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ORIGINAL ARTICLE

Optimization of additive content and their combination to improve the quality of pure barley bread

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AQ1 **Abstract** The objective of this study was to model the influence of pregelatinized OSA starch (OSA), wheat gluten (Gl) and xylanase (Xyl) on breadmaking potential of barley flour by using response surface methodology. Addition of these ingredients had significant effect on specific bread volume, crust and crumb lightness, crumb texture, average cell size and crumb density. OSA showed the most pronounced effect on specific bread volume, average cell size, AQ2 crumb density and hardness. Interaction between OSA and Gl, as well as Gl and Xyl affected the specific bread volume increase and crumb chewiness decrease, while the interaction between OSA and Xyl affected the specific volume decrease and crumb chewiness increase. An optimal barley bread formulation appeared to be the one containing 9.68% OSA, 2.0% Gl and 4.51 g/100 kg Xyl. This optimal barley bread formulation predicted an increment of 14–28% bread volume and a decrease of 105–217% crumb chewiness in comparison to formulations containing medium amounts of AQ3 single improvers (1% Gl, 5% OSA, 2.5 g/100 kg Xyl).

Keywords Barley bread · Bread-making · Gluten · OSA starch · Xylanase

Introduction

Although barley (*Hordeum vulgare* L.) has been used in human nutrition from ancient time, its potential to be used in the creation of novel barley-based foodstuff has not yet been sufficiently explored (Sullivan et al. 2013; Newman and Newman 2008). The renewed consumers' interest in the consumption of barley-based food is associated with valuable nutritional profile of barley and a number of associated health benefits (Rahaie et al. 2014; Bird et al. 2008; Li et al. 2003). Barley flour is not appropriate for the production of leavened bread due to its weak dough viscoelasticity and gas retention capability (Collar and Angioloni 2014; Newman and Newman 2008). Therefore, the formulation of composite wheat-barley bread has been more common than pure barley bread (Collar and Angioloni 2014; Rieder et al. 2012; Sullivan et al. 2010, 2011; Baik and Ullrich 2008; Trogh et al. 2004; Gill et al. 2002).

Cereal scientists have been facing the challenge to formulate non-wheat breads of such sensory attributes that will correspond to those of white wheat bread to the greatest possible extent. Long-established fact that maximal amount of barley flour up 20% in combination with wheat flour does not have detrimental effect on the quality of yeast bread is valid more than 80 years. Therefore, recent research in the field has been focused on the improvement of barley bread quality (Newman and Newman 2008). Supplementation of wheat by barley flour (in most cases up to 30%) is primarily performed to enhance the nutritional profile of baked products, but in that case the decrease of bread volume and the increase of crumb firmness and cohesiveness were observed (Rieder et al. 2012; Sullivan et al. 2010; Gill et al. 2002). Pure barley bread is largely produced as traditional products in some parts of the world. According to recent scientific literature only Kinner et al. (2011) reported the formulation of pure barley bread optimized with the addition of malt flour, pre-gelatinized flour and acidifier.

The objective of this study was to optimize the formulation of pure barley bread of satisfying appearance, textural and structural attributes by application of selected

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improvers: pre-gelatinized starch sodium octenyl succinate (OSA), wheat gluten (Gl) and xylanase (Xyl).

Materials and methods

Materials

Hull-less barley was purchased from agricultural cooperative Agrodunav, Karavukovo, Serbia and milled by a laboratory roller mill MLU 202 (Bühler, Switzerland) using a procedure previously reported by Andersson et al. (2003) without preconditioning. Six flour mill streams were obtained (three break and three reduction flour streams) and mixed to obtain straight-run white barley flour. The selected improvers were: OSA starch C*EmTex 12688 (pre-gelatinized starch sodium octenyl succinate (Cargill, France), wheat gluten (Fidelinka, Serbia) and xylanase VERON 191 S (AB Enzymes, Germany). Other ingredients used for experimental baking (salt, sucrose and fresh yeast) were purchased from local store.

Breadmaking procedure

Barley bread doughs were prepared by mixing all ingredients in a 300-g Farinograph (Brabender, Germany) bowl until the consistency of 800 ± 10 BU was reached plus 1 min. The content of OSA, Gl and Xyl was assessed according to a Box–Behnken experimental design, while the content of salt, yeast and sucrose was kept constant at 2.5, 3.0 and 3.0%, respectively. The content of all ingredients was expressed in % on flour weight basis. After mixing, the dough was subjected to bulk fermentation (30 °C, 80% RH, 45 min) for first proving. After that, the dough was divided into two dough pieces (150 g), rounded, shaped into loaves, placed in pans (L × W × H:95 × 65 × 50 mm) and proofed (30 °C, 85% RH) until the optimal volume was reached. The dough was baked using a MIWE deck baking oven (MIWE Condo, Germany) at 220 °C until the mass loss of 8% was reached. The baked bread was cooled for 3 h at ambient temperature and bread volume was measured. Bread was sealed into plastic bags and stored at 23 °C for 24 h for further analysis.

Determination of bread volume

The specific bread volume (ml/g) was determined by laser-based VolScan Profiler (Stable Micro Systems, UK) and expressed as average value of four loaves.

Crumb texture

Bread loaves were sliced by electric slicer KRUPS 372-75 (KRUPS International) to 12.5 mm-thick slices. The round crumb pieces (R = 45 mm) were taken from the central slices of the loaf 24 h after baking for the texture profile analysis (TPA) using a Texture Analyser TA-XT2i (Stable Micro Systems, UK) and a 75 mm diameter aluminium probe. Crumb pieces were double compressed at 5 mm/s to 40% of its thickness. Textural properties recorded in eight replicates were: hardness, cohesiveness, springiness, chewiness and resilience.

