Effects of yeast, carboxymethylcellulose, yoghurt, transglutaminase and cyclodextrinase on mixing properties of oat dough

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Abstract The effects of yeast, carboxymethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) and cyclodextrinase (CG) on the mixing properties of oat dough were investigated through the use of DoughLab. A 2^{5-2} fractional factorial design resolution III with yeast (1.25, 3.25 %), CMC (1, 2 %), YG (10.75, 33.75 %), TG (0.5, 1.5 %) and CG (10, 40 μ l) as independent variables was implemented. The parameters measured were water absorption, arrival time, stability, energy at peak, peak resistance, development time, departure time, softening and bandwidth at peak. CMC significantly ($p < 0.05$) increased stability, energy at peak, development and departure times, but significantly ($p < 0.05$) decreased water absorption, peak resistance, softening and bandwidth at peak. TG significantly increased water absorption, peak resistance and softening, but significantly decreased energy and development time. YG significantly ($p < 0.05$) decreased all the parameters measured, with the exception of softening, which was significantly increased. In contrast, yeast and cyclodextrinase did not significantly affect the oat dough during mixing. Principal component analysis indicated that 85.5 % of the variation in the data could be explained by two components. Component 1 explaining 52.3 % of the variation loaded highly on dough strength (stability and departure time). Component 2 contributing 33.2 % of the variation loaded on dough resistance (water absorption and peak resistance). CMC significantly increased dough strength while yoghurt reduced it significantly. TG

significantly ($p < 0.05$) increased the resistance of the dough to mixing while CMC and yoghurt reduced it significantly ($p < 0.05$). Hence, CMC, TG and yoghurt are ingredients of choice when modifying oat dough mixing properties.

Keywords Oat dough · Yeast · CarboxyMethylCellulose · Yoghurt · Transglutaminase · Cyclodextrinase · Response surface methodology · Mixing properties

Introduction

In spite of oats being used as breakfast food, it is currently receiving increased interest due to its nutritional value (e.g., high in soluble dietary fibre, proteins, unsaturated fatty acids, vitamins, minerals and other nutrients). A substantial amount of dietary fibre in oats is found in the cell wall. It is mostly composed of a polysaccharide called β -glucan (mixed linkage (1–3) (1–4)- β -D-glucan) (Huang et al. 2010). β -glucan reduces the concentration of serum cholesterol, attenuate blood glucose levels, slow insulin response in the blood and maintain the balance of intestinal flora (Krauss et al. 2000; Huang et al. 2010). As reported by the US Food and Drug Administration (FDA), the positive function of soluble dietary fibre found in oats is its ability to reduce the risk for coronary heart diseases (FDA 1996).

The dough making performance of oat (*Avena sativa*) flour is mainly based on the swelling properties of endogenous pentosans. Pentosans are able to bind water and increase the viscosity of the dough at low pH, thus improving the flow properties and shape of the dough during proofing and baking. In contrast to wheat proteins, oat proteins are not capable of forming three dimensional structures. Two possible reasons are assumed to be responsible for the different behaviour of oat and wheat proteins. Firstly, oat and wheat proteins show qualitative as well

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as quantitative differences. For example the wheat gluten network consists of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits stabilized by intermolecular disulphide bonds (Wieser and Kieffer 2001), whereas oat proteins lack LMW glutenin subunits. Although oat also contains HMW subunits, the ability of forming intermolecular disulphide bonds is inferior as compared to the HMW glutenin subunits of wheat (Koehler and Wieser 2000). The second reason for the limited aggregation behaviour of oat proteins is the low pentosan fraction, which leads to the formation of a gel-like layer on the flour particles thereby hindering protein aggregation (Wang 2003). This low pentosan fraction of oat flour results in a dough with a higher plasticity than wheat dough and its surface is more moist and sticky. Consequently, manual dough processing and machinability of oat dough is more difficult compared to that of wheat dough.

The mixing properties of oat dough may be modified by any one of the following components: yeast, carboxymethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) or cyclodextrinase (CG). *Saccharomyces cerevisiae* is considered to be one of the major yeasts used in dough fermentation and has an important effect on dough rheological properties. Research has shown that the effect of the yeast on rheological properties is similar to the effect of hydrogen peroxide (Mirsaeedghazi et al. 2008). This fact indicates that the effect of yeast on rheological properties is due to the production of hydrogen peroxide by the yeast. The carbon dioxide produced during fermentation dissolves in water, resulting in a decrease in pH. Hence, the carbon dioxide produced will also affect the rheological properties of the fermented dough (Spies 1997).

The main whey proteins contained in yoghurt, the α -lactalbumin (four disulphide bonds) and the β -lactoglobulin, which can be a monomer, dimer and an oligomer (depending on the pH, ionic strength and temperature), have a globular structure and a hydrophobic, compact folded polypeptide chain, which results in a decrease in the water absorption of dough (Houben et al. 2012). Some of the useful properties of dairy proteins, however, are the emulsifying and stabilizing ability of caseinates, the gelling properties of whey protein concentrates and isolates, which is evident in the water-absorption capacity of high-heat non-fat dry milk (Chandan 1997).

