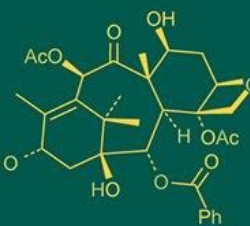
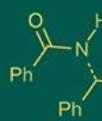
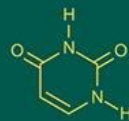
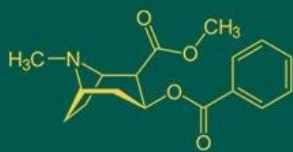


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Development of protein rich pasta supplemented with WEP and SMP using response surface methodology

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Abstract

The objectives of the present study were to optimize the level of supplementation with 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) and implementing Central Composite Design. Outcomes were demonstrated through various cooking parameters, namely Minimum Cooking Time (MCT), Volume Expansion (VE), Water Uptake Ratio (WUR), Gruel Solid Loss (GSL), Color Analysis (L, a*, and b*), Texture Analysis (Firmness and Toughness), and Sensory Analysis (Overall Acceptability OA). It was found that increases in WEP and SMP percentages in the formulation resulted in a significant ($p < 0.05$) increase in MCT, GSL, firmness, toughness, and a* and b* values, whereas VE, WUR, L value, and OA showed a declining trend. The best-suited formulation conditions predicted by the models were 5% WEP and 10% SMP.

Keywords: Response surface methodology, central composite design, protein fortified pasta

Introduction

The snack food market is constantly evolving in terms of product types. Although most snacks are not primarily consumed for their nutrients, many are created with a nutrition perspective in mind. Consumers desire tasty snacks that smell good, feel good, and look appealing, while also being nutritionally sound and healthy. Extrusion cooking is a modern food processing technology used for preparing a variety of snacks, specialties, and supplementary foods. This technology provides the opportunity to process various food products through minor changes in ingredients and processing conditions on the same machine. By making slight adjustments to the hardware and processing conditions, several different shapes, textures, colors, and appearances can be produced.

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 its wheat taste, smooth surface, and firmness (Kadam and Prabhasankar 2012) [19]. According to the International Pasta Organization Survey (2015), global pasta consumption increased by 2 million tons from 2013 to 2014. Pasta is a healthy food that serves as an important source of carbohydrates with virtually no fat (Foschia *et al.*, 2015) [16]. Pasta products are high in starch but low in proteins and dietary fibers, and they are mainly made up of wheat flour, which is deficient in an essential amino acid. Nowadays, it has become important to improve the quality of pasta by fortifying it with other essential ingredients that have proven nutritional qualities.

Pasta may be fortified with functional ingredients to alter its nutritional quality, such as essential amino acids, minerals, vitamins, phenolic compounds, and more. The addition of protein-containing ingredients in pasta caters to health-conscious consumers who prefer a product rich in essential proteins, healthy lipids, and other health benefits (Kadam and Prabhasankar 2012) [19]. Several studies have evaluated protein pasta fortification from different sources, such as bean flour, shrimp meat (Ramya *et al.*, 2015) [23], and fish proteins (Surasani *et al.*, 2019) [24].

Protein can be obtained from animal or vegetable sources. It is well known that not all proteins are alike. Proteins from different sources possess varying properties that affect their quality.

Egg protein is widely regarded 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. Eggs in general, and egg white in particular, also have very little flavor, thus not negatively impacting the organoleptic properties of foods, which eliminates or reduces the need for masking flavors. High levels of protein combined with other sources, such as egg and cheese powder, can provide the basis for a range of highly nutritious extruded snack products.

A modern active lifestyle necessitates the development of functional pasta for better health and disease prevention. With the appropriate selection and addition of food ingredients, there is a great opportunity to develop pasta products with health benefits when consumed. Semolina pasta is a widely consumed foodstuff, but its biological value is low because its protein is deficient in essential amino acids (Martínez-Villaluenga *et al.*, 2010) [21]. However, if semolina is extended with other ingredients rich in these essential proteins from milk, fish, eggs, and meat, not only are amino acids supplemented, but dietary vitamins and minerals can also be provided (Gull *et al.*, 2018) [18].

Egg is one of the most 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%. The proteins contained in egg whites, such as ovalbumin, ovotransferrin, and lysozyme, contain hydrolysates that have multifunctional properties such as antihypertensive, antioxidant, anti-inflammatory, and anti-diabetic properties (Garcés-Rimón *et al.*, 2016) [17]. However, the literature is almost silent on the fortification of pasta with whole egg powder.

Milk proteins have long been recognized for their nutritional and technological value. They boast high nutritional value compared to other proteins due to their relatively high content of essential amino acids and good digestibility (Meisel 1998) [22]. Caseins and whey proteins are the two main protein groups found in milk. Caseins, which account for about 80% of the protein content in bovine milk, are isolated from milk through acid or rennet precipitation. These proteins are flexible and heat-stable, containing a

higher proportion of amino acids such as histidine, methionine, and phenylalanine.

