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Article *in* Journal of Food Science and Technology -Mysore- · June 2014

DOI: 10.1007/s13197-013-1224-3

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ORIGINAL RESEARCH PAPER

EFFECT OF REDUCING AGENTS ON WHEAT GLUTEN AND QUALITY CHARACTERISTICS OF FLOUR AND COOKIES

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Received on 30th August 2013
Revised on 14th October 2013

The aim of the present study was to determine the effect of reducing agents (L-cystine, glutathione and proteases) on wheat gluten recovery and quality characteristics of dough and cookies. PBW-343 and RAJ-3765 wheat varieties were analysed for physico-chemical properties which indicated that wheat variety RAJ-3765 had superior quality characteristics in comparison to PBW-343. Wet gluten and dry gluten %yields were reduced with addition of reducing agents. As the concentration of reducing agents increased gluten, yield decreased further. The dough strength (resistance to extension) decreased, whereas extension of dough increased significantly with the addition of reducing agents. Upon addition of reducing agents, spread factor increased, whereas hardness decreased. Glutathione was found to be the most effective reducing agent out of the three reducing agents used in this study.

Keywords: wheat, gluten, L-cystine, glutathione, protease, cookies.

Introduction

Wheat is the most widely cultivated cereal crop in the world and mainly used for milling and baking. Some wheat varieties (e.g. *Triticum aestivum*) are suitable for bread making while others (e.g. *Triticum durum*) are suitable for biscuits and cooking making (Sapirstein *et al.,* 2007). The major factor for the suitability of wheat varieties for making different types of bakery products is the ability to form gluten network. Gluten, the protein component of flour which gives the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents (Wieser, 2007; Kaushik, *et al.,* 2013). In wheat products such as bread, gluten network formation is desirable for gas retention and better volume of product, while in products such as biscuits, extensibility is required, so gluten formation is undesirable. Getting the desired quality of wheat flour for making specific kinds of bakery products is a challenging task for bakery industries.

An alternative for the management of wheat flour is the modification of the gluten protein by different agents (Liu *et al.,* 1996). Gluten-modifying agents such as oxidizing and reducing agents are used in controlling the rheological properties of wheat flour (Sandhu *et al.*, 2011). Reducing agents cleave the intermolecular and intramolecular disulfide bonds in the gluten proteins. This cleavage results in reduced molecular weight for the proteins, and the extensibility of the dough is increased (Stauffer, 1994). The incorporation of reducing agents, such as L-cysteine hydrochloride (L-cysteine HCl), reduced the water absorption capacity (WAC) and stability of medium-strong wheat flour as well as of weak wheat flour (Ravi *et al.,* 2000).

Therefore, the aim of the present study was to investigate the comparative effect of L-cystine, proteases and glutathione at three different concentrations on wheat gluten extraction and gluten network. Two wheat varieties were milled and flour quality was evaluated after addition of reducing agents.

Materials and methods

Materials

Two wheat varieties *viz.* PBW-343 and RAJ-3765 were purchased from the wheat breading farm, Haryana Agriculture University, Hisar (India). Wheat grains were stored in airtight plastic containers with parad tablets (Himalya, India) enclosed in cloth for protection of wheat grains.

Chemicals

Reducing agents *viz*. L-cysteine hydrochloride, glutathione and protease enzyme employed in this study were of analytical grade and purchased from Rankem (Ranbaxy, New Delhi, India).

Physical parameters of grains

Test weight, thousand-kernel weight, length and breadth were determined using standard methods as described by AACC (2005).

Milling of wheat varieties

Wheat grains were tempered for 24 h to 16% moisture content and milled using a roller-mill (Chopin Laboratory CD-1 mill, France). Flour obtained was stored in airtight plastic containers under ambient conditions for further analysis. To ensure the purity of the roller-milled flour samples from each lot, mechanical and manual cleaning of the roller-mill, including air blasting, was applied between milling of each of the wheat samples.

Proximate analysis

Wheat grains and wheat flour samples of different wheat varieties were powdered in a Falling Number Mill (Model 3100, Sweden) to pass through a 100 mesh sieve and stored in airtight containers in a refrigerator till further chemical analysis. Samples were analyzed for moisture, ash and protein using methods as described by AACC (2005).

