Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.114322

Effect of enzyme compositions on the rheological properties of bread dough enriched in buckwheat flour

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Abstract

Buckwheat is a good source of dietary fibre, protein and minerals, which has many beneficial effects on human health. However, previous research has illustrated that the addition of buckwheat has detrimental effects on the dough rheology and final products quality. Thus, the objective of this study was to investigate the effects of α-amylase (6 and 10 ppm), xylanase (70 and 120 ppm) and cellulase (35 and 60 ppm) on the rheological properties of bread dough incorporated with buckwheat flour. The mixing property of dough was measured by using a DoughLAB. The results indicated that the addition of single enzyme decreased water absorption, development time, stability, extensibility and stickiness, whereas increased softening, MTI and resistance to extension. Compared to the single enzyme, the enzyme compositions showed lower development time, water absorption and stability, and higher softening, MTI, resistance to extension and extensibility. As a result, enzyme compositions were more efficient than the single enzyme due to the synergistic effect of α-amylase, xylanase and cellulase.

Keywords: dough rheology; doughlab; xylanase; cellulase; α-amylase.

Practical Application: This work investigates how the single enzyme and combined enzymes influence the rheological properties of buckwheat dough during bread making. It has a widespread practical application in baking industry.

1 Introduction

Food rheology is an essential part of food science, providing analysis of the flow and deformation of liquid and semi-solid foods. The rheology of dough is concerned with the complex interrelationship of different flours and additives (reducing agents, oxidizing agents, enzymes, emulsifiers, sugar and salt) that govern the flow and deformation of dough systems under external forces (Tebben et al., 2018). Several types of instruments have been employed to characterize the rheology of cereal products, such as Farinograph, DoughLAB, Alveograph, Extensograph and Texture Analyser (Parenti et al., 2021). In particularly, the doughLAB is an evolution of the current flour analysis equipment, which provides enhanced functions compared with common analysis with its higher speed and higher torque capabilities (Liu et al., 2017a).

Buckwheat (*Fagopyrum*) is gluten-free pseudocereal that belongs to the family of Polygonaceae and grown in many countries (China, Russia, Canada, USA and Italy) (Giménez-Bastida & Zieliński, 2015). According to the study, buckwheat grain contains certain high-level nutritional components, such as dietary fibre, proteins, lipids, and polyphenols (Koval et al., 2020). Buckwheat grain has many beneficial effects on human health, such as reduction of plasma cholesterol level, anti-inflammatory, anti-cancer, reducing glycaemic response and enhancing hypertension (Ninomiya et al., 2022). Additionally, many studies have pointed out that buckwheat is effective in the prevention and management of the diseases, such as diabetes, obesity, heart disease, diseases of the large bowel, and colon cancer (Gallo & Montesano, 2023; Graziano et al., 2022; Zhu, 2021).

However, previous studies have illustrated that the addition of buckwheat flour has detrimental effects on the dough rheology and final products quality. The effect of buckwheat flour on the dough rheology was investigated by Liu et al. (2017b) who indicated that buckwheat addition significantly influenced rheological properties of the bread dough. For instance, the addition of buckwheat hull decreased pasting properties of starch due to the water holding capacity of dietary fibre (Liu et al., 2022). The addition of buckwheat bran flour caused an increase in water absorption of steamed bread dough due to the high fibre and lipid content of buckwheat (Zhang et al., 2022). Alfaris et al. (2022) illustrated that dough formulated with wheat bran had a higher value of dough stability in comparison to dough with wheat flour only due to dilution of gluten and disruption of the gluten network structure.