Digital image analysis

Central slices of each loaf were scanned by a flatbed scanner CanoScanLiDE 100 (Canon, Japan) at 300 DPI and software MP Navigator EX, with default settings for brightness and contrast. The scans were saved in tiff format and cropped by ImageJ software (National Institutes Health, Bethesda, MD) to obtain 45 × 45 mm scan which were converted to an 8-bit greyscale images. The differentiation of gas cells and non-cells was carried out by means of the Otsu algorithm (Gonzales-Barron and Butler 2006). The obtained measurements included: the number of cells per cm^2 (cell density), mean cell area (cell size) and cell-to-total area ratio. Any form larger than 0.1 mm^2 was considered to be crumb cell.

Bread lightness

Crumb and crust lightness was measured by the colorimeter Minolta Chroma Meter (CR-400) (Konica Minolta Sensing Inc., Japan) with standard illuminant D65. The results for lightness were interpreted as follows: $L^* = 0$ for black and $L^* = 100$ for white The crumb lightness was recorded in five, while the crust lightness in 15 replicates per loaf on the central slices 24 h after baking.

Experimental design

Response surface methodology (RSM) using a three-level three-factor Box–Behnken experimental design was set up in a way to evaluate the effects of OSA starch (A), wheat gluten (B) and xylanase (C) on the selected responses (specific bread volume, Y1; hardness, Y2; springiness, Y3; cohesiveness, Y4; chewiness, Y5; resilience, Y6; cell density, Y7; cell size, Y8; cell-to-total area ratio, Y9; crumb and crust lightness, Y10 and Y11 and to determine their optimal levels to obtain barley bread of satisfactory textural and structural properties. A total of 17 experiments were designed based on the variation of the factors at three levels (Table 1). The central points corresponded to the factors at level 0. Each experiment was performed in duplicate and the average value was taken as the response (Y).

The obtained responses were subjected to multiple regression analysis to find out the relationship between the

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | LE | TYPESET
MS Code : JFST-D-15-01858 | CP | DISK

Table 1 Experimental factors and their levels in Box Behnken experimental design

Factor	Low value (+1)	Central value (0)	High value (−1)
OSA starch content (%)	0.0	5.0	10.0
Gluten content (%)	0.0	1.0	2.0
Xylanase content (g/100 kg)	0.0	2.5	5.0

factors used and the responses obtained. To obtain the regression equations, linear and second order polynomials were fitted to the experimental data. The mathematical relationship between the responses and studied factors (n = 3) was described by a second-degree polynomial (quadratic) model:

$$Y = b + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii}^2 X_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} a_{ij} X_i X_j$$

where Y is the modelled response for obtained response, b_0 is an intercept; X_i is the factor and a_i is the corresponding coefficient; X_{ii} is the quadratic factor; a_{ii} is the quadratic coefficient; X_{ij} is the two-factor interaction; and a_{ij} is the two-factor interaction coefficient. The analysis of variance (ANOVA) was used to determine the statistical significance of the terms in the regression equation for each response. The models performance was evaluated on the basis of coefficient of determination (R^2) and model *p* value.

Desirability function methodology was used for the simultaneous optimization of selected factors in order to achieve the optimal formulation of barley bread (Pourfarzad et al. 2014). The optimization criteria and goals were set to:

- Maximize the specific volume of bread (importance 4), the cohesiveness (importance 3), average cell size (importance 4), the crumb lightness (importance 2) and
- Minimize the hardness of bread crumb (importance 5), the chewiness (importance 5), cell density (importance 4), the crust lightness (importance 2)

The optimization criteria for all factors were selected in the range.

Results and discussion

The selection of factors and responses for experimental design

Since non-wheat cereals yield bread of poor quality, non-wheat bread is commonly formulated with ingredients able to improve the viscoelastic properties of dough and the overall bread quality (e.g. proteins, hydrocolloids, emulsifiers, etc.). Ingredients for this study were selected on the basis of preliminary baking trials and literature data. OSA starch (OSA) was selected due to its ability to increase the dough extensibility, improve the specific bread volume, decrease crumb hardness and modify the crumb structure—pore distribution and size (Dapčević Hadnađev et al. 2014a, b). Due to the fact that barley proteins lack the ability to form the structural network, the building of an elastic, extensible matrix for formation of bread structure was enabled by selection of gluten (Gl) as one of the ingredients. Supplementation with xylanase (Xyl) proved to be a good choice for the formulation of palatable breads with increased levels of dietary fibers (Newman and Newman 2008). The layout of performed Box–Behnken experimental design is given in Table 2.

All breads formulated by given design were characterized in terms of specific volume, textural and structural properties and lightness (Table 2). The effects of factors on selected responses were investigated and main effects and interaction effects plots are presented and interpreted (Figs. 1, 2, 3, 4). Regression equations obtained for selected responses are shown in the form of coded variables in Table 3.

Effect on selected ingredients on specific volume of barley bread

Specific volume of bread varied in the range between 1.35 and 1.80 ml/g depending on the factor combination (Table 2). The increasing content of OSA (A), Gl (B) and Xyl (C) as well as interaction between OSA and Gl (AB) affected the increase in specific bread volume, while the interaction between OSA and Xyl affected the opposite effect (Table 3). The obtained model was significant ($p < 0.0001$) with significant model terms A, AB and AC determined on the basis of calculated *p* values (data not shown). The predicted R^2 was in reasonable agreement with the R^2 adjusted (Table 3). The effects of A, AB and AC is shown by main and interaction effects plots in Fig. 1a–c.

