DEVELOPMENT AND QUALITY EVALUATION OF PASTA FORTIFIED WITH WHOLE EGG AND SKIM MILK POWDER

Thesis

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

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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 AND QUALITY EVALUATION OF PASTA FORTIFIED WITH WHOLE EGG AND SKIM MILK 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 **Ms. Jamadar Deepika (L-2019-V-29-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 present study was conducted to optimize the level of incorporation of whole egg powder (WEP) and skim milk powder (SMP) for the development of protein rich pasta. Thirteen different formulation combinations were devised for the preparation of protein rich pasta using Response Surface Methodology (RSM) implementing Central Composite Design. Outcomes were demonstrated by various cooking parameters viz. MCT, VE, WUR, GSL, Color analysis, Firmness and toughness and Overall acceptability. It was found that with increase in WEP and SMP percentages in the formulation resulted significant (p<0.05) increase in MCT, GSL, firmness, toughness, a* and b* value, whereas VE, WUR, L* value and OA showed declined trend. The best-suited formulation condition predicated by the models were 5% WEP and 10% SMP. The proximate compositions of fortified pasta was significantly improved as compared to control pasta. The control and developed fortified pasta were subjected for aerobic storage at (20-30±5°C) in aluminium foil-LDPE laminate pouches for 60 days ,and analysed 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, microbial counts were observed 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 fortified pasta than control pasta. The developed fortified pasta incorporated with combine levels of WEP (5%) and SMP (10%) 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 added protein (WEP & SMP) 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 127.70 Rs/Kg as compared to less nutritious control pasta with production cost of 72.00 Rs/Kg, Hence, it can be recommended as a profitable start up business venture. It was concluded that the highly nutritious protein and WEP and SMP rich pasta could be developed with acceptable shelf life of 60 days.

Keywords: Skim milk powder, whole egg powder, response surface methodology, fortified pasta

Signature of Major Advisor   Signature of Student

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ABBREVIATIONS USED

AOAC	:	Association of Official Analytical Chemists
APHA	:	American Public Health Association
CCD	:	Central composite design
CP	:	Control pasta
DP	:	Developed pasta
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
WEP	:	Whole Egg Powder
SMP	:	Skim Milk Powder
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre
mm	:	Millimetre
MT	:	Million tonnes
N	:	Newton
CP	:	Control Pasta
^{o}C	:	Degrees Celsius
ppm	:	Parts per million

RSM	:	Response Surface Methodology
s	:	Seconds
SE	:	Standard error
SPC	:	Standard plate count
SPSS	:	Software Package for Social Sciences
TBARS	:	Thiobarbituric acid reactive substances
TPA	:	Texture profile analysis
viz.	:	Videlicet
w/v	:	Weight by volume
w/w	:	Weight by weight
Wt	:	Weight
GSL	:	Gruel solid loss
MCT	:	Minimum cooking time
VE	:	Volume Expansion
OA	:	Overall Acceptibility
WUR	:	Water uptake ratio
PV	:	Peroxide value

CHAPTER I

INTRODUCTION

The snack food market is constantly evolving relative to product types and although most snacks are not primarily consumed for their nutrients, many snacks are made with nutrition perspective in mind. Consumers want snacks that are tasty, smell good, feel well, and look good, and also being nutritionally sound and healthy. Extrusion cooking is a modern food processing technology applied for preparation of a variety of snacks, specialty and supplementary foods (Harper & Jansen, 1985). Extrusion technology allows for minute variations in materials and processing conditions on the same machine to produce a wide range of food products. Minor adjustments in the hardware and processing conditions can produce a variety of distinct shapes, textures, colours, and appearances (Riaz, 2006).

Pasta is a cereal-derived staple food and the second most consumed food worldwide, due to its low cost, nutritional composition, versatility, easy preparation and storage, extended shelf life as well as desirable sensory attributes such as wheat taste, smooth surface and firmness (Kadam & Prabhasankar, 2012). Pasta is a healthy food which is an important source of carbohydrates with virtually no fat (Foschia et al., 2015). Pasta products are high in starch, but low in proteins and are mainly made up of wheat flour which is deficient in an essential amino acids. Now a days, fortification of pasta with essential ingredients improves the quality of pasta nutritionally.

Pasta may be fortified with functional ingredients to alter its nutritional quality, such as with essential amino acids, minerals, vitamin, phenolic compounds etc. The supplementation of protein-rich components to pasta is intended to appeal to health-conscious consumers who prefer a product that is high in proteins, healthy fats, and other nutrients.. Pasta is a cereal-derived staple food that is the world's second most consumed food, thanks to its low cost, nutritional content, variety, ease of preparation and storage, long shelf life, and sensory features such wheat flavour, smooth surface, and hardness. (Kadam & Prabhasankar, 2012). It has been brought to light from various studies that pasta can be fortified from different sources, such as bean flour (Gallegos-Infante et al., 2010), shrimp meat (Ramya et al., 2015), and fish proteins (Surasani et al., 2019).

Protein can be obtained from animal or vegetable sources. It is well known that all proteins are not alike. Proteins from different sources have different properties that affect the quality of protein. Egg protein is widely considered as the highest nutritional quality protein of all food sources, providing all the essential amino acids in amounts that closely match human requirements and is therefore a standard against which all other proteins are evaluated (Chernoff, 2004). Eggs in general, and egg white in particular also have very little flavor, and thus do not negatively impact the organoleptic properties of foods, and therefore reduces the need for masking flavors. High level of protein combinations with other sources such as egg and cheese powder can provide the basis for a range of highly nutritious extruded snack products (Őzer et al., 2004).

Modern active lifestyle necessitates development of fortified pasta 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, with a low biological value due to its protein's lack of necessary amino acids (Martínez-Villaluenga et al., 2010). However, if the semolina is extended with other ingredients rich in these essential proteins from milk, fish, egg and meat, not only amino acids are supplemented, but also the dietary vitamins and minerals can be provided (Gull et al., 2018).

Egg is versatile and near perfect foods in nature. It is rich in protein, fat, vitamins and mineral substances, the yolk and white components are all of high biological value and are readily digested. They are known to supply the best proteins besides milk. Egg proteins are an important source of amino acids and exhibit versatile functional and biological value i.e. about 100%. Egg white contains proteins like ovalbumin, ovotransferrin, and lysozyme that contain hydrolysates that have multifunctional benefits like antihypertensive, antioxidant, anti-inflammatory, and anti-diabetic properties (Garcés-Rimón et al., 2016). However, the literature is almost silent for the fortification of pasta with whole egg powder.

Since long milk proteins have been recognized for their nutritive and technological value. Milk proteins have high nutritional value related to other proteins because of their relatively high content of essential amino acids and good digestibility (Meisel, 1998). Caseins and whey proteins are the two main protein groups in milk. Caseins, representing about 80% of the protein content in bovine milk, are isolated from milk by acid or by rennet precipitation. Caseins are flexible and heat stable proteins and contains a higher portion of the amino acids like histidine, methionine, phenylalanine etc.

The fortification of pasta by replacing semolina with rich sources protein i.e. whole egg and casein in pasta will not only increase the nutritive value but will also make the pasta a functional food. Although a lot of work has been done to improve pasta's functional properties and nutritive benefit using changes in formulations and processing; possibility of fortification of whole egg powder and milk powder will open new era of functional protein rich pasta foods. Hence the present study was proposed keeping in view the following objectives.

1. To optimize the level of incorporation of egg protein i.e. whole egg powder and milk protein i.e. skim milk powder for the development of pasta and its quality evaluation thereof.
2. To evaluate the storage stability of the developed fortified pasta at ambient temperature (20-30±5ºC)
3. To calculate the production cost of developed fortified pasta.

CHAPTER II

REVIEW OF LITERATURE

Snacks are known to be a portion of food, smaller than a regular meal, generally consumed between meals (Falola et al., 2014). Variety of 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 & more portable than prepared diets food. 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 frequently consumed paste-product made from 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 & Matsuo, 1988). Italians, who are the largest consumers of pasta products in the world, call these products *‘pastaalimentare’* (alimentary paste) (Dick & Matsuo, 1988; Donnelly & Ponte, 2000). Pasta is a traditional food product with origins dating back to the first century B.C. (Dick & Matsuo, 1988; 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 & 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.

Pasta has grown in popularity in recent years as a result of its nutritional benefits and reputation as a low-glycemic-index food (Jenkins et al., 1988; Wolever, 1990; Björck 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 & Piazza, 2006). Pasta is also high in complex carbs, B vitamins, and iron while being low in salt and total fat (Douglass & Matthews, 1982). Normally when we eat pasta, it is accompanied by a series of adjuncts or sauces such as vegetables, fish, meat, eggs (source of protein), and/or legumes (source of protein and fibre) that enhance and improve its nutritional value through a sort of complementary process (Ferrari & Piazza, 2006).

Durum wheat is one of the toughest whea, and when milled, it creates a coarse particle known as "Semolina." (Sissons, 2008). Pasta products are made from wheat durum, which is the most common raw material used in their 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 throughout cooking and dough development during the mixing and extrusion steps (Feillet & Dexter, 1996). Pasta is considered as a suitable vehicle for the incorporation of nutrients by the WHO and FDA (Chillo et al., 2008; Gallegos-Infante et al., 2010; Ovando-Martinez et al., 2009; Cubadda, 1994). However, pasta is nutritionally unbalanced due to its low lipid and fiber content, and to the low biological value protein, owing to its low lysine content.

2.2 Fortification of pasta

To increase the bio-functional and nutritional properties of pasta, Prabhasankar et al. (2009) used Indian brown seaweed (S. *marginatum*) as an ingredient. To make seaweed-incorporated pasta, several degrees of seaweed (1.0, 2.5, and 5.0 percent; w/w) were substituted, while pasta without seaweed served as a control. Microstructure tests found that adding up to 2.5 percent of seaweed to pasta improved the gluten network. Up to 2.5 percent (w/w) of Indian brown seaweed (S. marginatum) can be used into pasta to improve bio functionality/quality as well as the utilitarian value of seaweeds as food products. When brown seaweed, U. *pinnatifida,* was integrated into semolina, different levels of U. *pinnatifida* were found.

According to Kadam et al. (2010), cereals provide dietary fibre, healthy protein, and lipids rich in important fatty acids in addition to a high starch content as an energy source. Vitamins, including several B vitamins, minerals, antioxidants, and phytochemicals are all important micronutrients found in grains. Cereals, in general, supply significant amounts of the majority of nutrients. Pasta is a multi-component system made up of biomacromolecules like carbs and lipids (Kill, 2008). Pasta is a carbohydrate source (74-77 percent, db) whose popularity is growing due to its nutritious qualities, particularly its low glycemic index (GI).

Petitot et al. (2010) developed nutritionally enriched spaghetti by combining durum wheat semolina with a high percentage (35%) of legume flour (split pea or faba bean). To avoid particle agglomeration during mixing, the manufacturing of fortified pasta necessitated a change in the pasta-making process (increased hydration level and mixing speed). Furthermore, the addition of bean flour resulted in a reduction in some pasta quality characteristics (e.g. higher cooking loss, lower breaking energy). This could be due to the addition of nongluten proteins and insoluble fibres, which degraded the pasta's overall structure. Some qualitative features of fortified pasta (e.g. lesser cooking loss) were increased by about.

Wani et al. (2011) tested the effects of adding cauliflower leaf powder to noodle formulations at 0, 10, 15, and 20% flour weight basis on chemical constituents (moisture, protein, fat, ash, and fibre). The results showed that samples of cauliflower leaf powder added noodles had higher protein, fibre, and ash than the control sample at all addition levels. The results revealed that integrating cauliflower leaf powder into roasted wheat flour up to 10% flour weight basis might yield satisfactory noodles in terms of physico-chemical and sensory qualities. Vijayakumar et al. (2010) also reported upsurge in fibre content with increase in millet flour blend incorporation. Eyidemir & Hayta (2009) exposed that increase in protein level in noodles incorporated with apricot flour as to control sample.

Using response surface approach, Badwaik et al. (2014) enriched semolina-based pasta by adding defatted peanut flour and carrot powder. The solid loss and stiffness of dry pasta were shown to decrease when the semolina to DPF and CP ratios increased.

In comparison to a control sample, Kulkarni et al. (2012) found that noodles made from wheat and malted ragi flour had greater protein, fibre, and minerals like calcium, iron, and phosphorus. With the addition of modified millet flour and pulse flour, nutritional analysis revealed an increase in protein, crude fibre, dietary fibre, and minerals components.

Kaur et al. (2013) examined at how varying degrees of plant protein supplementation from mushroom powder, Bengal gram flour, and defatted soy flour affected the nutritional quality of pasta. Supplementation of semolina wheat was done with mushroom powder (0–12%), Bengal flour (0–20%) and defatted soy (0–15%). The cooking time of pasta was increased by mushroom powder and defatted soy flour, however there was no significant difference in the cooking time of Bengal gramme supplemented pasta. On the basis of cooking and sensory value, pasta made with 8% mushroom powder, 15% Bengal flour, and 9% defatted soy was found to be of higher quality and nutritional value.

Sant'Anna et al. (2014) tested the cooking, nutraceutical, and sensory qualities of grape marc powder incorporated at 25, 50, and 75 g/kg in fettuccini pasta preparation. The results demonstrated that adding the dry by-product did not affect the water absorption or solid loss of the pasta after it was cooked. Regardless of the concentration of the dry residue applied, sensory research revealed that the addition of grape marc powder lowered the acceptance of aroma, aftertaste, flavour, and appearance. Furthermore, the addition of 25 g/kg of grape marc powder resulted in the highest overall acceptance and the least colour alterations.

Shukla & Srivastava (2014) assessed for nutrient conformation of 50% finger millet combined noodles and establish that 50% finger millet combined noodles contained highest quantity of fat (1.15%), ash (1.40%), fiber (1.28%) and carb (78.54%).

Yadav et al. (2014) improved wheat-based pasta formulations by adding pearl millet flour and veggies. Extruded was a mixture of wheat and pearl millet flour (9:1) with vegetable paste (2 percent dry solids). Because of the enhanced mineral content, using pearl millet flour and vegetables resulted in nutritionally dense pasta when compared to the control. The addition of vegetables improved the textural properties of the gruel, such as increased hardness and decreased stickiness, and resulted in a considerable reduction in gruel loss. The most nutritionally, texturally, and sensory pleasing pasta was spinach integrated pasta.

