significant. The "Lack of Fit F-value" of 3.44 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8521), being a measure of the goodness of fit of the model, indicated that 85.21% of the total variation was explained by the model. The value of hardness was first found to decrease with increase in % oil level but after reaching to a certain minima, it again increased with increase in % oil level and reached to its peak value i.e 100 N at 7.50 % oil level (Fig 1). Similar type of findings were reported by Bloukas *et al* (1997), who observed that in low-fat frankfurters with 9% fat, there was significant increase in hardness. Park *et al* (1993) reported maximum hardness in

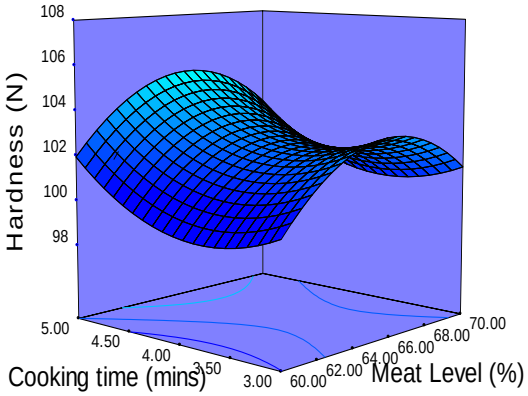
Table 14: Sum of squares of responses at 5% level of significance.

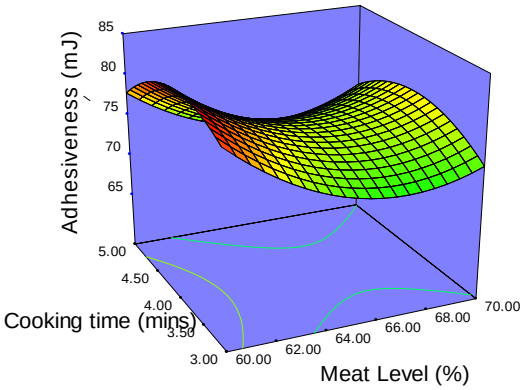
Source	Hardness (N)	Adhesiveness (mJ)	Adhesive force (N)	Stringiness (mm)	*L*	*a*	*b*	a_w	M (%)	CY (%)	CA	Flv	Csp	AT	MFI	OA
Model	283.25	371.04	3.61	0.50	126.86	5.19	8.14	0.039	8.06	37.91	0.31	0.77	0.11	0.63	1.20	0.62
A	4.53	48.72*	0.29	0.010	8.93	0.84*	0.23	0.022*	0.68	28.05*	0.077*	0.43*	2.551 E-005	0.14*	1.18*	0.61*
B	29.09	97.13*	0.073	0.072*	46.95*	0.016	0.12	1.188 E-003	4.500 E-004	4.87	5.740 E-003	2.551 E-003	2.551 E-003	0.016	0.010	2.551 E-003
C	8.64	4.98	0.35*	5.556 E-004	8.38	0.026	0.54	0.016*	1.20	4.99	0.010	0.064	2.066 E-003	0.023	5.740 E-003	6.378 E-004
AB	0.51	0.85	0.037	0.17*	21.11*	0.23	3.27*	-	0.56	-	0.032	0.020	0.000	0.020	-	-
AC	2.170 E-003	3.96	0.90*	0.063*	1.74	0.49	0.45	-	0.063	-	0.18*	0.13	0.049*	0.062	-	-
BC	162.49*	32.83*	0.35*	0.032	16.82	0.85*	3.53*	-	0.49	-	5.102 E-003	0.13	0.000	0.032	-	-
A^2	27.08	90.32*	0.64*	4.178 E-003	5.30	1.08*	-	-	2.23*	-	-	-	0.047*	0.036	-	-
B^2	40.97*	47.16*	0.53*	0.15*	15.36	1.18*	-	-	1.17	-	-	-	7.103 E-003	0.10*	-	-
C^2	12.71	53.39*	0.49*	7.299 E-004	0.59	0.22	-	-	1.15	-	-	-	5.921 E-003	0.20*	-	-
Lack of fit	35.43	13.19	0.16	0.045	9.20	0.26	3.39	0.021	0.92	24.78	0.060	0.23	5.663 E-003	0.032	0.26	0.52
R^2	0.8521	0.9103	0.8954	0.8695	0.8426	0.8456	0.6679	0.5231	0.8301	0.5725	0.7141	0.6965	0.8342	0.8395	0.6859	0.4913

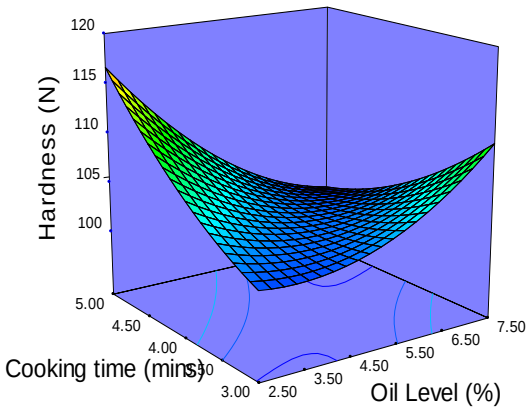
*Significant ($P < 0.05$), A = Meat level (%), B = Oil level (%), C = Cooking time (mins), R^2 = Coefficeint of Determination, *L*= Lightness, *a*= redness, *b*= yellowness, a_w= water activity, M= moisture %, CY= cooking yield %, CA = colour/appearance, Flv= flavour, Csp= crispiness, AT=After-taste, MFI=Meat flavour intensity and OA=overall acceptability.











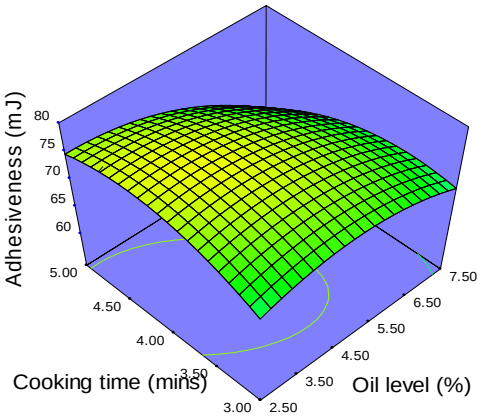


Fig 1: Surface plot (3-D) for Hardness. Fig 2: Surface plot (3-D) for Adhesiveness.

extruded beef products with highest level of meat and fat. Similar trend was observed with cooking time; where hardness reached to a maximum value of 102N during the longest cooking time i.e. 5 mins (Fig 1). This finding corresponds to the results of Bertola *et al* (1994), who showed that between 60 and 64°C, hardness decreased with cooking time until reaching the lowest asymptotic values which was related to protein denaturation. Between 66 and 68°C, hardness decreased at first but increased later due to actin denaturation; at the temperatures 81 and 90°C no modifications were observed and hardness remained at its higher values. At the same time hardness was initially found to increase with increase in % meat level and after reaching to the highest point, it decreased thereafter as shown in Fig 1. These findings were similar to the study of Smith *et al* (1991), who observed increase in shear force values with increasing levels of meat. Ba-Jaber *et al* (1993) also revealed that hardness of extrusion-cooked poultry meat incorporated with soy protein isolate and kappa-carrageenan increased with increase in meat level. Nurul *et al* (2009) also observed that in fish crackers, hardness increased with the increase in the ratio of the fish meat. With linear increase in cooking time and % oil level, hardness increased linearly (Fig 1). This might be due to loss of moisture and case hardening.

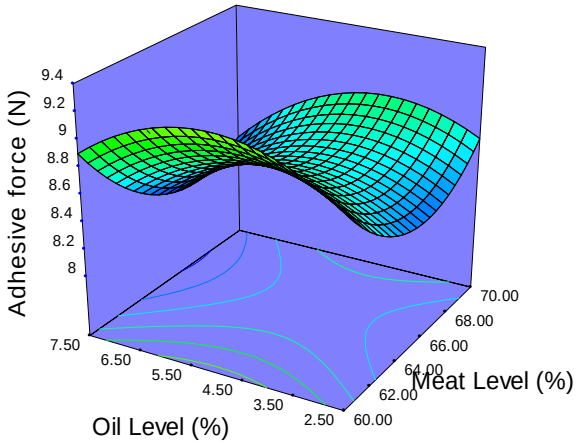
4.1.3.2 Adhesiveness

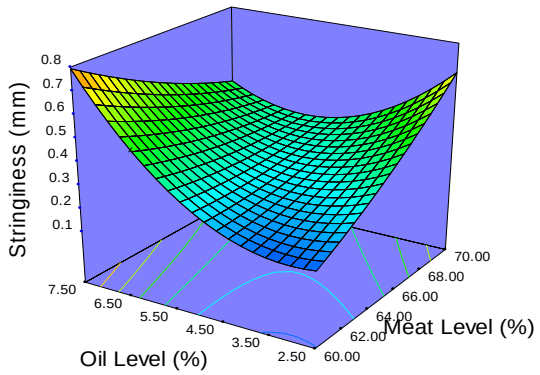
Adhesiveness is defined as the work necessary to pull the compression surface from the test piece after the first compression. Being a form of energy it is measured in mJ. In the present study, adhesiveness of CMC was found to have quadratic relationship with all the three variables. % Meat level (A), % oil level (B), % oil level * cooking time (BC), % meat level (A^2), % Oil level (B^2) and cooking time (C^2) were found to have significant effect ($P<0.05$) on adhesiveness at 5% level of significance (shown in Table 14). The Model F-value of 7.90 implied the model was significant. The "Lack of Fit F-value" of 0.75 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.9103) indicated that 91.03% of

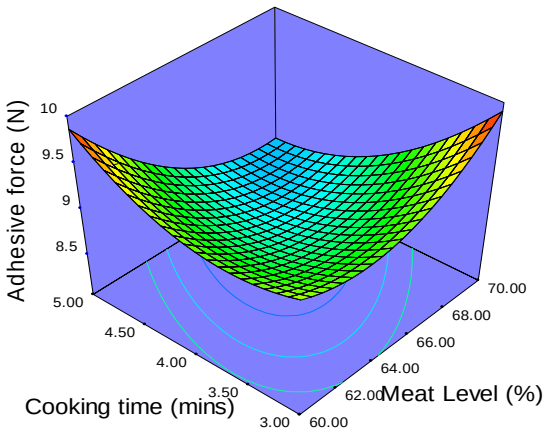
the total variation could be explained by the model. Adhesiveness was first found to increase with increase in % oil level but after reaching to its highest value, it decreased with increase in % oil level and reached to a value of 75mJ at oil level of 7.50%. Similar trend was observed with cooking time; where adhesiveness reached to a value of 75mJ during the longest cooking time i.e. 5mins (Fig 2). Cilla *et al* (2006) reported that values of adhesiveness in vacuum and modified atmosphere packaged dry cured hams were 52.74 and 54.26 gs respectively. However, with increase in meat level it first decreased and then again increased. With continuous increase in cooking time, increase in adhesiveness followed by decrease during the interaction of cooking time and meat level was observed whereas a steep increase in adhesiveness was observed with increase in cooking time during the interaction of cooking time and oil level was observed (Fig 2).

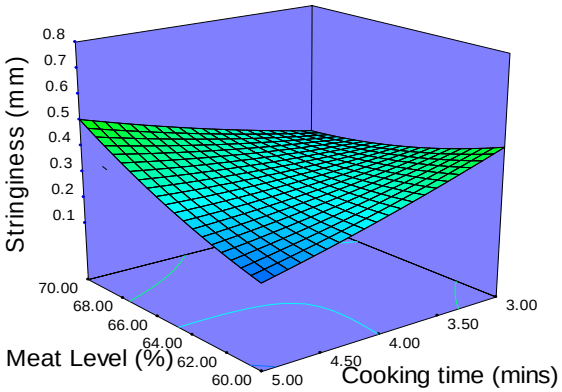
4.1.3.3 Adhesive force

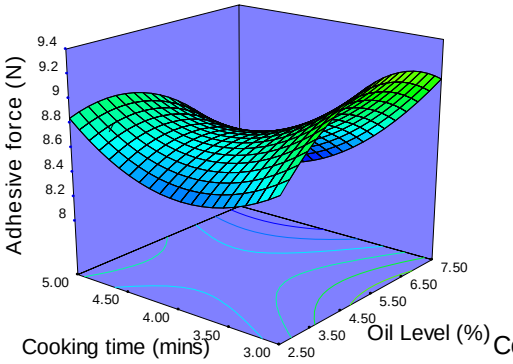
The maximum force required to separate teeth after biting sample is adhesive force. More technically, it can be defined as the maximum negative force generated during probe return. It is measured in Newton (N). In the present study, it was found to have quadratic relationship with the three variables. In this case, cooking time (C), % meat level * cooking time (AC), % oil level * cooking time (BC), % meat level (A^2), % Oil level (B^2) and cooking time (C^2) were significant model terms ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 6.66 implied the model was significant. The "Lack of Fit F-value" of 0.84 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8954), being a measure of the goodness of fit of the model, indicated that 89.54% of the total variation was explained by the model. Adhesive force was first increased with increase in % oil level but after reaching to its highest value, it decreased with increase in % oil level and reached to a value of 8.8N at oil level of 7.50%. However, it first decreased and later on increased with increase in % meat level. Adhesive force of CMC continuously increased











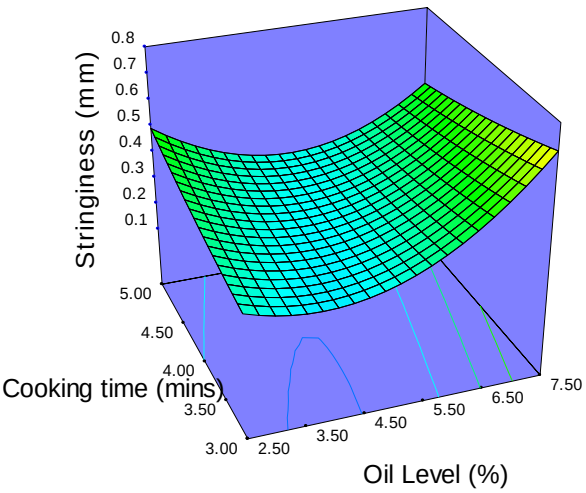


Fig 3: Surface plot (3-D) for Adhesive force. Fig 4: Surface plot (3-D) for stringiness.

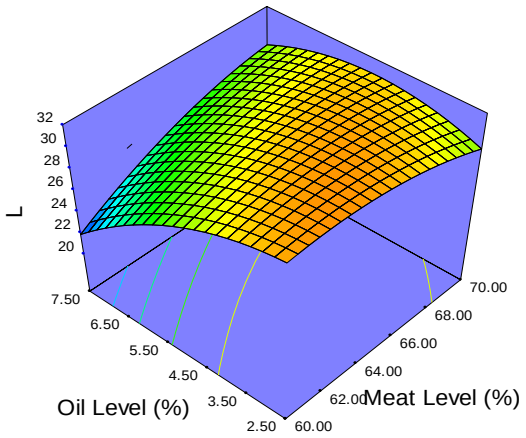
with increase in cooking time and at 5 mins cooking level, a value of more than 9.5N was observed. Exactly similar trend was followed with meat level. With increase in cooking time for CMC, after a short fall, adhesive force increased continuously (Fig 3).

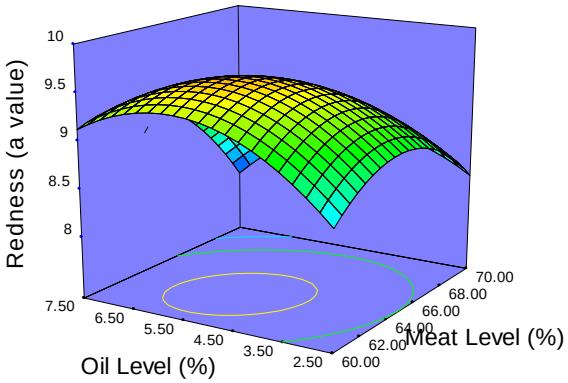
4.1.3.4 Stringiness

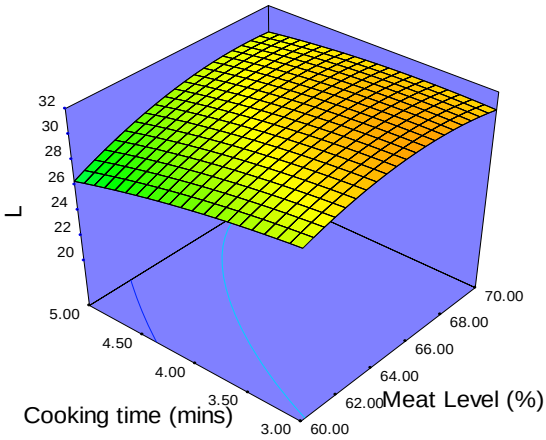
It is the elastic recovery of the food samples like in chewing gum and cream cheese. It can also be defined as the distance (expressed in millimeters) extended by the product during decompression before breaking off. It was found to have quadratic relationship with all the three variables. In this case, % oil level (B), % meat level * % oil level (AB), % meat level * cooking time (AC) and % Oil level (B^2) were significant model terms ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 5.18 implied the model was significant. The "Lack of Fit F-value" of 2.01 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8695), being a measure of the goodness of fit of the model, indicated that 86.95% of the total variation was explained by the model. A continuous increase in stringiness was observed with both increase in % oil level and meat level and a maximum value of 0.8mm was achieved at highest level (7.50%) of oil. A linear increase in % meat level and cooking time produced linear increment in the value of stringiness with the highest value of 0.5mm at 70% meat level. A cooking time of 5mins produced a stringiness of more than 0.4mm in the product (Fig 4).

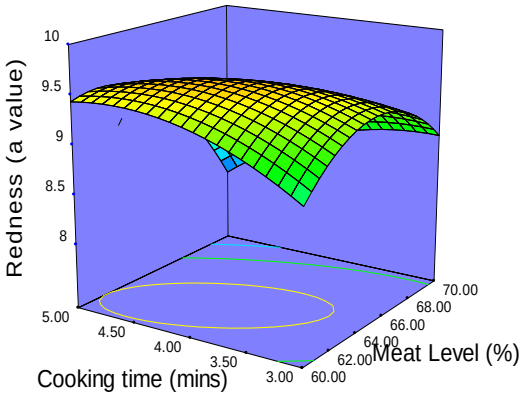
4.1.3.5 Lightness (*L* value)

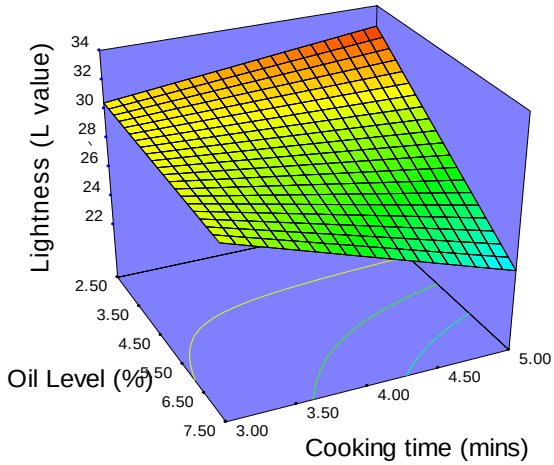
'*L*' value denotes (brightness 100) or lightness (0) values of the product. It was found to have quadratic relationship with the three variables. For lightness, % oil level (B) and % meat level * % oil level (AB) were found to have significant effect ($P<0.05$) on lightness at 5% level of significance (shown in Table 14). The Model F-value of 3.72 implied the model was significant. The "Lack of Fit F-value" of 1.48 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8426), being a measure of the goodness of fit of











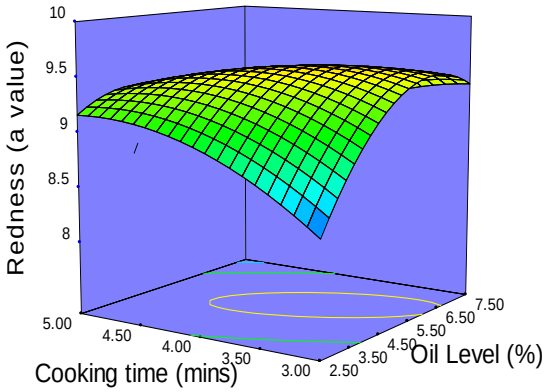


Fig 5: Surface plot (3-D) for *L* value.

Fig 6: Surface plot (3-D) for *a* value.

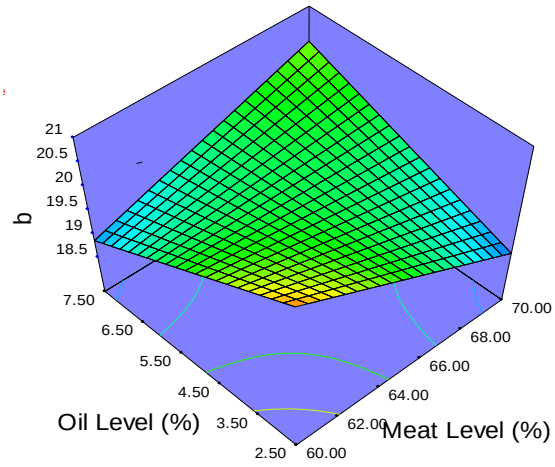
the model, indicated that 84.26% of the total variation was explained by the model. With increase in % oil level in CMC, there was a progressive decrease in *L* value and a maximum value of *L* (30) was observed at minimum % oil level i.e. 2.50% (Fig 5). Bloukas *et al* (1997) also reported that in low-fat frankfurters with 9% fat, there was significant increase in lightness. Increase in cooking time of CMC decreased the lightness and its value was slightly more than 24 at 5mins of cooking time. It may be due to the better development of brown colour on prolonged cooking. *L* value increased with regular increase in % meat level (Fig 5).

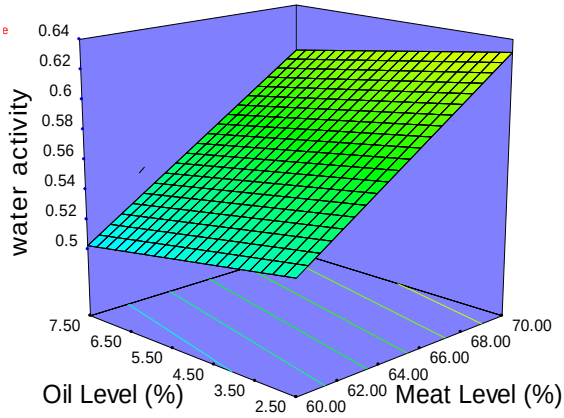
4.1.3.6 Redness (*a* value)

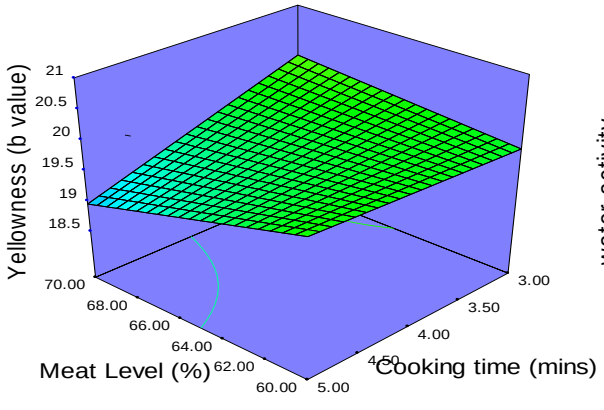
a -value indicates the redness or greenness of the product. It is an indicator of colour stability in meat and meat products. It was found to have quadratic relationship with the three variables. In this case, % meat levels (A), % oil level * cooking time (BC), % meat level (A^2) and % Oil level (B^2) were significant model terms ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 4.26 implied the model was significant. The "Lack of Fit F-value" of 0.50 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8456) indicated that 84.56% of the total variation was explained by the model. With the increase in % oil level, there was increase in *a* value which was followed by a slight decrease at higher oil levels. *a* value was 9 at 7.50% oil level. Also as the cooking time increased, *a* value also increased and it was maximum (>9) at 5mins level. However, with increase in % meat level, *a* value first increased and then decreased (Fig 6). Lee *et al* (2003) also reported that in chicken snacks, with increase in meat level there was increase in *a* value.