It was found that OSA concentration affected specific bread volume more than any other parameters. The obtained results confirmed our previous results obtained for white bread and gluten-free bread (Dapčević Hadnađev et al. 2014a, b). The positive impact of OSA on the specific volume of barley bread (Fig. 1a) can be primarily attributed to the morphology of OSA starch granules which were disrupted by drum drying pre-gelatinization and its greater

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | LE | TYPESET
MS Code : JFST-D-15-01858 | CP | DISK

Table 2 Box–Behnken experimental design for the optimization of barley bread formulation and responses obtained

Formulation	Factors			Responses									
	OSA (%)	Gl (%)	Xyl (g/100 kg)	SV (ml/g)	H (g)	Spr	Coh	Chew (g)	Res	Cells/cm^2	MCA (mm^2)	*L** crust	*L** crumb
1	10	2	2.50	1.80	3748	1.01	0.64	2426	0.43	57	0.44	46.79	69.94
2	0	1	5	1.48	8606	0.93	0.66	5302	0.38	105	0.30	55.71	72.93
3	0	0	2.5	1.50	8372	0.92	0.66	5094	0.39	120	0.27	57.38	73.25
4	5	2	5	1.71	5183	0.94	0.66	3229	0.39	77	0.41	50.87	71.20
5	0	1	0	1.39	10,008	0.93	0.70	6453	0.42	89	0.36	56.05	74.03
6	10	0	2.5	1.70	4252	0.97	0.63	2572	0.37	72	0.44	45.36	71.80
7	5	1	2.5	1.61	6869	0.90	0.65	4008	0.39	75	0.40	53.00	72.41
8	5	1	2.5	1.62	6604	0.92	0.65	3985	0.39	85	0.34	51.99	72.43
9	5	1	2.5	1.63	6422	0.91	0.65	3783	0.38	86	0.32	49.17	71.85
10	5	1	2.5	1.57	7255	0.93	0.65	4404	0.39	84	0.30	51.45	72.42
11	0	2	2.5	1.35	11,716	0.90	0.69	7265	0.40	115	0.26	56.29	74.24
12	5	0	0	1.56	6824	0.94	0.65	4178	0.39	108	0.27	51.37	72.66
13	5	0	5	1.61	7731	0.92	0.63	4495	0.38	92	0.30	51.29	73.01
14	10	1	0	1.80	3788	0.95	0.66	2361	0.40	84	0.34	48.63	69.30
15	10	1	5	1.64	4727	0.94	0.63	2783	0.37	46	0.40	48.81	71.25
16	5	1	2.5	1.61	7099	0.93	0.65	4274	0.38	83	0.34	50.70	72.27
17	5	2	0	1.65	5709	0.94	0.71	3780	0.43	107	0.34	51.11	70.99

OSA OSA starch, *Gl* gluten, *Xyl* xylanase, *SV* specific bread volume, *H* hardness, *Spr* springiness, *Coh* cohesiveness, *Chew* chewiness, *Res* resilience, *MCA* mean cell area, *L** lightness

capacity to develop bread structure and volume. The enhanced specific volume of barley bread affected by OSA can be attributed to its emulsifying properties and the presence of hydrophobic groups exhibiting increased interfacial activity in the dough during proofing and baking.

At lower concentrations of OSA, specific volume of barley bread was higher in the absence of Gl in the formulation (Fig. 1b). However, at higher concentrations of OSA and the presence of Gl in formulation, specific bread volume was higher than that without Gl. As reported by Dapčević Hadnađev et al. (2014b) OSA starch addition influences increase in dough extensibility. Addition of OSA starch in barley bread dough in lower amount affected dough extensibility thus having favourable effect on gas retention during fermentation, while increased amount of OSA starch yielded too sticky and weak dough. Therefore, addition of gluten to formulation with high amount of OSA prevented excessive dough weakening.

The interaction effect of OSA and Gl on the specific bread volume was in agreement with previously reported results of Hung and Morita (2004) who obtained higher loaf volume when wheat flour was substituted with cross-linked starches (5–15%) and vital wheat gluten for the compensation of gluten dilution.

The interaction effect of OSA and Xyl (Fig. 1c) showed that at lower concentration of OSA, barley bread formulated with Xyl was of higher specific volume than that without enzyme. However, formulation with higher content of OSA without Xyl yielded bread of higher specific volume than that with Xyl.

Since both Xyl (Butt et al. 2008) and pregelatinized OSA starch (Dapčević Hadnađev et al. 2014b) decrease dough strength, their interaction effect led to weakened dough which could not retain its form during fermentation and consequently produced bread with decreased specific volume.

The improving effect of xylanase in non-wheat bread formulation is attributed to its conversion of water-insoluble into soluble arabinoxylans with water binding capacity due to which dough firmness is decreased, bread volume increased and bread is characterized by finer and more uniform crumb (Ahmad et al. 2014; Al-Widyan et al. 2008; Butt et al. 2008). The obtained results were in accordance with the results of Schoenlechner et al. (2013), Shah et al. (2006) and Trogh et al. (2004) who reported the increase of bread volume of millet/wheat composite bread, whole wheat bread and barley/wheat composite bread, respectively influenced as by xylanase.

Effects of selected ingredients on textural properties of barley bread crumb

Hardness of bread crumb was in the range from 3748 to 11,716 g depending on factor combination (Table 2). The

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | ☐ LE | ☐ TYPESET
MS Code : JFST-D-15-01858 | ✔ CP | ✔ DISK