Response surface methodology (RSM) is a mathematical and statistical technique for testing multiple process variables and their interactive, linear, and quadratic effects, being useful in solving multivariable equations obtained from experiments simultaneously. It is a significant technique to determine the interaction between responses (dependent variables) and factors (independent variables) and to reduce the number of experimental trials compared to complete experimental design (Murphy *et al.* 2004; Tiwari *et al.* 2008; Ghodke *et al.* 2009) [1, 4, 7]. It has been used for the simultaneous analysis of the effects of process parameters in fresh meat processing (Jakobsen and Bertelsen 2000) [5] and in some meat products. In the present study, meat level (110-130 g) and processing conditions, including drying time (7-9 h) and steaming time (12-18 min), were independent variables, while physicochemical properties such as yield, water activity, WAI, WSI, hardness, overall acceptability, and total color change were the dependent variables. These independent variables were optimized using the central composite design of response surface methodology.

The fortification of pasta by replacing semolina with rich sources of protein, such as whole egg and casein, will not only increase the nutritive value but also make the pasta a functional food. Although much work has been done to improve the functional properties and nutritive value of pasta through changes in formulations and processing, the possibility of fortifying with whole egg powder and milk powder will open a new era of functional, protein-rich pasta foods. Hence, the present study was developed.

Materials and Methods

Chemicals, media and standards

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

Formulation of control and developed of pasta

The formulations of the control and developed functional pasta were standardized based on available literature and various laboratory preliminary trials. The standardized formulation is given in Table 1.

Table 1: Formulations of the Control pasta and Developed functional pasta

S. No.	Ingredients	Percentage (w/w)	
		Control pasta (%)	Developed functional 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

Pasta preparation

The various blends of whole egg powder and skim milk powder supplemented pasta were mixed in a mixing chamber of a pasta machine (Kent, New Delhi, India) with the required amount of water (based on trials) for 8-10 minutes to uniformly hydrate the flour particles. The pasta was extruded at 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 an industrial dryer (MAC industrial dryer, New Delhi, India) at 50 °C for 4-5 hours and was packed in polyethylene bags at 4 °C until further analysis.

Table 2: Coded and uncoded levels of independent variables used in Central Composite Design (CCD) for formulation of functional pasta

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

Table 3: Experimental design of Independent variables for optimization of level of incorporation of whole egg powder and skim milk powder for the development of protein fortified pasta.

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

Analytical techniques

Cooking Quality

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

Minimum cooking time

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

Water uptake ratio

Pasta (10 g) was cooked in 100 mL of 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 weight

gain after cooking, and results were reported as percent water absorption. Cooking water was collected to determine cooking loss (AACC, 2000).

Volume expansion

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

Gruel solid loss

Cooking loss was determined according to the approved AACC method (AACC 2000). Aliquots (50 mL) of the cooking water were drawn and placed in petri dishes for evaporation, drying in an oven at 105 °C. The weight of the solid residue obtained was expressed as the percentage of solid loss during cooking.

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

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) with a 75 mm probe. A 50 kg load cell was used to measure these parameters. Pre-test and post-test speeds were set to 2.0 mm/s and 10.0 mm/s, respectively, with 30 percent 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 from the resultant file.

Sensory evaluation

All product samples were coded and evaluated for the degree of liking or disliking on a 9-point scale, using descriptive categories ranging from "like extremely" to

"dislike extremely". 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 color, flavor, body and texture, and overall acceptability) by a panel of semi-trained judges. The following proforma was used for sensory evaluation.

Instrumental colour profile analysis

Colour profile was measured using the CR-400 Konica Chroma meter (Konica Minolta, Japan), set at 2° of cool white light (d65) and known as 'L', a, and b values. The 'L' value denotes brightness (100) or lightness (0), a (+ redness/- greenness), b (+ yellowness/- blueness). The instrument was calibrated using a light trap (black hole) and

a white tile provided with the instrument. Then, the above colour parameters were selected. The instrument was placed directly on the surface of the pasta at three different points.

Results and Discussion

The objectives of the present study were to optimize the level of fortification of egg protein, i.e., whole egg powder, and milk protein, i.e., skim milk powder, for the development of fortified pasta rich in protein. The developed pasta was evaluated for storage stability at ambient temperature ($20\text{--}30\pm 5^\circ\text{C}$) for 60 days, wrapped in aluminium foil-LDPE laminate pouches. The production cost for developing functional pasta was also calculated. This chapter details the results obtained from different experiments carried out according to the aforementioned objectives, and the results are presented in the text with the support of statistically analyzed Tables (1 to 6) and Figures (1-10). Critical analyses of the results, with suitable support from available literature to draw inferences, have been attempted in this chapter.

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

Experimental design

Response surface methodology (RSM) is used to optimize the level of incorporation of whole egg powder and skim milk powder as two composition coded/uncoded variables (Table 2). The experiments were formulated according to a Face Centered Central Composite Design, resulting in 13 experimental designs of independent variables (Table 3). In order to develop the most acceptable protein-fortified pasta by incorporating whole egg powder (WEP) and skim milk powder (SMP), responses were demonstrated by various cooking parameters, namely: minimum cooking time (MCT), volume expansion (VE), water uptake ratio (WUR), gruel solid loss (GSL), colour analysis (L, a^* , and b^*), texture analysis (firmness and toughness), and sensory analysis (overall acceptability; OA). The fitness of the polynomial model equation to the responses was evaluated by the coefficient of determination (R^2) as well as by the lack of fit using the F-test at a 5% level of significance.

