Original Research Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Optimization of rheological characteristics of bread dough supplemented with defatted wheat germ

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Abstract

Optimization of bread dough formulation with defatted wheat germ (DWG) in terms of dough rheological properties was the objective of this study. Fractional factorial design 2_V^{5-1} was applied in order to investigate influence of DWG granulation (<150 μm and 150–1000 μm), DWG content (10 and 20%), addition of gluten (0 and 5%), ascorbic acid (0.004 and 0.008%) and combined improver (0.1 and 0.3%) on farinograph and extensograph parameters. Responsive variables were water absorption and the degree of softening, obtained by farinograph, and resistance to extension and extensibility as well as their ratio, obtained by extensograph analysis. Gluten addition expressed dominant positive effect on water absorption and the degree of softening. The most pronounced negative effect on dough resistance exhibited DWG content (contribution of 57%). The combination of DWG with smaller particle size and 5% of gluten addition increased both resistance and extensibility, but R/E ratio was not affected. The optimal values for DWG granulation, DWG content, gluten content, ascorbic acid content and combined improver content were: < 150 μm, 14.24%, 4.83%, 0.004% and 0.1%, respectively, while predicted values for desired responsible variables were: water absorption 65.90%, degree of softening 84.05 FU, resistance 301.30 EU, extensibility 108.55 mm and R/E 2.5.

Keywords

Bread dough, defatted wheat germ, farinograph analysis, extensograph analysis

Date received: 3 December 2021; accepted 2 June 2022

INTRODUCTION

The aim of the wheat flour milling process is to obtain the best possible dissociation of the starchy endosperm from the other parts of the grain and achieve as high as possible flour extraction with the lowest contamination of bran and germ (Antoine et al., 2004). As a consequence, wheat germ alongside with the wheat bran represents the most important by-product of the wheat flour milling industry (Ghafoor et al., 2017). The germ is removed because the presence of germ adversely affects the keeping quality as well as the reprocessing quality of the flour (Ge et al., 2000). Generally, as a by-product, it is used in animal feed formulations (Shurpalekar & Haridas Rao, 1977); however the germ is the most nutritious part of the wheat kernel and has great potential as a valuable nutrient fortifier in food.

Germ is characterized by high protein content, mainly in the form of albumins and globulins (Miladi et al., 1972; Zhu et al., 2006). It is rich in 17 amino acids, especially the essential amino acids lysine, methionine, and threonine (Jensen & Martens, 1983). Wheat germ represents a rich source of minerals, dietary fiber, vitamins (Garcia et al., 1972; Sidhu et al., 2007) and particularly alpha and beta-tocopherols (Barnes, 1982; Engelsen & Hansen, 2009). It contains functional phytochemicals such as flavonoids,

Food Science and Technology International 0(0) 1–13

DOI: 10.1177/10820132221108708
journals.sagepub.com/home/fst
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sterols, octacosanols and glutathione (Nystrom et al., 2007), and has components with high antioxidant activity (Zhokhov et al., 2010; Zhu et al., 2011).

On the other hand, germ also has a considerable amount of fat characterized by high content of unsaturated fatty acids. The lipases and the lipoxygenases can hydrolyze lipids and initiate the oxidative rancidity process leading to the very short shelf life of raw germ (Sjovall et al., 2000), as well as flours containing germ particles (Galliard, 1986). Therefore, prior to being used as a food supplement germ is usually stabilized by inactivating the enzymes by heat treatment (Pinarli et al., 2004), or by removing the oil fraction from the germ by either mechanical pressing or solvent extraction (Karwowska & Kostrzewa, 1988). Pressing is usually preferred by consumers because wheat germ oil obtained this way is accepted as natural, although solvent extraction recovers more of the wheat germ oil (90%) than pressing (50%) (Dunford & Zhang, 2003). Defatted wheat germ (DWG) remains as a by-product from the germ oil removing process. It is rich in many nutritional ingredients and potentially it could be used as a valuable fortification substance (Duţă et al., 2018).

Because of wide acceptability, high consumption and low price wheat bread is an ideal supplementation vehicle for nutritional improvement (Tsen, 1980; Arshad et al., 2007). The addition of different quantities of wheat germ (raw or stabilized) at different particle sizes was studied for its effect on the rheological characteristics of dough and on the final product quality.

The results reported in literature considering the effect of germ addition on rheological properties of dough are partially contradictory probably due to the fact that different types of wheat germ samples were used (raw, heat stabilized, extruded, defatted). Srivastava et al. (2007) observed no variation after the addition of raw germ from 5 to 20%, while incorporation of heat treated germ caused increase in water absorption. Similar to this, Majzoobi et al. (2012a) and Gomez et al. (2012) reported the increase of water absorption following the increase of both raw and heat-treated germ while the increase being more pronounced with the addition of heat-treated germ. On the other hand, Ranga Rao et al. (1980) reported a decrease in this parameter after the germ addition but the decrease was minimal in the case of heat-treated germ. Majzoobi et al. (2012a) and Gomez et al. (2012) reported increase of dough development time and increase of softening values following the increase of both raw and heat-treated germ. On the other hand, Tsen (1980) and Srivastava et al. (2007) reported that germ supplements reduced developing time of wheat flour dough. The majority of the authors agree that germ addition reduces dough stability (Tsen, 1980; Srivastava et al., 2007; Majzoobi et al., 2012a; Gomez et al., 2012). Ge et al. (2001) studied the influence of adding different quantities of defatted germ flour on the stretch characteristics of noodles and determined that the maximum intensity, resistant intensity, extending nature and the proportional value of intensity/extend all increased after adding DWG flour.

In general, these studies showed that incorporation of wheat germ increases nutrition value, while at the same time dough rheological properties weakened. This is caused by the presence of glutathione in wheat germ and the gluten dilution following the higher germ content. Glutathione can reduce disulphide bonds in the gluten network and hence decrease the stability and strength of the dough resulting in reduction in bread quality (Shurpalekar & Haridas Rao, 1977; Al-Hooti et al., 2002; Majzoobi et al., 2012a; Bahal et al., 2013; Ma et al., 2014).