In order to overcome the negative effects of buckwheat, enzymes such as α-amylase and xylanase have been widely used to improve the dough handling and breadmaking in the bakery industry (Bueno et al., 2016; Dahiya et al., 2020a; Dahiya & Singh, 2019; Pourmohammadi & Abedi, 2021; Singhal et al., 2021). Fungal α-amylase is the most common enzyme used in bread making as anti-staling agents, which can randomly damage starch and reduce its water binding ability, thus increasing the gluten hydration (Alqah et al., 2022; Rebholz et al., 2021). Xylanase is

Received 11 Nov., 2022
Accepted 27 Nov., 2022
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the second most common enzyme used in food and feed, paper and pulp, textile, pharmaceuticals, which can attack the AX backbone and break the glycosidic linkages in AX, resulting in changing the functional and physicochemical properties of AX (Paul & Thatoi, 2022). Celluloses are widely used for extraction and clarification of fruit and vegetable juices, which can catalyze the hydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans (Singhal et al., 2021). Therefore, these enzymes have potential to improve the rheological behaviour of dough incorporated with buckwheat and the quality of final products (Dahiya et al., 2020b; Liu et al., 2017a). However, there is limited research about the effect of combination enzymes, especially, the combination of cellulase, xylanase and α-amylase on the rheological properties of bread dough with 15% buckwheat flour.

Hence, the objective of this study was to investigate the effect of α-amylase, xylanase and cellulase on the rheological properties of the bread dough with 15% content of buckwheat flour compared to regular bread dough without buckwheat flour.

2 Materials and methods

2.1 Materials

Wheat flour (Champion Flour Milling Ltd, Christchurch, New Zealand) and Buckwheat flour (Ceres Organics Ltd, Auckland, New Zealand) were purchased at the local supermarket. Fungamyl 2500 SG 3.2.1.1 (2-10 ppm), Pentopan Mono BG 3.2.1.8 (20-120 ppm) and Cellulast BG 3.2.1.4 (10-60 ppm) were purchased from Novozymes Australia Pty Ltd (Novozymes, North Rocks NSW, Australia).

2.2 Design of experiment

Two experimental designs were performed to investigate the individual and interactional effect of α-amylase, xylanase and cellulase on the rheological properties of the bread dough. Firstly, one-way analysis of variance (ANOVA) was used to analyse the effect of single enzyme on the rheological properties of regular dough and dough incorporated with 15% of buckwheat as buckwheat dough. According to the manufacture recommendations of Novozymes and previous research (Liu et al., 2017a; Serventi et al., 2016), the dosage of the Pentopan Mono BG, Cellulast BG and Fungamyl 2500 SG was added with 70 ppm, 35 ppm and 10ppm, respectively (Supplementary Material Table S1).

Second, a full factorial 2^3 design of experiments was used to investigate the effect of combined enzymes on the rheological properties of dough incorporated with 15% of buckwheat. Generally, there are three factors (α-amylase, xylanase and cellulase) at two levels (-1, 1) resulted in 8 different combinations of experiments and the coded values per each level of each factor are shown in Supplementary Material Table S2. In terms of the estimated coefficients (β_i, β_{ij} & β_{ijk}), the theoretical response function (W) was calculated as following polynomial model (Equation 1):

$$W = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{123} ABC \quad (1)$$

Factors: A – α-amylase; B – xylanase; C – cellulase; AB – α-amylase*xylanase; AC – α-amylase*cellulase; BC – xylanase*cellulase; ABC – α-amylase*xylanase*cellulase.

W – The theoretical response variable; β_0 – The global mean; β_i – The regression coefficient corresponding to main factor; β_{ij} and β_{ijk} –The regression coefficient corresponding to the interactions.

This multiple linear regression model with three independent variables describes the rheological property of dough is related to the α-amylase, xylanase and cellulase.

2.3 Rheological properties of dough

The rheological properties of dough were evaluated using a DoughLAB (Perten Instruments Australia, Macquarie Park, Australia) equipped with 300 g mixing bowl following AACC 54-21.02 standard method. The weight of the ingredients was adjusted according to the moisture content of the samples to obtain consistency in moisture contents, and water absorption (as is) was corrected to 500 FU. Dough development time, stability, softening, mixing tolerance index, and departure time were calculated using DoughLab software (version 1.3.0.185). Analysis was performed in triplicate.