Fitting the models

Each response was evaluated based on the linear, quadratic, and interaction effects of the independent variables, namely whole egg powder and skim milk powder. The experimental data were fitted to second-order polynomial equations, and the regression coefficients were calculated. The significance of the model coefficients was determined through analysis of variance (ANOVA), as summarized in Table 4-6. The quality of the generated model was assessed using ANOVA, R^2 , and the lack of fit. The ANOVA results in Table 4-6 and Fig. 1-10 indicated that the model exhibited very high F-values and very low p-values (<0.0001) for all ten studied responses. The coefficient of variation (CV) describes the extent of data dispersion. The CV values (ranging from 0.04 to 1.79) for the proposed models demonstrated the high precision and reliability of the experiments.

In addition, high R^2 values and non-significant 'Lack of Fit' ($p>0.05$) were observed in Tables 4-6, indicating that the second-order model was highly significant to the obtained data and capable of describing the relationship between the formulation conditions and the studied responses. The larger

regression coefficient in a model with a significant p-value indicated a more substantial effect on the respective response variables.

The coefficients of multiple 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 obtained for the responses of MCT, L, a^* , b^* , firmness, toughness, GSL, VE, WUR and OA, respectively. This indicated that the second-order polynomial models were adequately represented by the respective experimental data.

Minimum cooking time (MCT)

The evaluation of pasta characteristics was conducted as a function of cooking time to obtain a more complete description of the cooking quality of a sample. In particular, its behavior under overcooking conditions was considered for testing the resistance of the sample to hydrothermal disintegration. The MCT was determined as the time required for at least 95% of the white, opaque core of the samples to disappear. The response surface analysis, as shown in Table 4, demonstrated that whole egg powder and skim milk powder in relation to MCT is quadratic, with a good regression coefficient ($R^2 = 0.9999$), and the model equation exhibited the relationship as per equation as follows.

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

The ANOVA of the quadratic regression models indicated that the model was significant ($p<0.05$) with a p-value of <0.0001 . P-values less than 0.05 indicate that model terms are significant. In this case, A, B, AB, A^2 , and 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. The Lack of Fit F-value of 0.24 implies that the Lack of Fit is not significant relative to the pure error. There is an 86.31% chance that a Lack of Fit F-value this large could occur due to noise. Thus, the predicted model results suggested that whole egg powder and skim milk powder (%) significantly increased the minimum cooking times (Table 4). Sharma *et al.* (2013) [10] reported that protein hinders the hydration and swelling capacity of starch granules by surrounding them, resulting in an increase in the MCT of pasta.

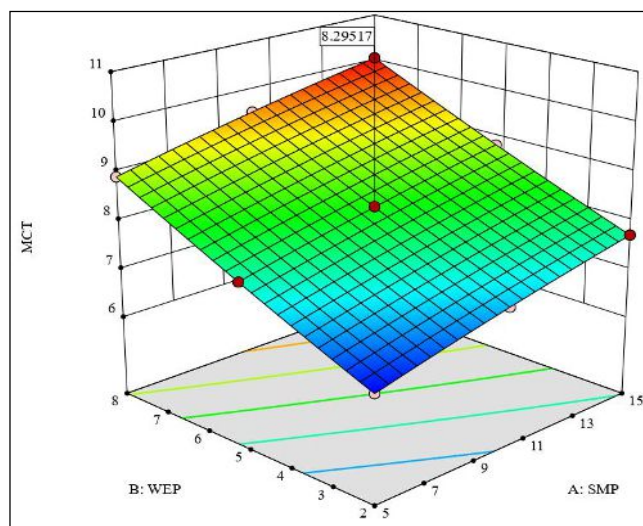


Fig 1: 3D Response Surface plots for MCT

3D graphs (Fig. 1) showed the effect of whole egg powder and skim milk powder on MCT. It was observed that MCT increased gradually with the increase in whole egg powder and skim milk powder. This was due to the addition of chicken meat powder, which restricted the supply of water to the starch granules (semolina) present in the pasta strands and delayed the swelling of the granules and possibly slowed down the start of the gelatinization process, resulting in an increase in MCT (Gupta *et al.*, 2021) [24].

Daud (2020) revealed that the minimum cooking time of chicken fortified pasta increased linearly with protein content, and high-protein-based chicken meat pasta became stronger and firmer internally when cooked compared to control pasta. The results of Kaur *et al.* (2013) [11] and Surasani *et al.* (2019) [24] agree with the present study. They reported that when protein-rich pasta is 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 5% whole egg powder and 10% skim milk powder.

Volume Expansion (VE)

Volume expansion relates to the increase in the volume of

the pasta when cooked for the optimum cooking time. The response surface analysis, as shown in ANOVA (Table 4) and Fig., demonstrates that the relationship between volume expansion and two independent variables (whole egg powder and skim milk powder) is quadratic. The model equation shows the relationship as per the following equation.

