DEVELOPMENT OF FUNCTIONAL PASTA INCORPORATED WITH CHICKEN MEAT AND MICROENCAPSULATED DOCOSAHEXAENOIC ACID POWDER

Thesis

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

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

By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"DEVELOPMENT OF FUNCTIONAL PASTA INCORPORATED WITH CHICKEN MEAT AND MICROENCAPSULATED DOCOSAHEXAENOIC ACID POWDER"** submitted for the degree of **M.V.Sc.** in the subject of **Livestock Products Technology** (Minor Subject: **Veterinary Public Health and Epidemiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Daud Masih (L-2018-V-25-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

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ABSTRACT

The objectives of the present study were to optimize the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional protein rich pasta. Thirteen different formulation combinations were devised for the preparation of functional pasta using Response Surface Methodology (RSM) implementing Central Composite Design. Outcomes were demonstrated by various cooking parameters viz. Minimum cooking time (MCT), Volume expansion (VE), Water uptake ratio (WUR), Gruel Solid loss (GSL), Color analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability OA). It was found that with increase in chicken meat powder percentages from 20 to 40 in the formulation there was significant (p<0.05) increase in MCT, GSL, firmness, toughness, *a** and *b** value, whereas VE, WUR, L value and OA showed declined trend. Among studied conditions, DHA powder showed least effect. The best-suited formulation condition predicated by the models were 30% chicken meat powder and 1.5 % DHA powder. The proximate compositions of developed pasta was significantly (p<0.05) improved as compared to control pasta. The control and developed functional pasta were subjected for aerobic storage at (20-30±5ºC) in aluminium foil-LDPE laminate pouches for 60 days, and analyzed at regular interval of 1, 15, 30, 45 and 60 days of storage, the production cost were also analyzed. Storage results showed increasing trend in TBARS, FFA, peroxide value and microbial counts in both the pasta samples, but the rate of increase was significantly lower (p<0.05) for the control products. Sensory scores were least for developed functional pasta than control pasta, might be due to the added chicken meat powder with the disadvantages of reddish color, strong fishy flavor of DHA powder and unacceptable textural properties. The developed functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) could be stored for 60 days without any marked loss in physico-chemical, color, textural, microbiological and sensory qualities. Microstructure studies revealed increased interaction between chicken protein and starch molecules, indicating a potential option to produce high quality pasta with enhanced nutritional and functional properties. The cost of production of developed pasta was 128 Rs/Kg as compared to less nutritious control pasta with production cost of 72 Rs/Kg, Hence, it can be recommended as a profitable start up business venture. It was concluded that the highly nutritious protein and DHA rich pasta could be developed with acceptable shelf life of 60 days.

Keywords: Chicken meat, DHA powder, response surface methodology, functional pasta

Signature of Major Advisor **Signature of Student**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS USED

AOAC	:	Association of Official Analytical Chemists
APHA	:	American Public Health Association
CBD	:	Central composite design
cfu	:	Colony forming unit
cm	:	Centimetre
FFA	:	Free fatty acids
Fig.	:	Figure
g	:	Gram
hrs	:	Hours
i.e.	:	That is
Kg	:	Kilogram
LDPE	:	Low density polyethylene
Lit	:	Litres
M	:	Molar
Meq	:	Miliequivalent
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre
mm	:	Millimetre
MT	:	Million tonnes
N	:	Newton
^{o}C	:	Degrees Celsius
MP	:	Meat Pasta
CP	:	Control pasta
ppm	:	Parts per million
RSM	:	Response Surface Methodology
s	:	Seconds
SE	:	Standard error
SPC	:	Standard plate count
TBARS	:	Thiobarbituric acid reactive substances
TPA	:	Texture profile analysis
viz.	:	Videlicet
w/v	:	Weight by volume
w/w	:	Weight by weight
Wt	:	Weight

CHAPTER I

INTRODUCTION

The world demand for snack based food is increasing due to growing trend towards fast food among younger generation, increase in the purchasing power of the people, ease of transportation, mechanization and convenience of cooking (Kumar *et al* 2019). However, nutritional specialists caution against the intake of extruded fast foods and brand them as "*junk food*" due to high content of fats, carbohydrate and lack of functional ingredients. Due to which, there is an ever-increasing awareness on changing the consumption pattern towards healthy foods. Out of many popular snacks based food items; pasta ranked topped with ease of convenience and cooking (Spinelli *et al* 2019).

Pasta is one of the important processed food items made from wheat semolina or fine flour and water. Pasta may have its origin in Asia and the Mediterranean but its growing popularity has made it truly a healthy food worldwide. Basically pasta is an Italian word for '*Paste'* and its different products such as macaroni, spaghetti and noodles are very popular in Europe and in the Western hemisphere. Approximately 14.3 million tons of pasta is produced worldwide with an estimate of 100,000 tons production in India (Anonymous 2020). The popularity of pasta product increased because they are simple to prepare, economical and can be stored after drying for a longer period without much deterioration in quality (Feillet *et al* 1996). Observing the increasing demand of pasta by all income and age group people, it can be used as a vehicle for supplementation of functional ingredients, protein etc. to overcome nutritional problems.

Modern active lifestyle necessitates development of functional pasta products for better health and disease prevention. With appropriate selection and addition of food ingredients there is a great opportunity for developing pasta products having health benefits when consumed. Semolina pasta is a highly consumed foodstuff, the biological value of which is low because it's less protein, owing to its low lysine content. However, if the semolina is supplemented with ingredients from fish, egg and meat, not only amino acids are supplemented, but also the dietary vitamins and minerals can be provided through the diet (Gull *et al* 2018). So, to impart nutritive value to these snacks, addition of meat based proteins is a promising step. However,

literature is almost scanty for incorporation of spent hen chicken meat into pasta products.

Chicken meat is the fastest growing component of global meat demand. In India, the consumption of chicken meat is higher as compared to other meats, as it is preferred over other meat for its health appeal because of its higher protein content as well as lower cholesterol and fat. The spent hens are old and culled chickens, which have completed their productive and reproductive phase of life (Barroeta 2007). The meat of such birds is tougher, less juicy due to high collagen contents and high degree of cross linkages as compared to broiler meat. By incorporation of spent hen meat into snacks foods like pasta formulation one can enhance nutritive value, palatability and can help in utilizing these poultry industry by-products (Kumar *et al* 2016).

Omega-3 fatty acids are classified as essential fatty acids, and are crucial for human health. However, they cannot be synthesized in human body and have to be acquired through the diet. Fish as well as algae oil is a good source of long chain omega-3 polyunsaturated fatty acids that include α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), later two acids have positive effects on the prevention and treatment of various chronic diseases (Simopoulos 2000).

A major challenge associated with the application of DHA/EPA in functional foods is the oxidation during processing and storage. Oxidation decreases the nutritional quality of the lipid and produces off flavour and aroma compounds due to the disruption of lipid hydroperoxides (Wagh and Chatli 2017). One commonly used method to minimize oxidation and off-odor production is encapsulation. Encapsulation is a process that creates a functional barrier between the core and wall material to prevent chemical and physical reactions and maintain the biological, functional, and physicochemical properties of the core material. Incorporation of novel microencapsulated DHA powder is promising way out to increase functionality of the food items (Krofa *et al* 2018). However, incorporation of microencapsulated DHA into pasta products is almost unexplored.

The possible utilization of chicken meat in pasta will not only increase the nutritive value but will also provide the poultry industry an alternate source of revenue generation. Despite a lot of work to improve the functional properties and nutritive value of pasta using the changes in formulations and processing; possibility of incorporation of spent hen chicken meat in pasta as a source of protein with

fortification of microencapsulated DHA will open a new era of functional meat based snacks foods. Hence, the present study was conducted keeping in view the following objectives:

1. To optimize the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta
2. To evaluate the storage stability of the developed functional pasta at ambient temperature
3. To calculate the production cost of developed functional pasta

CHAPTER II

REVIEW OF LITERATURE

Snacks are described to be a portion of food, smaller than a regular meal, generally consumed between meals (Falola *et al* 2014). Snacks come in different forms including packaged snack foods, processed snacks, and items made from fresh ingredients at home, super markets or local street vendors (Bucher *et al* 2016). Traditionally, snacks were prepared from household ingredients such as fruits, nuts, grains, leftovers etc. Processed snack foods, as a form of convenience foods, are designed to be less perishable, more durable, and more portable than prepared foods. However, they often contain substantial amount of preservatives, sweeteners, and other ingredients that makes them energy dense and nutritionally poor (Nelson 2014).

2.1 Pasta

Pasta is the most commonly consumed paste-product made from durum wheat. The term 'pasta' has generally been reserved to describe paste products fitting the Italian style of extruded foods such as spaghetti or lasagna, and is usually distinguished from the oriental style of sheeted and cut foods called 'noodles', which are commonly made from wheat other than durum (Dick and Matsuo 1988). Italians, who are the largest consumers of pasta products in the world, call these products '*pastaalimentare*' (alimentary paste) (Dick and Matsuo 1988, Donnelly and Ponte 2000). Pasta is a traditional food product with origins dating back to the first century B.C. (Agnesi 1998), which is becoming increasingly popular worldwide because of its convenience, nutritional quality and palatability (Cubadda 1994). Pasta can be categorized into four main groups or types: (i) Long-goods such as spaghetti, vermicelli, and linguine, (ii) Short-goods include elbow macaroni, rigatoni, and ziti, (iii) Egg noodles consists of pasta made with egg, (iv) Specialty items such as lasagna, manicotti, jumbo shells, and stuffed pasta.

Over 600 pasta shapes are produced, however, the number of sizes and shapes that can be produced is virtually unlimited and depends on the shape of the die from which the product is extruded or the cutter with which it is cut. Spaghetti which is in the form of solid rods, elbow macaroni, lasagna, shells, and various noodle shapes are among the most popular shapes (Dick and Matsuo 1988). Although pasta is traditionally manufactured using only durum wheat semolina, pasta is sometimes also

made from non-durum wheat flour, or farina or mixtures of durum and common wheat because common wheat is traded at a lower price than durum wheat.

In recent years, pasta has become more popular due to its nutritional properties, and regarded as a product with low glycemic index (Jenkins *et al* 1988, Wolever 1990, Björk *et al* 2000). The nutritional value of pasta derives from its high energy value (around 350 kcal per 100 g), reasonable protein content (11-12%) and its digestibility. Its mineral content is unbalanced with a marked prevalence of potassium (Ferrari and Piazza 2006). Pasta also provides significant quantities of complex carbohydrates, proteins, B-vitamins, and iron and is low in sodium and total fat (Douglass and Matthews 1982). Normally when we eat pasta, it is accompanied by a series of adjuncts or sauces such as vegetables (source of Vit. A and C), fish, meat, eggs (source of protein), and/or legumes (source of protein and fiber) that enhance and improve its nutritional value through a sort of complementary process (Ferrari and Piazza 2006).

Durum wheat is the hardest wheat that produces a coarse particle called "Semolina" during milling (Sissons 2008). Pasta products are manufactured from durum wheat semolina, which is known to be the prominent raw material suitable for its production (Nedeljković *et al* 2014). Pasta production involves mixing of durum wheat semolina with water, kneading, extrusion, shaping, and drying. Pasta prepared from durum semolina provides typical viscoelastic behavior to pasta that helps in maintaining a desirable firm texture during cooking and optimal dough formation during the mixing and extrusion phases (Feillet and Dexter 1996). The World Health Organization (WHO) and the Food and Drug Administration (FDA) consider pasta as a suitable vehicle for the incorporation of nutrients (Chillo *et al* 2008, Gallegos-infante *et al* 2010, Ovando-Martinez *et al* 2009, Cubadda 1994). However, pasta is not nutritionally balanced due to its low lipid and fiber content, and to the low biological value of its protein, owing to its low lysine content.

2.2 Fortification in pasta

Kaur *et al* (2013) assessed the effects of supplementation of pasta with plant proteins from mushroom powder, bengal gram flour and defatted soy flour at different levels on its nutritional quality. Supplementation of wheat semolina was done with mushroom powder (0–12 per cent), bengal gram flour (0–20 per cent) and defatted soy flour (0–15 per cent). Mushroom powder and defatted soy flour increased the

cooking time of pasta whereas non-significant variation was observed in cooking time of Bengal gram supplemented pasta. Significant correlation was observed between water absorption and volume expansion of pasta. On the basis of cooking and sensory quality, pasta in combination with 8 per cent mushroom powder, 15 per cent Bengal gram flour and 9 per cent defatted soy flour resulted in a better quality and nutritious pasta.

Shreenithee and Prabhasankar (2013) analyzed the interaction of yellow pea flour and *T. durum* semolina in pasta processing and the influence of different shapes of pasta on product quality and nutritional profiles. Results indicated that the noodles with 20 per cent yellow pea flour had favourable sensory attributes, protein content, texture, yellowness values, reduction in the glucose release and increased protein digestibility. Shell pasta, noodle and vermicelli were extruded with the optimized 20 per cent pea flour for comparison. Proximate composition results showed an increase in protein content for all the samples compared to control. Shell pasta had the lowest cooking loss and a good firmness.

Anna *et al* (2014) incorporated 25, 50 and 75 g/kg of grape marc powder in fettuccini pasta preparation and evaluated its effect over the cooking, nutraceutical and sensory properties of the resultant pasta. The results showed that the incorporation of the dried byproduct did not interfere with the water absorption and in the solid loss of the pasta after cooking. Sensory analysis showed that the incorporation of grape marc powder reduced the acceptance of aroma, aftertaste, flavour and appearance, regardless of the concentration of the dried residue added. Furthermore, the incorporation of 25 g/kg of grape marc powder presented the best overall acceptance, with lower changes of colour.

Sereewat *et al* (2014) prepared spaghetti from rice flour and defatted soy flour and examined the effects of barrel temperature on cooking quality and texture of extruded rice spaghetti. The substitution of rice flour with defatted soy flour (DSF) at 90:10 increased protein content and resulted in a more yellowish product with greater hardness of the cooked spaghetti strand compared to the product extruded from rice flour only. The addition of cross-linked modified starch (MS) (rice flour:DSF: MS at 86:10:4) could improve the texture of the cooked rice pasta. Sensory evaluation showed that rice spaghetti made from rice flour, DSF and the modified starch at the above ratio was comparable to commercial spaghetti made from durum semolina.

Yadav *et al* (2014) optimized the formulations for wheat-based pasta incorporated with pearl millet flour and vegetables. A blend of wheat and pearl millet flour (9:1) with vegetable paste (2 per cent dry solids) was extruded. Incorporation of pearl millet flour and vegetable resulted in nutritionally rich pasta as compared to control due to increased mineral content. Vegetable incorporation improved the textural attributes i.e., increased firmness and decreased stickiness significantly and caused significant reduction in gruel loss. Based on the nutritional, textural and sensory acceptability, spinach incorporated pasta was most acceptable. The effect of storage time was not significant on pasta quality parameters.

Gull *et al* (2015) replaced durum wheat semolina with finger millet flour (FMF), pearl millet flour (PMF) in pasta making at 10, 20, 30, 40, 50g/100g and carrot pomace powder (CPP) at 2, 4, 6, 8, 10g/100g (flour basis, 30g of water/100g) and evaluated the effects on cooking qualities, colour and textural properties of the developed pasta samples and compared with control sample. Significant differences in cooking quality characteristics, colour and texture of the pasta samples were observed. With increase in the substitution level of millet flours and CPP, solid loss increased whereas weight gain and firmness decreased. Colour analysis showed slight decrease in L* value for all raw and cooked pasta samples.

Mridula *et al* (2015) optimized the level of groundnut meal, carrot juice and refined wheat flour for the development of pasta using response surface methodology. Different experimental combinations were designed using box-benken design of experiments considering 10–20 g groundnut meal, 14–30 mL carrot juice and 80–90 g refined wheat flour. Pasta samples with higher level of groundnut meal and carrot juice showed higher antioxidant activity and overall sensory acceptability. The samples with higher groundnut meal resulted in higher protein content. Pasta samples with higher amount of carrot juice showed higher rehydration ratio and lesser cooking time with low solid loss in cooking water. Different levels of groundnut meal, carrot juice and wheat flour significantly affected the colour as well as cooking quality of pasta. The protein and antioxidant activity of pasta were increased with increasing level of groundnut meal and carrot juice in the sample.

Rathi *et al* (2004) studied nutritional parameters of pearl millet pasta. Results showed that protein, fat, ash and dietary fiber contents of pearl millet-based pasta were significantly ($p \leq 0.05$) higher compared to control pasta. The results of Shukla

and Srivastava (2014) indicated that noodles incorporated with 50% finger millet contained highest amount of crude fat 1.15%, ash 1.40%, crude fiber 1.28%, carbohydrate 78.54%, physiological energy 351.36 kcal, insoluble dietary fiber 5.45%, soluble dietary fiber 3.71%, iron 5.58 mg/100g and calcium 88.39 mg/100g, respectively. Kulkarni *et al* (2012) reported higher values of protein, fiber and minerals such as calcium, iron and phosphorous in noodles developed from wheat and malted ragi flour compared to control sample. Results of nutritional analysis showed an increase in protein, crude fiber, dietary fiber and minerals components with incorporation level of modified millet flour and pulse flour respectively.