Some of the studies showed that it is possible to achieve acceptable product quality by appropriate formula modifications or process optimization (Gomez et al., 2012; Sidhu et al., 2001; Srivastava et al., 2007). It is reasonable to assume that the dilution of the gluten, coming as a result of higher germ content, can be alleviated by adding gluten to the formulation. Bahal et al. (2013) reported that the addition of gluten along with the lipoxygenase had a synergistic effect on bread quality. Also, there are reports in literature (Tsen et al., 1974; Tsen, 1975; 1980) that the addition of surfactants, such as sodiumstearoyl-2 lactylate, athoxylated mono-glycerides, sucrose monopalmitate and tallowate, can be used to strengthen dough structure and improve baking performance of wheat flour fortified with germ.

Design of experiments (DoE) is an important tool that can be applied for the determination of the effects of main process parameters and their interactions, as well as for optimization of the process conditions (Montgomery, 2001). Full 2^k factorial design includes screening of the changes in responses obtained in all possible combinations of input factors, where each of them takes values on two levels ("high" and "low"). But, if the number of input factors is large ($k \geq 4$), the required number of experiments might be unreasonable. Thus, suggestion is to use fractional factorial design which includes the performing of limited number of experiments.

The aim of this study was to optimize the formulation of wheat flour dough supplemented with DWG flour based on the selected empirical rheological properties. Fractional factorial design was used to investigate the effect of level of DWG flour addition, DWG flour particle size, content of gluten, ascorbic acid content and bread improver addition on the following properties of the dough: water absorption and the degree of softening, obtained by farinograph, and resistance to extension and extensibility as well as their ratio, obtained by extensograph analysis. Empirical rheological measurements such as farinograph and extensograph analysis were applied since they are suitable for the determination of the optimal flour quality for a particular purpose. Moreover, the aforementioned empirical techniques used for dough quality control are also recognized as standard methods by ICC, AACC, ISO, and different national standards (Dapčević Hadnađev et al., 2011). The pharinographic parameters water absorption and degree of softening represent reliable indicators of flour quality (protein quality and activity of proteolytic enzymes). In addition, these parameters are very important from the aspect of bakery products producers. The yield of the dough and the yield of the bread depend directly on the water absorption, while the degree of softening affects the way the dough is processed, and based on this parameter the behavior of the dough during the technological process can be predicted. Thus, these two parameters were selected as a responsive parameters obtained from farinograph analysis.On the other hand, regarding the selected extensographic parameters, the additives used in this experiment (gluten, ascorbic acid) have a direct and pronounced effect on the selected parameters of resistance to extension and extensibility, while insight into changes in other extensographic parameters can be easily estimated based on values of these two parameters. Based on the previous researches conducted by different authors and results obtained in their experiments, the influence of 10% and 20% of DWG (on WF replacement basis) on the rheological properties of dough was chosen for examination. Higher addition of DWG would be deleterious not only to loaf volume and texture of bread, but also to dough handling, making it very sticky (Sidhu et al., 2001; Al-Hooti et al., 2002). Furthermore, introduction of DWG can negatively affect rheological characteristics of dough due to the dilution of gluten proteins. Therefore, in order to minimize mentioned negative effect, the influence of gluten addition (5%) on the rheological properties of the dough was studied. In addition, the weakening effect of the glutathione present in DWG on the gluten matrix can be minimized by adding different oxidizing agents that can reinforce the gluten network by the formation of disulfide bonds (Elkasabany & Hoseney, 1980). Published results from many authors (Hruškova & Novotna, 2003; Baratto et al., 2016) showed that adding ascorbic acid in low quantities (at ppm level) can contribute to better dough rheology and bread characteristics. Improved bread quality was noticed in the research of Thuy & Phuong (2017) where even higher amount of ascorbic acid (0.01%) was used in the experiment. If the higher amount of added ascorbic acid is included in the dough formulation, because of a limited amount of oxygen in the dough system, only the part of ascorbic acid is utilized as an oxidizing agent. Ascorbic acid added in excess, remains in the dough, and can serve as a source of antioxidant vitamin for consumers of bread (Sidhu et al., 2001). Regarding this, a higher amount of ascorbic acid (0.004 and 0.008%) was used in this study. Considering that this experiment also included an addition of combined improver (0.1 or 0.3%) which, among other ingredients, usually contains ascorbic acid, addition of a higher amount was considered unnecessary.

MATERIALS AND METHODS

Materials

A typical commercial wheat flour (WF) having moisture 13.3%, protein 11.1%, starch 79.9%, sugar 3.1%, fat 1.1%, total dietary fibre 3.1% and ash 0.46% was supplied by AD Danubius. Defated wheat germ containing moisture 5.0%, protein 30.8%, starch 21.0%, sugar 14.1%, fat 10.6%, total dietary fibre 13.3% and ash 4.39% was obtained from Hochdorf Nutrifood AG, Switzerland. Powdered vital gluten was purchased from Fidelinka (Serbia) and ascorbic acid from Prolabo (United Kingdom). Bread improver consisting of wheat flour, ascorbic acid, stabilizer, enzyme, and emulsifier was purchased from Puratos (Belgium) while yeast and salt were purchased from the local market. The reason for using three different improvers was due to the expectation that the impact of DWG on the deterioration of dough properties will be to the large extent that the only way to achieve optimal values of dough rheological properties is to use a combination of different additives and study the effect of their interaction.

Chemical analyses

The moisture, ash, crude fat, starch, total dietary fibre content, reducing and total sugar content of DWG and wheat flour were determined according to methods described in AOAC (AOAC, 2000). The total nitrogen content was detected using the Kjeldahl method and the percentages of nitrogen were transformed into protein content by multiplying by a conversion factor of 5.7 (AOAC, 2000).

Preparation of the flour – DWG blends

Blends were prepared by replacing the part of WF with two types of DWG, different only in granulation: VIOGERM 1055 (< 150 μm) and VIOGERM 1080 (150–1000 μm), marked as fine fraction (F) and coarse fraction (C), respectively. Quantity of DWG used for the substitution of WF was either 10 or 20%. All other ingredients as gluten (0 or 5%), ascorbic acid (0.004 or 0.008%) and combined improver (0.1 or 0.3) were added on the basis of the WF and DWG blends. Each blend formulation was mixed using the F-6-RVC agitator (Forberg International AS, Oslo, Norway).