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

The Model F-value of 813.13 implies that the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. In this case, ANOVA of the linear and interaction regression models for VE showed that A, B, AB, A², and B² are significant model terms. Table 4 shows that WEP (%) and SMP (%) have significant ($p < 0.005$) linear, quadratic, and interaction effects on VE. The Lack of Fit F-value of 0.4093 implies that the Lack of Fit is not significant relative to the pure error.

3D graphs, as shown in Fig 4, depict the effect of WEP (%) and SMP (%) on the VE. It was observed that VE significantly decreased with the increase of both WEP (%) and SMP (%) and nearly reached the lowest point with the highest WEP (%) of 15% and SMP (%) of 8%.

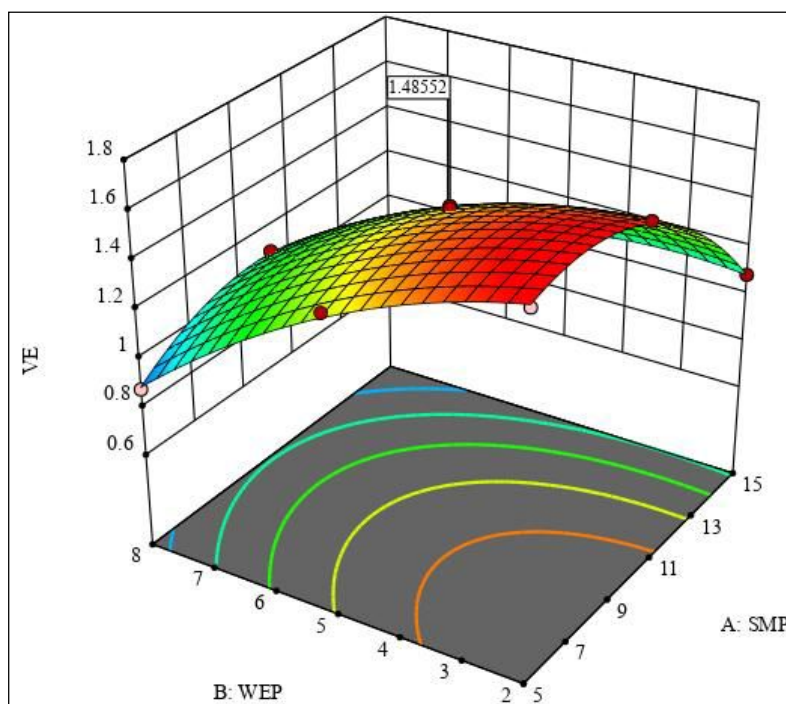


Fig 2: 3D Response Surface plots for VE

Hence, it was concluded that the increase in the level of whole egg powder and skim milk powder in the pasta decreased the volume expansion of pasta (Fig 2), which was due to a decrease in the starch content and an increase in the levels of protein and fiber in the pasta. The protein and fiber have lower swelling power compared to starch and swell less when cooked compared to starch, resulting in pasta with added egg and skim milk powder exhibiting lower volume expansion (Sharma *et al.*, 2013) [10].

The optimized model predicated by RSM showed a volume expansion of 1.49% under the formulation condition, 5% for

whole egg powder, and 10% for skim milk powder.

Water Uptake Ratio (WUR)

Table 4 shows the effects of whole egg and skim milk powder (%) on the water uptake ratio of fortified pasta. Additionally, Fig. 3 illustrates these effects as three-dimensional graphs (3D), allowing for the evaluation of how formulation variables influence WUR. The second-order regression model equation predicting the effect of formulation variables on WUR is as follows:

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

Table 4: 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		

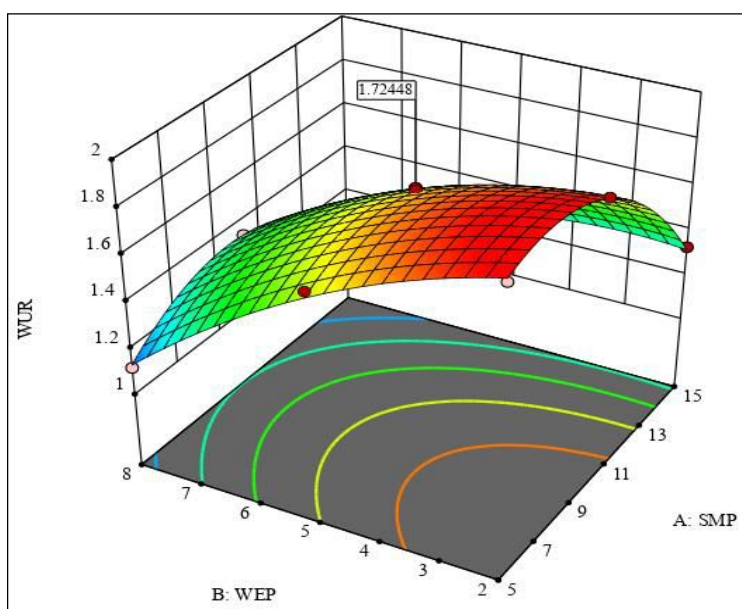
A- Chicken meat powder; B- DHA powder

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

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 R² value (0.9944), which measures the goodness of model fitting, indicated that 99.60% of the total variation was due to the more significant effect of WUR. A low CV value (1.20) indicated higher precision and reliability of the experiment. 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, A², and B² are significant model

terms. The Lack of Fit F-value of 0.08 implies that the Lack of Fit is not significant relative to the pure error.