Wani *et al* (2011) depicted increase in fiber and ash content of noodles substituted with cauliflower leaf powder at 0, 10, 15 and 20% level than control noodles. Increase in fiber content with increase in level of millet flour blend incorporation was also reported by Vijayakumar *et al* (2010). Similar studies of Eyidemir and Hayta (2009) showed an increase in protein and ash content in noodles incorporated with apricot flour compared to control sample.

2.3. Protein fortification of pasta

The level and composition of proteins in wheat flour are of great importance for the cooking and eating qualities of noodle and pasta products (Fu 2008, Hou 2010). The purpose of adding natural proteins to pasta and noodles is mainly for improving nutritional quality and maintaining a strong dough structure. Some exogenous proteins can impact gluten proteins in the dough, thereby enhancing the pasta and noodle dough structure and improving the chewiness of the final product (Maforimbo *et al* 2008).

The protein content and composition of the wheat semolina are important and they influence the pasta quality. The best type of hard wheat for pasta manufacturing is durum wheat (*Triticum durum*). Durum wheat consists of 12-16 % protein. The proteins in wheat semolina can be divided into five groups, albumins (15% of protein), globulins (3% of protein), monomeric gliadin (33% of protein), polymeric glutenin (16% of protein) and the residue 33% (Lamacchia *et al* 2006, Atwell 2001). Each amino acid has an amino group, carboxyl acid group and a hydrocarbon group (R-group). The carboxyl acid group and the amino acid group in the proteins are bound together with peptide bonds that link the amino acids together in long chains.

The R-group does not contribute to the peptide bond but is on the other hand involved in interactions with other protein chains.

In a study conducted be Liu *et al* (2016), semolina flour was substituted with beef emulsion (EM) at three different levels of 15, 30 and 45% (w/w) to develop a pasta with enhanced nutritional profile. The protein, fat, and water content significantly increased with addition of meat. The addition of meat enhanced the pasta gluten network. The redness and yellowness of cooked pasta increased with meat addition. Tensile strength increased from 0.018 N/mm²in the control sample to 0.046 N/mm² in 45 EM sample. All meat-containing samples had significantly higher elasticity than control (0.039 N/mm. Five essential amino acids (leucine, lysine, methionine, threonine, tryptophan) in pasta digesta increased significantly with increasing meat addition.

Few investigations have concentrated on expanding dietary benefits of pasta in terms of protein content (Adegunwa *et al* 2012, Fuad and Prabhasankar 2010). Cooking loss is an important parameter to measure the quality of pasta products. Low cooking loss is identified as the high quality of pasta (Lu *et al* 2016). The capability of gluten-starch network to retain the physical uprightness of pasta during cooking is responsible for the cooking loss. Protein network of pasta can be strengthened by thermal protein denaturation to improve the firmness of cooked pasta. As indicated by the Cappa and Alamprese (2017) the mechanical properties of cooked pasta can be improved by incorporation of egg white powder because of the strong protein matrix created by ovalbumin and the break load and strain of cooked pasta can be increased more significantly, than in pasta without egg white powder.

Correia *et al* (2017) have investigated the effect of mushroom powder as a protein supplement in fresh pasta development. According to the results solid loss in the cooking water has been within the recommended level which is 9% (Correia *et al* 2017). Increments of mushroom powder content and drying temperature has led to reduce the adhesiveness, internal, and external firmness of pasta. Desai *et al* (2018) have studied the incorporation of fish (*Pseudophycis bachus*) powder on the physiochemical attributes of pasta. It is demonstrated that incorporation of fish powder can improve the protein, lipid and ash content of pasta while reducing the moisture and carbohydrate contents.

Protein interactions in a constant matrix as well as the content and quality of protein are imperative requirements to develop an ideal network of protein and carbohydrate to improve the cooking quality of pasta Noni and Pagani (2010). Desai *et al* (2018) have revealed that incorporation of fish powder can decrease the cooking quality because of disruption and weakening of the gluten protein network. Similar results have been reported by Ramya *et al* (2015) who have considered the incorporation of shrimp meat powder in pasta products and have detailed that the cooking loss (leaching of solids) is increased when there is increase in the incorporated amount of shrimp meat powder. Due to the higher cooking loss and lower water absorption the optimum cooking time is decreased when the shrimp meat powder is added.

Alamprese and others (2005) indicated that adding egg albumin to pasta can give the product high resistance to breakage and good cooking tolerance, resulting in higher water absorption and lower cooking losses. The same effect can be achieved with casein (Chillo *et al* 2009, Sozer 2009).

Devi *et al* (2013) reported that incorporation of fish mince has increased the water absorption of pasta. This might be because of the strong protein-starch matrix formed by fish mince that have higher limits to absorb and hold water.

Firmness is an impression of the bond quality and the uprightness of the protein network in cooked pasta. Addition of fish powder (Desai *et al* 2018), shrimp meat powder (Kadam and Prabhasankar 2010), and beef meat (Liu *et al* 2016) has increased the firmness value of pasta. These results might be happening due to the low water absorption and low swelling index. Firmness can be reduced due to the higher swelling index and water absorption in pasta (Foschia *et al* 2015). Among the animal protein sources, fish protein concentrate has been subjected to several researches because of its nourishing qualities. Goes *et al* (2016) have studied the fresh pasta enrichment with protein concentrate of tilapia. Results have revealed that incorporation of fish concentrate can increase the mineral profile, lipid content and protein content and reduce the caloric value of the pasta products.

2.4 Meat Snacks products

The global Meat Snacks Market accounted for USD 5.0 billion in 2018 and is expected to register 3.7% CAGR during the forecast period, 2019 to 2024. The meat snacks market is highly fragmented, with a large number of players present in the

market. Meat snacks have gained popularity due to the demand for high-protein convenient snack products.. The manufacturers are spending highly on the development of innovative products and flavours considering, consumer preferences, which is further boosting the market growth. However, the increasing prevalence of heart diseases, obesity, and diet-related diseases have restrained the growth of the factor. The growing trend for veganism and decreasing demand for red meat are the major challenges faced technological, economic, ecological, social and organisational implications.

Cunningham (1993) prepared extruded meat-based snack food. A high protein, shelf stable, meat-containing snack product was prepared with at least about 15 per cent by weight meat and at least about 50 per cent by weight wheat flour were mixed and passed through an extruder under time and temperature conditions to yield an expanded, cellular extrudate having the general appearance of a bread stick.

Park *et al* (1993) investigated 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 response surface methodology. Contour plots showed a convex curve of expansion ratio (ER) with moisture, concave curves of bulk density (BD) and shear-force (SF) with moisture, and concave curves of SF with each of the four extrusion variables. Fat decreased ER and increased BD, whereas corn starch increased ER. Products with high ER and low BD and SF tended to have prominent air cells, continuous protein matrices, and smooth cell wall surfaces in scanning electron micrographs. 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.

Lee *et al* (2003) studied on the utilization of chicken meat and potato starch snack product that was increasingly crispy when starch concentration was increased. Furtaw (2006) received a patent for a baking and drying method designed to produce meat snacks with a crisp texture. Sofos *et al* (1995) examined the feasibility of extrusion cooking of hand-deboned chicken meat (HDCM) and mechanically-deboned turkey meat (MDTM) combinations in a single screw extruder with various non-meat binders.

Value added chicken meat noodles were developed by Verma *et al (*2014). Technology of chicken meat noodles preparation was standardized with various levels (0, 30, 40 and 50 per cent) of meat along with whole wheat flour and other necessary ingredients. The emulsion was initially prepared and thereafter this emulsion was moulded into noodles and dried in hot air oven over at 65°C for required time (7-8 hours). The analysis was performed on emulsion, physico-chemical qualities and sensory attributes using suitable method. They concluded that noodles having 30 per cent chicken meat represented an acceptable preference in term of sensory evaluation as compared to 40 and 50 per cent.

Liu *et al* 2016 studied the effects of meat addition on pasta based snacks products in which they substituted semolina flour with beef emulsion at three different levels of 15, 30 and 45% (w/w) to develop a pasta with enhanced nutritional profile. They concluded that protein, fat, and water content significantly increased with addition of meat. The addition of meat enhanced the pasta gluten network. The redness and yellowness of cooked pasta increased with meat addition. Tensile strength increased from 0.018 N/mm2 in the control sample to 0.046 N/mm2 in 45EM sample.

2.5 Storage studies of meat based extruded products

Foods are perishable by nature. Several reactions take place in foods during processing and storage. Upon storage one or more quality attribute of food may change to undesirable state (Singh 1994). Consumers increasingly demand high quality foods. The food should remain safe without any unwanted sensorial changes (Kilcast and Subramaniam 2000). Shelf life of the product is determined when the first significant change in overall quality is detected (Baixauli *et al* 2008). Food is exposed to various environmental conditions such as temperature, humidity, oxygen and light, which can trigger several reaction mechanisms that lead to deterioration. The changes in the food that occurs during storage can be categorized as physical changes which are caused by mishandling during food processing and storage.

There are two main physical parameters that determine the quality of dried pasta: colour/aesthetic appearance and mechanical strength (Feillet and Dexter 1996). Pasta colour is the result of yellow and brown components. During pasta drying, the protein-carbohydrate reactions, which involve the terminal amino group of free amino acids, proteins and reducing sugars, Maillard reactions, lead to a change in colour of pasta by non-enzymatic reactions (Acquistucci 2000). High occurrence of this

reaction in pasta making is undesirable because it increases the redness and brownness of the pasta. Mechanical strength is the measurement of dry pasta products ability to withstand compression forces as an indication of the resistance of the product to handling and transportation. Having a strong gluten matrix and an unchecked product is essential to for high mechanical strength (Feillet and Dexter 1996). Presence of non-traditional ingredients have shown to have an effect in decreasing the mechanical strength of non-traditional pasta product in comparison to the traditional, however this effect can be relatively compensated by applying an ultra-high drying treatment to the product (Abecassis *et al* 1989).

Rhee *et al* (1999) prepared a cooked- puffed snack from blends of corn starch (81.72-84.86%) and ground meat (goat meat, lamb, mutton, spent hen meat, beef 15.14-18.28%) with target moisture level of 26.5% (with no added water) using a single-screw extruder. All extrudates were well expanded and low in fat (< 1.5%), aw (< 0.12), bulk density, and shear force. 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). Most panelists did not perceive "meat" note or species-related meat flavour. Total polyunsaturated fatty acid percentage was similar for extrudates with beef, lamb and mutton and highest for those with chicken. When extrudates were stored aerobically at 37°C for up to 120 days, lipid oxidation (as measured in meq. peroxides/kg fat) was lower for products containing goat meat, lamb, or mutton than for those with beef or chicken. The degree of polyunsaturation or unsaturation of their fat only partly accounted for the lipid oxidation differences.

Lee *et al* (2003) studied the physical evaluation of popped cereal snacks with spent hen meat. Various blends of spent hen meat and grains (potato starch, corn starch, and rice flour) were popped using a popping machine. Lowest bulk density was observed in the snack with 1:2 ratio of meat and potato starch. 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. All popped snacks were significantly different ($P<0.001$) in bulk density, colour, and breaking force. As the grain content of snacks increased, the size of the air cells also increased. Results of scanning electron microscopic and optical microscopic

observations revealed the popping degree of snack with starch and spent hen meat was affected by the presence of meat. 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.

A study was conducted by Raja *et al* (2014) to evaluate the effect of ambient storage on the quality attributes of aerobically packaged fish curls incorporated with optimum levels of different flours. The curls were developed by extrusion technology using fish meat (Catla catla). The fish curls containing optimum levels of different flours viz. 20 percent corn flour, 10 percent black gram flour and 10 percent peanut flour were compared with the control snacks containing 30 percent rice flour and assessed for storage quality and shelf life at ambient temperature. The curls were aerobically packaged in LDPE (low density polyethylene) pouches and evaluated for various physicochemical, microbiological and sensory parameters. Mean values of pH of all the curls showed significantly ($p < 0.05$) decreasing trend with increasing days of storage (6.34 on day 0 and 5.90 on day 28 for control samples, 6.41 on day 0 and 6.11 on day 28 for corn flour incorporated samples, 6.36 on day 0 and 6.14 on day 28 for black gram flour incorporated samples, 6.57 on day 0 and 6.34 ± 0.01 on day 28 for peanut flour incorporated samples). TBARS (mg malonaldehyde/kg), total plate count (log cfu/g) and yeast and mould count (log cfu/g) for the control as well as treatment samples showed significantly ($p < 0.05$) increasing trend with storage. Coliform counts (log cfu/g) were not detected until day 28 in all the products. The mean scores of sensory parameters i.e. appearance and colour, flavour, crispiness, texture and overall acceptability for control as well as treatment samples showed significantly ($p < 0.05$) decreasing trend with storage period. The decrease was significantly ($p < 0.05$) highest on 21st and 28th day of storage. The mean values for all the quality and storage parameters up to the day 21 of the storage were within the acceptable limits. Thus, based on various physicochemical and sensory parameters, the curls incorporated with optimum level of different flours were acceptable up to 21 days of ambient storage within the LDPE pouches.

Ranganna *et al* (2014) attempted to develop millets' based cold extruded products (vermicelli and pasta) and to study their storage. Five small millets' (barnyard, foxtail, kodo, little and proso) were used in the study. Small millets flour, wheat and soy flours were used in the ratio of 50: 40: 10 for the development of cold

extruded products. Vermicelli kheer and pasta masala were prepared from all the five millets and were subjected to sensory evaluation along with control (wheat based vermicelli). Sensory results showed that the millets kheer were more acceptable and foxtail millet kheer was better preferred followed by kodo and proso millet kheers. The millets pasta masalas were also very much acceptable compared to control. Among the millets, proso millet pasta masala was more preferred for its sensory attributes followed by kodo millet. Nutritional analysis of stored vermicelli showed not much variation in composition before and after storage irrespective of packaging material. Both the gauges of package (300 and 400 PE) were found suitable for storing vermicelli up to two months without affecting the quality.

Devi *et al* (2013) studied processing, packaging and storage of pasta prepared from proso millet blended with wheat flour different combinations. Effect of low density polyethylene (LDPE) and poly propylene (PP) on sensory, physico-chemical and biochemical quality of pasta during storage of three months at ambient atmosphere condition were analyzed. Pasta prepared under equal proportions of millet and wheat flour got maximum overall acceptance in the sensory panel and the rate of loss of most quality attributes was low in pasta stored under low density polyethylene (LDPE) samples compared to samples packaged under polypropylene (PP). It was concluded that pasta could be best preserved up to three months at ambient atmospheric condition under LDPE without appreciable quality loss.

Studies of Yadav *et al* (2014) specified that effect of storage time was not significant on pasta quality parameters, indicating that pasta was acceptable up to three months stored in polyethylene bags without any preservative under ambient conditions. Results of Kaur *et al* (2012) also revealed that pasta prepared from durum wheat semolina enriched with 15% level of wheat, rice and oat bran and 10% barley bran packed in high density polyethylene packs was highly acceptable up to four months of storage at ambient condition with respect to quality. Effect of storage conditions on quality characteristics of noodles was investigated by Pangloli *et al* (2000). Noodles were packed in plastic bags under partial vacuum or air and stored at 4.4°C or 22 to 30°C for six months. Results showed that noodles prepared from wheat flour, 10% defatted soy flour and 10% sweet potato flour or 15% puree can be stored successfully under air with greater quality retention at 4.4°C for six

months.

Studies of Manthey *et al* (2008) reported that packaging conditions have little or no effect on cooking quality of flax seed flour blended semolina pasta. Parameters evaluated during storage were cooking quality, colour and sensory attributes. Results revealed that storage has non -significant effect on the measured parameters.

2.6 Response surface methodology

Response surface methodology (RSM) was reported to be an effective tool for optimizing the process, as highlighted by various researchers (Thakur and Saxena 2000, Vatsala *et al* 2001).

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 modelling 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 (Myers and Montgomery 2009).

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. In addition, 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 (Montgomery 2005).

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 behaviour 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 (Montgomery 2005). 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.

Acosta *et al* (2006) applied response surface methodology (BoxBehnken design) to assess and model effects of 3 factors, sweetener, low methoxyl pectin, and calcium content (each at 3 levels), on the overall acceptability of a blackberry (*Rubus irasuensis* Liebm.) jelly, as determined by 100 consumers. Jelly was produced using clarified juice, obtained from a cross-flow microfiltration process. Results showed that the model fit was significant, and there was satisfactory correlation between actual and fitted values (R^2= 0.925 and adjusted R? = 0.791). The model presented no significant lack of fit (p=0.096). Sweetener level had a significant effect on overall acceptability (p<0.05), but low methoxyl pectin and calcium levels did not. The statistical model was further used to optimize the factor levels for highest acceptability, in order to obtain a jelly that provided less than 8 calories per serving, making it possible to label the product as "low calorie."

CHAPTER III

MATERIALS AND METHODS

The present study "Development of functional pasta incorporated with chicken meat and microencapsulated docosahexaenoic acid powder" was carried out in the Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

3.1 Source of raw materials

3.1.1 Source of meat and development of chicken meat powder

Spent hen chicken meat was procured from the University Poultry Farm, Department of Livestock Production Management, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The birds were slaughtered as per standard procedure in the experimental slaughter house of Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab with due consideration of the animal welfare and ethical aspects. The dressed carcasses were brought to the laboratory immediately, chilled at 4±1 º C for 12-18 h, followed by manual deboning. The skin, external fascia, fat and all separable connective tissues were removed and boneless meat was recovered. The boneless meat and fat were packed separately in low density polyethylene (LDPE) bags in the unit pack of 1 Kg and subsequently stored in a deep freezer at -18±1ºC till further use. The required quantity of frozen meat packs were taken out, thawed overnight in a refrigerator (4±2 º C), and used whenever needed.

