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CHANGES OF PROTEIN FRACTIONS IN WHEAT FLOUR CAUSED BY ADDITIVES

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Abstract

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The influence of different combinations of reducing and oxidising agents (L-cysteine hydrochloride monohydrate + L-ascorbic acid, inactivated dry yeast + L-ascorbic acid, L-threonine + L-ascorbic acid, L-tryptophan + L-ascorbic acid) on the change in the proportion of glutenins, gliadins, albumins, globulins in wheat flour was investigated.
Different concentrations of amino acid combinations were added to wheat flour. By means of Size Exclusion High performance liquid chromatography (SE-HPLC), the changes in protein fractions caused by individual concentrations of amino acid combinations were evaluated against the control sample (pure wheat flour).
It was detected that the mixture of flour + L-ascorbic acid + L-cysteine hydrochloride monohydrate had the stronger reducing effect than the mixture of flour + L-ascorbic acid + inactivated dry yeast. On the other hand, the mixture of flour + L-ascorbic acid + L-tryptophan had the stronger oxidising effect than the mixture of flour + L-ascorbic acid + L-threonine.

amino acid, oxidising agent, reducing agent, flour, mixture

Flour protein content is probably the most important property of wheat flour. Wheat endosperm proteins are divided into two main fractions, monomeric and polymeric proteins. The monomeric fraction includes gliadins, albumins, and globulins. Albumins and globulins are considered functional proteins, whereas gliadins are referred to as storage proteins. Polymeric fraction mainly consists of glutenin polymers composed of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) associated together by intermolecular disulfide bonds (Rhazi *et al.*, 2009).

Gliadins and glutenins constitute the gluten proteins that are considered the most important fractions responsible for variations in bread-making quality (Weegels *et al.*, 1996). The breadmaking quality of wheat flour is largely determined by the quality of the gluten fraction. The glutenin polymer structure, size distribution and subunit composition and the gliadin/glutenin ratio are major factors in determining gluten quality and, hence, the breadmaking potential of wheat flour (Khatkar *et al.*, 1995; Ciaffi *et al.*, 1996; Janssen *et al.*, 1996; Veraverbeke *et al.*, 1998; Uthayakumaran *et al.*, 1999; D´Ovidio and Masci, 2004; Joye *et al.*, 2009). Gliadins are monomeric proteins that consist of single chain polypeptides and contribute to the viscous properties of dough; glutenins are polymeric proteins in which the individual subunits are linked by disulphide bonds (Field *et al.*, 1983a; 1983b) and are thought to have molecular weights ranging from a few hundred thousand to many millions dalton (Da). These glutenin polymers have been described as "nature's largest polymers" (Wrigley, 1996) and they are the main components responsible for differences in end-use quality among different flour cultivars (Weegels *et al.*, 1996; Mendichi *et al.*, 2008). Glutenin subunits, of both low (LMW-GS) and high (HMW-GS) molecular weight, exhibit both inter- and intra-chain disulphide bonding, and are bound together in glutenin polymers, the size of which is strongly correlated with gluten quality (Gupta *et al.*, 1993; Gupta *et al.*, 1995). Protein strength is an inherent characteristic, but the amount of protein present can be influenced by the conditions under which wheats are grown (e.g. sulphur deficiency, heat or water stress). Non-gluten proteins include albumins (water extractable) and globulins (dilute salt medium extract-

able) which comprise metabolic proteins (enzymes or inhibitors) and minor storage proteins (Joye *et al.*, 2009).

Several agents present in wheat can potentially affect the breadmaking performance of the flour (Joye *et al.*, 2009). Although the dough and bread characteristics strongly depend on the breadmaking recipe and procedure, the type and properties of the flour used also play an important role. The dough properties and breadmaking quality of flour are affected by variation in the levels of natural reducing or oxidising agents in wheat flour. Oxidising agents generally promote SS bond formation and, hence, minimise SH/SS interchange with positive impacts on loaf volume, oven rise and oven rise time. In contrast, reducing agents promote SH/SS interchange reactions, and result in weaker dough, reduced mixing time and improved dough machinability (Joye *et al.*, 2009). Baking quality can be accelerated by improvers that modify the physical properties of gluten during fermentation. Dough improvers are often added to flour to improve dough elasticity or extensibility and baking quality. For better acting, a dough improver often consists of oxidising and reducing agents. Reducing agents, such as glutathione, which is tripeptide (γ-glutamylcysteinylglycine) containing two carboxylic acid groups, one free amino group and a SH group (Joye *et al.*, 2009), and cysteine (Morita *et al.*, 1996), reduce the kneading force and dough fermentation time by accelerating gluten disrupture and then the number of sulfhydryl groups increases (Biebaut, 1991).