Empirical rheological measurements

The empirical rheological properties of dough samples were determined using Brabender farinograph (Brabender SEW, Duisberg, Germany) and extensograph (Brabender EXEK/7, Duisberg, Germany) according to AACC method 54-21 and 54-10.01, respectively (AACC, 2000). The parameters obtained from the corresponding curves were analyzed as follows: water absorption and degree of softening (farinographic data), resistance and extensibility of the dough (extensographic data). For every dough blend, three samples were prepared, and each of them is analyzed in replicate. This way, every dough blend was analyzed six times, after which the average result was calculated and used as response value.

Statistical analysis

In the frame of the analysis of rheological characteristic, the influences of five input factors (A-DWG granulation, B-DWG content, C-gluten content, D-ascorbic acid content, E-combined improver content) on four responses (R1-water absorption, R2-degree of softening, R3-resistance to extension and R4-extensibility) were studied (Table 1). Fractional factorial design 2_V^{5-1} was used, where 16 experiments (runs) were needed instead of 32 required for full factorial design. By this design a meaningful model was obtained, providing the estimation of significance of influence of all main effects and their two-factor interactions.

For calculation of statistical significance of the influence of input factors, analysis of variance (ANOVA) and residual analysis were used. Regression model was given by the following equation:

$$y = \beta_0 + \sum_{i=1}^{5} \beta_i x_i + \sum_{1 \le i \le 4,\ i<j \le 5} \beta_{ij} x_i x_j \ , \qquad (1)$$

where y represent a response, x_i and x_j are input factors in coded form and β_i, β_{ij} are unknown parameters for main factors and their interaction, respectively. The analysis of residuals is used to check the model validity. For graphical representation of significance and direction of single and combined factor influences, one factor graphs were used. Water absorption, degree of softening, resistance to extension, extensibility, and their (R/E) ratio were set as response variables in order to obtain optimal conditions for dough formulation. Optimal values were determined according to desirability functions (Myers et al., 2016):

$$0 \le d_i \le 1, \ i = 1, 2, \ \ldots, \ m, \qquad (2)$$

Table 1. Independent experimental factors and their levels.

Factor	Type	Low	High
A: DWG granulation†	Categoric	F	C
B: DWG content (%)	Numeric	10	20
C: Gluten content (%)	Numeric	0	5
D: Ascorbic acid content (%)	Numeric	0.004	0.008
E: Combined improver (%)	Numeric	0.1	0.3

†DWG granulation: fine fraction (F) – <150 μm, coarse fraction (C) – 150 – 1000 μm.

, which describe the set limits on outputs, and the goal is to maximize the overall desirability function:

$$D = \sqrt[m]{d_1 \cdot d_2 \cdots d_m}. \qquad (3)$$

When setting the output, it was possible to mark certain outputs as a priority. Afterward, the input parameters were set to best meet the first priority response, and later on the remaining responses.

All analysis was carried out using Design-Expert 11 (Design-Expert 11 (Stat-Ease, Minneapolis, MN, USA).

RESULTS AND DISCUSSION

A total of 16 experimental runs were determined by the Fractional factorial design 2_V^{5-1}. Recommended order of values of input factors which define dough formulations and the obtained dough properties, i.e. water absorption (R1), degree of softening (R2), resistance to extension (R3) and extensibility (R4), along with corresponding coefficients of determination (R^2) are presented in Table 2.

Regression plots obtained by experimental and predicted values were given in Figure 1.

Regression coefficients are represented in Table 3, where by star are denoted input factors and their interactions terms which expressed statistically significant influence on the observed responses, according to p values from ANOVA table ($p < 0.05$).

Farinographic data

Water absorption (r1). The influences of input factors with their contributions are presented in Table 3. It can be observed that gluten content, DWG granulation and DWG content had strong influence on dough water absorption (gluten being the most important). Although interactions of DWG and gluten content, DWG granulation and gluten content, as well as ascorbic acid and combined improver content had also statistically significant influence ($p < 0.05$), their contribution was rather moderate.

High value of coefficient of determination $R^2 = 0.9896$, represented in Table 2, suggests that proposed model adequately represents observed experimental data. Direction of the influence of main factors are illustrated by one factor graph, where the rest factors are set on the intermediate level and granulation is of type F (Figure 2). In order to reduce number of presented graphs in this paper, authors have decided to present only one factor graphs for granulation type F, in cases when statistically significant factors exhibited similar intensity and same trend of influence on the observed response for both DWG granulation type used. When statistically significant factors exhibited opposite intensity or different trend of influence on the observed response for different DWG granulation type, one factor graphs were presented for

Table 2. The fractional factorial design 2_V^{5-1} with natural values of input factors and obtained responses with corresponding coefficients of determination.

	Input factors					Responses			
Run	A (type)	B (%)	C (%)	D (%)	E (%)	R1 (%)	R2 (FU)	R3 (EU)	R4 (mm)
1	C	10	0	0	0.1	62.4	90	220	92
2	C	10	5	0	0.3	69.9	90	280	72
3	F	10	0	0.01	0.1	56.7	100	310	94
4	F	10	5	0.01	0.3	63.9	90	470	100
5	C	20	0	0.01	0.1	62.5	100	175	85
6	F	20	0	0	0.1	59.6	110	120	103
7	C	20	5	0	0.1	74.3	80	130	66
8	F	10	5	0	0.1	64.2	80	380	110
9	C	10	5	0.01	0.1	69.5	90	290	79
10	F	20	5	0.01	0.1	67.2	100	210	95
11	F	20	5	0	0.3	67.8	90	220	95
12	C	10	0	0.01	0.3	62.3	110	210	93
13	C	20	0	0	0.3	60.0	100	150	85
14	F	10	0	0	0.3	56.9	100	280	94
15	F	20	0	0.01	0.3	59.4	130	160	80
16	C	20	5	0.01	0.3	74.5	90	65	68
R^2 – coefficient of determination						0.9896	0.9840	0.9785	0.9635

both types of granulation. For the following response it is apparent that three main factors A, B and C had most pronounced influence on dough water absorption (increase of B and C caused the increase of the response).