4.1.3.7 Yellowness (*b* value)

b –value indicates the yellowness or blueness of the product. It was found to fit with the three variables as per 2FI model. In this case, % meat level * % oil level (AB) and % oil level * cooking time (BC) were found to have significant effect ($P<0.05$) at 5% level of significance (shown in Table







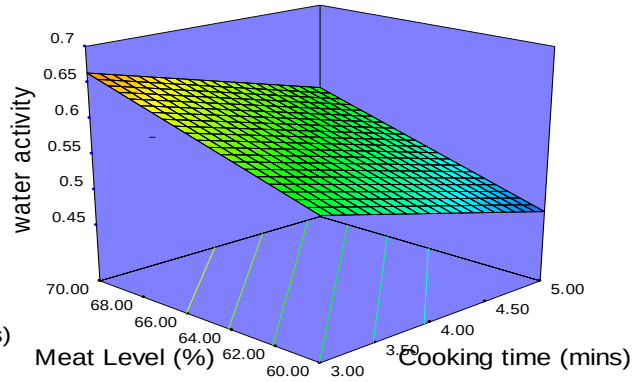


Fig 8: Surface plot (3-D) for water activity.

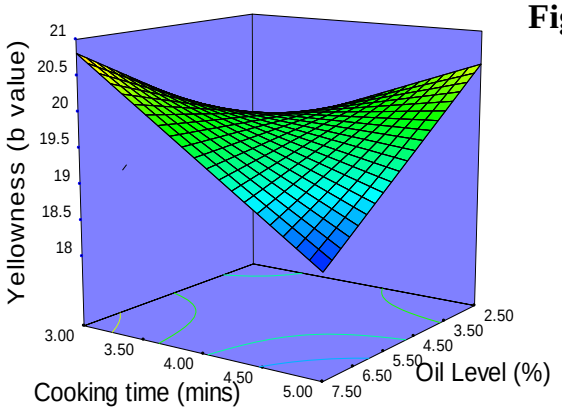


Fig 7: Surface plot (3-D) for *b* value.

14). The Model F-value of 3.35 implied the model was significant. The "Lack of Fit F-value" of 3.41 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.6679) indicated that 66.79% of the total variation was explained by the model. As per this model all the dependent variables were negatively correlated with *b* value. With increase in % oil level of CMC, there was decrease in *b* value and it was only 18.5 at 7.50 oil level. *b* value also decreased linearly with increase in % meat level and its value was <19 at 70% meat level (Fig 7). This observation is in contradiction with results of Lee *et al* (2003), who reported that in chicken snacks, with increase in meat level there was increase in *b* value. Exactly similar trend was observed with increase in cooking time levels. Moreover, *b* value was found to be > 20.5 at minimum cooking time level i.e. 3 min (Fig 7).

4.1.3.8 Water activity (a_w)

Water activity is a physical property that has a direct implication for microbiological safety of food. It is the amount of water available to microbes for their growth. It was found to have linear relationship with the three variables. In this case, % meat level (A) and cooking time (C) were found to have significant effect ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 4.75 implied the model was significant. The "Lack of Fit F-value" of 0.66 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.5231), being a measure of the goodness of fit of the model, indicated that only 52.31% of the total variation was explained by the model. As per model there was linear increase in a_w with increase in % meat level (Positive Linear relationship) while it declined with increase in % oil level (Negative Linear relationship). It may be because meat being rich in water content increases the water activity. At 70% meat level, a_w was highest (>0.60) but at 7.5% oil level, it was even less than 0.5. Water activity also decreased linearly with increase in cooking time (Fig 8). Rhee *et al* (1999b) water activity for all the extrudates was very low (0.11-0.12)

and no notable differences were found among the products from different meat sources.

4.1.3.9 Moisture (%)

It is the total amount of water (free, immobilized and bound) present in meat sample. It was found to have quadratic relationship with the three variables. In this case, % meat level (A^2) was the single factor found to be significant ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 3.80 implied the model was significant. The "Lack of Fit F-value" of 1.69 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8301), being a measure of the goodness of fit of the model, indicated that 83.01% of the total variation was explained by the model. With increase in % meat level, moisture % first decreased and then increased. At highest (70%) meat level, > 8.5% moisture was observed (Fig 9). The finding is in accordance with the observation of Sharma and Nanda (2002), who observed maximum moisture in chicken chips with highest meat level. Similar type of trend was observed with increase in % oil level and at 7.5% oil level, moisture % was >7. However, with increase in cooking time, moisture % first decreased and then increased (Fig 9). This is in agreement with the statement that cooking lowers the moisture content particularly on the surface (Pearson and Gillett 1997). M**urphy *et al* (2001) also reported decrease in moisture % of** cooked chicken breast patties with increase in the cooking temperature.

4.1.3.10 Cooking yield (%)

It is the amount of cooked CMC relative to the amount of raw product and expressed in percentage. It was found to have linear relationship with the three variables. As per this model only one variable i.e. % meat level (A) was found to be significant ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 5.80 implied the model was significant. The "Lack of Fit F-value" of 3.13 implied the Lack of Fit was not significant relative to the pure error. The R^2 value

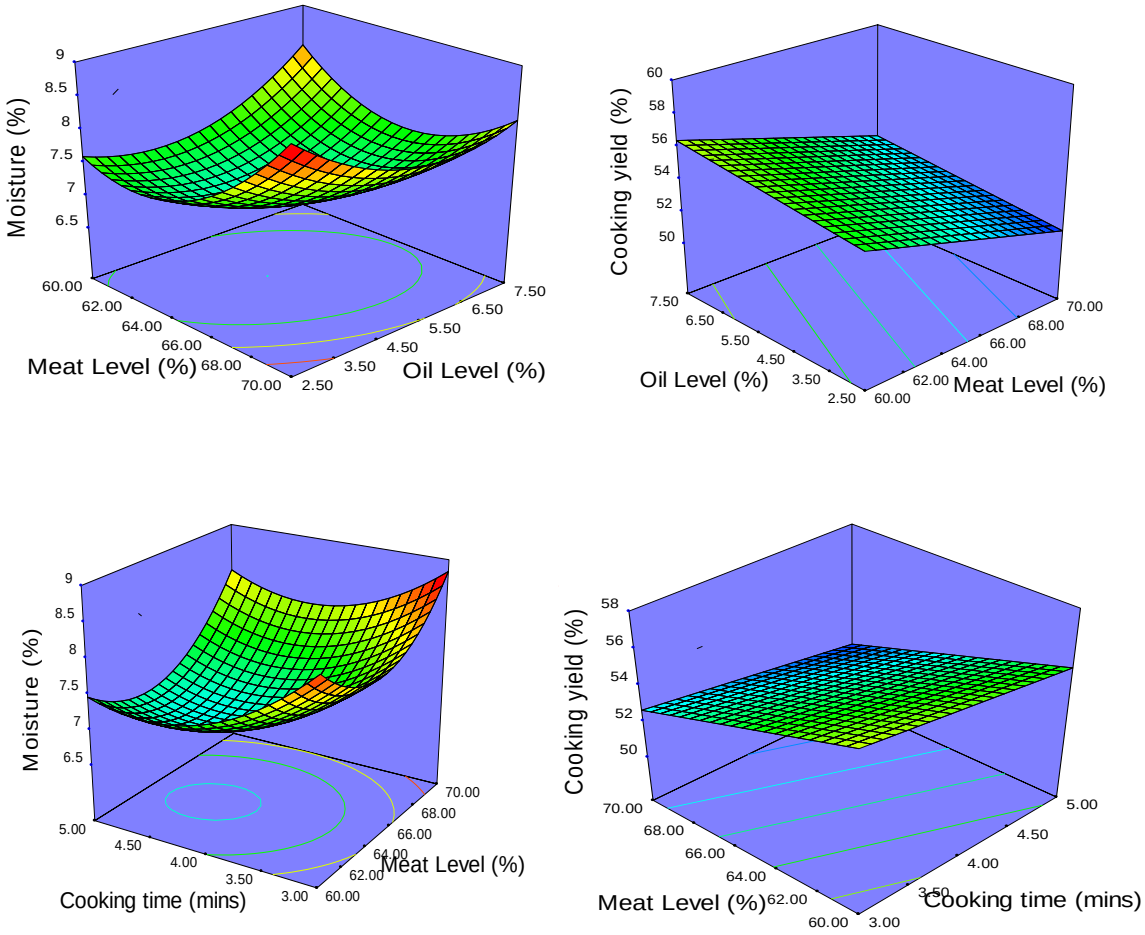


Fig 10: Surface plot (3-D) for cooking yield (%).

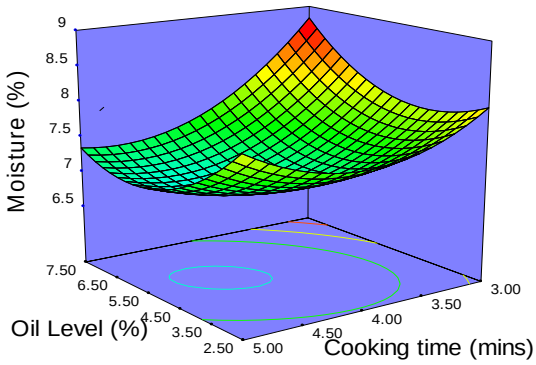
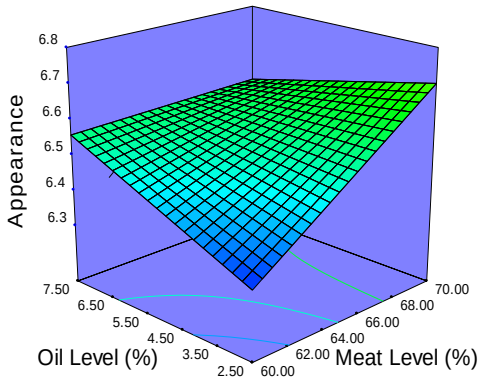


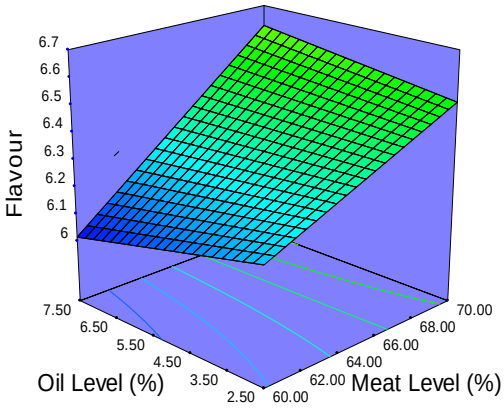
Fig 9: Surface plot (3-D) for moisture (%).

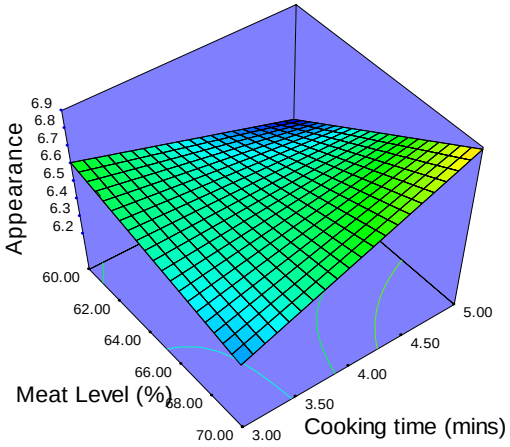
(0.5725), being a measure of the goodness of fit of the model, indicated that 57.25% of the total variation was explained by the model. With linear increase in % meat level, cooking yield decreased linearly (Negative Linear relationship). This result is in agreement with the results reported by Sharma and Nanda (2002), who showed that in chicken chips with highest meat level, cooking yield was significantly lower ($P<0.05$). With decrease in % oil level, cooking yield decreased. Similar trend for this observation was reported by Hughes *et al* (1998) in frankfurters. They reported that decreasing the fat content decreased the cooking yield, emulsion stability and product lightness. About 56% cooking yield was observed at 7.5% oil level. As the cooking time increased, cooking yield decreased (Fig 10). It is a matter of fact that higher cooking times decrease moisture content and produce shrinkage. The observation is in accordance with the results reported by Barbanti and Pasquini (2005), who showed that increase in cooking time and temperature result in increased cook losses (thus decreased cooking yield) from chicken breast meat samples.

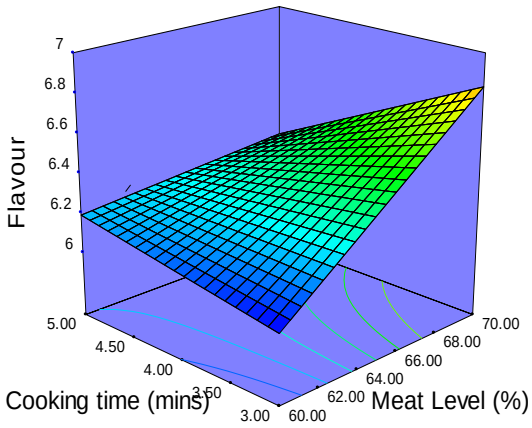
4.1.3.11 Colour / Appearance

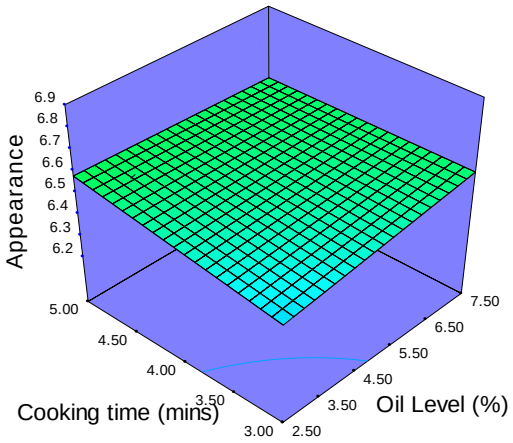
A colour is the result of a combination of a couple of factors. The most important factor that contributes to meat colour is the pigments that absorb certain wavelengths of light and reflect others. It was found to fit with the three variables as per 2FI model. In this case, % meat level (A) and % meat level * cooking time (AC) were found to have significant effect ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 4.16 implied the model was significant. The "Lack of Fit F-value" of 0.62 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.7141) indicated that 71.41% of the total variation was explained by the model. With increase in % meat level, appearance increased during the interaction of meat level (A) with oil level (B). At the same time, with % increase in meat level, appearance decreased during the interaction of meat level and cooking time.











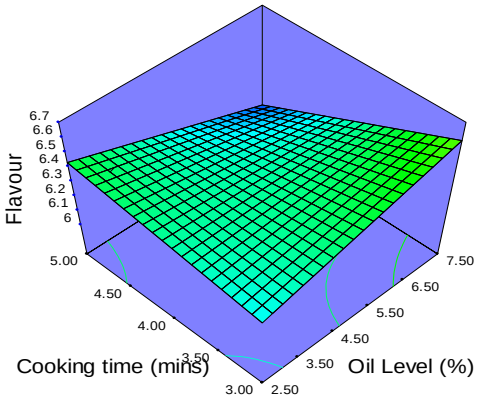


Fig 11: Surface plot (3-D) for Appearance. **Fig 12: Surface plot (3-D) for Flavour.**

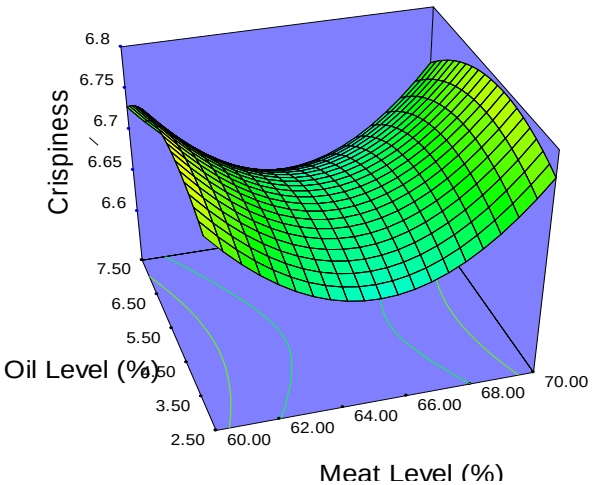
However, with increase in cooking time, appearance always improved. At 5mins cooking time, the sensory rating for appearance was 6.5. Similar trend of increase in appearance was found while % oil level was increased (Fig 11). This finding is in accordance with the results reported by **Muguerza *et al* (2002), who prepared dry fermented pork sausages and observed that sausages containing more fat were having higher values of appearance.**

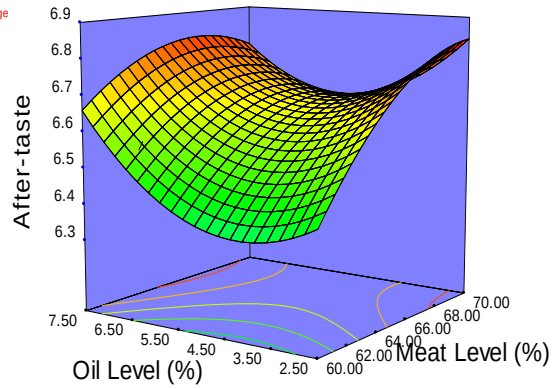
4.1.3.12 Flavour

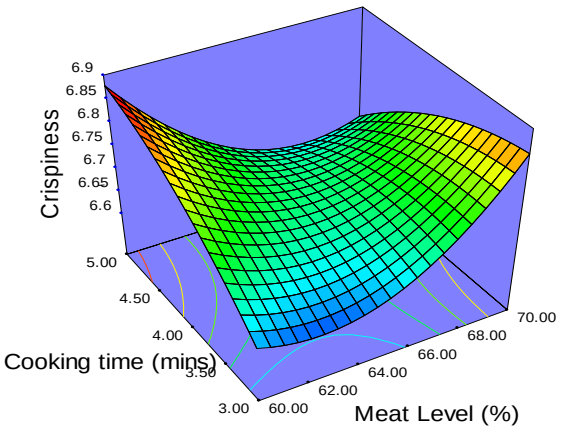
It is a complex attribute of meat palatability which includes odour and taste (Calkins and Hodgen 2007). It is an important factor affecting consumer's meat purchase habits and preferences when tenderness was held constant (Sitz *et al* 2005). It was found to fit with the three variables as per 2FI model. In this case, only % meat level (A) was found to have significant effect ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 3.82 implied the model was significant. The "Lack of Fit F-value" of 1.45 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.6965) indicated that 69.65% of the total variation was explained by the model. With continued increase in % meat level, flavour improved throughout the case (Fig 12). The observation strongly agrees with the study of Singh *et al* (2002), who observed that in chicken snacks with highest meat level, flavour scores were maximum. However, with increase in % oil level flavour decreased during the interaction of meat level and oil level. At the same time, with increase in % oil level flavour increased during the interaction of oil level and cooking time. But with increased cooking time, flavour also increased (Fig 12). It is in agreement with statement of Pearson and Gillett (1997), who documented that cooking always intensifies the flavour of meat.

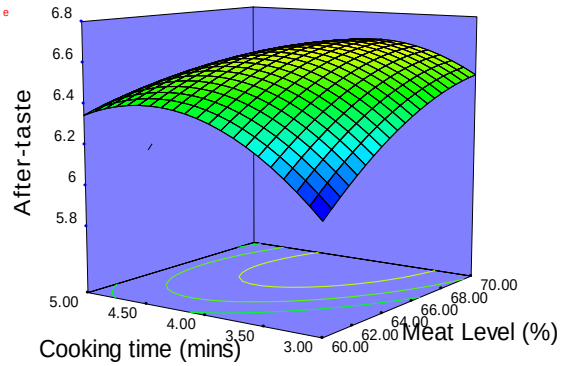
4.1.3.13 Crispiness

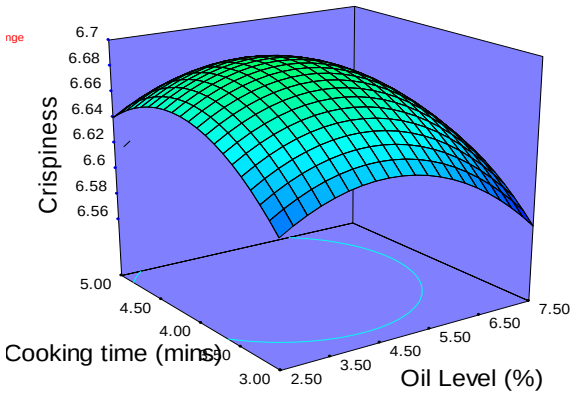
Crispiness (Csp) of CMC was found to have quadratic relationship with the three variables. In this case, % meat level * cooking time (AC) and % meat levels (A^2) were significant model terms











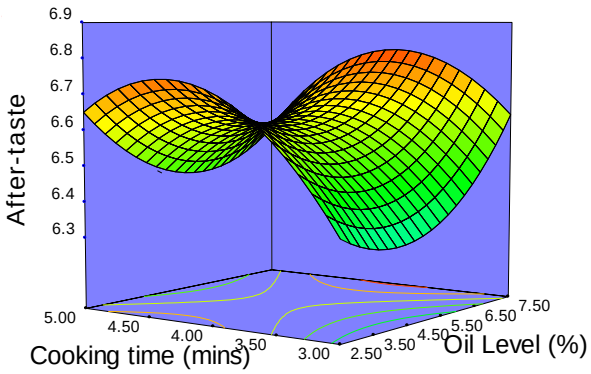


Fig 13: Surface plot (3-D) for crispiness. **Fig 14: Surface plot (3-D) for After-taste.**

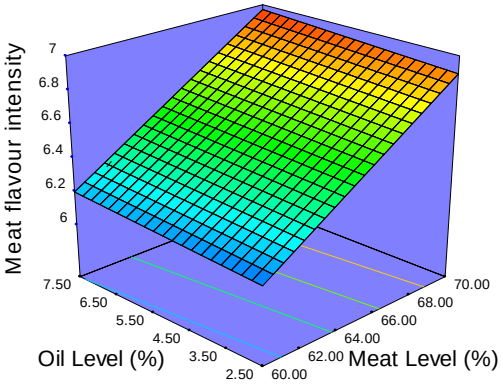
(P<0.05) at 5% level of significance (shown in Table 14). The Model F-value of 3.91 implied the model was significant. The "Lack of Fit F-value" of 0.46 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8342) indicated that 83.42% of the total variation was explained by the model. With increase in % meat level, crispiness first decreased and then increased (Fig 13). The finding was similar to the one reported by Sharma and Nanda (2002), who showed that crispiness was significantly higher in chicken chips with minimum level of meat %. But with increase in % oil level, crispiness first increased and then decreased and a crispiness value of more than 6.7 was observed at 7.5% oil level. Increased cooking times always improved the crispiness of product and it was highest at 5mins of cooking period (Fig 13).

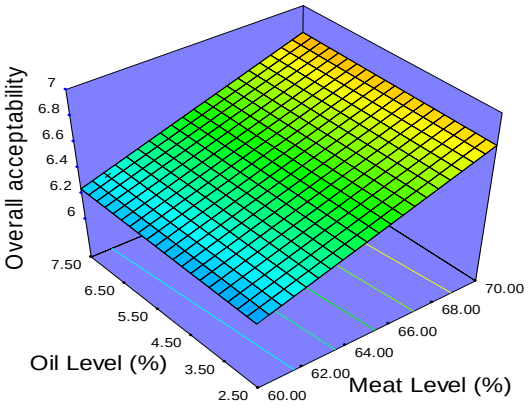
4.1.3.14 After-taste

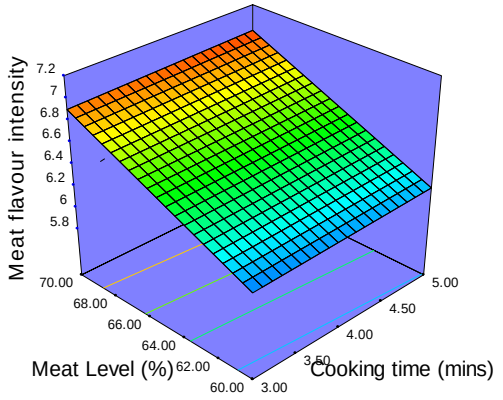
After-taste (AT) in CMC was found to have quadratic relationship with the three variables. In this case, % meat level (A), % Oil level (B^2) and cooking time (C^2) were significant model terms (P<0.05) at 5% level of significance (shown in Table 14). The Model F-value of 4.07 implied the model was significant. The "Lack of Fit F-value" of 0.48 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.8395) indicated that 83.95% of the total variation was explained by the model. With increase in meat level after taste also increased (Fig 14). The observation is in accordance with the study of Singh *et al* (2002), who observed that in chicken snacks with highest meat level, after-taste scores were maximum. With increase in % oil level, after taste first decreased and then increased. At 7.5% oil level, it was more than 6.6. With increase in cooking time, it first increased and then decreased. At 5mins of cooking time, after taste was more than 6.2 (Fig 14).

