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Development and evaluation of gluten free pasta from amaranth flour blended with soya flour and egg white

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Abstract
This research work presents the investigational studies on "Development & evaluation of gluten free pasta from amaranth flour blended with soya flour and egg white." Products formulation optimized using response surface methodology (RSM). This introductory chapter presents an overview of dissertation which include introduction to research topic, raw materials, justification and objectives of research work. It mainly focuses on status and contribution raw materials i.e. amaranth flour, soya flour and egg albumin to the final product. It also highlighted on gluten intolerance (celiac disease). It also overlooks on justification i.e. why we gone to be utilized the selected raw materials in the final product. Finally, the objectives of project were designed with respect to project title.

Keywords: gluten, pasta, amaranth, soya, egg white

1. Introduction
Gluten intolerance i.e. celiac disease is an autoimmune disorder of the small intestine that occurs in genetically pre-disposed people of all ages from middle infancy onward. Peoples with celiac disease owing to the reduced ability of the small intestine to properly absorb the nutrients from the food. Celiac disease is caused by a reaction to gliadin, a prolamin (gluten protein) found in wheat, and similar proteins found in the crops of the tribe Triticeae (which includes other common grains such as barley and rye). The increased prevalence of celiac disease has lead to an increased demand for gluten-free products, thus products not containing wheat, rye, barley or spelt wheat. At present, the only effective treatment is a lifelong gluten-free diet. No medication exists that will prevent damage or prevent the body from attacking the gut when gluten is present.

Amaranth is a reasonably well-balanced pseudocereal with functional properties that have been shown to provide nutraceutical benefits. The health attributed include decreasing plasma cholesterol levels, stimulating the immune system, exerting an antitumor activity, reducing blood glucose levels and improving conditions of hypertension and anemia. In addition, it has been reported to posses anti-allergic and antioxidant activities. Although several species are often considered weeds, people around the world Value amaranth as vegetable, cereal, and ornamental (Wikipedia.org). Amaranth grain contains no gluten and is to consume for individuals with celiac disease. It is found that this fibrous grain contains three times more fiber than wheat and five times more iron than wheat. Amaranth scores high protein value when compared to other grains. When amaranth is combined with other grains, the protein value score approaches the ideal amino acid reference pattern established in 1973 by the FAO/WHO of United Nation (National Research council, 1984) [18].

Soybeans contain isoflavones called genistein and daidzein, which are one source of phytoestrogens in the human diet. Because most naturally occurring estrogenic substances show weak activity, normal consumption of foods that contain these phytoestrogens should not provide sufficient amounts to elicit a physiological response in humans. Soybeans and processed soy foods are among the richest foods in total phytoestrogens (wet basis per 100g), which are present primarily in the form of the isoflavones daidzein and genistein.

Egg whites have become very popular due to their high protein and low cholesterol content. Once a popular breakfast staple, the whole egg was shown to be high in cholesterol which made it a food to be avoided.

It was for this reason that egg sales began to plummet. However, egg whites, once separated from the yolk, have very little cholesterol and one cup has twenty-six grams of protein. Since egg whites are so versatile, they're used in all kinds of food dishes such as egg white omelets, breakfast sandwiches, and salads. Studies developed and other than being a good source of protein, egg whites were shown to also contain essential minerals including; Potassium, Magnesium, Calcium, Phosphorus, Copper, Zinc, Iron (http://the-health-benefit-nutritional-value-egg-white). The egg white is also a good source of riboflavin and selenium along with essential vitamins such as folate, B12, niacin betaine and choline.

Pasta is a basic product of the Italian tradition with increasing worldwide popularity. It has substantial nutritional value. The WHO and the FDA consider it a suitable vehicle for the incorporation of nutrients. Pastas may be divided into two broad categories, dried (*pasta secca*) and fresh (*pasta fresca*). Most dried pasta is commercially produced via an extrusion process. In modern times to meet the demands of both health conscious and celiac sufferers the use of rice, maize and amaranth has become commercially significant. Grain flours may also be supplemented with cooked potatoes.