CMC is a derivative of cellulose with carboxymethyl groups bound to some of the hydroxyl groups present in the glucopyranose monomers that form the cellulose backbone. The addition of hydrocolloids such as CMC increases the water absorption and dough development time (DDT) of rice dough, and has been attributed to the hydrophilic character of these polymers (Leon et al. 2002).

Commercial TG preparations for food applications have been available for several years. In industrial processes, this enzyme is mostly used in the meat and fish processing industry for the production of restructured meat as well as for dairy and tofu products (Herrero et al. 2008). Transglutaminases are

a family of enzymes (EC 2.3.2.13) that catalyses an acyl-transfer reaction between the γ -carboxamide group of peptide bound glutamine residues (acyl donor) and a variety of primary residues (acyl-acceptors) (Jaros et al. 2006). Formation of an isopeptide bond between a free amine group (e.g., protein- or peptide-bound lysine residues) and the γ -carboxamide group of protein- or peptide-bound glutamine residues causes the formation of high molecular weight polymers. It seems to be the predominant reaction caused by TG in nature (Jong and Koppelman 2002). In the absence of primary amines, water molecules are used as acyl-acceptors. The γ -carboxamide side chains are de-amidated, forming glutamic acid residues (Motoki and Seguro 1998). TG therefore improves the water-holding capacity and reduces the required work input during mixing (Basman et al. 2002).

Cyclodextrinase or cyclodextrin glycosyl transferase (EC 2.4.1.19) catalyses four different reactions: cyclization, coupling, disproportionation and hydrolysis (Ohnishi et al. 1997). Cyclodextrins are the end-products from these reactions. They are formed due to the hydrolysis and cyclization of starch, releasing closed circular molecules of six, seven, or eight glucose units that are referred to as α -, β -, or γ - cyclodextrin, respectively (Gujral et al. 2003). These molecules possess a polar surface responsible for the aqueous solubility and a hydrophobic inner core (Gujral et al. 2003). Thus, they are typically characterized as having a hydrophilic exterior. This property allows them to dissolve in water, while the hydrophobic cavity allows for the formation of inclusion complexes with a wide variety of hydrophobic guest molecules. The cyclodextrins form complexes with fatty acids and emulsifiers influencing the rheological properties of starch and functionality of the resultant starch (Rosell 2009). Although it is reported that the optimum activity of cyclodextrinase is 70 °C, it must be acting on the damaged starch during the mixing process and also during the proofing stage (30 °C), bringing about some hydrolysis, which affects the dough rheology.

The application of yeast, CMC, YG, TG and CG still remain to be evaluated with oat dough formulation. Monitoring the enzymatic reaction by screening accurate measurement of the dough properties, while using relevant processing conditions, is consequently important in the development of improved oat dough. Therefore, the aim of the present study was to investigate the effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough, with a view to establish the combination for improved mixing properties.

Materials and methods

Source of materials

Commercial oat flour was purchased from Health Connection Wholefoods (Cape Town, South Africa). Cyclodextrinase

(CG) and Transglutaminase (TG) (Activa WM) were donated by Novozymes (Johannesburg, South Africa) and Maccallum & Associates (Cape Town, South Africa; representing Ajinomoto Company in South Africa), respectively. Among all the available hydrocolloids, carboxymethylcellulose (CMC) was selected for this work because it significantly increases the water absorption capacity of dough in cold water, reduces the stickiness of dough, as well as increases the plasticity and elasticity of dough leading to improved machinability and raising-power of the dough (Walocel 2015). CMC and DATEM (diacetyl tartaric acid ester of mono- and diglycerides or E472e) and fat were kindly donated by Danisco/Dupont (Cape Town, South Africa). Plain yoghurt (YG), sugar, instant dry yeast and salt were purchased from a local supermarket. All ingredients used in this study were of food grade.

Proximate analysis of oat flour

All chemical analyses of the oat flour were performed in triplicate. Moisture, total ash and protein contents were respectively determined according to the standard methods AACC 44–15A (AACC 1994a), AACC 08–02 (AACC 1994b) and AACC 46–30 (AACC 1994c).

Particle size distribution of oat flour

Particle size distribution of oat flour was determined by using a U.S. standard sieve (212 μm mesh). A known weight of oat flour (100 g) was placed on the sieve and the weight of samples retained on a sieve was recorded after 10 min of shaking at 1400 rpm (Chen et al. 1988). The particle size distribution was expressed as the percentage of particles retained on each sieve (Toma et al. 1979). The particle size distribution analysis was performed in triplicates.

Determination of protein concentration of TG and CG

The protein concentration of the TG and CG was determined using the standard Bradford's protein assay (Bradford 1976). It is a spectroscopic analytical method used to determine the concentration of protein in a solution and is dependent on the amino acid composition of the measured protein. This method is mainly characterized by the binding of Coomassie Brilliant Blue G-250 dye to proteins. This blue protein-dye form is detected at 595 nm in the assay using a spectrophotometer or microplate reader. At different stages of purification, the enzyme samples of TG and CG were diluted (1/10, 1/100 and 1/1000). Bovine Serum Albumin (BSA) (1.0 ml) and dye (0.5 ml) reagents were added to 0.1 ml of the enzyme sample. Protein concentration was determined from a standard curve generated from the absorbance detected for known

concentrations of bovine serum albumin (BSA). This analysis was performed in triplicates.