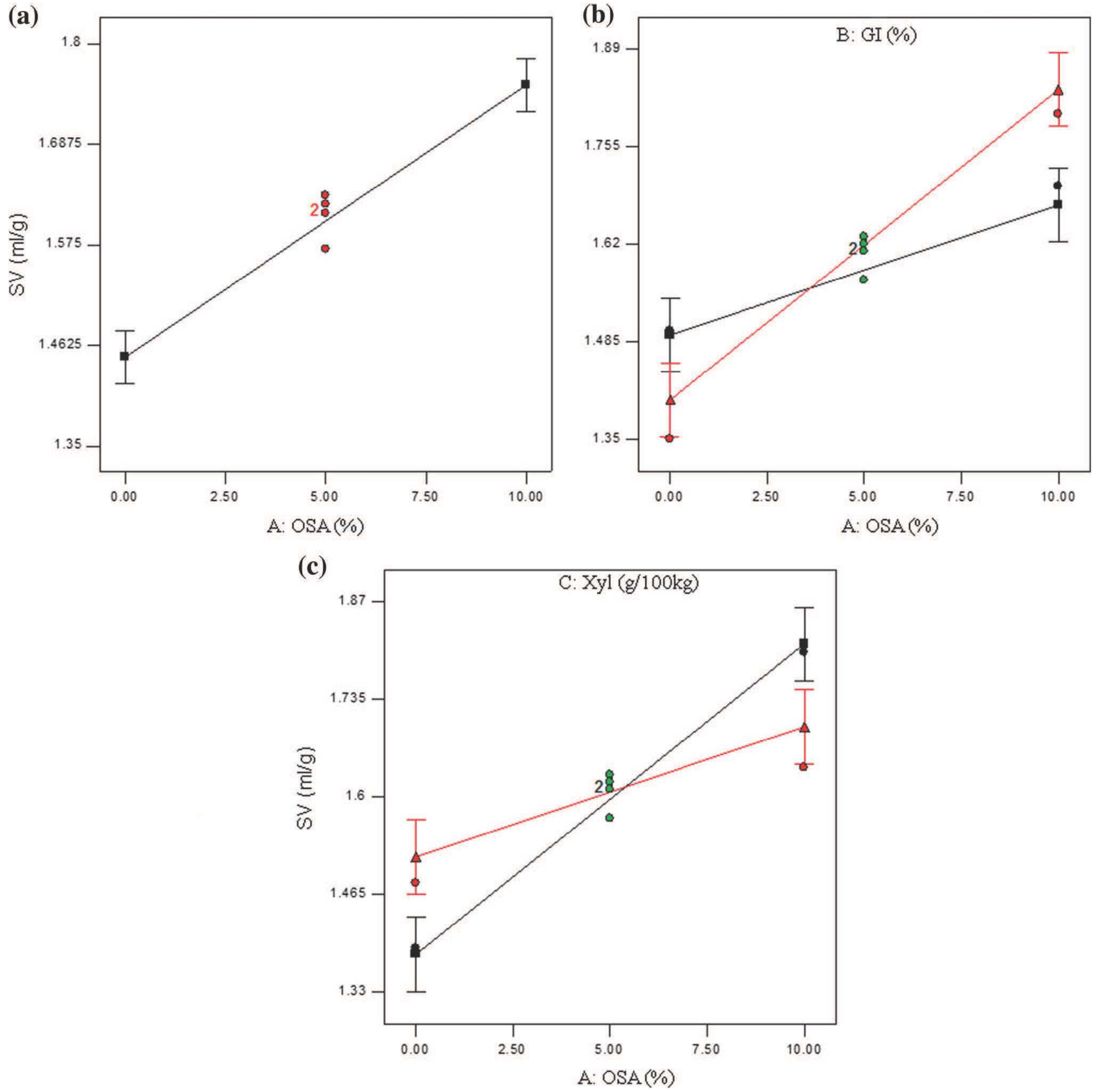

Fig. 1 Effects of factors on specific bread volume [OSA starch (**a**), interaction effects of OSA starch and gluten (**b**) and OSA starch and xylanase (**c**)]

increasing content of OSA (A), Gl (B) and Xyl (C) affected the decrease in crumb hardness (Table 3). The obtained model was significant ($p < 0.0001$) with A as significant model term; its effect on the bread hardness is shown in Fig. 2a. The values for predicted (0.645) and adjusted (0.787) R^2 values were in reasonable agreement.

Model for springiness of bread crumb could not be established, since no significant linear or quadratic effects were present ($p < 0.05$).

Cohesiveness of bread crumb was in the range from 0.63 to 0.71 (Table 2) depending on the applied formulation. From the linear model established it is obvious that increasing content of OSA and Xyl, as indicated with negative sign, affected the decrease of cohesiveness, while gluten demonstrated the opposite effect. The established model was significant ($p < 0.0001$), where A, B, and C were significant model terms, while OSA expressed more pronounced effect on cohesiveness than other factors

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | □ LE | □ TYPESET
MS Code : JFST-D-15-01858 | ✔ CP | ✔ DISK