2. Materials and Methods

This carried with actual requirement for the development of gluten free pasta, preliminary trials to find out desirable product variable level, implementation of RSM design into the product formation, evaluation of pasta samples. It also carried with optimization of pasta and finally evaluation of final optimized pasta product. Procure raw materials of good quality i.e. amarath, soybean, egg. Amaranth and soybean are grind to make flour, egg white from the egg is separated at the time of preparation.

2.1 Preparation of pasta product

2.1.1 Preliminary trials

The preliminary trials was conducted to find out the reliable proportion of amaranth flour, soya flour, egg white and moisture content for pasta preparation.

2.1.2 Selection of optimum Moisture content of composite for extrusion

Before conducting the experiments the moisture content of mixed composite was determined by gravimetric method. Preliminary trials were carried out at different moisture content of mixed composite by addition of fresh water. The extrusion of mixed composite was done by keeping the final moisture content as 26, 30, and 34%. The water to be added to the mix was calculated by Pearson's square method. The calculated amount of water was added slowly to the mixed composite to maintain the above said moisture content. From the above combination 26% moisture content composite were not given proper shapes and 30% moisture content composite produces pasta strand that were given proper shapes. 34% moisture content strands were not proper as 30% moisture content. Therefore 30% moisture content of composite was selected to carry out the experiments.

2.1.3 Selection of optimum proportion of soya flour in mixed composite

Preliminary trials were conducted to prepare the pasta from various proportion of soya flour in mixed flour while the Moisture content was kept constant (30%). Trials were conducted in proportions of 15%, 20%, and 25% of soya flour in mixed composite. Then the prepared pasta were evaluated for sensory analysis and cooking loss.

2.1.4 Selection of optimum level of egg white

Similarly preliminary trials were conducted to obtain the optimum amount of egg white in pasta for proper binding of pasta. Pasta was prepared by the addition of 20, 25, and 30g of egg white in composite. Among these three combinations, pasta containing 20g egg white were not given proper binding and structure, whereas pasta containing 25g and 30g egg white were given proper binding, good structure.

2.1.5 Experimental Design

For optimization of pasta product, the experiments were conducted according to Central Composite rotatable Design (CCRD) with two variables at five levels each. The independent variables were soya flour concentration, egg white concentration for preparation of pasta product.

Response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The method was introduced by Box and Wilson, 1951. Response surface methodology (RSM) was adopted in the experimental design as it emphasizes the modeling and analysis of the problem in which the response of interest is influenced by several variables and the objective is to optimize this response. The experiments were conducted according to composite rotatable design with percent proportion of soya flour and egg white as independent variables.

Total no of experiments = $2^{(\text{number of variables})}$ + 2 (Number of variables) + central points. For two variables,

Total number of experiments = $(2)^2 + 2\ (2) + 5 = 13$

The five levels of the process variables were coded as -α, -1, 0, +1 and +α.

2.1.5.1 Selection of independent variables

1. Soya flour (%) : 5 - 25
2. Egg white (%) : 10 – 30

2.1.5.2 Range and levels of the independent variables in a CCRD design.

Independent Variables	Symbol	Levels in coded form				
	Uncoded	-1.41	-1	0	+1	+1.41
Soya flour (%)	A	0.86	5	15	25	29.14
Egg white (%)	B	5.86	10	20	30	34.14

Proportions of soya flour and egg white variables have been selected by conducting preliminary experiments. The independent variables were percentage of soya flour and amount of egg white (g/100 g). The low and high levels of the independent variables were 10% and 25% soya flour and 15% and 30% of egg white per 100 g of mixed composite. Proportion of amaranth flour in the mixed composite was varied with respect to independent variable. The optimization was done with the aim to get minimum cooking loss, color characteristics of pasta, and sensory evaluation in pasta.