Using response surface approach, Mridula et al. (2016) optimise the levels of groundnut meal, capsicum juice, and refined wheat flour for the manufacture of pasta with increased protein and antioxidant activity. Different experimental combinations were designed using the Box–Behnken design of trials, with groundnut meal ranging from 10 to 20 g, capsicum juice ranging from 14 to 30 mL, and refined wheat flour ranging from 80 to 90 g, respectively. With increasing levels of groundnut meal and capsicum juice in the samples, antioxidant activity and phenolic content increased. Pasta samples with a higher percentage of groundnut meal contained more protein. To obtain the greatest experience, optimization was carried out.

Mridula et al. (2017) used response surface methods to standardise the amount of groundnut meal, carrot juice, and refined wheat flour used in the manufacture of pasta. Using the Box–Behnken design of experiments, different experimental combinations were created using 10-20 g groundnut meal, 14-30 mL carrot juice, and 80-90 g refined wheat flour. Pasta samples containing a larger percentage of groundnut meal and carrot juice had increased antioxidant activity and sensory appeal. Protein content was increased in the samples with more groundnut meal. Pasta samples with more carrot juice had a greater rehydration ratio and took less time to cook, with no solid loss in the cooking water.

Fortified pasta was made by replacing durum wheat semolina with 0, 5, and 10 g/100 g of olive pomace (OP), according to Simonato et al. (2019). Both cooked and uncooked pasta were fortified with OP, which dramatically boosted TPC and antioxidant capacity. The addition of OP reduced the ideal cooking time while increasing the swelling index, water absorption, and cooking loss. Firmness and adhesiveness both increased dramatically as OP levels climbed. These findings suggest that pasta fortified with OP could be a healthful product as well as a technological alternative for repurposing food industry by-products.

2.3 Protein fortification of pasta

Protein content and composition in wheat flour have a significant impact on the cooking and eating quality of noodle and pasta products (Hou, 2010). Natural proteins are added to pasta and noodles for a variety of motives, including boosting nutritional quality and maintaining a solid dough structure. Some exogenous proteins can affect gluten proteins in dough, improving the structure of pasta dough and the chewiness of the product (Maforimbo et al., 2008).

Protein interactions in a continuous matrix, as well as protein amount and quality, are critical prerequisites for developing an optimum protein and carbohydrate network to optimise pasta cooking quality (Barbiroli et al., 2013). Desai et al. (2018) discovered that adding fish powder to a recipe reduced the cooking quality by disrupting and weakening the gluten protein network. Ramya et al. (2015) published similar findings, claiming that adding shrimp flesh powder to pasta products causes cooking loss (leaching of solids). When shrimp flesh powder was added, the optimum cooking time was reduced due to higher cooking loss and lower water absorption.

Marti et al. (2014) investigated the effects of incorporating egg albumen and whey proteins into parboiled rice flour pasta (PR). Egg albumen improved the aesthetic of the pasta and resulted in a product that was lower in cooking loss, firmer, and more nutritionally useful than the others. Small starch granules are uniformly surrounded by a protein network in albumen-enriched pasta. The addition of 15% liquid albumen to PR improves the texture and structural characteristics of rice-based gluten-free pasta significantly.

According to Cappa and Alamprese (2017), adding egg albumin to pasta increased product breaking resistance and cooking tolerance, resulting in higher water absorption and fewer cooking losses. Correia et al. (2017) explored the use of mushroom powder as a protein supplement in the manufacture of fresh pasta. Increases in mushroom powder amount and drying temperature resulted in decreased pasta adhesiveness, internal firmness, and exterior firmness. Desai et al. (2018) investigated the effects of fish powder (*Pseudophycis bachus*) on the physiochemical properties of pasta. They showed that adding fish powder to pasta can improve the protein, fat, and ash content.

In cooked pasta, firmness refers to the quality of the bonds and the uprightness of the protein network. Fish powder is added. Adding of powder fish (Desai et al., 2018) & powder of shrimp meat (Kadam & Prabhasankar, 2010) amplified the firmness value of pasta. These findings could be explained by the low water absorption and swelling index. Due to the higher swelling index and water absorption in pasta, firmness might be reduced (Foschia et al., 2015).

2.4 Egg based extruded products

The egg is a very unique biological entity, and egg products are useful polyfunctional components and good sources of key nutrients when used in different food systems. For a human protein efficiency ratio (PER) of 3.2, egg products contain protein that contains all of the necessary amino acids. They are high in important vitamins and minerals, as well as being easily digestible. The polyfunctional qualities of egg products, which make them so valuable and important in many final foods, are mostly due to their proteins.

Julianty et al. (1994) tested four amounts of egg white powder (EWP) added to a fish cracker formulation consisting of tapioca starch, fish paste, and water: 0, 1.5, 3.0, and 4.5 percent. The addition of 1.5 and 3.0 percent EWP had no significant effect (p0.01) on diametral and longitudinal expansion, whereas the addition of 4.5 percent EWP reduced them significantly (p0.01). As the percentage of EWP in the formulation grew, the final product's bulk density and protein content increased. At 4.5 percent EWP, the total colour difference measurement revealed a significant increase (p0.01) in brown coloration.

Khouryieh et al. (2006) looked at the quality of fresh egg noodles made with total or partial egg substitute replacement. Without sacrificing quality, none of the egg alternatives evaluated could completely replace whole egg in egg noodles. In terms of the physical and sensory qualities assessed, partial substitution with eggs was possible. The variation in protein level in egg substitutes had no significant impact on the cooking quality of noodles.

2.5 Skim milk powder

Niturkar et al. (1992) combined wheat semolina, white flour, and skim milk powder to create a seviah. At 2.4 and 6.0 percent, dried skim milk proteins were supplemented. Vermicelli made from semolina fractions combined with milk proteins had an average thread length of 40 to 42 cm, whereas those made from white flour combinations had a thread length of 47 to 50 cm, indicating a larger thread length. The inclusion of milk protein at a rate of 6% in either flour fraction resulted in a reduction in the maximum thread length.

Yadav et al. (2014) made and tested non-wheat pasta with pearl millet flour, barley, and whey protein concentrates. Using response surface methodology (RSM) and a CCD, pasta was made with pearl millet enriched with 10–30% barley flour, 5–15% whey protein concentrate, 2.5–4% carboxymethyl cellulose, and 27–33% water. The results revealed that barley flour and whey protein concentrate (WPC) had a substantial beneficial influence on pasta lightness and a negative effect on stickiness, improving overall acceptability.

Ribanar et al. (2015) evaluated the addition of roasted Bengal gram and SMP as high protein and energy sources, finding that the addition of SMP was acceptable. When compared to the addition of almond and cashew nut combination, there was an upsurge in hardness affecting the organoleptic score as the amount of roasted Bengal gram and SMP rose, indicating higher suitability of snack bar with almond and cashew nut combination. Ruiz-Armenta et al. (2019) evaluated the physicochemical, phytochemical, and antioxidant changes in different stages during the production of indirectly expanded snacks by microwave.

2.6 Response Surface Methodology

Response surface methodology is a mathematical and statistical tool for simultaneously assessing many process variables and their interaction, linear, and quadratic effects, as well as solving multivariable equations. It is a useful strategy for determining the interaction between responses (dependent variable) and factors (independent variable), as well as for reducing the number of experimental trials as compared to a full experimental design. (Murphy et al., 2004; Tiwari et al., 2008; Ghodke et al., 2009). It has been used to analyse the effects of many process parameters in fresh meat processing at the same time (Jakobsen & Bertelsen, 2000). RSM was stated to be an effective instrument for optimizing the procedure, as highlighted by various academics (Vatsala et al., 2001).

RSM has several benefits, including assisting in determining the factor points that will simultaneously satisfy a set of desired specifications, determining the optimum grouping of factors that yields a desired reply and describing the response near the optimum, obtaining a quantitative understanding of the system behaviour over the tested region, and determining how a specific response is affected.

CHAPTER III

MATERIALS AND METHODS

The present study "Development and quality evaluation of pasta fortified with whole egg and skim milk powder" was conducted at the Dept. of LPT, COVS, GADVASU, Ludhiana, PB, India.

3.1 Source of raw materials

3.1.1 Semolina, WEP and SMP powder

Semolina, Skim milk powder and Whole egg powder was obtained from local market Ludhiana.

Table 1: Proximate composition of raw materials used

Proximate composition (%)	Semolina	Whole egg powder	Skim milk powder
Moisture	13.25±0.15	12.92±0.17	4.51±0.5
Protein	11.36±0.09	41.76±0.32	35.21±0.5
Fat	1.08±0.04	10.48±0.23	1.01±0.5
Ash	0.47±0.01	2.47±0.26	9.2±5.25

3.1.2 Packaging material

LDPE (100 to 120 gauge) and Aluminium foil-low density polyethylene (LDPE) laminates were utilised for AP.

3.1.3 Chemicals and media

Various analytical grade reagents and compounds, culture and fine grade performance standards needed for assessing the quality of the reviewed items, obtained from standard companies such as Sisco Research Laboratories, Fisher Scientific, Central Drug House, Hi-Media and Sigma-Aldrich etc.

3.2 Formulation of control & developed pasta

Formulation of the control and developed fortified pasta were standardized on the basis of review of the literature and some preliminary trials conducted in laboratory. The standardized formulation is showed in Table 4.

Table 2: Formulations of the Control and WEP incorporated pasta

S. No	Ingredients	Percentage (w/w)			
		Control (%)	WEP pasta (2%)	WEP pasta (5%)	WEP pasta (8%)
1.	**Semolina**	100.0	98	95	92
2.	**Water**	90 mL	90 mL	90 mL	90 mL
3.	**WEP**	-	2	5	8
	Total	100	100	100	100

Table 3: Formulations of the Control and SMP incorporated pasta

S. No	Ingredients	Percentage (w/w)			
		Control (%)	SMP pasta (5%)	SMP pasta (10%)	SMP pasta (15%)
1.	**Semolina**	100.0	95	90	85
2.	**Water**	90 mL	90 mL	90 mL	90 mL
3.	**SMP**	-	5	10	15
	Total	100	100	100	100

Table 4: Formulations of the Control pasta and Developed fortified pasta

S. No.	Ingredients	Percentage (w/w)	
		Control pasta (%)	Developed fortified pasta (%)
1.	**Semolina**	100.00	85
2.	**Water**	90 mL	90 mL
3.	**Whole egg powder**	-	5
4.	**Skim milk powder**	-	10
	Total	100	100

3.2.1 Preparation of blends

The semolina was supplemented with whole egg powder and skim milk powder as per shown in Table 2, 3 and 4, was weighted carefully and mixed properly by passing twice through the sieve (10 mesh) to prepare blends.

Fig. 1: Pasta making machine (Kent Ltd, New Delhi)

3.2.2 Pasta preparation

The various blends of whole egg powder and skim milk 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 as per Table 2, 3 and 4. 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

Control **T-2** **T-1** **T-3**

Fig. 2: Control and Developed protein fortified pasta (raw) photographs

Fig. 3: Photographs of Packaged Control and Developed protein fortified pasta

3.3 Analytical techniques

3.3.1 Cooking Quality

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

3.3.1.1 Minimum cooking time

Weighed 10g of sample was heated in 100 mL DW 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 (AACC, 2000). MCT was evaluated 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.

3.3.1.2.4 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 (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, 1973). 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, Flavor. 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	Flavor	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° of cool white light (d65) and known as 'L', *a**, and *b** values.

'L' value denotes (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/-blueness). The light trap (black hole) and white tile that come with the device were used to calibrate it. Then the colour characteristics listed above were chosen. At three different points, the instrument was positioned directly on top of the pasta.

3.7 Peroxide value; Koniecko (1979)

5 g of the sample

⇩

blended for 2 min with 30 ml chloroform in the presence of anhydrous sodium sulphate

⇩

Filtered through filter paper (Whatman filter paper No. 1)

⇩

25 ml chloroform extract (filtered) +30 mL of glacial acetic acid +2 ml. of saturated potassium iodide solution

⇩

Added 100 mL of distilled water + 2 mL of fresh 1 percent starch solution

⇩

titrated immediately against 0.1N sodium thiosulphate till the end point (non-aqueous layer turned to colorless).

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

3.8 Free fatty acids (FFA); Koniecko (1979)

5 g of the sample

⇩

blended into anhydrous sodium sulphate 30 ml of chloroform

⇩

Filtered through filter paper (Whatman filter paper No. 1)

⇩

Added 2 or 3 drops of 0.2 % phenolphthalein indicator to chloroform extract

⇩

titrated against 0.1N alcoholic potassium hydroxide

⇩

pink colour (end point)

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

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

3.9 Thiobarbituric acid reactive substances (TBARS) value: Witte et al. (1970)

10 g of the sample

⇩

5 ml of precooled 20% trichloroacetic acid (TCA) + 2 M orthophosphoric acid (2 min)

⇩

Filtered through filter paper (Whatman filter paper No. 1)

⇩

3 mL of TCA extract (filtrate)

⇩

Mixed with equal amount of 2-thiobarbituric acid (TBA) reagent

⇩

Placed in a dark cabinet for 16 hrs.

⇩

Blank: 3 mL of 10% TCA and 3 mL of 0.005 M TBA reagent

⇩

Absorbance (O.D.): 532 nm using UV-VIS spectrophotometer

⇩

Multiplying O.D. value with K factor of 5.2.

3.10 Proximate composition

Using a hot air oven, Kel Plus, Socs Plus, and Muffle furnace, the moisture, protein, fat, fibre, and ash composition of the pasta was calculated.

3.11 Microbiological quality

SPC, coliform and YM count in the the samples were counted according to the American Public Health Association's guidelines (APHA, 1984).

3.11.1 Preparation of sample and serial dilution

laminar flow (Model: RH-58-03, Rescholar equipment, Ambala, India)

10 g of the sample (aseptic weighing)

⇩

transferred to mortar containing 90 mL of sterile 0.1% peptone water

⇩

uniform dispersion and to get a 10 dilution of the sample

⇩

1 mL of solution with micropipette into test tube containing 0.1% peptone water

⇩

further dilution to a level of 10^2 prepared.