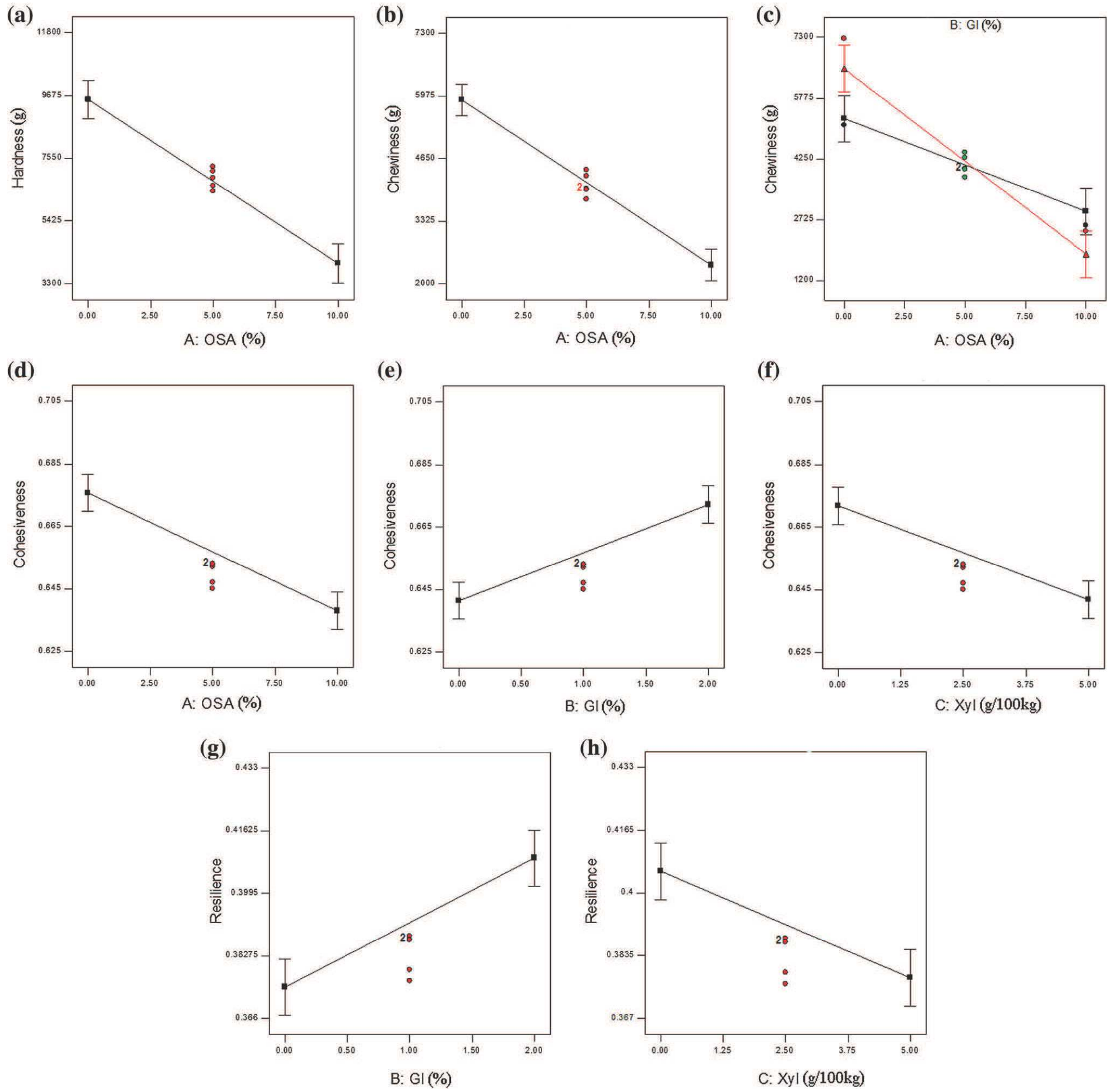

Fig. 2 Effects of factors on textural properties of bread [OSA starch on hardness (**a**), chewiness (**b**), cohesiveness (**d**), interaction effects of OSA starch and gluten on cohesiveness (**c**); gluten on cohesiveness (**e**) and resilience (**g**); xylanase on cohesiveness (**f**) and resilience (**h**)]

(Fig. 2d–f). The values for predicted (0.742) and adjusted (0.827) R^2 values were in reasonable agreement.

Chewiness of bread crumb was in the range from 2572 to 7265 g depending on the formulation (Table 2). The obtained model indicated that the increasing content of OSA and Xyl affected the decrease in crumb chewiness, while Gl expressed the opposite effect. Moreover, the interaction between OSA and Gl, as well as Gl and Xyl affected the decrease in crumb chewiness, while the interaction between OSA and Xyl showed the opposite effect. The obtained model was significant ($p < 0.0001$), where A and AB were significant model terms, with more pronounced effect of OSA on chewiness than other factors (Fig. 2b, c). The interaction effect of OSA and Gl on crumb chewiness indicated that lower content of OSA without Gl in formulation yielded crumb with lower chewiness, whilst formulation with higher OSA content and Gl yielded crumb with lower chewiness. The values for predicted (0.605) and adjusted (0.866) R^2 were not as closed as normally expected.

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | ☐ LE | ☐ TYPESET
MS Code : JFST-D-15-01858 | ✔ CP | ✔ DISK