Experimental design for mixing properties

A 2^{5-2} fractional factorial resolution III design was used to determine the main effects of independent variables on the mixing properties of oat dough. The independent variables (yeast (X_1), CMC (X_2), plain yoghurt (X_3), transglutaminase (X_4) and cyclodextrinase (X_5)), and their quantities are detailed in Table 1. The outline of the experimental design (11 runs) with the coded levels (-1 =low, 0 =middle, $+1$ =high values of independent variables) are summarized in Table 2. Each point of the design was performed in triplicates with the centre in four replicates. Following the combination of the ingredients as per the design, dough samples were produced following the process described in the next paragraph. The experiments were carried out in randomized order. The dependent variables of the experimental design were mixing variables were water absorption, arrival time, stability, energy at peak, peak resistance, development time, departure time, softening and bandwidth at peak as presented in Table 3.

The mixing properties of the oat dough were determined using DoughLab (Perten Instruments, Warriewood, Australia) following the method reported by McCann and Day (2013) with some modification. Following the design in Table 2, all the ingredients were placed in the DoughLab mixing bowl, and were mixed. After tempering the solids, the water required for optimum consistency was added. Particular attention was given to the determination of water absorption to ensure the complete hydration of all the components. The settings used as required by the equipment in the test were 200 g as base flour amount, 13.10 % as sample moisture, 30 % expected absorption, 500 FU as target peak resistance. Water absorption and peak resistance were automatically adjusted at the end of analysis. The amount of solid material added, the total amount of solid material added and amount of liquid material added

Table 1 Process variables and their levels used in the 2^{5-2} fractional factorial design for oat dough preparation^{1, 2}

Factor (/100 g flour)		Lower level (-1)	Upper level ($+1$)
Yeast (g)	X_1	1.25	3.25
CMC (g)	X_2	1	2
YG (g)	X_3	10.75	33.75
TG (g)	X_4	0.5	1.5
CG (μl)	X_5	10	40

¹ Transformation of coded variable (x_i) to uncoded variable (X_i) levels could be obtained from: $X_1 = x_1 + 2.25$; $X_2 = 0.5x_2 + 1.5$; $X_3 = 11.50x_3 + 22.25$; $X_4 = 0.5x_4 + 1$; $X_5 = 15x_5 + 25$

² CMC, Carboxymethylcellulose; YG, Plain yoghurt; TG, Transglutaminase; CG, Cyclodextrinase

Table 2 Independent variables and quantities used for the 2^{5-2} fractional factorial design for oat dough formulations^{1, 2}

Run	Ingredients				
	Yeast	CMC	YG	TG	CG
1	−1	−1	−1	+1	−1
2	+1	−1	−1	+1	+1
3	−1	+1	−1	−1	+1
4	+1	+1	−1	−1	−1
5	−1	−1	+1	−1	+1
6	+1	−1	+1	−1	−1
7	−1	+1	+1	+1	−1
8	+1	+1	+1	+1	+1
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0

¹ Coded levels of the quantity of ingredients (−1, 0, +1) corresponds to lower, middle and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 µl) per 100 g of oat flour

² CMC, Carboxymethylcellulose; YG, Plain yoghurt; TG, Transglutaminase; CG, Cyclodextrinase

were dependent on each point of the experimental design. The mixing time of each assay was 20 min.

Each design point was performed in triplicates with the centre in four replicates. The experiment was carried out in randomized order. The parameters described in Table 3 were obtained from the software version DLW 1.0.7.58 to assess the dough mixing properties.

Statistical analysis

Multivariate Analysis of Variance (MANOVA) was used to determine the differences between treatments for determining significant effects. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS 2010).

The system behavior was described by a linear factorial model regression, carried out using Design-Expert software (Trial version 7.0.0, Static Made Easy, Minneapolis, Minnesota, USA) and given by:

$$Y = \beta_0 + \sum_{k=1}^5 \beta_i X_i + \sum_{k=1}^5 \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where Y is the response variable, β_0 is a constant; β_i and β_{ij} are the linear and interactive coefficients, respectively; X_i and X_j are the levels of the ingredients and ε is the random error. The quality of the fit of the linear model equation was evaluated by R^2 , adjusted R^2 , adequate precision (AP) and lack of fit. The significance of the regression coefficient was verified by determining the p -value.

Optimizing the amount of yeast, CMC, YG, TG and CG was required to obtain the parameters required for the optimal machinability of oat dough, a vital aspect in the making of gluten-free bread. The optimization objective was to minimise energy at peak and development time of oat dough while maximising water absorption and peak resistance of oat dough.