3.11.2 Standard plate count (SPC); Pour plate method

23.5 g of plate count agar +1000 mL of distilled water
⇩
boiled to dissolve the medium
⇩
The plates were allowed to get solidify
⇩
inverted and incubated at 35±2 °C for 24 h
⇩
pH (7.0±0.1) was adjusted
⇩
sterilized by autoclaving at 15 pressure (121 °C) for 15 min
⇩
20 mL molten media kept at 45±2 °C was inoculated in duplicate set+1mL of aliquots
⇩
Avg. no. of colonies were multiplied by the reciprocal of the dilution (log10 cfu/g of sample)

3.11.3 Coliform count

51.53 g of media (Violet Red Bile Agar) +1000 mL of distilled water
⇩
boiled to dissolve and cooled to 45 °C (pH-7.4±0.2)
⇩
inoculation of the suitable sample dilution
⇩
plates were incubated at 35±2 °C for 24 h (Log10 cfu/g of sample)

3.11.4 Yeast and mould count

39.0 g of potato dextrose agar +1000 mL distilled water
⇩
Boiled to dissolve the medium
⇩
sterilized by autoclaving at 15 lbs pressure at 121 °C for 15 min
⇩
pH 3.5 by acidification with 10% tartaric acid solution
⇩
inoculation of suitable sample dilution
⇩
incubated at 25 °C for 5 days
⇩
Red, black, greenish black, white colored colonies counted (Log10 cfu/g)

3.12 Microstructure

The structural morphology of 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 x 10-5 Pa.

3.13 Statistical analysis

Design Expert 11.0 (Stat-Ease Inc., USA) applications was used to validate the experimental findings of the RSM. The level of significance was derived using a 95% confidence level (Myers & Montgomery, 2016). Standard statistical approaches were used to examine the data using IBM SPSS Statistics-20.0 software, USA packages (Snedecor & Cochran, 1994). For each attribute, duplicate samples were drawn and reproduced three times (n=6). A panel of seven judges conducted sensory evaluations, with a total of 21 (n=21) observations. Two-way analysis of variance (ANOVA) and the critical difference test were used to compare means across storage periods, among treatments, and between treatments. Duncan's multiple range method was used to test the statistical significance was estimated at 5% level ($p<0.05$). The data was presented in the form of Mean±S.E.

CHAPTER IV

RESULTS AND DISCUSSION

The aim of the current research project was to standardize the level of fortification of egg protein i.e. whole egg powder (WEP) and milk protein i.e. skim milk powder (SMP) for the development of fortified 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 fortified pasta was also calculated. The current section gives information about the results gained from various experiments conducted in accordance with the above-mentioned aim. The results displayed in the text is presented with the support of statistically studied Tables (5 to 21) and Figures (4-22). 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 level of incorporation of WEP and SMP for development of protein fortified pasta (PFP)

4.1.1 Optimization of the level of incorporation of whole egg powder for development of protein fortified pasta

To optimize the level of incorporation of whole egg powder (WEP), four different treatments i.e. Control samples (without WEP), T-1: 2% WEP, T-2: 5% WEP and T-3: 8% WEP according to the given formulation in **Table 5**. These levels were finalized on the basis of literature available and preliminary trials conducted in the department. Developed pasta products were estimated for various cooking parameters like Minimum cooking time (MCT), Volume expansion (VE), Gruel Solid loss (GSL), L, *a** and *b**, Sensory attributes (Overall acceptability; OA), textural characteristics (Firmness and toughness). Results are presented in Table 9 to 11 with suitable findings justified with available references.

There was significant variation in optimum time of cooking of pasta by supplementation of WEP. Control pasta showed least time for cooking tailed by T-1,T-2 and T-3. A study conducted by Daud in 2020 revealed that meat protein enriches the quality of pasta. It was also found that greater the protein in pasta, greater is the cooking time. Water infiltration and starch gelatinization are major cause for

MCT (Edwards et al., 1993).

A glance at Table 5 had given a relation between high supplementation level of WEP and major increment in the GSL of pasta. GSL of control sample of pasta was 12.15, which increased steadily to 16.04, 17.11 and 18.75 at 2, 5 and 8 per cent level of incorporation of WEP. GSL was reported maximum at 6 per cent level of WEP supplementation. This much increase in GSL of pasta may be attributed to higher protein content of supplement. Parallel trend of significant decline in Volume expansion scores of pasta was observed as depicted from Table 5, control sample showed highest score while WEP-5% pasta product showed the best scores among all treated pasta products incorporated with whole egg powder, the reason might be the fiber and protein which have lower swelling power in contrast to starch and they swell less when cooked in comparison to starch.

Table 5: Effect of different levels of fortification of whole egg powder (WEP) on pasta products

Treat.	MCT	GSL	VE	OA	Firmness	Toughness	L*	*a**	*b**
Control	7.01± 0.02 a	12.15± 0.11 a	1.85± 0.02 d	8.52± 0.08 d	0.52± 0.04 a	0.62± 0.01 a	73.32± 0.12 d	1.32± 0.02 a	10.03± 0.11 a
WEP 2%	7.31± 0.04 b	16.04± 0.12 b	1.78± 0.01 c	8.09± 0.06 c	1.21± 0.06 b	1.12± 0.02 b	68.08± 0.23 c	2.14± 0.04 b	13.67± 0.15 b
WEP 5%	7.89± 0.02 c	17.11± 0.10 c	1.47± 0.04 b	7.86± 0.09 b	1.32± 0.04 c	1.23± 0.01 c	61.17± 0.19 b	2.81± 0.02 c	18.61± 0.10 c
WEP 8%	8.25±0.04 d	18.75± 0.09 d	1.15± 0.02 a	6.71± 0.07 a	2.11± 0.08 d	2.04± 0.01 d	59.50± 0.17 a	3.11± 0.05 d	23.22± 0.12 d

Overall acceptability of WEP fortified pasta decreased significantly with increasing level of WEP, this might be due to impartment of unattractive dark yellowish colour due to WEP. Pasta products with WEP-5% was chosen best as it showed high acceptability for color and appearance, flavour, texture and other sensory attributes. Beyond WEP-5% level of incorporation, resulted in dark yellowish colour along with egg flavour which limits sensorial acceptability. Both firmness and toughness showed increasing values, with WEP treatment as the pasta texture mainly depends on the protein's interactions with the insoluble networks of semolina, which forms stable matrix structure leads to higher firmness and toughness. The L* displayed declining values with incorporation of WEP, while *a** and *b** values showed increasing trend with increasing levels of WEP.

The conclusion of the study was that pasta fortified with WEP-5% level showed best sensory attributes and physico-chemical and cooking qualities.

4.1.2 Optimization of the level of incorporation of SMP for development of protein fortified pasta

To optimize the level of incorporation of SMP, four different groups of pasta samples were prepared i.e. Control samples (without SMP), T-1= 5% SMP, T-2 = 10% SMP and T-3= 15% SMP as per the formulation shown in **Table 6**. These levels were finalized on the basis of literature available and preliminary trials conducted in the department. Developed pasta products were evaluated for various cooking parameters like Minimum cooking time (MCT), Volume expansion (VE), Gruel Solid loss (GSL), Colour analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability; OA). Results are presented in Table 9 to 11 with suitable findings justified with available references.

Table 6: Effect of different levels of fortification of skim milk powder (SMP) on pasta products

Treat.	MCT	GSL	VE	OA	Firmness	Toughness	L*	*a**	*b**
Control	7.08± 0.02[a]	12.21± 0.11[a]	1.88± 0.02[d]	8.56± 0.08[d]	0.51± 0.04[a]	0.61± 0.01[a]	73.41± 0.12[d]	1.36± 0.02[b]	10.05± 0.11[a]
SMP 5%	7.55± 0.04[b]	17.27± 0.02[b]	1.62± 0.06[c]	8.41± 0.06[c]	1.28± 0.06[b]	1.02± 0.02[b]	62.24± 0.04[a]	1.42± 0.04[d]	12.69± 0.02[b]
SMP 10%	8.10± 0.02[c]	17.92± 0.04[c]	1.48± 0.04[b]	8.36± 0.02[b]	1.52± 0.04[c]	1.27± 0.06[c]	63.09± 0.02[b]	1.40± 0.02[c]	16.41± 0.01[c]
SMP 15%	10.25± 0.04[d]	18.95± 0.06[d]	1.37± 0.02[a]	7.09± 0.04[a]	2.81± 0.08[d]	2.17± 0.05[d]	65.07± 0.05[c]	1.34± 0.05[a]	19.20± 0.06[d]

The critical evaluation of Table 6 showed that minimum cooking time (MCT) of control was least amongst the studied pasta samples, while SMP treated samples showed increasing MCT with increasing levels of SMP. Highest MCT was reported for pasta supplemented with 15% SMP i.e.10.25 and least was for control samples i.e. 7.08 min. GSL, VE, firmness and toughness linearly increased with increased levels of SMP in treatments, while control pasta samples showed least values for the studied parameters. These findings were justified by Daud, (2020) in pasta incorporated with varying levels of meat proteins.

Colour of the pasta is crucial attribute as it affects the market demand and consumer acceptability, SMP fortified pasta samples showed, higher L* value as compared to that of control samples. The reason for this could be because of inherent white color of skim milk powder. As the amount of fortification of SMP increased, a decline in trend of *a** and *b** values observed. Among all the treatments pasta samples with SMP-10% showed higher consumer acceptability. The textural attributers i.e. firmness and toughness of the WEP treated pasta samples showed increasing trend with increasing levels of WEP linearly, as the pasta texture mainly depends on the protein's interactions with the insoluble networks of semolina, which forms stable matrix structure leads to higher firmness and toughness.

It was concluded that pasta fortified with SMP-10% level showed best sensory attributes along with physico-chemical and cooking qualities.

4.1.3 Proximate composition of developed pasta

Developed pasta was subjected for proximate estimation. It was found that Moisture Moisture (%), Carbohydrate (%), Protein (%), Fat (%) and Ash (%) in control was 10.86±0.11, 70.08±0.06, 10.27±0.09, 1.02±0.04 and 0.55±0.01 respectively. Whereas, developed fortified pasta showed 13.00±0.05 Moisture (%), 50.00±0.02 Carbohydrate (%), 23.25±0.02 Protein (%), 2.61±0.02 Fat (%) and 3.36±0.08 Ash (%) contents.

It was evident that incorporation of WEP-5% and SMP-10% significantly (p<0.05) increased moistness (p<0.05) in developed fortified pasta (added with WEP and SMP) as related to CP (without WEP and SMP). Water is attracted to charged molecules like protein as it is a dipolar molecule. During the pasta-making process, starch granules swell with water and a protein network film were developed around them. The addition of WEP and SMP increased the charged protein content, resulting in improved water holding capacity and higher moisture content. The moisture content of control pasta and developed fortified pasta samples were 10.86±0.11 (%), 13.00+0.05 (%), respectively.

When WEP-5% and SMP-10% was added to the pasta formulation, protein and ash content remained high (p<0.05) compared to that of control pasta. Therefore, the pasta with high protein quality and quantity could be prepared by addition of protein rich sources i.e. WEP and SMP. Fat composition enhanced significantly (p<0.05) with increasing whole egg powder. Higher protein and fat content of the WEP and SMP accounts for the rising fat and protein values. The increasing ash concentration in protein enriched pasta indicates a higher mineral composition. Fortification of both WEP and SMP significantly improved the nutritional as well as functional properties of the developed pasta, which justifies the committed objectives.

4.1.4 Design of Experimental

The level of inclusion of WEP and SMP as two composition coded/uncoded factors were optimized using RSM (Table 7). The trials were designed using a Face Centered Central Composite Design, which resulted in 13 designs (Table 8). Several cooking parameters were used such as (Minimum cooking time (MCT), Volume expansion (VE), Water uptake ratio (WUR), Gruel Solid loss (GSL), Colour L*, *a** and *b**, Firmness and toughness and Overall acceptability; OA, in order to illustrate the most acceptable protein fortified pasta by using WEP and SMP responses.

Table 7: Coded and uncoded levels of independent variables used in Central Composite Design (CCD)

Symbols	Independent variables	Coded levels		
		-1	0	+1
A	Whole Egg Powder	2	5	8
B	Skim Milk Powder	5	10	15

Table 8: Experimental design of Independent variables

Run	Independent variables (Factors)	
	Skim Milk Powder (%)	Whole Egg Powder (%)
1	**15**	**5**
2	**10**	**5**
3	**5**	**5**
4	**5**	**2**
5	**10**	**2**
6	**10**	**5**
7	**10**	**8**
8	**15**	**8**
9	**15**	**2**
10	**5**	**8**
11	**10**	**5**
12	**10**	**5**
13	**10**	**5**

4.1.5 Fitting the models

Each outcome was calculated as a response of linear-quadratic-interaction effects of independent derivatives namely WEP and SMP. The regression coefficients were generated after the experimental values were fitted into 2nd equations (polynomial), and the relevance of the model coefficients was verified using ANOVA, as shown in Table 9-11. The adequacy of the developed model was assessed using ANOVA, R^2, and the model's LOF. For all 10 examined responses, the ANOVA results in Table 9-11 and Fig. 4-13 revealed that the model had very high *F*-values and very low *p*-values (<0.0001).The degree under which the data were spread is described by the coefficient of variation (CV). The proposed models' CV values (varying from 0.04 to 1.79) showed that the tests were extremely credible and accurate.

The coefficients of numerous determinations (R^2) 0.999, 0.9844, 0.9962, 0.9995, 0.9997, 0.9993, 0.9999, 0.9971, 0.9960 and 0.9770 were gained for the response of MCT, L, *a**, *b**, firmness, toughness, GSL, VE, WUR and OA respectively. It shown that the experimental data adequately represented the second-polynomial models were adequately represented by the respective experimental data.

Minimum cooking time (MCT)

The pasta quality was assessed as a function of cooking time in order to generate a comprehensive report on cooking quality; in particular, its response under overcooking circumstances was evaluated in order to assess the sample's resistance to hydrothermal degradation. The MCT was defined as the amount of time it took for at least 95% of the white, opaque core of the samples to vanish. The examination of the response surface, as illustrated in Table 9 demonstrated that WEP and SMP with relation to MCT is quadratic with a good regression coefficient (R^2= 0.9999), and model equation exhibited the relationship as per equation as follows.

$$\mathbf{MCT=18.02+0.58A+1.18B-0.01AB-0.04A^2+0.05B^2}$$

The ANOVA of the models (quadratic-regression) showed that the model was significant. In this case A, B, AB, A^2, B^2 are significant model terms. The statistical analyses revealed that both whole egg powder and skim milk powder had significant linear and interaction effects (p<0.0001) on the MCT of fortified pasta. Thus, predicated model results suggested that a WEP and SMP (%) significantly increased the MCT (Table 9). Sharma et al., (2013) reported that protein hinders the hydration-swelling capacity of granules of starch by surrounding them, resulting in upsurge in the MCT.