For preparation of spent hen chicken meat powder, minced meat was cooked in a pressure cooker at 15 psi for 30 min, and then drying was carried out in temperature controlled Industrial dryer (MAC industrial drier, New Delhi, India) at 60±1°C for 15 h. Then grinding was done using a mixer grinder and subsequently sieved by passing through a sieve with a pore size of about 500 µ to obtain the chicken meat powder and subsequently stored in a refrigerator at 4±1ºC till further use.

3.1.2 Semolina and DHA powder

Semolina was procured from the local market, Ludhiana, Punjab. Food grade microencapsulated DHA Powder with 20 % active content was purchased from V.B. Medicare Pvt. Ltd., Hosor, (T. N.), India and stored at -18°C.

Table 1: Proximate composition of raw materials used

Proximate composition (%)	Semolina	Chicken meat powder
Moisture	13.25±0.15	4.52±0.05
Protein	11.36±0.09	76.14±0.24
Fat	1.08±0.04	9.36±0.41
Ash	0.47±0.01	4.22±0.08

3.1.3 Packaging material

Low density polyethylene (LDPE 100-120 gauge) and Aluminium foil-low density polyethylene (LDPE) laminates were used for aerobic packaging.

3.1.4 Chemicals, media and standards

Different analytical grade chemicals, media, and high purity standards required for analysing the quality of studied products were procured from standard firms like Sisco Research Laboratories, Fisher Scientific, Central Drug House, Hi-Media and Sigma-Aldrich etc.

3.2 Formulation of control and developed pasta

Formulation of the control and developed functional pasta were standardized on the basis of available literature and various preliminary trails conducted in laboratory. The standardized formulation is given in Table 2.

Table 2: Formulations of the Control pasta and Developed functional pasta

S. No.	Ingredients	Percentage (w/w)	
		Control pasta (%)	Developed functional pasta (%)
1.	**Semolina**	100.00	68.50
2.	**Water**	90 mL	90 mL
3.	**Chicken meat powder**	-	30.00
4.	**DHA powder**	-	1.50
	Total	100	100

3.2.1 Preparation of blends

The semolina was supplemented with DHA and chicken meat powder as shown in Table 2, was weighted carefully and mixed properly by passing twice through the sieve (10 mesh) to prepare blends.

Fig. 1: Pasta and noodle making machine (Kent)

3.2.2 Pasta preparation

The various blends of DHA and chicken meat powder supplemented pasta was mixed in a mixing chamber of pasta machine (Kent, New Delhi, India) with the required amount of water (based on trials) for 8-10 min to hydrate the flour particles uniformly. The pasta was extruded with a speed of 60 rpm. The temperature of the extruded dough was maintained at 37-40 °C. The pasta was cut into a desirable length of 3 cm. The drying of Pasta (Penne/tube-shaped) was carried out in Industrial dryer (MAC industrial drier, New Delhi, India) at 50 °C for 4-5 h and was packed in polyethylene bags at 4 °C till further analysis.

Flow diagram for preparation of pasta

Blend

↓

Optimum water

↓

Mixing (10 min)

↓

Extrusion using Kent Pasta maker

↓

Cutting (into desired shape and size)

↓

Drying (40-50 °C, 5-6% moisture)

↓

Dried product

↓

Packaging

(a) **(b)**

Fig. 2: Control (a) and developed functional pasta (b)

3.3 Analytical techniques

3.3.1 Cooking Quality

Cooking quality of the control and developed functional pasta samples were analyzed for the following cooking characteristics.

3.3.1.1 Minimum cooking time

Weighed sample (10 g) of pasta was cooked in 100 mL distilled water for required time. Samples of cooked pasta were removed at interval of 30 s. The pasta was placed between a pair of glass plates and their inner core was observed by tightly pressing plates together (approved method 16-50 AACC, 2000). Minimum cooking time was determined by the point at which the white core of the sample disappeared, indicating the penetration of water in the core of pasta.

3.3.1.2 Water uptake ratio

Pasta (10 g) was cooked in 100 mL distilled water for the minimum cooking time (sec). The cooked pasta was rinsed with cold water and excess water was blotted with paper towels. Water absorption was determined from the gain in weight after cooking and results were reported as per cent water absorption. Cooking water was collected for determination of cooking loss (AACC, 2000).

3.3.1.3 Volume expansion

10 g of sample was immersed in 200 mL distilled water contained in a 250 mL measuring cylinder. Increase in volume was recorded as initial volume of the product. The sample boiled in 100 mL distilled water for minimum cooking time, washed, blotted and again added to 200 mL water contained in 200 mL measuring cylinder. Increase in volume was recorded and expressed as mL/g volume expansion.

3.4.1.2.4 Gruel solid loss

Cooking loss was determined according to the approved AACC method (AACC 2000). Aliquots (50 mL) from the cooking water were drawn and placed in petri-dishes for evaporation to dry in an oven at 105 °C. Weight of solid residue obtained was expressed as per cent solid loss during cooking.

$$\text{Gruel solid loss (\%)} = \frac{\text{Wt. of solid residue} \times \text{Total vol. of cooking water}}{\text{Vol. of aliquot taken for evaporation} \times \text{Wt. of sample}} \times 100$$

3.4 Textural attributes

Textural attributes i.e. firmness and toughness of samples were evaluated using the Texture Analyzer (Model: TA-XT plus, Stable Micro Systems, USA) using (75 mm) probe. For measuring these parameters 50 kg load cell was used, Pre-test and Post-test speed were set to 2.0 mm/s and 10.0 mm/s, respectively with 30 per cent compression of the original height. The maximum force required to shear the pasta was taken as firmness from the force-time graph. Toughness values were also noted by resultant file.

3.5 Sensory evaluation

All product samples were coded and evaluated for degree of liking or disliking on a 9-point scale, using descriptive categories ranging from like extremely, to dislike extremely (Larmond 1970). The samples were presented randomly in identical containers and the panelists were asked to check the appropriate category on the scale. Cooked pasta was evaluated for sensory attributes (Appearance and colour, Flavour, Body and Texture and Overall acceptability) by a panel of semi-trained judges. The following Performa was used for sensory evaluation.

SENSORY EVALUATION PERFORMA

Product: Date:

Sample No.	Appearance and colour	Flavour	Body and Texture	Overall acceptability	Comment (if any)

SCORING:

Liked extremely- 9	Liked slightly – 6	Disliked moderately - 3
Liked very much – 8	Neither liked nor disliked – 5	Disliked very much – 2
Liked moderately -7	Disliked slightly – 4	Disliked extremely – 1

3.6 Instrumental colour profile analysis

Colour profile was measured using CR-400 Konica Chroma meter (Konica Minolta, Japan) set at 2^{o} of cool white light (d_{65}) and known as '*l*', *a*, and *b* values. '*l*'

value denotes (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/-blueness). 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 pasta at three different points.

3.7 Peroxide value

The peroxide value was measured as per the procedure described by Koniecko (1979), with suitable modifications. Five gram of pasta sample 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).

$$\text{PV (meq/Kg sample)} = \frac{\text{0.1x mL 0.1N sodium thiosulphate}}{\text{Wt. of sample (g)}} \times 1000$$

3.8 Free fatty acids (FFA)

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

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

3.8.1 Thiobarbituric acid reactive substances (TBARS) value

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

3.9 Proximate composition

The moisture, protein, fat, fiber and ash content of the pastawere estimated by using hot air oven, Kel Plus, Socs Plus and Muffle furnace, respectively.

3.9.1 *Moisture*

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

3.9.2 *Protein estimation*

The protein content of the samples 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). Approximately 0.2-0.3 g moisture free pasta sample 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 ºC in the digestion unit. The appearance of clear green coloured liquid indicated the completion of digestion. The sample was cooled and then diluted with distilled water (10-20 mL). Exactly 40 mL of 40 percent sodium hydroxide was added to the aliquot to make it alkaline. Distillation was carried out automatically in the distillation unit. The ammonia liberated during the process gets collected in boric acid containing an 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 the light pink end point. The percentage crude protein was calculated using the following formula. A parallel blank was run to eliminate the error.

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

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

3.9.3 Fat estimation

Fat content was estimated as Ether Extraction following AOAC 1995 method using Socs Plus (SCS-6-AS, Pelican Industries, Chennai, India). Dried, ground and weighed pasta sample was taken in an extraction thimble (Whatman No.1 filter paper) fitted in a specially designed beaker. The Initial weight of the empty beakers was noted (W_1). The thimble with the sample was 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^{o}C) for 20-30 min. The beakers were removed and cooled in a desiccator. The final weight of the beakers was noted as W_2. The fat percentage in the sample was calculated using the following formula.

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

3.9.4 Ash estimation

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

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

3.9.5 Fatty acid estimation

Fatty acid profile estimation was carried out at *Opal Research & Analytical Services*, Ghaziabad, Uttar Pradesh, India 201007 having Reg. No. UPS052827002630.

3.10 Microbiological quality

Standard plate count, yeast count, mould count and coliform count in the samples were enumerated following the methods as described by American Public Health Association (APHA 1984).

3.10.1 Preparation of sample and serial dilution

The sample bags were opened in a laminar flow (Model: RH-58-03, Rescholar equipment, Ambala, India) pre-sterilized by ultra-violet (UV) radiation. 10 g of sample 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. 1mL of this diluted solution was taken with a micropipette having a sterile tip into a sterile test tube containing presterilized 0.1% peptone water for further dilution to a level of 10^{-2}. Similarly, further dilutions were prepared according to the requirement. Preparations of the sample and serial dilutions were done near flame in a horizontal laminar flow observing all possible aseptic conditions.

3.10.2 Standard plate count (SPC)

23.5 g of plate count agar (Hi-Media Laboratories Pvt. Ltd. Mumbai (M091S)) was suspended in 1000 mL of distilled water. It was boiled to dissolve the medium completely and final pH (7.0±0.1) was adjusted. It was sterilized by autoclaving at 15 lb pressure (121 ^{o}C) for 15 min. The pour plate method was followed for enumeration of bacterial colonies. About 20 mL of the sterilized molten media kept at 45±2 ^{o}C was inoculated aseptically to each duplicate set of Petri plates with 1mL of aliquots. Then, these were gently stirred for uniform distribution of the aliquots. The plates were allowed to stand for some time till the agar media got solidified. The plates were then inverted and incubated at 35±2 ^{o}C for 24 h. Following incubation, the plates showing 30-300 colonies were counted. The average number of colonies were multiplied by the reciprocal of the dilution and expressed as $\log_{10}$cfu/g of sample.

3.10.2 Coliform count

Coliform count were enumerated using selective media, Violet Red Bile Agar (VRBA, M581A) procured from Hi-Media Laboratories Pvt. Ltd. Mumbai. A total of 51.53 g of media was suspended in 1000 mL of distilled water, boiled to dissolve the medium completely and cooled to 45 ^{o}C. The final pH of the medium was adjusted to 7.4±0.2. Pour plate technique was followed for inoculation of the suitable sample dilution and the plates were incubated at 35±2 ^{o}C for 24 h and results were expressed as $\log_{10}$cfu/g of sample.

3.10.3 Yeast and mould count

39.0 g of potato dextrose agar (PDA- M096, Hi-Media Laboratories Pvt. Ltd., Mumbai, India) was suspended in 1000 mL distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure at temperature 121 °C for 15 min. The pH of the sterilized medium was set to pH 3.5 by acidification with 10% tartaric acid solution. 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 appearing on the plates were counted and expressed as Log cfu/g.

3.11 Microstructure

The structural morphology of raw and cooked pasta was studied using a scanning electron microscope (SEM) (Model JSM6100 (Jeol) with Image Analyser) at Sophisticated Analytical Instrumentation Facility, CIL and UCIM, Punjab University, Chandigarh, Punjab, India. The cross-section of the dried pasta was transferred onto a holding pan and sputter-coated with gold using a vacuum evaporator for 2-3 min. Sputter coated pasta sample was transferred to the microscope stage where it was examined at an accelerating voltage of 15 kV and a vacuum of 9.75 × 10-5 Pa.

3.12 Statistical analysis

The experimental results of the response surface design were analysed using Design Expert® 11.0 (Stat-Ease Inc., Minneapolis, MN, USA) programs. The significance level was based on a confidence level of 95 % (Myers and Montgomery 2009). Data were analyzed statistically on IBM SPSS Statistics-20.0 software, USA packages as per standard methods (Snedecor and Cochran 1994). Duplicate samples

were drawn for each parameter and replicated thrice (n=6). Sensory evaluation was performed by a panel of seven judges, total observations were 21 (n=21). Means between the periods of storage, between treatments, and within treatments were compared by two-way analysis of variance (ANOVA) and critical difference test. The statistical significance was estimated at 5% level (p<0.05) and evaluated with Duncan‘s multiple range test. The results were presented in the form of Mean±S.E.

CHAPTER IV

RESULTS AND DISCUSSION

The objectives of the present study were to optimize the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta rich in protein. The developed pasta was evaluated for the storage stability at ambient temperature (20-30±5 ºC) for 60 days wrapped in aluminium foil-LDPE laminate pouches. The production cost for development of functional pasta was also calculated. The present chapter details the results obtained from different experiments carried out in accordance with the above-mentioned objectives and the results are presented in the text with the support of statistically analyzed Tables (3 to 18) and Figures (3-23). The critical analyses of the results with suitable support of available literature to draw the inference have been attempted in the present chapter.

4.1 Experiment No. 1: Optimization of the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta

4.1.1 Experimental design

Response surface methodology (RSM) used to optimize the level of incorporation of chicken meat powder and microencapsulated docosahexaenoic acid (DHA) powder as two composition coded/uncoded variables (Table 3). The experiments were formulated according to a Central Composite Design (CCD) resulting in 13 experimental design of Independent variables (Table 4). In order to develop the most acceptable functional pasta by incorporating chicken meat powder and DHA powder responses were demonstrated by various cooking parameters viz. (Minimum cooking time (MCT), Volume expansion (VE), Water uptake ratio (WUR), Gruel Solid loss (GSL), Colour analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability; OA). The fitness of the polynomial model equation to the responses was evaluated by the coefficient of R square as well as by the lack of fit using the F-test with 5% level of significance.

Table 3: Coded and uncoded levels of independent variables used in Central Composite Design (CCD) for formulation of functional pasta

Symbols	Independent variables	Coded levels		
		-1	0	+1
A	Chicken meat powder	20	30	40
B	Docosahexaenoic acid (DHA)	1	1.5	2.0

Table 4: Experimental design of Independent variables for optimization of level of incorporation of chicken meat powder and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta

Run	Independent variables (Factors)	
	Chicken meat powder (%)	DHA (%)
1	30	1.5
2	30	1.5
3	40	1.0
4	40	1.5
5	20	1.0
6	30	2.0
7	40	2.0
8	30	1.5
9	30	1.5
10	20	2.0
11	30	1.5
12	30	1.0
13	20	1.5

4.1.2 Fitting the models

Each response was evaluated as a function of linear, quadratic and interaction effect of the independent variables viz. chicken meat powder and DHA powder. The experimental data were fitted into the second-order polynomial equations and the regression coefficients were calculated, the significance of the coefficients of the models were determined by analysis of variance (ANOVA) as summarized in Table 5-7. The quality of the generated model was evaluated by ANOVA, R^2, and the lack of fit of the model. The ANOVA results in Table 5-7and Fig. 3-12 suggested that the model had very high *F*-values and very low *p*-values (<0.0001) for all 10 studied responses. Coefficient of variation (CV) describes the extent to which the data were dispersed. The CV values (ranging from 0.0755 to 28.02) for the proposed models indicated the high precision and reliability of the experiments.

In addition, high R^2 values and non-significant 'Lack of Fit' (p>0.05) were observed in Table 5-7, indicating that the quadratic model was highly significant to obtained data and capable of describing the relationship between the formulation conditions and studied responses. The larger regression coefficient in a model with significant *p*-value indicated a more significant effect on the respective response variables.

The coefficients of multiple determinations (R^2) 0.9990, 0.5225, 0.7947, 0.8690, 0.7986, 0.5699, 0.9977, 0.8959, 0.9327 and 0.6187 were obtained for the response of MCT, L, *a**, *b**, firmness, toughness, GSL, VE, WUR and OA respectively. It indicated that the second-order polynomial models were adequately represented by the respective experimental data.

Minimum cooking time (MCT)

The response surface analysis as shown in Table 5 demonstrated that the relationship between chicken meat and DHA powder with relation to MCT is quadratic with a good regression coefficient (R^2= 0.9998), and model equation exhibited the relationship as per equation as follows.

$$\mathbf{MCT=18.03+1.42A+0.02B+0.00A-0.06A^2-0.00B^2}$$

The ANOVA of the quadratic regression models showed that the model was

significant (p<0.05) with p-values of <0.0001. The statistical analyses revealed that chicken meat (%) had both significant linear and interaction effects (p<0.0001) on the MCT of formulated pasta followed by the linear term effects of DHA powder. Thus, predicated model results suggested that a chicken meat powder (%) significantly increased the minimum cooking times. Furthermore, there was non-significant (p>0.05) quadratic term effect of chicken meat powder (%) and DHA powder (Table 5).