4.1.3.15 Meat flavour intensity

It was found to have linear relationship with the three variables. As per this model only one variable i.e. % meat level (A) was found to be significant (P<0.05) at 5% level of significance (shown







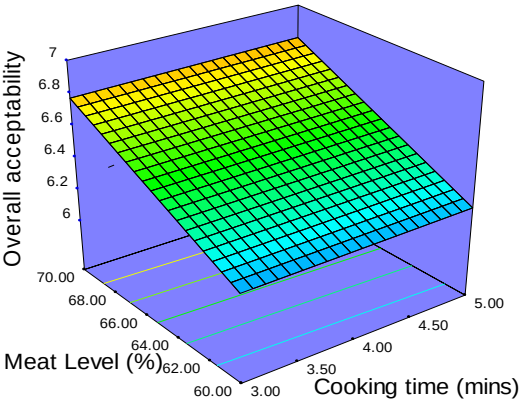


Fig 15: Surface plot (3-D) for meat flavour intensity.

Fig 16: Surface plot (3-D) for overall acceptability.

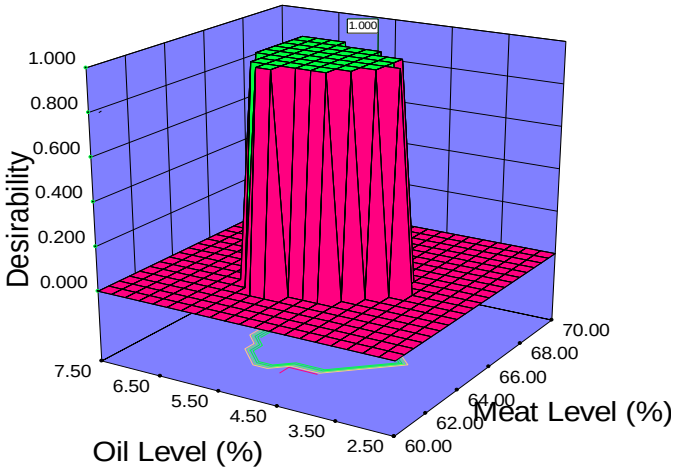


Fig 17: Surface plot (3-D) for Desirability (Oil Level vs Meat Level).

in Table 14). The Model F-value of 9.46 implied the model was significant. The "Lack of Fit F-value" of 0.41 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.6859), being a measure of the goodness of fit of the model, indicated that 68.59% of the total variation was explained by the model. Meat flavour intensity (MFI) increased linearly, with increase in % meat level. The finding supports the study of Singh *et al* (2002), who observed that in chicken snacks with highest meat level, meat flavour intensity scores were maximum. Also with increase in % oil level and cooking time, meat flavor intensity always increased linearly. At highest levels of meat (70%) and oil (7.50%) meat flavour intensity was 6.8 and 6.0 respectively (Fig 15).

4.1.3.16 Overall acceptability

Overall acceptability (OA) of CMC was found to have linear relationship with the three variables. As per this model only one variable i.e. % meat level (A) was found to be significant ($P<0.05$) at 5% level of significance (shown in Table 14). The Model F-value of 4.19 implied the model was significant. The "Lack of Fit F-value" of 1.95 implied the Lack of Fit was not significant relative to the pure error. The R^2 value (0.4913), being a measure of the goodness of fit of the model, indicated that 49.13% of the total variation was explained by the model. With increase in % meat level, % oil level and cooking time overall acceptability always increased linearly. This contradicts the study of Sharma and Nanda (2002), who reported significantly higher overall acceptability in chicken chips with minimum meat level. At highest levels of meat (70%) and oil (7.50%) overall acceptability was >6.6 and >6.0 respectively (Fig 16).

4.1.4 Desirability

In RSM, the most useful approach to optimization of multiple responses is to use the simultaneous optimization technique popularized by Derringer and Suich (1980). Their procedure makes use of desirability functions. Essentially, the approach is to translate the functions to a

common scale (0 and 1), combine them using the geometric mean and optimize the overall metric. By using this technique, instead of optimizing each outcome separately, settings for the predictor variables sought to satisfy all of the outcomes at once. On the basis of ranges of different responses, a total of 43 solutions were found out of which, the product with 65% meat level, 5% oil level and 4 mins cooking time was having desirability of 1.0 and it was selected. Surface plot (3-D) for Desirability (Oil level vs Meat level) has been shown in Fig 17.

4.1.5 Optimization of process parameters

Responses were optimized individually in combination using Box-Behnken Design (Design Expert 8.0.4.1, 2010). In response surface analysis, the selected model was used to calculate the stationary point. A stationary point is a point at which the slope of the response surface is zeroed in all the directions. Since the optimum response for each variable were not all in exactly the same region in the space formed by the processing variables. So, constraints were set (shown in Table 15) such that the selected meat level (%), oil level (%) and cooking time (mins) was optimum for most important attributes and close to optimum for the others. These constraints were met in the region where meat level was 65 %, oil level was 5% and cooking time was 4 mins. The model equation for the response variables predicted values under the identified optimum conditions which were experimentally verified to be in general agreements with the model.

4.2 Experiment No.2: Optimization of the level of rice flour, tapioca starch and potato starch in chicken meat caruncles.

4.2.1 Optimization of level of rice flour in chicken meat caruncles.

4.2.1.1 Physico-chemical quality of chicken meat caruncles prepared by using rice flour

Four different batches of chicken meat caruncles were prepared viz. control (without rice flour, RF), T_1 = 22.75% RWF + 12.25% RF; T_2 = 17.50% RWF + 17.50% RF and T_3 = 12.25% RWF +

Table 15: Values of constraints fed to software for optimization.

Constraints	Lower limit	Higher limit
A:Meat Level (%)	60	70
B:Oil Level (%)	2.5	7.5
C:Cooking Time (%)	3	5
Hardness (N)	100.275	105.683
Adhesiveness (mJ)	72.79	78.683
Adhesive force (negative, N)	8.35	8.91667
Stringiness (mm)	0.233333	0.433333
L value	28.3267	33.3133
a value	9.04	9.89
b value	19.0033	19.98
water activity (a_w)	0.4755	0.645
Moisture (%)	6.27	7.24
Cooking yield (%)	52.8	54.925
Appearance	6.35714	6.64286
Flavour	6	6.35714
Crispiness	6.64286	6.78571
After taste	6.5	6.85714
Meat flavour intensity	6.35714	7
Overall acceptability	6.28571	6.64286

22.75% RF and their quality was evaluated. Data pertaining to emulsion stability (E.S.) %, cooking yield (C.Y.) %, pH, water activity (a_w), Hydratability, WAI and WSI are presented at Table16. It was observed that there was no significant difference (P>0.05) of emulsion stability % and CY % among the control and treated CMC. The emulsion stability % and CY % in treated sample are almost comparable with the control samples. These findings were exactly in agreement with the results of Singh *et al* (2002) who did not found any significant difference in emulsion stability % and CY % of chicken snacks prepared by using rice flour. Kale (2009) also observed similar results for emulsion stability % and CY % in chicken snacks sticks.

There was a significant decrease (P<0.05) in pH of CMC in treated sample but it did not significantly vary among T_1, T_2 and T_3 samples. There was marginal decrease in pH as the rice flour content was increased in the product formulation. Singh *et al* (2002) did not observe any significant difference in pH of chicken snacks when rice flour was used. The a_w of control sample was significantly lower (P<0.05) than the treated sample but it did not significantly vary among T_1, T_2 and T_3 batches. The higher a_w in the treated CMC might be due to more absorption of water into the rice flour. The hydratability significantly (P<0.05) decreased in T_1, T_2 and T_3 samples (0.96-1.27) as compared to control batch (1.63). But it did not significantly vary among the treated samples. The highest WAI was found in control samples (4.04) which was also significantly higher (P<0.05) than T_1 (3.79), T_2 (3.80) and T_3 (3.53) samples. The WAI was significantly lower in T_3 as compared to other treated and control samples. There was no significant difference in WSI among the control, T_2 and T_3 while it was significantly lower in T_1 than control and T_3 CMC. Kale (2009) also observed that there was no significant difference of hydratability, WAI and WSI of chicken snack sticks. Lower values of hydratability, WAI and WSI of treated samples than control samples may be due to presence of rice flour. This observation is in agreement with the report of Anna

Table 16: Effect of incorporation of rice flour on the physico-chemical properties of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Emulsion stability (%)	97.98±0.35[a]	97.47±0.20[a]	98.06±0.19[a]	97.58±0.19[a]
Cooking yield (%)	52.13±1.07[a]	51.70±0.56[a]	51.89±0.80[a]	52.03±0.41[a]
pH	5.88±0.05[b]	5.79±0.02[a]	5.76±0.02[a]	5.75±0.03[a]
Water activity (a_w)	0.29±0.01[a]	0.34±0.01[b]	0.35±0.00[b]	0.34±0.01[b]
Hydratability	1.63±0.11[b]	1.25±0.13[a]	0.96±0.11[a]	1.27±0.13[a]
WAI	4.04±0.05[c]	3.79±0.08[b]	3.80±0.05[b]	3.53±0.08[a]
WSI	0.05±0.00[b]	0.04±0.00[a]	0.05±0.00[ab]	0.05±0.00[b]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 22.75% RWF+ 12.25% RF, T_2= 17.50% RWF+ 17.50% RF and T_3= 12.25% RWF+ 22.75% RF.

Table 17: Effect of incorporation of rice flour on the proximate composition of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Moisture (%)	4.96±0.11^{a}	5.34±0.17^{b}	5.50±0.11^{bc}	5.79±0.11^{c}
Protein (%)	30.41±1.64^{c}	24.41±0.29^{a}	28.67±0.89^{bc}	26.90±0.65^{ab}
Fat (%)	10.16±0.14^{b}	9.76±0.07^{a}	10.15±0.04^{b}	9.79±0.05^{a}
Crude Fiber (%)	3.00±0.37^{ab}	1.83±0.31^{a}	1.83±0.31^{a}	3.67±0.56^{b}
Ash (%)	3.77±0.13^{bc}	3.25±0.13^{a}	4.15±0.08^{c}	3.48±0.22^{ab}
Carbohydrates (%)	47.70±1.74^{a}	55.42±0.44^{b}	49.70±0.85^{a}	50.37±0.83^{a}
Moisture: Protein ratio	0.17 ±0.01^{a}	0.22±0.01^{c}	0.19±0.00^{b}	0.22±0.01^{c}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 22.75% RWF+ 12.25% RF, T_2= 17.50% RWF+ 17.50% RF and T_3= 12.25% RWF+ 22.75% RF.

Anandh *et al* (2005) who also observed similar trends with increase in corn flour level in buffalo meat snacks. Non-significant (P>0.05) differences for the above quality parameters among different variants of CMC might be due to the same level of incorporation of refined wheat flour i.e. 35% (alone or in combination with rice flour). The observations reported by the above research workers comply with the present study.

4.2.1.2 Proximate composition of chicken meat caruncles prepared by using rice flour

Data pertaining to proximate composition of CMC are presented at Table 17. The moisture % of control sample (4.96) showed significantly lower (P<0.05) value whereas it was significantly higher in T_3 batch (5.79). It seems as the rice flour content was increased in product, moisture % also increased. Anna Anandh *et al* (2005) also reported that as the corn flour content was increased, moisture % of buffalo meat snacks increased linearly. Jean *et al* (1996) reported final moisture content of less than 5% for extrudates. These findings support the present study.

The protein % was significantly lower in T_1 and T_3 batch as compared to control CMC. But there was no significant difference between T_2 and control batch. The decrease in protein content of RF batches may be due to lower protein content of RF as compared to RWF which was being replaced in the formulation. Sharma and Nanda (2002) also reported significantly higher protein % in control chicken chips as compared to treated groups. This is in agreement with the present study. The fat % did not significantly vary between control and T_2 or between T_1 and T_3 batches. Crude fiber % was found to be significantly lower (P<0.05) in T_1 and T_2 samples as compared to T_3 CMC. But there was no significant difference between T_3 and control samples in respect of crude fiber %. It varied from 1.83-3.67 in treated samples. The ash % was significantly higher in T_2 batch (4.15) as compared to T_1 and T_3 batches but it did not significantly vary with respect to control sample (3.77). Carbohydrates % was significantly higher (P<0.05) in T_1 batch as compared to other CMC variants.

Moisture: Protein ratio was similar in T_1 and T_3 batch (0.22) but was significantly lower in control (0.17) and T_2 (0.19). However, Anna Anandh *et al* (2005) also reported that as the corn flour content was increased, there was linear increase in fat, protein and ash content of buffalo meat snacks. This is in contradiction with the present study.

4.2.1.3 Texture Profile of chicken meat caruncles prepared by using rice flour

Data pertaining to texture profile of CMC are presented at Table 18. It was observed that hardness was significantly increased ($P<0.05$) in T_2 (84.33) as compared to control (61.39) and T_1 (66.67) while it did not significantly vary from T_3 sample (70.69). Lee *et al* (2003) documented the popped cereal snacks prepared from spent hen meat and rice flour showed the highest breaking force as compared to corn starch and potato starch combinations. The adhesiveness, adhesive force and stringiness of CMC did not show any significant variation among control and three different treated CMC.

4.2.1.4 Colour Profile of chicken meat caruncles prepared by using rice flour

Data pertaining to colour profile viz. *L* value, *a* value, *b* value, hue and chroma are presented at Table 19. The *L* value of control and T_3 batches did not vary between them. Same observation was found among T_1 and T_2 batches with respect to *L* value. The *L* value of T_1 (42.16) and T_2 (41.79) were found to be significantly higher ($P<0.05$) as compared to control (36.81) and T_3 (38.25) batches. The *a* value showed no significant variation among different treated and control samples showing that replacement of RF did not affect the appealing colour of CMC. The *b* value was significantly higher in T_1 batch (26.13) than only control batch (24.31) whereas it did not significantly vary between T_2 (25.31) and T_3 (24.98) samples. The result of hue angle was similar to *b* value of CMC in different batches. Hue angle of T_1 batch showed significantly higher value than control but it did not show any significant difference between treated CMC. The chroma of CMC could not bring about any significant change within the control (27.13) and treated (27.39-28.34) CMC. Comparatively higher *L*

Table 18: Effect of incorporation of rice flour on texture profile of chicken meat caruncles.

Mean ± S.E

Parameters	Treatments			
	Control	T_1	T_2	T_3
Hardness (N)	61.39±3.52[a]	66.67±5.02[a]	84.33±5.70[b]	70.69±6.98[ab]
Adhesiveness (mJ)	44.40±4.26[a]	46.53±4.60[a]	34.42±3.84[a]	46.94±5.92[a]
Adhesives force (N)	12.48±1.24[a]	15.50±1.69[a]	12.84±1.22[a]	15.07±1.45[a]
Stringiness (mm)	1.61±0.38[a]	1.58±0.43[a]	0.65±0.20[a]	1.45±0.41[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 22.75% RWF+ 12.25% RF, T_2= 17.50% RWF+ 17.50% RF and T_3= 12.25% RWF+ 22.75% RF.

Table 19: Effect of incorporation of rice flour on colour profile of chicken meat caruncles.

Mean ± S.E

Parameters	Treatments			
	Control	T_1	T_2	T_3
L	36.81±1.02[a]	42.16±1.50[b]	41.79±0.99[b]	38.25±1.25[a]
a	12.03±0.59[a]	10.90±0.58[a]	10.90±0.60[a]	11.04±0.84[a]
b	24.31±0.53[a]	26.13±0.67[b]	25.31±0.50[ab]	24.98±0.41[ab]
Hue angle	63.80±0.71[a]	67.41±0.89[b]	66.78±0.99[ab]	66.31±1.55[ab]
Chroma	27.13±0.72[a]	28.34±0.77[a]	27.59±0.61[a]	27.39±0.57[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 22.75% RWF+ 12.25% RF, T_2= 17.50% RWF+ 17.50% RF and T_3= 12.25% RWF+ 22.75% RF.

and *b* values and lower *a* values in treated groups than control are in agreement with the results of Lee *et al* (2003) who observed that increase in rice flour content in popped chicken snacks also increased *L* and *b* value but decreased *a* value.

4.2.1.5 Sensory attributes of chicken meat caruncles prepared by using rice flour

Data pertaining to various sensory attributes of CMC incorporated with RF are presented at Table 20. All the sensory attributes namely colour, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability did not show significant variation between T_2 and T_3 batches. The colour scores remained significantly lower in T_2 and T_3 batches whereas it was significantly higher in T_1 and control samples. This indicates that RF at higher level affected the appealing colour of product. All the sensory attributes of T_1 sample are comparable with the control sample. Moreover, among the treated groups, T_1 batch got higher scores for almost all the attributes so it was considered most acceptable. Sharma and Nanda (2002) also observed that there was no significant difference between in colour and meat flavour intensity of control and treated groups. Singh *et al* (2002) also documented higher sensory scores (colour, texture, crispiness and overall acceptability) for chicken snacks with 50% meat level.

4.2.2 Optimization of level of tapioca starch in chicken meat caruncles

4.2.2.1 Physico-chemical quality of chicken meat caruncles prepared by using tapioca starch

Four different batches of CMC were prepared viz. control (without TS), T_1 = 17.50% RWF + 17.50% TS; T_2 = 14.00% RWF + 21.00% TS and T_3 = 10.50% RWF + 24.50% TS and their quality was evaluated. Data pertaining to emulsion stability (E.S.) %, cooking yield (C.Y.) %, pH, water activity (a_w), Hydratability, WAI and WSI are presented at Table 21. There was no significant difference ($P>0.05$) in emulsion stability % of control and treated samples. The emulsion stability of treated CMC ranged from 96.71-97.93 and was comparable with control CMC. The cooking yield % was

Table 20: Effect of incorporation of rice flour on the sensory attributes of chicken meat caruncles. **Mean±S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Colour/Appearance	6.83±0.14^{b}	6.89±0.07^{b}	6.33±0.12^{a}	6.39±0.14^{a}
Flavour	6.56±0.13^{bc}	6.61±0.14^{c}	6.22±0.12^{ab}	6.11±0.07^{a}
Crispiness	6.28±0.09^{a}	6.33±0.14^{a}	6.56±0.18^{a}	6.72±0.15^{a}
After-taste	6.56±0.13^{ab}	6.61±0.14^{b}	6.22±0.09^{a}	6.22±0.09^{a}
Meat flavour intensity	6.50±0.14^{bc}	6.61±0.14^{c}	6.11±0.07^{a}	6.22±0.09^{ab}
Overall acceptability	6.72±0.12^{bc}	6.89±0.11^{c}	6.44±0.13^{ab}	6.28±0.12^{a}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 22.75% RWF+ 12.25% RF, T_2= 17.50% RWF+ 17.50% RF and T_3= 12.25% RWF+ 22.75% RF.

Table 21: Effect of incorporation of tapioca starch on the physico-chemical properties of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Emulsion stability (%)	98.03±0.16^{a}	97.09±0.37^{a}	97.93±0.36^{a}	96.71±0.64^{a}
Cooking yield (%)	53.50±0.84^{a}	55.12±0.56^{ab}	55.68±0.58^{b}	54.90±0.66^{ab}
pH	5.96±0.15^{b}	5.91±0.12^{b}	5.82±0.12^{ab}	5.71±0.08^{a}
Water activity (a_w)	0.36±0.02^{a}	0.35±0.00^{a}	0.32±0.00^{a}	0.36±0.02^{a}
Hydratability	1.42±0.07^{bc}	1.52±0.04^{c}	1.30±0.05^{ab}	1.19±0.06^{a}
WAI	4.26±0.18^{a}	4.78±0.20^{b}	5.03±0.45^{b}	4.93±0.38^{b}
WSI	0.06±0.05^{a}	0.05±0.01^{a}	0.06±0.00^{a}	0.05±0.01^{a}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 17.50% RWF+ 17.50% TS, T_2= 14.00% RWF+ 21.00% TS and T_3= 10.50% RWF+ 24.50% TS.

significantly higher ($P<0.05$) in T_2 batch (55.68) than control (53.50) but it was marginally higher than T_1 (55.12) and T_3 (54.90) batches. There were less cooking losses in all the treated groups than the control group. Hughes *et al* (1998) also reported that addition of 3% tapioca starch in low-fat frankfurters from lean pork and beef significantly reduced the cooking losses. Knight and Perkin (1991), McAuley and Mawson (1994) also observed less cooking losses with dry addition of tapioca starch in restructured meat products. Berry (1997) also got similar results on addition of tapioca starch in low fat beef patties. These findings are in agreement with the present study.

The pH of T_3 batch was significantly lower ($P<0.05$) than control and T_1. Among the treated samples, pH did not vary significantly between T_1, T_2 and T_3 but it decreased continuously as the content of tapioca starch was increased. This is in contradiction with the study of Mittal and Usborne (1986) who reported decrease in pH of snacks with increase in level of meat and decrease in starch content. The a_w did not showed significant variation among the different variants of CMC. For treated samples it ranged from 0.32-0.36. Hydratability of T_3 sample was significantly lower ($P<0.05$) than control and T_1. Among the treated batches, hydratability of T_1 (1.52) was significantly higher than T_2 (1.30) and T_3 (1.19) and there was a continuous decrease in the value as the content of tapioca starch was increased in the formulation. WAI of control samples was significantly lower ($P<0.05$) than the treated samples. However, among the treated samples there was no significant variation of WAI. WAI of T_2 was marginally higher than T_1 and T_3 batches. The increase in WAI of treated samples may be due to increased gelatinization of tapioca starch as documented by Davidson *et al* (1984) and Cheftel (1986). There was no significant variation between WSI of control and treated CMC. The WSI of treated samples were almost comparable with that of control sample.

4.2.2.2 Proximate composition of chicken meat caruncles prepared by using tapioca starch

Data pertaining to proximate composition of CMC prepared by using tapioca starch are presented at Table 22. There was no significant variation of moisture % and protein % between control and treated samples. However, the highest value of both moisture % (5.71) and protein % (28.51) was found in control samples. Among the treated samples the values for both the parameters were comparable with control. The fat % of T_1 (12.08) was significantly higher ($P<0.05$) than T_2 (9.17) and T_3 (9.83) batch. There was no significant variation of fat % between control, T_1 and T_3 samples. There was no significant difference between crude fiber % of control and T_1 as well as T_2 and T_3 samples. However, among the treated CMC, crude fiber % was found to be significantly lower ($P<0.05$) in T_1 than T_2 and T_3 samples. There was a continuous increase in crude fiber % of the samples as the content of tapioca starch increased in the formulation. There was no significant variation of ash % among control and treated samples of CMC. However, there was a marginal decrease in ash % as the content of tapioca starch increased in the formulation. Carbohydrates % did not vary significantly among control and treated batches. Among treated groups the carbohydrates % was marginally higher in T_2 (52.15) than T_1 (50.09) and T_3 (49.69). There was no significant variation of moisture: protein ratio among control and treated samples. All the values were comparable to each other.

4.2.2.3 Texture Profile of chicken meat caruncles prepared by using tapioca starch

Data pertaining to texture profile of CMC are presented at Table 23. There was no significant variation between hardness of control, T_1 and T_3 samples. However, the hardness of control batch was lower than all the treated groups. This is in agreement with the study of Hachmeister and Herald (1998) who also observed increase in value of hardness in tapioca starch added turkey meat batters as compared to control samples. The adhesiveness of control batch (57.15) was significantly

Table 22: Effect of incorporation of tapioca starch on the proximate composition of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Moisture (%)	5.71±0.25^{a}	5.55±0.08^{a}	5.49±0.11^{a}	5.56±0.25^{a}
Protein (%)	28.51±1.14^{a}	25.52±2.74^{a}	24.45±1.27^{a}	25.38±0.73^{a}
Fat (%)	11.58±0.45^{bc}	12.08±0.81^{c}	9.17±0.44^{a}	9.83±0.84^{ab}
Crude Fiber (%)	2.30±0.34^{a}	2.33±0.61^{a}	4.83±0.60^{b}	5.83±1.14^{b}
Ash (%)	4.85±0.08^{a}	4.43±0.66^{a}	3.91±0.15^{a}	3.72±0.40^{a}
Carbohydrates (%)	47.06±1.28^{a}	50.09±2.91^{a}	52.15±1.93^{a}	49.69±2.38^{a}
Moisture: Protein ratio	0.20±0.02^{a}	0.23±0.02^{a}	0.23±0.01^{a}	0.22±0.01^{a}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 17.50% RWF+ 17.50% TS, T_2= 14.00% RWF+ 21.00% TS and T_3= 10.50% RWF+ 24.50% TS.