Design Expert-8 was used to estimate desirability, an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimisation found a point that maximizes the desirability function. The variations observed in the mixing parameters of oat dough such as water absorption,

Table 3 Mixing parameters assessed by DoughLab

Parameters	Definition
Water absorption	Percentage of water required for the dough to produce a torque of 1.1 ± 0.07 Nm or Water absorption corrected for target peak resistance and actual flour moisture content (typically to 14 % moisture basis).
Arrival time	The time required for the top (maximum) curve to reach the peak resistance. This value is related to the rate at which water is taken up by the flour.
Stability	The elapsed time at which the torque produced is 1.1 ± 0.07 or the difference between the arrival and departure times. Stability indicates the flour's tolerance to mixing.
Development time	The time to reach the maximum torque at 30 °C or the time taken for the dough to reach the peak resistance, between times T1 and T2. Development time is related to the protein content and quality of the flour sample, and the test conditions used.
Departure time	The required time for the top (maximum) curve to fall below the peak resistance. A longer departure time indicates stronger flour.
Softening	The difference in torque between the peak resistance and the middle (average) curve at the specified time after the development time (typically 12 min).
Bandwidth at peak	The difference in torque between the top (maximum) and bottom (minimum) curves at the development time.
Peak resistance	The maximum torque attained, as measured from the middle (average) curve, between times T1 and T2.
Energy at peak	Maximum energy required to stretch the test piece to its rupture point or the accumulated mechanical energy applied to the dough up to the development time.

Source: DoughLab (2009)

Table 4 Effect of Yeast, CMC, YG, TG and CG on the water absorption, arrival time, stability, energy at peak and peak resistance of oat dough^{1, 2}

Run	Ingredients					Response variables				
	Yeast	CMC	YG	TG	CG	Water absorption (%)	Arrival time (min)	Stability (min)	Energy at peak (Wh/kg)	Peak resistance (FU)
1	-1	-1	-1	+1	-1	38.45±0.49	2.95±0.21	7.40±0.85	11.45±2.33	40.30±19.09
2	+1	-1	-1	+1	+1	37.70±0.57	2.80±0.71	7.50±2.69	10.45±0.46	816.00±20.08
3	-1	+1	-1	-1	+1	35.75±0.07	3.60±0.85	16.40±0.85	20.65±1.91	736.50±4.24
4	+1	+1	-1	-1	-1	35.15±0.07	2.90±0.14	16.90±0.42	15.55±4.03	712.90±2.83
5	-1	-1	+1	-1	+1	34.80±0.99	1.55±0.07	2.05±0.64	3.75±0.78	696.40±40.87
6	+1	-1	+1	-1	-1	33.70±0.14	1.95±0.07	5.75±0.35	4.25±0.21	652.00±5.66
7	-1	+1	+1	+1	-1	33.90±0.57	2.25±0.21	7.21±2.69	5.65±0.78	663.95±21.99
8	+1	+1	+1	+1	+1	34.80±0.71	2.00±0.42	10.06±10.82	4.85±0.78	699.90±25.60
9	0	0	0	0	0	35.77±0.55	2.92±0.31	4.22±0.83	9.17±1.39	737.77±22.44

¹ Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 µl)

² CMC, Carboxymethylcellulose; YG, Plain yoghurt; TG, Transglutaminase; CG, Cyclodextrinase

arrival time, stability, development time, departure time, softening, bandwidth at peak, peak resistance and energy at peak were examined by principal component analysis (PCA) with the computer software SIRIUS (Pattern Ret System A/S, N-5015, Bergen, Norway). The procedure of cross-validation was used to determine the significance of the principal components (PC) (Weld 1978). The results of the PCA are presented as a plot where the treatments (PC-scores) and attributes (PC-loadings) are represented on the same plot.

Results and discussion

Proximate composition and particle size distribution of oat flour

The proximate composition analysis showed that oat flour was consisted of moisture (13.10±0.00 %), ash (1.04±0.02 %), protein (9.13±0.06 %), and particle size <212 µm (67.4±0.41 %) and 212 µm (31.68±0.64 %). All these values were not within the range highlighted in the literature (Flander et al. 2007). It could be explained by the use of different varieties of oats cereals and some processing factors such as degree of milling.

Protein concentrations of TG and CG

The protein concentrations of transglutaminase (TG) and cyclodextrinase (CG) were respectively 35.75±0.40 and 51.75±0.42 µg/ml. The low protein concentration of TG could be due to the presence of insoluble protein polymers inhibiting the proteolytic activity whereas CG contains soluble proteins. CG proteins were easily degraded during the proteolysis and hence yielding higher protein concentration.

Effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

Model adequacy

The effects of independent variables on the mixing properties of oat flour are outlined in Tables 4 and 5. The linear model regression coefficients for mixing properties of oat dough are detailed in Table 6. The model *p*-value ranged from <0.0001 to 0.0105 indicating that the linear models were significant (*p*<0.05) for each response in explaining the variation between the independent and dependent variables. The adequacy of precision (estimation of the signal to noise ratio) ranged from 7.12 to 22.35. A ratio of four is desirable. These values being greater to 4 indicated that the models were adequate. The significant lack of fit ranged from 0.09 to 8.07 indicated that lack of fit was not significant (*p*>0.05) and hence the model was adequate. The adjusted *R*² ranged from 0.43 to 0.91 indicating variation in the fit for the models. The adjusted coefficients of correlation (*R*²_{adj}) of water absorption (0.84), energy at peak (0.89), peak resistance (0.84) and development time (0.91) exhibited better goodness of fit compared to other parameters (Table 6). In general the models were adequate in explaining the variation between the independent and dependent variables and could be used to navigate the design space. However, for optimising the effects models with higher *R*² were used.