With increase of gluten concentration from 0 to 5%, water absorption increased (Figure 2(c)). This is rather expected due to gluten capability to bind water. These results were in accordance to MacRitchie (1984) who reported that with greater protein content, the flour absorbs more water. Also, with higher wheat germ content water absorption increased (Figure 2(b)), which could be explained by high content of proteins and fibers in wheat germ and their capability to bind water (from 146 to 229%, as reported by Vani and Zayas (1995) and Ge et al. (2000). Even though, the similar was reported by Qarooni (1996) where substituting of raw wheat germ (1–3%) with flour increased water absorption of the flour, obtained results were rather unexpected. Substitution of the WF with the same amount of DWG in the dough formulations should lead to gluten dilution and hence, decreasing the water absorption of the dough. According to Pomeranz (1988) gluten can bind water up to 60% of its weight, while hydrocolloids, sugars, albumins and globulins from wheat germ can absorb less amounts of water (Majzoobi et al., 2012a). Moreover, the remaining glutathione in the DWG can degrade the gluten network reducing its water absorption ability (Srivastava et al., 2007). Furthermore, results showed that addition of germ with larger particle size (fraction C) gave dough with higher water absorption ability when compared with addition of germ with smaller particle size (fraction F) (Figure 2(a)). This is in compliance with work of the other authors (Petrović et al., 2015) who determined that the use of larger wheat germ particles in the blends formulation requires larger quantity of water for obtaining the optimal consistency of dough.

Degree of softening (r2). Every linear term of observed factors expressed statistically significant influence on the degree of softening ($p < 0.05$) (Table 3). As in the case of water absorption, the most dominant influence had gluten content, which contributed with 45%. Dough with higher quantity of gluten had denser and stronger network, which had capability to sustain long mechanical processing treatments (Dapčević Hadnađev et al., 2011). Furthermore, the interaction of DWG granulation and DWG content showed the most significant contribution to the changing of analyzed parameter. High value of coefficient of determination $R^2 = 0.9840$, represented in Table 2, suggests that proposed model adequately represents observed experimental data.

The content of gluten manifested a strong negative influence on the degree of softening (Figure 3(d)), while the influence of ascorbic acid content and combined improver addition was positive (Figure 3(e) and f, respectively). When observing DWG content, its influence was both positive and negative, depending on the DWG granulation. As presented in Figure 3(a), the addition of DWG with a smaller particle size (fraction F) gave a higher degree of softening of the dough in comparison when DWG with larger particle size (fraction C) was added. Moreover, a higher amount of DWG with smaller particle sizes negatively influenced the dough structure increasing its degree of softening (Figure 3(b)). On the contrary, with the

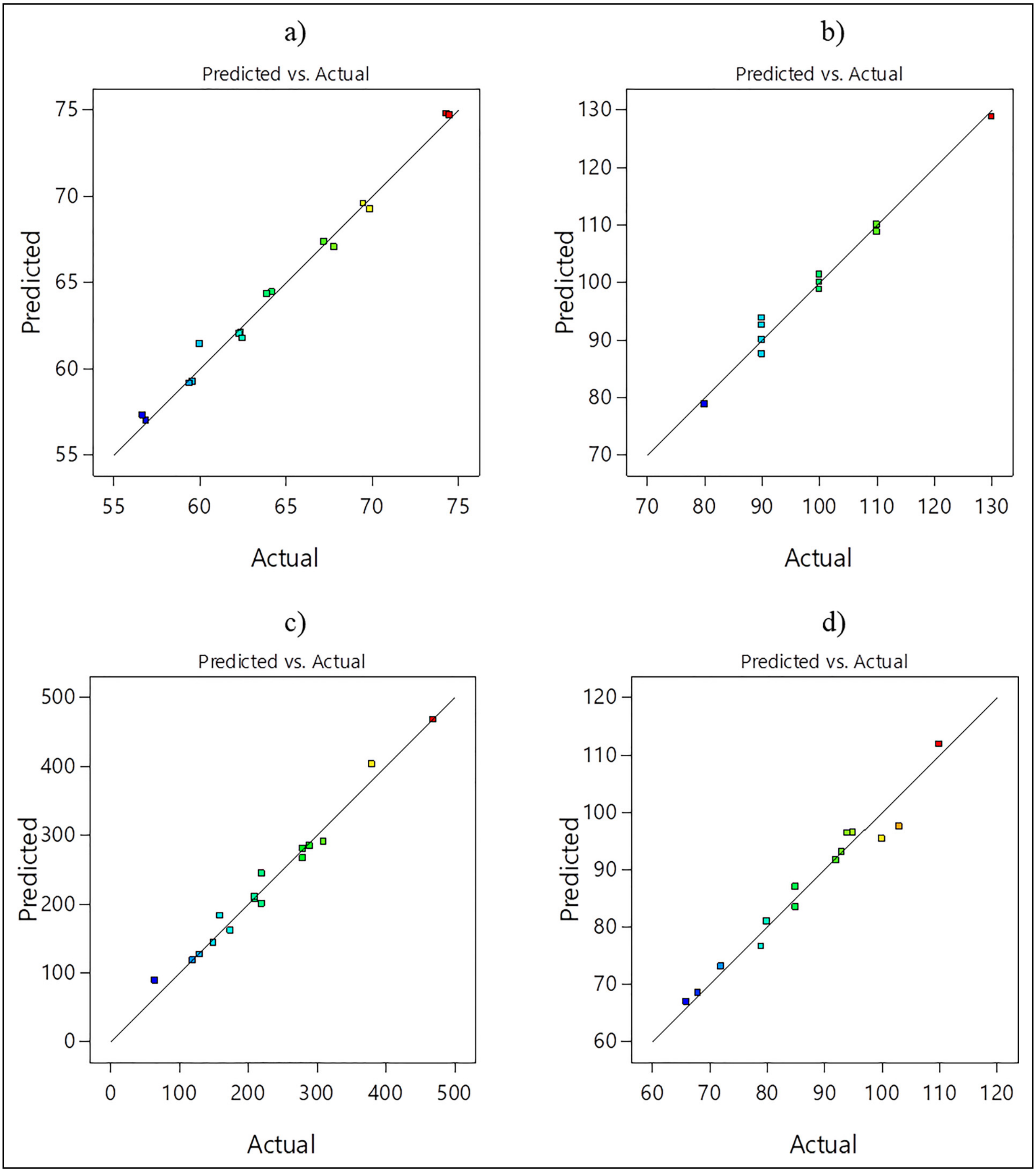

Figure 1. Comparison of experimentally obtained responses and predicted values for the following responses a) Water absorption, b) Degree of softening c) Resistance to extension d) Extensibility.

addition of the higher amount of DWG with larger particle sizes, the dough degree of softening was slightly decreased (Figure 3(c)). This effect can be noticed also in the interaction of input factors A and B (Figure 3(g)). This may occur due to a possible higher amount of damaged starch granules present in the DWG of smaller granulation (fraction F). Petrović et al. (2015) reported that the content of total non-fiber carbohydrates in the DWG fractions VIOGERM 1055 (which corresponds to fraction F) and VIOGERM 1080 (which corresponds to fraction C) was 36.3 and 33.7%, respectively. Also, according to the analysis conducted in this research, starch content in the

Table 3. Input parameters p-values and contributions for all responses.