Table 23: Effect of incorporation of tapioca starch on texture profile of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Hardness (N)	58.37±4.46^{a}	66.98±5.09^{ab}	73.84±4.36^{b}	72.14±5.39^{ab}
Adhesiveness (mJ)	57.15±6.14^{c}	28.96±2.74^{a}	40.93±5.98^{ab}	49.12±5.96^{bc}
Adhesives force (N)	16.79±2.14^{a}	14.31±1.24^{a}	15.78±1.48^{a}	14.34±0.95^{a}
Stringiness (mm)	2.43± 1.16^{b}	0.50±0.19^{a}	0.98±0.33^{ab}	1.02±0.24^{ab}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 17.50% RWF+ 17.50% TS, T_2= 14.00% RWF+ 21.00% TS and T_3= 10.50% RWF+ 24.50% TS.

higher than T_1 (28.96) and T_2 (40.93). However, among the treated groups there was no significant variation between T_1, T_2 and T_3 samples but there was a continuous increase in the adhesiveness as the content of tapioca starch increased in the formulation. There was no significant variation between adhesive force and stringiness of control, T_1, T_2 and T_3 samples. All the values were comparable for both the parameters.

4.2.2.4 Colour Profile of chicken meat caruncles prepared by using tapioca starch

Data pertaining to colour profile of CMC are presented at Table 24. The *L* and *a* value of control batch was significantly lower ($P<0.05$) than T_1, T_2 and T_3 samples. Among the treated groups there was no significant variation in both *L* and *a* values and all the values were comparable to each other. There was no significant variation of *b* value among the control and treated batches. Both control and T_1 were having the same *b* value i.e. 26.43. Hue angle was significantly higher ($P<0.05$) in control batch than the treated batches. There was no significant variation of hue angle among the treated groups. Chroma did not differ significantly among the control and treated batches. The values of chroma ranged from 28.75-30.65.

4.2.2.5 Sensory attributes of chicken meat caruncles prepared by using tapioca starch

Data pertaining to various sensory attributes of CMC incorporated with TS are presented at Table 25. All the sensory attributes namely colour, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability did not show significant variation between control, T_1, T_2 and T_3 batches. Among the treated groups T_2 got marginally higher scores than T_1 and T_3 groups, so it was considered most acceptable. Sajilata and Singhal (2004) documented that incorporation of modified starches into snacks can have a high degree of mouth melt, less waxiness, improved texture and increased crispiness.

Table 24: Effect of incorporation of tapioca starch on colour profile of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
L	38.32±0.61[a]	43.00±1.12[b]	43.11±0.72[b]	42.32±1.08[b]
a	11.20±0.63[a]	13.64±0.30[b]	13.47±0.41[b]	13.69±0.46[b]
b	26.43±0.71[a]	26.43±0.49[a]	27.50±0.62[a]	25.86±0.51[a]
Hue angle	67.06±1.18[b]	62.68±0.58[a]	63.86±0.88[a]	62.14±0.63[a]
Chroma	28.75±0.74[a]	29.76±0.49[a]	30.65±0.57[a]	29.28±0.61[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 17.50% RWF+ 17.50% TS, T_2= 14.00% RWF+ 21.00% TS and T_3= 10.50% RWF+ 24.50% TS.

Table 25: Effect of incorporation of tapioca starch on the sensory attributes of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Colour/Appearance	7.11±0.23[a]	6.89±0.20[a]	7.00±0.12[a]	6.89±0.18[a]
Flavour	6.89±0.11[a]	6.89±0.14[a]	6.89±0.16[a]	6.78±0.12[a]
Crispiness	7.22±0.25[a]	6.83±0.08[a]	7.22±0.09[a]	6.94±0.06[a]
After-taste	6.89±0.16[a]	6.67±0.14[a]	6.89±0.22[a]	6.44±0.18[a]
Meat flavour intensity	6.83±0.14[a]	6.72±0.15[a]	6.56±0.18[a]	6.56±0.15[a]
Overall acceptability	7.17±0.19[b]	6.72±0.12[ab]	6.89±0.23[ab]	6.56±0.13[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 17.50% RWF+ 17.50% TS, T_2= 14.00% RWF+ 21.00% TS and T_3= 10.50% RWF+ 24.50% TS.

4.2.3 Optimization of level of potato starch in chicken meat caruncles

4.2.3.1 Physico-chemical quality of chicken meat caruncles prepared by using potato starch

Four different batches of CMC were prepared viz. control (without PS), T_1 = 14.00% RWF + 21.00% PS; T_2 = 7.00% RWF + 28.00% PS and T_3 = 35.00% PS and their quality was evaluated. Data pertaining to emulsion stability (E.S.) %, cooking yield (C.Y.) %, pH, water activity (a_w), Hydratability, WAI and WSI are presented at Table 26. There was no significant variation of emulsion stability %, CY % and pH between control and treated groups. The values for all the three parameters ranged from 97.01-97.65, 53.05-55.21 and 5.78-5.90 respectively. All the values in treated groups were comparable to control groups. Shand (2000) also reported that there was no significant difference of cooking yield (%) between control, potato starch (4%) and κ-carrageenan (0.25%) groups. The a_w of control group was significantly higher (P<0.05) than the treated groups. a_w was equal in all the treated batches. Hydratability did not differ significantly between control and treated batches. But the value was marginally higher in control (1.19) as compared to T_1 (1.05), T_2 (0.98) and T_3 (1.10). WAI of control samples was significantly lower (P<0.05) than the treated samples. This may be due to increased gelatinization of potato starch in treated groups as proposed by Davidson *et al* (1984) and Cheftel (1986). Park e*t al* (1993) also reported that high corn starch and low fat level resulted in higher WAI of extrudates. However, among the treated samples WAI of T_3 (5.09) was significantly higher (P<0.05) than T_1 (4.60) and T_2 (4.79). There was no significant variation between WSI of control and treated CMC. The WSI of treated samples were almost comparable with that of control sample.

Table 26: Effect of incorporation of potato starch on the physico-chemical properties of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Emulsion stability (%)	97.01±0.17[a]	97.64±0.18[a]	97.65±0.33[a]	97.63±0.26[a]
Cooking yield (%)	55.21±0.66[a]	54.79±0.81[a]	53.05±0.68[a]	54.56±0.53[a]
pH	5.80±0.05[a]	5.88±0.09[a]	5.78±0.03[a]	5.90±0.06[a]
Water activity (a_w)	0.29±0.00[b]	0.25±0.01[a]	0.25±0.00[a]	0.25±0.01[a]
Hydratability	1.19±0.12[a]	1.05±0.08[a]	0.98±0.13[a]	1.10±0.07[a]
WAI	4.16±0.08[a]	4.60±0.03[b]	4.79±0.04[b]	5.09±0.12[c]
WSI	0.10±0.01[a]	0.06±0.00[a]	0.06±0.00[a]	0.12±0.08[a]

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1= 14.00% RWF+ 21.00% PS, T_2= 7.00% RWF+ 28.00% PS and T_3= 35.00% PS.

Table 27: Effect of incorporation of potato starch on the proximate composition of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Moisture (%)	4.72±0.09[a]	4.38±0.20[a]	4.41±0.02[a]	4.51±0.24[a]
Protein (%)	35.49±2.09[b]	25.43±0.55[a]	23.19±0.53[a]	25.72±1.42[a]
Fat (%)	11.46±0.70[a]	13.78±0.70[a]	12.99±0.96[a]	12.64±0.90[a]
Crude Fiber (%)	2.30±0.45[a]	2.02±0.35[a]	3.52±0.70[a]	2.53±0.71[a]
Ash (%)	3.51±0.52[b]	2.98±0.32[ab]	4.57±0.13[c]	2.23±0.24[a]
Carbohydrates (%)	42.53±2.04[a]	51.41±1.48[b]	51.33±2.04[b]	52.37±2.29[b]
Moisture: Protein ratio	0.13±0.01[a]	0.17±0.00[b]	0.19±0.00[b]	0.18±0.02[b]

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 14.00% RWF+ 21.00% PS, T_2= 7.00% RWF+ 28.00% PS and T_3= 35.00% PS.

4.2.3.2 Proximate composition of chicken meat caruncles prepared by using potato starch

Data pertaining to proximate composition of CMC prepared by using PS are presented at Table 27. There was no significant variation of moisture %, protein %, fat %, crude fiber %, carbohydrates % and moisture: protein ratio between T_1, T_2 and T_3 samples. The protein % was significantly higher ($P<0.05$) in control group than the treated groups. The ash % was significantly higher ($P<0.05$) in T_2 batch (4.57) than control (3.51), T_1 (2.98) and T_3 (2.23). Carbohydrates % was significantly lower ($P<0.05$) in control group than all the treated groups. Moisture: protein ratio was significantly lower ($P<0.05$) in control samples than T_1, T_2 and T_3 samples. This may be due to higher amount of protein in it.

4.2.3.3 Texture Profile of chicken meat caruncles prepared by using potato starch

Data pertaining to texture profile of CMC are presented at Table 28. Hardness of control batch (63.78) was significantly lower ($P<0.05$) than T_1 (97.07), T_2 (100.55) and T_3 (119.27) batches. Among the treated groups, hardness did not vary significantly but it increased continuously as the content of PS increased in the formulation. The finding confirms the result of Garcia-Garcia and Totosaus (2008) who reported that addition of 10% potato starch in low fat sausages produced much harder and resilient product. Bloukas *et al* (1997) also observed increase in hardness and skin strength of low fat frankfurters incorporated with 3.5% PS. Hachmeister and Herald (1998) also observed increase in value of hardness in potato starch added turkey meat batters as compared to control samples. Bushway *et al* (1982) reported that frankfurters with 1.5% PS were more tender and juicy than those made with 3% PS. There was no significant variation of adhesiveness, adhesive force and stringiness between the control and treated groups. There was a marginal decrease in the value of adhesiveness, adhesive force and stringiness from T_1 to T_2 and then increase from T_2 and T_3. For all these parameters comparatively lower values were found in T_2 than T_1 and T_3.

Table 28: Effect of incorporation of potato starch on texture profile of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Hardness (N)	63.78±3.88[a]	97.07±8.92[b]	100.55±8.96[b]	119.27±7.14[b]
Adhesiveness (mJ)	37.48±4.46[a]	34.92±5.25[a]	34.13±4.03[a]	40.87±5.87[a]
Adhesives force (N)	13.19±1.02[a]	14.09±1.85[a]	12.29±1.79[a]	12.51±1.56[a]
Stringiness (mm)	1.52±0.61[a]	1.54±0.58[a]	0.70±0.22[a]	1.05±0.22[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 14.00% RWF+ 21.00% PS, T_2= 7.00% RWF+ 28.00% PS and T_3= 35.00% PS.

Table 29: Effect of incorporation of potato starch on colour profile of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
L	44.92±0.96[a]	42.87±1.21[a]	42.71±2.55[a]	43.53±1.62[a]
a	12.93±0.96[a]	13.56±0.82[a]	13.54±0.37[a]	12.35±0.55[a]
b	26.91±0.37[a]	25.98±0.49[a]	25.07±1.64[a]	25.66±0.59[a]
Hue	64.57±1.54[a]	62.57±1.50[a]	61.03±1.53[a]	64.36±0.70[a]
Chroma	29.94±0.66[a]	29.38±0.59[a]	28.57±1.52[a]	28.49±0.73[a]

Mean ± SE with different superscripts in the same row differ significantly (P<0.05). Control= 35.00% RWF; T_1 = 14.00% RWF+ 21.00% PS, T_2= 7.00% RWF+ 28.00% PS and T_3= 35.00% PS.

4.2.3.4 Colour Profile of chicken meat caruncles prepared by using potato starch

Data pertaining to colour profile of CMC are presented at Table 29. No significant differences were found for *L* value, *a* value, b value, hue and chroma of control and treated groups. All the values were comparable. There was a marginal but continuous decrease in the *a* value and chroma of treated CMC as the content of PS increased in the formulation. Among treated groups, with increase in PS content *b* value first decreased marginally (T_2) and then again increased in T_3. The finding is again in accordance to Lee *et al* (2003) who also observed exactly similar trend in *b* value (first decreased marginally and then increased) with increase in PS in popped chicken snacks.

4.2.3.5 Sensory attributes of chicken meat caruncles prepared by using potato starch

Data pertaining to various sensory attributes of CMC incorporated with PS are presented at Table 30. All the sensory attributes namely colour, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability did not show significant variation between control, T_1, T_2 and T_3 batches. Colour, after-taste and meat flavour intensity were significantly higher ($P<0.05$) in control groups than T_1. Moreover, among the treated groups the scores for all the sensory attributes increased marginally as the content of PS was increased in the formulation. The marginal increase in crispiness as the content of PS increased was in agreement with report of Carey *et al* (1998), which showed that potato starch controls crisp and crunchy texture of snack foods. Altunakar *et al* (2004) also revealed that addition of starch for the preparation of deep-fat fried chicken nuggets increased the crispness significantly at the last stage of frying and the moisture content decreased with the increase in frying time. Also the scores for almost all sensory attributes were comparatively higher for T_3 group than for T_1 and T_2. Hence T_3 was considered most acceptable among the treated groups.

Table 30: Effect of incorporation of potato starch on the sensory attributes of chicken meat caruncles. **Mean ± S.E**

Parameters	Treatments			
	Control	T_1	T_2	T_3
Colour/Appearance	6.94±0.10^{b}	6.50±0.14^{a}	6.67±0.17^{ab}	6.83±0.14^{ab}
Flavour	6.94±0.18^{a}	6.44±0.13^{a}	6.67±0.17^{a}	6.56±0.18^{a}
Crispiness	6.83±0.20^{ab}	6.39±0.16^{a}	6.83±0.19^{ab}	7.06±0.18^{b}
After-taste	6.94±0.18^{b}	6.33±0.12^{a}	6.61±0.16^{ab}	6.67±0.14^{ab}
Meat flavour intensity	6.94±0.18^{b}	6.33±0.12^{a}	6.56±0.18^{ab}	6.61±0.18^{ab}
Overall acceptability	7.00±0.17^{a}	6.56±0.06^{a}	6.89±0.18^{a}	6.89±0.14^{a}

Mean ± SE with different superscripts in the same row differ significantly ($P<0.05$). Control= 35.00% RWF; T_1 = 14.00% RWF+ 21.00% PS, T_2= 7.00% RWF+ 28.00% PS and T_3= 35.00% PS.

4.3 Experiment No. 3: Comparative study of natural preservatives on the quality characteristics of chicken meat emulsion.

Four different batches of CME i.e. control (without natural preservative), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP were prepared and stored for 9 days at refrigeration temperature of 4±1°C to evaluate the quality changes in CME. Data pertaining to their physico-chemical quality are presented at Table 31.

4.3.1 Physico-chemical quality of chicken meat emulsion prepared by using selected levels of clove powder, ginger and garlic pastes.

It was found that pH of CME did not show any significant change up to day 5 among the control and three treated samples. While on day 7, highest pH in T_3 (5.85) and on day 9, it was in control (5.74). During the storage period, control and CP treated samples could maintain the pH of CME without significant change till the end of the storage. On the other hand, ginger paste batch (T_2) showed a significantly lower pH (5.38) on day 9 as compared to day 1 (5.65). The garlic paste batch (T_3) showed a significantly higher pH (5.85) on day 7 but again it was decreased on day 9 to 5.53 which did not significantly vary with the pH of day 1. Kumar and Tanwar (2011) observed a non-significant effect on the pH value after incorporation of clove powder in chicken nuggets as compared to the control batch. They also reported a significant increase in the pH of both control and treated batches with the advancement of the storage period. Verma and Sahoo (2000) also reported that as the storage time increased the pH of α-tocopherol preblended ground chevon (stored at 4±1°C for 9 days) increased linearly ($P<0.05$). The increase in pH during the storage period may be due to growth of Gram-negative bacteria such as Pseudomonas, Moraxella, Acinetobacter etc. (Kirsch *et al* 1952 and McDowell *et al* 1986).

At the beginning of storage, titrable acidity of ginger paste batch (0.04) was significantly higher than the clove powder batch but did not significantly vary with control and T_3 batch (garlic

Table 31: Effect of different natural preservatives on the physico-chemical quality of chicken meat emulsion stored at 4±1°C. **Mean ± S.E**

Tmts/ Days	Day 1	Day 3	Day 5	Day 7	Day 9
			pH		
C	5.70 ± 0.10^{Aa}	5.50 ± 0.12^{Aa}	5.82 ± 0.10^{Aa}	5.61 ± 0.11^{Aa}	5.74 ± 0.07^{Ab}
T_1	5.62 ± 0.11^{Aa}	5.59 ± 0.13^{Aa}	5.76 ± 0.09^{Aa}	5.77 ± 0.02^{Aab}	5.63 ± 0.13^{Aab}
T_2	5.65 ± 0.10^{Ba}	5.64 ± 0.05^{Ba}	5.69 ± 0.06^{Ba}	5.54 ± 0.09^{ABa}	5.38 ± 0.11^{Aa}
T_3	5.76 ± 0.07^{ABa}	5.75 ± 0.03^{ABa}	5.67 ± 0.08^{ABa}	5.85 ± 0.03^{Bb}	5.53 ± 0.12^{Aab}
			Titrable acidity (% lactic acid)		
C	0.04 ± 0.00^{Aab}	0.04 ± 0.00^{Aa}	0.05 ± 0.00^{ABa}	0.07 ± 0.01^{BCa}	0.08 ± 0.01^{Ca}
T_1	0.03 ± 0.00^{Aa}	0.03 ± 0.00^{Aa}	0.05 ± 0.00^{ABa}	0.07 ± 0.01^{Ba}	0.07 ± 0.01^{Ba}
T_2	0.04 ± 0.00^{Ab}	0.04 ± 0.00^{Aa}	0.05 ± 0.00^{Aa}	0.09 ± 0.02^{Ba}	0.09 ± 0.02^{Ba}
T_3	0.03 ± 0.00^{Aab}	0.03 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.07 ± 0.02^{Ba}	0.07 ± 0.01^{Ba}
			Extract Release Volume (ml)		
C	22.33 ± 1.65^{Ca}	20.33 ± 1.17^{BCa}	19.33 ± 0.84^{ABCa}	18.33 ± 0.99^{ABa}	16.33 ± 0.80^{Aa}
T_1	24.17 ± 1.99^{Ba}	22.83 ± 1.70^{ABa}	21.83 ± 1.56^{ABa}	20.33 ± 1.56^{ABa}	18.50 ± 1.67^{Aa}
T_2	23.00 ± 1.79^{Aa}	21.50 ± 1.73^{Aa}	20.67 ± 1.67^{Aa}	19.67 ± 1.61^{Aa}	18.50 ± 1.34^{Aa}
T_3	23.33 ± 1.54^{Ba}	22.17 ± 1.45^{ABa}	21.17 ± 1.45^{ABa}	20.33 ± 1.20^{ABa}	18.83 ± 1.20^{Aa}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3%GiP and T_3 = 2% GaP.

paste). On day 3, 5, 7 and 9, there was no significant change in titrable acidity among control and three treated CME samples. This indicates that natural preservatives did not affect the titrable acidity of CME. As the storage period increased, titrable acidity also increased significantly ($P<0.05$) in control, T_1, T_2 and T_3 samples which recorded the titrable acidity values 0.08, 0.07, 0.09 and 0.07 from the initial values 0.04, 0.03, 0.04 and 0.03 respectively. Stoltenberg *et al* (2006) while preparing beef snack sticks found that titrable acidity of the raw batter (with each 10% and 25% citric acid and lactic acid) was ranged from 0.85% to 0.94% but after heat processing to an internal temperature of 64.4°C, the titrable acidity increased to 1.6% to 1.8%. ERV did not vary significantly within control and treated batches of CME throughout the storage period. However, all the treated samples showed a marginal increase in ERV in all the storage intervals. In general the ERV decreased as the storage period increased in all CME samples. Kumar *et al* (2007) observed that values for ERV of chicken patties prepared from spent hen meat (stored at 4±1°C for 6 weeks under vacuum-packaging conditions) were comparable to its day 0 value in early stage decreased significantly ($P<0.05$) on subsequent storage without any noticeable defects even on day 42.

4.3.2 Effect of different natural preservatives on the oxidative stability of chicken meat emulsion.

Data pertaining to oxidative stability parameters such as FFA, PV, TBARS number, DPPH % inhibition and ABTS % inhibition are presented at Table 32. On day 1 i.e. beginning of the storage, FFA content was almost similar in the natural preservative treated samples whereas the control sample (0.11) showed a significantly higher ($P<0.05$) value than T_3 batch. On all the storage days i.e. 3, 5, 7, 9 FFA was significantly higher in control as compared to treated CME batches. Among the treated batches, garlic paste batch (T_3) showed significantly ($P<0.05$) lower FFA during all the storage intervals up to day 9. In general FFA increased as the storage period increased. It reached to 0.20, 0.18, 0.17 and 0.15 on day 9 from initial value of 0.11, 0.10, 0.10 and 0.08 respectively. Das *et al*

Table 32: Effect of different natural preservatives on the oxidative stability of chicken meat emulsion stored at 4±1°C. **Mean ± S.E**

Tmts/ Days	Day 1	Day 3	Day 5	Day 7	Day 9
			Free Fatty acids (%)		
C	0.11 ± 0.01^{Ab}	0.16 ± 0.00^{Bc}	0.17 ± 0.00^{Bc}	0.19 ± 0.00^{Cc}	0.20 ± 0.00^{Cc}
T_1	0.10 ± 0.01^{Aab}	0.14 ± 0.01^{Bb}	0.15 ± 0.00^{BCb}	0.16 ± 0.00^{Cb}	0.18 ± 0.01^{Db}
T_2	0.10 ± 0.01^{Aab}	0.15 ± 0.01^{Bbc}	0.15 ± 0.00^{Bb}	0.17 ± 0.00^{BCb}	0.17 ± 0.01^{Cb}
T_3	0.08 ± 0.00^{Aa}	0.10 ± 0.01^{Ba}	0.12 ± 0.00^{Ca}	0.14 ± 0.00^{Da}	0.15 ± 0.00^{Da}
			Peroxide value (meq/kg)		
C	1.03 ± 0.24^{Aa}	1.13 ± 0.21^{ABa}	1.30 ± 0.13^{ABCb}	1.60 ± 0.05^{BCb}	1.73 ± 0.04^{Cb}
T_1	0.50 ± 0.11^{Aa}	0.63 ± 0.17^{ABa}	0.77 ± 0.10^{ABa}	1.00 ± 0.18^{BCa}	1.27 ± 0.10^{Ca}
T_2	0.73 ± 0.15^{Aa}	0.73 ± 0.15^{Aa}	0.73 ± 0.15^{Aa}	0.97 ± 0.12^{Aa}	1.13 ± 0.18^{Aa}
T_3	0.87 ± 0.31^{Aa}	0.93 ± 0.38^{Aa}	1.03 ± 0.57^{Aab}	1.33 ± 1.08^{Aab}	1.40 ± 1.02^{Aab}
			TBARS number (mg MDA/kg)		
C	1.98 ± 0.13^{Ac}	2.10 ± 0.14^{Ab}	2.33 ± 0.07^{Ab}	2.36 ± 0.07^{Ac}	2.77 ± 0.20^{Bb}
T_1	1.30 ± 0.03^{Aa}	1.47 ± 0.12^{ABa}	1.62 ± 0.12^{ABCa}	1.66 ± 0.07^{BCa}	1.86 ± 0.17^{Ca}
T_2	1.49 ± 0.14^{Aab}	1.61 ± 0.23^{Aa}	1.75 ± 0.11^{ABa}	1.66 ± 0.05^{Aa}	2.16 ± 0.18^{Ba}
T_3	1.75 ± 0.08^{Abc}	1.91 ± 0.08^{Aab}	2.18 ± 0.05^{Bb}	1.98 ± 0.14^{ABb}	2.23 ± 0.01^{Ba}
			DPPH (% inhibition)		
C	27.02 ± 4.60^{BCa}	29.83 ± 2.96^{Ca}	19.98 ± 1.53^{ABa}	16.60 ± 0.87^{Aa}	12.88 ± 2.60^{Aa}
T_1	29.39 ± 4.97^{Aa}	28.61 ± 6.61^{Aa}	43.93 ± 4.74^{Ab}	39.00 ± 5.67^{Ab}	35.17 ± 3.80^{Ab}
T_2	26.16 ± 6.54^{Aa}	28.75 ± 5.07^{Aa}	24.76 ± 7.40^{Aa}	17.00 ± 3.03^{Aa}	18.13 ± 1.41^{Aa}
T_3	29.43 ± 5.28^{Ba}	28.28 ± 3.26^{Ba}	21.08 ± 3.44^{ABa}	13.58 ± 2.57^{Aa}	11.86 ± 2.26^{Aa}

	ABTS (% inhibition)				
C	41.93±3.08^{Da}	35.98±3.55^{CDa}	29.81±1.32^{BCa}	26.21±1.06^{ABa}	19.10±2.90^{Aa}
T_1	63.36±1.73^{Dc}	60.74±1.62^{CDc}	56.24±0.96^{Cc}	47.62±2.47^{Bc}	37.55±2.73^{Ac}
T_2	48.69±3.33^{Dab}	43.74±2.71^{CDab}	38.93±2.77^{BCb}	33.31±2.41^{ABb}	27.88±1.41^{Ab}
T_3	51.41±3.33^{Cb}	48.33±2.64^{Cb}	43.79±3.13^{BCb}	37.69±3.09^{ABb}	32.36±2.67^{Abc}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3%GiP and T_3 = 2% GaP.

