

FUNCTIONAL PROPERTIES OF MAIZE FLOUR AND ITS BLENDS WITH WHEAT FLOUR: OPTIMIZATION OF PREPARATION CONDITIONS BY RESPONSE SURFACE METHODOLOGY

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Abstract

The functional properties such as water holding capacity (WHC), oil holding capacity (OHC), least gelation capacity (LGC), emulsifying activity (EA) and foaming capacity (FC) of full fat and defatted maize flour were studied using response-surface methodology. A positive significant linear effect ($p > 0.05$) of concentration of maize flour in the blend on WHC, OHC and EA and a negative linear effect on LGC and FC was observed. Drying temperature had a positive significant linear effect while the quadratic and interaction effects of both variables were found to be non-significant in each case. The optimum conditions of maize concentration in blend (g/100g blend) and drying temperature (°C) generated from the statistical model for different functional properties were: WHC; 75 and 125, OHC; 25 and 75, LGC and FC; 75 and 75 and EA; 75 and 125 respectively.

Introduction

Maize and wheat flours are frequently used in various pharmaceutical and food formulations all over the world. These flours are used either in their pure form or in the form of their blends with other seed flours such as groundnut and soy bean (Akapapnam & Darbe, 1994; Akubor & Onimawo, 2003). The industrial and food applications of these flours significantly depend on their functional characteristics. Functional properties such as water absorption, oil absorption, gelation, foaming and emulsifying capacities are the intrinsic physicochemical properties which illustrate the structural behaviour of the food systems. The investigations about the functional properties of seed flours provide an advanced knowledge for their use in the preparation of different pharmaceutical and food products. The beneficial uses of plant flours in the pharmaceutical and food industry directly depend on their functional properties (Ogungbenle *et al.*, 2002). Any change in these properties during processing, transport and storage may significantly influence the nutritional and consumption importance of the food materials. The factors which affect the functional properties of food materials during storage and processing should be optimized to improve the functional characterization of the food materials.

A number of statistical designs are available to study the relationship between a dependent and one or more factors or input variables. Response Surface Methodology (RSM) is used to create response-surface models for the prediction of changes in response variables as a function of changes in input variables (Montgomery, 2009). RSM is a set of statistical and mathematical tools that are useful for designing, developing, improving and optimizing the process under study. RSM has extensively been used in the field of industry and chemical engineering to study the yield of a system as it varies in response to the changing levels of one or more input variables. The response

variable is the measured quantity of the output of a trial whose value is assumed to be affected by changing the levels of input variables.

It is also used to find the optimum levels of the input variables leading to the desired goals of response variable (Khuri & Cornell, 1987). Moreover, in RSM, a central composite design (CCD) is often used for building a second order quadratic model for the response variable. CCD has been acknowledged as a useful practice to optimize the process variable and the objective is to optimize the response. It reduces the number of experimental runs necessary to establish a mathematical trend in experimental design allowing the determination of the optimum levels of experimental factors required for a given response. Being an effective statistical technique for optimization of process conditions, RSM (CCD) are useful as an economical way of obtaining maximum information in a short time period and lesser number of experiments (Aslan, 2007; Anarjan *et al.*, 2010). RSM has been also used previously for the optimization of preparation conditions to study the effect of preparation variables on functional properties of blends and product characteristics of different flours (Asare *et al.*, 2004; Ikegwu & Okoli, 2011).

Previously, some work has been reported on the functional properties of maize flour and its blends with soy bean, Nile tilapia and groundnut seed flour (Akubor & Onimawo, 2003; Fasasi *et al.*, 2007; Akapapnam & Darbe, 1994). However, no data has been found to be available on the effect of preparation conditions on the functional properties of blend of maize flour with wheat flour. Therefore, the present study was planned to study the effect of defatting on the functional properties of maize flour as well as the evaluation of the effect of concentration of maize flour and drying temperature on the functional properties of blends of maize flour with wheat flour using response surface methodology.