2.1.5.3. Coded and Un-coded values of variables for pasta products:

Sr. no.	Soya flour (%)		Egg white (%)	
	Coded	Uncoded	Coded	Uncoded
1	-1	5	-1	10
2	+1	25	-1	10
3	-1	5	+1	30
4	+1	25	+1	30
5	-1.41	0.86	0	20
6	+1.41	29.14	0	20
7	0	15	-1.41	5.86
8	0	15	+1.41	34.14
9	0	15	0	20
10	0	15	0	20
11	0	15	0	20
12	0	15	0	20
13	0	15	0	20

2.2 Preparation of pasta

Pasta was prepared by a laboratory level single screw extruder (La Monferrina, Italy). Amaranth flour and soya flour were mixed together into different proportions, and the desired amount of egg white added to mix. Then water was added to mixed flour up to the moisture 30%. Then mixing and kneading the mass for 15 minutes to produce a stiff, plastic, homogeneous dough, and then applying pressure so as to extrude the dough through a series of die orifices. The extrude pasta product (Macaroni, 0.92 mm thickness, 5.3 mm external diameter and 32.0 mm length) were cut into pieces of uniform size by means of knife moving over the outer die surface. After extrusion, the shaped pieces were dried at 60 °C for 30 minutes to a final moisture content ≈ 12% and packed in commercially available low-density polyethylene pouches.

3. Result and Discussion

This presents actual experiment on the basis of response surface methodology (RSM) were carried out to optimize the formulation of pasta from amaranth flour, soya flour and egg white. As no literature was available on pasta formulation from amaranth flour, soya flour and egg white, preliminary trials were conducted to obtain approximate level of variables ranged from 5 to 25% for soya flour and 10 to 30 for egg white; whereas the proportion of amaranth flour varied with respect to soya flour and egg white. Effect of addition of soya flour and egg white were studied on physico-chemical, cooking quality, textural and sensory characteristics of pasta i.e. bulk density, protein content, cooking time, cooking loss, percent expansion, water absorption, color, and overall acceptability, etc. The central composite rotatable design (CCRD) for two variables at five levels was used for deciding experimental combinations. A second order polynomial model for the dependent variables was established to fit the experimental data. ANOVA test was carried out using Design expert 6.0 to determine the significance at different levels.

3.1 Optimization of gluten free pasta prepared from amaranth flour, soya flour and egg white by response surface methodology

The pasta was prepared according to central composite rotatable design and was analyzed for cooking loss (%), water absorption (%), percent expansion, adhesivness, cohesiveness after cooking, protein content (%), bulk density, hardness, color difference (ΔE) and overall acceptability.

3.1.1 Diagnostic checking of fitted model and surface plots for protein content

In this study, Protein content was an important determinant factor because pasta was fortified with soya flour to increases the nutritional value of gluten free pasta and to increase the protein content of product.

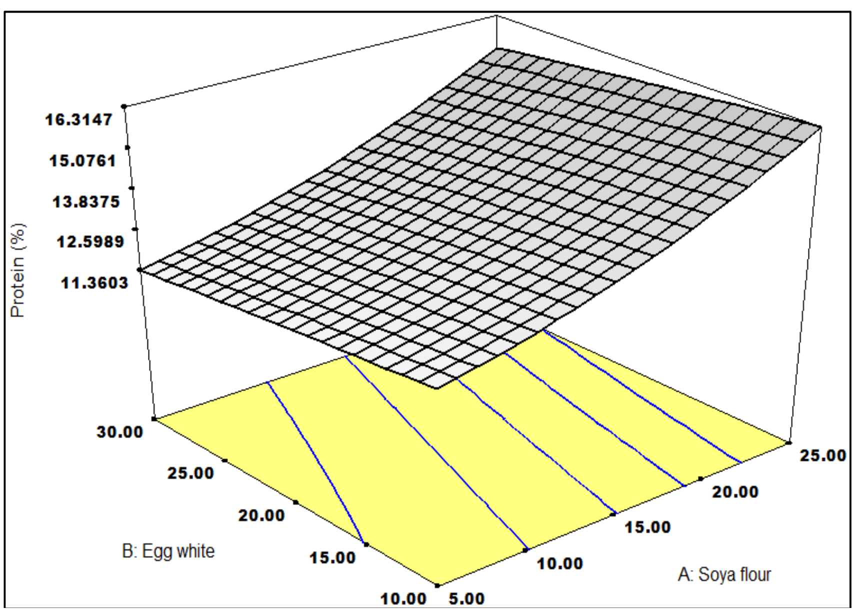

Fig 1: Response surface plot for the variation of protein content of pasta as a function of Soya flour and Egg white

However, coefficient of Egg white (B) was not so much effective to increase protein content. Where; protein content were slightly decreased as egg white concentration increased. This result was similar to the, Rebello and Schaich (1999) who observed; extrusion process causes breakage of the protein matrix, and, as a result, the product does not have a compact and continuous protein network on wheat flour proteins and reported the fragmentation and cross-linking of these proteins.