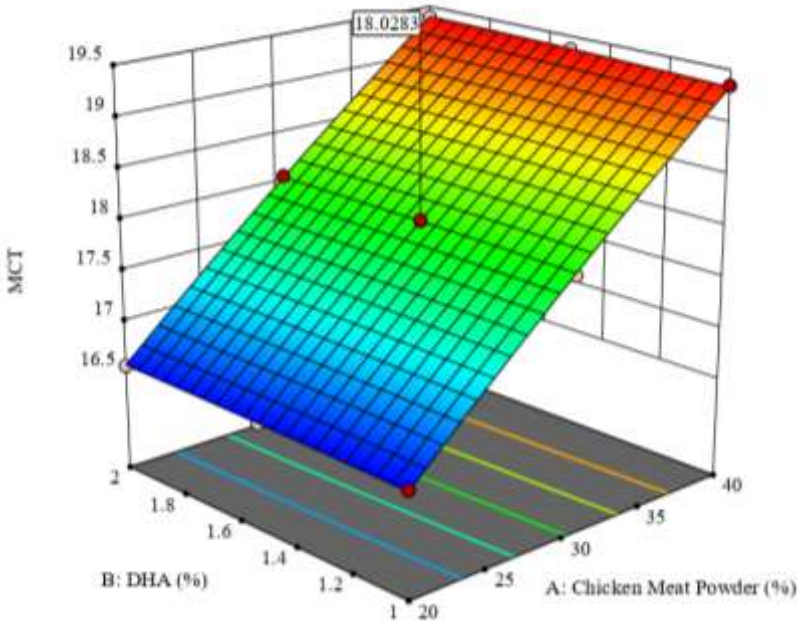

Fig. 3: 3D Response Surface plots for MCT

3D graphs (Fig. 3) showed the effect of chicken meat and DHA powder on MCT. It was observed that MCT increased gradually with the increase in chicken meat (%) with least effect of DHA powder. Increase in the level of chicken powder in the pasta increased the minimum cooking time. This was due to addition of chicken meat powder, which restricted supply of water to starch granules (semolina) present in the pasta strands and delays the swelling of the granules and possibly slowed down the start of the gelatinization process resulted in increase in MCT(Gupta *et al* 2020).

Oh *et al* (1985) observed a linear relationship between protein content and MCT of pasta. Results of Kaur *et al* (2013) and Surasani *et al* (2019) agree with the present study. They reported when protein-rich pasta gets cooked, it becomes firmer and stronger internally than pasta with low protein content. Sharma *et al* (2013)

reported that protein hinders the hydration and swelling capacity of starch granules by surrounding them, resulting in increase in the MCT of pasta. The optimized MCT predicted by response surface methodology was 18.02% under the formulation condition with chicken meat powder of 30% and DHA powder of 1.5%.

Volume Expansion (VE)

RSM as demonstrated (Table 5) followed a high regression value (R^2 =0.9675) for the VE in the functional pasta and second order polynomial equation explained the relationship as follows,

$$\mathbf{VE= 1.48-0.05A-0.01B+0.00AB+0.02A^2+0.00B^2}$$

ANOVA of the linear and interaction regression models for VE showed that the model was significant *(p*<0.05) with p-values of <0.0001. Table 5 showed that chicken meat powder (%) had significant *(p*<0.0001) linear effects as well as interaction effect on VE. The ANOVA table showed that there was non-significant quadratic term effect for both the variables i.e. chicken meat (%) and DHA powder. The Model F-value of 1.48 implies the model is significant. There is only a 0.01% chance that an F-value such large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case A, B, A^2 and B^2 are significant model terms. The Lack of Fit F-value of 0.4093 implies the Lack of Fit is not significant relative to the pure error.

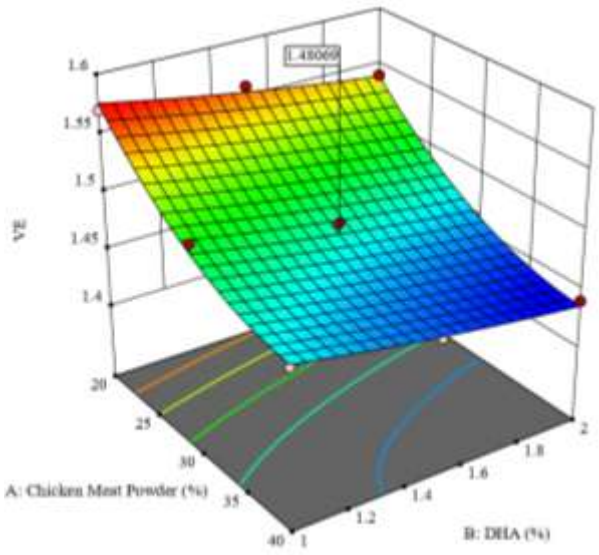

Fig. 4: 3D Response Surface plots for VE

Table 5: ANOVA and regression coefficients of the second-order polynomial model for the response variables of developed pasta

Source	df	*MCT*			VE			WUR			GSL		
		Coefficient	**Sum of squares**	**p-Value**	**Coefficient**	**Sum of Squares**	**p-Value**	**Coefficient**	**Sum of squares**	**p-Value**	**Coefficient**	**Sum of squares**	**p-Value**
Model	5	18.03	12.06	<0.0001	1.48	0.0198	<0.0001	1.72	0.1535	<0.0001	17.62	3.49	<0.0001
Linear													
A	1	1.42	12.04	<0.0001	-0.0517	0.0160	<0.0001	-0.1433	0.1233	<0.0001	0.7583	3.45	<0.0001
B	1	0.0267	0.0043	0.0031	-0.0183	0.0020	0.0005	0.0100	0.0006	0.1282	0.0250	0.0037	0.0024
Quadratic													
$A_1 B_2$	1	0.0025	0.0000	0.7447	0.0000	0.0000	1.000	-0.0025	0.0000	0.7351	0.0075	0.0002	0.2955
Interaction													
A_1^2	1	-0.0690	0.0131	0.0001	0.0226	0.0014	0.0014	-0.0993	0.0272	<0.0001	-0.0991	0.0271	<0.0001
B_2^2	1	-0.0090	0.0002	0.3463	0.0026	0.0000	0.5799	0.0107	0.0003	0.2511	0.0009	2.053E-06	0.9170
Residual	7		0.0015			0.0004			0.0014			0.0012	
Lack of fit	3		0.0011	**0.1172**		0.0002	**0.4093**		0.0011	**0.0687**		0.0007	**0.2823**
Pure error	4		0.0004			0.0002			0.0003			0.0005	
Total	12		12.06			0.0202			0.1549			3.49	
Adj. R^2		**0.9998**			**0.9675**			**0.9844**			**0.9994**		
Pred. R^2		**0.9990**			**0.8959**			**0.9327**			**0.9977**		
C.V. %		0.0820			0.4963			0.8480			0.0755		

A- Chicken meat powder**;** B- DHA powder

MCT(min)**:-**Minimum cooking time, VE(mL/ g**:-**Volume expansion, WUR(g/100 g)**:-**Water uptake ratio,GSL (g/ 100 g)**:-**Gruel solid loss.

3D graphs as shown in Fig 4 depicts the effect of chicken meat powder (%) and DHA powder on the VE. It was observed that VE decreased with the increasing chicken meat powder (%) and nearly reached at lowest point with highest chicken meat powder (%) tested i.e. 40%. The increase in DHA powder showed less significant effect on VE. Hence, it was concluded that, increase in the level of chicken meat powder in the pasta decreased the volume expansion of pasta, which was due to decrease in the starch content and increase in the level of protein and fiber in the pasta (Liu *et al* 2016). The protein and fiber have lower swelling power in comparison to starch and they swell less when cooked in comparison to starch, due to which the pasta with meat exhibits lower volume expansion (Sharma *et al* 2013).

Optimized model predicated by RSM showed 1.48% of volume expansion under the formulation condition; chicken meat of 30% and DHA powder of 1.5%.

Water Uptake Ratio (WUR)

Table 5 shows the effects of chicken meat powder (%) and DHA powder on the water uptake ratio of developed pasta. In addition, Fig. 5 illustrates these effects as three-dimensional graphs (3D), where direction of the effects of formulation variables on WUR can be evaluated. The second-order regression model equation predicting effect of formulation variables on WUR is as follows:

$$\mathbf{WUR=1.72-0.14A-0.01B-0.00AB-0.09A^2+0.01B^2}$$

The Model F-value of 1.72 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case, only A and A^2 are significant model terms, which implies that chicken meat powder (%) significantly affected WUR. The Lack of Fit F-value of 0.068 implies the Lack of Fit is not significant relative to the pure error.

The Fig. 5 demonstrated the effect of chicken meat powder (%) and DHA powder on WUR. It was observed that with the increase in chicken meat powder (%) from 20 to 40% there is significant decrease in WUR, whereas DHA powder showed least or non-significant effect on WUR as predicated by the model terms. This effect of chicken meat powder on the WUR is due to lower swelling power and swelling index of chicken meat (Ghumman *et al* 2016)).

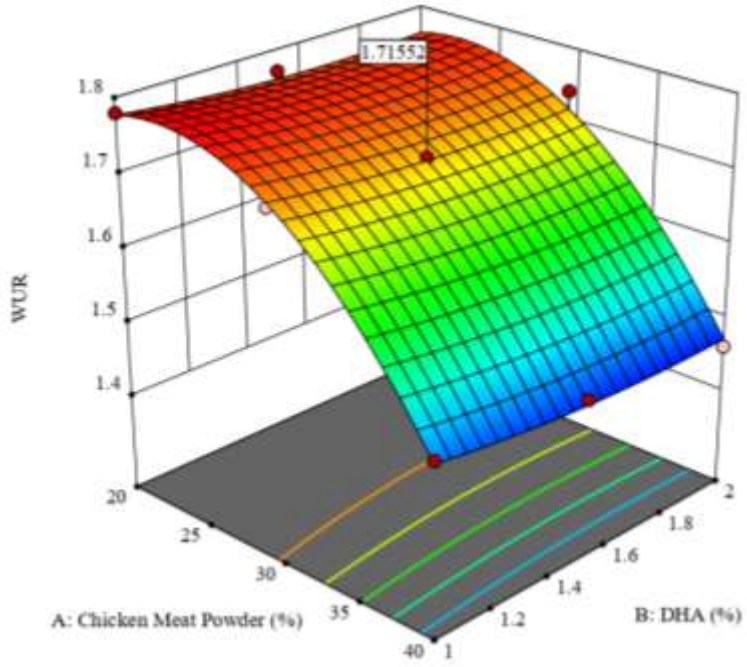

Fig. 5: 3D Response Surface plots for WUR

RSM indicated 1.71 % of water uptake ratio under the formulation condition; chicken meat of 30% and DHA powder of 1.5 %.

Gruel Solid Loss (GSL)

Cooking loss, related to solid leaching during cooking, is widely used as an indicator of the overall cooking performance (Gull *et al* 2015). The response surface analysis as shown in Table 5, demonstrated that the relationship between chicken meat and DHA powder with relation to GSL is linear and interaction term with a good regression coefficient (R^2= 0.9994), and model equation exhibited the relationship as per equation as follows.

$$\mathbf{GSL=17.62+0.75A+0.02B+0.00AB-0.09A^2+0.00B^2}$$

The linear and interaction regression models showed that the model was significant (p<0.05) with p-values of <0.0001. The statistical analyses revealed that chicken meat powder (%) had both significant linear and interaction effects (p<0.0001) on the GSL of formulated pasta followed by the linear term effects of DHA powder. Thus, predicated model results suggested that chicken meat powder (%) significantly affected the GSL. DHA powder showed non-significant (p>0.05) quadratic and interaction term effect of chicken meat (%) and DHA powder (Table 5).

Table 6: ANOVA and regression coefficients of the second-order polynomial model for the response variables of developed pasta

Source	Df	*Firmness*			Toughness			*OA*		
		Coefficient	Sum of squares	p-Value	Coefficient	Sum of Squares	p-Value	Coefficient	Sum of squares	p-Value
Model	2	1.48	11.45	<0.0001	1.31	2.49	0.0021	7.91	6.76	0.0018
Linear			9.89							
A	1	1.28	1.57	<0.0001	0.6003	2.16	0.0010	-0.2000	0.2400	0.1677
B	1	0.5108		0.0128	0.2335	0.3271	0.1032	0.2000	0.2400	0.1677
Quadratic										
$A_1 B_2$								0.0750	0.0225	0.6518
Interaction										
A_1^2								-1.17	3.81	0.0005
B_2^2								-0.4241	0.4968	0.0624
Residual	10		1.71			1.02			0.7092	
Lack of fit	1.13		1.13	**0.4223**		0.3437	**0.8849**		0.3292	**0.4295**
Pure error	0.5855		0.5855			0.6740			0.3800	
Total	13.17		13.17			3.51			7.47	
Adj. R^2	**0.8438**		**0.8438**		**0.6518**			**0.8373**		
Pred. R^2	**0.7986**		**0.7686**		**0.5699**			**0.6187**		
C.V. %	**28.02**		28.02		24.30			4.44		

A- Chicken meat powder**;** B- DHA powder; Firmnes**s** (N)**,**Toughness(N) O.A**:-**Overall acceptability**.**

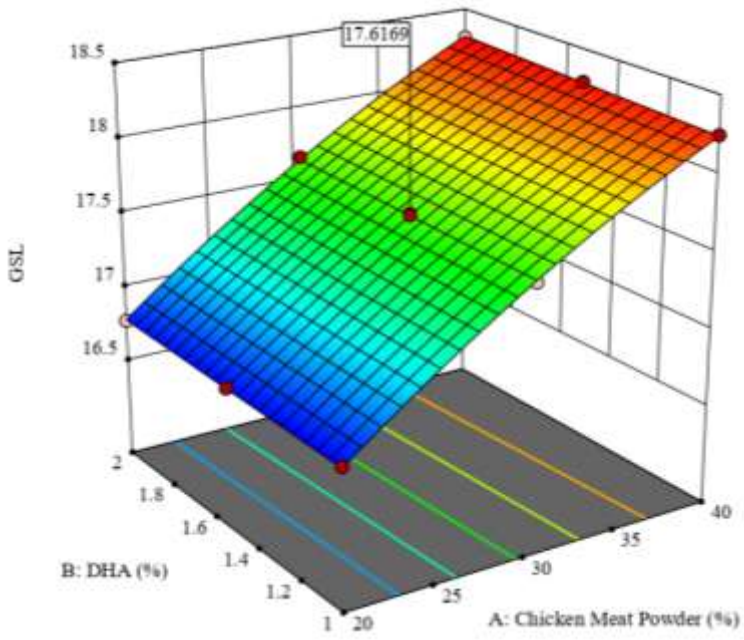

Fig. 6: 3D Response Surface plots for GSL

Three-dimensional graphs (Fig. 6) showed the effect of chicken meat and DHA powder on GSL. It was observed that GSL increased significantly with the increase in chicken meat powder (%) with non-significant effect of DHA powder on GSL. Increase in GSL with increasing levels of chicken meat powder (%) was due to the weak meat protein and starch matrix (semolina) interaction that destroyed during cooking) (Gopalakrishnan *et al* 2011). The higher gruel solid loss can also be attributed to the dilution of gluten protein and thereby weakening of starch and protein matrix. The model predicated 17.61% of GSL by keeping 30% of chicken meat and DHA powder of 1.5%.

Firmness

Texture has been defined as one of the important attribute of pasta structure and textural characteristics are recognized as more important for consumers. Functional properties determine the quality characteristics of pasta including firmness, gruel loss and overall acceptability. High quality pasta has a good cooking resistance and firmness, does not release an excessive amount of organic matter into the cooking water and does not show stickiness. Moreover, the pasta quality is related to a low breakage susceptibility to dry conditions (Chillo *et al* 2010).

Table 6 shows the effects of chicken meat powder (%) and DHA powder on the firmness of developed pasta. In addition, Fig. 7 illustrate these effects as three-

dimensional graphs (3D), where direction of the effects of formulation variables on firmness can be predicated. The second-order regression model equation predicting effect of formulation variables on firmness is as follows:

Firmness=1.48+1.28A+0.51B

The Model F-value of 1.48 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case, only linear term effect of A and B are significant model terms, which imply that chicken meat powder (%) significantly affected firmness. The Lack of Fit F-value of 0.068 implies the Lack of Fit is not significant relative to the pure error.