Main effect of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

The linear model regression coefficients for mixing properties of oat dough for each response variable are shown in Table 6. Yeast did not have significant (*p*>0.05) effect on all the

Table 5 Effect of yeast, CMC, YG, TG and CG on the development time, departure time, softening and bandwidth at peak of oat dough^{1, 2}

Run	Ingredients					Response variables			
	Yeast	CMC	YG	TG	CG	Development time (min)	Departure time (min)	Softening (FU)	Bandwidth at peak (FU)
1	-1	-1	-1	+1	-1	4.95±0.78	10.40±0.71	67.75±11.95	51.25±0.49
2	+1	-1	-1	+1	+1	4.75±0.21	10.25±1.91	73.50±22.20	54.35±10.25
3	-1	+1	-1	-1	+1	9.45±0.78	20.00±0.00	0.00±0.00	45.90±3.25
4	+1	+1	-1	-1	-1	7.80±1.56	19.80±0.28	10.70±15.13	47.85±1.34
5	-1	-1	+1	-1	+1	2.10±0.28	3.65±0.78	93.20±0.57	44.15±4.17
6	+1	-1	+1	-1	-1	2.65±0.21	7.50±0.14	39.40±2.26	40.50±0.71
7	-1	+1	+1	+1	-1	3.35±0.35	9.40±2.55	43.10±0.99	41.00±0.28
8	+1	+1	+1	+1	+1	2.80±0.57	12.05±11.24	58.85±26.80	41.70±2.97
9	0	0	0	0	0	4.59±0.55	7.14±0.95	73.04±13.31	45.42±2.20

mixing properties of oat dough. However, it slightly decreased the water absorption ($F(1, 24)=1.98$, $p=0.1773$) (Fig. 1) and peak resistance ($F(1, 24)=1.64$, $p=0.2160$) (Fig. 2) of oat dough when its level was increased from 1.25 to 3.25 g (Tables 4 and 5). The yeast consumed water as a nutrient to achieve the fermentation process of glucose and therefore it slightly ($p>0.05$) decreased the water absorption (38.45–37.70 %) and peak resistance (840.30–816.00 FU) of oat dough (Fig. 2). As the level of yeast decreased from 3.25 to 1.25 g, the energy at peak slightly ($F(1, 24)=3.96$, $p=0.0629$) increased (10.45–11.45 Wh/kg) (Tables 4 and 5, Fig. 3) while the development time of the oat dough slightly

($F(1, 24)=2.31$, $p=0.1472$) increased (2.10–2.65 min) as yeast level varied from 1.25 to 3.25 g (Tables 4 and 5, Fig. 4). These variations could be explained by the YG addition decreasing dough pH and limiting starch degradation of oat flour into dextrins or simple sugars used as substrates by yeast during the mixing process. Therefore, the YG acidity could be slowing down the significant effect of yeast.

The mixing parameters such as stability ($F(1, 24)=12.49$, $p=0.0024$), energy at peak ($F(1, 24)=26.27$, $p=0.0001$), development ($F(1, 24)=49.69$, $p<0.0001$) and departure ($F(1, 24)=23.66$, $p=0.0001$) times, water absorption ($F(1, 24)=21.10$, $p=0.0003$), peak resistance ($F(1, 24)=19.34$,

Table 6 Regression coefficients of linear model for mixing properties of oat dough^{1, 2}

Coefficients	Response variable								
	Water absorption (%)	Arrival time (min)	Stability (min)	Energy at peak (Wh/kg)	Peak resistance (FU)	Development time (min)	Departure time (min)	Softening (FU)	Bandwidth at peak (FU)
Linear									
β_0	41.1127	3.0080	6.7408	15.9406	910.0339	5.0751	8.4989	94.1279	52.0907
β_1	-1.0000	0.3625	0.8937	1.2250	-37.4375	0.7500	0.7687	-30.6375	-0.9375
β_2	-2.9510 ¹	0.6333	4.4833 ¹	4.8208 ¹	-93.4531 ¹	3.6156 ¹	4.5083 ¹	-59.4979 ¹	-1.9167
β_3	-0.1071 ¹	-0.0489 ¹	-0.2516 ¹	-0.4304 ¹	-4.2766 ¹	-0.1755 ¹	-0.3027 ¹	0.8978	-0.3478 ¹
β_4	1.3625 ¹	–	-2.2375	-2.9500 ¹	55.5875 ¹	-1.5625 ¹	–	24.9750 ¹	2.4750
β_5	-0.0133	-0.0258	-0.1592	-0.1217	0.6637	–	-0.1808	1.9008	0.2458
Interaction									
β_{12}	0.5375	-0.3000	–	-1.3500	20.2625	-0.6625 ¹	–	18.6250	0.8000
β_{25}	0.01917	0.0167	0.0992	0.0967	–	5.5000E-003	0.1142	-0.9083	-0.1333
R^2	0.8883	0.6582	0.5697	0.9228	0.8808	0.9328	0.6061	0.6817	0.6587
Model p -value	<0.0001	0.0017	0.0105	<0.0001	<0.0001	<0.0001	0.0020	0.0026	0.0044
Adjusted R^2	0.8423	0.5443	0.4263	0.8910	0.8411	0.9104	0.5025	0.5507	0.5181
AP	15.2500	9.0450	7.124	18.5830	16.1950	22.3500	8.5740	8.4060	7.4390
Lack of fit	1.0600	3.6100	8.0700	0.3600	1.0700	0.2200	5.0000	18.7500	0.0900