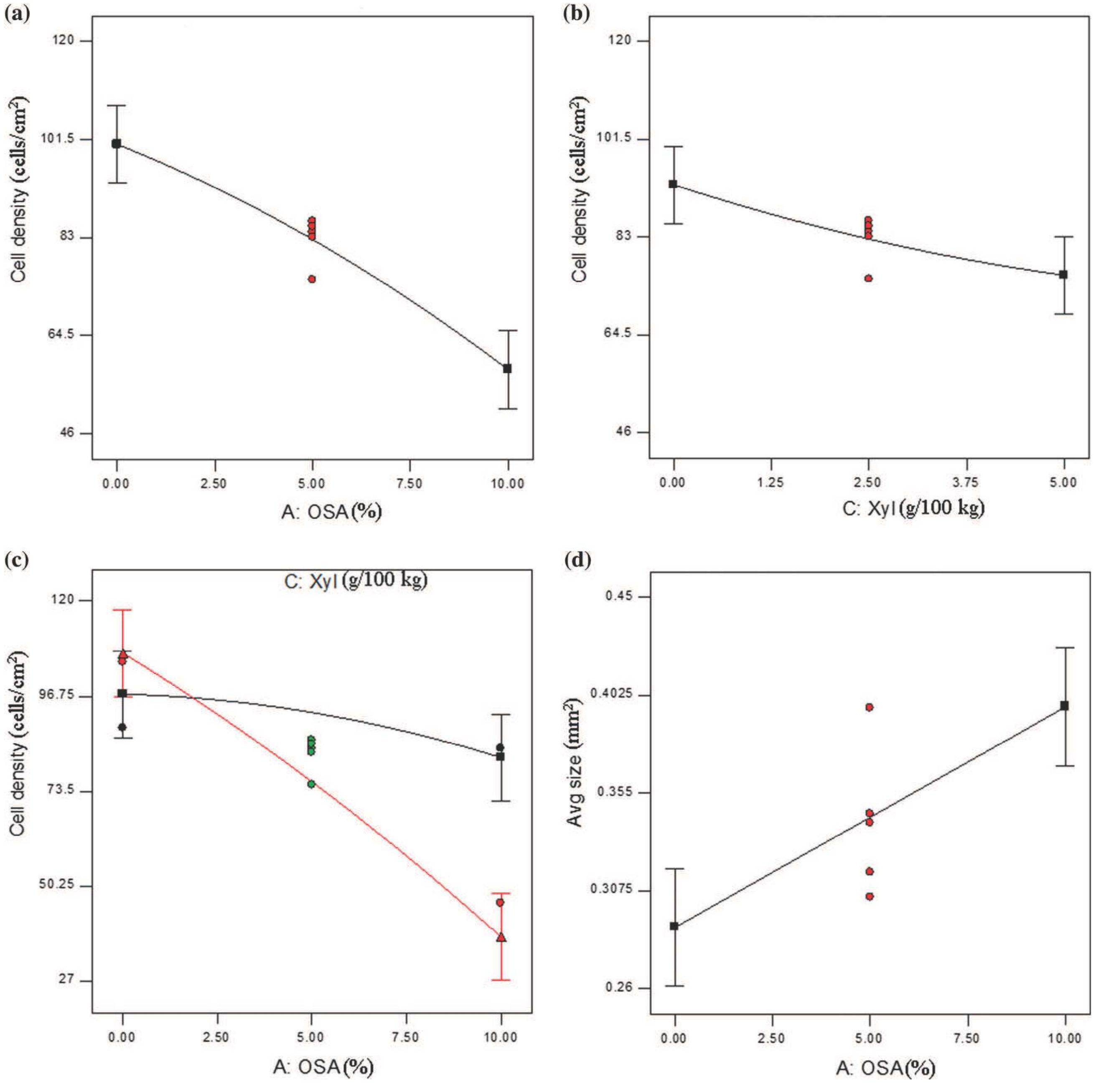

Fig. 3 Effects of factors on structural properties of bread crumb [OSA starch on cell density (**a**) and average cell size (**d**), interaction effects of OSA starch and xylanase on cell density (**c**); xylanase on cell density (**b**)]

Resilience of bread crumb was in the range from 0.37 to 0.43 depending on the formulation (Table 2). The obtained model indicated that increasing content of OSA and Xyl affected the decrease in bread crumb resilience, while gluten expressed the opposite effect. The significant model terms were B and C, where Gl expressed more pronounced effect than other parameters. The resilience increased with the increasing Gl content, and decrease with increasing Xyl concentration (Fig. 2g, h). The values for predicted (0.454) and adjusted (0.633) R^2 values were in reasonable agreement.

The wheat bread crumb texture softening effect was already documented for OSA starch (Dapčević Hadnađev et al. 2014b) and xylanase (Al-Widyan et al. 2008). The fact that gluten addition affected decrease in crumb hardness and increase in crumb cohesiveness, chewiness and resilience can be associated with the influence of bread volume and density of bread loaves on large-scale deformation hardness measurements (Goesaert et al. 2008). Therefore, the decrease in crumb hardness with the addition of gluten could be associated with it influence on bread volume increase and cell density decrease.

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | ☐ LE | ☐ TYPESET
MS Code : JFST-D-15-01858 | ☑ CP | ☑ DISK