(2011) reported increasing trend of FFA during refrigeration storage of raw ground meat for 9 days. Other workers also suggested similar trend in FFA of buffalo meat (Rao and Kowale 1988), goat meat (Verma and Sahoo 2000) and goat meat patties (Das *et al* 2008) during 9 days of refrigeration storage.

Peroxide value (Fig 18) of control sample remained significantly higher on day 5, 7 and 9 as compared to natural preservative treated samples. PV did not show significant variation on day 1 and 3 among control and treated CME batches. Within the treated batches PV did not significantly vary. Ginger paste batch (T_2) showed marginally lower PV than CP and garlic paste batches. As the storage period increased, there was increase in PV in control and T_1 batch but T_2 and T_3 batch did not show any significant difference. TBA value (Fig 19) was significantly lower in T_1 (1.30) and T_2 (1.49) as compared to control (1.98) and T_3 (1.75) at the beginning of the storage. Clove powder (T_1) maintained lowest TBARS number in all the storage intervals till the end of the storage among the natural preservatives tried. The finding is very well in accordance with the study of Vasavada *et al* (2006) who also documented that antioxidant activity of cloves in cooked ground beef (stored at 2°C for 15 days) was highest in terms of TBA value than ginger, cinnamon, caraway, fennel, nutmeg and other spices. In all the CME batches, TBARS number significantly increased. At the end of storage i.e. day 9, reaching to 2.77, 1.86, 2.16 and 2.23 from the initial value of 1.98, 1.30, 1.49 and 1.75 in case of control, T_1, T_2 and T_3 batches respectively. Also at the end of storage period, highest TBARS number was found in T_3 and lowest in T_1 batch among the treated samples but they did not significantly vary among themselves showing that all the three preservatives are potential antioxidants. At day 9 of storage, comparatively lower TBA values shown by ginger (2.16) than control (2.77) and garlic (2.23) is in accordance with the study of Stoilova *et al* (2007) who

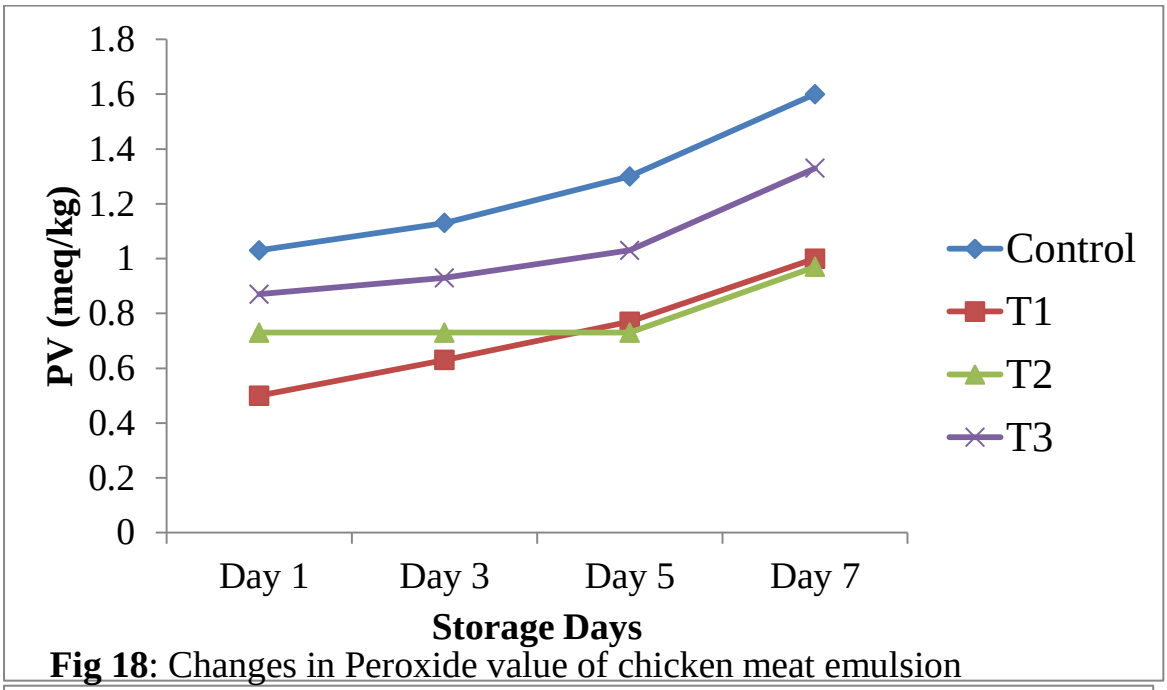


Fig 18: Changes in Peroxide value of chicken meat emulsion during refrigeration storage at 4±1°C.

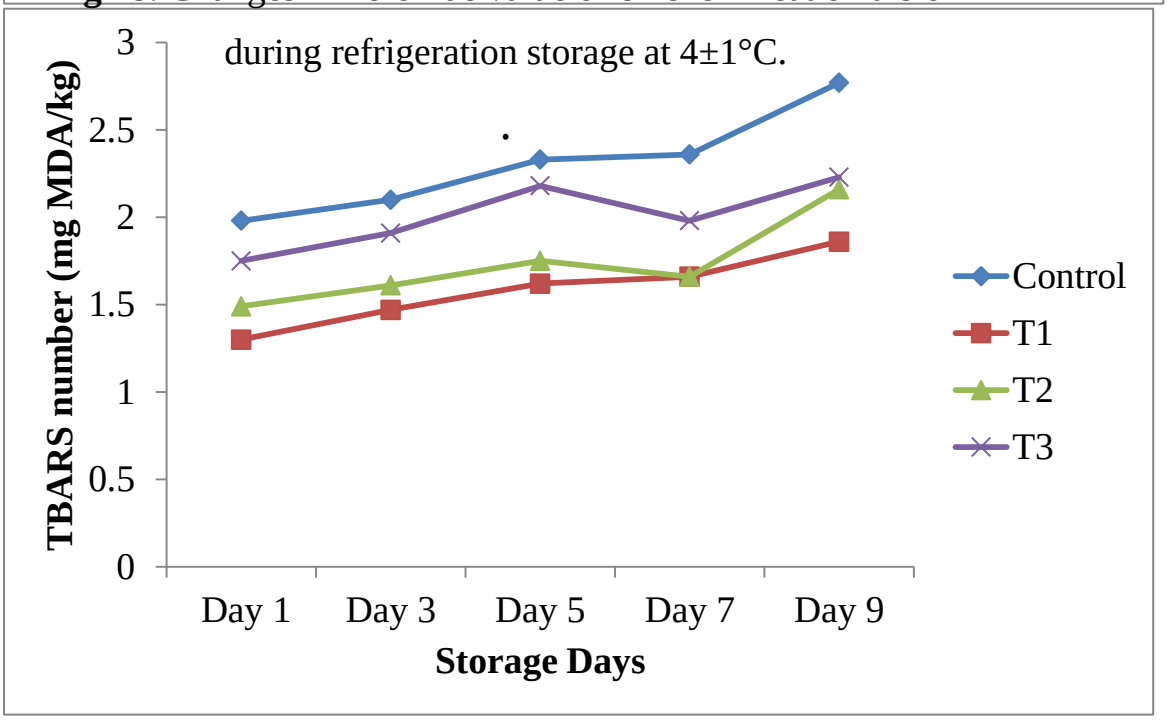


Fig 19: Changes in TBARS number of chicken meat emulsion during refrigeration storage at 4±1°C.

Control (without natural preservatives), T_1 (0.2% clove powder), T_2 (3% Ginger paste) and T_3 (2% Garlic paste).

documented that samples with 0.05% ginger extract showed lower TBA values as compared to control and BHT samples. Shan *et al* (2009) also revealed that out of clove, cinnamon stick, oregano, pomegranate peel and grape seed, clove exhibited strongest antioxidant activity in terms of TBA value in raw pork at room temperature. Bali *et al* (2011) observed that there was a significant ($p<0.01$) increase in TBA value of chicken sausages (stored at 4±1°C for 21 days) incorporated with garlic and coriander throughout the storage period. Sallam *et al* (2004) also revealed that addition of fresh garlic paste to chicken sausage (stored at 3°C for 21 days) significantly delayed lipid oxidation (both in terms of PV and TBA value) than the control samples.

DPPH % inhibition (Fig 20) did not significantly vary among control and treated CME batches up to day 3 thereafter it was found to be significantly higher ($P<0.05$) in T_1 batch showing that clove powder is better inhibitor of free radicals formation. The maximum % inhibition in terms of DPPH was shown by CP batch on day 5, 7 and 9 indicating that it is potentially superior to ginger and garlic paste in scavenging the free radicals. As the storage period progressed, DPPH % inhibition significantly decreased in control and T_3 samples whereas the natural preservative groups (T_1 and T_2) exhibited no significant change in DPPH till the end of storage period. Both CP and ginger showed better results in terms of DPPH % inhibition but the effect of CP was double than that of ginger. This finding is in accordance with the results of Gulcin *et al* (2010) who reported a significant decrease ($P<0.01$) in the concentration of DPPH radical due to scavenging its scavenging ability. The scavenging effect of clove oil and standards on the DPPH radical decreased in the order of clove oil > BHT > α-tocopherol > BHA > trolox, which were 83.6, 67.8, 64.9, 62.5 and 29.4%, at the

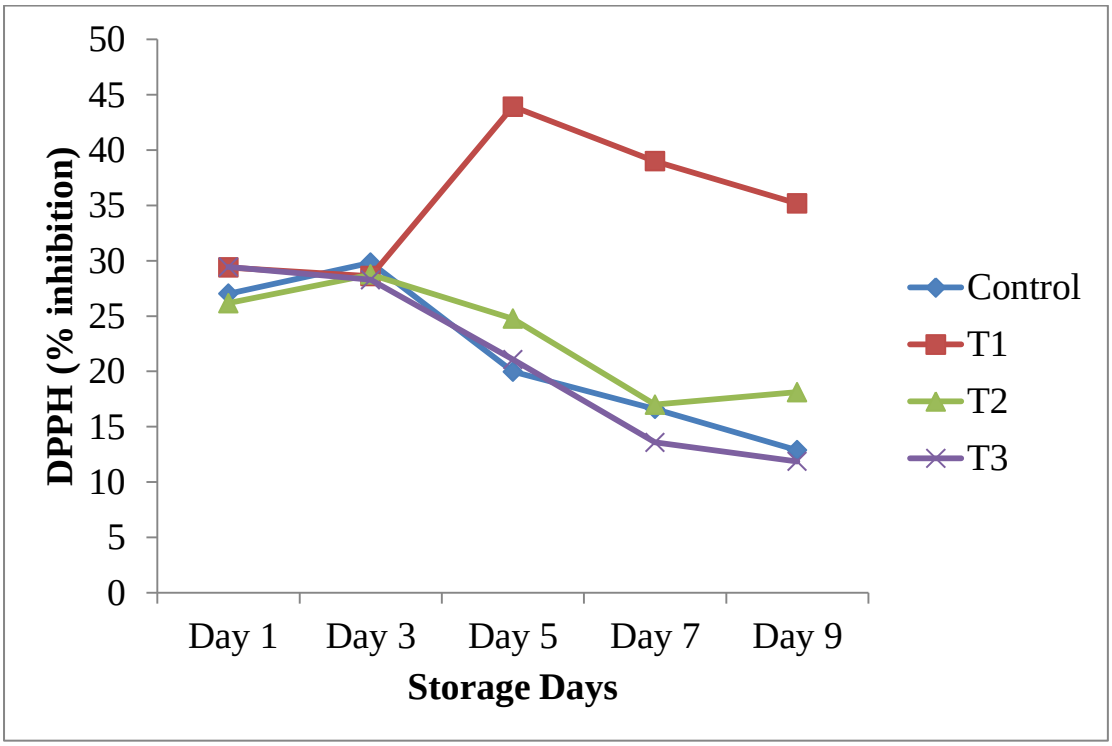
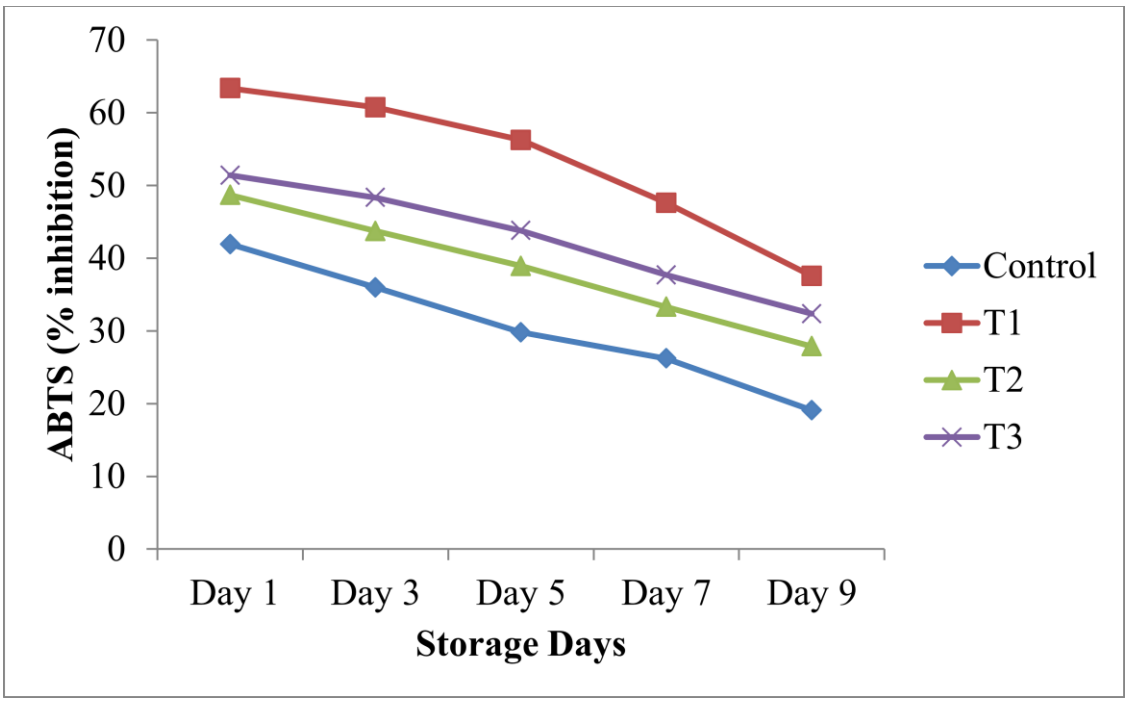


Fig 20 : Changes in DPPH (% inhibition) of chicken meat emulsion during refrigeration

Fig 21: Changes in ABTS (% inhibition) of chicken meat emulsion



. Control (without natural preservatives), T_1 (0.2% clove powder), T_2 (3% Ginger paste) and T_3 (2% Garlic paste).

during refrigeration storage at 4±1°C.

concentration of 45 µg/ml, respectively. In an another study, Gulcin *et al* (2004) also reported that DPPH radical decreased in the order of ethanol extract of clove buds > water extract of clove buds = BHA > BHT > ethanol extract of lavender > water extract of lavender > α-tocopherol and were 74%, 62%, 62%, 60%, 50%, 45% and 31% at the concentration of 60µg/ml, respectively. At day 9 of storage, comparatively higher DPPH (% inhibition) shown by ginger (18.13) as compared to control (12.88) and garlic (11.86) is exactly in accordance with the study of Stoilova *et al* (2007) who documented that ginger extract showed significant effect in inhibition of DPPH as compared to control and BHT samples.

On day 1, ABTS % inhibition (Fig 21) was significantly higher in T_1 batch as compared to other treated and control batches and same trend was continued in all other storage intervals till the end of storage period showing that CP is potentially much better than ginger and garlic in scavenging the free radicals. At the end of storage maximum ABTS % inhibition (37.55) was found in T_1 batch as compared to T_2 (27.88), T_3 (32.36) and control (19.10) samples. In general ABTS significantly decreased as the storage period increased in all the CME batches. Gulcin *et al* (2010) also reported that there was a significant decrease ($P<0.01$) in the concentration of $ABTS^{\cdot+}$ due to potent radical scavenging action of clove oil than BHT, α-tocopherol and trolox. The scavenging effect of clove oil and standards on the $ABTS^{\cdot+}$ decreased in the order of BHA = clove oil ≈ BHT > α-tocopherol > trolox, which were 100, 98.7, 97.8, 86.3 and 4.4 %, at the concentration of 45µg/ml, respectively.

4.3.3 Effect of different natural preservatives on the colour profile of chicken meat emulsion

The results on different colour profiles such as *L*, *a*, *b* values, hue, chroma and metmyoglobin % of different CME variants are presented at Table 33. *L* value was lowest

(33.29) in T_2 batch on day 1. There was no significant difference in *L* value between control, T_1 and T_3 batches at the beginning of the storage. *L* value did not change significantly on day 3, 7 and 9 among control and three different CME batches. While on day 5, the lowest *L* value (31.27) was observed in T_1 batch but it was not significantly different from T_2 and T_3 batches. On day 1, the highest *a* value was found in T_1 batch

Table 33: Effect of different natural preservatives on the colour profile of chicken meat emulsion stored at 4±1°C. **Mean ± S.E**

Tmts/ Days	Day 1	Day 3	Day 5	Day 7	Day 9
			***L* value**		
C	34.32 ± 0.13^{Aab}	35.29 ± 1.60^{Aa}	37.41 ± 1.60^{Ab}	38.62 ± 2.41^{Aa}	36.84 ± 2.18^{Aa}
T_1	37.63 ± 0.86^{Bb}	36.93 ± 1.38^{Ba}	31.27 ± 1.83^{Aa}	35.60 ± 2.43^{ABa}	35.85 ± 1.96^{ABa}
T_2	33.29 ± 1.16^{Aa}	38.64 ± 1.99^{Aa}	33.54 ± 1.53^{Aab}	38.56 ± 2.10^{Aa}	38.43 ± 2.30^{Aa}
T_3	37.73 ± 2.04^{ABb}	39.93 ± 1.53^{Ba}	34.99 ± 0.32^{Aab}	40.04 ± 1.60^{Ba}	35.71 ± 1.66^{ABa}
			***a* value**		
C	0.90 ± 0.14^{Aa}	0.59 ± 0.14^{Aa}	0.79 ± 0.18^{Aa}	0.59 ± 0.13^{Aa}	0.46 ± 0.13^{Aa}
T_1	1.29 ± 0.05^{Ab}	1.13 ± 0.08^{Ab}	0.97 ± 0.10^{Aa}	1.25 ± 0.17^{Ab}	1.11 ± 0.12^{Ab}
T_2	0.65 ± 0.18^{Aa}	0.64 ± 0.16^{Aa}	0.66 ± 0.11^{Aa}	0.76 ± 0.20^{Aa}	0.53 ± 0.13^{Aa}
T_3	0.75 ± 0.07^{Aa}	0.69 ± 0.18^{Aa}	0.60 ± 0.17^{Aa}	0.67 ± 0.14^{Aa}	0.39 ± 0.04^{Aa}
			***b* value**		
C	8.61 ± 0.31^{Aa}	8.83 ± 0.41^{Aa}	9.42 ± 0.59^{Ab}	9.09 ± 0.09^{Ab}	8.99 ± 0.22^{Aa}
T_1	8.73 ± 0.22^{Ba}	8.48 ± 0.33^{ABa}	7.71 ± 0.41^{Aa}	8.34 ± 0.10^{ABa}	8.55 ± 0.35^{ABa}
T_2	8.99 ± 0.25^{Aab}	9.78 ± 0.53^{Aa}	8.86 ± 0.49^{Aab}	9.40 ± 0.29^{Ab}	9.43 ± 0.14^{Aa}
T_3	9.70 ± 0.24^{Ab}	9.78 ± 0.47^{Aa}	8.98 ± 0.30^{Aab}	9.68 ± 0.35^{Ab}	9.22 ± 0.45^{Aa}
			Hue angle		

C	84.17±0.72^{Ab}	86.27±0.76^{ABb}	85.39±0.76^{ABb}	86.29±0.84^{ABb}	87.16±0.74^{Bb}
T_1	81.57±0.54^{Aa}	82.44±0.46^{Aa}	82.88±0.57^{Aa}	81.43±1.25^{Aa}	82.67±0.63^{Aa}
T_2	86.01±1.02^{Ab}	86.44±0.71^{Ab}	85.68±0.70^{Ab}	85.32±1.28^{Ab}	86.80±0.77^{Ab}
T_3	85.53±0.47^{Ab}	86.12±0.88^{Ab}	86.33±0.97^{Ab}	86.14±0.66^{Ab}	87.59±0.23^{Ab}
			Chroma		
C	8.66±0.32^{Aa}	8.85±0.42^{Aa}	9.46±0.60^{Ab}	9.12±0.09^{Aab}	9.00±0.23^{Aa}
T_1	8.83±0.22^{Ba}	8.55±0.33^{ABa}	7.77±0.41^{Aa}	8.44±0.08^{ABa}	8.63±0.36^{ABa}
T_2	9.02±0.26^{Aab}	9.81±0.54^{Aa}	8.89±0.49^{Aab}	9.44±0.28^{Ab}	9.45±0.14^{Aa}
T_3	9.73±0.24^{Ab}	9.81±0.48^{Aa}	9.01±0.31^{Aab}	9.71±0.35^{Ab}	9.23±0.45^{Aa}
			Metmyoglobin (%)		
C	79.58±1.68^{Aa}	80.13±1.59^{Ab}	82.13±1.97^{Aa}	82.93±1.55^{Ab}	84.09±2.00^{Aa}
T_1	71.03±1.92^{Aa}	72.55±1.66^{Aa}	74.03±1.04^{Aa}	74.94±0.88^{Aa}	79.05±1.67^{Aa}
T_2	73.32±1.63^{Ba}	74.92±1.07^{Bab}	76.31±1.17^{Ba}	78.01±1.74^{Ab}	81.12±1.12^{Ba}
T_3	75.76±1.19^{Aa}	76.48±1.65^{Ab}	78.38±1.43^{Aa}	79.44±1.56^{Ab}	81.45±1.14^{Aa}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3%GiP

and T_3 = 2% GaP.