Gluten extraction

Gluten extraction was carried out by adopting the procedure as described by AACC (2005). Reducing agents were added to flour and mixed properly. The reducing agents were mixed in flour at three different concentrations (50, 75 and 100 ppm) and dough was prepared using 2% sodium chloride solution at the rate of 60% of the weight of flour. The prepared dough was kept immersed in water for 45 min and then it was placed in the gluten washer bowl and washed automatically for 5 min with water to recover wet gluten. The wet gluten was dried using gluten dryer.

Wet and dry gluten obtaining

The wet gluten yield of wheat varieties was determined. The dough was washed and gluten retained was collected and weighed for the determination of wet gluten yield. The wet gluten yield was calculated by the formula given below:

Wet gluten yield (%) = (weight of wet gluten obtained × 100) / weight of flour

The dry gluten yield was determined by drying wet gluten in gluten dryer (Infracant Kft, Focus Engineering GMK, Hungary).

Dry gluten yield (%) = (weight of dry gluten obtained × 100) / (weight of flour)

Sodium dodecyl sulfate (SDS Solution) sedimentation volume of flour samples was estimated according to the method as described by Axford *et al.* (1978).

Falling number test

The falling number was determined by the approved method as described by AACC (2005) and the results were expressed as time in seconds.

Dough resistance to extensibility and extensibility at break

Dough resistance to extensibility and extensibility at break of dough samples was determined using a Texture Analyzer TA.XT2i (Stable Micro System, Surrey, UK), operated according to the American Society for Testing and Materials (ASTM) standard method. Three specimens (40mm wide) of each film were measured and cut using a cutter. The peak loads and extension at break were recorded for testing film specimens. Resistance to extensibility and extensibility at break were calculated according to the ASTM method. Each test piece was placed centrally on the sample platform of Kieffer with the extension hook previously positioned beneath. Extensibility at break was determined by the following equation:

Extensibility = (Distance Sample Stretched ×100) / (Original length of sample)

Preparation of Cookies

Cookie dough consisted of shortening (91.2g), sugar (126.16g), and salt (1.86 g) sodium bicorbonate (2.22), dextrose solution (29 ml), flour (200g) and water (14 ml). The processing parameters used for preparation of cookies were creaming time (402 s), mixing time (100 s), cookie thickness (10 mm), baking temperature (180 ºC) and baking time (17 min). Cookies were prepared by mixing fat and sugar first for creaming, then by adding dextrose solution, sodium bicarbonate and salt with reducing agents and mixed for 2.5 min. The flour was then added, mixed for 1.66

min, sheeted and molded (60 mm diameter) and afterwards baked at 180 ºC for 17 min, cooled and packed in air tight containers.

Spread factor of cookies

Cookie dough, when heated, spreads at a rate presumably governed by the viscosity of the heated matrix. Spreading continues until some molecular event occurs that "sets" the structure, by causing a sudden large increase in dough viscosity (Abboud *et. al.,* 1985a). The final cookie diameter results from these two factors; a high spread rate plus a delayed set time gives the largest diameter, and the other three possible combinations give lower diameters. Spread factor was calculated with the following equation:

Spread factor = Diameter of cookies / Thickness of cookies

Hardness of biscuits / cookies

Cookie hardness was determined with a Texture Analyser TAXT2i (Stable Micro Systems Ltd., Surrey, UK) equipped with a 5 kg load cell in compression mode with a Knife Edge (HDP/BS) attached to the load cell carrier and lowered into the slotted insert. The Heavy Duty Platform (HDP/90) is repositioned so that there was no contact between the blade and slot surfaces and a 'blank' test run as a check. The blade was then raised to allow placement of the sample. Pre- and post-test speeds were 1.5 mm/s, while test speed was 2.0 mm/s. The maximum force reading (i.e. highest peak) was observed within the first seconds of the test. At this point the biscuit fractured into two major pieces. Hardness of cookies (Eight cookies) was determined after 30min. of baking with the Texture Analyser as the peak force of the three-point-bending test.

Statistical analysis

The data was analyzed using the method described by Kaushik et al. (2014). Means (n=3), standard error mean (SEM), linear regression analysis with 95% confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data was subjected to a single way analysis of variance (ANOVA).