2.4 Dough extension analysis

Dough extension test were conducted by a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). The Texture Analyser equipped with Kieffer dough and gluten extensibility rig was used to perform the extension tests. The resistance to extension (g) and extensibility (mm) was determined in tension mode by recording the peak force and the distance at the maximum and the extension limit. Dough produced with only wheat flour was considered as the regular. Dough was formulated with wheat flour and buckwheat (15 g/100 g) following the previous research of Liu et al. (2017b). As the optimum dough, the formulations were prepared following the factorial design. The test settings were: pre-test speed: 2.0 mm/s; test speed: 3.3 mm/s; post-test speed: 10.0 mm/s; distance: 75 mm; trigger force: 5 g (5 kg load cell).

2.5 Dough stickiness

Analysis of dough stickiness was carried out by Chen-Hoseney's method and the dough was placed into the chamber of Stable Micro system/Chen–Hoseney Dough Stickiness Cell, and then closed with a die by screwing for test. The test settings were: pre-test and test speed: 0.5 mm/s; post-test speed: 10.0 mm/s; distance: 4 mm; time: 0.1 s; trigger force: 5 g (5 kg load cell).

2.6 Statistical analysis

All data were treated by ANOVA and multiple regression analysis using Minitab 17 statistical software, version 17. 2. 1 (Minitab Pty Ltd, Sydney) at a significance level $p < 0.05$.

3 Results and discussion

3.1 Effect of single enzyme on the rheological properties of buckwheat dough

The effects of single enzyme on the rheological properties of dough incorporated with 15% buckwheat flour are presented in Table 1. As a result, the incorporation of α-amylase to buckwheat dough decreased water absorption, development time, stability,

Table 1. Effect of single enzyme on rheological properties of buckwheat dough.

Sample	15% buckwheat	+ Amylase	+ Xylanase	+ Cellulase
WA %	65.07 ± 0.03A	62.10 ± 0.17B	62.23 ± 0.06B	62.43 ± 0.15B
Development time (min)	7.12 ± 0.13A	6.53 ± 0.15B	6.83 ± 0.06B	6.23 ± 0.06B
Stability (min)	8.73 ± 0.45A	8.07 ± 0.06B	6.50 ± 0.30C	5.03 ± 0.06D
Softening (FU)	53.37 ± 5.31C	88.07 ± 2.63A	71.63 ± 1.93B	90.46 ± 0.45A
Departure time (min)	12.21 ± 0.36A	10.20 ± 0.35B	10.50 ± 0.10B	9.00 ± 0.10C
MTI (FU)	31.31 ± 0.11D	52.17 ± 2.65B	41.46 ± 1.46C	60.70 ± 0.10A
Extension (g)	17.54 ± 1.24C	38.23 ± 0.36B	42.21 ± 0.39A	42.58 ± 1.18A
Extensibility (mm)	34.60 ± 0.21A	18.43 ± 0.35B	17.15 ± 0.73C	16.07 ± 0.22C
Stickiness (g)	77.59 ± 5.39A	62.35 ± 0.26C	65.48 ± 0.11B	52.93 ± 1.68D

Means ± standard deviations (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). WA-Water absorption; MTI-Mixing tolerance index; FU-unit; DT-Development time.

departure time, extensibility and stickiness, whereas raised the softening, MTI and resistance to extension. Similar results were observed by Sahnoun et al. (2013) who reported that the addition of α-amylase reduced development time, water absorption, and stability of bread dough. According to the research of Atalay et al. (2013), the addition of transglutaminase decreased the water absorption and extensibility, while increased maximum resistance of flour blended with buckwheat milling products. Additionally, Patel et al. (2012) illustrated that the addition of fungal α-amylase to wheat flour resulted in decrease of the water absorption, development time, stability and extensibility and an increase of resistance due to the presence of a low molecular weight dextrin produced by α-amylase hydrolysis.