Figure 3 demonstrates the effect of whole egg and skim milk powder (%) on WUR. It was observed that with the increase in whole egg powder and skim milk powder (%), there is a significant decrease in WUR, as predicted by the model terms. This effect of whole egg and skim milk powder (%) on the WUR is due to the lower swelling power and swelling index of the added protein sources, i.e., whole egg and skim milk powder (Ghumman *et al.*, 2016) [11].

**Fig 3:** 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 major technological properties for assessing the quality of pasta; solid loss indicates the

resistance of pasta to disintegrate during boiling. Thus, a low amount of solids in the cooking water suggests good cooking quality. Cooking loss, related to solid leaching during cooking, is widely used as an indicator of overall cooking performance (Gull *et al.*, 2018) [18]. The response surface analysis, as shown in Table 4, demonstrates that the relationship between whole egg and skim milk powder (%)

concerning GSL is linear, with the interaction term showing a good regression coefficient ($R^2=0.999$). The model equation exhibits the relationship as per the following equation.

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

The linear and interaction regression models indicated 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. Thus, the predicted model results suggested that whole egg and skim milk powder (%) significantly affected the GSL (Table 4).

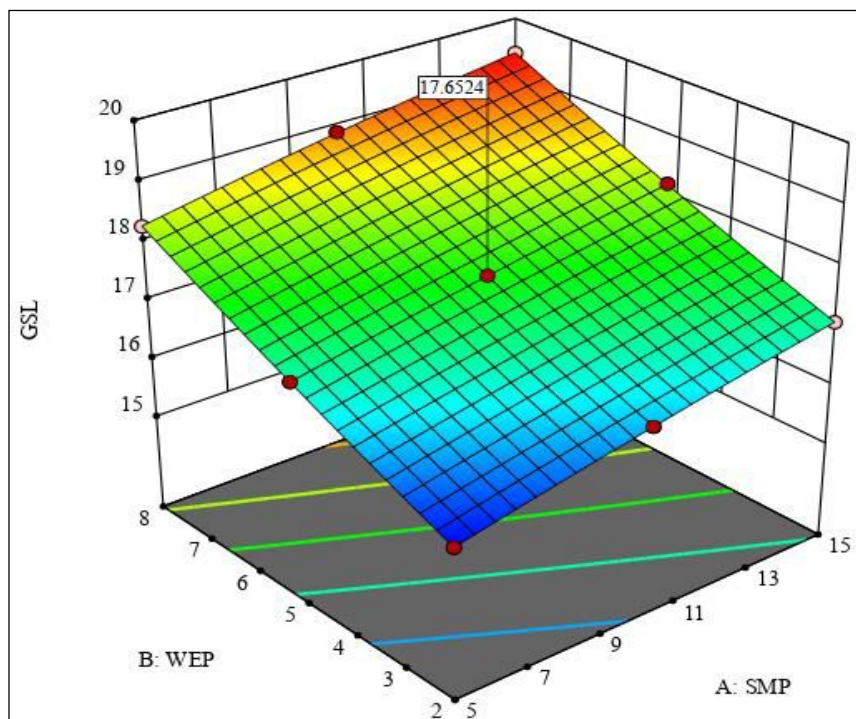


Fig 4: 3D Response Surface plots for GSL

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

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

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

Three-dimensional graphs (Fig. 4) showed the effect of whole egg and skim milk powder (%) on GSL. It was observed that GSL increased significantly with the increase in whole egg as well as skim milk powder (%). The increase in GSL with rising levels of added protein-rich whole egg and skim milk powder (%) was due to the weak interaction between the protein and starch matrix (semolina) that was destroyed during cooking (Gopalakrishnan *et al.*, 2011) [12]. The higher gruel solid loss can also be attributed to the

dilution of gluten protein, which weakened the starch and protein matrix. The model predicted 17.65% of GSL by keeping 5% of whole egg and 10% of skim milk powder (%).

Firmness

Texture has been defined as one of the important attributes of pasta structure, and textural characteristics are recognized as being more important for consumers. Functional

properties determine the quality characteristics of pasta, including firmness, gruel loss, and overall acceptability. High-quality pasta has good cooking resistance and firmness, does not release an excessive amount of organic matter into the cooking water, and does not show stickiness. Moreover, pasta quality is related to a low susceptibility to breakage under dry conditions. The texture of pasta is greatly dependent on the protein network, and the presence of a strong protein network makes pasta firmer.