(treated with CP) which was also significantly higher ($P<0.05$) than control, T_2 and T_3 batches showing that CP induced better appeal. The same trend was observed on day 3, 7 and 9 showing superiority of CP over ginger and garlic in maintaining the colour of the product. At the end of storage on day 9, *a* value of T_1 was 1.11 while in T_2 it was 0.53 and in T_3 0.39 showing that effect of CP was double than ginger paste and about triple as compared to garlic paste in maintaining the good colour of the product. There was no significant change in the *a* value at different storage intervals in all the control and treated samples. There was no significant difference of *b* value between control, T_1, T_2 and T_3 on day 1 whereas *b* value of T_3 (9.70) was significantly higher than control (8.61) and T_1 (8.73). On day 3, *b* value did not show any significant results between different batches and the similar trend was observed at the end of storage. On day 5, highest b value was obtained in control sample and lowest value was in T_1 batch. It was significantly lower ($P<0.05$) than control, T_2 and T_3 batches. This indicates that CP is preferred preservative among the natural preservatives used. Hue angle for T_1 (81.57) was significantly lower ($P<0.05$) than control (84.17), T_2 (86.01) and T_3 (85.53) on day 1 at the beginning of the storage. Same trend was continued on all other storage intervals i.e. on day 3, 5, 7 and 9. This speaks of CP as a preservative of ingredient as compared to ginger and garlic. There was no significant difference of chroma between control, T_1 and T_2 CME whereas it was significantly higher ($P<0.05$) in T_3 (9.73) as compared to control and T_1 batches on day 1. On day 3, no significant variation was observed among four different CME batches. Similar trend was also observed on day 9 whereas on day 5 chroma of control (9.46) was significantly higher ($P<0.05$) than T_1 (7.77) but without any variation from T_2 and T_3 samples. Chroma also remained lowest on T_1 batch which was significantly lower than T_2 and T_3 batches. Naveena *et al* (2006) also observed that *L* values of buffalo meat steaks (incorporated with lactic acid, clove and vitamin C and stored at 4±1°C for 12 days) were significantly ($P < 0.05$) higher compared to the control throughout the display period. The *a* values were significantly ($P < 0.05$) higher

for control samples up to the 3rd day of display and thereafter decreased significantly compared to other

samples. All the treatments also had significantly increased *b* values as compared to the control. It was also evident that colour was significantly ($P < 0.05$) stabilized by the inclusion of Vit C along with lactic acid + clove mixture. Treatment with lactic acid + clove + Vit C resulted in an intense red colour after third day of display showing *a* value of about 14. The chroma was observed to be lowest in all treated samples and highest in control on day 0. However, as the display period increased LA + clove + Vit C treated samples exhibited significantly ($P<0.05$) higher chroma values than the others. Sahoo and Anjaneyulu (1997) also reported a significant increase in chroma values in ground buffalo meat preblended with 500 ppm of sodium ascorbate during 10 day refrigerated storage.

There was no significant change in metmyoglobin % among control (79.58), T_1 (71.03), T_2 (73.32) and T_3 (75.76) at the beginning of the storage on day 1. On day 3, metmyoglobin % was significantly lower ($P<0.05$) in T_1 than control and T_3 but it was not significantly different from ginger paste batch. On day 5, no significant change in metmyoglobin % between all the variants was observed and same trend was observed on day 9. Whereas on day 7, T_1 (74.94) batch showed lowest metmyoglobin % which was also significantly lower ($P<0.05$) than control (82.93), T_2 (78.01) and T_3 (79.44). Marginally or significantly lower values were obtained in T_1 batch. This might be due to the potent antioxidant action of clove powder than ginger and garlic. All the values increased marginally throughout the storage period which might be due to decreasing oxidative stability of all the batches. Here overall the values of metmyoglobin % were on higher side than the normal values. The probable reason may be due to the effect of tenderizing agents (papain and $CaCl_2$) on the intact structure of proteins or there may be denaturation of myoglobin. Marginally lower values of metmyoglobin % were observed in treated than the control groups. Kumudavally *et al* (2011) also concluded that application of 95% clove extract on fresh mutton (stored at 25±2°C for 4 days) lead to significantly ($P<0.05$) lower increase in metmyoglobin % as compared to the control without clove treatment as the storage period progressed.

4.3.4 Effect of different natural preservatives on the colour and odour scores of chicken meat emulsion

Data pertaining to colour and odour scores of CME incorporated with natural preservatives are presented at Table 34. Colour scores of all different CME batches were almost similar in control (4.25) and treated (4.00-4.17) batches. Colour scores of CP batch (T_1) remained highest on all the storage intervals till the end of storage i.e. day 9. This indicates that CP is a potent preservative having better function in maintaining the colour of CME. As the storage period progressed, colour scores decreased in all the CME batches. Odour scores also did not differ significantly among the treated batches at the beginning of the storage but was significantly higher than control batch. On day 3, T_1 and T_3 showed significantly higher ($P<0.05$) odour scores than control and T_2 batch. But no effect on day 5, due to the effect of preservatives. On day 7, T_1 (CP batch) showed significantly higher ($P<0.05$) odour scores (3.33) than control (2.83) but it did not differ from T_2 (2.92) and T_3 (2.92). Similar trend was observed on day 9, highest odour score showing that it consistently maintained the odour of CME and thus remained preferred preservative. These results are in agreement with the study of Das *et al* (2011) who also proposed that there was no significant difference in colour and odour scores of control and curry leaf powder treated raw ground goat meat (stored at 4±1°C for 9 days). Throughout the storage period, both colour and odour scores declined linearly. Similar trend in colour and odour scores were found by Verma and Sahoo (2000) in tocopherol preblended ground chevon (stored at 4±1°C for 9 days).

Table 34: Effect of different natural preservatives on the colour and odour scores of chicken meat emulsion stored at 4±1°C. Mean ± S.E

Tmts/ Days	Day 1	Day 3	Day 5	Day 7	Day 9
			Colour scores (5 Point scale)		
C	4.25 ± 0.11^{Da}	4.00 ± 0.00^{Db}	3.50 ± 0.13^{Cbc}	3.00 ± 0.13^{Ba}	2.25 ± 0.11^{Aa}
T_1	4.08 ± 0.08^{Da}	4.00 ± 0.00^{Db}	3.67 ± 0.11^{Cc}	3.08 ± 0.08^{Ba}	2.75 ± 0.11^{Ab}
T_2	4.00 ± 0.13^{Ca}	3.42 ± 0.20^{Ba}	2.92 ± 0.15^{ABa}	2.75 ± 0.21^{Aa}	2.42 ± 0.15^{Aab}
T_3	4.17 ± 0.11^{Da}	3.50 ± 0.18^{Ca}	3.17 ± 0.11^{BCab}	2.92 ± 0.15^{Ba}	2.42 ± 0.20^{Aab}
			Odour scores (5 Point scale)		
C	3.50 ± 0.18^{Ca}	3.33 ± 0.11^{Ca}	3.08 ± 0.08^{BCa}	2.83 ± 0.11^{ABa}	2.50 ± 0.22^{Aab}
T_1	4.08 ± 0.20^{Cb}	3.92 ± 0.20^{BCb}	3.58 ± 0.30^{BCa}	3.33 ± 0.17^{ABb}	2.92 ± 0.15^{Ab}
T_2	4.00 ± 0.00^{Db}	3.58 ± 0.15^{Ca}	3.17 ± 0.17^{Ba}	2.92 ± 0.15^{ABab}	2.58 ± 0.08^{Aab}
T_3	4.42 ± 0.08^{Eb}	3.83 ± 0.11^{Db}	3.33 ± 0.21^{Ca}	2.92 ± 0.15^{Bab}	2.25 ± 0.11^{Aa}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$). C = Control (without natural preservatives),

T_1 = 0.2% CP, T_2 = 3%GiP and T_3 = 2% GaP.

4.3.5 Effect of different natural preservatives on the microbiological quality of chicken meat emulsion

Data pertaining to microbiological parameters such as SPC, coliform count and Yeast and mold count are presented at Table 35. It was observed that SPC (Fig 22) was marginally higher in control CME (log_{10} 4.65) than T_1 (log_{10} 4.18), T_2 (log_{10} 4.18) and T_3 (log_{10} 3.61) at the beginning of the storage on day 1 without showing any significant difference among them. On subsequent storage intervals, the control samples exhibited significantly higher microbial load as compared to treated emulsion batches till the end of the storage. Among the three different treated batches, there was no significant difference on day 3, 5, 7 and 9. However, at the end of the storage T_1 showed the lowest microbial load (log_{10} 4.63). This indicates that all three natural preservatives are effective in checking the microbial growth during the storage period. Among them CP was proved to be a preferred preservative ingredient. Coliform count showed no significant variation among control and treated batches at the beginning of the storage with marginally higher microbial load in control batch. There was hardly any significant variation in coliform count on day 5 and 7 whereas on day 9, control samples showed significantly higher coliform count as compared to T_1 (log_{10} 2.47), T_2 (log_{10} 2.63) and T_3 (log_{10} 2.64). It was further noticed that coliform count did not significantly vary among three treated batches with marginally lower values in CP treated batches. This indicates that CP is preferred preservative out of three. The microbial load in terms of coliform count significantly increased at the end of storage reaching to log_{10} 2.90 in control sample and log_{10} 2.47 in T_1, log_{10} 2.63 in T_2 and log_{10} 2.64 in T_3 on day 9. Throughout the storage period, comparatively lower values of both SPC and coliform counts were detected in CP batch than GiP and GaP batches which is in accordance with the

findings of Leuschner and Lelsch (2003) who revealed that out of ground clove, fresh garlic and red chilli, ground clove exhibited strongest antimicrobial systems in broth model systems. *S. aureus* was not detected in any of the CME batch throughout the storage period of 9 days. Yeast and mold count (Fig 23) did not show any significant difference among different batches but control batch had marginally higher load (log_{10} 1.51) as compared to treated (log_{10} 1.05 to log_{10} 1.10) batches. On day 3, 5, 7 and 9 yeast and mold count did not bring any significant variation among different emulsion batches. However, it always remained highest in all the storage intervals for control emulsion batch. At the end of storage yeast and mold reached to log_{10} 2.66, log_{10} 2.52, log_{10} 2.43 and log_{10} 2.40 from the initial values of log_{10} 1.51, log_{10} 1.10, log_{10} 1.05 and log_{10} 1.05 in case of control, T_1, T_2 and T_3 batches respectively. This indicates that natural preservatives used in the present study could not inhibit yeast and mold successfully. However, yeast and mold remained marginally lower than control samples during all the storage intervals. Bali *et al* (2011) observed that in chicken sausages (incorporated with garlic and coriander and stored at 4±1°C for 21 days) total plate count of garlic treated sausages was lower than control and coriander batches. Yeast and mold were not detected initially but after 7 days onwards there was significant increase in all the groups throughout the storage period.

Table 35: Effect of different natural preservatives on the microbiological quality of chicken meat emulsion stored at 4±1°C. Mean ± S.E.

Tmts/ Days	Day 1	Day 3	Day 5	Day 7	Day 9
			Standard Plate Count (log_{10}cfu/g)		
C	4.65±0.09^{Aa}	4.83±0.08A^{Bb}	4.85±0.05^{Bb}	4.92±0.02^{Bb}	4.97±0.04^{Bb}
T_1	4.18±0.09^{Aa}	4.31±0.07A^{Ba}	4.42±0.08^{ABCa}	4.51±0.10^{BCa}	4.63±0.10^{Ca}
T_2	4.18±0.09^{Aa}	4.41±0.11A^{Ba}	4.58±0.09^{Ba}	4.62±0.10^{Ba}	4.64±0.09^{Ba}
T_3	3.61±0.72^{Aa}	4.43±0.06A^{Ba}	4.41±0.05^{ABa}	4.48±0.09^{ABa}	4.72±0.11^{Bab}
			Coliform Count (log_{10}cfu/g)		
C	2.38±0.05^{Aa}	2.49±0.09^{ABb}	2.61±0.09^{ABa}	2.68±0.12^{BCa}	2.90±0.07^{Cb}
T_1	1.38±0.44^{Aa}	1.54±0.49^{ABa}	2.35±0.09^{Ba}	2.44±0.08^{Ba}	2.47±0.09^{Ba}
T_2	1.87±0.39^{Aa}	2.37±0.10^{ABb}	2.46±0.10^{ABa}	2.51±0.11^{Ba}	2.63±0.10^{Ba}
T_3	1.72±0.35^{Aa}	2.22±0.11^{Bab}	2.43±0.09^{Ba}	2.56±0.07^{Ba}	2.64±0.05^{Ba}
			Yeast and Mold Count(log_{10}cfu/g)		
C	1.51±0.48^{Aa}	2.28±0.06^{Ba}	2.36±0.09^{Ba}	2.50±0.08^{Ba}	2.66±0.09^{Ba}
T_1	1.10±0.49^{Aa}	1.80±0.37^{ABa}	2.18±0.09^{Ba}	2.23±0.11^{Ba}	2.52±0.10^{Ba}
T_2	1.05±0.47^{Aa}	1.38±0.44^{ABa}	1.82±0.37^{ABa}	2.15±0.07^{Ba}	2.43±0.14^{Ba}
T_3	1.05±0.47^{Aa}	1.72±0.35^{ABa}	1.87±0.38^{ABa}	1.92±0.38^{ABa}	2.40±0.10^{Ba}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05). C = Control (without natural preservatives),

T_1 = 0.2% CP, T_2 = 3%GiP and T_3 = 2% GaP.

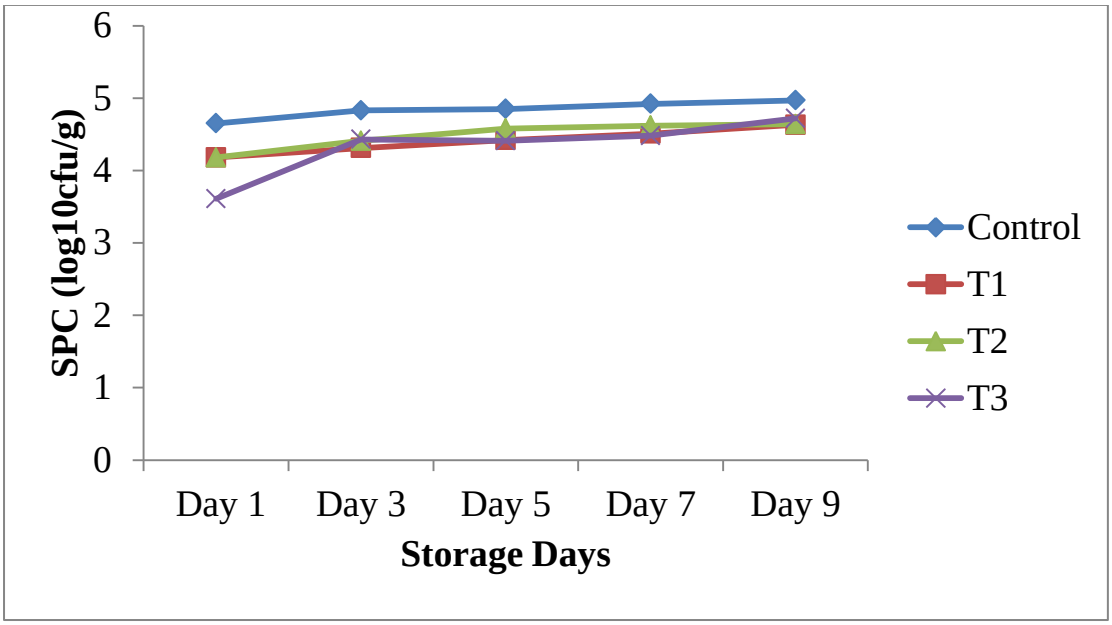


Fig 22: Changes in SPC (log_{10}cfu/g) of chicken meat emulsion during refrigeration storage at 4±1°C.

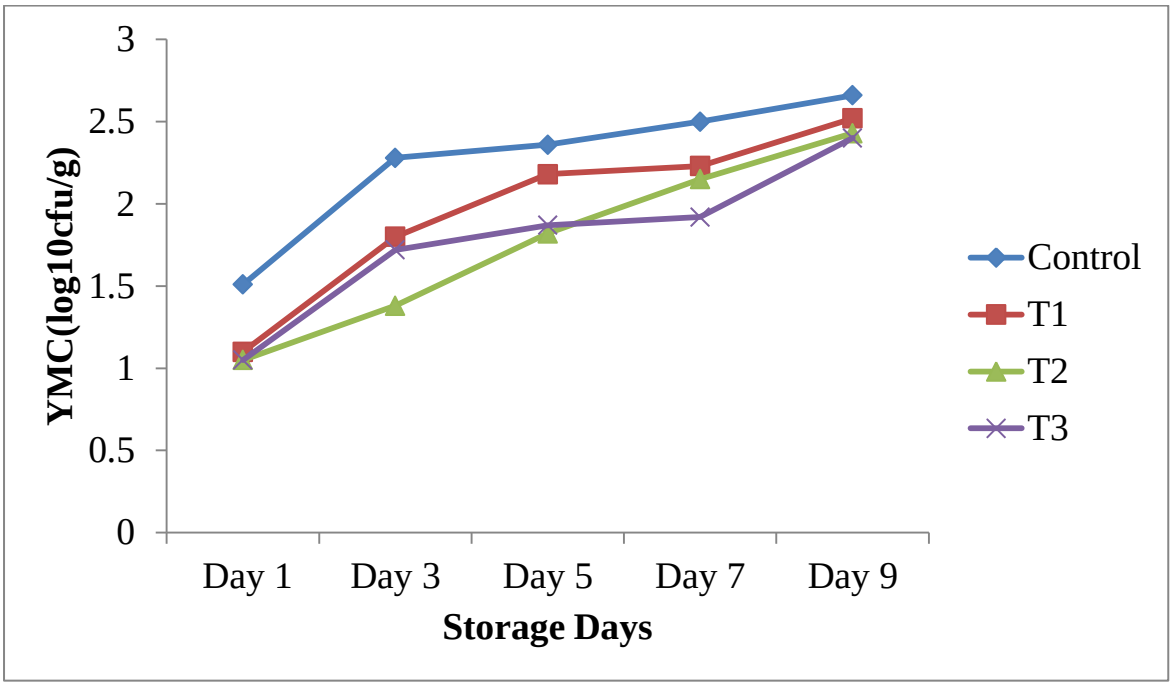


Fig 23: Changes in YMC (log_{10}cfu/g) of chicken meat emulsion during refrigeration storage at 4±1°C.

Control (without natural preservatives), T_1 (0.2% clove powder), T_2 (3% Ginger paste) and T_3 (2% Garlic paste).

4.4 Experiment No. 4: Storage stability of chicken meat caruncles incorporated with clove powder as a natural preservative with different packaging conditions at room temperature (35±2°C, 70% R.H).

Two batches i.e. Control and Treated (0.2% CP) were prepared following procedure as mentioned in experiment No. 2. The first group was packaged in Low density Polyethylene for aerobic packaging and second group was packaged in MAP laminated pouches using 50:50 CO_2/N_2 gas mixture. The latter batch was named as modified atmosphere packaged sample. Finally four different variants of CMC were prepared viz. Control aerobic (CA), Control modified (CMAP), treated aerobic (TA) and treated modified (TMAP) and were analyzed for different parameters.

4.4.1 Effect of clove powder and modified atmosphere packaging on oxidative stability of chicken meat caruncles.

Data pertaining to the mean values of pH, FFA %, PV, TBARS number, DPPH % inhibition and ABTS % inhibition are presented at Table 36. It was observed that pH of the product was significantly higher in CA (5.75) and significantly lower in TMAP (5.47). There was no significant difference between CMAP (5.66) and TA (5.62) samples in respect of pH at the beginning of the storage i.e. day 0. The highest value of pH was maintained in CA sample throughout the storage period whereas there was decrease in pH in the treated batches (TA and TMAP). The findings are very well in agreement with the report of Anna Anandh *et al* (2005) who observed progressive decrease in the pH of buffalo meat snacks (stored at 30±2°C for 30 days under aerobic packaging conditions) with increase in the storage period. Pexara *et al* (2002) also reported decrease in pH of cured turkey fillets packaged under vacuum and MAP (80% CO_2+ 20% N_2) conditions. Modi *et al* (2007) also observed a gradual

decrease ($P \leq 0.05$) in pH from 5.8 to 5.5 in dehydrated chicken kebab mix stored at (27±2°C) for 6 months under MAP conditions. The decrease in pH of product during storage might be due to the activity of acid producing bacteria (Raj 2002). At the end of storage it was observed that there was no significant change in pH of CMAP, TA and TMAP samples. Singh *et al* (2011) observed highly significant difference (P<0.01) in pH of chicken snacks on both day 0 and 6 and non-significant differences in pH on the last three days during the storage period of 30 days at 30±2°C under aerobic and vacuum packaging conditions. However, CA samples show marginal decrease in pH of product on day 60. While comparing the packaging mode, MAP of both control and treated samples show significant lower (P<0.05) pH than aerobic samples at beginning. This might be due to higher concentration of CO_2 in MAP samples which get absorbed into the product and formed carbonic acid as documented by Dixon and Kell (1989) in the storage study of dry cured hams. On subsequent days, similar trend was observed on day 10 for both control and treated samples and on day 30 only for control sample. Thereafter there was no significant difference of MAP till the end of storage period.

No significant change in FFA was noticed among the four products on day 0 and 10. On day 20, TMAP has significantly lower FFA %. On day 30 and 40, treated batches showed significantly lower (P<0.05) FFA than two control batches showing that CP could check the lipid degradation in the product. On day 40, both CMAP and TMAP had significantly lower (P<0.05) FFA as compared to their aerobic counterparts (CA and TA) showing that MAP was more effective over aerobic packaging to prevent the lipid changes during storage. On day 50, only TMAP sample had significantly lower FFA than CA sample and had marginally lower FFA as compared to CMAP and TA samples showing that there was a synergistic effect of MAP and CP. At the end of storage, TMAP also showed a marginally lower FFA than TA and CMAP and significantly lower (P<0.05) than CA sample. Modi *et al* (2007) reported that freshly prepared dehydrated chicken kebab mix had FFA values of 0.99 %, which gradually (P <

0.05) increased to 1.74 % during 6 months of MAP storage. Finally it can be suggested that CP and MAP treatment could greatly help in protecting the lipid changes during storage.

PV (Fig 24) of different variants of CMC at the beginning of the storage period showed a significantly lower ($P<0.05$) value in TA (0.27) and TMAP (0.20) than CA (0.93) and CMAP (0.47). There was no significant difference between TA and TMAP indicating that MAP could not show its efficacy on day 0 in treated samples. On subsequent storage intervals, the treated batches (TA and TMAP) had significantly lower PV or had marginally lower PV than their control counterparts (CA and

Table 36: Effect of clove powder and modified atmosphere packaging on oxidative stability of chicken meat caruncles stored at 35±2°C and 70% R.H.

Mean ± S.E

Tmts/ Days	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
				pH			
CA	5.75±0.01^{Ec}	5.71±0.02^{DEc}	5.62±0.01^{CDa}	5.53±0.01^{Bc}	5.54±0.04^{BCb}	5.47±0.06^{ABa}	5.39±0.03^{Ab}
CMAP	5.66±0.04^{Eb}	5.59±0.02^{DEb}	5.54±0.02^{CDa}	5.49±0.00^{Cbc}	5.52±0.01^{CDb}	5.39±0.05^{Ba}	5.30±0.02^{Aa}
TA	5.62±0.02^{Db}	5.64±0.03^{Dbc}	5.49±0.07^{Ca}	5.38±0.02^{Ba}	5.44±0.02^{BCa}	5.33±0.05^{ABa}	5.24±0.00^{Aa}
TMAP	5.47±0.02^{Ba}	5.50±0.04^{Ba}	5.48±0.05^{Ba}	5.43±0.04^{Bab}	5.49±0.03^{Bab}	5.39±0.05^{ABa}	5.29±0.02^{Aa}
				Free Fatty acids (%)			
CA	0.08±0.01^{Aa}	0.10±0.01^{Ba}	0.12±0.01^{BCc}	0.13±0.00^{CDb}	0.14±0.00^{Dd}	0.18±0.01^{Eb}	0.21±0.01^{Fb}
CMAP	0.07±0.01^{Aa}	0.09±0.01^{ABa}	0.10±0.01^{BCbc}	0.11±0.01^{CDb}	0.13±0.00^{Dc}	0.16±0.01^{Eab}	0.18±0.01^{Ea}
TA	0.06±0.01^{Aa}	0.08±0.01^{ABa}	0.09±0.01^{Bab}	0.09±0.01^{BCa}	0.11±0.00^{Cb}	0.16±0.01^{Dab}	0.17±0.01^{Da}
TMAP	0.06±0.01^{Aa}	0.08±0.01^{ABa}	0.08±0.01^{BCa}	0.09±0.01^{BCa}	0.10±0.00^{Ca}	0.14±0.01^{Da}	0.16±0.01^{Da}
				Peroxide value (meq/kg)			
CA	0.93±0.04^{Ac}	1.03±0.06^{ABc}	1.07±0.^{04ABc}	1.27±0.08^{Bc}	1.57±0.12^{Cc}	2.10±0.18^{Dc}	2.700±0.100^{Ec}
CMAP	0.47±0.04^{Ab}	0.60±0.05^{Ab}	0.80±0.07^{Bb}	0.87±0.04^{Bb}	1.03±0.03^{Cb}	1.33±0.07^{Db}	1.73±0.07^{Eb}
TA	0.27±0.04^{Aa}	0.47±0.04^{Bab}	0.53±0.04^{Ba}	0.73±0.04^{Cab}	0.87±0.04^{Cab}	1.10±0.05^{Dab}	1.33±0.07^{Ea}
TMAP	0.20±0.00^{Aa}	0.40±0.07^{Ba}	0.47±0.07^{Ba}	0.57±0.06^{Ba}	0.77±0.06^{Ca}	0.97±0.08^{Da}	1.27±0.04^{Ea}

Table 36: continued.....