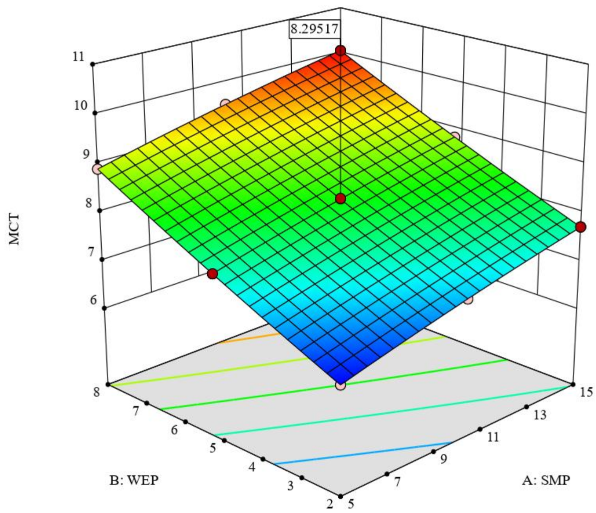

Fig. 4: 3D Response Surface plots for MCT

3D graphs (Fig. 4) showed the effect of whole egg powder and skim milk powder on MCT. It was observed that MCT levels gradually increased when whole egg powder and skim milk powder levels increased. This was due to addition of protein sources i.e. SMP and WEP, which restricted supply of water to starch particles (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., 2021).

Daud (2020) revealed that MCT of chicken fortified pasta increased linearly with protein content & pasta became firmer internally when subjected for cooking than control pasta. Results of Kaur et al. (2012) 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. The optimized MCT predicted by response surface methodology was 8.29% under the formulation condition with whole egg powder of 5% and skim milk powder of 10%.

Volume Expansion (VE)

Volume expansion relates to the increase in the volume of the pasta when cooked for optimum cooking time. The ANOVA (Table 9) and Fig.5 showed the interaction between VE with two independent variables (WEP and SMP) and equation as follows.

$$\mathbf{VE= 1.49-0.17A-0.27B+0.12AB-0.24A^2+0.11B^2}$$

In this case, ANOVA of the linear and interaction regression models for VE showed A, B, AB, A^2, B^2 are significant. Table 9 showed that WEP (%) and SMP (%) had significant *(p*<0.005) linear, quadratic as well as interaction effect on VE.

3D graphs as shown in Fig 5 depicts the effect of WEP (%) and SMP (%) on the VE. It was observed that VE significantly decreased with the increasing both WEP (%) and SMP (%) and nearly reached at lowest point with highest WEP (%) i.e. 15% and SMP (%) i.e. 8%.

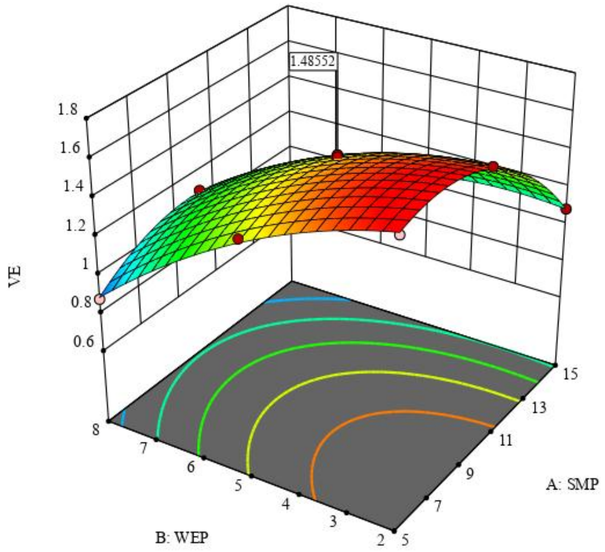

Fig. 5: 3D Response Surface plots for VE

Hence, it was postulated, rise in WEP and SMP in the pasta decreased the volume expansion of pasta (Fig 5), which was due to decrease in the starch content and increase in the level of protein-fiber of 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 added egg and skim milk powder exhibits lower volume expansion (Sharma et al., 2013).

Optimized model predicated by RSM showed 1.49% of volume expansion under the formulation condition; whole egg powder of 5% and skim milk powder of 10%.

Water Uptake Ratio (WUR)

Table 9 shows the effects of whole egg and skim milk powder (%) on the water uptake ratio of fortified pasta. In addition, Fig. 6 illustrates these effects as three-dimensional graphs (3D), where direction of the effects of formulation variables on WUR can be evaluated. The model equation predicting effect of formulation variables on WUR is as follows:

$$\mathbf{WUR=1.72-0.18A-0.27B+0.12AB-0.26A^2-0.11B^2}$$

Table 9: 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	8.30	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-SMP**;** B- WEP; MCT(min)**:-**Minimum cooking time, VE(mL/ g**:-**Volume expansion, WUR(g/100 g)**:-**Water uptake ratio, GSL (g/ 100 g)**:-**Gruel solid loss.

The ANOVA of the quadratic regression models showed that the model was significant ($p<0.05$) with p-values of <0.0001 for WUR. The statistical analyses revealed that WEP and SMP (%) had both significant ($p<0.05$) linear and interaction effects ($p<0.0001$) on WUR. In this case A, B, AB, A^2, B^2 are significant model terms.

The Fig. 6 confirmed the effect of whole egg and skim milk powder (%) on WUR. It was perceived that with rise in WEP and SMP (%), there was significant decline in WUR. This effect on the WUR is due to lower swelling index of added protein source (Ghumman et al., 2016).

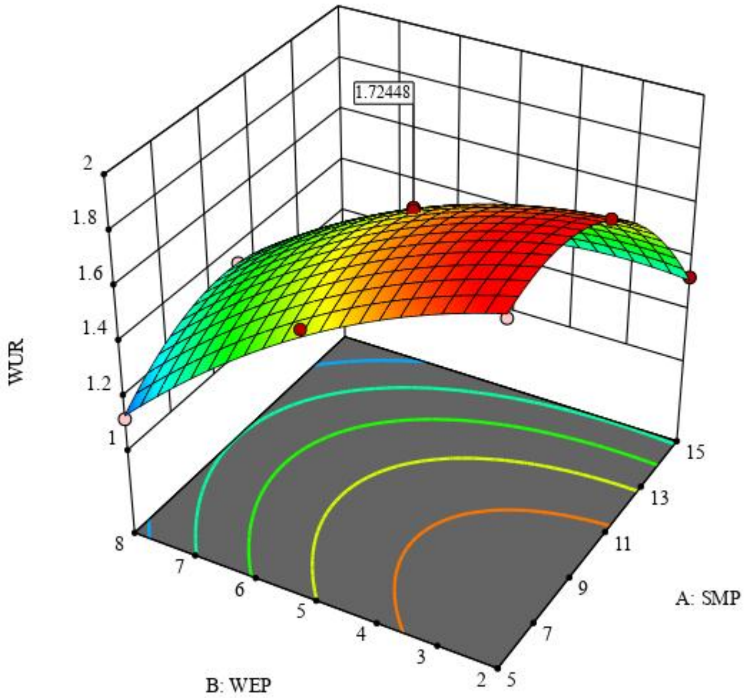

Fig. 6: 3D Response Surface plots for WUR

RSM indicated 1.72 % of water uptake ratio under the formulation condition; whole egg powder of 5% and skim milk powder of 10 %.

Gruel Solid Loss (GSL)

Gruel solid loss is one of the most important technological features for evaluating pasta quality. Solid loss indicates the pasta's resistance to breakdown during boiling, so a low amount of solids in the cooking water denotes good cooking quality. Cooking loss, which is induced by solid leaching during cooking, is used as a measure of total cooking quality (Gull et al., 2018). The RSM as shown in Table 9, confirmed that the relationship between WEP & SMP (%) and model equation as follows.

$$GSL=17.65+0.58A+1.18B+0.022AB-0.04A^2+0.05B^2$$

The analyses revealed that both i.e. WEP & SMP (%) had significant linear and interaction effects (p<0.0001) on the GSL of formulated pasta. Thus, predicated model results suggested that WEP & SMP (%) significantly affected the GSL (Table 9).

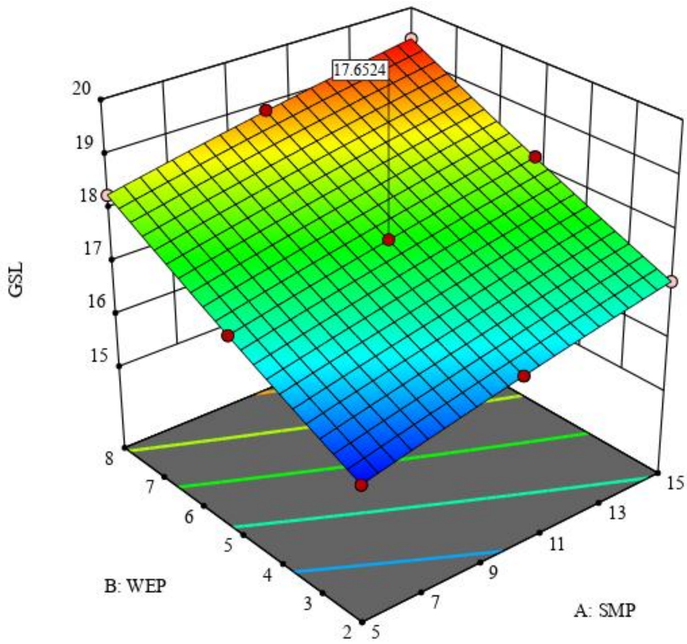

Fig. 7: 3D Response Surface plots for GSL

Table 10: 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- SMP; B- WEP; Firmness (N), Toughness(N) O.A:-Overall acceptability.

Three-dimensional graphs (Fig. 7) showed the effect of whole egg and skim milk powder (%) on GSL. GSL improved significantly with the upsurge in WEP & SMP (%). Increase in GSL with increasing levels of added protein rich whole egg and skim milk powder (%) was due to the weak protein-starch matrix (semolina) which was broken 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 17.65% of GSL by keeping 5% of whole egg and skim milk powder (%) of 10%.

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 like firmness, GSL and OA. 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). Texture of pasta is greatly dependent on the protein network and presence of strong protein network makes pasta more firmer.

Table 10 shows the effects of fortified whole egg powder and skim milk powder (%) on the firmness of fortified pasta. In addition, Fig. 8 illustrate these effects as three-dimensional graphs, where direction of the effects of formulation variables on firmness can be predicated. The regression model equation is as follows:

$$\textbf{Firmness}=1.79+0.58A+1.19B+0.05-0.03A^2+0.05B^2$$

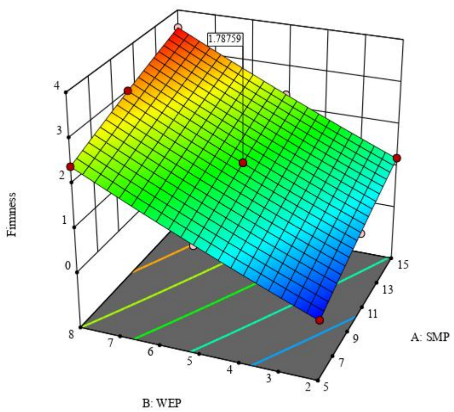

Fig. 8: 3D Response Surface plots for firmness

The Fig. 8 established the effect of WEP and skim milk powder % on firmness. Increased WEP & SMP (%) from 2 to 8 % and 5 to 10%, respectively, resulted increased firmness. In comparison to control pasta, egg and milk proteins interacted with the insoluble networks of pasta, establishing stable matrix structures and culminating in enhanced firmness and toughness. These results were justified by SEM analysis in our study (Liu et al., 2016). Breaking samples with higher protein fortified content needed more power, demonstrating that whole egg powder and skim milk powder were efficient ingredients for fortifying the pasta structure network. Higher protein content in pasta has been shown in studies to result in higher textural intensity, including hardness and toughness (Chang & Wu, 2008). Daud (2020), studied the textural qualities of pasta fortified with meat proteins. The RSM models showed 1.78% of firmness under the formulation condition; WEP of 5% and SMP of 10%.

Toughness

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 & Swanson, 2013). The textural characteristics of pasta play a crucial role in determining the final consumer acceptability. The tuning of texture parameters is a vital step in ensuring consumer acceptance of items. Textural qualities of pasta are primarily affected by the matrix structural network of starches, glutens, extra proteins, and other components (Mudgil et al., 2016).

Table 10 shows the effects of whole egg powder and skim milk powder (%) on the toughness of fortified pasta. In addition, Fig. 9 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:

$Toughness=1.26+0.29A+0.49B+0.08AB+0.24A^2-0.55B^2$

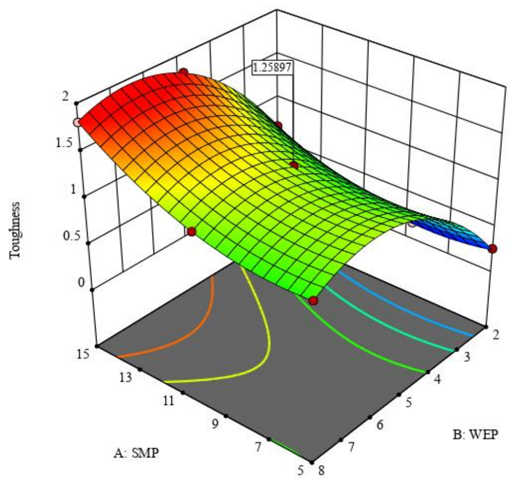

Fig. 9: 3D Response Surface plots for toughness

The Fig. 9 verified the effect of WEP and SMP (%) on toughness. It was evident that with rise in both (p<0.05) increase in toughness was noted as In compared to control pasta, proteins interacted with the insoluble networks of semolina, generating stable matrix structures and resulting in enhanced firmness and toughness (Liu et al., 2016). The RSM models showed 1.25% of toughness under the formulation condition; whole egg powder (%) of 5% and skim milk powder of 10%. The SEM analysis of cooked pasta studies justified the increase in firmness and toughness of developed pasta.