In terms of buckwheat dough with xylanase, xylanase decreased the water absorption, development time, stability, departure time, extensibility and stickiness, whereas increased softening, MTI and resistance. Xue et al. (2020) reported that addition of xylanase decreased the water absorption, development time and extensibility of bread dough due to the depolymerization of pentosane by xylanase. According to Jia et al. (2011), the addition of xylanase to the dough incorporated with almond skin flour led to a decrease in development time and stability. Moreover, Dahiya & Singh (2019) pointed out that xylanase addition reduced the water absorption and stickiness of whole wheat flour. Thus, this observation could be attributed to the enzymatic hydrolysis of soluble pentosane. Table 1 also shows that the effect of cellulase on the rheological properties of buckwheat dough has the same trend with xylanase and α-amylase. Similar results were observed by Yang et al. (2021) who indicated that the addition of cellulase had negative effects on extensibility, tenacity and stability of buns dough. Additionally, Altınel and Ünal (2017) illustrated that hemicellulase addition presented a decrease in extensibility of wheat meal bread dough.

3.2 Effect of enzymes combination on rheological properties of buckwheat dough

Full factorial design 2^3 was used to investigate the effect of α-amylase, xylanase and cellulase combinations on the rheological properties of buckwheat dough. The analytical results and the interaction of α-amylase, xylanase and cellulase on parameters of buckwheat dough rheology are presented in Table 2. Table 3 illustrates regression coefficients and R^2 obtained from the full factorial design in dough rheology. The coefficients that showed significant difference ($p < 0.05$) in Table 3 were fitted to the following empirical model (Equantions 2-10):

$$W\left(WA\,\%\right) = 61.32 + 0.1A - 0.53C + 0.18AB - 0.33AC + 0.09BC + 0.08ABC\ \left(R^2 = 0.95\right) \quad (2)$$

$$W\left(Development\ time\right) = 6.35 + 0.17AB + 0.15AC\ \left(R^2 = 0.66\right) \quad (3)$$

$$W\left(Stability\right) = 5.07 + 0.12AC + 0.08ABC\ \left(R^2 = 0.63\right) \quad (4)$$

$$W\left(Softening\right) = 108.51 + 2.41A + 5.92B + 1.62AB - 2.85AC - 2.98BC - 2.59ABC\ \left(R^2 = 0.91\right) \quad (5)$$

$$W\left(Departure\ time\right) = 8.83 - 0.43A - 0.12B + 0.10C + 0.24AC\ \left(R^2 = 0.86\right) \quad (6)$$

$$W\left(MTI\right) = 64.38 + 3.53A - 1.44C + 1.11AB - 3.00AC - 1.04BC\ \left(R^2 = 0.86\right) \quad (7)$$

$$W\left(Extension\right) = 32.63 - 6.82A - 2.31B - 5.42C - 1.85AB + 1.83AC - 1.55BC + 4.93ABC\ \left(R^2 = 0.99\right) \quad (8)$$

$$W\left(Extensibility\right) = 18.45 + 1.24A + 0.55B + 0.21C - 0.32AB - 0.25BC - 0.35ABC\ \left(R^2 = 0.98\right) \quad (9)$$

$$W\left(Stickiness\right) = 74.58 - 7.07A + 4.61B + 0.28C - 3.88AB + 0.81AC - 2.38BC + 1.65ABC\ \left(R^2 = 0.99\right) \quad (10)$$

Factors: A – α-amylase; B – xylanase; C – cellulase; AB – α-amylase*xylanase; AC – α-amylase*cellulase; BC – xylanase*cellulase; ABC – α-amylase*xylanase*cellulase.

Compared to the single enzyme, combined enzymes reduced water absorption of buckwheat dough to the minimum value (60.9%) when the enzymes were added with the concentration (6, 120, 60 ppm). Table 3 illustrates that

Table 2. Effect of combined enzymes on rheology of buckwheat dough.