The oxidants increase the elastic/viscous ratio in dough. The type of action is dependent on the type of oxidant. Ascorbic acid, although being itself a reducing agent, can exert an oxidising effect on the dough properties after its oxidation by atmospheric oxygen. The oxidation product is dehydro-$_L$-ascorbic acid (Hrušková and Novotná, 2003). Ascorbic acid reformes gluten to a three-dimensional matrix and oxidizes the SH group to SS bond, which increases the dough strength by retaining gas, and thus enlarges the bread volume. It is generally accepted that the rheological properties of dough and its three-dimensional network are dependent on the arrangement and number of disulfide bonds and sulfhydryl groups of the protein. A small amount of cysteine or reduced glutathione dramatically increase the extensibility of dough (Bloksma, 1972; Dong and Hoseney, 1995). However, there has not been found much information about the influence of amino acids as L-threonine and L-tryptophan on the rheological properties of dough. Amino acids as L-threonine and L-tryptophan are normally present in minor amount in wheat flour. These amino acids belong to essential amino acids and their addition to the dough strengthen final bakery product thereby improve their quality (nutritional value). Hence, these two amino acids were investigated with the combination of ascorbic acid and their influence on the changes in HMW-GS and LMW-GS groups was analyzed. Good nutrition can lead to an impressive range of benefits. From the perspective of human capital, these include improvement in health, cognitive development and work capacity. From the viewpoint of development, they include greater economic and agricultural productivity, better education, improved workforce development as well as greater resilience to shocks induced by social, economic and natural causes. The evidence suggests that these benefits can be achieved at high levels of economic efficiency for a wide range of nutrition policy instruments (Golian, 2009).

The aim of this study was to evaluate the oxidising and reducing effect as the change on the proportion of the glutenins, gliadins, albumins, globulins, between pure wheat flour and wheat flour with the addition of different amounts of reducing and oxidising agent.

MATERIALS AND METHODS

Flour

For the assessment, common commercial wheat flour T 530 (quantitative parameters: moisture content (MC) = 14.3 %, gluten content in dry matter (GC) = 36.4 %, Falling number (FN) = 339 s), provided by Penam, s.r.o. Kroměříž, Czech Republic, was used. It was used 1 g flour for each analysis.

Other information about flour was found by alveoconsistograf analysis (Chopin – Tripette & Renauld, France) according to the methods AACC 54-30A and 54-50, respectively.

Alveograph and consistograph characteristics of investigated untreated flour are shown in table I.

I: *Alveograph and consistograph characteristics of investigated untreated flour*

Alveograph characteristic	Value	Consistograph characteristic	Value
P[a]	95 mmH_2O	PrMax[e]	2237 mb
L[b]	89 mm	TPrMax[f]	173 s
P/L[c]	1.06	Tol[g]	241 s
W[d]	287 10E-4J	D250[h]	197 mb
		D450[i]	742 mb

P[a] – tenacity, L[b] – extensibility, P/L[c] – configuration ratio, W[d] – deformation energy, PrMax[e] – maximum pressure, TPrMax[f] – time to reach maximum pressure, Tol[g] – time that pressure is higher than PrMax minus 20%, D 250[h] – the drop in pressure at 250 seconds from PrMax minus 20%, D 450[i] – the drop in pressure at 450 seconds from PrMax minus 20%.
*mb (millibar) – the device is calibrated on values mb

II: *The composition of individual samples (% w/w)*

Sample	Mixture composition (% w/w)		
	Flour	**L-ascorbic acid**	**L-threonine/L-tryptophan**
TH1/TR1[a,w]	62.50	18.75	18.75
TH2/TR2[b,x]	55.56	16.67	27.78
TH3/TR3[c,y]	50.00	15.00	35.00
TH4/TR4[d,z]	45.45	13.64	40.91
	Flour	**L-ascorbic acid**	**L-cysteine hydrochloride monohydrate**
C1[e]	75.19	22.56	2.26
C2[f]	73.53	22.06	4.41
C3[g]	72.46	21.74	5.80
	Flour	**L-ascorbic acid**	**Inactivated dry yeast**
IDY1[h]	62.50	18.75	18.75
IDY2[i]	55.56	16.67	27.78
IDY3[j]	50.00	15.00	35.00