L* value

The colour of a product is an important quality criterion that is closely linked to consumer perception. The effects of WEP and SMP on L* of developed pasta showed in Table 11. In addition, Fig. 10 illustrates these effects as 3D graphs, where direction of the effects of formulation variables on L* can be evaluated. The model equation predicting effect of formulation variables on L value is as follows:

$$\mathbf{L=62.87+0.73A-1.27B+0.29AB}$$

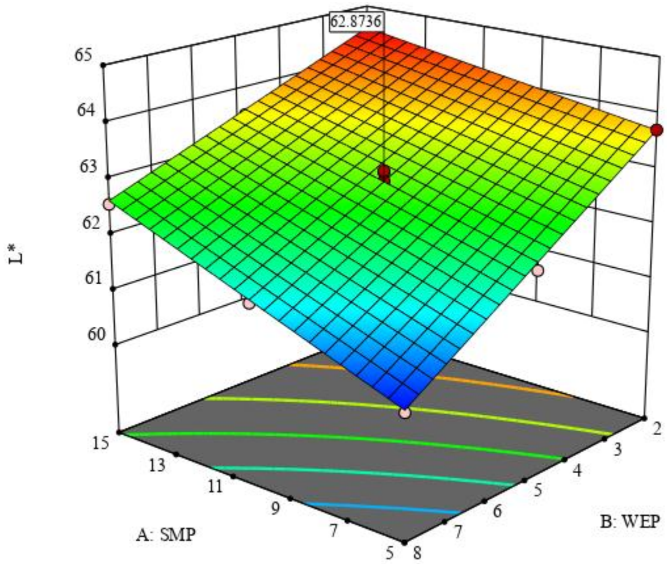

Fig. 10: 3D Response Surface plots for L value

Table 11: 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- SMP**;** B- WEP; *L** (lightness), *a** (green to redness) and *b** (blue to yellowness)

The Fig. 10 validated the effect of WEP and SMP on L*, which implicating that with increase in SMP (%) there was upsurge in L* of resultant product, because inherent colour of raw ingredients i.e. skim milk powder colour as it has high L* value and low *a** and *b** values. It is also evident from Fig. 10 that increase in whole egg protein content from 2 to 8 % resulted significant decrease in L* value. Higher L* due to egg yolk colour (Savita et al., 2013), moreover the whole egg powder itself was yellowish in colour. RSM indicated 62.87 of L* value under the formulation condition; WEP of 5% and SMP of 10%.

The *a and *b** value**

The RSM (Table 11) demonstrated a high regression value (R^2 =0.9962) for *a** value and (R^2 =0.9995) for *b** value. The equation is as follows;

$$a^* = 1.71 - 0.1A + 0.19B + 0.03AB - 0.03A^2$$

$$b^* = 18.62 + 0.03A + 1.02B + 0.08AB - 0.39A^2 + 0.2B^2$$

It was observed that both WEP and SMP (%) had significant (p<0.0001) linear term effects on *a** and *b** value (Table 11). The effect of formulation parameters on *a** and *b** value and their interactions are shown in Fig. 11 and Fig. 12.

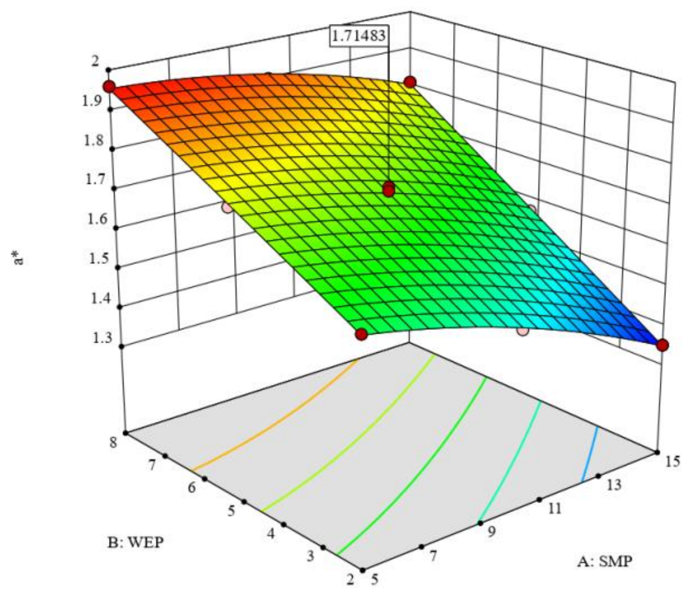

Fig. 11: 3D Response Surface plots for a* value

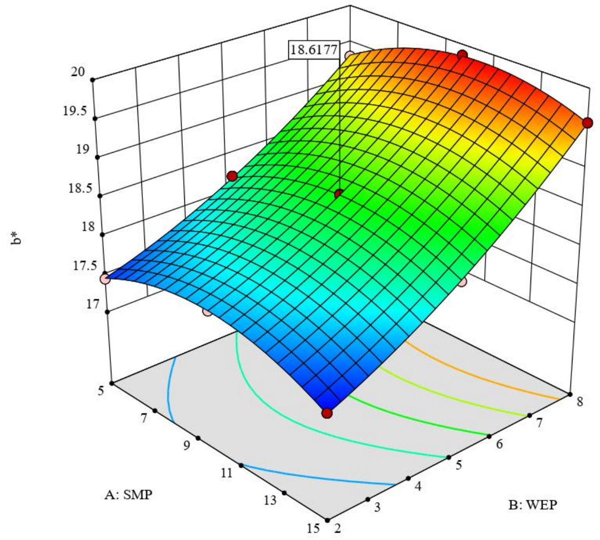

Fig. 12: 3D Response Surface plots for b* value

Increasing WEP percent showed linear escalation in *a** & *b** values as per Fig. 11 and 12. It was found that higher yellowishness resulted in upsurge in *b** value (Savita et al., 2013). The optimal formulation levels of selected variables determined by RSA were WEP of 5% and SMP powder of 10% exhibiting *a** value of 1.71 and 18.61 *b** value.

Overall acceptability (OA)

The most reliable test is sensory evaluation, particularly general acceptability, because it allows for overall features of any foods. RSM as demonstrated (Table 10) followed a high regression (R^2 =0.83) for the OA in the developed pasta and equation explained the relationship as follows,

$$\mathbf{OA= 8.13+0.07A-0.52B-0.15AB-.93A^2-0.84B^2}$$

ANOVA of models (interaction regression) for OA displayed that the model was significant. Table 10 indicated that WEP and SMP (%) had significant (p<0.0001) effects on OA.

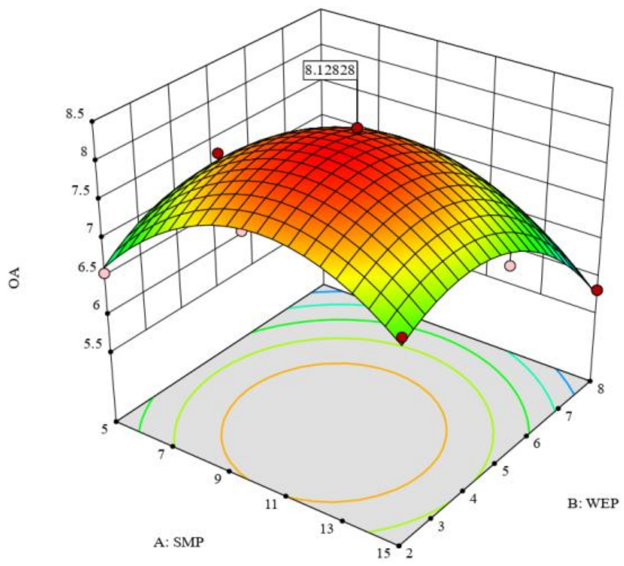

Fig. 13: 3D Response Surface plots for Overall acceptability

Fig. 13 represents the WEP (%) and SMP powder on the OA. It was observed that OA increased with the increasing WEP (%) and nearly reached at highest point at 5% tested and then followed declined trend afterwards. Similar trend were reported for SMP (%). Yadav et al. (2014) reporting this may be due to unattractive dark yellowish colour of cooked pasta, which limits the wider acceptability of the developed pasta products. Optimized model predicated by RSM showed 8.12% of overall acceptability under the formulation condition; WEP of 5% and SMP of 10%.

Optimization of level of incorporation of whole egg powder and skim milk powder for development of protein fortified pasta

The numerical optimization processes for determining the optimum level of WEP and SMP for creating highly acceptable protein supplemented pasta were performed using Design-Ex. 12 software. The optimal combination as: WEP: 5% and SMP: 10% were standardized. As a result, for additional testing, created protein enriched pasta with optimal formulation conditions of WEP (5%) and SMP (10%) were used.

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

In the present experiment fortified pasta T-1 (WEP-5%), T-2 (SMP-10%), T-3 (WEP:SMP; 5:10%) and control (without whole egg and skim milk powder) were prepared as per the formulations shown in Table 4 and stored at ambient temperature (20-30±5 °C) for 60 days under aerobic packaging conditions wrapped in aluminium foil-LDPE laminate pouches. The samples were drawn at regular interval of 15 days.

MCT, OA, firmness and toughness, TBARS, FFA and peroxide-value were estimated to assess the storage quality of the pasta samples. Microbiological qualities were also evaluated on the basis of enumeration of SPC , YM Counts and Coliforms Counts. The findings are presented in the text with the support of statistically analyzed Tables (12 to 21) and Figures (14 to 22). 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 12 depicted the minimum cooking time of control and developed fortified pasta. Significant variation was observed with respect to minimum cooking time of all the studied pasta. On 1st day of storage, control pasta (without WEP and SMP) required 7.13 min for complete gelatinization of starch on cooking, whereas, fortified pasta T-1 required 7.86 minutes, T-2 required 8.08 minutes and T-3 required 8.34 minutes. The same trend were followed during period of storage (60days) for all samples. Amongst treatments T-3 showed longest MCT value as related to remaining pasta samples. This longer cooking time in T-3 could be linked to more established protein networks, due to higher levels of protein content i.e. 15%. The optimum cooking time is measured by the amount of water penetration and starch gelatinization (Edwards et al., 1993).

SEM investigation publicised that enriched pasta had a composite protein structure than CP (Fig 17-22), which could have hindered water entrance into the starch granule and delayed the initiation of the gelatinization procedure, leading in higher cooking time.

Yamazaki et al. (1977) explained the principle of protein hydrophilicity, in which they postulated that while cookery, protein and starch fight 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 as a result, a longer time required for gelatinization. The increase in cooking time is because of the enhanced protein level of the pasta. Oh et al. (1986) revealed that protein content and overall cooking time of pasta have a linear relationship. Results of Kaur et al. (2013) and Surasani et al. (2019) both concur with the findings of the study.

Storage period had non-significant effect on the minimum cooking time of stored control as well as developed fortified pasta i.e. T-1, T-2 and T-3. As the storage period progressed, the time required to cook pasta increased, however, only a slight (non-significant) increase was noted. For control pasta sample, the cooking time slightly increased from 7.13 to 7.16 and for developed fortified pasta sample T-1 increased from 7.86 min to 7.89 min, T-2 increased from 8.08 to 8.12 min and for T-3 increased from 8.34 to 8.37. The fact that the best cooking time is mostly determined by the rates of water penetration and starch gelatinization could account for this rise. (Edwards et al., 1993).

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

Color is significant quality criteria which is tied directly to food acceptability. It acts as an indicator of quality, flavor expectation and commercial value (Fradique et al., 2010). Extrudate colour is a reflection of its thermal history and accounts for non-enzymatic browning resulting by Maillard browning during the extrusion process (Yadav et al., 2014). During storage period color of the product may change due to undesirable reactions such as Maillard browning and degradation of color pigments, depending upon storage conditions viz., temperature, packaging material etc. (Borrelli et al., 2008). The Mean values of the colour of the control and fortified pasta presented in Table 12. On the 1st day of storage, significantly higher L* values were reported in control pasta followed by T-2>T-3 and least were noted for T-1 i.e. pasta fortified with WEP. It might be due to Maillard browning reaction resulting in darkness of products, which must have accounted for the decreased L* values during the storage period. The critical analysis of Table 12 showed that, with advancement of storage time L* value decreased in all of the samples, statistically significant declined was witnessed after 45th day onwards during storage period.

According to Kadam & Prabhasankar (2012), addition of 10-30 g/100 g shrimp meat into pasta reduced the lightness (L*) value when compared to control. Desai et al. (2018) and Surasani et al. (2019) reported reduced lightness values in pasta after the addition of fish powder and pangas protein isolate, respectively. Liu et al. (2016), and Phongthai et al. (2017) observed similar observations during their storage investigations on pasta incorporated with green mussel powder, beef, and egg albumen, respectively.

Table 12: Effect of WEP and SMP fortification on MCT and instrumental colour profile of control and developed pasta during storage (20-30±5ºC) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Minimum cooking time (Min)					
CP	$7.13±0.11^{a}$	$7.14±0.18^{a}$	$7.15±0.14^{a}$	$7.15±0.26^{a}$	$7.16±0.12^{a}$
T-1	$7.86±0.12^{b}$	$7.87±0.24^{b}$	$7.87±0.25^{b}$	$7.88±0.11^{b}$	$7.89±0.27^{b}$
T-2	$8.08±0.14^{c}$	$8.09±0.13^{c}$	$8.10±0.17^{c}$	$8.11±0.14^{c}$	$8.12±0.25^{c}$
T-3	$8.34±0.22^{d}$	$8.35±0.26^{d}$	$8.36±0.27^{d}$	$8.36±0.23^{d}$	$8.37±0.12^{d}$
L*					
CP	$72.39±0.73^{Bc}$	$72.30±0.52^{Bc}$	$72.28±0.54^{Bc}$	$72.11±0.77^{Bc}$	$71.38±0.75^{Ac}$
T-1	$62.77±0.69^{Da}$	$62.64±0.65^{Da}$	$61.71±0.46^{Ca}$	$60.68±0.84^{Ba}$	$58.53±0.92^{Aa}$
T-2	$63.87±0.92^{Bb}$	$63.65±0.83^{Bb}$	$63.41±0.92^{Bb}$	$62.98±0.91^{Ab}$	$62.86±0.81^{Ab}$
T-3	$63.41±0.78^{Bb}$	$63.40±0.66^{Bb}$	$63.16±0.77^{Bb}$	$62.35±0.82^{Ab}$	$62.31±0.97^{Ab}$
a*					
CP	$1.36±0.04^{Aa}$	$1.37±0.02^{Aa}$	$1.37±0.05^{Aa}$	$1.39±0.07^{Ba}$	$1.39±0.02^{Ba}$
T-1	$2.87±0.02^{Ad}$	$2.87±0.01^{Ad}$	$2.93±0.06^{Bd}$	$3.12±0.05^{Cd}$	$3.19±0.03^{Dd}$
T-2	$1.41±0.05^{Ab}$	$1.42±0.04^{Ab}$	$1.42±0.05^{Ab}$	$1.46±0.07^{Bb}$	$1.51±0.04^{Cb}$
T-3	$1.82±0.06^{Ac}$	$1.82±0.06^{Ac}$	$1.84±0.07^{Bc}$	$1.86±0.02^{Cc}$	$1.88±0.07^{Dc}$
b*					
CP	$9.21±0.02^{Aa}$	$9.20±0.15^{Aa}$	$9.17±0.14^{Aa}$	$9.12±0.17^{Aa}$	$9.11±0.15^{Aa}$
T-1	$18.47±0.11^{Bd}$	$18.41±0.26^{Bd}$	$18.39±0.26^{Bd}$	$17.51±0.25^{Ad}$	$17.42±0.24^{Ad}$
T-2	$16.36±0.24^{Bc}$	$16.21±0.17^{Bb}$	$16.14±0.11^{Bb}$	$15.58±0.15^{Ab}$	$15.54±0.12^{Ab}$
T-3	$17.67±0.18^{Bb}$	$17.58±0.55^{Bc}$	$17.49±0.16^{Bc}$	$16.76±0.22^{Ac}$	$16.68±0.14^{Ac}$

n=6**, CP**: Control pasta, **T-1**: Whole egg powder (5%); **T-2**: skim milk powder (10%) and **T-3**: whole egg powder and skim milk powder (5:10). *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly (p<0.05).