¹ significant at $p<0.05$, β = constant, β_1 = effect of yeast, β_2 = effect of CMC, β_3 = effect of YG, β_4 = effect of TG, β_5 = effect of CG, R^2 : regression coefficient, AP, Adequate Precision

² CMC, Carboxymethylcellulose; YG, Plain yoghurt; TG, Transglutaminase; CG, Cyclodextrinase

Fig. 1 Effect of CMC and Yeast on the water absorption of oat dough based on 100 g of oat flour

Fig. 2 Effect of CMC and Yeast on the peak resistance of oat dough based on 100 g of oat flour

$P=0.0004$) and softening ($F(1, 24)=16.91$, $p=0.0007$) were significantly ($p<0.05$) affected by CMC (Table 6). Stability (7.40–16.40 min), energy at peak (11.45–20.65 Wh/kg), development (4.95–9.45 min) and departure (10.40–20.00 min) times, increased with increase in CMC from 1 to 2 g (Tables 4 and 5). Conversely, water absorption (38.45–35.75 %), peak resistance (840.30–736.50 FU), softening (67.75–0.00 FU) and bandwidth at peak (51.25–45.90 FU) decreased with increased amounts of CMC from 1 to 2 g (Tables 4 and 5). The addition of CMC lowered water absorption (38.45–35.75 %) as its level increased from 1 to 2 g (Tables 4 and 5, Fig. 1). These changes were caused by a decrease of the absorption capacity of oat flour indicating that CMC resulted in flour hydrating processes that were slower, causing the dough to have a lower hydrating ability. Lazaridou et al. (2007) showed that the addition of CMC increased the water absorption of rice flour. This was attributed to the hydrophilic character of CMC (Leon et al. 2002). However, the results presented here differ. CMC effectively bound water molecules through hydrogen bond formation at $6.5 \leq \text{pH} \leq 9$. Furthermore, the addition of non-starch polysaccharides such as CMC to starch-water systems limited the hydration of the starch and, since water has a plasticizing effect in amorphous regions of the

Fig. 3 Effect of CMC and Yeast on the energy at peak of oat dough based on 100 g of oat flour

starch, the mobility of the plasticizer will also be restricted. Thus, the non-starch polysaccharides might have an “anti-plasticizing” effect (Bertolini et al. 2005). A decrease in pH was noted during the mixing process and was attributed to the lactic acid present in the yoghurt. The lactic acid could inhibit any hydrogen bond formation and therefore resulted in limited water absorption by CMC. This decrease in water absorption lead to increased energy at peak (Fig. 3), a decreased peak resistance (Fig. 2) as well as an increased development (Fig. 4) and departure times, due to the fact that the oat flour required less water, less resistance and more energy to reach a dough consistency of 500 FU. These findings were not consistent with the study of Lazaridou et al. (2007) which was based on rice flour. According to Lazaridou et al. (2007), the addition of CMC increased the water absorption capability of rice dough. This was attributed to the ability of CMC to bind large amounts of water in a gluten-free system. In addition, the rice dough exhibited an increase in dough development time (Lazaridou et al. 2007). This increase of development time is consistent with the studies performed by Sivaramakrishnan et al. (2004) on rice flour fortified with 4.5 % hydroxypropylmethycellulose (HPMC). The stability of oat dough measured by DoughLab improved flour strength, with higher values being related to stronger doughs (Rosell et al. 2001) such as wheat dough as

the level of CMC increased from 1 to 2 g. Therefore, stability of oat dough was clearly positively affected by CMC, indicating that CMC acted as a gluten replacer (Tables 4 and 6). Therefore, CMC rendered the oat flour dough tolerant to mixing. The dough softening was significantly decreased by the presence of CMC as the dough water absorption decreased.

Yoghurt significantly ($p < 0.05$) decreased all responses (Table 6), with the exception that it caused significant ($F(1, 24) = 4.44, p = 0.05$) increase in softening (10.70–93.20 FU) as its level increased from 10.75 to 33.75 g. The decreased water absorption (38.45–33.90 %) and increased dough softening (10.70–93.20 FU) were respectively due to the decreased pH caused by the lactic acid in the yoghurt and dairy proteins acting as gluten replacers. In addition, the main whey proteins contained in yoghurt, the α -lactalbumin (four disulphide bonds) and the β -lactoglobulin, which can be a monomer, dimer and an oligomer depending on pH value, ionic strength and temperature, have a globular structure and hydrophobic, compact folded polypeptide chain, hence decreasing water absorption of oat dough (Houben et al. 2012). Conversely, dairy products such as yoghurt, that have been used in gluten-free bread formulas, increased water absorption as its level decreases and, therefore, enhanced the softening properties of gluten-free dough

Fig. 4 Effect of CMC and Yeast on the development time of oat dough based on 100 g of oat flour

(Gallaher et al. 2004; Nunes et al. 2009). According to Gallagher (2009), dairy proteins possess functional properties similar to gluten, as they are able to form networks and have good swelling properties. Some of the useful properties of dairy proteins are the emulsifying and stabilizing ability of caseinates, the gelling properties of whey protein concentrates and isolates, as well as the water-absorption capacity of high-heat, non-fat dry milk (Chandan 1997).