Functional properties

Water holding capacity (WHC) and oil holding capacity (OHC): The WHC and OHC of the samples were determined by using the methods suggested by Beuchat (1977). The flour/blend (1 g) was vortexed with distilled water (10 mL) in pre-weighed centrifuge tube for 30 minutes. After standing at room temperature for 30 minutes, the sample was centrifuged for 25 min at 3000 × g. The sediments were weighed after complete removal of the supernatant. For the determination of OHC, the flour/blend (0.5 g) was homogenized with canola oil (5 mL) in pre-weighed centrifuge tube and proceeded further as described for WHC. The WHC and OHC (%) were calculated as:

$$WHC \text{ or } OHC (\%) = [(W_2 - W_1) / W_0] \times 100$$

where W_0 is the weight of the sample, W_1 is the weight of centrifuge tube plus sample and W_2 is the weight of the centrifuge tube plus sediments.

Least gelation concentration (LGC): The LGC of the flours and blends were determined using the method described by Coffman and Garcia (1977). A series of sample suspensions of increasing concentrations such as 2, 4, 6, 8, 12, 14, 16, 18 and 20% (m/v) were prepared in distilled water (10 mL). All the suspensions were heated gently for 1 hour in a boiling water bath followed by cooling at 4°C for 2 hours. The suspensions were inverted and the LGC was taken as the concentration at which the inverted suspension did not fall or slip.

Foaming capacity (FC): Foaming capacity of the flours and blends was determined by the method of Coffman and Garcia (1977). The flour/blend (2g) was dispersed in distilled water (100 mL) and homogenized properly for two minutes in a kitchen blender. Volumes were recorded before and after homogenization and percent increase in the volume was calculated as FC of the flour by using the following formula:

$$FC (\%) = [(V_2 - V_1) / V_1] \times 100$$

where, V_1 = Initial volume of the solution and V_2 = volume of solution after homogenization.

Emulsifying activity (EA): Emulsifying activity of flours and blends were determined by the method as described by Neto *et al.*, (2001). The flour/blend (2g) was dispersed in distilled water (10mL) and height of solution in the cylinder was measured. The solution was homogenized with refined canola oil (5mL) and the resulting emulsion was centrifuged at 1100 × g for 5 minutes. The height of the emulsified layer was measured and the emulsifying activity was calculated as the percent increase in the height of the solution by following equation:

$$EA (\%) = [H_2 / H_1] \times 100$$

where H_1 is the initial height of solution before emulsification, H_2 is height of the emulsified layer.

Statistical analysis: The results for the functional properties of full fat maize flour, defatted maize flour, full fat wheat flour and blend of full fat flours of maize and wheat (1:1) were expressed as means ± standard deviation of three parallel replicates. The data were statistically analyzed by one way analysis of variance (ANOVA) using statistical software (SPSS, version 14.0) and the means were separated by applying Tukey's multiple range tests at a confidence interval of $p \leq 0.05$.

Response-surface methodology was used to study the effect of concentration of maize flour in the blend and drying temperature on the functional properties of blends of maize flour with wheat flour. Response-surface models were created for the prediction of change in functional properties of blends as a function of preparation conditions and for the optimization of independent variables to achieve the desired values of response variables. The quadratic polynomial models for the response variables were predicted by determining the regression coefficients using least-squares technique (Myers & Montgomery, 2002). The generalized polynomial model for predicting the variation of the response variables is given below:

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$$

where Y_i is the predicted response, β_0 is a constant, β_1 and β_2 are the regression coefficients for the main variable effects, β_{11} and β_{22} are quadratic effects and β_{12} is the interaction effect of independent variables.