Table 12 depicts the increase in a* values for control as well as T-1, T-2 and T-3 fortified pasta with the increasing storage period. The rise in *a** value could be attributed to increased non-enzymatic browning during storage (Wani & Kumar, 2016). It can further be noted that the change in *a** value was more pronounced in T-1 (WEP-5%) fortified pasta products followed by T-3 which showed slight less then T-1 i.e. 1.82, which followed by T-2 i.e. 1.41 and the lowest showed in control i.e. 1.36. It could be because of the addition of whole egg powder which causes slight yellowness in the treated T-1 sample. Sunita & Chauhan (2008) and Pua et al. (2007) reported a comparable increase in enzymatic browning for cereal flakes and jackfruit, snacks, respectively that may lead to the increment in *a** value during storage. The *b** value showed a declining trend during storage (Table 12). For control pasta products, it decreased from the initial value of 9.21 to 9.11 and 18.47 to 17.42 for T-1, 16.36 to15.54 for T-2 and 17.67 to 16.68 for T-3 fortified pasta products.

Firmness and Toughness

The textural properties of pasta affect the final consumer acceptability, which play a crucial role in determining quality assessment of pasta. The results for the firmness & toughness are presented in Table 13. The strength of the linking and the integrity of the protein matrix present in pasta after cooking are measured by firmness. Firmness of the control and fortified pasta (T-1, T-2 and T-3) dropped linearly as the storage progressed and T-3 pasta product showed highest firmness value compare to control as well as T-1 and T-2 on day 1 storage period. The reason might be addition of high protein content (15%), which contributed thermal denaturation of the protein during cooking that enhances the firmness. Higher firmness is due to a greater number of chains of polypeptide in combination with increased protein content, which raises the likelihood of proteins interacting to form an intractable network (Surasani et al., 2019).

The result is in good compliance with the findings reported by (Alireza Sadeghi & Bhagya, 2008; Duda et al., 2019; Liu et al., 2016) in pasta fortified with varying levels of protein sources. Elevated protein incorporation in banana pasta increased firmness (Rachman et al., 2019). Egg protein exhibited higher hardness values than soy protein, which is consistent with earlier investigations on the incorporation of egg and soy protein into pasta (Larrosa et al., 2016; Marti & Pagani, 2013; Phongthai et al., 2017; Sarawong et al., 2014).

Table 13, depicted the firmness values of studied pasta samples with respect to storage period of 60 days. It was observed that, toughness values were significantly varied (p<0.05) between treatments and CP samples. T-3 showed higher toughness value among all the treatments. This may due to presence of large amount of chains of polypeptide linked to higher levels of protein in developed fortified pasta (whole egg powder and skim milk powder). This insoluble protein structure can abduct bloated and gelatinized starch granules, prohibiting pasta from becoming tangled (Chillo et al., 2010).

Table 13: Effect of WEP and SMP fortification on instrumental texture profile of control and developed 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	$0.43±0.01^{a}$	$0.43±0.02^{a}$	$0.43±0.02^{a}$	$0.42±0.06^{a}$	$0.41±0.01^{a}$
T-1	$1.31±0.02^{Eb}$	$1.30±0.03^{Db}$	$1.28±0.04^{Cb}$	$1.26±0.02^{Bb}$	$1.23 ±0.06^{Ab}$
T-2	$1.46±0.05^{Dc}$	$1.45±0.04^{Cc}$	$1.45±0.08^{Cc}$	$1.44±0.06^{Bc}$	$1.42±0.04^{Ac}$
T-3	$1.83±0.09^{Dd}$	$1.83±0.07^{Dd}$	$1.82±0.07^{Cd}$	$1.81±0.08^{Bd}$	$1.80±0.06^{Ad}$
Toughness					
CP	$0.63 ±0.02^{a}$	$0.62±0.05^{a}$	$0.61±0.04^{a}$	$0.61±0.05^{a}$	$0.60±0.06^{a}$
T-1	$1.21±0.06^{Db}$	$1.19±0.07^{Cb}$	$1.19±0.04^{Cb}$	$1.18±0.08^{Bb}$	$1.16 ±0.06^{Ab}$
T-2	$1.26±0.05^{Cc}$	$1.26±0.06^{Cc}$	$1.25±0.05^{Bc}$	$1.24±0.02^{Ac}$	$1.24±0.04^{Ac}$
T-3	$1.28±0.04^{Cd}$	$1.28±0.05^{Cd}$	$1.27±0.04^{Bd}$	$1.26±0.06^{ABd}$	$1.25±0.06^{Ad}$

n=6**, CP**: Control pasta, **T-1**: Whole egg powder (5%); **T-2**: skim milk powder (10%) and **T-3**: whole egg powder and skim milk powder (5:10). *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly (*p*<0.05).

Toughness (Table 13) followed a similar declining trend throughout storage period as that of firmness. It decreased from the initial value of 0.63 to 0.60 for CP samples and 1.21 to 1.16 for the T-1, 1.26 to1.24 for T-2 and 1.28 to 1.25 for the T-3 fortified pasta. As the storage period progressed, control pasta showed statistically non-significant change in toughness scores. Whereas, T-2 and T-3 fortified pasta exhibited a significant drop in toughness scores from the 15th day onwards, while T-1 pasta showed a significant drop in scores from the 30th day onwards.

Sensory evaluation

Sensory evaluation is important not only for new product creation but also for determining a product's shelf life and acceptability. By measuring the total experience of eating quality, sensory evaluation symbolises product approval. In the present study, preference for all sensory attributes decreased throughout the storage days (Table 14). On the 1st day of storage, control pasta showed the highest scores (8.48) for appearance and color, which declined to 7.40 on the 60th day of storage Whereas, on the 1st day of storage developed pasta (T-1, T-2 and T-3) displayed (p<0.05) lower appearance & colour scores as compared to control pasta and also followed similar declined trend with progress of storage. Appearances and colour of both control as well as fortified pasta (T-1, T-2 & T-3) decreased gradually as the storage progressed, at the end of the storage highest scores were reported for control pasta samples followed by T-2>T-3 and least scores were reported for T-1 samples. This was probably due to the supplementation of whole egg powder into T-1 pasta, which had a slightly dark yellow color. Dalbon et al. (1996) stated that the egg yolk gives a deep yellow color to fresh pasta. Where as skim milk powder fortification in T-2 and T-3 gives slight whitish colour. Decrease in color 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 snacks added with in fish powder.

The mean body and texture scores declined as storage progressed for control and developed fortified pasta at ambient temperature for 60 days (Table 14). Control pasta showed least change (non-significant) in body and texture scores throughout the storage period. Within treatments fortified pasta T-1, T-2 and T-3 showed declined values as the protein fortification increased i.e. T-3>T-2>T-1 and all the three fortified pasta sample showed progressive declined trend in the scores as the storage days increases up to 60th day. 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 14: Effect of WEP and SMP fortification on sensory attributes of control and developed pasta during storage (20-30±5ºC) under ambient atmospheric conditions

Treat/ Days	Day 1	Day 15	Day 30	Day 45	Day 60
Appearance and colour					
CP	8.48 ± 0.05^{Ac}	8.47 ± 0.04^{Ac}	8.43 ± 0.05^{Ac}	8.41 ± 0.06^{Ac}	7.40 ± 0.06^{Bd}
T-1	8.03 ± 0.05^{Da}	8.01 ± 0.05^{Da}	7.90 ± 0.06^{Ca}	7.51 ± 0.05^{Ba}	6.17 ± 0.06^{Aa}
T-2	8.46 ± 0.05^{Cc}	8.44 ± 0.05^{Cc}	8.41 ± 0.06^{BCc}	8.21 ± 0.02^{Bb}	7.13 ± 0.02^{Ac}
T-3	8.21 ± 0.06^{Db}	8.15 ± 0.02^{CDb}	8.09 ± 0.01^{Cb}	7.50 ± 0.04^{Ba}	6.92 ± 0.05^{Ab}
Body and texture					
CP	8.45 ± 0.12^{c}	8.44 ± 0.11^{c}	8.41 ± 0.15^{d}	8.37 ± 0.12^{c}	8.32 ± 0.12^{c}
T-1	8.01 ± 0.10^{Da}	7.92 ± 0.08^{CDa}	7.86 ± 0.09^{Ca}	6.38 ± 0.10^{Ba}	6.07 ± 0.09^{Aa}
T-2	8.43 ± 0.11^{Cc}	8.41 ± 0.06^{Cc}	8.38 ± 0.11^{BCb}	7.49 ± 0.09^{Bb}	7.13 ± 0.07^{Ab}
T-3	8.18 ± 0.09^{Cb}	8.16 ± 0.12^{Cb}	8.01 ± 0.10^{Bc}	7.46 ± 0.09^{ABb}	7.11 ± 0.08^{Ab}
Flavour					
CP	8.58 ± 0.09^{c}	8.57 ± 0.11^{c}	8.53 ± 0.11^{c}	8.51 ± 0.14^{c}	8.50 ± 0.12^{d}
T-1	6.43 ± 0.12^{Ca}	6.30 ± 0.15^{BCa}	6.26 ± 0.13^{Ba}	6.10 ± 0.12^{ABa}	6.07 ± 0.11^{Aa}
T-2	8.26 ± 0.14^{Ac}	8.24 ± 0.13^{Ac}	8.21 ± 0.12^{Ac}	8.20 ± 0.09^{Ab}	8.18 ± 0.17^{Ac}
T-3	8.19 ± 0.12^{Db}	8.17 ± 0.14^{Db}	8.09 ± 0.11^{Cb}	7.81 ± 0.13^{Ba}	7.02 ± 0.18^{Ab}
Overall acceptability					
CP	8.49 ± 0.13^{Ac}	8.48 ± 0.12^{Ac}	8.43 ± 0.13^{Ac}	8.42 ± 0.10^{Ac}	7.41 ± 0.14^{Bd}
T-1	8.04 ± 0.18^{Da}	8.03 ± 0.14^{Da}	7.91 ± 0.11^{Ca}	7.52 ± 0.11^{Ba}	6.18 ± 0.10^{Aa}
T-2	8.47 ± 0.14^{Cc}	8.45 ± 0.12^{Cc}	8.42 ± 0.15^{BCc}	8.23 ± 0.16^{Bb}	7.14 ± 0.14^{Ac}
T-3	8.24 ± 0.12^{Db}	8.17 ± 0.09^{CDb}	8.11 ± 0.14^{Cb}	7.52 ± 0.10^{Ba}	6.93 ± 0.11^{Ab}

n=6**, CP**: Control pasta, **T-1**: Whole egg powder (5%); **T-2**: skim milk powder (10%) and **T-3**: whole egg powder and skim milk powder (5:10). *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small 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 all four tested products decreased gradually ($p>0.05$) as the days of storage progressed (Table 14). The decline in flavor scores was comparatively less in control and T-2 pasta samples, while T-1 and T-3 showed more pronounced decline for flavour score. This trend of decrease in flavor scores can be corroborated with increase in TBA value, which indicates lipid oxidation that in turn leads to off flavor development, thus decreasing sensory scores of the product. The reduction in flavour increased microbial load (Sahoo & Anjaneyulu, 1997). Similar reports have been published by Raja et al. (2014) during storage studies of aerobically packaged fish curls.

Khouryieh et al. (2006) studied the sensory properties of egg noodles, reported that addition of whole egg provided firmer texture to the noodles. Studies revealed that addition of SMP at different rate did not show significant ($p>0.05$) effected the expansion index, color & appearance and flavor score of the snack (Yadav et al., 2020).

The critical analysis of Table 14 indicated that control and fortified pasta products i.e. T-1, T-2and T-3 followed a declined trend in overall acceptability scores as the storage days progressed. The decline in overall acceptability scores could be attributed to changes in scores of color and appearance, flavour, texture and other sensory attributes, which in terms are good indicators of physiochemical changes during storage period (Surasani et al., 2019). On the 1st day, significantly higher score reported for control pasta samples followed by T-2>T-3 and least scores were reported in T-1 pasta samples. T-1 had the lowest acceptability score at the conclusion of the 60-day storage period when compared to the other treatment samples. This might be due to the added WEP with the drawbacks of dark yellowish red colour, strong flavor of yolk, resulted pasta was less satisfactory to the sensory board. Alike results have also been described by Kumar & Sharma (2004) for various snack based products.

TBARS, FFA and PV

Thiobarbituric acid (TBA) value was used to assess the significance of lipid oxidation in the extruded snack throughout storage period. The results with respect to change in TBA value for developed fortified pasta and control samples are illustrated in Fig. 14.

The TBA value of control pasta, increased from initial value from 0.57 to 0.82, while in the developed fortified pasta (T-1, T-2 & T-3) it increased from 0.71 to 0.87 during the conclusion of the storage time . The overall mean values of TBARS of developed fortified pasta and control pasta samples increased significantly ($p<0.05$) over storage period of 60 days.