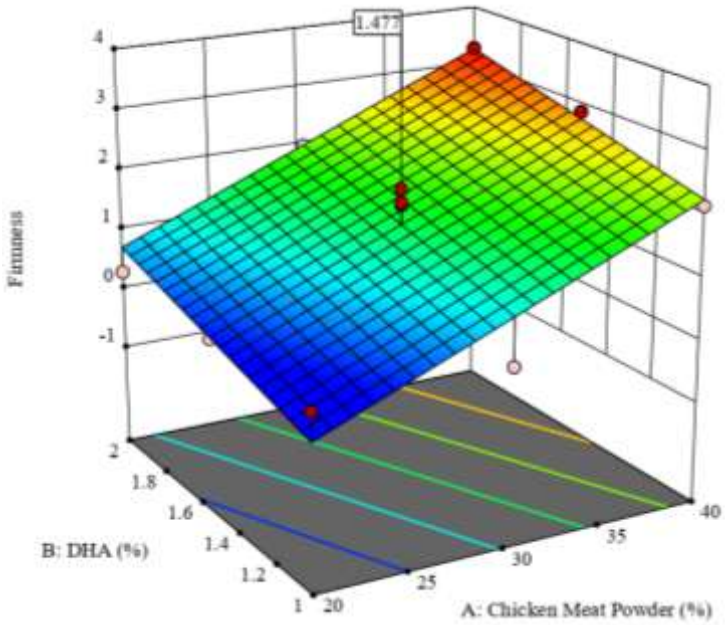

Fig. 7: 3D Response Surface plots for firmness

The Fig. 7 demonstrated the effect of chicken meat powder (%) and DHA powder on firmness. It was observed that with increase in chicken meat powder (%) from 20 to 40%, there was a significant increase in firmness. This was due to the fact that meat proteins interacted with the insoluble networks of pasta, forming stable matrix structures, and leading to higher firmness and toughness of pasta with chicken meat in comparison to control pasta, these results were justified by SEM analysis in our study (Liu *et al* 2016). More force was required to break samples with higher meat content, indicating that meat powder functioned as an effective ingredient to fortify the pasta structure network. Studies demonstrated that incorporation of a higher amount of protein in pasta could cause higher textural intensity including

firmness and toughness (Chang & Wu, 2008). The RSM models showed 1.47% of firmness under the formulation condition; chicken meat of 30% and DHA powder of 1.5%.

Toughness

The textural characteristics of pasta play a crucial role in determining the final consumer acceptability. The optimization of texture parameters is a critical point to ensure the acceptance of products by the consumers. The matrix structural network of starches, glutens, additional proteins and other ingredients mainly affects textural properties of pasta (Mudgil *et al* 2016). High quality pasta has a good cooking resistance and firmness resulted in less or no release of an excessive amount of organic matter into the cooking water without stickiness (Day and Swanson 2013)

Table 6 shows the effects of chicken meat powder (%) and DHA powder on the toughness of developed pasta. In addition, Fig. 8 illustrates these effects as three-dimensional graphs (3D), where direction of the effects of formulation variables on toughness can be predicated. The second-order regression model equation predicting effect of formulation variables on firmness is as follows:

Toughness=1.31+0.60A+0.23B

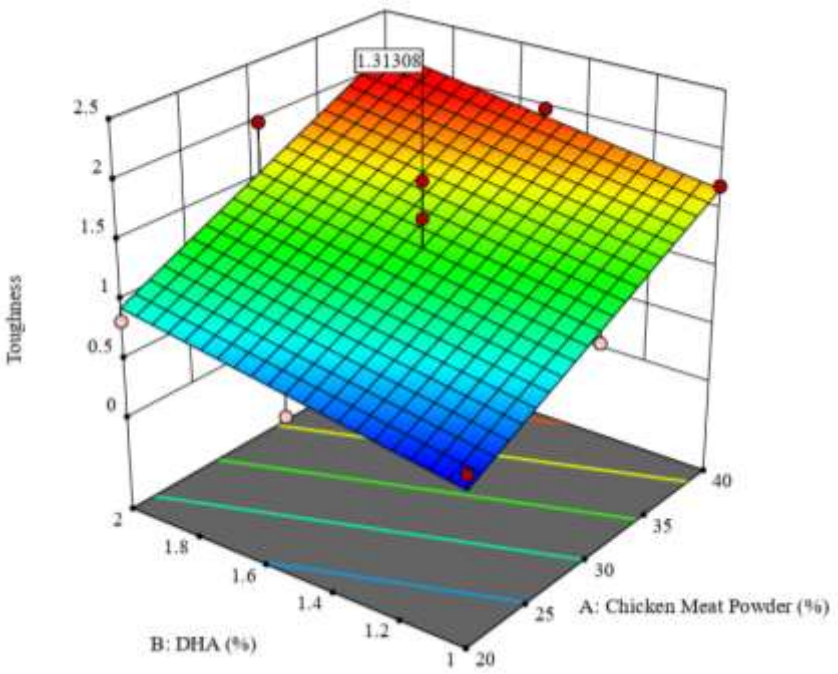

Fig. 8: 3D Response Surface plots for toughness

The Model F-value of 1.31 implies the model is significant. In this case only linear term effect of A is significant model terms, which implies that chicken meat powder (%) significantly affected toughness. The Lack of Fit F-value of 0.88 implies the Lack of Fit is not significant relative to the pure error. The Fig. 8 demonstrated the effect of chicken meat powder (%) and DHA powder on toughness. It was observed that with the increase in chicken meat powder (%) from 20 to 40% resulted in significant (p<0.05) increase in toughness. These results are in accordance to firmness, which might be due to the fact that meat proteins interacted with the insoluble networks of semolina, forming stable matrix structures, and leading to higher firmness and toughness of pasta with chicken meat in comparison to control pasta (Liu *et al* 2016). The RSM models showed 1.31% of toughness under the formulation condition; chicken meat of 30% and DHA powder of 1.5%. The SEM analysis of cooked pasta studies justified the increase in firmness and toughness of developed pasta.

L value

The colour is an important parameter in determining the quality and acceptability of pasta. The effects of chicken meat powder (%) and DHA powder on L value of developed pasta showed in Table no 7. In addition, Fig. 9 illustrates these effects as 3D graphs, where direction of the effects of formulation variables on L value can be evaluated. The second-order regression model equation predicting effect of formulation variables on L value is as follows:

$$L=58.27-2.47A-1.09B-0.82AB+1.82A^2+1.49B^2$$

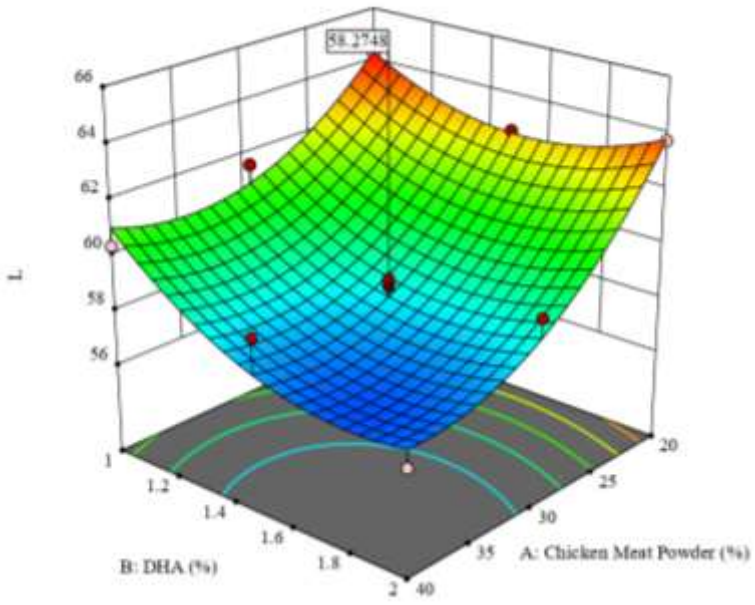

Fig. 9: 3D Response Surface plots for L value

Table 7: ANOVA and regression coefficients of the second-order polynomial model for the response variables of developed pasta

Source	Df	*L*			a*			*b**		
		Coefficient	Sum of squares	p-Value	Coefficient	Sum of Squares	p-Value	Coefficient	Sum of squares	p-Value
Model	2	58.27	71.05	0.0035	3.23	20.75	<0.0001	19.63	32.02	<0.0001
Linear										
A	1	-2.47	36.67	0.0012	1.84	20.38	<0.0001	2.25	30.34	<0.0001
B	1	-1.09	7.16	0.0525	0.2470	0.3661	0.2773	0.5300	1.69	0.0273
Quadratic										
$A_1 B_2$		-0.8260	2.73	0.1931						
Interaction										
A_1^2		1.82	9.13	0.0338						
B_2^2		1.49	6.12	0.0681						
Residual	10		9.22			2.77			2.53	
Lack of fit	1.13		3.94	**0.4800**		2.04	**0.2869**		1.93	**0.2424**
Pure error	0.5855		5.27			0.7341			0.6030	
Total	13.17		80.27			23.52			34.55	
Adj. R^2	**0.8438**	**0.8032**			**0.8585**			**0.9122**		
Pred. R^2	**0.7986**	**0.5225**			**0.7947**			**0.8690**		
C.V. %	**28.02**	1.92			16.32			**2.56**		

A- Chicken meat powder**;** B- DHA powder; *L** (lightness), *a** (green to redness) and *b** (blue to yellowness)

The high Model F-value of 58.27 implies the model is significant. In this case, only linear term and interaction term effect of A had significant effect on L value of developed pasta, which implies that chicken meat powder (%) significantly affected L value. The Lack of Fit F-value of 0.048 implies the Lack of Fit is not significant relative to the pure error. The Fig. 9 demonstrated the effect of chicken meat powder (%) and DHA powder on L value, which implicating that with the increase in chicken meat (%) there is significant decrease in L value, which might be due to the inherent colour of raw ingredients i.e. chicken meat colour as it has low L value and higher *a** and *b** values. It was found that higher redness and corresponding decrease in lightness of colour were probably due to the colour of myoglobin in chicken meat powder that changes to the bright red oxymyoglobin (Desai *et al* 2018). RSM indicated 58.27 of L value under the formulation condition; chicken meat of 30% and DHA powder of 1.5%.

_a*_ and _b*_ value

The response surface analyses (Table 7) demonstrated a high regression value (R^2 =0.8585) for *a** value and second order polynomial equation explained the relationship.

$$a^* = 3.23 + 1.84A + 0.24B$$

It was observed that chicken meat powder (%) had significant (p<0.0001) linear term effects on *a** value. DHA powder had least significant effect on *a** value as shown in Table 9. The effect of formulation parameters on *a** value and their interactions are shown in Fig. 10.

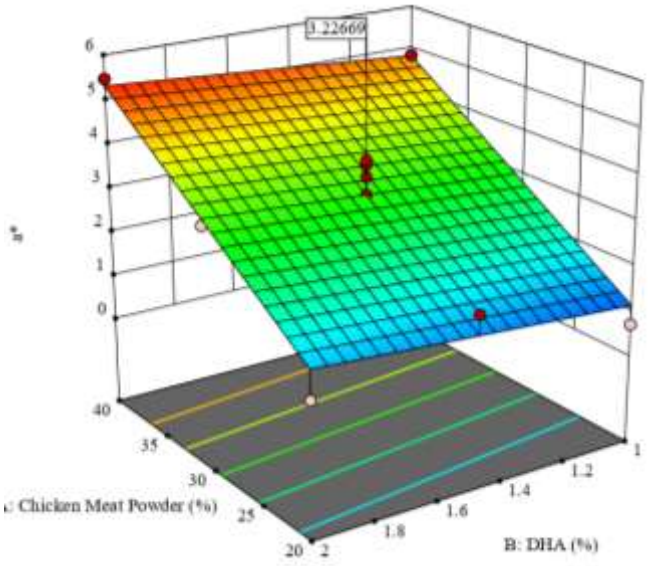

Fig. 10: 3D Response Surface plots for a* value

Increasing chicken meat percent showed linear increase in *a** values as per Fig. 7. It was found that higher redness and corresponding decrease in lightness of colour were probably due to the colour of myoglobin in chicken meat powder that changes to the bright red oxymyoglobin (Desai *et al* 2018). The optimal formulation levels of selected variables determined by RSA were chicken meat of 30% and DHA powder of 1.5% exhibiting a* value of 3.22.

b* **value**

The response surface analyses (Table 7) demonstrated a high regression value (R^2 =0.91) for *b** value and second order polynomial equation explained the relationship.

$$b^* = 19.63 + 2.25A + 0.53B$$

It was observed that chicken meat powder (%) had significant (p<0.0001) linear term effects on *b** value. DHA powder had least significant effect on *b** value as shown in Table 9. The effect of formulation parameters on *b** value and their interactions are shown in Fig. 11. Increasing chicken meat percent showed linear increase in b* values as per Fig. 7. The optimal formulation levels of selected variables determined by RSA were chicken meat of 30% and DHA powder of 1.5% exhibiting b* value of 19.63.

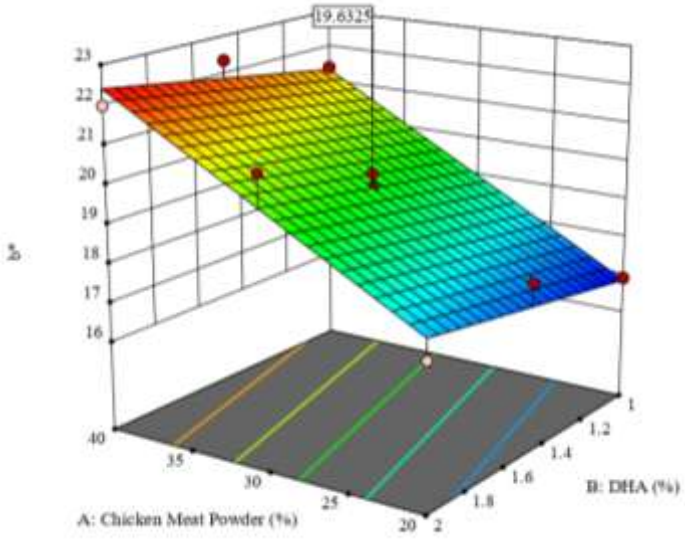

Fig. 11: 3D Response Surface plots for b* value

Overall acceptability (OA)

Sensory evaluation particularly overall acceptability is most reliable test as it allows overall characteristics of any foods.RSM as demonstrated (Table 6) followed a

high regression value (R^2 =0.83) for the OA in the developed pasta and second order polynomial equation explained the relationship as follows,

$$\mathbf{OA= 7.91-0.20A+0.20B+0.07AB-1.17A^2-0.42B^2}$$

ANOVA of interaction regression models for OA showed that the model was significant (p<0.05) with p-values of <0.0001. Table 6 showed that chicken meat powder (%) had significant (p<0.0001) interaction effects on OA. The ANOVA table showed that there was non-significant(p>0.05) effect for DHA powder. The Model F-value of 1.48 implies the model was significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case only A^2 were significant model terms. The Lack of Fit F-value of 0.42 implies the Lack of Fit is not significant relative to the pure error.

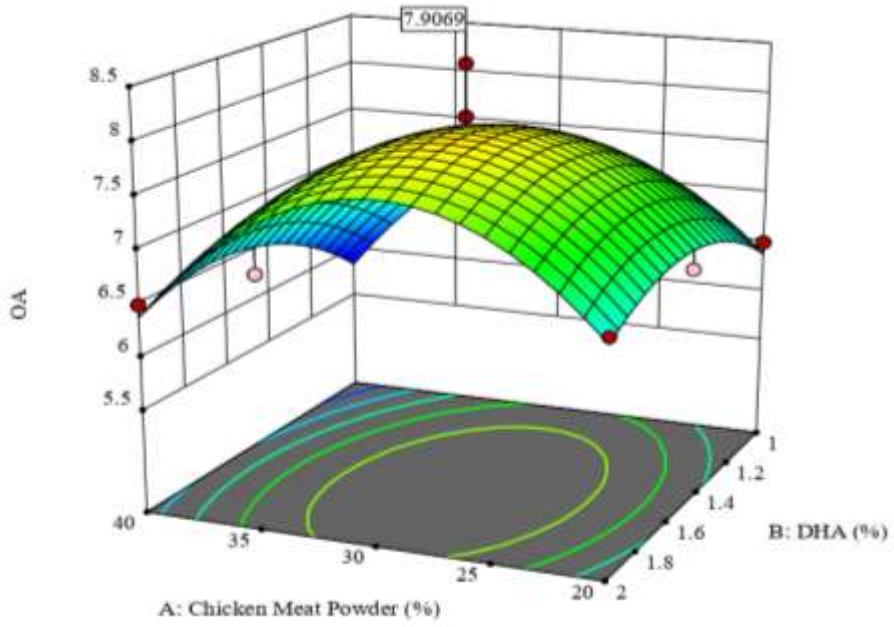

Fig. 12: 3D Response Surface plots for Overall acceptability

Fig. 12 represents the effect of chicken meat powder (%) and DHA powder on the OA. It was observed that OA decreased with the increasing chicken meat powder (%) and nearly reached at lowest point with highest chicken meat powder (%) tested i.e. 40%. Similar findings were recorded by Yadav *et al* (2014) reporting this may be due to unattractive dark colour of cooked pasta, which limits the wider acceptability of the developed pasta products. Optimized model predicated by RSM showed 7.91% of overall acceptability under the formulation condition; chicken meat of 30% and DHA powder of 1.5%.

4.1.3 Proximate and fatty acids profile of developed pasta.

Developed pasta from Experiment 1 was subjected for proximate and fatty acids estimation. Table 8 depicted the proximate composition of both control and developed pasta. It was found that Moisture (%), Protein (%), Fat (%) and Ash (%) in control was 10.89±0.11, 10.28±0.09, 1.01±0.04 and 0.57±0.01, respectively. Whereas, developed pasta showed 13.28±0.06 Moisture (%), 26.25±0.02 Protein (%), 2.41±0.02 Fat (%) and 3.34±0.08 Ash (%) contents.