Blocks	A	B	C	WA %	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Buckwheat	0	0	0	65.1	7.1	8.7	53.3	12.2	31.3	17.5	34.6	77.6
1	6	70	35	61.6	6.7	5.2	98.9	9.6	59.8	40.7	16.3	68.6
2	6	70	60	61.2	6.4	5.1	104.7	9.3	63.9	39.23	16.5	75.6
3	6	120	60	60.9	6.2	5.0	112.8	9.0	61.0	25.3	18.5	86.5
4	6	120	35	61.2	6.3	5.2	107.9	9.2	58.8	52.5	17.6	96.0
5	10	70	35	62.2	6.0	5.1	101.2	8.1	69.6	36.8	18.6	66.3
6	10	120	35	62.3	6.2	4.6	126.8	8.1	75.8	21.7	20.28	66.8
7	10	120	60	60.9	6.7	5.3	109.6	8.5	63.0	21.5	19.6	67.5
8	10	70	60	60.2	6.2	5.1	105.8	9.0	63.8	22.6	20.3	69.8

All values are means. A (factor) – α-amylase; B (factor) – xylanase; C (factor) – cellulase. Regular – wheat flour dough; Buckwheat – wheat flour dough with 15% buckwheat flour. WA-Water absorption; MTI-Mixing tolerance index; FU-unit; DT-Development time..

Table 3. Estimated regression coefficients of the factors of the rheological properties of buckwheat dough.

Coefficients	WA%	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Constant	61.32	6.35	5.07	108.51	8.83	64.38	32.63	18.45	74.58
Amylase	0.10	NS	NS	2.41	-0.43	3.53	-6.82	1.24	-7.07
Xylanase	NS	NS	NS	5.92	-0.12	NS	-2.31	0.55	4.61
Cellulase	-0.53	NS	NS	NS	0.10	-1.44	-5.42	0.21	0.28
Amylase*Xylanase	0.18	0.17	NS	1.62	NS	1.11	-1.85	-0.32	-3.88
Amylase*Cellulase	-0.33	0.15	0.12	-2.85	0.24	-3.00	1.83	NS	0.81
Xylanase*Cellulase	0.09	NS	NS	-2.98	NS	-1.04	-1.55	-0.25	-2.38
Amylase*Xylanase*Cellulase	0.08	NS	0.08	-2.59	NS	NS	4.93	-0.35	1.65
R^2	95.49%	66.36%	63.11%	90.88%	86.21%	85.90%	99.25%	98.19%	99.89%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1, β_2 and β_3 – regression coefficients corresponding to main factors; β_{12}, β_{13}, β_{23} and β_{123} – regression coefficients corresponding to interactions; '+' – positive effect; '-' – negative effect. WA-Water absorption; MTI-Mixing tolerance index; FU-unit; DT-Development time.

the interaction of α-amylase*xylanase, xylanase*cellulase and α-amylase*xylanase*cellulase have a positive synergistic effect on the water absorption, while α-amylase*cellulase shows negative effect. Therefore, the buckwheat dough incorporated with combined enzymes need less water than buckwheat dough with single enzyme during dough mixing. Similar observation was reported by Atalay et al. (2013), who pointed out the combination of transglutaminase and sodium stearoyl-2-lactylate decreased the water absorption of dough incorporated with 20% buckwheat milling product due to synergistic effect between the additives. Additionally, Yang et al. (2021) reported that incorporation of xylanase, glucose oxidase and cellulase can improve the negative effects of the single enzyme on the dough in terms of extensibility, tenacity and stability. This observation may be attributed to the interactions among enzyme activities and their coupled reactions (Altuna et al., 2016; Eugenia Steffolani et al., 2012; Kriaa et al., 2016).