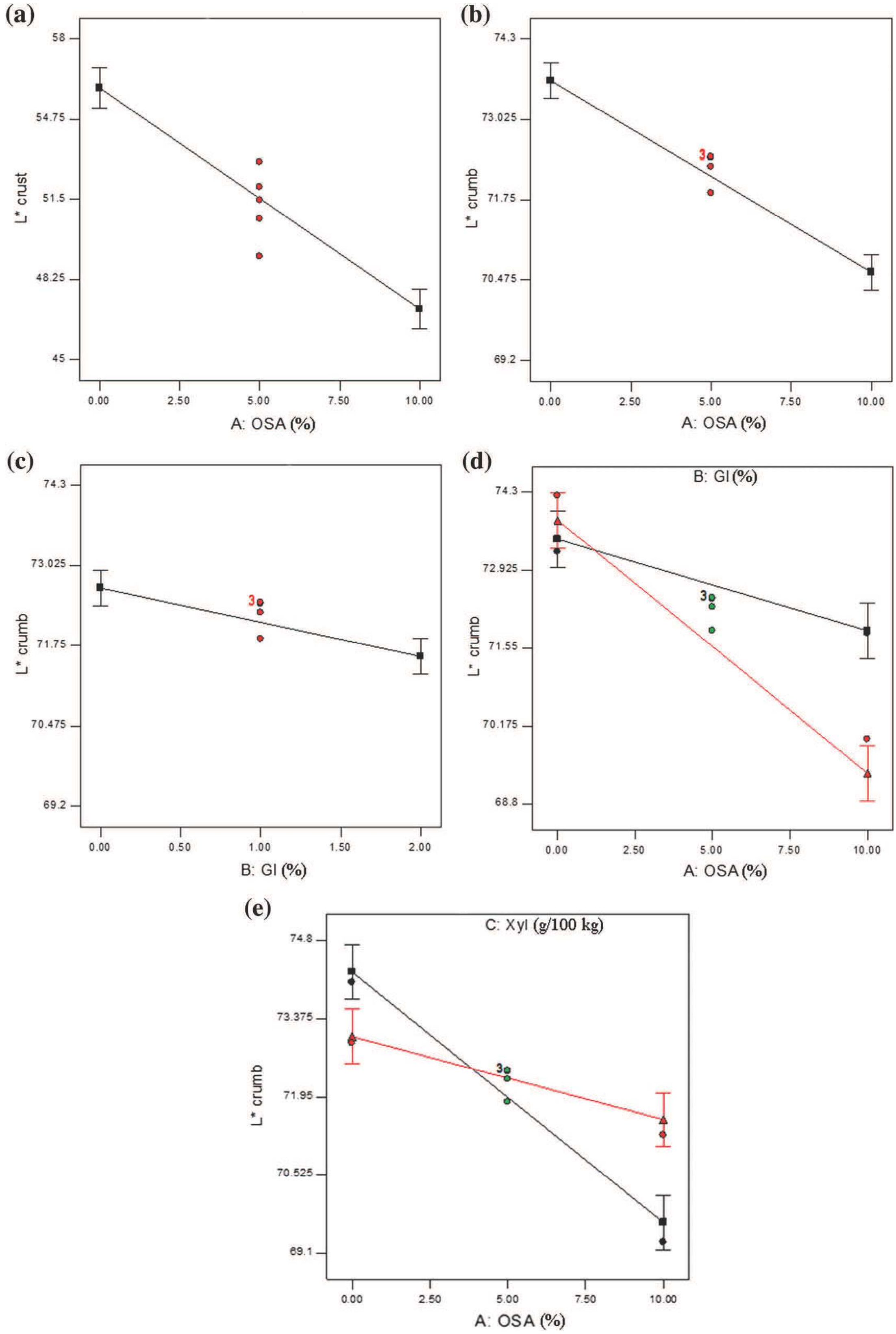

Fig. 4 Effects of factors on crust [OSA starch (**a**)] and crumb lightness [OSA starch (**b**), gluten (**c**), interaction effects of OSA starch and gluten (**d**) and OSA starch and xylanase (**e**)]

Journal : **Large 13197**	Dispatch : **17-12-2016**	Pages : **12**
Article No. : **2435**	□ LE	□ TYPESET
MS Code : **JFST-D-15-01858**	✔ CP	✔ DISK

Table 3 Regression models obtained for selected responses together with ANOVA outputs and summary statistics for the models

Parameter	Equation	F value	*p* value	R^2_{adj}	R^2_{pred}
Specific volume	SV = 1.60 + 0.15A + 0.018B + 0.005 C + 0.062AB − 0.063AC	22.72	<0.0001	0.872	0.703
Hardness	H = 6759.53 − 2773.25A − 103.00B − 10.25C	20.74	<0.0001	0.787	0.645
Cohesiveness	Coh = 0.66 − 0.019A + 0.015B − 0.015C	26.49	<0.0001	0.827	0.742
Chewiness	Chew = 4140.71 − 1746.58A + 45.20B − 120.42C − 579.27AB + 393.40AC − 217.25BC	18.23	<0.0001	0.866	0.605
Resilience	Res = 0.39 − 0.00275A + 0.017B − 0.014C	10.22	0.0010	0.633	0.454
Cell density	CD = 82.60 − 21.25A − 4.50B − 8.50C − 2.50AB − 13.50AC − 3.50BC − 3.30A^2 + 11.70B^2 + 1.70C^2	12.10	0.0017	0.862	0.215
Cell size	CS = 0.34 + 0.054A + 0.020B + 0.013C	4.87	0.0174	0.421	0.108
L* crust	L* crust = 51.53 − 4.48A − 0.043B − 0.060C	35.05	<0.0001	0.865	0.816
L* crumb	L* crumb = 72.12 − 1.52A − 0.54B + 0.18C − 0.71AB + 0.76AC − 0.035BC	24.19	<0.0001	0.897	0.724

A OSA starch content, B gluten content; C xylanase concentration

Effects of selected ingredients on structural properties of barley bread crumb

Cell density of bread crumb was in the range from 46 to 120 (cells/cm^2) depending on the formulation (Table 2). The obtained model indicated that increasing content of OSA, Gl and Xyl as well as their mutual interactions affected the decrease of cell density. The model was significant ($p < 0.05$) with significant model terms A, C, AC and B^2 (Fig. 3a–c). The content of OSA exhibited the most pronounced effect on cell density. The increasing OSA content affected the decrease in the cell density, as was the case with Xyl (Fig. 3a–b). R^2 predicted (0.2147) was not as close to the R^2 adjusted (0.8619) as expected.

The interaction effect of OSA and Xyl indicated that at lower concentrations of OSA, formulation without Xyl yielded denser crumb, whereas at higher concentrations of OSA with Xyl in formulation yielded crumb with lower cell density. The values for predicted (0.215) and adjusted (0.862) R^2 values were not in reasonable agreement.

Average cell size of bread crumb was in the range from 0.26 to 0.44 mm^2 g depending on the formulation (Table 2). The increasing content of OSA, Gl and Xyl affected the increase in average cell size, wherein the content of OSA appeared to be the only significant term (Fig. 3d). The average cell size increased with the increasing content of OSA in the formulation. The model was significant ($p < 0.05$). The values for predicted R^2 (0.108) was not as close to the adjusted R^2 of 0.4206 as expected, which can be attributed to the difficulties in determination of cell density and average cell size due to too dense crumbs obtained for certain formulations.

The poor quality of the models for parameters of bread crumb structures can be attributed to the difficulties in the determination of cell density and average cell size due to too dense crumbs yielded by certain formulations within the given experimental design.

The obtained results were in accordance with previously reported results where pre-gelatinized OSA starch influenced the formation of bread crumbs with the largest mean gas cell area (Dapčević Hadnađev et al. 2014a). The positive impact of OSA starch on crumb structure was associated with its ability to provide greater gas production, which consequently lessened the cell density and increase bread loaf volume (Dapčević Hadnađev 2013). Moreover, all improvers used in this study increased the specific barley bread volume which influenced increased porosity of the crumb.

Bread lightness

Colour is a huge factor in consumer acceptance of barley foods in general and barley bread in particular, especially

Journal : Large 13197 | Dispatch : 17-12-2016 | Pages : 12
Article No. : 2435 | ☐ LE | ☐ TYPESET
MS Code : JFST-D-15-01858 | ☑ CP | ☑ DISK

Table 4 Optimum formulations and predicted values of the response variables

Solutions	OSA (%)	Gl (%)	Xyl (g/100 kg)	SV (ml/g)	Hardness (g)	Cohesiveness	Chewiness (g)	Avg. size (mm^2)	Cell density	L* crust	L* crumb	Desirability
1	9.68	2.00	4.51	1.78	4052	0.64	2033	0.42	46.00	47.24	70.17	0.728
2	9.80	2.00	4.40	1.78	3988	0.64	1984	0.43	46.00	47.14	70.09	0.728
3	9.74	1.96	4.39	1.78	4023	0.64	2033	0.42	46.00	47.19	70.16	0.727
4	10.00	0.10	0.43	1.73	4088	0.64	2488	0.37	90.73	47.14	70.93	0.590

due to the fact that barley flours and barley products are darker than that produced from wheat (Newman and Newman 2008; Quinde-Axtell et al. 2006). Due to dark appearance of barley food which may be negatively perceived by the consumers, it is particularly important to improve this aspect of quality. Discoloration in barley bread is associated with polyphenols auto oxidation in the presence of oxygen and/or by polyphenols oxidation influenced by polyphenol oxidase or metals (Quinde-Axtell et al. 2006).