Table 8: Proximate composition of control and developed functional pasta

Proximate composition	Control pasta	Developed functional pasta
Moisture (%)	10.89±0.11[a]	13.28±0.06[b]
Protein (%)	10.28±0.09[a]	26.25±0.02[b]
Fat (%)	1.01±0.04[a]	2.41±0.02[b]
Ash (%)	0.57±0.01[a]	3.34±0.08[b]

n=6**,** *Mean ± SE. with different superscripts column wise (capital alphabets) differ significantly (p<0.05).

It was evident from Table 8 that incorporation of 30% chicken meat significantly (p<0.05) increased moisture content (p<0.05) in developed functional pasta (added with chicken meat pasta) and DHA) as compared to control pasta (without chicken meat and DHA). The increase in moisture content was due to the interaction of meat protein in the pasta. Water, as dipolar molecule, is attracted to charged compounds like protein. Myofibril proteins in chicken meat are the major compounds responsible for the high water holding capacity of chicken meat (Huff-Lonergan and Lonergan 2005).

During pasta making, starch granules swells with water and a protein network film were formed surrounding the starch granules. The addition of chicken meat increased the charged protein content, resulting in improved water holding capacity and higher moisture content. The moisture content of control pasta and developed functional pasta samples were 10.89±0.11 and 13.28±0.06 (%), respectively.

Protein and ash content increased significantly (p<0.05) when 30% chicken meat powder was added to the pasta formulation, which was almost 1.2 fold more

than control pasta. Therefore, the pasta with high protein quality and quantity could be prepared by 30 per cent chicken meat. Fat content increased significantly (p<0.05) with increasing meat content. The increasing values in fat and protein are due to the high protein and fat content of the chicken meat. The increasing ash content indicates a higher mineral content in meat enriched pasta. The fatty acid estimation showed that Oleic (%), Linoleic (%), Linolenic (%), Palmitic (%) and Stearic (%) was 1.20, 3.90, 6.28, 1.29 and 7.30 respectively. Incorporation of DHA powder significantly improved the nutritional as well as functional properties of the developed pasta, which justifies the committed objectives.

4.2 Experiment No. 2: Storage stability of the developed functional pasta at ambient temperature under aerobic packaging.

In the present experiment developed functional pasta and control (without chicken meat and microencapsulated DHA powder) was stored under aerobic packaging conditions wrapped in aluminium foil-LDPE laminate pouches at ambient temperature (20-30±5 ºC) for 60 days as shown in plate 1. The samples were drawn at regular interval of 15 days. The storage quality was evaluated on the basis of cooking time, colour, textural, sensory parameters, Thiobarbituric acid reactive Substances (TBARS), free fatty acids content, peroxide value etc. Microbiological qualities were also be evaluated on the basis of enumeration of Standard Plate Count, Yeast and Mould Count, Coliforms Counts etc. The results are presented in the text with the support of statistically analyzed Tables (9 to 13) and Figures (12 to 14). The critical analyses of the results with suitable support of available literature to draw the inference have been attempted in the present chapter.

Minimum Cooking Time (MCT)

Table 9 reflected the cooking quality of control and developed functional pasta. Significant variation was observed with respect to minimum cooking time of both studied pasta. Control pasta (without chicken meat and DHA powder) required 6.15 min for complete gelatinization of starch on cooking, whereas, developed functional pasta showed significantly higher MCT i.e. 17.35 min on 1st day of storage.

The optimum cooking time depends primarily on the rates of water penetration and starch gelatinization (Edwards *et al* 1993). As shown in Fig (15-22) a more complex protein network showed in developed functional pasta than control pasta that

may limit the water entry into the starch granule, and possibly slowed down the start of the gelatinization process, resulting into increased cooking time. Yamazaki and Graetz (1977) explained the principle of protein hydrophilicity, in which they postulated that during cooking, there is a competition between starch and protein for water. It is well known fact that when less amount of protein surrounds starch granules, they swell and gelatinize faster. Hence, it is postulated that, pasta with higher protein content, results in slower starch swelling and subsequently a longer time required for gelatinization.

Storage period had no significant effect on the minimum cooking time of stored control as well as developed functional pasta. As the storage period progressed, the time required to cook pasta increased, however, only a slight increase was noted. For control pasta sample, the cooking time slightly increased from 6.15 to 7.18 and for developed functional pasta sample increased from 17.35min to 18.56min. This increase could be attributed the fact that the optimum cooking time primarily depends on the rates of water penetration and starch gelatinization (Edwards *et al* 1993).

L, *a and *b**value**

Colour is an important quality attribute directly related to the acceptability of the food. Colour of the product may change during storage due to undesirable reactions such as Maillard browning and degradation of colour pigments, depending upon storage conditions viz., temperature, packaging material etc. (Borrelli *et al* 2008). Table 9 depicted Mean±SE values of the L, *a** and *b** attributes of the control as well as developed functional pasta. It was observed that L* value decreased in both the samples, but statistically significant difference was noticeable only after 30^{th} days of storage which was then remained consistent throughout the chicken meat fortified pasta showed decreased L value from 74.05 to 68.52 during 2 months of storage period (Table 5). Maillard browning resulting in darkness of products must have accounted for the decrease in L* values during the storage period (Jan *et al* 2017).

Kadam and Prabhasankar (2012) reported that the addition of 10-30 g/100 g shrimp meat into pasta decreased the lightness (L*) value compared to control. Similar observations were also made by Vijaykrishnaraj *et al* (2016), Liu *et al* (2016) and Phongthai *et al* (2017) during their storage studies on pasta incorporated with green mussel powder, meat and egg albumen, respectively.

Table 9: Effect of chicken meat and DHA powder on minimum cooking time and instrumental colour properties of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Minimum cooking time					
CP	6.15 ± 0.04^{Aa}	6.38 ± 0.05^{Aa}	6.57 ± 0.02^{Aa}	7.12 ± 0.01^{Ab}	7.18 ± 0.04^{Ab}
MP	17.05 ± 0.05^{Ba}	17.12 ± 0.05^{Ba}	18.16 ± 0.04^{Bb}	18.31 ± 0.06^{Bb}	18.46 ± 0.03^{Bb}
L*value					
CP	74.05 ± 0.09^{Bc}	70.05 ± 0.06^{Bb}	69.70 ± 0.07^{Bb}	68.63 ± 0.06^{Ba}	68.52 ± 0.06^{Ba}
MP	60.49 ± 0.07^{Ad}	59.70 ± 0.07^{Ad}	49.63 ± 0.06^{Ac}	45.72 ± 0.06^{Ab}	38.67 ± 0.06^{Aa}
a value*					
CP	1.63 ± 0.03^{Aa}	2.13 ± 0.05^{Ab}	2.19 ± 0.06^{Abc}	2.21 ± 0.06^{Ac}	2.46 ± 0.07^{Ad}
MP	3.15 ± 0.05^{Ba}	3.18 ± 0.06^{Ba}	3.57 ± 0.06^{Bb}	3.92 ± 0.07^{Bc}	4.17 ± 0.07^{Bd}
b value*					
CP	9.18 ± 0.07^{Ab}	9.08 ± 0.06^{Ab}	8.64 ± 0.06^{Aa}	8.58 ± 0.05^{Aa}	8.46 ± 0.04^{Aa}
MP	16.56 ± 0.07^{Bb}	16.32 ± 0.06^{Bb}	15.72 ± 0.06^{Ba}	15.60 ± 0.06^{Ba}	15.48 ± 0.05^{Ba}

n=6**,** CP: Control pasta, MP**:** Meat pasta,*Mean ± SE. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($p<0.05$).

Table 9 shows the increase in *a** values for control as well as developed functional pasta with the storage increasing period. The increase in a* value may be because of a gradual increase in non-enzymatic browning during storage (Wani and Kumar 2016). It can further be noted that the change in *a** value was more pronounced in developed functional pasta products in comparison to control samples. Rai and Chauhan (2008) and Pua *et al* (2008) reported a similar increase in enzymatic browning for cereal flakes and jackfruit, snacks, respectively which may be attributed to the increase in *a** value during storage.

The *b** value showed a declining trend during storage (Table 9). For control pasta products, it decreased from the initial value of 9.18±0.07 to 8.46±0.04 and 16.56±0.07 to 15.48±0.05 for the developed functional pasta. Within treatments, significant difference ($p<0.05$) was observed in *b** values, these might be due to inherent reddish yellow colour of added chicken meat (Wani and Kumar 2016)

observed the similar pattern of decrease in *b**values of composite extruded snack added with protein sources (meat) during storage.

Firmness and Toughness

The textural characteristics of pasta play a crucial role in determining the final consumer acceptability. The results for the mean values of firmness and toughness are presented in Table10. Firmness of the control and developed functional pasta decreased linearly as the storage progressed and the scores were comparable ($p>0.05$) up to 30th day of storage in control and 15th day in developed pasta samples. The decrease in scores was significant ($p<0.05$) on 45thday onwards in control and 30th day in developed pasta samples. Within treatments, significant difference ($p<0.05$) was observed in firmness values, these might be due higher protein content in developed functional pasta samples (30% chicken meat), which showed high firmness values as compared to control samples (without chicken meat).

Table 10: Effect of chicken meat and DHA powder on textural properties of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Firmness					
CP	0.36 ± 0.02^{Ab}	0.34 ± 0.01^{Ab}	0.31 ± 0.03^{Ab}	0.27 ± 0.04^{Aa}	0.26 ± 0.02^{Aa}
MP	0.65 ± 0.01^{Bc}	0.63 ± 0.03^{Bc}	0.52 ± 0.01^{Bb}	0.51 ± 0.02^{Bb}	0.43 ± 0.01^{Ba}
Toughness					
CP	0.57 ± 0.01^{Ab}	0.56 ± 0.03^{Ab}	0.54 ± 0.02^{Ab}	0.43 ± 0.02^{Aa}	0.41 ± 0.01^{Aa}
MP	0.83 ± 0.04^{Bc}	0.82 ± 0.01^{Bc}	0.76 ± 0.01^{Bb}	0.75 ± 0.01^{Bb}	0.68 ± 0.03^{Ba}

n=6**,** CP: Control pasta, MP**:** Meat pasta,*Mean ± SE. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($p<0.05$).

Texture of pasta is greatly dependent on the protein network and presence of strong protein network makes pasta more firm (Foo *et al* 2011). Kreger *et al* (2012) observed decrease in energy required to break the pasta samples (having 28% fish protein) as measured by texture analysis over 6 months of storage time. Decline in hardness of the product during storage maybe correlated with the increase in moisture content.

Toughness (Table 10) showed similar declining trend throughout storage period as that of firmness. It decreased from the initial value of 0.57±0.01 to 0.41±0.01 for control pasta samples and 0.83±0.04 to 0.68±0.03 for the developed functional pasta. Within treatments, significant difference (p<0.05) was observed in toughness values, This has been attributed to the higher number of polypeptide chains associated with higher protein levels in developed chicken meat pasta that increase the chance for proteins to interact to form an insoluble network. This insoluble protein network can entrap swollen and gelatinized starch granule, which prevents pasta from disruption (Chillo *et al* 2010).

Sensory evaluation

Sensory evaluation plays a vital role not only in new product development but also in determining the shelf life and acceptability of a product. Sensory evaluation is a measure of the overall impression of eating quality and depicts product acceptability. Preference for all sensory attributes decreased throughout the storage days (Table 11). On the 1st day of storage, control pasta showed the highest scores (8.43) for appearance and colour, which declined to 7.98 on the 60th day of storage. Whereas, on the 1st day of storage developed pasta showed significantly (p<0.05) lower appearance & colour scores as compared to control pasta and also followed similar declined trend with progress of storage.

This was probably due to the incorporation of chicken meat and DHA powder into the developed functional pasta, having slightly dark reddish colour. Decrease in sensory colour and appearance score during storage are in corroboration with the result of L^* value, and the product appeared dull and darker. Raja *et al* (2014) also reported decline in sensory scores in fish snacks.

The mean body and texture scores for control and developed functional pasta at ambient temperature for 60 days are shown in Table 11. The body and texture scores were 8.19, 8.11, 7.83, 7.75 & 7.73 for 1st, 15th, 30th, 45th and 60th day of storage, respectively for control pasta. For developed functional pasta the scores were 8.16, 7.98, 7.76 7.56 and 7.44 for 1st, 15th, 30th, 45th and 60th day of storage, respectively.

The analysis of variance revealed that mean body and texture scores decreased significantly (p<0.05) during storage period, but were in acceptable range (above 6 i.e. better than like slightly) during the whole period of 2 months. The mean body and texture score was highest for control pasta products and lowest for

developed functional pasta. This might be due to weakening of protein starch matrix during the storage study. Chin *et al* (2012) also reported similar finding in noodles during storage at ambient temperature for 6 months.

Table 11: Effect of chicken meat and DHA powder on sensory properties of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Appearance and colour					
CP	8.43 ± 0.05^{Bc}	8.42 ± 0.06^{Bc}	8.20 ± 0.02^{Bb}	8.18 ± 0.01^{Bb}	7.98 ± 0.04^{Ba}
MP	8.25 ± 0.04^{Ad}	7.95 ± 0.05^{Ac}	7.93 ± 0.05^{Abc}	7.92 ± 0.02^{Ab}	7.83 ± 0.06^{Aa}
Body and texture					
CP	8.19 ± 0.08^{Ac}	8.11 ± 0.06^{Bbc}	7.83 ± 0.01^{Bb}	7.75 ± 0.02^{Ba}	7.73 ± 0.06^{Ba}
MP	8.16 ± 0.04^{Ae}	7.98 ± 0.04^{Ad}	7.76 ± 0.02^{Ac}	7.56 ± 0.03^{Ab}	7.44 ± 0.05^{Aa}
Flavour					
CP	8.25 ± 0.02^{Bb}	8.23 ± 0.01^{Bb}	7.94 ± 0.07^{Ba}	7.93 ± 0.02^{Ba}	7.91 ± 0.03^{Ba}
MP	7.74 ± 0.06^{Ad}	7.58 ± 0.03^{Acd}	7.56 ± 0.05^{Ac}	7.13 ± 0.06^{Ab}	6.46 ± 0.02^{Aa}
Overall acceptability					
CP	8.33 ± 0.02^{Bc}	8.32 ± 0.03^{Bc}	8.11 ± 0.03^{Bb}	8.09 ± 0.02^{Bb}	7.89 ± 0.02^{Ba}
MP	8.18 ± 0.02^{Ac}	8.09 ± 0.05^{Ac}	7.82 ± 0.01^{Ab}	7.77 ± 0.01^{Aa}	7.76 ± 0.06^{Aa}

n=21**,** CP: Control pasta, MP**:** Meat pasta,*Mean ± SE. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly ($p<0.05$).

Flavour is a multifaceted sensory quality, which includes both taste and odour of product and is a principal indicator of product quality. Flavour of the both tested products decreased gradually ($p>0.05$) as the days of storage progressed and on 30^{th} day of storage the scores were significantly ($p<0.05$) declined till the end of storage period of 60 days (Table 11). The decline in flavour scores was comparatively less in control pasta samples than developed functional pasta samples and also the product was acceptable by the panelists. Statistically prominent ($p<0.05$) drop in flavour scores, within treatment as well as storage period, was observed from 15^{th} day onwards. This trend of decrease in flavour scores can be corroborated with increase in TBA value, which indicates lipid oxidation that in turn leads to off flavour development, thus decreasing sensory scores of the product. The reduction in flavour could be attributed to the increased lipid oxidation, liberation of fatty acids and

increased microbial load (Sahoo and Anjaneyulu 1997). Similar reports have been published by Raja *et al* (2014) during storage studies of aerobically packaged fish curls.

Overall acceptability scores of control as well as chicken meat & DHA powder added functional pasta decreased linearly as the storage days progressed. The scores showed a non-significant decrease up to 15^{th} day of the storage in almost both the products. The overall acceptability scores were significantly ($p<0.05$) higher in the control pasta as compared to the treatments. The decline in overall acceptability scores could be attributed to changes in scores of colour and appearance, flavour, texture and other sensory attributes. Similar findings have also been reported by Kumar and Sharma (2004), for various snacks based products.

As shown in Table 11, the sensory scores among the studied products viz. appearance & colour, flavour and overall acceptability scores were significantly least for developed functional pasta than control pasta. This might be due to the added chicken meat with the disadvantages of reddish colour, strong fishy flavour of DHA powder and unacceptable textural properties, therefore the developed pasta was less unacceptable to the sensory panel. Besides, the strong flavour mainly referred to fishy odor, this was because the added DHA powder was rich in unsaturated fatty acids in particular omega 3-fatty acids, which were readily oxidized to the volatile compounds with fishy odor (Fang *et al* 2017).

TBARS, FFA and PV

The extent of lipid oxidation in the extruded snack during storage was measured in terms of Thiobarbituric acid (TBA) value. The results with respect to change in TBA value (in terms of absorbance) for developed functional pasta and control samples are illustrated in Fig. 13. The TBA value of control pasta, increased from initial value from 0.57 to 0.82, while in the developed functional pasta, it increased from 0.71 to 0.87 at end of the storage period. The overall mean values of TBARS of developed functional pasta and control pasta samples increased significantly ($p<0.05$) over storage period of 60 days.