3.1.2 Diagnostic checking of fitted model and surface plots for Bulk density

Bulk density is a major physical property of the products. Bulk density is the measurement of density of the material when packed or stacked. So this parameter is very much important when the material is going to be produced in a large scale. The bulk density of packed materials depends on the geometry, size, and surface properties of individual particles. The bulk density mainly depend on exapansion of pasta.

Fig. showed that the effect of changing Soya flour and Egg white concentration on the bulk density of pasta. Coefficient of Soya flour (A) was shown positive effect on bulk density. It was mean that as concentration of Soya flour increased, the bulk density was also increased. It might be due high starch gelatinization temperature resulted in the larger extent of volume of the pasta increased and the bulk density increased. Showed that increased in amaranth flour increased the bulk density. However, coefficient of Egg white (B) was shown positive effect on the bulk density. Where, a bulk density increased as concentration of egg white increased. Bulk density is mainly depend on expansion of pasta. With the increase of egg white concentration expansion also increased and it will leads to increased in bulk density.

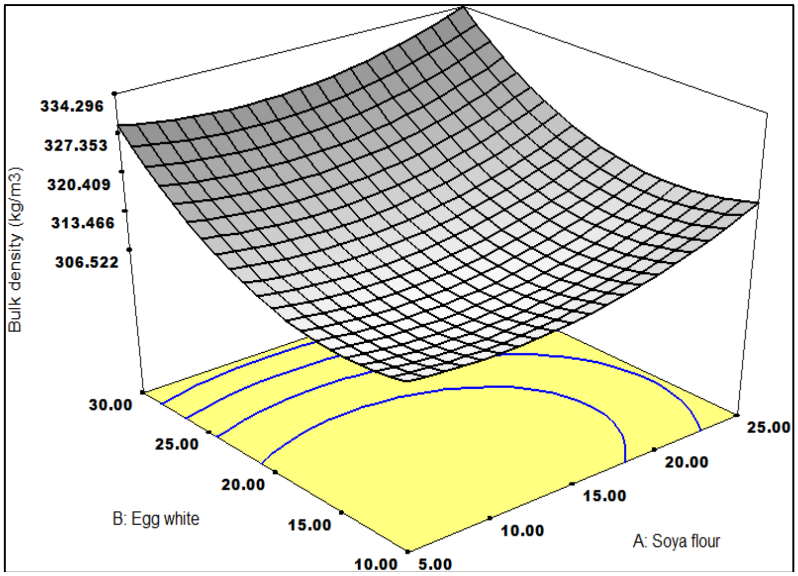

Fig 2: Response surface plot for the variation of bulk density of pasta as a function of Soya flour and Egg white

3.1.3 Diagnostic checking of fitted model and surface plots for hardness

Texture analysis is primarily concerned with measurement of the mechanical properties of a product, often a food product, as they relate to its sensory properties detected by humans. Sensory definition of hardness is Force required to compress a food between the molars. Defined as force necessary to attain a given deformation. While the instrumental definition is Peak force of the first compression cycle.

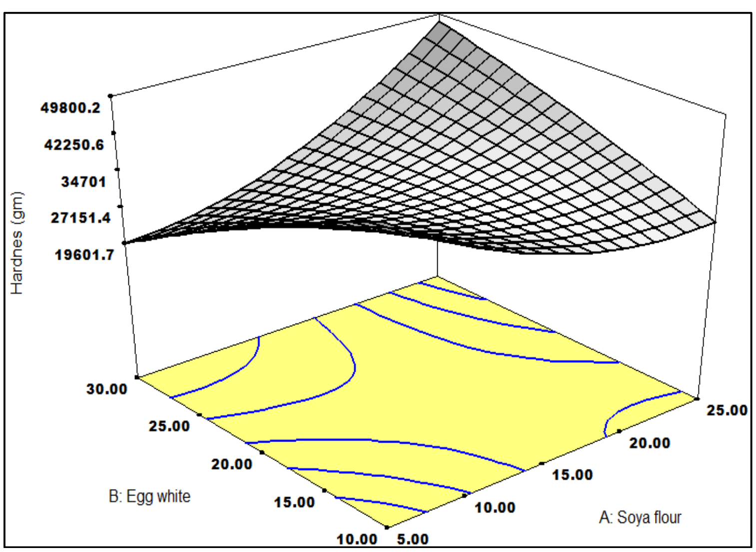

Fig 3: Response surface plot for the variation of hardness of pasta as a function of Soya flour and Egg white