Response	R1		R2		R3		R4	
	p-value	contribution (%)	p-value	Contribution (%)		Contribution (%)	p-value	Contribution (%)
Model	< 0.0001*	–	0.0001*	–	0.0015*	–	0.0002*	–
A	< 0.0001*	20.9186	0.0025*	6.66667	0.0018*	15.5288	< 0.0001*	45.3239
B	0.0006*	5.04686	0.0025*	6.66667	< 0.0001*	57.2831	0.0048*	8.58094
C	< 0.0001*	67.8522	<0.0001*	45.0667	0.0103*	6.90168	0.0224*	4.43969
D	0.7955	0.0107507	0.0004*	13.0667	0.3423	0.47341	0.1458	1.39714
E	0.6267	0.0383575	0.0025*	6.66667	1	0	0.0338*	3.61567
AB	–	–	0.0004*	13.0667	0.0629	2.44532	–	–
AC	0.0164*	1.46329	0.024*	2.4	0.0125*	6.26003	0.0002*	25.8854
AD	–	–	–	–	0.1298	1.41242	0.0183*	4.8834
AE	–	–	–	–	0.09	1.89366	0.078	2.22117
BC	0.0058*	2.27769	0.024*	2.4	0.0152*	5.64967	–	–
BD	–	–	–	–	–	–	–	–
BE	–	–	–	–	–	–	–	–
CD	–	–	–	–	–	–	–	–
CE	–	–	0.024*	2.4	–	–	–	–
DE	0.0194*	1.35393	–	–	–	–	–	–

*Statisticaly significant at $p < 0,05$.

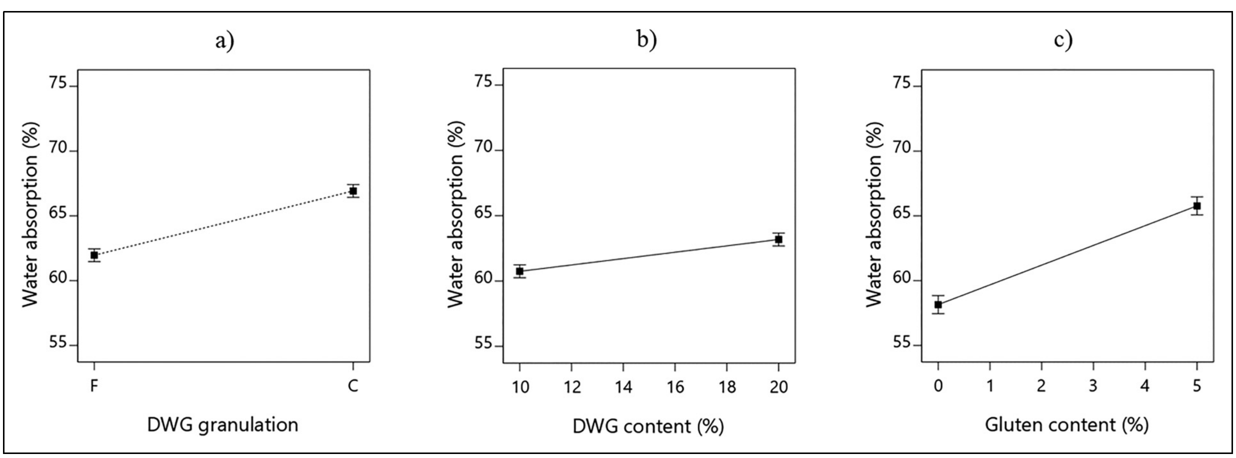

Figure 2. The influence of a) DWG granulation, b) DWG content and c) gluten content on dough water absorption.

DWG was 21%. Thus, it may be expected that both fractions of DWG contain damaged starch and that a higher amount of damaged starch is present in DWG of smaller granulation (fraction F). Such increase in the content of the damaged starch can prolong the development time of the dough. Hence, the weaker gluten network is formed and such dough is not capable to sustain intensive mechanical processing treatments (Dapčević Hadnađev et al., 2011).

With the addition of ascorbic acid degree of softening significantly increased (Figure 3(e)). This was unexpected, since ascorbic acid represents oxidizing agent, reinforcing the gluten network by the formation of disulfide bonds (Baratto et al., 2016). Increasing of the degree of softening was also noticed when combined improver was added (Figure 3(f)). This trend probably occurs as a consequence of the composition of combined improver itself, which may contain monoglyceride and diglyceride fatty acid. Lipids