The significance of the estimated regression coefficient for each response variable was assessed by lack of fit test (F -ratio) at a probability (p) of 0.05. The lack of fit measures the degree of failure of a model to fit the data in experimental domain particularly for reduced points in a randomized experiment. A non-significant value of lack of fit indicates the adequacy of the model in describing the response variable (Montgomery, 2001). The corresponding variables with larger F -values and smaller p -values were considered more significant (Amin & Anggoro, 2004). The reduced model contained only those terms which were found statistically significant ($p < 0.05$). The adequacy of the response surface models was determined by the analysis of coefficient of determination (R^2) (Weng *et al.*, 2001). The adjusted coefficient of determination (R^2_{adj}) was also determined to measure the fairness of fit of the regression equation (Yang *et al.*, 2009). The coefficient of variation (CV) was determined to check the precision and reliability of the experiments carried out. A low value of CV suggests a better precision and reliability of the experiments (Thana *et al.*, 2008). The development of experimental design, data analysis and optimization procedure were performed using the statistical software Design Expert 8.0.4.1 (Stat-Ease, Inc.).

For graphical optimization of independent variables, the three-dimensional plots were constructed between response and the two independent variables. The adequacy of the response-surface models was verified by plotting the experimental values versus those predicted by the final reduced models.

Results and Discussion

In present study the functional properties of maize flour and its blends with wheat flour were evaluated. Table 2 presents the results for functional properties of full fat maize flour, defatted maize flour, full fat wheat flour and blend of full fat flours of maize and wheat (1:1). The WHC, OHC, LGC, EA and FC of full fat flour, defatted flour and blend of maize with wheat flours ranged from 39.80 ± 2.60 to 311 ± 5.00 , 95.50 ± 3.50 to 216 ± 7.00 , 4.00 ± 0.00 to 6.33 ± 0.41 , 10.45 ± 0.52 to 20.05 ± 0.72 and 13.78 ± 0.22 to $16.29 \pm 0.65\%$ respectively. The statistical analysis of the data showed a significant difference ($p < 0.05$) in all of the functional properties among different flours. The full fat flour of maize showed highest WHC, OHC and EA but lowest values of LGC and FC. On the other hand, the full fat flour of wheat was

found to possess an opposite behavior. The result showed that defatting of maize flour significantly increased its WHC, OHC and FC but decreased EA values. No significant effect of defatting was observed on LGC values. These findings are in accordance with those reported for corn germ proteins and *Cassia fistula* seed flours (Zayas & Lin, 1989; Akinyede & Amoo, 2009). The increase in WHC, OHC and FC in defatted flour may be due to the fact that the removal of oil from the flour results in the exposure of hydrophilic groups in the proteins molecules to the outer environment which facilitates the maximum absorption of water on the surface of proteins (Lin *et al.*, 1974). The blend of maize flour with wheat flour prepared at room temperature (25°C) resulted in a decrease in WHC, OHC and EA but an increase in LGC and FC as compared to those of full fat flour of maize and wheat.

Table 2. Functional properties of full fat and defatted flour of maize, full fat flour of wheat and their blend as determined at 25°C .

Functional property	Maize flour (full fat)	Maize flour (defatted)	Wheat flour (full fat)	Blend of full fat flours of maize and wheat in 1:1 ratio
WHC (%)	149.00 ± 4.00	311.00 ± 5.00	63.80 ± 2.10	89.80 ± 2.60
OHC (%)	107.00 ± 1.00	216.00 ± 7.00	72.50 ± 2.00	95.50 ± 3.5
LGC (%)	4.00 ± 0.00	4.00 ± 0.00	10.00 ± 0.56	6.33 ± 0.41
EA (%)	20.05 ± 0.72	16.24 ± 0.41	6.50 ± 0.46	10.45 ± 0.52
FC (%)	13.78 ± 0.22	15.28 ± 0.57	19.56 ± 0.97	16.29 ± 0.65

The WHC and OHC capacities are essential functional properties of protein which depend on pore size and the charges on the protein molecules. High WHC determines the hydrophilic nature and high hydrogen bonding of protein molecules (Altschul & Wilcke, 1985). A range of WAC from 149.1 to 471.5% is considered favorable for the preparation of viscous foods such as soups, gravies and bakery products (Anon, 1990). High WHC of defatted maize flour make it superior to wheat flour to be used in such food products. The preparation of blend of maize flour with wheat flour would improve the texture and quality of those food products which are currently prepared from wheat flour only. The WHC of maize flour has been found to be lower than that reported earlier for maize flour and jack bean starch (Fasasi *et al.*, 2007; Yusuf *et al.*, 2007).