Tmts/ Days	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
				TBARS number (mg MDA/kg)			
CA	0.89 ± 0.09^{Ab}	0.93 ± 0.07^{ABb}	0.99 ± 0.07^{ABb}	1.10 ± 0.05^{Bc}	1.29 ± 0.04^{Cc}	1.36 ± 0.06^{Cc}	1.44 ± 0.06^{Cc}
CMAP	0.84 ± 0.08^{Aab}	0.88 ± 0.06^{ABab}	0.92 ± 0.06^{ABab}	1.01 ± 0.06^{BCbc}	1.13 ± 0.03^{CDb}	1.21 ± 0.02^{DEb}	1.30 ± 0.01^{Eb}
TA	0.67 ± 0.09^{Aab}	0.79 ± 0.06^{ABab}	0.82 ± 0.06^{ABab}	0.86 ± 0.06^{Bab}	1.03 ± 0.02^{Cab}	1.11 ± 0.03^{CDab}	1.21 ± 0.03^{Dab}
TMAP	0.61 ± 0.10^{Aa}	0.70 ± 0.06^{ABa}	0.75 ± 0.05^{ABa}	0.79 ± 0.05^{Ba}	0.96 ± 0.03^{Ca}	1.03 ± 0.03^{CDa}	1.13 ± 0.03^{Da}
				DPPH (% inhibition)			
CA	22.39 ± 1.92^{Aa}	25.05 ± 3.88^{Aa}	18.28 ± 1.10^{Aa}	24.10 ± 2.40^{Aab}	19.50 ± 2.73^{Aab}	19.30 ± 1.49^{Aab}	20.12 ± 1.61^{Aa}
CMAP	21.27 ± 1.53^{Ba}	22.93 ± 3.83^{Ba}	15.15 ± 2.16^{ABa}	17.91 ± 3.04^{ABa}	11.67 ± 4.12^{Aa}	14.34 ± 2.84^{ABa}	16.86 ± 2.20^{ABa}
TA	28.46 ± 2.40^{Ab}	28.46 ± 4.48^{Aa}	20.42 ± 1.99^{Aab}	26.50 ± 2.30^{Aab}	23.21 ± 3.27^{Ab}	21.27 ± 1.78^{Ab}	21.89 ± 2.86^{Aa}
TMAP	32.14 ± 2.13^{Bb}	31.03 ± 3.62^{Ba}	24.29 ± 1.37^{ABb}	27.51 ± 3.24^{ABb}	20.96 ± 2.63^{Aab}	21.79 ± 2.13^{Ab}	22.24 ± 2.18^{Aa}
				ABTS (% inhibition)			
CA	56.93 ± 0.81^{Fa}	53.81 ± 1.36^{Ea}	52.36 ± 0.89^{DEa}	50.17 ± 0.64^{CDa}	48.17 ± 1.10^{BCa}	46.69 ± 0.94^{Ba}	43.05 ± 0.43^{Aa}
CMAP	71.43 ± 2.51^{Db}	67.93 ± 1.35^{CDb}	65.43 ± 0.77^{CDb}	62.69 ± 1.24^{BCb}	58.93 ± 3.01^{ABb}	56.41 ± 2.74^{ABb}	54.19 ± 2.35^{Ab}
TA	88.26 ± 0.51^{Ec}	83.17 ± 1.20^{Dc}	80.43 ± 1.03^{CDc}	78.48 ± 0.96^{BCc}	77.17 ± 0.70^{Bc}	74.17 ± 1.16^{Ac}	71.81 ± 0.96^{Ac}
TMAP	89.81 ± 0.62^{Ec}	86.88 ± 1.57^{DEc}	85.45 ± 1.29^{Dd}	84.29 ± 1.28^{CDd}	81.64 ± 0.86^{BCc}	79.00 ± 0.99^{ABd}	77.12 ± 1.33^{Ad}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$).
CA = Control aerobic, CMAP = Control modified atmosphere packaging (without preservative); TA = Treated aerobic and TMAP = Treated modified atmosphere packaging (0.2 % CP).

CMAP). This is due to the presence of clove powder which has prevented iron catalysed

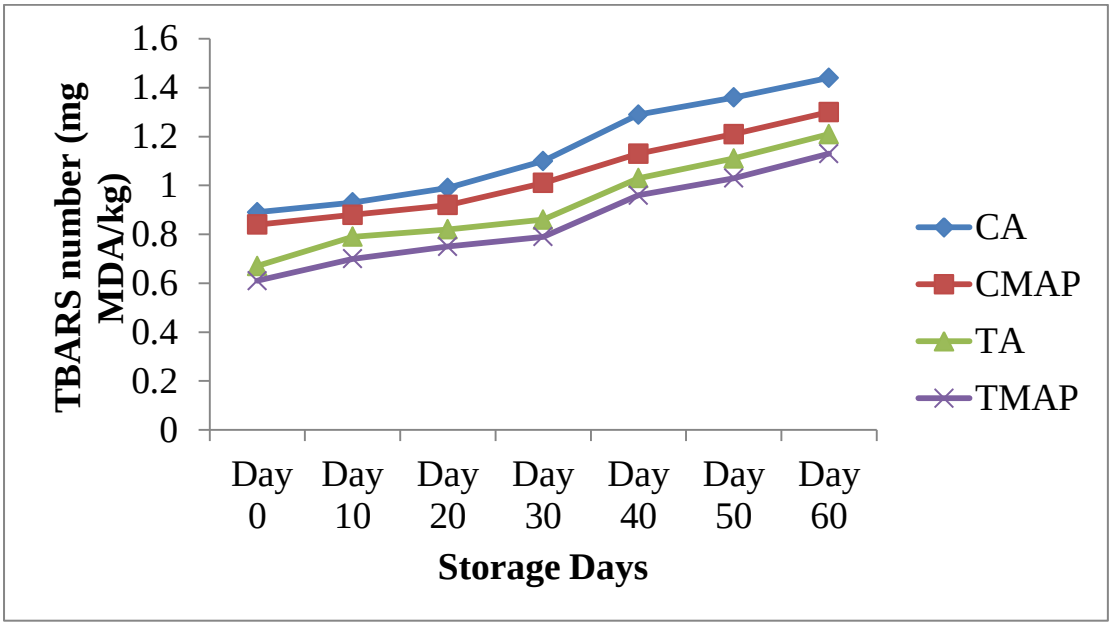


Fig 24: Changes in Peroxide value of chicken meat caruncles during storage at 35±2°C.

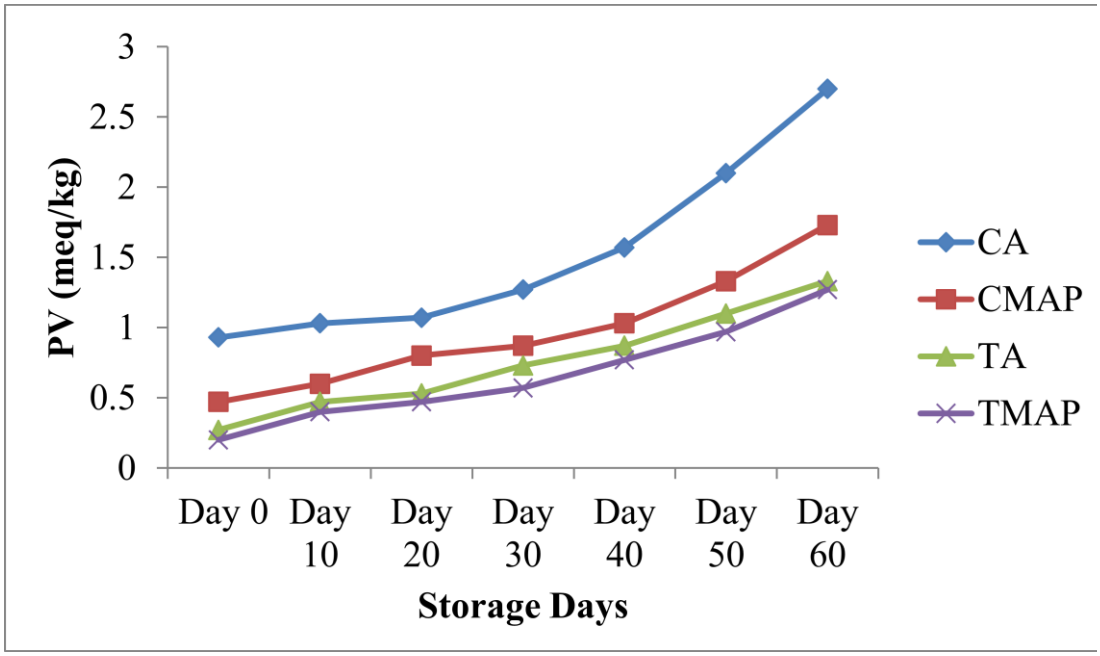


Fig 25: Changes inTBARS number of chicken meat caruncles during storage at 35±2°C.

CA = Control aerobic, CMAP = Control modified atmosphere packaging (without preservative); TA = Treated aerobic and TMAP = Treated modified atmosphere packaging (0.2 % CP).

oxidation in treated batches. PV of CA sample remained significantly higher throughout the storage till day 60 whereas the treated (TA) and MAP (CMAP and TMAP) had lower PV as compared to CA sample indicating that treatment of CP and MAP was quite effective in preventing lipid peroxidation of product. MAP (50%CO_2+50%N_2) gas mixture is also helpful in retarding peroxidation of fats by exclusion of oxygen from the pack. Hence comparatively lower PV values were observed in MAP samples than the aerobically packaged samples. The lowest PV was maintained by TMAP throughout the storage period indicating the synergistic effect of CP and MAP. At the end of storage, PV values reached to 2.70, 1.73, 1.33 and 1.27 from their initial values of 0.93, 0.47, 0.27 and 0.20 in case of CA, CMAP, TA and TMAP samples respectively. It was noticed that there was significant increase in PV of different variants of CMC irrespective of treatment and MAP as the storage period progressed. Rhee *et al* (1999b) also reported the steady increase in PV of meat extrudates stored at 37°C under aerobic conditions for 4 months. However, treatment with CP and MAP could be effective to a great extent in preventing lipid peroxidation as evidenced by lower PV in TA and TMAP samples.

TBARS (Fig 25) are formed as a by-product of lipid peroxidation. There was no significant difference in TBARS number among CMAP, TA and TMAP samples at the beginning of the storage. TBARS number of TMAP sample was significantly lower ($P<0.05$) than CA sample and was marginally lower than CMAP and TA sample on day 0. This indicated that CP and MAP treatment was very much effective from the beginning of storage to prevent lipid oxidation in the product. Similar trend was observed in treated samples (TA and TMAP) on subsequent storage intervals till the end of storage on day 60 whereas no significant difference was observed in TBARS number between CA and CMAP was found on day 30 but subsequently on day 40, 50 and 60, CMAP samples exhibited significantly lower TBARS number till the end of the storage. It seems that MAP is more effective in reducing TBARS number than aerobic packaging. Similar results were reported by Pastoriza *et al* (1996) in hake slices packaged in MAP (stored at 2±1°C) where shelf life was extended for

about three weeks without any loss in the sensory attributes. Cilla *et al* (2006) reported that the composition of the modified atmospheres significantly affected TBARS values after 60 and 120 days of refrigeration storage of ham slices. Moreover, as the storage period advanced TBARS number in all the batches

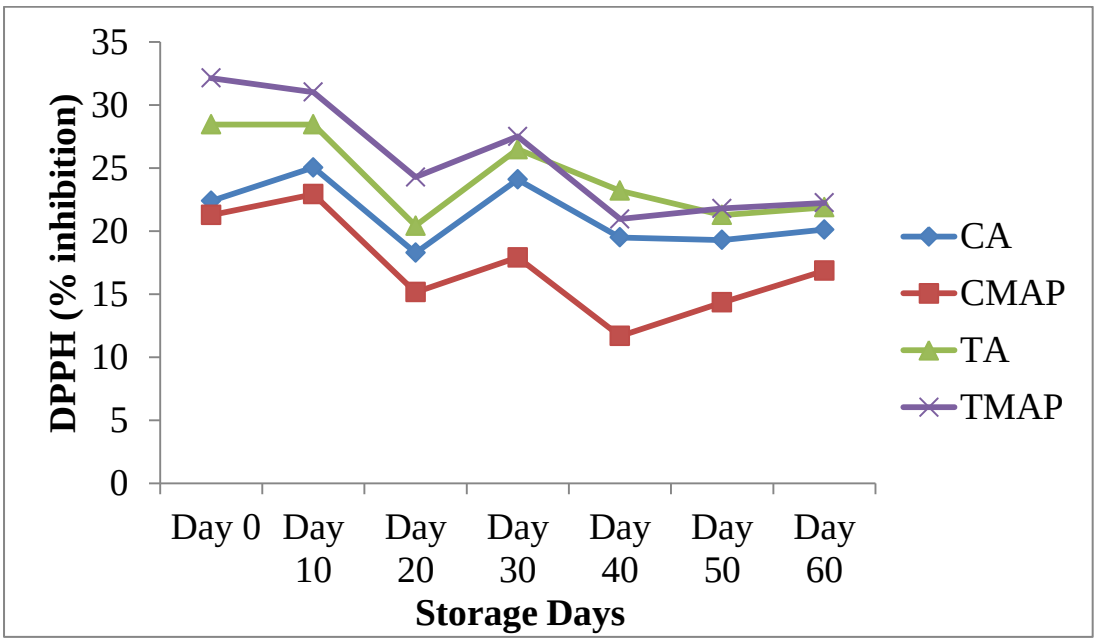


Fig 26: Changes in DPPH (% inhibition) of chicken meat caruncles during storage at 35±2°C.

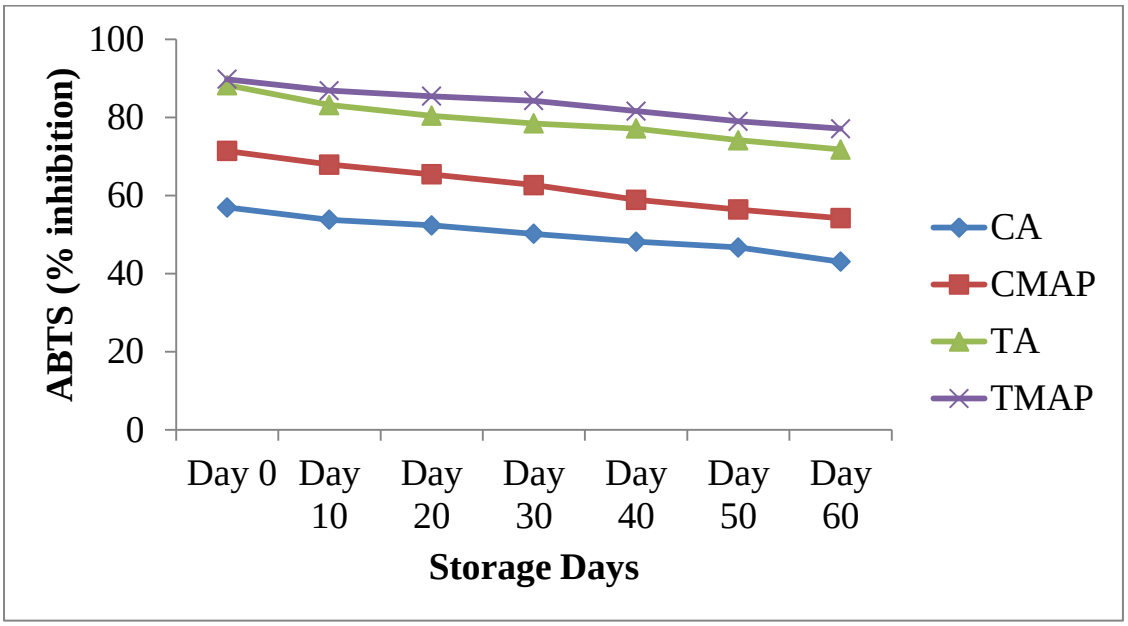


Fig 27: Changes in ABTS (% inhibition) of chicken meat caruncles during storage at 35±2°C.

CA = Control aerobic, CMAP = Control modified atmosphere packaging (without preservative); TA = Treated aerobic and TMAP = Treated modified atmosphere packaging (0.2 % CP).

increased marginally irrespective of type of packaging. This finding is very well in agreement with the study of Jin *et al* (2010) who revealed that TBARS values of dry cured pork (stored at 4°C for 90 days under vacuum, 100%N_2 and MAP: 20%CO_2 + 80%N_2) increased significantly ($P<0.001$) in all the packaging systems at 60 and 90 days of storage. Singh *et al* (2011) also reported that TBA value of chicken snacks (stored at 30±2°C for 30 days under aerobic and vacuum packaging) initially decreased up to the 12th and 18th day and thereafter increased. Park *et al* (1993) also reported that there were no consistent or marked differences in TBA values of beef snacks when stored for 15-210 days. From the observations as mentioned above it is very much clear that MAP was very much effective in both control and treated samples against oxidation problems of the product.

There was no significant variation in DPPH % inhibition (Fig 26) between TA and TMAP as well as between CA and CMAP samples whereas both the treated batches were having significantly higher ($P<0.05$) DPPH % inhibition than the control samples. This indicated that there was good effect of CP and MAP packaging to scavenge the free radicals even at the beginning of the storage on day 0. All through the storage period TA and TMAP had significantly higher ($P<0.05$) or marginally higher DPPH % inhibition as compared to control counterparts (CA and CMAP). Study of MAP showed that it was effective both in control and in treated samples to protect the food product against lipid changes during storage. This is because in all storage intervals both CMAP and TMAP showed higher or marginally higher DPPH % inhibition than CA and TA samples. Further it was noticed that combination of natural preservative (CP) and MAP produced synergistic effect as evidenced by significantly higher or marginally higher DPPH % inhibition in TMAP samples as compared to other variants (CA, CMAP and TA). Kong *et al* (2010) observed that clove extract was having the highest DPPH activity among other five spice extracts and their activity decreased in order; clove > cassia bark > nutmeg > rosemary > liquorice > round cardamom having activity of 77.5%, 63.5%, 54.8%, 48.3%, 37.2% and 29.2% respectively.

The ABTS % inhibition (Fig 27) was significantly higher ($P<0.05$) in TA (88.26) and TMAP (89.81) as compared to CA (56.93) and CMAP (71.43). The MAP sample of both control and treated batches showed higher ABTS % inhibition showing that MAP was effective in scavenging the free radicals even at very beginning of the storage. Similar trend was maintained on subsequent storage intervals. The treated CMC showed better results in terms of ABTS % inhibition than both the control batches (CA and CMAP). This indicated that CP is very much effective to scavenge the free radicals during the storage period of 60 days at room temperature. All through the storage period TMAP samples showed either marginally or significantly higher ABTS % inhibition than the TA sample indicating that there is synergistic effect of CP and MAP in terms of scavenging the free radicals produced during storage. In general it was observed that there was a significant decrease in ABTS % inhibition at the end of storage on day 60 as compared to beginning of storage i.e. day 0.

4.4.2 Effect of clove powder and modified atmosphere packaging on microbiological quality of chicken meat caruncles.

Different microbiological parameters such as SPC, *S. aureus*, Coliform count and Yeast and mold count were determined in the four variants (CA, CMAP, TA and TMAP) of CMC during storage period of 60 days at room temperature of 35±2°C and 70% R.H. Coliform count, *S. aureus* and Yeast and mold count were not detected in any of the test sample throughout the storage period of 60 days. Data pertaining to SPC during the above storage period of different CMC are presented at Table 37. It was found that there was a significant lower ($P<0.05$) SPC in TA ($\log_{10}$ 4.00) and TMAP ($\log_{10}$ 4.00) than their control counterparts CA ($\log_{10}$ 4.36) and CMAP ($\log_{10}$ 4.39) at the beginning of the storage on day 0. The above observation indicated that CP and MAP treatment of product was quite effective from the beginning of storage in decreasing the microbial load of CMC. Similar trend was observed on

subsequent storage intervals of day 10, 30, 40, 50 and 60 in TA and TMAP samples as compared to control samples. While on day 20, no significant variation was found between CA (log_{10} 4.56) and CMAP (log_{10} 4.58) samples while TMAP (log_{10} 4.00) had a significantly lower ($P<0.05$)

Table 37: Effect of clove powder and modified atmosphere packaging on microbiological quality of chicken meat caruncles stored at 35±2°C and 70% R.H. **Mean ± S.E**

Tmts/ Days	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
				Standard Plate Count (log_{10}cfu/g)			
CA	4.36 ± 0.04^{Ab}	4.50 ± 0.02^{Bb}	4.56 ± 0.03^{BCc}	4.62 ± 0.02^{CDb}	4.68 ± 0.03^{DEb}	4.72 ± 0.03^{EFb}	4.78 ± 0.03^{Fb}
CMAP	4.39 ± 0.04^{Ab}	4.49 ± 0.05^{Bb}	4.58 ± 0.04^{BCc}	4.61 ± 0.03^{CDb}	4.65 ± 0.02^{CDEb}	4.70 ± 0.02^{DEb}	4.74 ± 0.02^{Eb}
TA	4.00 ± 0.00^{Aa}	4.10 ± 0.06^{ABa}	4.15 ± 0.07^{ABb}	4.15 ± 0.07^{ABa}	4.25 ± 0.05^{BCa}	4.36 ± 0.04^{CDa}	4.43 ± 0.06^{Da}
TMAP	4.00 ± 0.00^{Aa}	4.00 ± 0.00^{Aa}	4.00 ± 0.00^{Aa}	4.10 ± 0.06^{Aa}	4.25 ± 0.05^{Ba}	4.33 ± 0.03^{BCa}	4.41 ± 0.05^{Ca}

Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$). CA = Control aerobic, CMAP = Control modified atmosphere packaging, TA = Treated aerobic and TMAP = Treated modified atmosphere packaging.

SPC than TA (log_{10} 4.15). In general it was observed that there was increase in SPC as storage period progressed in all the control and treated samples reaching to log_{10} 4.78, log_{10} 4.74, log_{10} 4.43 and log_{10} 4.41 from the initial values of log_{10} 4.36, log_{10} 4.39, log_{10} 4.00 and log_{10} 4.00 in case of CA, CMAP, TA and TMAP samples respectively. Low microbial load in MAP samples may be due to presence of CO_2 in the pack which acts as bacteriostatic. Wongwicharn *et al* (2009) also observed that in roasted chicken (stored at 4±1°C under aerobic and MAP conditions) Coliform count, *S. aureus* and Yeast and mold count were not detected. MAP was effective for inhibiting growth of total viable counts than the aerobic samples. Low bacterial count in CP treated samples has already been documented.

4.4.3 Effect of clove powder and modified atmosphere packaging on sensory attributes of chicken meat caruncles.

Data pertaining to different sensory attributes of CMC viz. colour, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability are presented in Table 38. The colour or appearance were significantly higher ($P<0.05$) in TA batch (7.62) than CMAP (7.33) and TMAP (7.31) but did not significantly vary from CA (7.48) sample at the beginning of storage on day 0. The effect of CP in improving the colour was evidenced even at the beginning of storage but on subsequent storage intervals colour scores did not significantly vary among control and treated batches. Starting from day 10 to day 60, CP treated (TA) showed a marginally lower colour scores as compared to its control counterpart (CA) showing that CP treatment could not signify change in improving the colour and appearance of the product. The TMAP sample had marginally higher colour scores as compared to CMAP sample from day 30 onwards till end of the storage. This speaks that MAP improved the colour and appearance of CMC to some extent. This observation is very well in agreement with the findings of Gok *et al* (2008) who concluded that pastrimas (stored at 4°C for 120 days) packaged by MAP (35% CO_2 + 65% N_2) were given higher sensory ratings and also preserved color better than those packaged under vacuum or aerobic conditions. Also they concluded that as the storage time increased, color scores declined with the lowest scores observed on day 120. The treated CMC (TA and TMAP) remained good to very good and the two controls (CA and CMAP) remained fair to good at the end of the storage period.

Table 38: Effect of clove powder and modified atmosphere packaging on sensory attributes of chicken meat caruncles stored at 35±2°C and 70% R.H.