Table 5 shows the effects of fortified whole egg powder and skim milk powder (%) on the firmness of fortified pasta. In addition, Fig. 5 illustrates these effects as three-dimensional graphs (3D), where the direction of the effects of formulation variables on firmness can be predicted. The second-order regression model equation predicting the effect of formulation variables on firmness is as follows:

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

The Model F-value of 9455.03 indicates that the model is significant. There is only a 0.01% chance that an F-value of this magnitude could result from noise. P-values less than 0.05 suggest that the model terms are significant. The Lack of Fit F-value of 0.068 indicates that the Lack of Fit is not significant compared to the pure error.

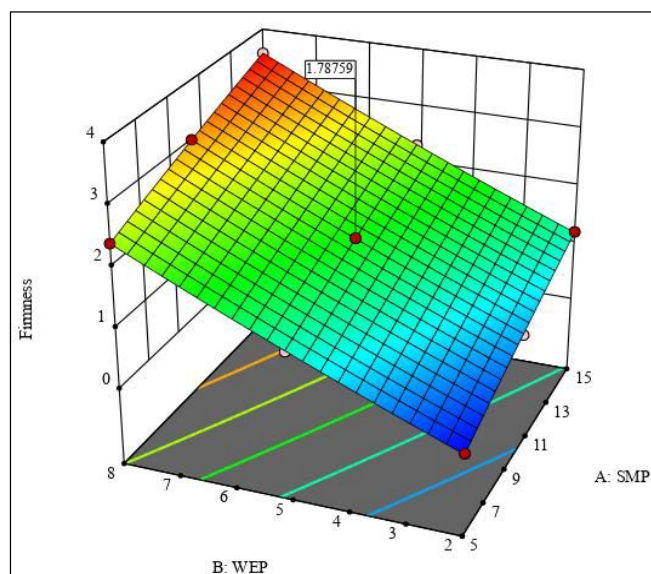


Fig 5: 3D Response Surface plots for firmness

Figure 5 demonstrated the effect of whole egg powder and skim milk powder percentages on firmness. It was observed that as the whole egg powder and skim milk powder percentages increased from 2% to 8% and 5% to 10%, respectively, there was a significant increase in firmness. This was due to the interaction of egg and milk proteins with the insoluble networks of pasta, forming stable matrix structures that resulted in higher firmness and toughness compared to the control pasta. These results were supported by SEM analysis in our study. More force was required to break samples with higher protein content, indicating that whole egg powder and skim milk powder functioned as effective ingredients for fortifying the pasta structure network. Studies demonstrated that incorporating a higher amount of protein in pasta could lead to increased textural intensity, including firmness and toughness (Chang & Wu, 2008) [13]. These results align with those of Daud (2020),

who studied the textural qualities of pasta fortified with meat proteins. The RSM models showed a firmness of 1.78% under the formulation conditions of 5% WEP and 10% SMP.

Toughness

High-quality pasta has good cooking resistance and firmness, resulting in less or no release of excessive organic matter into the cooking water, without stickiness (Day and Swanson 2013) [15]. The textural characteristics of pasta play a crucial role in determining final consumer acceptability. Optimizing texture parameters is critical to ensuring product acceptance by consumers. The matrix structural network of starches, glutes, additional proteins, and other ingredients mainly affects the textural properties of pasta (Mudgil *et al.*, 2016) [14].

Table 5 shows the effects of whole egg powder and skim milk powder (%) on the toughness of fortified pasta. In addition, Fig. 6 illustrates these effects as three-dimensional graphs (3D), where direction of the effects of formulation variables on toughness can be predicted. The second-order regression model equation predicting effect of formulation variables on firmness is as follows:

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

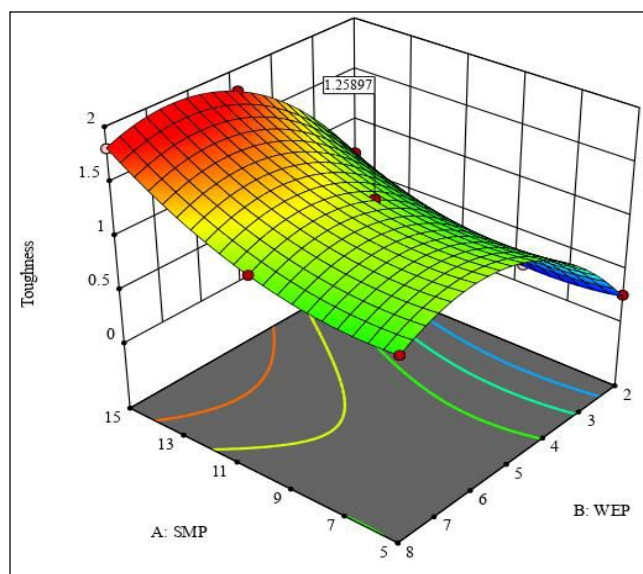


Fig 6: 3D Response Surface plots for toughness

The Fig. 6 demonstrated the effect of whole egg powder and skim milk powder (%) on toughness. It was observed that increasing the percentages of whole egg powder and skim milk powder resulted in a significant ($p < 0.05$) increase in toughness. These results align with firmness, which might be attributed to the interaction of whole egg and skim milk proteins with the insoluble networks of semolina, forming stable matrix structures and leading to higher firmness and toughness of pasta compared to control pasta. The RSM models indicated 1.25% toughness under the formulation condition of 5% whole egg powder and 10% skim milk powder. The SEM analysis of cooked pasta justified the increase in firmness and toughness of the developed pasta.