High OHC indicates the enhanced hydrophobic character of proteins in the flours. OHC is exhibited by the proteins in the flour which physically bind to fat by capillary attraction. These proteins expose more non-polar amino acids to the fat and enhance hydrophobicity as a result of which flours absorb oil. OHC play a significant role in ground meat formulations like sausages and to increase the shelf life of meat products (Akinyede & Amoo, 2009). Therefore, the defatted flour with comparatively higher OHC may be favorable in food formulations where an improvement in oil holding capacity is required. The OHC of full fat flour of maize was found to be lower than that reported earlier for maize flour (Fasasi *et al.*, 2007).

LGC is the ability of flour to form gel which provide structural matrix for holding water and other water soluble materials like sugars and flavors. It may vary from flour to flour depending on the relative ratios of their structural constituents like proteins, carbohydrates and lipids (Sathe *et al.*, 1982). The gelation property of the flour provides consistency in food preparations especially the semi-solid products (Bhat & Srithsr, 2008). The increasing concentration of proteins enhances the interaction among the binding forces which in turn increases the gelling ability of flour (Lawal *et al.*, 2004). The lower the LGC value the better the gelling ability of the flour. The maize flour was found to be used in food preparations due to its low LGC value as compared to wheat flour. The blend of maize flour with wheat flour would improve the gelation properties of wheat flour. LGC of maize flour was found to be comparatively lower than that reported earlier for maize flour (Fasasi *et al.*, 2007).

FC is much important in manufacturing and maintaining structure of different food products like ice creams and bakery products during and after processing. The ability of the flours to form foam depends on the presence of the flexible protein molecules which may decrease the surface tension of water (Sathe *et al.*, 1982). The FC value of maize flour was found to be higher than that reported earlier for maize flour (Akubor & Onimawo, 2003). Due to low FC of maize flour it would be better to use its blend with wheat flour to maintain the structure of food products like ice creams.

EA is the maximum amount of oil emulsified by protein in the given amount of flour. The EA value of maize flour were found to be higher than those reported earlier for wheat flour (Akubor *et al.*, 2000) but lower than that reported earlier for maize (Akubor & Onimawo, 2003). The comparatively higher EA of full fat flour of maize makes it better to be used in food formulations like snacks, pastries, coffee whiteners and frozen desserts.

Response surface analysis and optimization of results:

The optimization process predicted an optimum level for

each of the independent variables that resulted in the desirable goals. The non-significant terms were dropped in the initial model and the experimental data were fitted again only to the significant parameters to obtain the final reduced model. However, the non-significant linear terms were included in the final reduced model if the quadratic or interaction terms containing these variables were found to be significant (Mirhosseini & Tan, 2009). The results for functional properties of blends prepared at random levels of preparation variables as per chosen by the experimental design are given in Table 3.

Table 3. The observed values of functional properties of blends of maize flour with wheat flour at random levels of experimental conditions as per chosen by central composite design.