The higher TBARS values in developed pasta samples than control might be due to the high protein and fat content and more unsaturated fatty acid content (DHA powder) in meat. Nayak and Tanwar (2005) also reported increase in TBARS values during the storage study because of increased lipid oxidation and production of volatile metabolites.

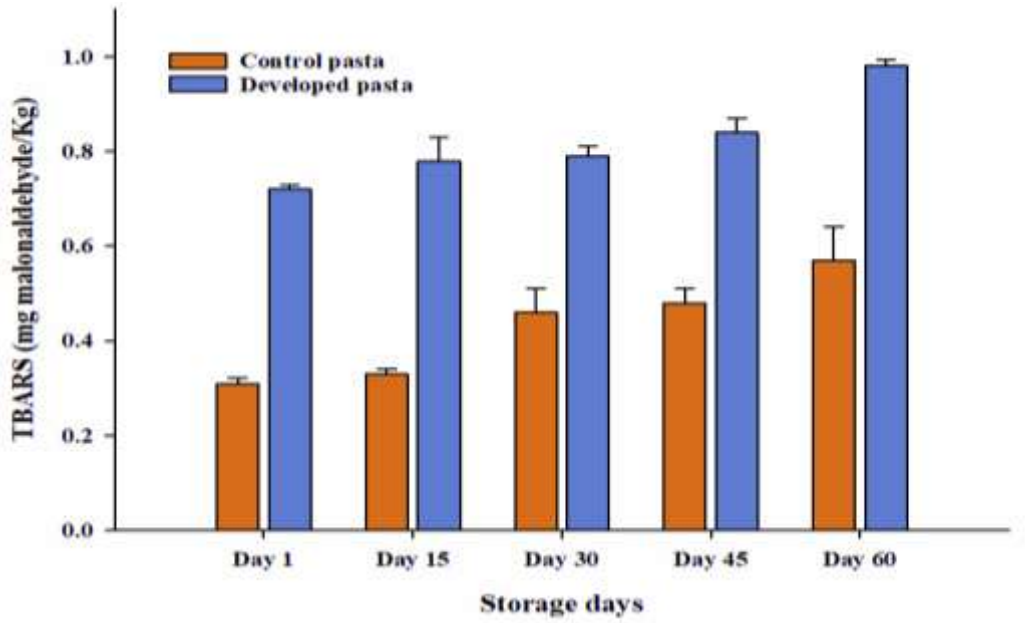

Fig. 13: Effect of chicken meat and DHA powder on TBARS values of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

The FFA and peroxide value in developed functional pasta samples were significantly (p<0.05) higher than control. The FFA values increased significantly (p<0.05) in control and treatment till 60thdays of storage (Fig. 14).

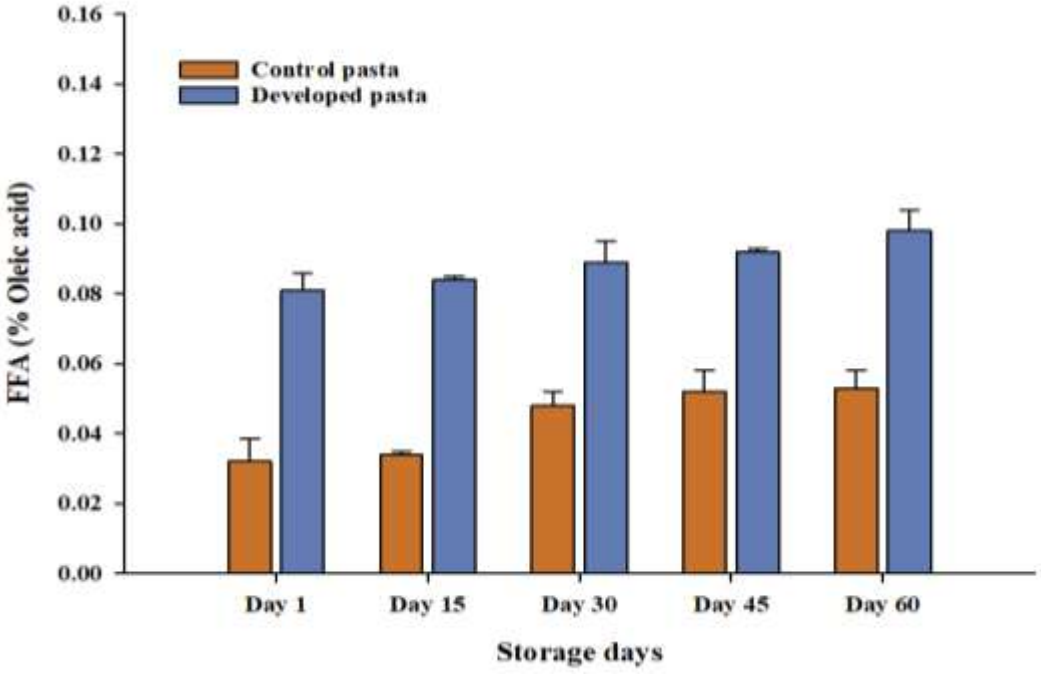

Fig. 14: Effect of chicken meat and DHA powder on FFA values of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

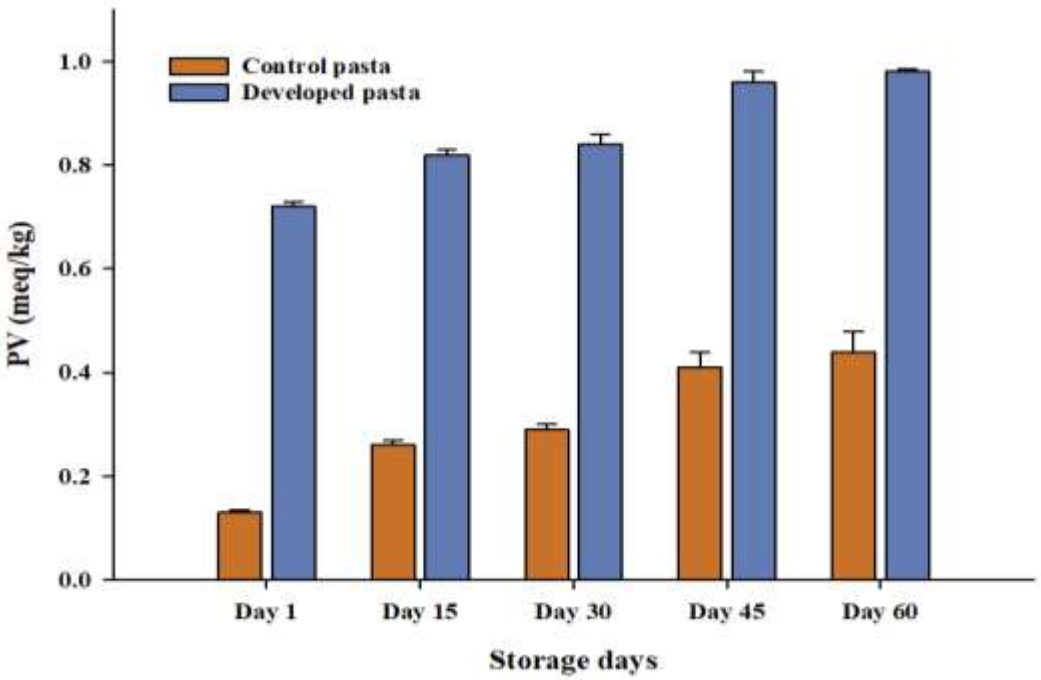

Fig. 15: Effect of chicken meat and DHA powder on PV values of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

It might be due to the presence of unsaturated fatty acid in developed functional pasta added with chicken meat and DHA powder than control, as unsaturated fats are more prone to oxidation.

Free fatty acids and peroxide content (Fig. 14) in both groups increased with storage study, but the values remained in the acceptable limit. Similar results were reported by Kaur *et al* (2013) in pasta.

PV followed increasing trend throughout storage period in both treated as well as control samples (Fig. 15). It might be due to the formation of hydroperoxides during storage than their degradation into secondary oxidation products. These results are in consonance with Botsoglou *et al* (2014) who studied effect of olive leaf (*Olea europea* L.) extracts on protein and lipid oxidation of frozen n-3 fatty acids-enriched chevon patties.

Microbial Quality Analysis

The mean values of various microbiological characteristics of aerobically packaged control and developed functional pasta containing chicken meat and DHA powder are presented in Table 13 during storage of 60 days. SPC in control ranged

from 0.95 to 1.89 and in developed meat pasta from 0.86 to 2.95. The SPC values increased significantly (p<0.05) in both control and chicken meat incorporated pasta during the whole storage study (Table 13). SPC values were slightly higher in chicken meat incorporated pasta than control which could be due to higher nutritive value of meat (easy availability of protein-rich nutrients to favour microbial growth) than semolina based control pasta. Similar finding were observed by the Singh *et al* (2011) in chicken snack.

Table 12: Effect of chicken meat and DHA powder on microbiological properties of control and developed functional pasta during storage (20-30±5 °C) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Standard plate count (log CFU/g)					
CP	N.D	N.D	0.95^{Aa}	1.45^{Ab}	1.89^{Ac}
MP	N.D	0.86	1.36^{Ba}	2.06^{Bb}	2.95^{Bc}
Coliform count (log CFU/g)					
CP	N.D	N.D	N.D	N.D	N.D
MP	N.D	N.D	N.D	N.D	N.D
Yeast and mould count (log CFU/g)					
CP	N.D	N.D	N.D	0.69^{Aa}	1.06^{Ab}
MP	N.D	N.D	0.81^{a}	1.39^{Bb}	1.64^{Bc}

n=6**,** CP: Control pasta, MP: Meat pasta,*Mean ± SE. with different superscripts row wise (small alphabets) and column wise (capital alphabets) differ significantly (p<0.05).

The SPC counts were much lower than maximum acceptable limit i.e. (log 6 CFU/g) for the SPC in the fresh extruded based products like noodles and pasta (Jay, 1996). It may be considered as reference point between spoiled and unspoiled extruded products. Table 13 revealed that throughout the storage period of 60 days, no coliforms were detected, it might be due to the lower water activity and hygienic handling and packaging of products.

Yeast and mould count were detected from 30th day and 45th day onwards in developed functional pasta and control pasta, respectively. Perusal of Table 13 showed that there was significant increase in Yeast and mould count beyond 45 days of storage in both studied treatments (p<0.05) but was much lower than acceptable limit for fresh pasta i.e. 4 log CFU/g (World Health Organization, 2000). It may be due to hygienic handling and packaging of products.

Microstructure of raw and cooked pasta

The SEM of cross-sections of raw and cooked control pasta at different resolution i.e. 150X and 2000X are shown in Figure 15 to 22. The oval-shaped starch granules were found in cooked and uncooked control pasta samples at both 150X and 2000X resolution (Figure15 to 18), whereas, the internal structure of uncooked developed functional pasta at150X and 2000X resolution (Figure 19 to 22) showed a homogenous and porous structure where starch granules are deeply embedded in a protein matrix. The addition of chicken meat decreased the porosity up to some extent resulting into protein network that wrapped the starch granules, which resulted in the restricted swelling of starch resulting from less absorption.

The replacement of semolina by chicken meat and DHA powder enhanced the protein matrix around the starch granules. The SEM results of pasta samples are in close agreement with the results reported by other researchers i.e. Gupta *et al* (2020) in pasta fortified with quinoa protein isolates and Kadam and Prabhasankar (2010) in shrimp meat pasta.

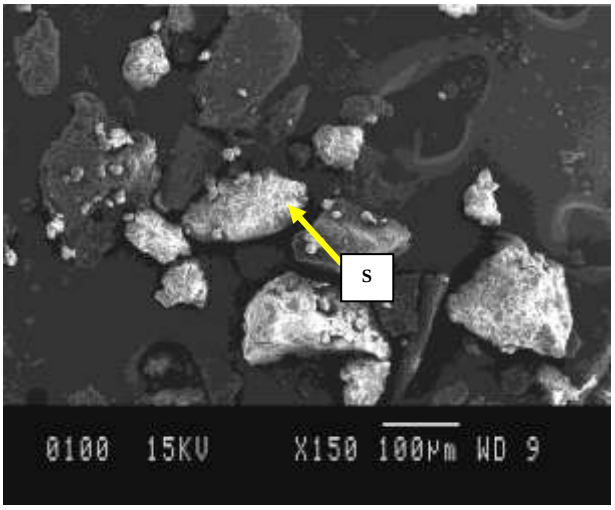

Fig. 16: Uncooked control pasta samples (150X resolution)
S: Starch

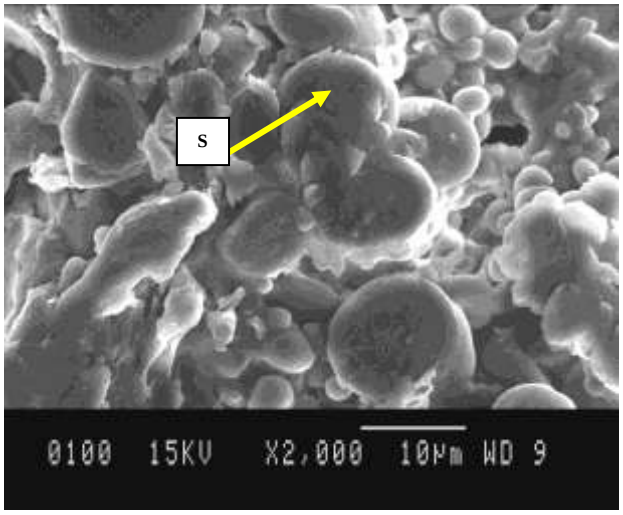

Fig. 17: Uncooked control pasta samples (2000X resolution)
S: Starch

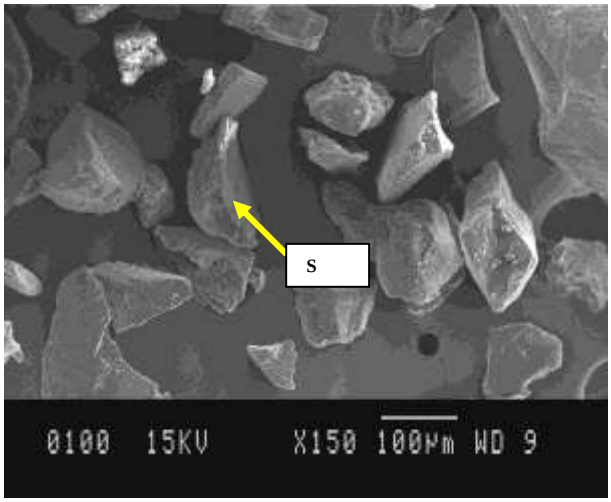

Fig. 18: Cooked control pasta samples (150X resolution)
S: Starch

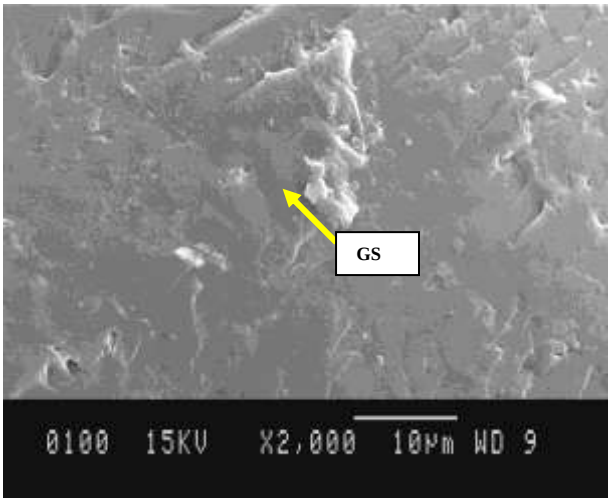

Fig. 19: Cooked control pasta samples (2000X resolution)
GS: Gelatinization of starch

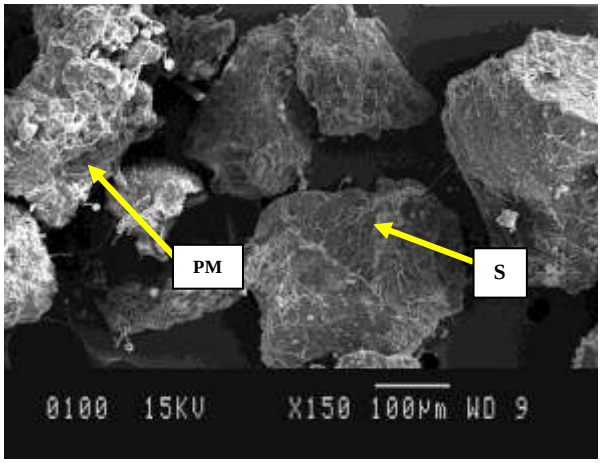

Fig. 20: Cooked functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) (150X resolutions)

(S: Starch, PM: Protein matrix)