TH1/TR1[a] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-threonine (18.75% w/w)/ L-tryptophan (18.75% w/w), TH2/TR2[b] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-threonine (27.78% w/w)/ L-tryptophan (27.78% w/w), TH3/TR3[c] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-threonine (35.00% w/w)/ L-tryptophan (35.00% w/w), TH4/TR4[d] mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-threonine (40.91% w/w)/ L-tryptophan (40.91% w/w), C1[e] mixture of flour (75.19% w/w + L-ascorbic acid (22.56% w/w) + L-cysteine hydrochloride monohydrate (2.26% w/w), C2[f] mixture of flour (73.53% w/w + L-ascorbic acid (22.06% w/w) + L-cysteine hydrochloride monohydrate (4.41% w/w), C3[g] mixture of flour (72.46% w/w + L-ascorbic acid (21.74% w/w) + L-cysteine hydrochloride monohydrate (5.80% w/w),IDY1[h] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + inactivated dry yeast (18.75% w/w), IDY2[i] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + inactivated dry yeast (27.78% w/w), IDY3[j] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + inactivated dry yeast (35.00% w/w)

Additives

Different combinations of reducing and oxidising agents were selected.

Oxidising agents; amino acids as L-threonine and L-tryptophan (Merck KGaA, Darmstadt, Germany).

Reducing agents; amino acids as L-ascorbic acid (Merck KGaA, Darmstadt, Germany), L-cysteine hydrochloride monohydrate and inactivated dry yeast (Ireks GmbH, Eppelborn, Germany).

Mixtures always included wheat flour, L-ascorbic acid and selected additive (L-threonine, L-tryptophan, L-cysteine hydrochloride monohydrate, inactivated dry yeast). Constant ratio of wheat flour and L-ascorbic acid (23:77) was kept for each analysis.

The composition of individual samples is seen in table II.

These combinations of reducing and oxidising agents, especially combinations (L-ascorbic acid + L-tryptophan, L-ascorbic acid + L-threonine) has not been used for practical application yet.

Methods

The flour was extracted for 2 hours at 60 °C in 0.1M phosphate buffer (pH 6.9) containing 2% SDS. After centrifugation (at 15.000 rev./min.) supernatant was applied to a column Phenomenex BioSep-SEC-S4000 (300 × 7.8 mm). The analysis was performed on a liquid chromatograph Shimadzu by means of SE-HPLC (Size exclusion High performance liquid chromatography) analysis according to (Dachkevitch and Autran, 1989). The mobile phase consisted of phosphate buffer (pH 6.9) with the addition of 0.1% SDS. The spectra were detected at 214 nm.

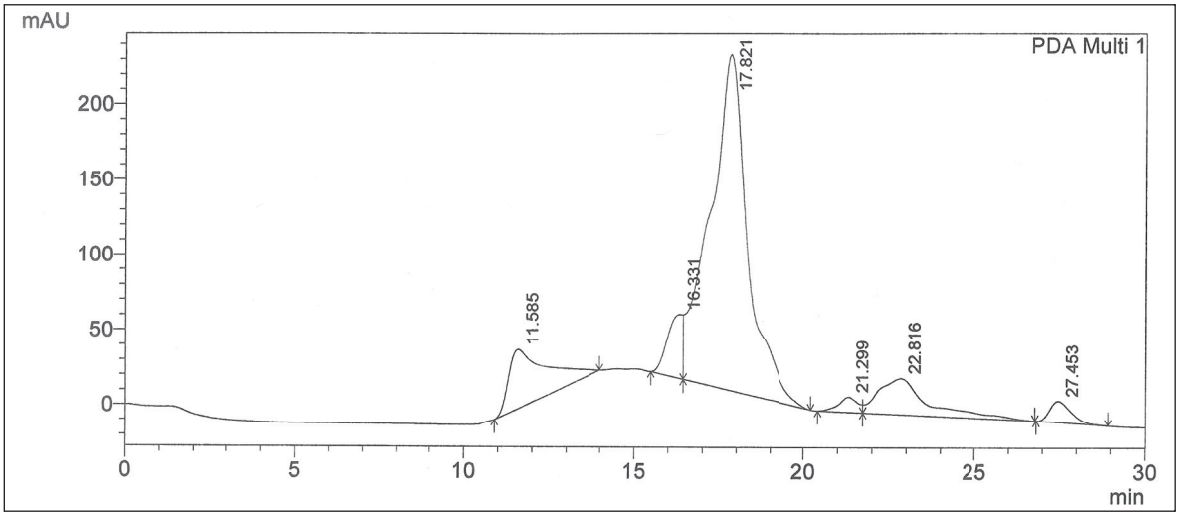
Proteinaceous fractions present in the sample were identified by means of standards of known molecular weights.

Example of chromatogram of the control sample (pure wheat flour) can be seen in figure 1.