Fig. showed that the effect of changing Soya flour and Egg white concentration on the bulk density of pasta. Coefficient of Soya flour (A) was shown positive effect on hardness. It was mean that as concentration of Soya flour increased, the hardness was also increased. This may be due to the fortification by soya flour attributed to the structural changes in the protein network. However, coefficient of Egg white (B) was shown negative effect on the hardness. Where, a hardness decreased as concentration of egg white increased. This may be due to low extrusion temperature. At low temperature protein coagulates and product hardness decreases.

3.1.4 Diagnostic checking of fitted model and surface plots for water absorption

The water absorption index of pasta is an indicator of the water absorbed by the starch and proteins during cooking, which is utilized for the gelatinization of starch and the hydration of protein. Water absorption (%) is the difference in weight of cooked pasta and uncooked pasta, expressed as the percentage of the weight of uncooked pasta.

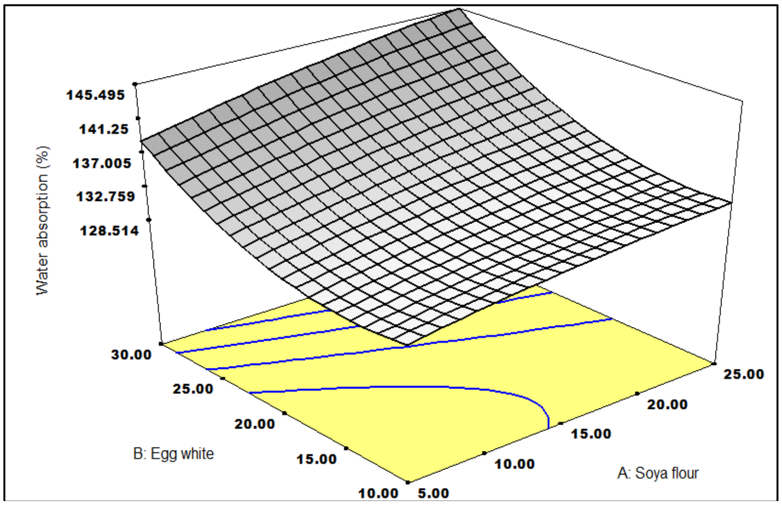

Fig 4: Response surface plot for the variation of water absorption of pasta as a function of Soya flour and Egg white

This was agreed with Enwere, McWalters, & Phillips, 1998 [8]; Granito, Guerra, Torres, & Guinand, 2004 reported greater water absorption capacity in thermally processed legumes; they claim that this behaviour is associated to the denaturation of proteins, particularly albumins. However, coefficient of Egg white (B) was shown positive effect on the water absorption; where water absorption increased as concentration of egg white increased. This was agreed with M. Chalamaiah & K. Balaswamy 2011, showed that Water absorption capacity of defatted egg protein concentrate in this study was high compared to those of Labeo rohita fish egg protein concentrate. The high water absorption of *mrigal* defatted egg protein concentrate may be due to the presence of polar groups such as COOH and NH2.

3.1.5 Diagnostic checking of fitted model and surface plots for percent expansion

Volume expansion after cooking is a quality characteristic of pasta. Volume expansion is proportional to the water absorption. It indicates the water holding capacity of pasta for starch gelatinization.

Fig. showed the effect of changing soya flour and egg white concentration on the percent expansion of pasta. Coefficient of Soya flour (A) was shown positive effect on percent expansion. It means that as concentration of soya flour increased the percent expansion was increased. This was agreed with Savita *et al.* (2013) who were found that Supplementation of pasta with different protein sources (legumes, milk proteins, egg protein) increased volume expansion and water absorption of pasta. However, coefficient of Egg white (B) was shown negative effect on the percent expansion; where a percent expansion decreased as concentration of egg white increased. This happens because, the non homogeneous heating of the product can create a collapse of the structure instead of its expansion.