Mean ± S.E

Tmts/ Days	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
				Colour/Appearance			
CA	7.48±0.10^{Eab}	7.10±0.07^{Da}	6.76±0.10^{Ca}	6.71±0.09^{Ca}	6.57±0.09^{Ca}	6.19±0.13^{Ba}	5.88±0.08^{Aa}
CMAP	7.33±0.09^{Ea}	7.02±0.07^{Da}	6.71±0.10^{Ca}	6.67±0.10^{Ca}	6.50±0.10^{BCa}	6.24±0.12^{Ba}	5.93±0.09^{Aa}
TA	7.62±0.10^{Db}	7.14±0.13^{Ca}	6.74±0.10^{Ba}	6.69±0.10^{Ba}	6.50±0.09^{Ba}	6.07±0.13^{Aa}	6.02±0.09^{Aa}
TMAP	7.31±0.09^{Da}	7.12±0.07^{Da}	6.52±0.10^{BCa}	6.76±0.08^{Ca}	6.62±0.09^{Ca}	6.29±0.13^{ABa}	6.04±0.08^{Aa}
				Flavour			
CA	7.19±0.07^{Ca}	7.07±0.06^{Ca}	6.62±0.13^{Ba}	6.64±0.06^{Ba}	6.67±0.09^{Ba}	6.26±0.13^{Aa}	6.02±0.06^{Aa}
CMAP	7.26±0.07^{Dab}	7.00±0.08^{Ca}	6.62±0.13^{Ba}	6.52±0.05^{Ba}	6.45±0.10^{Ba}	6.36±0.12^{Ba}	6.07±0.08^{Aa}
TA	7.48±0.09^{Db}	7.33±0.07^{Db}	6.62±0.13^{Ca}	6.50±0.06^{BCa}	6.62±0.09^{Ca}	6.24±0.13^{ABa}	6.12±0.08^{Aa}
TMAP	7.33±0.08^{Dab}	7.17±0.06^{Dab}	6.69±0.12^{Ca}	6.52±0.06^{BCa}	6.41±0.09^{ABa}	6.26±0.12^{ABa}	6.14±0.09^{Aa}
				Crispiness			
CA	7.24±0.06^{Eab}	7.05±0.07^{DEa}	6.93±0.11^{CDa}	6.69±0.09^{BCa}	6.55±0.10^{Ba}	6.50±0.11^{Ba}	6.00±0.09^{Aa}
CMAP	7.31±0.05^{Dab}	7.10±0.07^{Da}	6.81±0.12^{Ca}	6.64±0.10^{BCa}	6.50±0.09^{Ba}	6.55±0.10^{BCa}	6.10±0.08^{Aa}
TA	7.19±0.05^{Fa}	7.02±0.07^{EFa}	6.86±0.12^{DEa}	6.71±0.08^{CDa}	6.57±0.08^{BCa}	6.33±0.11^{ABa}	6.12±0.08^{Aa}
TMAP	7.38±0.06^{Eb}	6.98±0.06^{Da}	7.10±0.10^{Da}	6.74±0.08^{Ca}	6.50±0.09^{Ba}	6.31±0.10^{ABa}	6.14±0.09^{Aa}

Table 38: continued……..

Tmts/ Days	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
				After-taste			
CA	7.19±0.12^{Ca}	7.14±0.07^{Ca}	6.57±0.10^{Ba}	6.48±0.09^{Ba}	6.52±0.09^{Ba}	6.29±0.14^{ABa}	6.12±0.07^{Aa}
CMAP	7.26±0.07^{Ea}	7.07±0.09^{Ea}	6.76±0.09^{Da}	6.45±0.10^{BCa}	6.52±0.09^{CDa}	6.24±0.13^{ABa}	6.14±0.08^{Aa}
TA	7.12±0.11^{Ca}	7.02±0.07^{Ca}	6.62±0.10^{Ba}	6.45±0.10^{ABa}	6.48±0.09^{ABa}	6.31±0.14^{Aa}	6.21±0.07^{Aa}
TMAP	7.24±0.11^{Ca}	7.07±0.06^{Ca}	6.50±0.10^{ABa}	6.62±0.10^{Ba}	6.57±0.08^{Ba}	6.38±0.13^{ABa}	6.21±0.08^{Aa}
				Meat flavour intensity			
CA	7.36±0.07^{Da}	6.93±0.07^{Ca}	6.98±0.09^{Ca}	6.74±0.09^{BCa}	6.60±0.10^{Ba}	6.19±0.12^{Aa}	6.12±0.08^{Aa}
CMAP	7.33±0.06^{Ea}	7.05±0.07^{Da}	6.98±0.09^{Da}	6.71±0.10^{Ca}	6.48±0.10^{BCa}	6.26±0.12^{ABa}	6.17±0.07^{Aa}
TA	7.36±0.06^{Ea}	7.10±0.06^{Da}	7.02±0.06^{Da}	6.69±0.09^{Ca}	6.50±0.10^{BCa}	6.29±0.11^{ABa}	6.17±0.08^{Aa}
TMAP	7.33±0.07^{Da}	7.07±0.06^{Ca}	6.86±0.10B^{Ca}	6.67±0.09^{Ba}	6.41±0.10^{Aa}	6.33±0.12^{Aa}	6.19±0.08^{Aa}
				Overall acceptability			
CA	7.29±0.07^{Fa}	7.17±0.06^{EFa}	6.98±0.10^{DEa}	6.79±0.06^{CDa}	6.60±0.10^{BCa}	6.45±0.10^{Ba}	6.19±0.08^{Aa}
CMAP	7.33±0.08^{Da}	7.19±0.06^{CDa}	6.98±0.11^{Ca}	6.69±0.06^{Ba}	6.41±0.10^{Aa}	6.45±0.11^{ABa}	6.26±0.06^{Aa}
TA	7.45±0.08^{Ea}	7.24±0.10^{Ea}	6.98±0.10^{Da}	6.69±0.06^{Ca}	6.52±0.10^{BCa}	6.43±0.11^{ABa}	6.26±0.06^{Aa}
TMAP	7.29±0.07^{Ca}	7.26±0.07^{Ca}	7.05±0.10^{Ca}	6.71±0.07^{Ba}	6.38±0.09^{Aa}	6.41±0.11^{Aa}	6.29±0.06^{Aa}

The sensory attributes were based on 8- point hedonic scale. Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$). CA = Control aerobic, CMAP = Control modified atmosphere packaging, TA = Treated aerobic and TMAP = Treated modified atmosphere packaging.

Flavour score of TA sample (7.48) was significantly higher ($P<0.05$) than CA sample but did not vary significantly from CMAP and TMAP batches. This indicates that CP treatment induced better flavor even at the beginning of storage on day 0 whereas MAP did not show its effect in improving flavour score on the same day. There was no significant difference in flavor score among the two control and two treated batches on subsequent storage intervals till the end of storage period. However, at the end of storage period (day 60) TA (6.12) and TMAP (6.14) samples showed a marginally higher flavour score as compared to their control counterparts i.e. CA (6.02) and CMAP (6.07). This indicates that natural preservative CP improved the flavour score to some extent and MAP further marginally improved flavour score. The finding is again in support of the fact given by Gok *et al* (2008) who reported that with increase in the storage period of pastrimas (as described above), taste scores decreased and became lowest on day 120. Ray *et al* (1993) also reported that frozen turkey strips stored in MAP up to 84 days had highest sensory scores in respect of aroma, appearance and structure.

Crispiness of CMC showed highest sensory scores in TMAP batches on day 0 but it was not significantly varied from CA and CMAP samples. While the crispiness score of TMAP was significantly higher ($P<0.05$) than TA showing that MAP was effective in improving the crispiness even at beginning of the storage. On day 10 to 60 crispiness score did not significantly vary among CA, CMAP, TA and TMAP samples. But on day 60, crispiness score was marginally higher in TA (6.12) and TMAP (6.14) samples as compared to CA (6.00) and CMAP (6.10). All the CMC samples were good to very good with respect to crispiness at the end of storage period.

After-taste score of four different variants of CMC did not significantly vary among themselves on day 0 and it ranged from 7.12 to 7.26 on 8 point descriptive scale. On subsequent storage days, similar trend was observed showing no significant change in after-taste score due to the treatment of CP as natural preservative and MAP. However, towards the end of storage on day 50 and 60, two treated (TA and TMAP) showed marginally higher

flavour scores than control counterparts (CA and CMAP). All the samples were maintained at good to very good at the end of storage period. In general, after-taste scores decreased as the storage period advanced in all the CMC samples.

Meat flavour intensity did not show any significant change due to CP and MAP from the beginning of the storage till the end. In general meat flavour intensity decreased in all the samples as the storage period progressed. However, the meat flavour intensity remained marginally higher in TMAP samples on day 50 and 60 as compared to CA, CMAP and TA. This indicates that CP and MAP were helpful in enhancing the meat flavour intensity to some extent during storage. All the CMC samples were good to very good even after two months of storage period.

The overall acceptability scores ranged from 7.29-7.45 between four different batches without any significant variation among them. On subsequent storage till day 60 no significant variation in overall acceptability scores were noticed among two control and two treated batches of CMC. However, at the end of storage on day 60, TAMP showed marginally higher overall acceptability scores as compared to other variants indicating that MAP was useful in improving the overall acceptability scores of product to some extent. In general overall acceptability scores decreased as the storage period advanced irrespective of CP and MAP treatment. Gok *et al* (2008) also showed similar decreasing trends of overall acceptability of pastrimas packaged under MAP conditions (as described above) with increase in the storage period.

With the advancement of storage period, all the sensory attributes namely colour, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability decreased irrespective of the type of product. The statement is strongly supported by the findings of Singh *et al* (2011) who reported that sensory attributes of chicken snacks showed insignificant decreasing trend during the whole of the storage period of 30 days irrespective of the type of product. Jin *et al* (2010) observed that sensory scores of dry cured pork (stored at 4°C for 90 days under vacuum packaging, 100% N_2 packaging and MAP: 25%CO_2 +

75%N_2) reduced significantly at day 60 and 90. Sharma and Nanda (2002) also stated that sensory scores of chicken chips (stored under nitrogen atmosphere) showed a progressive decrease during the storage period of 12 weeks. Kalra *et al* (1987) also observed similar trend in sensory scores of colour and texture of snacks during storage at room temperature for a period of 6 months.

Chapter-V

SUMMARY AND CONCLUSIONS

Snack food industry is rapidly growing all over the world and its increasing trend is not only because of convenient nature but also its ability to satisfy short term hunger. Very few experimental studies have been conducted on the process of development of shelf stable meat based snacks. Incorporation of meat in cereal snacks improves nutritive value and sensory attributes. Spent hen meat is a good source of proteins and omega-3 fatty acids. Hence the development of health oriented functional meat based snacks from spent hen meat is important. To improve crispiness and to inhibit lipid oxidation and growth of yeast and mold various flours and starches such as rice flour, tapioca starch and potato starch; natural preservatives such as clove powder, ginger and garlic and different packaging conditions seem to have an important effect on shelf life. In view of all these aspects, an attempt was made in the present study to develop health oriented chicken meat caruncles with longer shelf life at room temperature. Deboned spent hen meat obtained from the White Leghorn spent hen carcasses following standard slaughtering procedure, were used in the development of chicken meat caruncles.

5.1 Experiment No.1: Standardization of the formulation and processing conditions for the development of chicken meat caruncles.

The experiments were conducted as designed by using Box-Behnken design of RSM including factors as meat level (%), oil level (%) and microwave cooking time (mins). Under this experiment 17 runs were conducted using three meat levels (60, 65 and 70%), three oil levels (2.5%. 5.0% and 7.5%) and three cooking times (3, 4 and 5 mins) as variables. Data pertaining to each run along with different physico-chemical parameters (texture profiles, colour profiles, moisture, cooking yield and

water activity) and sensory characteristics (appearance, flavour, crispiness, after-taste, meat flavour intensity and overall acceptability) were analyzed by model fitting using second order polynomial. After fitting the equation several targets of response were given through the software for achieving the best combination of variables which resulted in required product. The final product selected was with 65% meat level, 5% oil level and cooking time 4 min.

5.2 Experiment No.2: Optimization of the level of rice flour, tapioca starch and potato starch in chicken meat caruncles.

5.2.1 Optimization of level of rice flour in chicken meat caruncles

For RF batch, Four different batches of CMC were prepared viz. control (without RF), T_1 = 22.75% RWF + 12.25% RF; T_2 = 17.50% RWF + 17.50% RF and T_3 = 12.25% RWF + 22.75% RF. It was observed that there was no significant difference ($P>0.05$) of emulsion stability % and CY % among the control and treated CMC. There was a significant decrease ($P<0.05$) in pH of CMC in treated sample but it did not significantly vary among T_1, T_2 and T_3 samples. There was marginal decrease in pH as the rice flour content was increased in the product formulationThe higher a_w in the treated CMC might be due to more absorption of water into the rice flour. The hydratability significantly ($P<0.05$) decreased in T_1, T_2 and T_3 samples (0.96-1.27) as compared to control batch (1.63). The moisture % of control sample (4.96) showed significantly lower ($P<0.05$) value whereas it was significantly higher in T_3 batch (5.79). It seems as the rice flour content was increased in product, moisture % also increased. The protein % was significantly lower ($P<0.05$) in T_1 and T_3 batch as compared to control CMC. The fat % did not significantly vary between control and T_2 or between T_1 and T_3 batches. Crude fiber % was found to be significantly lower ($P<0.05$) in T_1 and T_2 samples as compared to T_3 CMC. The ash % was significantly higher in T_2 batch (4.15) as compared to T_1 and T_3 batches. In texture profile, hardness was significantly increased ($P<0.05$) in T_2 (84.33) as

compared to control (61.39) and T_1 (66.67) while it did not significantly vary from T_3 sample (70.69). The *L* value of control and T_3 batches did not vary between them. The *L* value of T_1 (42.16) and T_2 (41.79) were found to be significantly higher ($P<0.05$) as

compared to control (36.81) and T_3 (38.25) batches. The *b* value was significantly higher in T_1 batch (26.13) than only control batch (24.31) whereas it did not significantly vary between T_2 (25.31) and T_3 (24.98) samples. Among the treated groups, T_1 batch got higher scores for almost all the attributes so it was considered most acceptable.

5.2.2 Optimization of level of tapioca starch in chicken meat caruncles

Four different batches of CMC were prepared viz. control (without TS), T_1 = 17.50% RWF + 17.50% TS; T_2 = 14.00% RWF + 21.00% TS and T_3 = 10.50% RWF + 24.50% TS. There was no significant difference ($P>0.05$) in emulsion stability % of control and treated samples. The cooking yield % was significantly higher ($P<0.05$) in T_2 batch (55.68) than control (53.50). There was a continuous decrease in the value of hydratability as the content of tapioca starch was increased in the formulation. WAI of control samples was significantly lower ($P<0.05$) than the treated samples. The fat % of T_1 (12.08) was significantly higher ($P<0.05$) than T_2 (9.17) and T_3 (9.83) batch. There was a marginal increase in fiber % and decrease in ash % as the content of tapioca starch increased in the formulation. In texture profile, there was no significant variation between hardness of control, T_1 and T_3 samples. Among the treated groups, there was no significant variation between T_1, T_2 and T_3 samples but there was a continuous increase in the adhesiveness as the content of tapioca starch increased in the formulation. In colour profile, the *L* and *a* value of control batch was significantly lower ($P<0.05$) than T_1, T_2 and T_3 samples. Among the treated groups T_2 got marginally higher scores than T_1 and T_3 groups, so it was considered most acceptable.

5.2.3 Optimization of level of potato starch in chicken meat caruncles

Four different batches of CMC were prepared viz. control (without PS), T_1 = 14.00% RWF + 21.00% PS; T_2 = 7.00% RWF + 28.00% PS and T_3 = 35.00% PS. There was no significant variation of emulsion stability %, CY %, pH, Hydratability and WSI between control and treated groups. The a_w of control group was significantly higher ($P<0.05$) than the treated groups but it was equal in all the treated batches. WAI of control samples was

significantly lower ($P<0.05$) than the treated samples. There was no significant variation of moisture %, protein %, fat %, fiber %, carbohydrates % and moisture: protein ratio between T_1, T_2 and T_3 samples. The ash % was significantly higher ($P<0.05$) in T_2 batch (4.57) than control (3.51), T_1 (2.98) and T_3 (2.23). In texture profile, hardness of control batch (63.78) was significantly lower ($P<0.05$) than T_1 (97.07), T_2 (100.55) and T_3 (119.27) batches. There was a marginal decrease in the *a* value and chroma of treated CMC as the content of PS increased in the formulation. Among the treated groups, the scores for T_3 group were comparatively higher than for T_1 and T_2. Hence T_3 was considered most acceptable.

5.3 Experiment No.3: Comparative study of natural preservatives on the quality characteristics of chicken meat emulsion.

From comparative study between control (100% RWF), RF (35% of RWF), TS (60% of RWF) and PS (100% of RWF), it was known that product with TS (60% of RWF) was most acceptable. TS (60% of RWF) acted as control in this experiment. Four different batches of CME i.e. control (without natural preservative), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP were prepared and stored for 9 days at refrigeration temperature of 4±1°C to evaluate the quality changes in CME. During the storage period, control and CP treated samples could maintain the pH of CME without significant change till the end of the storage. On day 3, 5, 7 and 9, there was

no significant change in titrable acidity among control and three treated CME samples. This indicates that natural preservatives did not affect the titrable acidity of CME. ERV did not vary significantly and ERV decreased as the storage period increased in all CME samples. On all the storage days, FFA was significantly higher in control as compared to treated CME batches. Peroxide value of control sample remained significantly higher on day 5, 7 and 9 and it increased in all the samples as the storage period progressed increased. Clove powder (T_1) maintained lowest TBARS number in all the storage intervals till the end of the storage among the natural preservatives tried. DPPH % inhibition did not significantly vary among control and treated CME batches. On day 1, ABTS %

inhibition was significantly higher in T_1 batch as compared to other treated and control batches. CP is potentially much better than ginger and garlic in scavenging the free radicals. In colour profile, there was no significant difference in *L* value between control, T_1 and T_3 batches at the beginning of the storage. On day 5, highest *b* value was obtained in control sample and lowest value was in T_1 batch. It was significantly lower ($P<0.05$) than control, T_2 and T_3 batches. This indicates that CP is preferred preservative among the natural preservatives used. There was no significant change in metmyoglobin % among control, T_1, T_2 and T_3 at the beginning of the storage but on day 7, T_1 showed lowest metmyoglobin % which was also significantly lower ($P<0.05$) than control, T_2 and T_3. As the storage period progressed, colour and odour scores decreased in all the CME batches. In microbiological profile, the SPC was marginally higher in control than treated batches and at the end of the storage T_1 showed the lowest microbial load ($\log_{10}$ 4.63). Coliform count showed no significant variation among control and treated batches at the beginning of the storage with marginally higher microbial load in control batch. *S. aureus* was not detected in

any of the CME batch throughout the storage period of 9 days. Yeast and mold count did not show any significant difference among different batches.

5.4 Experiment No. 4: Storage stability of chicken meat caruncles incorporated with clove powder as a natural preservative with different packaging conditions at room temperature (35±2°C, 70% R.H).

Two batches i.e. Control and Treated (0.2% CP) were prepared and packaged in Low density Polyethylene for aerobic packaging and two layered laminated pouches using 50:50 CO_2/N_2 gas mixture. Finally four different variants of CMC were prepared viz. Control aerobic (CA), Control modified (CMAP), treated aerobic (TA) and treated modified (TMAP). It was observed that pH of the product was significantly higher in CA (5.75) and significantly lower in TMAP (5.47) and no significant difference between CMAP (5.66) and TA (5.62) samples at the beginning of the storage i.e. day 0. No significant change in FFA was noticed

among the four products on day 0 and 10. PV of different variants of CMC at the beginning of the storage period showed a significantly lower ($P<0.05$) value in TA and TMAP than CA and CMAP. TBARS number of TMAP sample was significantly lower ($P<0.05$) than CA sample and was marginally lower than CMAP and TA sample on day 0. There was no significant variation in DPPH % inhibition between TA and TMAP as well as between CA and CMAP samples whereas both the treated batches were having significantly higher ($P<0.05$) DPPH % inhibition than the control samples. The MAP sample of both control and treated batches showed higher ABTS % inhibition showing that MAP was effective in scavenging the free radicals even at very beginning of the storage. Coliform count, *S. aureus* and Yeast and mold count were not detected in any of the test sample throughout the storage period of 60 days. It was found that there was a significant lower ($P<0.05$) SPC in TA ($\log_{10}$ 4.00) and

TMAP (log_{10} 4.00) than their control counterparts CA (log_{10} 4.36) and CMAP (log_{10} 4.39) at the beginning of the storage on day 0 and SPC increased in all batches as storage period progressed. The effect of CP in improving the colour was evidenced even at the beginning of storage but it did not vary significantly thereafter. Flavour score of TA sample (7.48) was significantly higher ($P<0.05$) than CA sample but did not vary significantly from CMAP and TMAP batches. Crispiness of CMC showed highest sensory scores in TMAP batches on day 0, but it was not significantly varied from CA and CMAP samples. While the crispiness score of TMAP was significantly higher ($P<0.05$) than TA showing that MAP was effective in improving the crispiness even at beginning of the storage. After-taste score of four different variants of CMC did not significantly vary among themselves on day 0 and it ranged from 7.12 to 7.26 on 8 point descriptive scale. MFI remained marginally higher in TMAP samples on day 50 and 60 as compared to CA, CMAP and TA. However, at the end of storage on day 60, TAMP showed marginally higher overall acceptability scores as compared to other variants indicating that MAP was useful in improving the MFI and OA scores of product to some extent.

Conclusions

1. The final product selected was with 65% meat level, 5% oil level and cooking time 4 mins in the first experiment.

2. Optimum level of incorporation of rice flour (35%), tapioca starch (60%) and potato starch (100%) replacing refined wheat flour was standardized for the formulation of CMC.

3. Among natural preservatives such as clove powder (0.2%), ginger paste (3%) and garlic paste (2%) used in CMC, clove powder (0.2%) was selected as most appropriate for the development of CME.

4. Final product along with natural preservative (0.2% clove powder) and MAP greatly enhanced the shelf life of CMC and maintained freshness of quality better than their control counterparts up to a storage period of 60 days at room temperature.

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APPENDIX - I

DEPARTMENT OF LIVESTOCK PRODUCTS TECHNOLOGY
COVS, GADVASU, LUDHIANA

Name: _____

Expt. No. :_____

Products: _____ **Scoring guide**

Date: _____

Scale of descriptive attribute of product

Attributes	8	7	6	5	4	3	2	1
Colour / Appearance	Excellent	Very Good	Good	Fair	Slightly poor	Moderately poor	Very poor	Extremely poor
Flavour	Extremely Slightly desirable	Very Moderately desirable	Moderately Very desirable	Slightly Extremely desirable	undesirable	undesirable	undesirable	undesirable
Crispiness	Extremely Slightly crispy	Very Moderately crispy	Moderately Very crispy	Slightly Extremely crispy	soft	soft	soft	soft
After-taste	Extremely Slightly pleasant	Very Moderately pleasant	Moderately Very pleasant	Slightly Extremely pleasant	repugnant	repugnant	repugnant	repugnant
Meat flavour Intensity	Extremely Slightly strong	Very Moderately strong	Strong Very	Fair Extremely	mild	mild	mild	mild
Overall acceptability	Extremely Slightly acceptable	Very Moderately acceptable	Moderately Very acceptable	Slightly Extremely acceptable	unacceptable	unacceptable	unacceptable	unacceptable

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Sample code Colour/ Appearance Flavour Crispiness After-taste
Meat flavour intensity Overall acceptability

_____
_____

Remarks:
Signature

VITA

Name of the Student	:	Parminder Singh
Father's name	:	S. Saroop Singh
Mother's name	:	Smt. Amarjeet Kaur
Nationality	:	Indian
Date of Birth	:	May 07, 1984.
Permanent home address	:	House No. F-11/655, Jawahar Nagar chowk, Near Bright Days School, Batala Road Amritsar. (Punjab). Pin-143001.

EDUCATIONAL QUALIFICATION

Bachelor's degree	:	B.V.Sc. & A.H.
University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
Year of Award	:	2009
OCPA	:	7.92/10.0
Scholarship	:	Merit scholarship holder throughout the degree.
Master's Degree	:	M.V.Sc. (Livestock Products Technology)
OCPA	:	8.84/10.00
University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
Year of Award	:	2011
Fellowship/Distinction	:	Merit Fellowship holder throughout the degree.