TG significantly ($p < 0.05$) affected water absorption ($F(1, 24) = 24.49$, $p = 0.001$), peak resistance ($F(1, 24) = 25.60$, $p < 0.001$), softening ($F(1, 24) = 6.50$, $p = 0.0208$), energy at peak ($F(1, 24) = 13.47$, $p = 0.019$) and development time ($F(1, 24) = 24.61$, $p = 0.001$) (Table 6). Water absorption (34.80–38.45 %) and peak resistance (696.40–840.30 FU) increased with the increase of TG from 0.5 to 1.5 g. Conversely, the energy at peak (11.45–3.75 Wh/kg) and development time (4.95–2.10 min) decreased when TG concentration was decreased from 1.5 to 0.5 g. In addition, TG decreased the dough softening (93.20–67.75 FU) as its level varied from 0.5 to 1.5 g. The rise in water-holding capacity was attributed to the cross-linking that occurred after TG addition, which caused changes in secondary structure or, possibly, due to changes in protein hydrophobicity from the formation of glutamic acid residues from glutamine hydrolysis (Gerrard

et al. 1998). However, this increase in water absorption is not consistent with the findings of Basman et al. (2002), Huang et al. (2010) and Han et al. (2011) whose studies were based on wheat dough, oat dough and buckwheat dough, respectively. This increase of water absorption of oat dough could be due to the added yoghurt. According to Huang et al. (2010), TG decreased the water absorption of oat dough. This decrease of water absorption was attributed to acyl-transfer reactions that introduced new functional groups leading to changes in the structure, charge, and hydrophobicity of the proteins (Han et al. 2011). The increased development time and peak resistance of oat dough increased the dough extensibility because of the modification of the cross-link between the oat proteins catalysed by TG. Similar findings were reported by Huang et al. (2010) on oat flour. These findings are mainly attributed to the cross-linking catalysed by TG. TG did not significantly affect cooking stability of the oat dough because no significant difference was observed in the oat starch after TG treatment. Similar results are reported by Siu et al. (2002) from research based on oat flour. Hence, TG did improve the mixing properties of oat dough.

CG did not significantly ($p > 0.05$) affect water absorption ($F(1, 24) = 2.82$, $p = 0.1113$), arrival time ($F(1, 24) = 0.014$, $p = 0.9084$), stability ($F(1, 24) = 0.025$, $p = 0.8757$), energy at peak

($F(1, 24)=0.76, p=0.3960$), peak resistance ($F(1, 24)=3.28, p=0.0866$), softening ($F(1, 24)=2.72, p=0.1177$), bandwidth at peak ($F(1, 24)=0.70, p=0.4150$) of oat dough during the mixing process (Table 6). As its level increased from 10 to 40 μl , the water absorption (38.45–34.80 %), arrival time (2.95–1.55 min), stability (7.40–2.05 min), energy at peak (11.45–3.75 Wh/kg), peak resistance (840.30–696.40 FU) and bandwidth at peak (51.25–44.15 FU) slightly ($p>0.05$) decreased (Tables 4 and 5) while softening of oat dough increased from 67.75 to 93.20 FU. CG did not have any effect on development time of oat dough (Table 4). These variations were attributed to the decreased dough pH limiting starch degradation of oat flour into dextrins or simple sugars caused by CG activity. This decreased the availability of simple sugars which were used as substrates by yeast during the mixing process. The optimum pH range of CG is between 7.5 and 8.5. As the yoghurt was added to oat flour, the lactic acid present decreased the pH, thereby inhibiting the significant action of the CG.

Interaction effect of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

There were two types of interactions during the mixing of oat dough. The first one was between yeast and CMC and the second one between CMC and CG. The combined effect of yeast and CMC was significantly ($p<0.05$) decreased the development time of oat dough during the mixing process (Table 6). However, yeast and CMC slightly ($p>0.05$) increased water absorption, peak resistance, softening and

bandwidth at peak and decreased arrival time, energy at peak of oat dough (Tables 4 and 5). Their interaction effect did not affect the departure time of oat dough. The efficiency of the interaction between yeast and CMC might be explained by the hydrophilic character of CMC and production of carbon dioxide and ethanol by yeast, thereby increasing dough water absorption (Table 6).

In combination CMC and CG slightly ($p>0.05$) increased water absorption, arrival time, stability, energy at peak, development time and departure time but decreased softening and bandwidth at peak of oat dough (Tables 4 and 5). This could be due to the hydrophilic character of CMC and starch degradation by CG, increasing dough water absorption (Table 6).