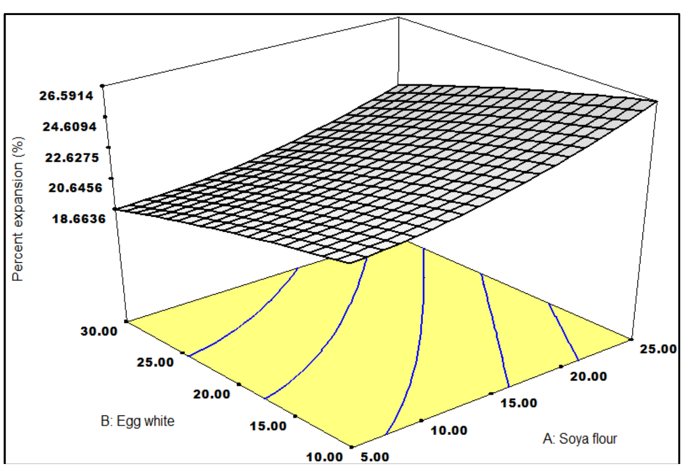

Fig 5: Response surface plot for the variation of percent expansion of pasta as a function of Soya flour and Egg white

3.1.6 Diagnostic checking of fitted model and surface plots for cooking loss

Cooking properties play an important role in indicating the quality of pasta. Cooking loss is the percentage of dry solids lost into the cooking water. The quantity of solids going into water during cooking of pasta is a determinant of pasta quality and compact textured pasta leads to lower cooking loss.

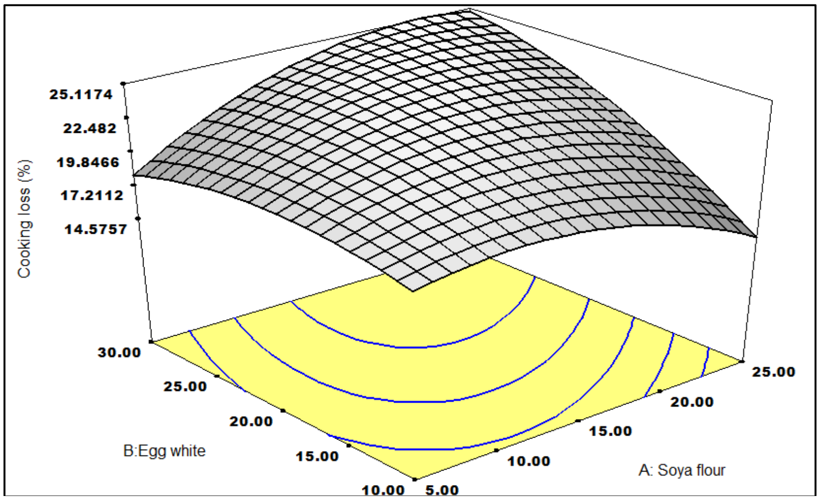

Fig 6: Response surface plot for the variation of cooking loss of pasta as a function of Soya flour and Egg white

Fig. showed the effect of changing Soya flour and Egg white concentration on the cooking loss of pasta. Coefficient of soya flour (A) was shown negative effect on cooking loss. As concentration of soya flour increased the cooking loss was slightly decreased. However, coefficient of Egg white (B) was shown positive effect on the cooking loss. It means that as concentration of egg white (B) increased, cooking loss was also increased. D' Egidio *et al*. (1981) [7] reported that the distilled water is unable to distinguish pasta products in terms of their quality, whereas tap water is very efficient in doing so. Several workers have show that, with increase water hardness cooked pasta has higher stickiness values (Dexter *et al*., 1983) [9], higher total organic matters in the rinse and cooking water (D'Egidio *et al*. 1981) [7] and higher cooking losses. According to those author this behaviour was mainly due to the higher pH and higher calcium–magnesium ions content of tap water compared to distiller water.