Results and discussion

General grain characteristics and flour yield

Physical parameters of grains were determined prior to milling and data is presented in Table 1. The grain length, grain width and crease depth of wheat variety RAJ-3765 were significantly higher ($P<0.05$) than PBW-343, suggesting that grains of wheat variety RAJ-3765 had larger size and density than PBW-343. The ash content of PBW-343 was significantly higher ($P<0.05$) than RAJ-3765. The test weight is the rough index of kernels density and regularity of size. The test weights of wheat varieties were 79.50 and 82.31 kg hl^{-1} for PBW-343 and RAJ-3765, respectively. The ash content of pure endosperm ranged between 0.30 to 0.35% (de Man, 1999). The grains parameters such as 1000 kernel weight, test weight and extraction rate of varieties did not vary significantly.

Quality parameters of wheat flour

Quality characteristics of flour samples of both varieties are summarized in Table 2. The protein content of flour is an important parameter for a wheat variety as it affects the final product quality. Non-significant difference (*P>0.05*) was observed in the protein contents of wheat varieties.

Table 1. Physico-chemical Parameters of Wheat Grains

Physico-chemical characteristics	Wheat varieties	
	PBW-343	RAJ-3765
Grain length (mm)	6.57±0.05[a]	6.83±0.03[b]
Grain width (mm)	3.06±0.02[a]	3.62±0.02[b]
Crease depth (mm)	1.62±0.01[a]	1.67±0.01[b]
1000 kernel weight (g)	40.7±0.69[a]	42.0±0.56[a]
Test weight (kg/hl)	79.5±0.91[a]	82.3±0.94[a]
Moisture content (%)	12.9±0.14[a]	12.7±0.13[a]
Ash (%)	0.78±0.01[b]	0.69±0.02[a]
Extraction rate (%)	71.70±0.66[a]	72.01±0.46[a]

Data are presented as mean ± SEM (n=3).

[a-b] Means with the same superscript in column do not vary significantly (P<0.05) from each other.

However, there was a significant difference in the SDS sedimentation values of the varieties. The SDS sedimentation value of RAJ-3765 wheat variety flour was significantly higher (*P<0.05*) than that of PBW-343. The falling number of RAJ-3765 wheat variety flour was also significantly higher (*P<0.05*) than that of PBW-343. The quantity and quality of gluten depend mainly on genotype (variety), growing conditions (soil, climate, fertilization, etc.).

Gluten is an important constituent of wheat because it provides strength to dough and texture to baked wheat products. Higher gluten content in wheat flour is recommended for bread and lower gluten content is found better for biscuits and cookies. The wet gluten yield was 24.10 and 30.05% for PBW-343 and RAJ-3765, respectively. Significant difference (*P<0.05*) in wet gluten yields was observed between both wheat varieties. Similar trend was observed in case of dry gluten yields. Autran *et al.* (1997) observed that pentosans and hemicelluloses in flours have a strong effect on gluten yield and flour processing properties are strongly determined by the way flour milling fractions are blended. In a response surface study on gluten extraction from low-grade flour and durum flour, it was found that the protein concentration in protein fraction increased as the water content in the dough increased from 400g/Kg to 710g/Kg (Dik *et. al.,* 2002).

Effect of reducing agents on gluten recovery

Three reducing agents L–cysteine HCL, glutathione and protease were used at 50, 75 and 100 ppm level. The effects of reducing agents on gluten recovery and % loss of gluten were determined. With addition of reducing agents, the wet gluten recovery decreased in comparison to control but the decrease was non-significant (*P>0.05*) (Table 3). The wet gluten recovery decreased with the increase in the

concentration of reducing agents. The maximum reduction in wet gluten yield was induced by glutathione.

Table 2. Physico-chemical Parameters of Wheat Flour

Flour characteristics	Wheat varieties	
	PBW-343	RAJ-3765
Moisture content (%)	13.91±0.13[a]	14.61±0.15[b]
Ash (%)	0.55±0.01[b]	0.42±0.02[a]
Protein content (%)	10.52±0.28[a]	11.21±0.10[a]
SDS sedimentation value (ml)	26.43±0.35[a]	33.02±0.64[b]
Falling number (s)	317±6.92[a]	412±9.24[b]
Wet gluten (%)	24.10±0.14[a]	30.05±0.16[b]
Dry gluten (%)	7.9±0.12[a]	9.83±0.06[b]

Data are presented as mean ± SEM (n=3).

[a-b] Means with the same superscript in column do not vary significantly ($p<0.05$) from each other.

The % loss of wet gluten was also determined. All samples had significant difference in % loss of wet gluten. The % loss of wet gluten increased with the increase in the concentration of reducing agents. The highest loss in wet gluten was induced by glutathione.