Experimental runs	X_1	X_2	WHC (%)	OHC (%)	LGC (%)	FC (%)	EA (%)
1	-1	-1	142.05	131.99	8.00	18.73	11.197
2	0	0	181.36	164.54	7.33	18.77	13.451
3	1	1	241.4	199.65	6.00	19.27	15.975
4	-2	0	151.21	107.87	10.67	21.66	10.732
5	0	0	181.36	164.54	7.33	18.77	13.451
6	2	0	210.44	190.48	5.33	16.73	16.751
7	1	-1	169.32	160.7	4.67	17.43	13.012
8	-1	1	173.52	180.32	9.33	20.07	12.809
9	0	2	220.93	203.95	8.67	20.37	14.887
10	0	0	181.36	164.54	7.33	18.77	13.451
11	0	-2	103.7	130.93	6.67	17	11.432

The application of RSM yielded the polynomial regression equations showing an empirical relationship between the functional properties of blends of maize flour with wheat flour and preparation variables. The polynomial regression equation includes the coefficient for intercept, main effects, interaction terms and quadratic effects. The influence of each factor on the response is shown by the sign and magnitude of the main effect. The RSM indicated that the relationship between preparation conditions and the functional properties of blends could be explained by significant second order polynomial regression models. The following polynomial regression equations were obtained to show the relationship between the functional properties of blends and preparation variables:

$$\text{WHC (\%)} = 183.51 + 17.80 X_1 + 28.17X_2 - 0.268 X_1^2 - 4.896X_2^2 + 10.15X_1X_2$$

$$\text{OHC (\%)} = 167.38 + 17.77X_1 + 19.44X_2 - 4.019X_1^2 + 0.548X_2^2 - 2.345X_1X_2$$

$$\text{LGC (\%)} = 7.0847 - 1.445X_1 + 0.555X_2 + 0.1828X_1^2 + 0.1003X_2^2 + 0.00X_1X_2$$

$$\text{FC (\%)} = 18.778 - 0.997X_1 + 0.827X_2 + 0.1057X_1^2 - 0.0218X_2^2 + 0.125X_1X_2$$

$$\text{EA (\%)} = 13.366 + 1.418X_1 + 0.957X_2 + 0.0779X_1^2 - 0.0676X_2^2 + 0.338X_1X_2$$

The main, quadratic and interaction effects of concentration of maize flour in the blend and drying temperature on functional properties of blends as determined by analysis of variance (ANOVA) are given in Table 4. The significance and adequacy of the model

was measured in terms of *F*-value and *p*-value at 5% significance level ($p \leq 0.05$). The measurement of *F*-value and *p*-values indicated that the concentration of maize flour in the blend has positive significant linear effect on WHC, OHC and EA but a negative significant linear effect on LGC and FC of the blends. The drying temperature, on the other hand, showed a positive significant linear effect on each of the functional properties of the blends. The interaction effects were found to be non-significant in each case. The quadratic effects of both of the independent variables on each of the functional properties of the blends were found to be non-significant. It is clear from RSM results that WHC, OHC and EA are increased by increasing the concentration of maize flour in the blend while LGC and FC are decreased in response to an increase in the concentration of maize flour in the blend. However, all the functional properties are increased by increasing the drying temperature.

The correlation coefficient (R^2) measures the variability of the model in the observed response values. A value of R^2 closer to unity gives better prediction of the response and high significance of the model. The values of R^2 for WHC, OHC, LGC and FC of the blends indicated that more than 96% of the variability in functional properties could be explained by the suggested model. However, the variability in EA was explained up to 93.85% by the model. The values of adjusted R^2 for these responses also advocate the significance of the model. The relatively low values of CV such as 4.44, 5.10, 6.68, 2.35 and 2.75% for WHC, OHC, LGC, FC and EA respectively showed a better precision and reliability of the experiments performed.

Table 4. Analysis of variance (ANOVA), Coefficient of variation (CV) and correlation coefficients (R^2) as calculated by response surface model for functional properties of blends of maize flour with wheat flour.