RESULTS AND DISCUSSION

Tables III–IV illustrate the peak characteristics of SE-HPLC spectra of the samples observed. The tables show the retention times and peak areas of the individual proteinaceous fractions as glutenins, gliadins, albumins and globulins. The tables show the values of pure flour along with the flour with different combinations of reducing and oxidising agents added.

As it is clear from table III, in the samples (C1-C3), different changes in spectrum characteristics occurred depending on the concentration of L-cysteine hydrochloride monohydrate. The peak areas detected in the 11th minute were the largest in sample C1, i.e. in the sample with the lowest concentration (2.26% w/w) of L-cysteine hydrochloride monohydrate. The smallest peak area was found in sample C2. The peak areas detected in the 11th minute in samples C3 has almost identical values. The peak area detected in the 16th minute was decreasing (in comparation with C1; C2 23%, C3 27%) with the increasing concentration of L-cysteine hydrochloride monohydrate. On the other hand, the peak area of gliadins detected in the 18th minute was gra-



1: *Chromatogram of the control sample (pure wheat flour), peak RT 11.585 = glutenins, peak RT 16.331 = gliadins, peak RT 17.821 = gliadins, peak RT 21.299 = albumins + globulins, peak RT 22.816 = albumins + globulins, peak RT 27.453 = albumins + globulins*

III: *Peak characteristics (retention time, area) for combination reducing and oxidising agent*

Sample	Ret. Time (min)	Control sample	C1[e]	C2[f]	C3[g]
Peak characteristics/Protein fraction		**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**
glutenins	11.	3 023	41	21	35
gliadins	16.	1 312	7 944	6 102	5 793
gliadins	18.	17 192	22 460	23 160	24 265
albumins + globulins	22.	391	31 328	34 981	35 954

C1[e] mixture of flour (75.19% w/w + L-ascorbic acid (22.56% w/w) + L-cysteine hydrochloride monohydrate (2.26% w/w)
C2[f] mixture of flour (73.53% w/w + L-ascorbic acid (22.06% w/w) + L-cysteine hydrochloride monohydrate (4.41% w/w)
C3[g] mixture of flour (72.46% w/w + L-ascorbic acid (21.74% w/w) + L-cysteine hydrochloride monohydrate (5.80% w/w)

IV: *Peak characteristics (retention time, area) for combination reducing and oxidising agent*

Sample	Ret. Time (min)	Control sample	IDY1[h]	IDY2[i]	IDY3[j]
Peak characteristics/Protein fraction		**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**
glutenins	12.	3 023	1 408	1 162	855
gliadins	18.	17 192	22 391	26 378	27 408
albumins + globulins	22.	391	23 446	21 289	21 123

[k]Note: The value of the inactivated dry yeast sample is 10 times higher so that its reducing effect could be compared with that of L-cysteine hydrochloride monohydrate
IDY1[h] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + inactivated dry yeast (18.75% w/w)
IDY2[i] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + inactivated dry yeast (27.78% w/w)
IDY3[j] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + inactivated dry yeast (35.00% w/w)

dually increasing (in comparation with C1; about 7%) in the sample C3 with the highest concentration (5.80% w/w) of L-cysteine hydrochloride monohydrate. Comparison with the control sample of flour showed that the presence of L-cysteine hydrochloride monohydrate in the sample caused a decrease (about 99%) in the peak areas of the corresponding glutenins. In contrast, the peaks of gliadins in the 18th minute (C1 23%, C2 26%, C3 29%), albumins and globulins (about 99%) increased. L-cysteine hydrochloride monohydrate is able to reduce the molecular weight of glutenin polymers by SH/SS interchange reactions. Similar information about ability of reducing agents to promote SH/SS interchange reactions was mentioned by authors (Morita *et al.*, 1996; Joye *et al.*, 2009).

Table IV shows that with the increasing amount of inactivated dry yeast, there is a decrease in the peak areas of glutenins (in comparation with IDY1; IDY2 17%, IDY3 39%), albumins and globulins (in comparation with IDY1; IDY2 9%, IDY3 10%). On the contrary, there was an apparent increase (in comparation with IDY1; IDY2 15%, IDY3 18%) in the peak areas of gliadins.

The values in Tables III and IV show that the mixture of flour + L-ascorbic acid + L-cysteine hy-

drochloride monohydrate had a stronger oxidising effect and thus a more significant impact on the viscosity and extensibility of dough. Increased compactness of dough and thus better rheological properties can be expected in the dough which is prepared from the mixture of flour + L-ascorbic acid + inactivated dry yeast.