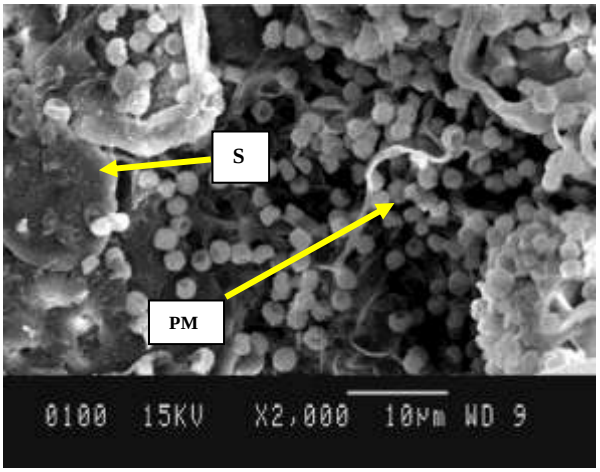

Fig. 21: Cooked functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) (200X resolutions)

(S: Starch, PM: Protein matrix)

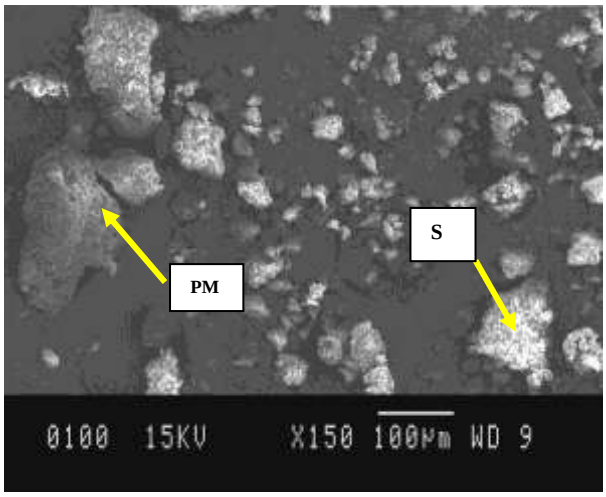

Fig. 22: Uncooked functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) (150X resolutions)

(S: Starch, PM: Protein matrix)

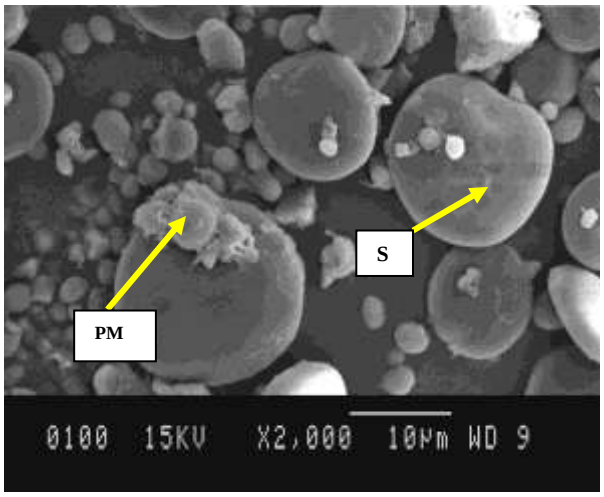

Fig. 23: Uncooked functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) (2000X resolutions)

(S: Starch, PM: Protein matrix)

4.3 Experiment No. 3: Calculation of the production cost of developed functional pasta.

4.3.1 Economics of production of functional pasta incorporated with chicken meat and microencapsulated DHA powder.

Development of any technology can be identified as successful until it is used for the benefit of the society. Technology for food products depends not only on its taste, appearance, colour, aroma etc., but also on its nutritive value and cost of production. For this, the economics including the cost of production of functional pasta incorporated with chicken meat and microencapsulated Docosahexaenoic acid powder was worked out.

The economy was worked out with the following assumptions

1) Per day production of functional pasta incorporated with chicken meat and microencapsulated Docosahexaenoic acid powder is 100 kg.
2) The unit remains in production for 25 days in a month therefore monthly production target of functional pasta incorporated with chicken meat and microencapsulated acid powder is 100 x 25 = 2500 kg/ month.
3) Cost of ingredients is calculated on the basis of prevalent market rate (January to June, 2020) in the local market.
4) To estimate accurate cost of production of functional pasta incorporated with chicken meat and microencapsulated Docosahexaenoic acid powder under commercial conditions, the expenditure incurred in terms of recurring items, labor charges, water and electricity charges, depreciation on machineries, rent paid, capital investment and its interest, was also taken under consideration.
5) Receipt is from the sale of functional pasta incorporated with chicken meat and microencapsulated Docosahexaenoic acid powder and not from by-product/recovery during processing of raw material. Hence it can be an additional profit for entrepreneur.

Table 13: Cost of production of formulation ingredients

Name of ingredients	Quantity (g)	Rate (Rs/Kg)	Approx. Cost (Rs)
Semolina	685	66	45.27
Chicken meat	300	156	46.80
DHA powder	15	2000	30.00
Total	1000		Rs 122.07

4.3.2 Cost of 1 Kg deboned spent hen meat

Price of live spent hen	Rs 60.00/Kg
Dressing percentage	69.94± 1.23
Cost of 1 Kg dressed carcass	Rs 85.78
Average recovery of deboned meat (%)	55.02 ±1.18
Cost of 1 Kg deboned meat	Rs 155.90/Kg (≈Rs 156/Kg)

4.3.3 Formulation cost of control and developed functional pasta

The cost of formulation for the control and developed functional pasta of 100 kg is given in Table 14.

Table 14: Formulation cost of 100 kg control and developed functional pasta

Ingredients	Rate Rs/kg	Control		Developed functional pasta	
		Qt. (Kg)	Amount (Rs)	Qt. (Kg)	Amount (Rs)
Semolina	66	100.00	6600	68.5	4521.00
Chicken meat	156		-	30	4680.00
DHA powder	2000		-	1.5	3000.00
Total (Rs)	-	-	**6600.00**		**12201.00**

A) Cost of formulation of 100 kg control pasta = Rs. 6600.00

B) Cost of formulation of 100 kg developed functional pasta= Rs. 12201.00

C) Overhead production cost for 100 Kg chicken meat pasta

a. Labor charges

Unskilled worker (5-daily paid laborers) (Rs 220.00/day × 6) = Rs. 1320/-

b. Electricity charges

Table 15: Electricity charges

Equipment	Watt × hrs.	KWH Unit
Pasta maker (5No.s)	2 ×200 × 24	9.60
Industrial dryer (2No.s)	2000 × 15.00	30.00
Packaging machine	100 × 2.0	0.20
Light, fan etc.	400 × 10	4.00
Total		Rs 43.8

Electricity charge (Rs 6/Unit) (43.8× Rs. 6.0) = Rs. 262.80

c. Equipment depreciation

Table 16: Equipment depreciation

Equipment	**Cost (Rs)**
Pasta maker	12000.00
Meat mincer	30000.00
Sealing machine	5000.00
Industrial dryer	100000.00
Total	**Rs. 1,47,000/-**

Depreciation @10% per annum = **Rs 14700/-**

Depreciation cost per day = **Rs. 40.27**

a) **Water charges (1000 litres) = Rs. 10/day**

b) **Cost of packaging material (pack 250g each)** = Rs. **240.00**

   a. (8"×6" Alum-LDPE Pouches @ Rs 0.6/pouch) (400 × 0.60)

c) **Room rent (Rs 3000/ month) = Rs.120.00 per day**

d) **Miscellaneous (Detergent, transportation etc.) = Rs.250.00 per day**

E. Total overhead cost (a+ b+ c+ d) = Rs.620.00/

Table 17: Production cost control and developed functional pasta

Total cost of production obtained from 100 Kg formulation
Total cost of production of control pasta = **(A) + (E) =**6600+620 =**Rs.7220/-**
Total cost of production of developed functional pasta = **(B) + (E)**= Rs. 12201+ 620 =**Rs. 12821.00/-**

Table 18: Production cost of 1Kg control and developed functional pasta

Control pasta	**= Rs. 72.00**
Developed functional pasta	**= Rs. 128.21**

CHAPTER IV

SUMMARY AND CONCLUSIONS

The present study planned to optimize the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta. The developed pasta evaluated for the storage stability at ambient temperature (20-30±5ºC) for 60 days wrapped in aluminium foil-LDPE laminate pouches. The production cost for development of functional pasta was also calculated. The present chapter details the summary and conclusions obtained from different experiments carried out in accordance with the above-mentioned objectives.

5.1 Optimization of the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder for the development of functional pasta

Response surface methodology (RSM) used to optimize the level of incorporation of chicken meat and microencapsulated docosahexaenoic acid (DHA) powder as two composition coded/uncoded variables. The experiments were formulated according to a Central Composite Design (CCD) resulted in 13 experimental design of Independent variables. In order to develop the most acceptable functional pasta by incorporating chicken meat and DHA powder responses were demonstrated by various cooking parameters viz. (Minimum cooking time (MCT), Volume expansion (VE), Water uptake ratio (WUR), Gruel Solid loss (GSL), Colour analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability OA). The fitness of the polynomial model equation to the responses was evaluated by the coefficient of R square as well as by the lack of fit using the F-test with 5% level of significance.

The ANOVA of MCT showed the quadratic regression models showed that the model was significant (p<0.05) with p-values of <0.0001. The statistical analyses revealed that chicken meat (%) had both significant linear and interaction effects (p<0.0001) on the MCT of formulated pasta followed by the linear term effects of DHA powder. Thus, predicated model results suggested that a chicken meat (%) significantly increased the minimum cooking times. Furthermore, there was non-significant (p>0.05) quadratic term effect of chicken meat (%) and DHA powder. The

optimized MCT predicted by response surface methodology was 18.02% under the formulation condition with chicken meat (%) of 30% and DHA of 1.5%. The ANOVA of the VE showed that there was non-significant quadratic term effect for both the variables i.e. chicken meat (%) and DHA powder. The Model F-value of 1.48 implies the model is significant. Optimized model predicated by RSM showed 1.48 % of volume expansion under the formulation condition; chicken meat of 30% and DHA powder of 1.5 %. WUR showed only A and A^2 significant model terms, which implies that chicken meat (%) significantly affected WUR. The Lack of Fit F-value of 0.068 implies the Lack of Fit is not significant relative to the pure error. RSM indicated 1.71 % of water uptake ratio under the formulation condition; chicken meat of 30% and DHA powder of 1.5 %. The ANOVA model of GSL showed linear and interaction regression models showed that the model was significant ($p<0.05$) with p-values of <0.0001. The statistical analyses revealed that chicken meat (%) had both significant linear and interaction effects ($p<0.0001$) on the GSL of formulated pasta followed by the linear term effects of DHA powder. The model predicated 17.61% of GSL by keeping 30% of chicken meat and DHA powder of 1.5%.

RSM models showed 1.47 % of firmness under the formulation condition; chicken meat of 30% and DHA powder of 1.5 % and 1.31 % of toughness under the formulation condition; chicken meat of 30% and DHA powder of 1.5 %.

The effect of chicken meat (%) and DHA powder on L value showed that increase in chicken meat (%) there is significantly decreased in L value, which might be due to the inherent colour of raw ingredients i.e chicken meat colour as chicken meat has low L value and higher a^* and b^* values. RSM indicated 58.27 of L value under the formulation condition; chicken meat of 30 % and DHA powder of 1.5 %.RSA were chicken meat of 30% and DHA powder of 1.5% exhibiting a* value of 3.22.Optimized model predicated by RSM showed 7.91 % of overall acceptability under the formulation condition; chicken meat of 30% and DHA powder of 1.5 %.

5.2 Proximate and fatty acids profile of developed pasta.

It was found that Moisture (%), Carbohydrate (%), Protein (%), Fat (%) and Ash (%) in control was 13.89±0.11, 70.92±0.06, 12.26±0.09, 1.82±0.04 and 0.57±0.01 respectively. Whereas, developed pasta showed 10.08±0.06 Moisture (%), 52.04±0.03 Carbohydrate (%), 33.25±0.02 Protein (%), 2.41±0.02 Fat (%) and 3.34±0.08 Ash (%) contents. The fatty acid estimation showed that Oleic (%),

Linoleic (%), Linolenic (%), Palmitic (%) and Stearic (%) was 1.20, 3.90, 6.28, 1.29 and 7.30 respectively.

5.3 Storage stability of the developed functional pasta at ambient temperature under aerobic packaging

In the present experiment developed functional pasta and control (without chicken meat and microencapsulated DHA powder) was stored under aerobic packaging conditions wrapped in aluminium foil-LDPE laminate pouches at ambient temperature (20-30±5ºC) for 60 days. The samples were drawn at regular interval of 15 days. The storage quality was evaluated on the basis of cooking time, colour, textural, sensory parameters, Thiobarbituric acid reactive Substances (TBARS), free fatty acids content, peroxide value etc. Microbiological qualities were also be evaluated on the basis of enumeration of Standard Plate Count, Yeast and Mould Count, Coliforms Counts etc.

Storage period had no significant effect on the minimum cooking time of stored control as well as developed functional pasta. As the storage period progressed, the time required to cook pasta increased, however, only a slight increase was noted. For control pasta sample, the cooking time slightly increased from 6.15 to 7.18 and for developed functional pasta sample increased from 17.35min to 18.56min.

L* value decreased in both the treatments, but statistically significant difference was noticeable only after 30^{th} days of storage which then remained consistent throughout. Increase in chicken meat content in the pasta decreased its L value from 74.05 to 68.52 during 2 months of storage period. It was found that there was increase in *a** values for control as well as developed functional pasta with the storage period. The *b** value showed a declining trend during storage. For control pasta products, it decreased from the initial value of 9.18±0.07 to 8.46±0.04 and 16.56±0.07 to 15.48±0.05for the developed functional pasta. Within treatments, significant difference ($p<0.05$) was observed in *b** values, these might be due to inherent reddish yellow colour of added chicken meat.

Firmness of the control and developed functional pasta decreased linearly as the storage progressed and the scores were comparable ($p>0.05$) up to 30^{th} day of storage in control and 15^{th} day in developed pasta samples. The decrease in scores was significant ($p<0.05$) 45^{th} day onwards in control and 30^{th} days in developed pasta samples. Within treatments, significant difference ($p<0.05$) was observed in firmness

values, these might be due higher protein content in developed functional pasta samples (30% chicken meat), which showed high firmness values as compared to control samples (without chicken meat). Toughness showed similar declining trend throughout storage period as that of firmness. It decreased from the initial value of 0.57±0.01 to 0.41±0.01for control pasta samples and 0.83±0.04 to 0.68±0.03 for the developed functional pasta.

The sensory scores among the studied products viz. appearance & colour, flavour and overall acceptability scores were significantly least for developed functional pasta than control pasta, this might be due to the added chicken meat with the disadvantages of reddish colour, strong fishy flavour of DHA powder and unacceptable textural properties, therefore the developed pasta was unacceptable to the sensory panel. Besides, the strong flavour mainly referred to fishy odor, this was because the added DHA powder was rich in unsaturated fatty acids in particular omega 3-fatty acids, which were readily oxidized to the volatile compounds with fishy odor.

The TBA value of control pasta, increased from initial value from 0.57 to 0.82, while in the developed functional pasta, it increased from 0.71 to 0.87 at end of the storage period. The overall mean values of TBARS of developed functional pasta and control pasta samples increased significantly ($p<0.05$) over storage period of 60 days. The FFA values increased significantly ($p<0.05$) in control and treatment till 60^{th} days of storage. Free fatty acids and peroxide content in both group pasta increased with storage study, but the values remained in the acceptable limit.

SPC in control ranged from 0.95 to 1.89 and in developed meat pasta from 0.86 to 2.95. The SPC values increased significantly ($p<0.05$) in both control and chicken meat incorporated pasta during the whole storage study. Yeast and mould count were detected from 30th day and 45^{th} days onwards in developed functional pasta and control pasta, respectively. Throughout the storage period of 60 days, no coliforms were detected; it might be due to the lower water activity and hygienic handling and packaging of products.

The SEM of cross-sections of raw and cooked control pasta at different resolution i.e. 150X and 2000X showed the oval-shaped starch granules and homogenous and porous structure of protein matrix where starch granules are deeply embedded in a protein. The addition of chicken meat decreased the porosity up to

some extent resulting into protein network that wrapped the starch granules which resulted in the restricted swelling of starch resulting from less absorption.

5.4 Economics of production of functional pasta incorporated with chicken meat and microencapsulated docosahexaenoic acid powder.

The results showed that production of control pasta was Rs. 72.00 and that of developed functional pasta was Rs. 128.21.

CONCLUSIONS

- ✓ Formulations protocol was standardized for the preparation of functional pasta using chicken meat and DHA powder with successful implementation of Response Surface Methodology (RSM) executing Central Composite Design (CCD).
- ✓ Standardized protocol of Response Surface Methodology (RSM) showed that functional pasta can be developed using 30% chicken meat and 1.5% DHA powder with highest scores for various physico-chemical, cooking and textural attributes.
- ✓ The developed functional pasta incorporated with chicken meat (30%) and DHA powder (1.5%) could be stored at 20-30±5°C temperature under ambient atmospheric conditions for 60 days without any marked loss in physico-chemical, colour, textural, microbiological and sensory qualities.
- ✓ Microstructure studies revealed increased interaction between chicken protein and starch molecules, indicating a potential option to produce high quality pasta with enhanced nutritional and functional properties.
- ✓ The cost of production of developed pasta was 128 Rs/Kg as compared to less nutritious control pasta with production cost of 72Rs/Kg, Hence, it can be recommended as a profitable start up business venture.

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