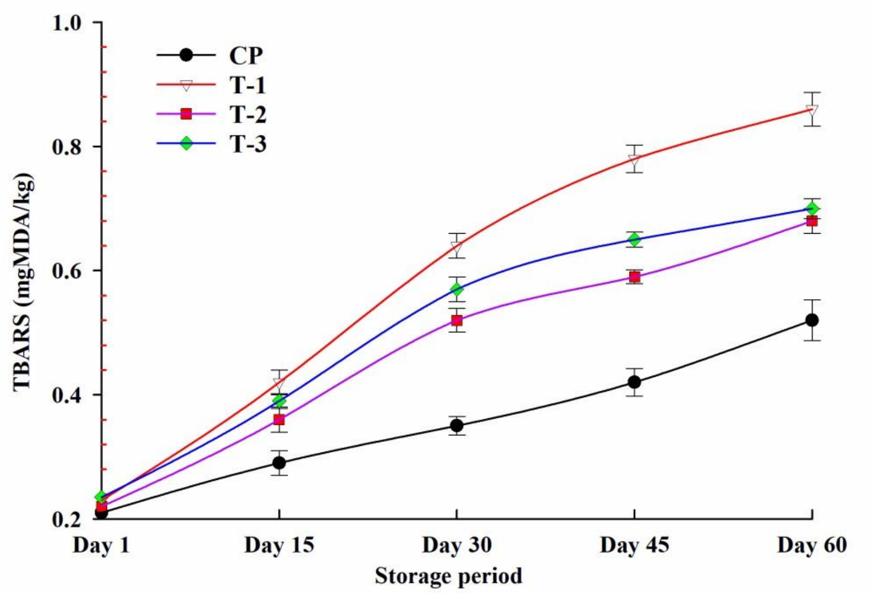

Fig. 14: Effect of WEP and SMP fortification on TBARS values of control and developed fortified pasta during storage (20-30±5 °C) under ambient atmospheric conditions

It could be related to the existence of unsaturated fatty acid in developed fortified pasta (T-1, T-2 and T-3) added with Whole egg powder and SMP than CP, as unsaturated fats are much more susceptible to oxidation than control.

The higher TBARS values in developed pasta samples compared to control the reason could be the higher protein and fat content. Nayak & Tanwar (2005) also have investigated that increase in TBARS values during the storage study because of increased fat degradation and production of volatile metabolites.

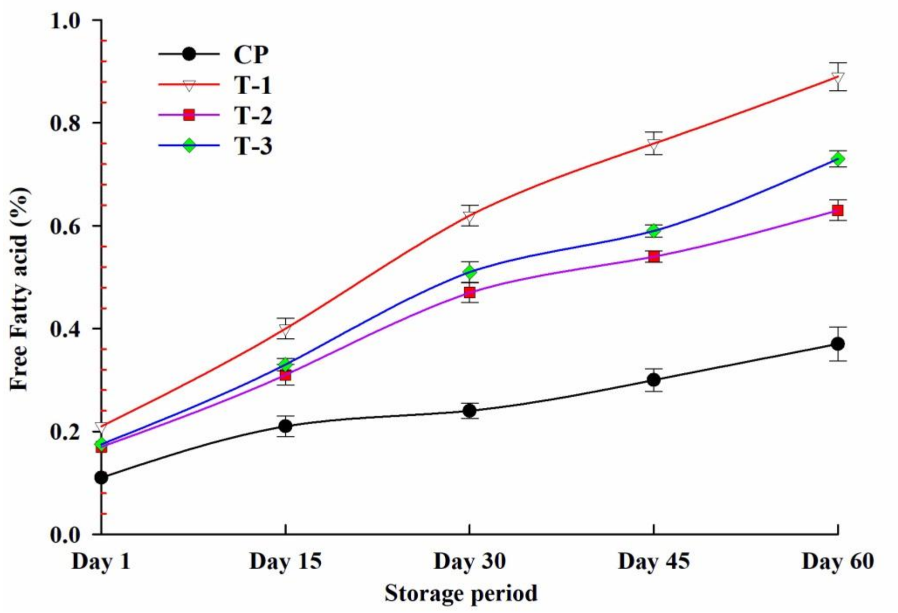

Fig. 15: Effect of WEP and SMP fortification on FFA values of control and developed fortified pasta during storage (20-30±5 °C) under ambient atmospheric conditions

The FFA content of the produced fortified pasta samples was substantially greater ($p<0.05$) greater than control. The FFA values showed significantly ($p<0.05$) increasing trend with progression of storage days (Fig. 15).

The Fig. 16 showed that PV values of CP and also developed fortified pasta samples.

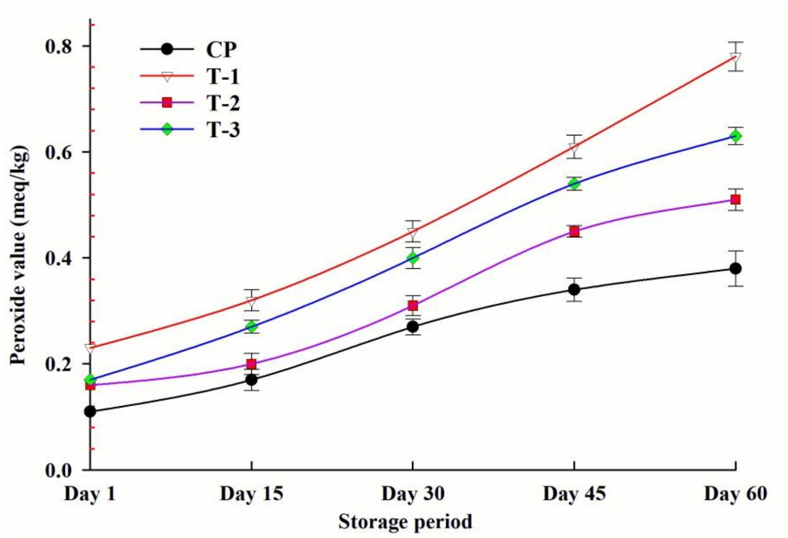

Fig. 16: Effect of WEP and SMP fortification on PV values of control and developed fortified pasta during storage (20-30±5 °C) under ambient atmospheric conditions

It was noted that PV followed increasing trend with progress of time of storage in both treated as well as control samples. The significantly higher PV values were noted in T-1 followed by T-3>T-2 and least were noted for control pasta samples. It could be because of hydroperoxide production during storage rather than their breakdown into subsequent oxidation products. 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 fortified pasta containing WEP and SMP powder are presented in Table 13 during storage of 60 days. SPC in control pasta was ranged from 0.94 to 1.23 and in developed pasta T-1 from 0.85 to 2.76, T-2 showed 1.22 to 1.78 and T-3 from 1.25 to 2.04.

The SPC increased ($p<0.05$) in both control and fortified pasta during the whole storage study (Table 15). During storage the significant increase in SPC observed on 15th day onwards in T-1 and on 30th day onwards in control, T-2 and T-3. SPC values were slightly higher in fortified pasta than control which could be due to higher nutritive value of added egg powder and skim milk powder (easy availability of protein-rich nutrients to favour microbial growth) than semolina based control pasta. Similar finding were observed by the Singh et al. (2021).

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. It may be considered as reference point between spoiled and unspoiled extruded products. Table 15 revealed that no coliforms were discovered during the 60-day storage period, which could be attributable to lower water activity and water activity and hygienic handling and packaging of products.

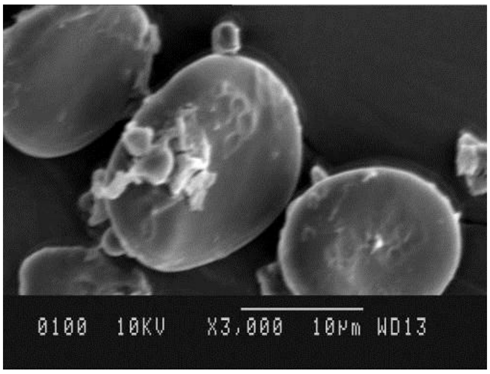

Fig. 17: control pasta samples (3000X resolution)

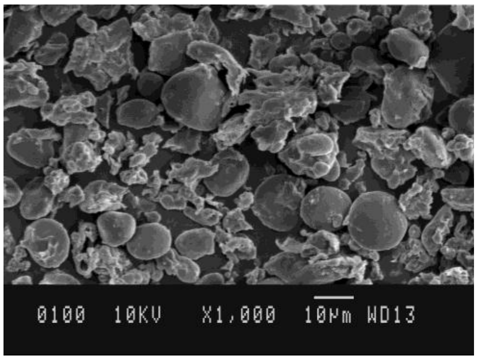

Fig.18: control pasta samples (1000X resolution)

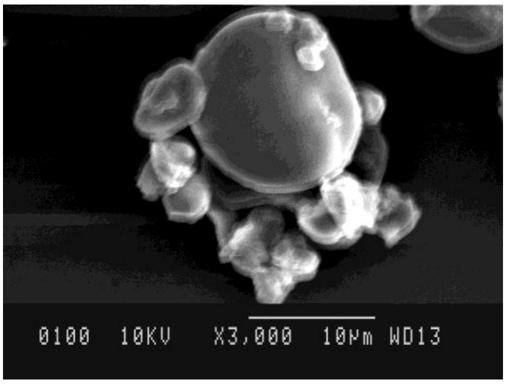

Fig. 19: WEP fortified pasta (3000X resolution)

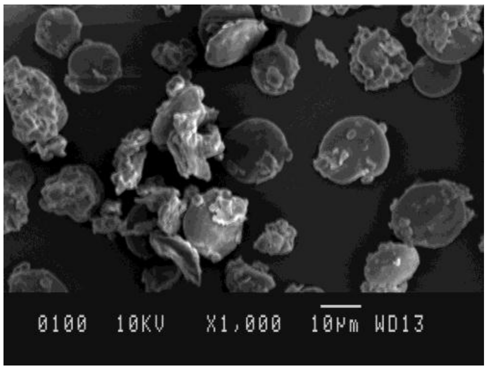

Fig. 20: WEP fortified pasta (1000X resolution)

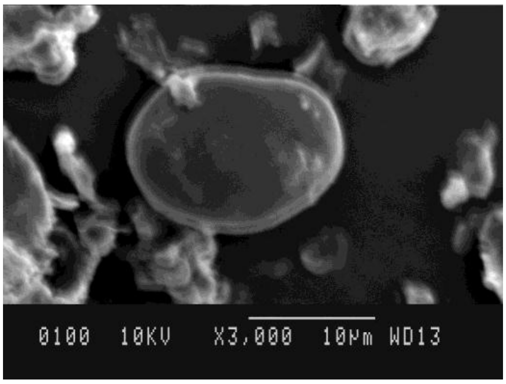

Fig. 21: SMP fortified pasta (3000X resolution)

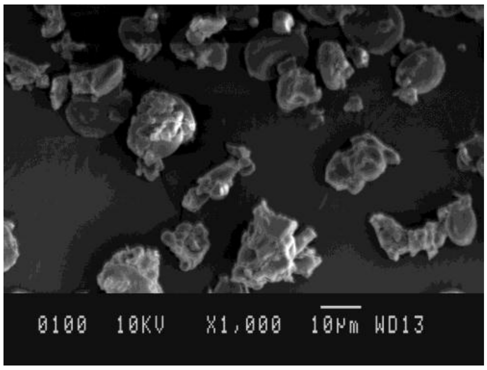

Fig. 22: SMP fortified pasta (1000X resolution)

Table 15: Effect of WEP and SMP fortification on microbiological qualities of control and developed fortified pasta during storage (20-30±5ºc) under ambient atmospheric conditions.

Treat/days	Day 1	Day 15	Day 30	Day 45	Day 60
Standard plate count (log cfu/g)					
CP	ND	ND	$0.94±0.01^{Aa}$	$1.16±0.02^{Aa}$	$1.23±0.01^{Aa}$
T-1	ND	$0.85±0.01^{Ac}$	$1.37±0.02^{Bc}$	$1.89±0.01^{Cc}$	$2.76±0.01^{Dd}$
T-2	ND	ND	$1.22±0.01^{Ab}$	$1.24±0.02^{Ab}$	$1.78±0.02^{Bb}$
T-3	ND	ND	$1.25±0.01^{Ab}$	$1.29±0.01^{Ab}$	$2.04±0.01^{Bc}$
Coliform count (log cfu/g)					
CP	ND	ND	ND	ND	ND
T-1	ND	ND	ND	ND	ND
T-2	ND	ND	ND	ND	ND
T-3	ND	ND	ND	ND	ND
Yeast and mold count (log cfu/g)					
CP	ND	ND	ND	ND	ND
T-1	ND	ND	ND	ND	ND
T-2	ND	ND	ND	ND	ND
T-3	ND	ND	ND	ND	ND

n=6**, CP**: Control pasta, **T-1**: Whole egg powder (5%); **T-2**: skim milk powder (10%) and **T-3**: whole egg powder and skim milk powder (5:10). *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly (*p*<0.05).

Complete absence of yeast and mould count has been showed by developed fortified pasta and control pasta. It may be due to hygienic handling and packaging of products.

Microstructure of raw pasta

The SEM of cross-sections of raw control and developed fortified pasta i.e T-1 fortified with 5% WEP and T-2 fortified with 10% SMP at different resolution i.e. 1000X and 3000X are shown in Figure 17 to 22. In control pasta samples, oval-shaped starch granules were found. at both 1000X and 3000X resolution (Figure 17 to 18), whereas the interior structure of raw produced fortified pasta (Figure 19 to

22) revealed a porous structure with starch-granules firmly embedded in a protein matrix. The addition of protein rich powder of WEP and SMP decreased the porosity up to some extent resulting into protein network that wrapped the starch granules, which resulted in the restricted starch swelling resulting from less absorption.

The protein matrix around the starch granules was improved by replacing semolina with WEP and SMP powder. The findings of Scanning Electron Microscopy on pasta samples are very similar to those reported by other researchers i.e. Gupta et al. (2021) in pasta fortified with quinoa protein isolates and Kadam & Prabhasankar (2010) in shrimp meat pasta.

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

4.3.1 Economics of production of fortified pasta incorporated with Skim milk powder and whole egg powder

Any technology development is not considered successful unless it is brought to use for the benefit of society. Food product technology is determined by various factors, including flavour, appearance, colour, aroma, and nutritional content, as well as the cost of production. The economics, which included the cost of production of fortified pasta incorporated with SMP and WEP, were calculated for this.

The following hypotheses were being used to estimate the economy

1) 100 kg of fortified pasta incorporated with whole egg powder and skim milk powder is produced per day.

2) As this facility operates for 25 days per month, the monthly production goal of fortified pasta incorporated with WEP and SMP is 100 x 25 = 2500 kg/ month.

3) Ingredient costs are estimated by using current market cost in the local market (from January to June 2021).

4) To evaluate the exact manufacturing costs of fortified pasta incorporated with whole egg powder and skim milk powder investment made in terms of recurrent goods, labour charges, water and electricity bills, devaluation on machines and

equipment, lease paid, capital expenditure and its annual percentage rate, was put into account under consideration.