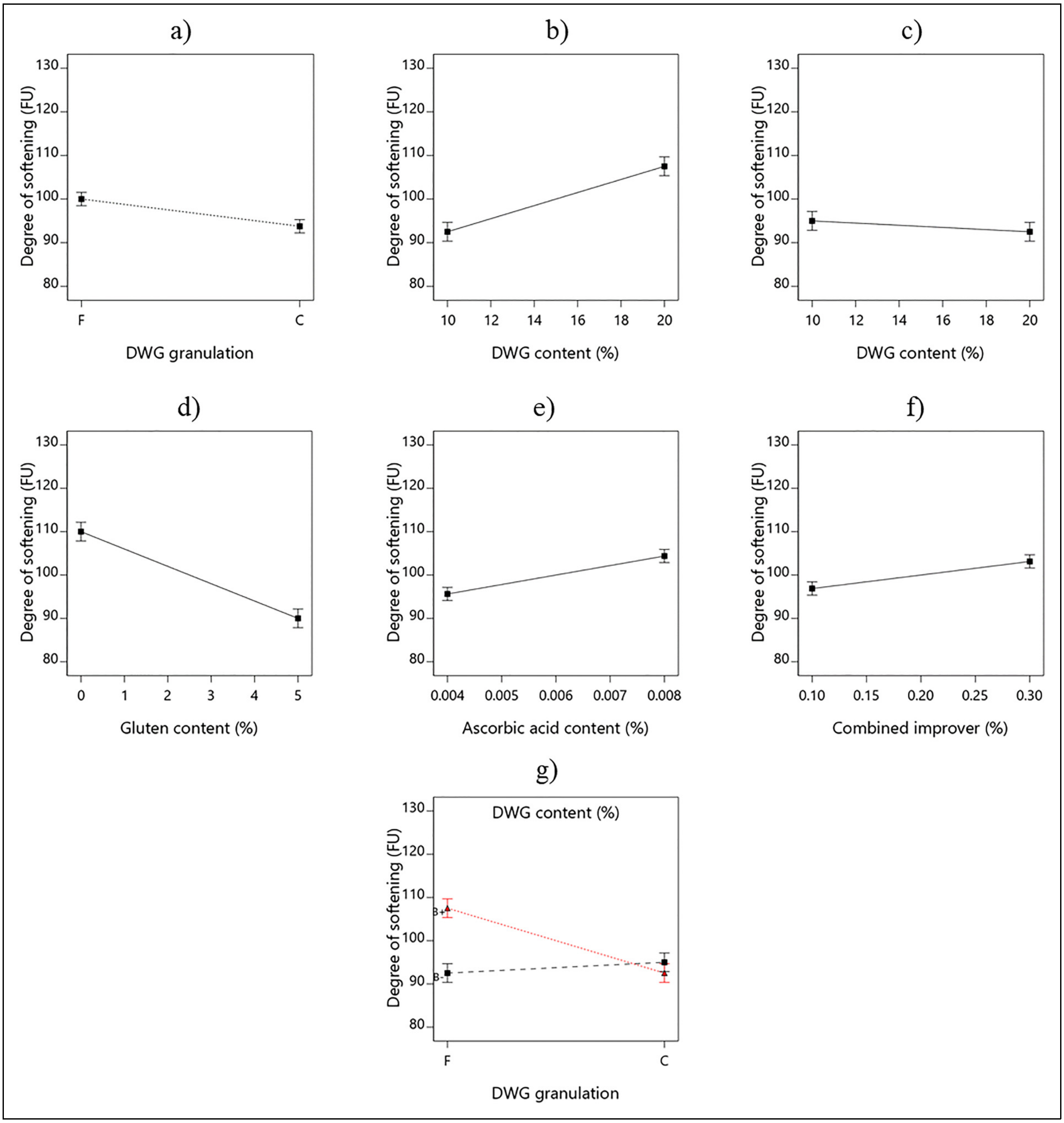

Figure 3. The influence of a) DWG granulation, b) DWG content (granulation type f), c) DWG content (granulation type c), d) gluten content, e) ascorbic acid content, f) combined improver content and g) interaction of DWG granulation and DWG content on dough degree of softening.

can interact with gluten and can soften the dough (Pomeranz, 1988).

Extensographic data

Resistance to extension (r3). The influence of input factors on resistance to extension of dough is presented in Table 3. DWG content was proved to be the most dominant input factor, followed by DWG granulation and gluten content. Moreover, interactions of DWG granulation and gluten content, as well as DWG and gluten content had a significant influence ($p < 0.05$) on analyzed parameter. High value of coefficient of determination $R^2 = 0.9785$, represented in Table 2, suggests that proposed model adequately represents observed experimental data.

From one factor graph (Figure 4(b)) it can be seen that DWG content had negative influence on resistance to extension. Decrease of resistance is associated with greater