A similar trend was observed in case of dry gluten yield and % loss of dry gluten of wheat varieties. The effect of reducing agents was more prominent on wheat variety PBW-343 as compared to RAJ-3765. It shows that wheat variety RAJ-3765 had stronger gluten network in comparison to PBW-343.

The decreasing effect of reducing agents on wheat gluten recovery was attributed to increased thiol - disulphide interchange reaction. The disulfide bonds in gluten were broken down chemically by a series of reactions with cysteine or glutathione known as disulfide interchange. The reactions are shown here with R and R' representing the two gluten molecules and with cysteine as the reducing agent:

$$R–S–S–R' + cys–SH \rightarrow R–S–S–cys + R'–SH$$

$$R–S–S–cys + cys–SH \rightarrow cys–S–S–cys + R–SH$$

These reactions reduced the number of cross-links between the gluten subunits proportional to the number of cysteine or glutathione molecules added and were reversible.

Wieser (2007) reported that number and type of SS bonds between gluten proteins have a major effect on the properties of the three-dimensional glutenin network and the dough rheological properties. Although gluten proteins contain only a few CSH residues (~2% of total amino acid composition), they are very important for the structure and functionality of gluten proteins. Joye *et al.* (2009) reported that reducing agents promote SH/SS interchange reactions, and result in weaker dough, reduced mixing time and improved dough machinability.

Effect of reducing agents on wheat dough and gluten extensibility

The effects of reducing agents on resistance to extensibility and extensibility of dough were determined. With addition of reducing agents, the resistance to extensibility decreased in comparison to control and decrease was significant ($P<0.05$) (Table 4 and Fig. 1). The resistance to extensibility also decreased with the increase in concentration of reducing agents. The maximum reduction in resistance to extensibility was induced by glutathione in comparison to L – cysteine and proteases. The extensibility of dough was also determined using extensigraph to check the effect of reducing agents. All samples had significant difference ($P<0.05$) in extensibility of dough. The extensibility of dough increased with the increase in concentration of reducing agents. The highest loss in % wet gluten was induced by glutathione in comparison to L – cysteine and proteases.

Joye *et al.* (2009) reported that reducing agents promote SH/SS interchange reactions, and result in weaker dough, reduced mixing time and improved dough machinability. Pareyt *et al.* (2008) reported that the extractabilities of both glutenin and gliadin decreased during baking. This confirms that gluten is not functionally inert during cookie baking (Gaines, 1990), and that both glutenin and gliadin influence baking. From above, it is clear that protein aggregation occurs during baking, threrefore, reducing agents play important role in cookies preparation.

Increasing the level of reducing agents considerably reduced the resistance to extension, and the extensibility of dough increased in both varieties. Thus, the incorporation of reducing agents decreased the strength of dough. The weakening effect of dough was attributed to the increasing rate of thiol – disulphide interchange reaction. Reducing agents mainly acted upon disulphide linkage and caused their breakage. Thus, reducing agents made the dough weaker, softer and more extensible.

Effect of reducing agents on quality of cookies

Spread factor

Cookies were prepared by using wheat flour of both varieties i.e. PBW 343 and RAJ 3765. Effect of L-cysteine HCL and glutathione were determined at different concentration (50, 100 and 100 ppm). After adding reducing agent to cookie batter, 30 min resting time was used. The effect of reducing agents on cookie spread is shown in **table 5**. It is evident from the results that the thickness of cookies decreased while the diameter and the spread factor increased. As the concentration increased there was further increase in spread of cookies. Effect of L – cysteine HCL was more pronounced as compared to glutathione. Cracks are also better in reducing agents treated cookies. This increasing effect of reducing agents was due to increased extensibility of dough. Reducing agents increased the thiol disulphide interaction. They inhibited the gluten network formation in dough. Hence the cookie dough spread more and diameter of cookies increased. PBW 343 showed higher spread factor than RAJ 3765, as RAJ 3765 had higher protein content than PBW 343.