With respect to development time, the addition of mixture enzymes showed a lower development time than single enzyme when the combined enzymes added with 10, 70, 35 ppm. The interaction of α-amylase*xylanase and α-amylase*cellulase had a significantly synergistic effect on the development time. Previous research found that combined xylanase and arabinofuranosidase was more effective in reducing the resistance to extension, softening degree, water absorption and development time (Xue et al., 2020). According to the research of Shafisoltani et al. (2014), who indicated that the combination of xylanase and glucose oxidase had an inverse effect on the development time. Moreover, Altuna et al. (2016) indicated the combination of transglutaminase, xylanase and glucose oxidase had synergistic effects on rheological properties of bread dough with high resistant starch.

In terms of stability, combined enzymes decreased the stability of buckwheat dough from 8.7 min to 4.6 min. The interaction of α-amylase*cellulase and α-amylase*xylanase*cellulase indicated a significant positive effect on the stability. For the softening, the enzyme combination had a higher value of softening than single enzyme. Similar results were reported byAltuna et al. (2016), the addition of xylanase, α-amylase and cellulase decreased dough stability and increased softening. This result is consistent with our previous research that the combination of α-amylase, xylanase and cellulase decreased the dough stability and increased dough softening (Liu et al., 2017a).

The combination of α-amylase, xylanase and cellulase significantly influenced the resistance to extension, extensibility and stickiness. For the extension tests, the addition of combined enzymes increased resistance to extension and reduced the extensibility of buckwheat dough. Similar observation was reported by Altuna et al. (2016) who found that the combination of transglutaminase (0-8 mg/100 g), glucose-oxidase (0-5 mg/100 g) and xylanase (0-1 mg/100 g) resulted in a decrease in extensibility and increase in resistance to extension. Yang et al. (2021) also pointed out that the mixture of xylanase, glucose oxidase and cellulase resulted in a softer gluten matrix. Stickiness of buckwheat dough was varied form 66.3 g to 96.0 g when combined enzymes were added with different concentrations. Table 2 shows that the minimum stickiness (66.3 g) was observed when the blended enzymes were added with 10, 70, 35 ppm. However, the buckwheat dough had the highest stickiness when the combined enzymes were added with the concentration (6, 120, 35 ppm). According to the research of Altuna et al. (2016), who found that the combination of transglutaminase, glucose-oxidase and xylanase increased the stickiness of dough incorporated with resistant starch. Previous research also suggested that the optimum combination of glucose oxidase, α-amylase and xylanase could be used to minimize dough stickiness (Eugenia Steffolani et al., 2012). These observations may be due to the degradation of cell wall components and higher water absorption of bran resulting in altered the water distribution among starch, protein and bran particles (Barrera et al., 2016; Pourmohammadi & Abedi, 2021; Altuna et al., 2016).

4 Conclusion

In this work, the individual and interactional effect of α-amylase, xylanase and cellulase on rheological properties of dough incorporated with 15% buckwheat flour were investigated. From the findings, it can be concluded that both single enzyme and blended enzymes had significant influence of bread dough rheology. The individual addition of α-amylase, xylanase and cellulase into buckwheat dough reduced water absorption, development time, stability, extensibility and stickiness, whereas increased softening, MTI and resistance to extension. In comparison with single enzyme, the enzymes combination showed lower development time, water absorption and stability, and higher softening, MTI, resistance to extension and extensibility. The results obtained from 2^3 full factorial design suggested that the combined enzymes were more efficient than the single enzyme due to the synergistic effect of α-amylase, xylanase and cellulase.

Therefore, the combination of enzymes revealed a better improvement of buckwheat dough rheology than single enzyme.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJQN202200836); and the Natural Science Foundation of Chongqing Technology and Business University (412/950322028).

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Function of enzymes (from Novozymes Biotechnology Company)

Table S2. Description of experimental factors at two level

This material is available as part of the online article from https://doi.org/10.5327/fst.114322