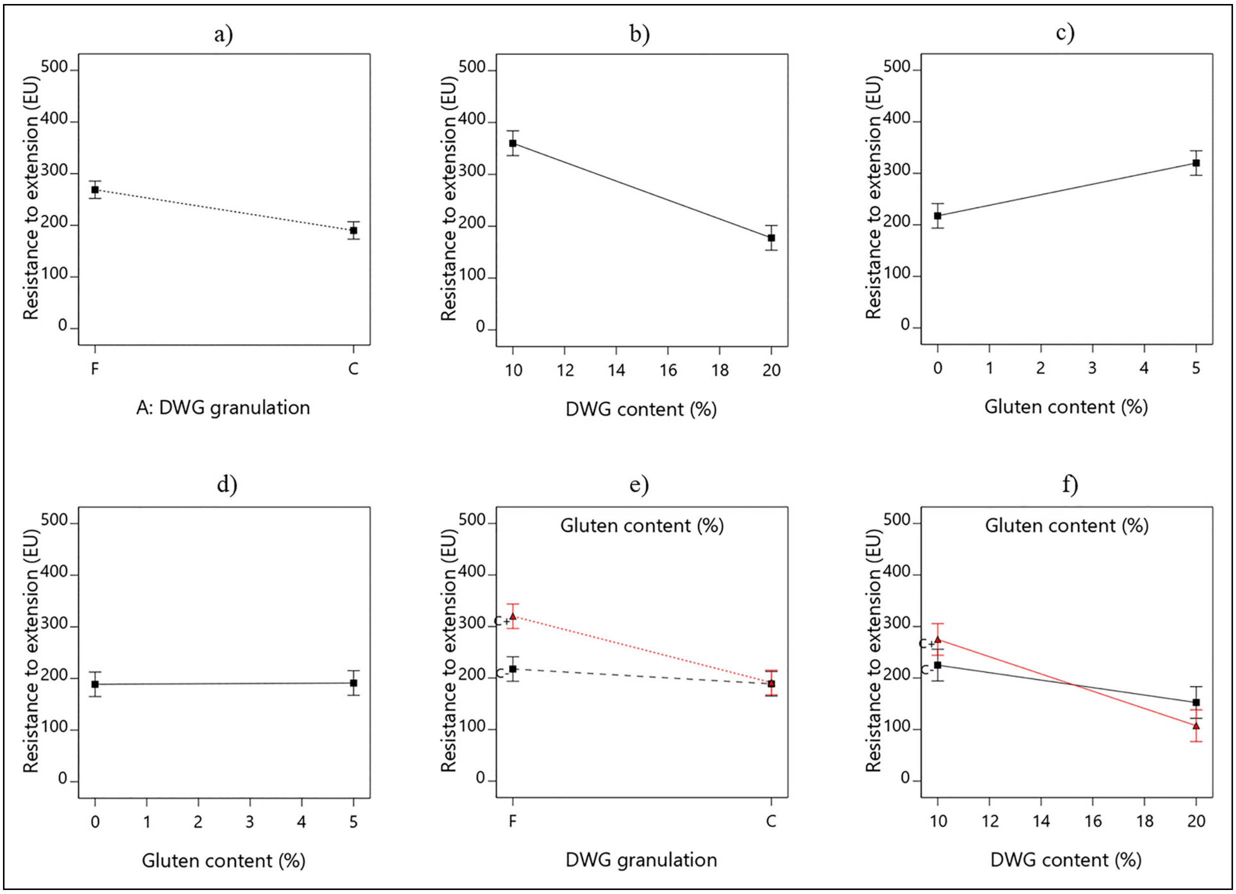

Figure 4. The influence of a) DWG granulation, b) DWG content, c) gluten content (granulation type f), d) gluten content (granulation type c) e) interaction of DWG granulation and gluten content and f) interaction of DWG content and gluten content on dough resistance to extension.

dilution of the gluten matrix when a large amount of DWG is added, which weakens the dough and makes it less resistant to extension (Gomez et al., 2012). The DWG granulation also significantly contributed to the changing of dough resistance (Table 3). Significantly lower value for this response was obtained when germ with larger particle size (fraction C) was added (Figure 4(a)). Decrease in this parameter with an increase of germ particle size was also reported in the work of Petrović et al. (2015). This was explained in the report of Zucco et al. (2011) who determined that differences in particle size have impact on the forming of chemical bonds between constituents of dough, mostly proteins and starch. Majzoobi et al. (2012b) similarly concluded that large particles of the dough form larger air bubbles in the dough structure during mixing, which can impact the forming of the chemical bonds between dough constituents.

The same explains why the addition of gluten only had a positive influence on dough resistance when germ with smaller particle size was added (Figure 4(c)). When dough was constituted of the germ with larger particle size, one factor graph showed that dough resistance to extension remains almost unchanged, although gluten is added (Figure 4(d)). Furthermore, interaction of DWG granulation and gluten content also moderately contributed to changes in dough resistance (Table 3). From Figure 4(e) it can be observed that addition of gluten increased investigated response only when combined with DWG granulation type F. Gluten addition in combination with higher DWG content decreased the resistance of dough (Figure 4(f)), which was rather unexpected, since the gluten was added in order to minimize the negative effects of DWG addition on the rheological properties.

Extensibility (r4). The influences of input factors with their contributions to the extensibility of the dough are presented in Table 3. Every linear term of observed factors except ascorbic acid expressed a statistically significant influence on dough extensibility. As opposite to linear term of ascorbic acid, the interaction of ascorbic acid and DWG granulation managed to have a significant influence on the analyzed parameter, as well as the interaction of DWG granulation and gluten. DWG granulation had the

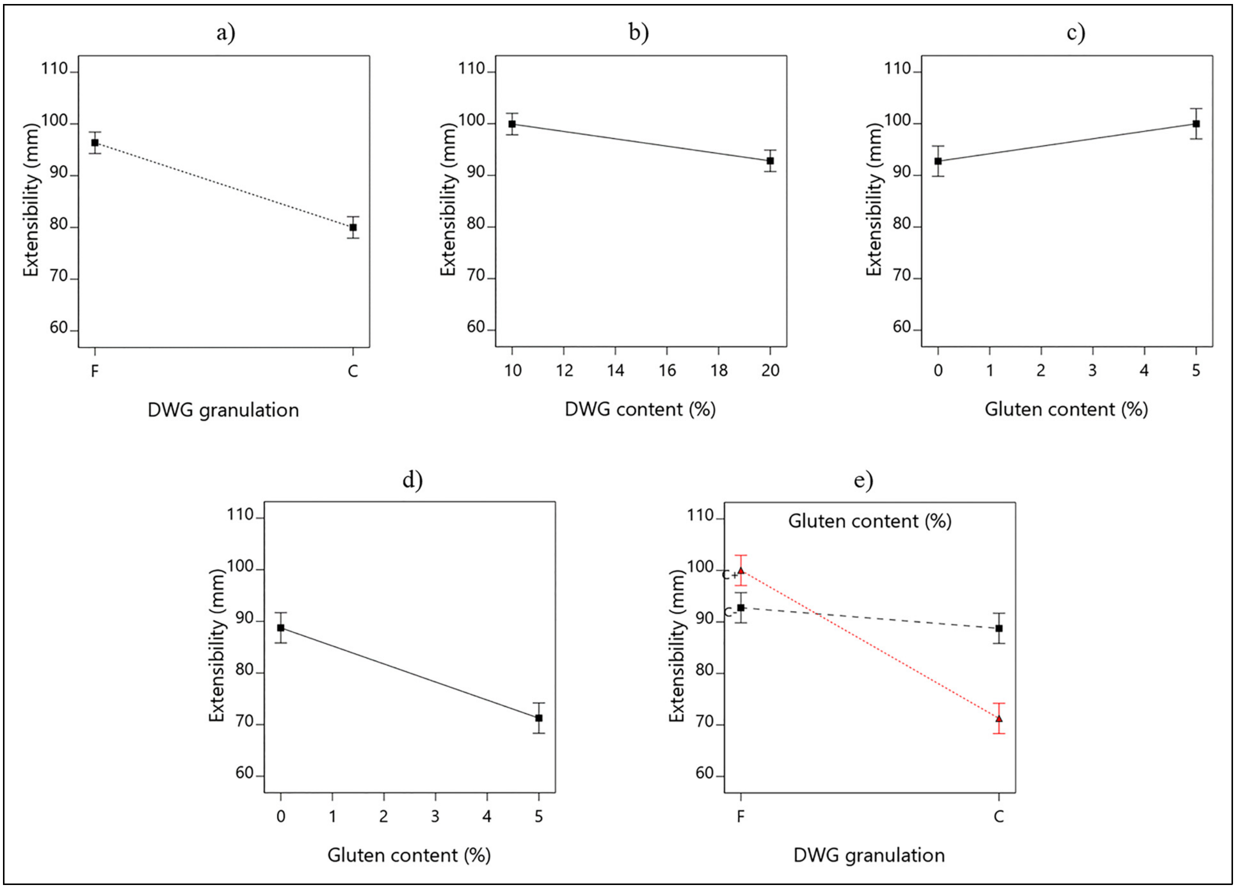

Figure 5. The influence of a) DWG granulation, b) DWG content, c) gluten content (granulation type f), d) gluten content (granulation type c) and e) interaction of DWG granulation and gluten content on dough extensibility.

most dominant contribution (45%), followed by interaction of DWG granulation and gluten content, while others had rather moderate contributions. High value of coefficient of determination $R^2 = 0.9635$, represented in Table 2, suggests that proposed model adequately represents observed experimental data.