The magnitude of cookie diameter reduction due to the blends addition was dependent on the amount of gluten in the blend. High gluten content in the protein blend resulted in smaller cookie spread. The 10% addition did not significantly affect the height of the cookies, but 15% and above replacement resulted in cookies with more height. Protein content significantly affected the diameter and the thickness of the cookies (Singh and Mohamed, 2007). This was in agreement with the earlier studies reported by McWatters, (1978) and Singh *et al.*, (1993), who also reported a decrease in spread factor with increased protein in the cookies. Wilderjans *et al.* (2008) for pound cake also showed that gluten is essential for product structure. Pareyt *et al.* (2008) reported that cookie diameter decreased with increasing gluten levels. Higher gluten levels increased spread onset time, while they had little impact on set time. This, together with the decrease in spread rate, accounts for the observed decrease in cookie diameter, and illustrates the importance of an early flow for cookie diameter. Reducing agents cleave intermolecular and intramolecular disulfide bonds in the gluten proteins. This cleavage results in reduced molecular weight for the proteins, and the extensibility of the dough was increased (Stauffer 1994). Wade (1970) reported that up to a level of about 450 ppm Sodium meta-bisulfite, the biscuit length was increased.

Hardness

The effect of reducing agents on cookies hardness was determined using texture analyser. Hardness decreased when reducing agents were incorporated (**table 6 and Figure 2**). Reducing agents made the cookies softer. As the concentration of reducing agents increased, the cookie hardness decreased. PBW 343 showed lower hardness than RAJ 3765, as PBW 343 had lower protein content than RAJ 3765.

Addition of proteases and reducing agents to dough significantly decreased rheological chracteristics (Monahar and Rao 1997; Oliver *et al.* 1995; Lindahl and Eliasson 1992; Gaines and Finney 1989). The dough weakening effect of L-cysteine and Glutathione is similar, due to the destruction / reduction of intermolecular SS bonds (Joye *et al.* 2009). Fustier *et al*. (2008), for semi-sweet biscuits, i.e. in a recipe with higher water and lower fat and sugar levels than used here, found a gradual increase in dough hardness with gluten concentration.

Table 3. Effect of Reducing Agents on Wheat Gluten Recovery

Reducing agent (ppm)	Wheat varieties							
	PBW-343				RAJ-3765			
	Wet gluten (%)	% loss in wet gluten	Dry gluten (%)	% loss in dry gluten	Wet gluten (%)	% loss in wet gluten	Dry gluten (%)	% loss in dry gluten
Control flour	24.12± 0.47^{a}		7.91± 0.05^{b}		30.05± 0.69^{a}		9.76± 0.07^{b}	
L – cysteine 50	24.01± 0.32^{a}	0.37± 0.01^{b}	7.76± 0.03^{ab}	1.77± 0.11^{c}	29.89± 0.61^{a}	0.53± 0.2^{b}	9.68± 0.14^{ab}	0.82± 0.05^{b}

Reducing agent (ppm)	Wheat varieties							
	PBW-343				RAJ-3765			
	Wet gluten (%)	% loss in wet gluten	Dry gluten (%)	% loss in dry gluten	Wet gluten (%)	% loss in wet gluten	Dry gluten (%)	% loss in dry gluten
75	23.88± 0.49^{a}	0.91± 0.01^{e}	7.62± 0.08^{ab}	3.79± 0.09^{g}	29.72± 0.10^{a}	0.76± 0.02^{d}	9.55± 0.20^{ab}	2.15± 0.06^{f}
100	23.72± 0.10^{a}	1.57± 0.03^{f}	7.52± 0.14^{a}	4.81± 0.10^{h}	29.55± 0.11^{a}	1.66± 0.02^{e}	9.43± 0.05^{a}	3.38± 0.10^{h}
Glutathione 50	23.98± 0.48^{a}	0.49± 0.01^{c}	7.72± 0.11^{ab}	2.22± 0.09^{e}	29.86± 0.10^{a}	0.63± 0.01^{c}	9.63± 0.06^{ab}	1.33± 0.05^{c}
75	23.87± 0.25^{a}	0.95± 0.01^{e}	7.63± 0.08^{ab}	3.42± 0.08^{f}	29.82± 0.08^{a}	0.76± 0.02^{d}	9.57± 0.05^{ab}	1.94± 0.07^{e}
100	23.60± 0.20^{a}	2.07± 0.03^{g}	7.49± 0.10^{a}	5.33± 0.07i	29.77± 0.10^{a}	1.66± 0.03^{e}	9.48± 0.07^{ab}	2.86± 0.10^{g}
Proteases 50	24.04± 0.16^{a}	0.24± 0.01^{a}	7.84± 0.10^{b}	0.75± 0.02^{a}	29.96± 0.24^{a}	0.29± 0.01^{a}	9.70± 0.07^{ab}	0.61± 0.20^{a}
75	23.99± 0.20^{a}	0.45± 0.01^{c}	7.82± 0.14^{b}	1.01± 0.01^{b}	29.90± 0.18^{a}	0.49± 0.01^{b}	9.67± 0.08^{ab}	0.92± 0.01^{b}
100	23.94± 0.52^{a}	0.66± 0.02^{d}	7.75± 0.08^{ab}	1.89± 0.03^{d}	29.85± 0.29^{a}	0.66± 0.02^{c}	9.60± 0.10^{ab}	1.63± 0.01^{d}

Data are presented as mean ± SEM (n=3).