Optimisation of ingredients

The optimisation goal was to maximise water absorption and peak resistance while minimising energy at peak and development time. The quantity of ingredients for preparing the optimal oat dough formulation per 100 g of oat flour was: yeast (1.25 g), CMC (1 g), yoghurt (20.66 g), TG (1.50 g) and CG (40 μl) with desirability of 0.83. Under this formulation, the model predicts a maximum of water absorption of 37.6 %; maximum peak resistance of 816.7 FU; minimum energy at peak of 6.3 Wh/kg and minimum development time of 2.3 min. To verify the results, an experiment was performed using optimal conditions. An average values of water absorption (36.15 ± 0.64 %), peak resistance (752.9 ± 24.46 FU), energy at peak (10.45 ± 0.35 Wh/Kg) and development time (5.10 ± 0.14 min) were obtained, which is close to the model

Fig. 5 Principal component analysis of dough mixing parameters

predicted values. This confirms that the model was adequate to predict the effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough.

Yeast, CMC, YG and CG might contribute to the strength of hydrophobic and electrostatic bondages of oat dough as these bondages are not sensitive to the high pressure levels exerted by yeast, CMC, YG and CG, thereby decreasing the water absorption of oat dough. However, small pressure levels promoted by TG weakened the protein network by dissociating and weakening hydrophobic and electrostatic bonds of oat dough, hence increasing the water absorption and the peak resistance of the dough (Houben et al. 2012).

Using Response Surface Methodology, the achieved mathematical models expressed in unscaled variables are presented in Table 6. Therefore, the water absorption, peak resistance, energy at peak and development time of oat dough can be predicted by the equations for the experimental area under consideration:

$$\begin{aligned}\text{Water absorption} &= 41.1127 - X_1 - 2.951 X_2 - 0.1071 X_3 + 1.3625 X_4 - 0.0133 X_5 + 0.5375 X_1 X_2 + 0.01917 X_2 X_5. \\ \text{Peak resistance} &= 910.0339 - 37.4375 X_1 - 93.4531 X_2 - 4.2766 X_3 + 55.5875 X_4 + 0.6637 X_5 + 20.2625 X_1 X_2. \\ \text{Energy at peak} &= 15.9406 + 1.225 X_1 + 4.8208 X_2 - 0.4304 X_3 - 2.95 X_4 - 0.1217 X_5 - 1.35 X_1 X_2 + 0.0967 X_2 X_5. \\ \text{Development time} &= 5.0751 + 0.75 X_1 + 3.6156 X_2 - 0.1755 X_3 - 1.5625 X_4 - 0.6625 X_1 X_2 + 5.5E-003.\end{aligned}$$

Components explaining the variation in mixing properties of oat dough as affected by yeast, CMC, YG, TG and CG

Principal component analysis (PCA) indicated that 85.5 % of the variation in the data could be explained by two components (Fig. 5). Component 1 explaining 52.3 % of the variation loaded highly on dough strength (stability and departure time). Component 2 contributing 33.2 % of the variation loaded on dough resistance (water absorption and peak resistance). CMC significantly increased dough strength while yoghurt reduced it significantly. TG significantly ($p < 0.05$) increased the resistance of the dough to mixing while CMC and yoghurt reduced it significantly ($p < 0.05$). Hence, CMC, TG and yoghurt were ingredients of choice for the modification of oat dough.

Conclusion

The aim of this study was to determine the effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough as well as to optimise the oat dough formulation through the establishment of the amount of yeast, CMC, YG, TG and CG

required, for optimal oat dough production. Among all the ingredients, only CMC, YG, and TG exhibited remarkable improvements on the machinability of oat dough. Yeast and CG did not have noteworthy effects on oat dough during mixing. The amounts of the ingredients were also optimized to obtain optimal process conditions. Optimized dosages of 1.25 g yeast/100 g flour, 1 g CMC/100 g flour, 20.66 g YG/100 g flour, 1.50 g TG/100 g flour, 40 µl CG/100 g flour (maximum of the respective response surface plot) led to maximized values for both water absorption (37.6 %) and peak resistance (816.7 FU), as well as minimized values for both energy at peak (6.3 Wh/kg) and development time (2.3 min), characteristics typically described as good indicators and predictors of the quality of fresh formulated oat dough to be obtained. TG when added alone resulted in a desirable increase in the water absorption and peak resistance of dough. The incorporation of both yeast and CMC into the dough formula is recommended because of the increase of the induced water absorption and peak resistance effects of the yeast/CMC formulated dough. YG and TG annulated the energy at peak and development time of dough when they were added at the dosage of 20.66 g YG/100 g flour and 1.50 g TG/100 g flour, respectively. Simultaneous presence of yeast/CMC is advisable because of the beneficial interactive effect on dough energy at peak and development time.

Formulations derived from statistical methods, such as response surface methodology, allow for the development of new gluten-free bread recipes. Such formulations in turn can be applied in the bread industry where there is a continuous need to meet the increasing consumer demands for bread products that is gluten-free, and yet aesthetically pleasing.

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