Direction of influence of main factors and their interactions on dough extensibility is depicted by one factor graphs (Figure 5). As can be seen, DWG granulation, DWG content and gluten addition influenced this parameter exactly in the same manner as above described effects on resistance. As expected, DWG content negatively affected the dough extensibility (Figure 5(b)). Furthermore, the extensibility of the dough was far higher when DWG granulation type F was added (Figure 5(a)). The addition of gluten increased extensibility of the dough when germ with smaller particle size (fraction F) was added (Figure 5(c)), while opposite effect occurred in the case of larger fraction (fraction C) of wheat germ (Figure 5(d)). Also, interaction of the DWG granulation and gluten content expressed high contribution on extensibility, which can be observed from Table 6 and from two-factor interaction graph of parameters A and C (Figure 5(e)). It is evident that the combination of the coarse fraction of DWG with gluten addition led to notably lower dough extensibility value. This might happen as a consequence of the presence of larger particles of DWG that are incorporated in the dough, which obstructed the forming of the strong protein matrix during mixing. Therefore, addition of the gluten should be considered when a coarse fraction of DWG is used for mixing of the dough, since it has not useful impact on the dough structure and it is rather economically unjustified.

Optimization

Given the complexity of the dough as a rheological system and the occurrence of changes in dough behavior due to changing of dough formulation, great care must be taken when considering the introduction of additional materials in dough composition. Not only is the analysis of influences that the additional material has on the rheological properties of the dough needed, but also finding the compromise between the simultaneously occurred positive and negative effects is necessary. Thus, models obtained in this work were used to perform the optimization in order to find optimal of dough formulation (DWG granulation,

Table 4. Optimization of bread dough formulation: optimal values of independent variables, predicted and validated values of targeted responses.

OPTIMIZATION CONDITIONS					PREDICTED VS VALIDATED RESPONSES	
variables and responses	goal	lower	upper	importance	predicted	validated
DWG granulation†	is in range	F	C	3	F	F
DWG content (%)	is in range	10	20	3	14.24	14.24
Gluten content (%)	is in range	0	5	3	4.83	4.83
Ascorbic acid content (%)	is in range	0.004	0.008	3	0.004	0.004
Combined improver content (%)	is in range	0.1	0.3	3	0.1	0.1
Water absorption (%)	minimize	56.7	74.5	1	65. 90	66.2
Degree of softening (FU)	minimize	80	130	2	84.05	80
Resistance to extension (EU)	is in range	300	470	4	301.30	305
Extensibility (mm)	maximize	66	110	4	108.55	115
R/E	is in range	2	2.5	5	2.5	2.65

†DWG granulation: fine fraction (F) – <150 μm, coarse fraction (C) – 150 – 1000 μm.

DWG content, gluten content, ascorbic acid content, and combined improver content) which would provide targeted levels for responses (water absorption, degree of softening, resistance to extension, and extensibility). In addition to the examined responses (particularly resistance and extensibility), resistance to extensibility ratio (R/E) was calculated and was considered when optimization process was performed, due to its importance in the assessment and predicting of the quality of bread as final product.

The extensibility represents important property of the wheat dough, which is heavily dependent on the gluten quality and allows obtaining characteristic structure and volume of the baked products. In addition, the interrelation between the resistance to extension and extensibility is indirectly responsible for the extent of the expansion during the fermentation process, thus influencing the baking performance and final product quality (Dapčević Hadnađev et al., 2011). Therefore, these responsive variables were given high value of coefficient of importance, while the same coefficient for the ratio of resistance to extensibility was set at maximum value. The pharinographic parameters water absorption and degree of softening represent reliable indicators of flour quality. Water absorption influence the yield of the final bakery product as well, while degree of softening affects the way the dough is processed. However, as obtained values for both parameters were in the appropriate range for the production of dough of satisfactory quality, these responsive variables were given minimum coefficients for importance.

The optimized input values within the investigated experimental domain and predicted, as well as validated values of investigated responses are presented in Table 4. The desirability function for specified combination was 0.86.

Conclusion

The results of this paper showed that the influence of replacing a part of wheat flour with DWG in the bread dough strongly depends on the characteristics of DWG itself. The significance of DWG content and granulation as well as their interaction was evident when considering the influence on farinographic indicators of flour-DWG blend quality. These two investigated variables had the most pronounced effect on the extensographic parameters. It is concluded that the addition of DWG leads to the weakening of the gluten network responsible for the maintenance of optimal extensibility and resistance of the dough. Furthermore, gluten addition highly contributed to water absorption increment and the reduction of the degree of softening, while also exhibited a positive effect on dough resistance and extensibility. The aforementioned fully justified the addition of gluten in such dough. However, the effect of gluten addition also showed dependence on DWG granulation used, so this interaction must necessarily be considered. Optimal formulation of bread dough with DWG was: DWG granulation type F, DWG content 14.24%, gluten content 4.83%, ascorbic acid content 0.004% and combined improver content 0.1%. Predicted values for desired physical and rheological properties of dough were: water absorption 65.90%, degree of softening 84.05 FU, resistance to extension 301.30 EU, extensibility 108.55 mm and R/E 2.5, while validated responses were: water absorption 66.2%, degree of softening 80 FU, resistance to extension 305 EU, extensibility 115 mm and R/E 2.65. Further research should be conducted to assess the effects on quality, nutritional profile and sensory analysis of bread obtained from dough with DWG as well as the consumer's acceptability of such product.

ACKNOWLEDGEMENTS

This research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. 451-03-68/2022-14/200134).

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the This research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, (grant number (Project No. 451-03-68/2022-14/200134)).

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