5) The invoice of the sale of fortified pasta incorporated with WEP and SMP, rather than a product recovery while raw material processing. As a result, it may be a source of additional profit for the business owner.

Table 16: Cost of production of formulation ingredients

Name of ingredients	**Quantity (g)**	**Rate (Rs/Kg)**	**Approx. Cost (Rs)**
Semolina	850	66	45.27
Whole egg powder	50	700	40.50
Skim milk powder	100	300	35
Total	**1000**		**Rs 120.77**

4.3.2 Formulation cost of control and developed fortified pasta

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

Table 17: Preparation expense of 100 kg CP and DP

Ingredients	**Rate Rs/kg**	**Control**		**Developed fortified pasta**	
		Qt. (Kg)	**Amount (Rs)**	**Qt. (Kg)**	**Amount (Rs)**
Semolina	66	100.00	6600	85	5610.00
Whole egg powder	700		-	5	3500.00
Skim milk powder	300		-	10	3000.00
Total (Rs)	-	-	6600.00		12110.00

A) Composition charges for 100 kg CP = Rs. 6600.00

B) Cost of formulation of 100 kg developed fortified pasta= Rs. 12110.00

C) Expense of overhead production for 100 kg of fortified pasta

a. Charges for labour

Underskilled employee (5-daily paid workers) (Rs 220.00/day x 6) = Rs. 1320/-

b. Electricity charges

Table 18: Electricity charges

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

Electric cost (Rs 6/Unit) (43.8x Rs. 6.0) =262.80

c. Equipment depreciation

Table 19: Equipment depreciation

Equipment	**Cost (Rs)**
Pasta maker	**12000.00**
Sealing machine	**5000.00**
Industrial dryer	**100000.00**
Total	**Rs 1, 17, 000/-**

Devaluation at a rate of 10% per year =**Rs 11700/-**

Depreciation expense per day **=Rs 40.27**

a) Charges for water supply (1000 litres) =Rs 10/day

b) Material charge of packaging (pack 250g each) = Rs. 240.00

a. (8"x6" Alum-LDPE Pouches @ Rs 0.6/pouch) (400 x 0.60)

c) Rental cost for a room (Rs 3000 per month) = Rs.120.00 per day

d) Miscellaneous =Rs.250.00 per day

E. The overall amount of expenditures (a + b + c+ d) =Rs.620.00/-

Table 20: Production cost control and developed fortified 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 fortified pasta = (B) + (E)= Rs. 12110+ 620= **Rs. 12730.00/-**

Table 21: Production cost of 1Kg control and developed fortified pasta

Control pasta	= Rs. 72.00
Developed fortified pasta	= Rs.127.30

CHAPTER V

SUMMARY AND CONCLUSIONS

The summary and conclusions of investigation entitled “DEVELOPMENT AND QUALITY EVALUATION OF PASTA FORTIFIED WITH WHOLE EGG AND SKIM MILK POWDER” as follows:

The objective of present study is to find the best level of incorporation of WEP and SMP for the production of fortified pasta. The developed pasta evaluated for the storage stability at ambient temperature (20-30± 5°C) for 60 days wrapped in aluminium foil LDPE laminate pouches. The production cost for development of fortified pasta was also calculated. The summary and results from several experiments carried out in accordance with the above given objectives are described in this chapter.

5.1 Optimization of the level of incorporation of WEP and SMP for the development of fortified pasta

5.1.1 Optimization of the level of incorporation of whole egg powder for development of protein fortified pasta

In this experiment, optimization of level of combination of whole egg powder (WEP), four different groups of pastas were formulated i.e. Control samples (without WEP), T-1: 3% WEP, T-2: 5% WEP and T-3: 7% WEP. Developed pasta products were evaluated for various cooking parameters like Minimum cooking time (MCT), Volume expansion (VE), Gruel Solid loss (GSL), Colour analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability; OA). There was significant variation in minimum cooking time of pasta by supplementation of WEP. Control pasta showed least time for cooking followed by T-1, T-2 and T-3. GSL for control pasta samples was 12.15, which increased steadily to 16.04, 17.11 and 18.75 at 2, 5 and 6 per cent level of incorporation of WEP. GSL was reported maximum at 6 per cent level of WEP supplementation. Pasta products with WEP-5% was chosen best as it showed high acceptability for color and appearance, flavour, texture and other sensory attributes. The L* values showed decreasing trend with increasing level of incorporation of WEP, while *a** and *b** values showed increasing trend with increasing levels of WEP. It was concluded that pasta fortified with WEP-5% level showed best sensory attributes and physico-chemical and cooking qualities.

5.1.2 Optimization of the level of incorporation of skim milk powder for development of protein fortified pasta

To optimize the level of incorporation of skim milk powder (SMP), four different groups of pasta samples were prepared i.e. Control samples (without SMP), T-1= 5% SMP, T-2 = 10% SMP and T-3= 15% SMP. These levels were finalized on the basis of literature available and preliminary trials conducted in the department. Developed pasta products were evaluated for various cooking parameters like Minimum cooking time (MCT), Volume expansion (VE), Gruel Solid loss (GSL), Colour analysis (L, *a** and *b**), texture analysis (Firmness and toughness) and Sensory analysis (Overall acceptability; OA). It was found that minimum cooking time (MCT) of control was least amongst the studied pasta samples, while SMP treated samples showed increasing MCT with increasing levels of SMP. Highest MCT was reported for pasta supplemented with 15% SMP i.e. 10.25 and least was for control samples i.e. 7.08 min. GSL, VE, firmness and toughness linearly increased with increased levels of SMP in treatments, while control pasta samples showed least values for the studied parameters.

It was concluded that pasta fortified with SMP-10% level showed best sensory attributes along with physico-chemical and cooking qualities.

5.1.3 Proximate composition of developed pasta

It was found that Moisture Moisture (%), Carbohydrate (%), Protein (%), Fat (%) and Ash (%) in control was 10.86±0.11, 70.08±0.06, 10.27±0.09, 1.02±0.04 and 0.55±0.01 respectively. Whereas, developed fortified pasta showed 13.00±0.05 Moisture (%), 50.00±0.02 Carbohydrate (%), 23.25±0.02 Protein (%), 2.61±0.02 Fat (%) and 3.36±0.08 Ash (%) contents.

5.1.4 Experimental design

RSM used to standardise the level of supplementation of two composition coded/uncoded factors i.e. Whole egg powder (WEP) and Skim milk powder (SMP).

The trials were designed using a Central Composite Design (CCD), which resulted in 13 independent variable experimental designs. Various cooking parameters, such as minimum cooking time (MCT), volume expansion (VE), water uptake ratio (WUR), Gruel Solid loss (GSL), colour analysis (L, *a** and *b**), and texture analysis, were used to produce the best suitable pasta fortified by incorporating WEP and SMP responses (Firmness and toughness) and Sensory attributes (Overall acceptability OA). The coefficient of R square as well as the lack of fit using an F-test with a 5% significant level were used to assess the polynomial model equation's fitness to the responses.

Minimum cooking time (MCT)

The ANOVA of the quadratic regression models showed that the model was significant (p<0.05) with p-values of <0.0001. P-values less than 0.05 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. The statistical analyses revealed that both whole egg powder and skim milk powder had significant linear and interaction effects (p<0.0001) on the MCT of fortified pasta. It was observed that MCT increased gradually with the increase in whole egg powder and skim milk powder. The optimized MCT predicted by response surface methodology was 8.29% under the formulation condition with whole egg powder of 5% and skim milk powder of 10%.

Volume Expansion (VE)

In this case, ANOVA of the linear and interaction regression models for VE showed A, B, AB, A^2, B^2 are significant model terms. It was observed that VE significantly decreased with the increasing both WEP (%) and SMP (%) and nearly reached at lowest point with highest WEP (%) i.e. 15% and SMP (%) i.e. 8%. Optimized model predicated by RSM showed 1.49% of volume expansion under the formulation condition; whole egg powder of 5% and skim milk powder of 10%.

Water Uptake Ratio (WUR)

The ANOVA of the quadratic regression models showed that the model was significant (p<0.05) with p-values of <0.0001 for WUR. The R^2 value (0.9944), being a measure of the goodness of model fitting, indicated that 99.60% of the total variation indicating more significant effect of WUR. A low value of CV (1.20) indicated higher precision and reliability of the experiment. According to model terms the increase in WEP and SMP (%), results in huge reduction in WUR.RSM indicated 1.72 % of water uptake ratio under the formulation condition; whole egg powder of 5% and skim milk powder of 10 %.

Gruel Solid Loss (GSL)

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 both i.e. whole egg and skim milk powder (%) had significant linear and interaction effects ($p<0.0001$) on the GSL of formulated pasta. It was observed that GSL increased significantly with the increase in whole egg as well as skim milk powder (%). The model predicated 17.65% of GSL by keeping 5% of whole egg and skim milk powder (%) of 10%.

Firmness

The Model F-value of 9455.03 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. The Lack of Fit F-value of 0.068 implies the Lack of Fit is not significant relative to the pure error. The RSM models showed 1.78% of firmness under the formulation condition; WEP of 5% and SMP of 10%.

Toughness

Enhancement in WEP and SMP (%) were found to reported in ($p<0.05$) increase in toughness. The RSM models showed 1.25% of toughness under the formulation condition; whole egg powder (%) of 5% and skim milk powder of 10%. The SEM analysis of cooked pasta studies justified the increase in firmness and toughness of developed pasta.

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

RSM indicated 62.87 of L* value under the formulation condition; WEP of 5% and SMP of 10%. The optimal formulation levels of selected variables determined by RSA were WEP of 5% and SMP powder of 10% exhibiting *a** value of 1.71 and 18.61 *b** value.

Overall acceptability (OA)

ANOVA of interaction regression models for OA showed that the model was significant ($p<0.05$) with p-values of <0.0001. Optimized model predicated by RSM showed 8.12% of overall acceptability under the formulation condition; WEP of 5% and SMP of 10%.

5.2 Storage stability of the developed fortified pasta at ambient temperature under aerobic packaging.

In the present experiment fortified pasta T-1 (WEP-5%), T-2 (SMP-10%), T-3 (WEP:SMP; 5:10%) and control (without whole egg and skim milk powder) were stored at ambient temperature (20-30±5 °C) for 60 days under aerobic packaging conditions wrapped in aluminium foil-LDPE laminate pouches. The samples were drawn at regular interval of 15 days.

On 1st day of storage, control pasta (without WEP and SMP) required 7.13 min for complete gelatinization of starch on cooking, whereas, fortified pasta T-1 required 7.86 minutes, T-2 required 8.08 minutes and T-3 required 8.34 minutes. The same trend were followed during storage period of 60days for all samples. Amongst treatments T-3 showed longest MCT value as compared to other pasta samples. On the 1st day of storage, significantly higher L* values were reported in control pasta followed by T-2>T-3 and least were noted for T-1 i.e. pasta fortified with WEP. The critical analysis of Table 12 showed that, with progression of storage period L* value decreased in all of the samples, statistically significant declined was witnessed after 45th day onwards during storage period. It can further be noted that the change in *a** value was more pronounced in T-1 (WEP-5%) fortified pasta products followed by T-3 which showed slight less then T-1 i.e. 1.82 , which followed by T-2 i.e. 1.41 and the lowest showed in control i.e. 1.36 while, *b** value showed a declining trend during storage.

In the present experiment fortified pasta T-1 (WEP-5%), T-2 (SMP-10%), T-3 (WEP:SMP; 5:10%) and control (without whole egg and skim milk powder) were stored at ambient temperature (20-30±5 °C) for 60 days under aerobic packaging conditions wrapped in aluminium foil-LDPE laminate pouches. The samples were drawn at regular interval of 15 days.

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Within treatments fortified pasta T-1, T-2 and T-3 showed declined values as the protein fortification increased i.e. T-3>T-2>T-1 and all the three fortified pasta sample showed progressive declined trend in the scores as the storage days increases up to 60^{th} day.

The decline in flavor scores was comparatively less in control and T-2 pasta samples, while T-1 and T-3 showed more pronounced decline for flavour score.

The critical analysis of Table 14 indicated that control and fortified pasta products i.e. T-1, T-2and T-3 followed a declined trend in overall acceptability scores as the storage days progressed. At the end of storage period of 60 days, T-1 showed least acceptability score as compared to other treatment samples. The overall mean values of TBARS of developed fortified pasta and control pasta samples increased significantly ($p<0.05$) over storage period of 60 days. The FFA value in developed fortified pasta samples were significantly ($p<0.05$) higher than control. The FFA values showed significantly ($p<0.05$) increasing trend with progression of storage days.

It was noted that PV followed increasing trend with progress of storage period in both treated as well as control samples. The significantly higher PV values were noted in T-1 followed by T-3>T-2 and least were noted for control pasta samples. The SPC values increased significantly ($p<0.05$) in both control and fortified pasta during the whole storage study. During storage the significant increase in SPC observed on 15th day onwards in T-1 and on 30th day onwards in control, T-2 and T-3. No coliforms were detected, Complete absence of yeast and mould count has been showed by developed fortified pasta and control pasta.

The SEM of cross-sections of pasta samples at different resolution i.e. 1000X and 3000X showed the oval-shaped starch granules and starch granules are deeply enmeshed in a protein network with an uniform and permeable structure. The addition of whole egg powder and skim milk powder 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.

5.3 Economics of production of fortified pasta incorporated with skim milk powder and whole egg powder.

The results showed that production of control pasta was Rs. 72.00 and that of developed fortified pasta was Rs. 127.30.

CONCLUSIONS

- With the effective execution of RSM performing Central Composite Design, the formulation procedure for the preparation of fortified pasta utilising whole egg powder and skim milk powder was standardised.
- Standardized procedure of RSM exhibited that fortified pasta can be developed using 10% SMP and 5% WEP with highest scores for various physico-chemical, cooking and textural attributes.
- The fortified pasta made with skim milk powder (10%) and whole egg powder (5%) may be stored for 60 days at storage (20-30±5°C) under ambient temperature conditions without any significant changes in physicochemical, colour, or textural, microbiological and sensory qualities

- Microstructure studies revealed increased interaction of semolina starch to that of skim milk powder and whole egg powder molecules, indicating a potential option to produce high quality pasta with enhanced nutritional and functional properties.
- The cost of production of developed pasta was 127.30 Rs/Kg as compared to less nutritious control pasta with production cost of 72.00 Rs/Kg, Hence, it can be recommended as a profitable start up business venture.

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