Source	WHC			OHC			LGC			FC			E.A		
	Rc ^a	F-value	p-value	Rc	F-value	p-value									
Model	1.835	45.69	0.0004	1.674	25.20	0.0015	7.085	24.11	0.0016	18.778	15.27	0.0048	13.366	72.98	0.0001
X_1	0.178	60.91	0.0006	0.178	54.47	0.0007	-1.445	102.67	0.0002	-0.997	44.42	0.0011	1.418	245.18	0.0001
X_2	0.283	152.51	0.0001	0.195	65.20	0.0005	0.555	15.15	0.0115	0.827	30.56	0.0027	0.957	111.65	0.0001
X_1X_2	0.102	6.60	0.0500	-0.024	0.32	0.5982	$1.1E^{-016}$	0.000	1.0000	0.125	0.23	0.6498	0.338	4.63	0.0839
X_1^2	-0.003	0.022	0.8873	-0.04	4.48	0.0879	0.183	2.64	0.1649	0.106	0.80	0.4110	0.078	1.19	0.3248
X_2^2	-0.049	7.41	0.0417	0.0055	0.083	0.7846	0.1004	0.80	0.4131	-0.022	0.034	0.8607	-0.068	0.89	0.3877
	CV (%) = 4.44			CV (%) = 5.10			CV (%) = 6.68			CV (%) = 2.35			CV (%) = 2.75		
	$R^2 = 0.9786$			$R^2 = 0.9618$			$R^2 = 0.9602$			$R^2 = 0.9865$			$R^2 = 0.9385$		
	R^2 (adjusted) = 0.9572			R^2 (adjusted) = 0.9237			R^2 (adjusted) = 0.9203			R^2 (adjusted) = 0.9730			R^2 (adjusted) = 0.8771		

*Rc: Regression coefficients

Three dimensional (3D) response surface plots were drawn to show the main and interaction effects of concentration of maize flour in the blend and drying temperature on functional properties of blends (Fig. 1). 3D surface plots showed that concentration of maize flour in the blend has a positive linear effect on WHC, OHC and EA and a negative linear effect on LGC and

FC. The drying temperature has a positive linear effect on each of the functional properties. The optimum levels of concentration of maize flour in the blend and drying temperature for different functional properties were found to be: WHC; 60 and 80, OHC; 40 and 100, LGC and FC; 0 and 40 and EA; 80 and 60 respectively.