$^{a-b}$ Means with the same superscript in column do not vary significantly (P<0.05) from each other.

Table 4. Effect of Reducing Agents on Wheat Gluten Extensibility

Reducing agent (ppm)	Wheat varieties							
	PBW-343				RAJ-3765			
	R (g)	E (mm)	R/E	% loss in R/E	R (g)	E (mm)	R/E	% loss in R/E
Control flour	19.12± 0.38^{e}	15.89± 0.39^{a}	1.2± 0.08^{e}		27.73± 0.80^{i}	12.55± 0.24^{a}	2.23± 0.03^{g}	
L – cysteine 50	15.7± 0.22^{c}	19.85± 0.48^{c}	0.79± 0.06^{c}	34.22± 0.23^{b}	16.22± 0.25^{c}	17.83± 0.46^{d}	0.91± 0.05^{c}	59.09± 0.32^{g}
75	12.11± 0.05^{ab}	24.35± 0.06^{f}	0.49± 0.03^{ab}	58.71± 0.22^{f}	14.44± 0.24^{b}	23.13± 0.28^{e}	0.62± 0.02^{b}	71.81± 0.33^{h}
100	11.40± 0.21^{a}	25.53± 0.49^{g}	0.44± 0.03^{a}	62.86± 0.38^{h}	12.81± 0.33^{a}	26.99± 0.47^{f}	0.47± 0.01^{a}	78.63± 0.35^{i}
Glutathione 50	13.2± 0.32^{b}	21.21± 0.20^{d}	0.62± 0.05^{b}	48.23± 0.11^{c}	21.66± 0.12^{g}	14.94± 0.29^{bc}	1.44± 0.04^{e}	34.54± 0.08^{b}
75	13.02± 0.32^{b}	21.68± 0.43^{de}	0.59± 0.01^{b}	50.12± 0.26^{d}	19.15± 0.21^{e}	15.89± 0.39^{c}	1.24± 0.07^{d}	45.45± 0.25^{e}
100	11.31± 0.16^{a}	24.53± 0.29^{fg}	0.46± 0.04^{ab}	61.69± 0.96^{g}	17.62± 0.03^{d}	18.31± 0.17^{d}	0.96± 0.05^{c}	56.36± 0.08^{f}
Proteases 50	18.12± 0.45^{d}	18.31± 0.25^{b}	0.98± 0.09^{d}	17.73± 0.39^{a}	24.21± 0.10^{h}	13.42± 0.21^{a}	1.82± 0.04^{f}	18.18± 0.19^{a}

Reducing agent (ppm)	Wheat varieties							
	PBW-343				RAJ-3765			
	R (g)	E (mm)	R/E	% loss in R/E	R (g)	E (mm)	R/E	% loss in R/E
75	13.23± 0.32^{b}	22.65± 0.35^{e}	0.58± 0.20^{ab}	51.54± 0.13^{e}	20.54± 0.28^{f}	14.45± 0.08^{b}	1.41± 0.02^{e}	35.90± 0.38^{c}
100	12.81± 0.18^{b}	26.99± 0.46^{h}	0.47± 0.10^{ab}	60.53± 0.12^{g}	20.23± 0.03^{f}	15.91± 0.44^{c}	1.26± 0.02^{d}	42.72± 0.35^{d}
	0.82	1.09	0.14	1.19	0.95	0.97	0.12	0.84

Data are presented as mean ± SEM (n=3). R – Resistance to extensibility, E – Extensibility

[a-b]Means with the same superscript in column do not vary significantly (P<0.05) from each other.