Crust lightness varied between 45.36 and 57.38 depending on the formulation (Table 2). The increasing content of OSA, Gl and Xyl affected the increase in darkness of bread crust. The significant model term was OSA content. The crust lightness decreased with increasing content of OSA (Fig. 4a). Sullivan et al. (2010) and Gill et al. (2002) reported the significant increase in *L** of bread crust with the increasing amount of barely flour in composite formulation.

The values for predicted (0.816) and adjusted (0.865) R^2 values were in reasonable agreement. The signal-to-noise ratio was found to be satisfactory as the observed adequate precision ratio was 15.147 (>4).

Crumb lightness varied between 69.30 and 73.25 depending on the formulation within given experimental design (Table 2). The increasing content of OSA and Gl affected the decrease of lightness, namely increasing darkness of barley bread crumb (Fig. 4b, c). The decrease of lightness was also observed due to interaction effect between OSA and Gl and Gl and Xyl, respectively. On the other hand, the increasing concentration of Xyl as well as interaction effect between OSA and Xyl affected the increase in crumb lightness. The model for crumb lightness was significant ($p < 0.0001$) with significant model terms A, B, AB and AC and the most dominant effect of OSA among the other factors. The R^2 predicted (0.724) was in the reasonable agreement with the R^2 adjusted (0.897). The interaction effect of OSA and Gl indicated that at lower content of OSA there were no significant differences in crumb lightness between the formulations with and without Gl. However, formulation with higher OSA content and Gl yielded crumb of significantly lower lightness than that without Gl (Fig. 4d).

The interaction effect of OSA and Xyl indicated that at lower OSA starch content the crumb was lighter in the formulation without Xyl, whereas higher content of OSA yielded lighter bread crumb with the supplemented Xyl (Fig. 4e).

According to the literature findings, finer bread crumb structure is characterized by larger number of cells of smaller average cell size per area resulting in higher light reflectance, while breads with increased crumb porosity (cell mean area) will express a reduction in crumb *L** values, due to higher absorption of the light (Esteller et al. 2006). In this paper, barley breads containing Gl and OSA were characterized with higher average cell sizes and thus expressed decrease in *L** values. On the contrary, addition of Xyl led to extensive hydrolysis of arabinoxylans thus resulting in lighter bread crumb (Butt et al. 2008).

Optimization of the formulation of barley bread

The optimization criteria were based on the importance of bread properties for the overall consumers' perception of bread—the freshness, colour, texture and biting properties (Gellynck et al. 2009; Heiniö 2006; Gomez et al. 2003). The constraints for optimization were set as described in Sect. 2.7. The results of optimization are shown in Table 4 by descending desirability.

The optimal barley bread formulations appeared to be those with the concentration of selected ingredients close to their maximal level (within the given experimental design). The optimal barley bread formulations (1–3) were characterized by lower cell density in relation to composite wheat/barley (60:40) breads reported by Rieder et al. (2015) and Collar and Angioloni (2014).

While optimizing the additive combination on the basis of sodium stearoyl-2-lactylate, transglutaminase and xylanase to improve soy-wheat bread quality, Ribotta et al. (2010) obtained more than 60% of bread volume increase and more than 70% of crumb hardness and chewiness decrease in comparison to the bread formulation without additives. Schoenlechner et al. (2013) optimized the quality of wheat/proso millet bread by addition of emulsifier, transglutaminase and xylanase. They observed that combined effect of transglutaminase and xylanase significantly affected the increase of bread volume and increase crumb firmness. Additionally, they reported that emulsifier addition affected the increase of bread volume and number of cells and decrease of crumb firmness and relative crumb elasticity.

In this paper, in comparison to formulations containing medium amounts of improvers (1% Gl, 5% OSA, 2.5% Xyl) the predicted optimal barley bread formulation (1) containing combination of improvers yielded bread of significantly higher specific volume, but lower hardness, chewiness and cell density. An increase in bread volume of 28.1%, 14.1 and 18.7% and decrease in crumb chewiness of 217, 105.5 and 150.6% was achieved in predicted optimal formulation (1) in comparison to breads containing 1% Gl, 5% OSA, 2.5% Xyl, respectively.

Conclusions

By varying the proportion and combination of pregelatinized OSA starch, wheat gluten and xylanase in barley bread formulation, a large variation in specific bread volume, crust and crumb lightness, crumb texture and structure could be observed.

OSA starch exhibited the most pronouncing effect on specific bread volume and average cell size. While interaction between OSA starch and gluten affected the increase in specific volume of barley bread, the interaction between OSA starch and xylanase affected its decrease. OSA starch and xylanase affected the decrease in crumb chewiness, while gluten expressed the opposite effect. Moreover, the interaction between OSA starch and gluten, as well as gluten and xylanase affected the decrease in crumb chewiness, while the interaction between OSA starch and xylanase showed the opposite effect being in agreement with decreased specific volume and excessive dough weakening effect. Additive incorporation mostly led to decrease in barley bread crumb lightness, except in the case of increased concentration of xylanase as well as interaction effect between OSA starch and xylanase. Overall, by combining the improvers, bread of acceptable quality could be produced from barley flour.

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