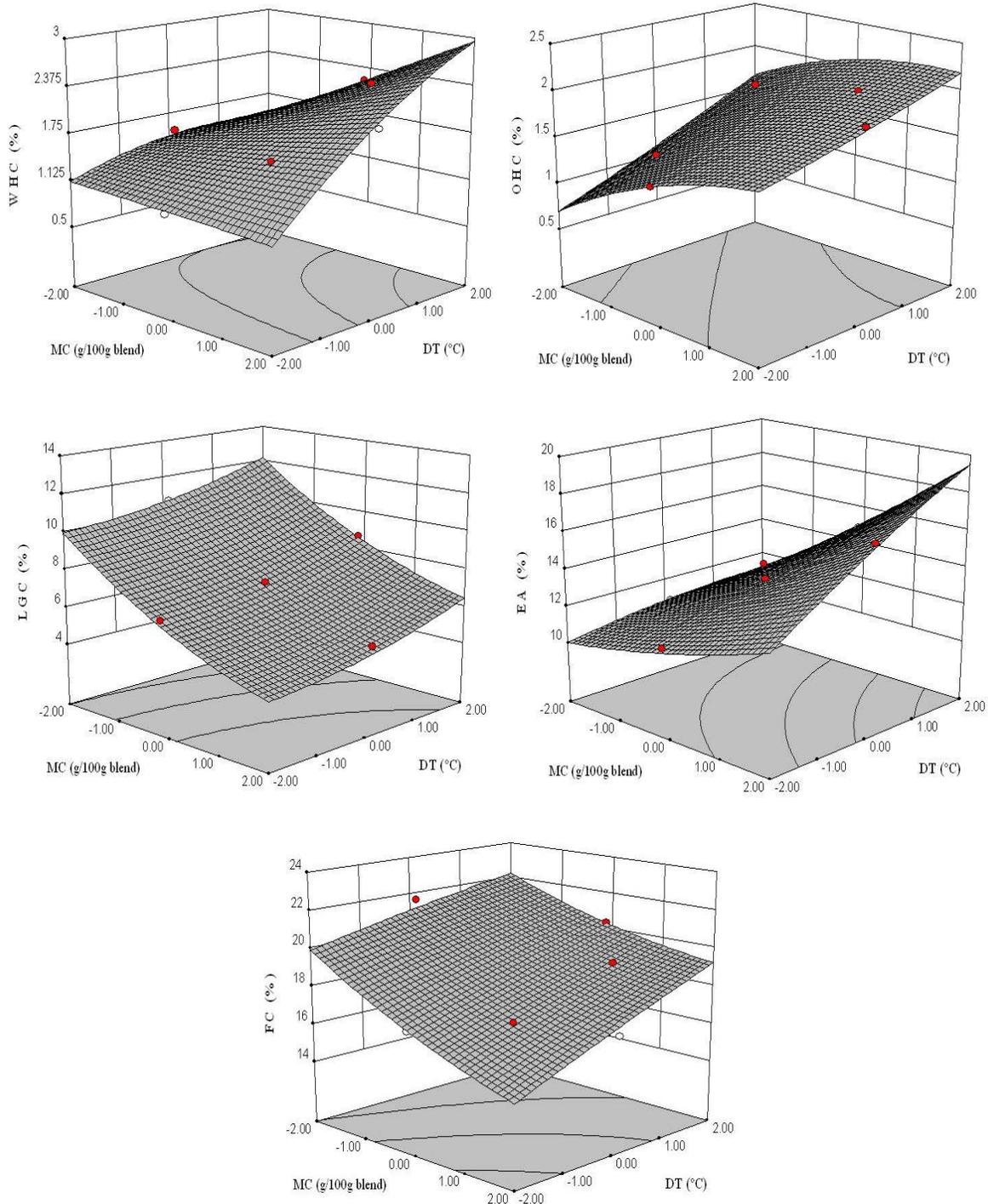


Fig. 1. Response surface plot of functional properties of blend of maize flour with wheat flour at various levels of preparation conditions. MC: Concentration of maize flour in the blend (g/100g blend), DT: Drying temperature (°C)

To test the applicability of the model, the predicted values of functional properties calculated from the polynomial regression equations were plotted against the experimental values (Fig. 2). A good agreement between the experimental and predicted values of responses was observed with high values of

coefficients of determination (R^2) such as 0.9789, 0.9603, 0.965, 0.9177 and 0.9869 for WHC, OHC, LGC, FC and EA respectively. The higher values of R^2 prove the applicability of proposed model with greater accuracy to study the effect of preparation conditions on the functional properties of the blends.