Table 5. Effect of reducing agents on spread factor of cookies

Reducing Agents (ppm)	Wheat Varieties					
	PBW 343			RAJ 3765		
	D (mm)	T (mm)	D/T	D(mm)	T(mm)	D/T
Control	83.2+0.64^{a}	11.71+0.13^{e}	7.11+0.02^{a}	72.8+0.38^{a}	10.7+0.06^{f}	6.8+0.01^{a}
L-cysteine 50	83.72+0.52^{ab}	11.16+0.05^{d}	7.50+0.01^{c}	73.05+0.21^{a}	10.42+0.04^{e}	7.01+0.01^{b}
75	84.0+0.48^{ab}	10.5+0.09^{b}	8.0+0.02^{e}	73.81+0.31^{b}	9.7+0.08^{c}	7.61+0.03^{d}
100	84.6+0.41^{b}	10.07+0.16^{a}	8.40+0.09^{g}	74.1+0.08^{b}	9.26+0.1^{a}	8+0.08^{f}
Glutathione 50	83.29+0.37^{a}	11.4+0.16^{d}	7.30+0.07^{b}	73.2+0.15^{a}	10.16+0.09^{d}	7.2+0.04^{c}
75	83.85+0.47^{ab}	10.75+0.11^{c}	7.80+0.04^{d}	73.89+0.10^{b}	9.47+0.09^{b}	7.8+0.07^{e}
100	84.02+0.76^{ab}	10.24+0.10^{a}	8.20+0.01^{f}	74.32+0.11^{b}	9.17+0.05^{a}	8.1+0.03^{g}

Data are presented as mean ± SEM (n=3). D – Diameter, T – Thickness, D/T - Spread Factor

[a-b] Means with the same superscript in column do not vary significantly (P<0.05) from each other.

Table 6. Effect of reducing agents on hardness of cookies

Reducing Agent (ppm)	Wheat Varieties	
	PBW 343	RAJ 3765
	Hardness	Hardness
Control	5351.5+298.65^{e}	6784.1+91.74^{g}
L-cysteine 50	4580.2+128.51^{d}	5132.7+95.09^{d}
75	4166.4+82.77^{c}	3949.7+74.31^{c}
100	2984.3+114.82^{a}	3706.5+94.00^{b}
Glutathione 50	4554.4+76.77^{d}	6388.4+30.77^{f}
75	4051.7+99.97^{bc}	5761+41.28^{e}
100	3803+58.19^{b}	3441.6+26.46^{a}

Data are presented as mean ± SEM (n=3).

[a-b] Means with the same superscript in column do not vary significantly (P<0.05) from each other.