L value

The color of the product is an important quality parameter that is strongly associated with consumer perception. The

effects of whole egg powder and skim milk powder on the L value of the developed pasta are shown in Table 6. In addition, Fig. 7 illustrates these effects as 3D graphs, allowing for the evaluation of the direction of the effects of formulation variables on the L value. The second-order

regression model equation predicting the effect of formulation variables on the L value is as follows:

$$L = 62.87 + 0.73A - 1.27B + 0.29AB$$

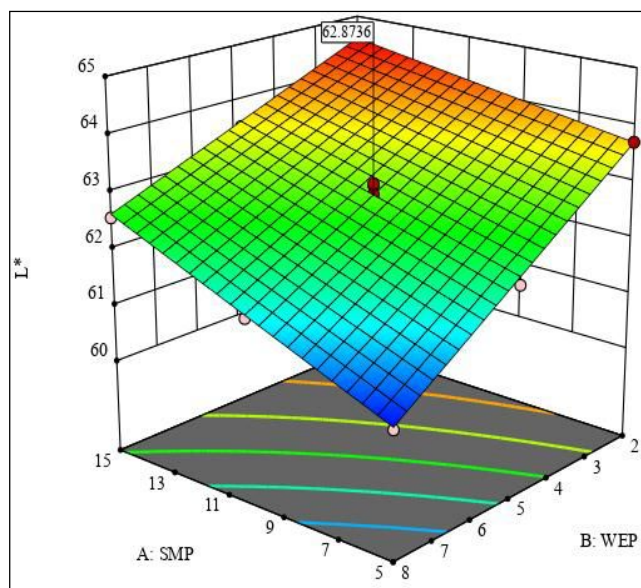


Fig 7: 3D Response Surface plots for L value

The Model F-value of 253.53 implies that 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 0.0500 indicate that the model terms are significant. In this case, A, B, and AB are significant model terms. The Lack of Fit F-value of 2.68 implies that the Lack of Fit is not

significant relative to the pure error. There is an 18.03% chance that a Lack of Fit F-value this large could occur due to noise. The Predicted R^2 of 0.9807 is in reasonable agreement with the Adjusted R^2 of 0.9844; that is, the difference is less than 0.2.

Table 6: 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_1B_2		-0.8260	2.73	0.1931						
Interaction										
A_1^2		1.82	9.13	0.0338						
B_2^2		1.49	6.12	0.0681						
Residual	10		9.22			2.77			2.53	
Lack of fit	1.13		3.94	0.4800		2.04	0.2869		1.93	0.2424
Pure error	0.5855		5.27			0.7341			0.6030	
Total	13.17		80.27			23.52			34.55	
Adj. R^2	0.8438	0.8032			0.8585				0.9122	
Pred. R^2	0.7986	0.5225			0.7947				0.8690	
C.V. %	28.02	1.92			16.32				2.56	

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

Figure 7 demonstrated the effect of whole egg powder and skim milk powder on L value, indicating that as the percentage of skim milk powder increases, there is a significant rise in the L value of the resultant product, likely due to the inherent color of the raw ingredients, namely the color of skim milk powder, which has a high L value and low a^* and b^* values. It is also evident from Figure 7 that an increase in whole egg protein content from 2% to 8% resulted in a significant decrease in L^* value. Higher yellowness and the corresponding decrease in lightness of

color were probably due to the color of the egg yolk in whole egg powder. Additionally, whole egg powder itself was yellowish. RSM indicated an L value of 62.87 under the formulation condition of 5% WEP and 10% SMP.

The a^* and b^* value

The response surface analyses (Table 6) demonstrated a high regression value ($R^2 = 0.9962$) for a^* value and ($R^2 = 0.9995$) for b^* value. The second-order polynomial equation explained the relationship.

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

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

It was observed that both WEP and SMP (%) had significant linear term effects on a^* and b^* values ($p < 0.0001$) (Table 6). The effects of formulation parameters on a^* and b^* values and their interactions are shown in Fig. 8 and Fig. 9.