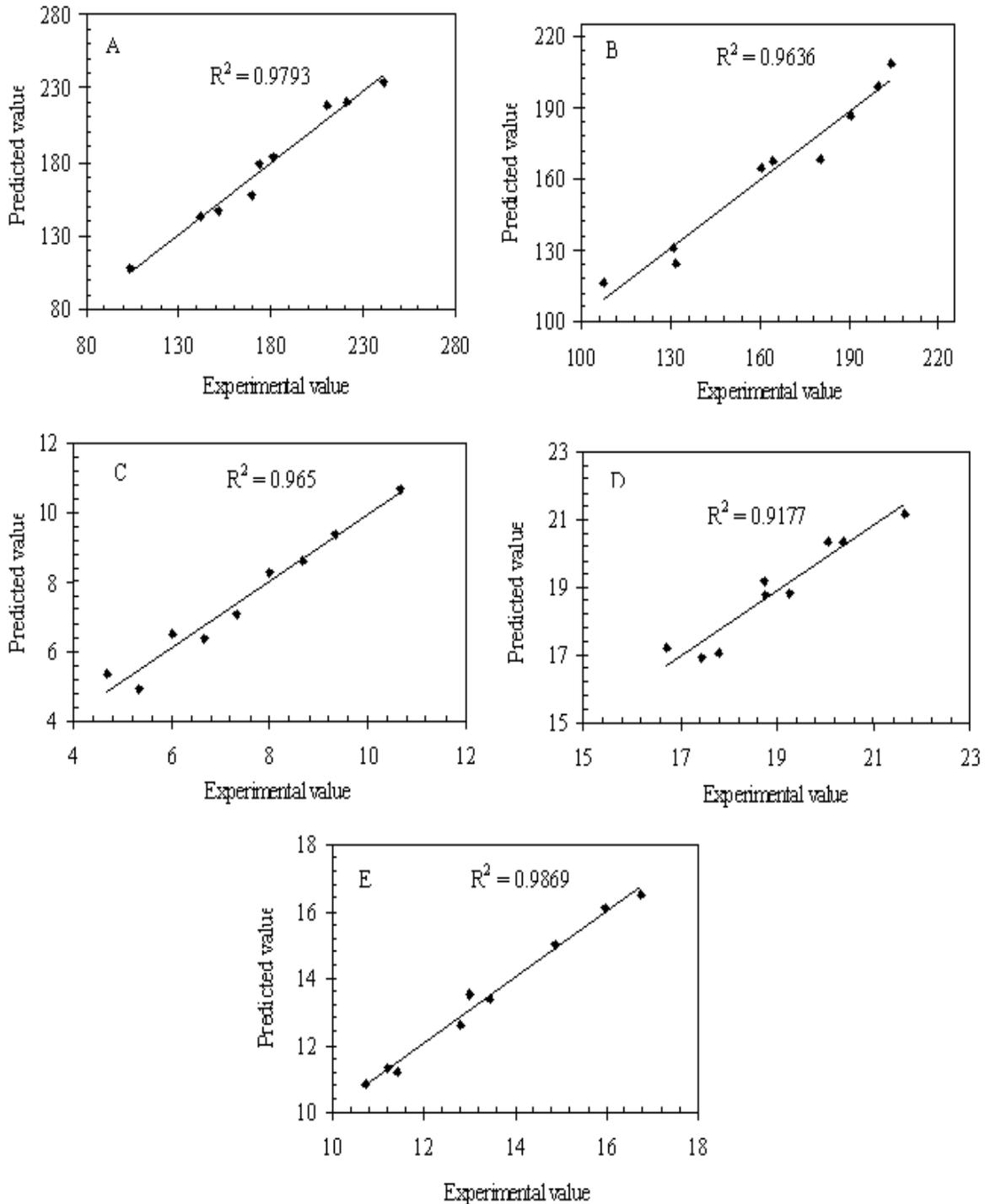


Fig. 2. Agreement between the actual values of functional properties of blend of maize flour with wheat flour and those predicted by the response surface analysis.

A: WHC, B: OHC, C: LGC, D: EA, E: FC

Conclusion

The defatting of maize flour significantly increased WHC, OHC and FC but decreased EA values. This suggests the suitability of defatted maize flour in soups, gravies and some bakery products. The application of RSM showed that WHC, OHC and EA are increased while LGC and FC are decreased in response to an increase in the concentration of maize flour in its blend with wheat flour. However, all the functional properties are increased by increasing the drying temperature. The optimum levels of concentration of maize flour in the blend (g/100g blend) and drying temperature (°C) for different functional properties were: WHC; 75 and 125, OHC; 25 and 75, LGC and FC; 75 and 75 and EA; 75 and 125 respectively. High values of R^2 suggest that the applicability and adequacy of the suggested model is very good. The data may provide valuable guidelines for the researchers, industrialists and manufacturers to achieve their desired goals.

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