3.1.7 Diagnostic checking of fitted model and surface plots for sensory evaluation

The sensory evaluation was carried out in order to get consumer response for overall acceptability of the gluten free pasta from amaranth flour supplemented with soya flour and egg white.

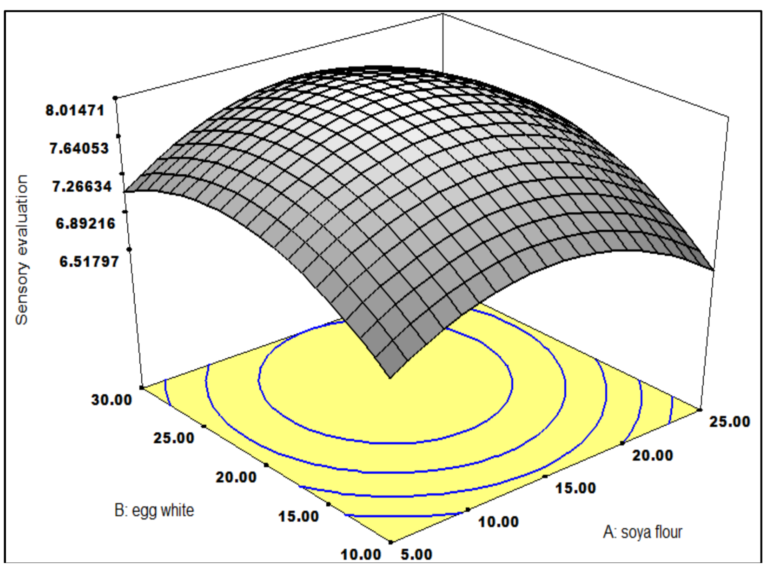

Fig 7: Response surface plot for the variation of sensory evaluation of pasta as a function of Soya flour and Egg white

Fig. showed the effect of changing soya flour and egg white concentration on the sensory evaluation of pasta. Coefficient of Soya flour (A) was shown negative effect on Sensory evaluation. It was mean that as concentration of Soya flour increased, the sensory evaluation was decreased. This might be due to decreased in cooking loss, increased hardness, water absorption, color difference and soya flour. However, coefficient of Egg white (B) was shown positive effect on the sensory evaluation; where sensory evaluation increased as concentration of egg white increased. This might be due to, increased water absorption, increased firmness, and improved color pasta.

3.2 Verification of results
The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses. The percent variations between predicted and actual value of experiments were below the 10% which indicate the accuracy of model.

3.2.1 Optimized experiment

Product variable		Uncoded value
1) Soya flour (A)	=	17.92%
2) Egg white (B)	=	19.88%

3.2.2 Proximate analysis of optimized pasta
After optimization, proximate analysis of pasta was estimated. Optimized pasta was evaluated for moisture, ash, protein, fat, crude fibre and total carbohydrate, etc. It was shown acceptable result as shown in Table.

Predicted and actual values of the responses at the optimized condition

Sr. No.	Response	Predicted value	Actual value	Variation (%)
1	Protein (%)	14.04	13.19	6.05
2	Bulk density (kg/m^3)	311.05	321.82	3.46
3	Hardness (g)	32323.8	32446.2	0.37
4	Water absorption (%)	133.98	129.68	3.21
5	Percent expansion (%)	22.54	21.63	4.04
6	Cooking loss (%)	23.10	22.18	3.98
7	Adhesiveness (g. sec)	0.44	0.46	4.54
8	Cohesiveness	0.77	0.74	3.89
9	Sensory evaluation	7.91	7.24	8.47

4. Conclusion
The overview of gluten free pasta pertinent literature on utilization of pseudocereal amaranth blended with different proportion of amaranth flour in extrusion technology, for production of gluten free nutritionally balanced blend of pasta by extrusion processing. Replacement of the gluten network to produce GF products is a major technological challenge, gluten being the essential structure-building protein. Optimised proportion was selected to get maximise water absorption, percent expansion, sensory evaluation, minimizes bulk density, adhesiveness, cohesiveness, cooking loss where as protein and hardness were kept in range. The finding of this research work demonstrate the feasibility of developing value added gluten free pasta from amaranth flour, soya flour and egg white by extrusion processing.

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