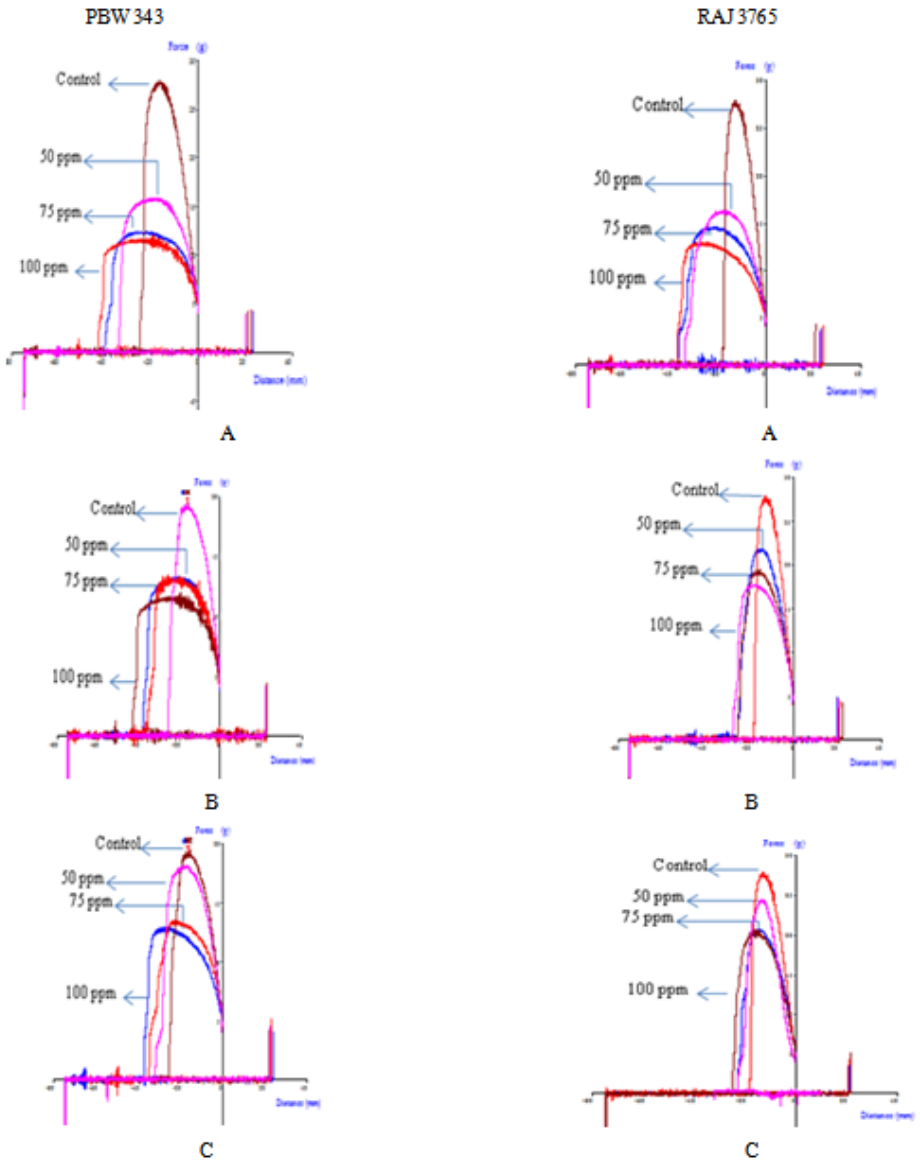

Figure 1. Effect of reducing agents on dough extensibility (A – L cysteine HCL, B– glutathione, C – proteases)

Conclusion

Reducing agents interfered with the gluten network formation, therefore the percentage of wet and dry gluten recovery was reduced. It was observed that reducing agents reduced dough strength and increased the extensibility of dough.

Glutathione was found to be most effective reducing agent in comparison to L-cysteine hydrochloride and protease enzyme.

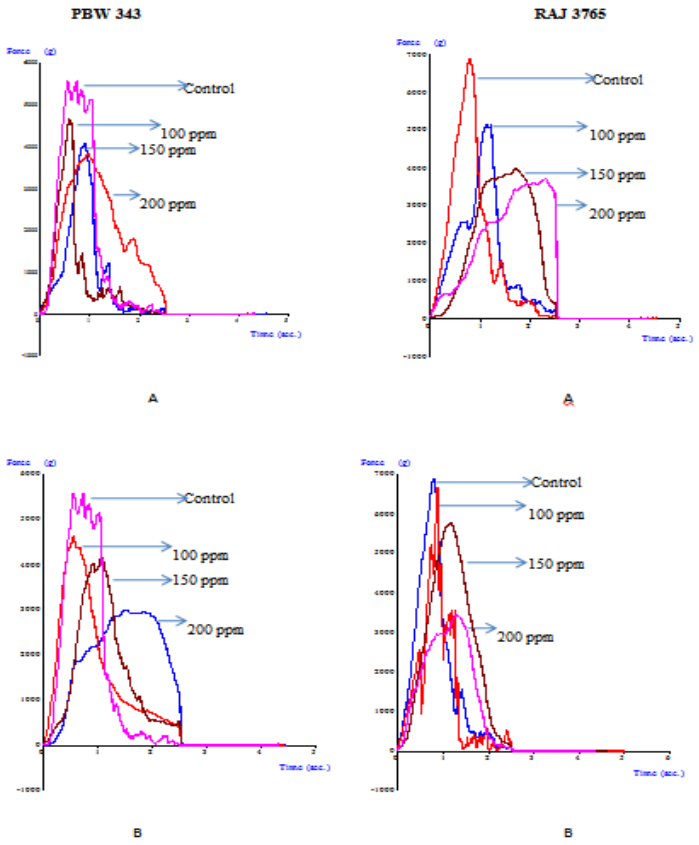

Figure 2. Effect of glutathione on hardness of cookies (A – L cysteine HCL, B – Glutathione)

Spread factor of cookies increased upon addition of reducing agents, whereas hardness decreased. It can be therefore concluded that reducing agents are useful in the reduction of dough strength and increasing the extensibility of the dough. Moreover the reducing agents increased the spread factor of cookies and reduced the hardness, which is considered desirable for processing of wheat flour for cookies and biscuits.

Acknowledgments

Authors acknowledge the financial support provided by Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, India.

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