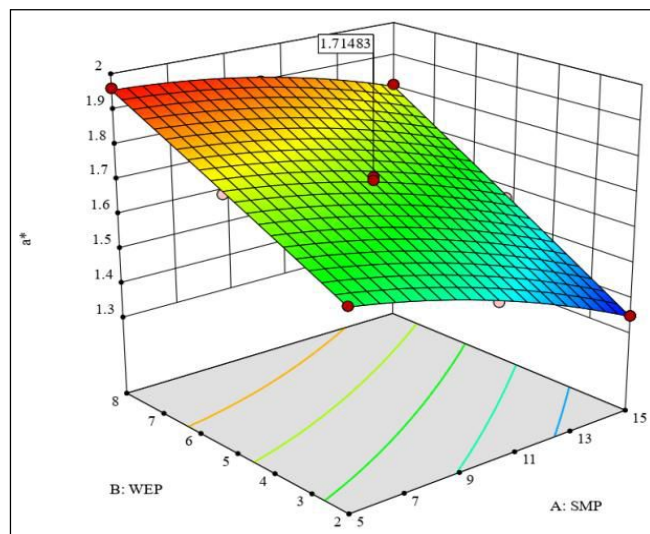


Fig 8: 3D Response Surface plots for a^* value

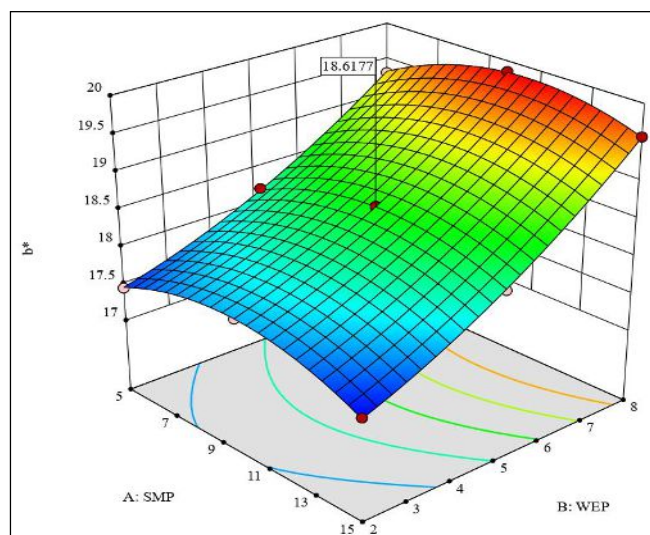


Fig 9: 3D Response Surface plots for b^* value

Increasing the percentage of whole egg powder showed a linear increase in a^* and b^* values, as indicated in Fig. 10 and 11. It was found that the higher yellowishness, along with a corresponding decrease in lightness and an increase in b^* value, was probably due to the color of the yolk in whole egg powder. The optimal formulation levels of selected variables determined by RSA were 5% WEP and 10% SMP powder, exhibiting an a^* value of 1.71 and a b^* value of 18.61.

Overall acceptability (OA)

Sensory evaluation, particularly overall acceptability, is the most reliable test as it allows assessment of the overall characteristics of any food. RSM, as demonstrated (Table 5), followed a high regression value ($R^2 = 0.83$) for the OA in the developed pasta, and a second-order polynomial equation explained the relationship as follows:

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

ANOVA of interaction regression models for OA indicated that the model was significant ($p < 0.05$) with p-values of < 0.0001 . Table 5 indicated that WEP and SMP (%) had significant ($p < 0.0001$) interaction effects on OA. The Model F-value of 1.79 suggests the model was significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.05 suggest model terms are significant. In this case, B, A^2 , and B^2 are significant model terms.

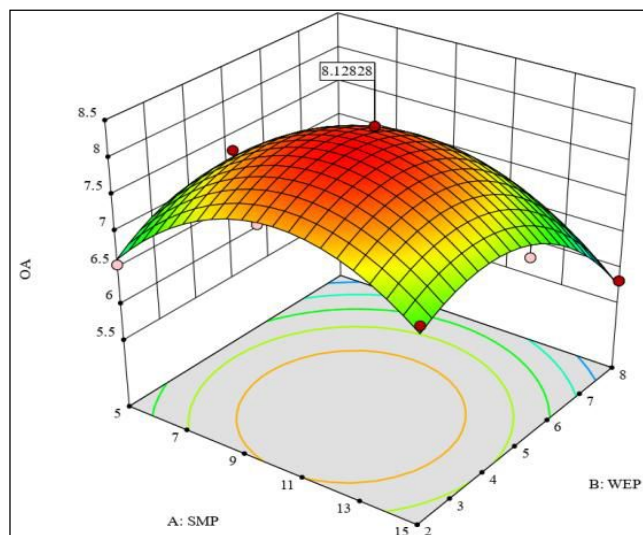


Fig 10: 3D Response Surface plots for Overall acceptability

Fig. 10 shows the WEP (%) and SMP powder on the OA. It was observed that the OA increased with the rising WEP (%) and nearly reached its highest point at 5% tested, before following a declining trend thereafter. Similar trends were reported for SMP (%). Yadav *et al.* (2014) reported that this may be due to the unattractive dark yellowish color of cooked pasta, which limits the wider acceptability of the developed pasta products. The optimized model predicted by RSM showed 8.12% overall acceptability under the formulation condition of WEP at 5% and SMP at 10%.

Summary and Conclusions

RSM is one of the useful techniques used for optimizing the basic formulation of protein-fortified pasta, namely, whole egg powder and skim milk powder. Both WEP and SMP levels, as well as processing conditions, significantly affect all the responses. The developed model equation can primarily be used to predict the quality of fortified pasta. Based on the three models employed in RSM and the response surface plots, the basic formulation and desired quality can be achieved by incorporating 5% whole egg powder and 10% skim milk powder, yielding the highest scores for various physico-chemical, cooking, and textural attributes. Microstructure studies revealed increased interaction between skim milk powder and whole egg powder molecules, indicating a potential option for producing high-quality pasta with enhanced nutritional and functional properties.

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