Table V illustrates the differences between SE-HPLC spectra of proteinaceous fractions for the same amount of combinations of oxidising and reducing agents added.

Table V illustrates that the proportion of the peak areas of glutenins (about 97%) and gliadins (about 12%) is always higher in the samples (IDY1-IDY3), which means that these doughs will be of better quality than those prepared from the mixture of flour + L-ascorbic acid + L-cysteine hydrochloride monohydrate. In contrast, the proportion of peak areas corresponding to albumins and globulins is higher in the samples (C1-C3; C1 25%, C2 39%, C3 41%).

Tables VI–VII illustrate the characteristics of SE-HPLC spectra of proteins in pure flour in comparison with different combinations of reducing and oxidising agents added.

Table VI shows that the peak areas of glutenins and gliadins decrease with the increasing amount of the mixture of flour + L-ascorbic acid + L-threonine. First, the peak areas of albumins and globulins increased rapidly (in comparation with control sample; about 98%) in sample TH1 and then decreased slowly in samples (TH2-TH4; in comparation with TH1; TH2 4%, TH3 13%, TH4 13%). Cross-linking of the gluten network was stronger with increasing amount of the mixture of flour + L-ascorbic acid + L-threonine. Its mean that it was difficult to extract protein fractions from the blend of flour and adddditives.

V: *Peak characteristics (retention time, area) for combination reducing and oxidising agent*

Samples	Ret. Time (min)	C1[e]	IDY1[h]	C2[f]	IDY2[i]	C3[g]	IDY3[j]
Peak characteristics/Protein fraction		**Area (10^3)**		**Area (10^3)**		**Area (10^3)**	
glutenins	12.	41	1 408	21	1 162	35	855
gliadins	18.	22 460	22 391	23 160	26 378	24 265	27 408
albumins + globulins	22.	31 328	23 446	34 981	21 289	35 954	21 123

[k]Note: The value of the inactivated dry yeast sample is 10 times higher so that its reducing effect could be compared with that of L-cysteine hydrochloride monohydrate

C1[e] mixture of flour (75.19% w/w + L-ascorbic acid (22.56% w/w) + L-cysteine hydrochloride monohydrate (2.26% w/w)
C2[f] mixture of flour (73.53% w/w + L-ascorbic acid (22.06% w/w) + L-cysteine hydrochloride monohydrate (4.41% w/w)
C3[g] mixture of flour (72.46% w/w + L-ascorbic acid (21.74% w/w) + L-cysteine hydrochloride monohydrate (5.80% w/w)
IDY1[h] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + inactivated dry yeast (18.75% w/w)
IDY2[i] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + inactivated dry yeast (27.78% w/w)
IDY3[j] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + inactivated dry yeast (35.00% w/w)

VI: *Peak characteristics (retention time, area) for combination reducing and oxidising agents*

Sample	Ret. Time (min)	Control sample	TH1[a]	TH2[b]	TH3[c]	TH4[d]
Peak characteristics/Protein fraction		**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**
glutenins	12.	3 023	342	577	540	396
gliadins	18.	17 192	11 452	10 110	9 067	8 193
albumins + globulins	22.	391	23 089	22 175	20 171	20 257

TH1[a] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-threonine (18.75% w/w)
TH2[b] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-threonine (27.78% w/w)
TH3[c] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-threonine (35.00% w/w)
TH4[d] mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-threonine (40.91% w/w)

VII: *Peak characteristics (retention time, area) for combination reducing and oxidising agents*

Sample	Ret. Time (min)	Control sample	TR1[w]	TR2[x]	TR3[y]	TR4[z]
Peak characteristics/Protein fraction		**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**	**Area (10^3)**
glutenins	12.	3 023	3 767	798	311	292
gliadins	18.	17 192	11 427	4 815	4 248	4 147
albumins + globulins	22.	391	21 652	9 585	8 850	7 989

TR1[w] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-tryptophan (18.75% w/w)
TR2[x] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-tryptophan (27.78% w/w)
TR3[y] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-tryptophan (35.00% w/w)
TR4[z] mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-tryptophan (40.91% w/w)

VIII: *Peak characteristics (retention time, area) for combination reducing and oxidising agents*

Samples	Ret. Time (min)	TH1[a]	TR1[w]	TH2[b]	TR2[x]	TH3[c]	TR3[y]	TH4[d]	TR4[z]
Peak characteristics/Protein fraction		**Area (10^3)**		**Area (10^3)**		**Area (10^3)**		**Area (10^3)**	
glutenins	12.	342	3 767	577	798	540	311	396	292
gliadins	18.	11 452	11 427	10 110	4 815	9 067	4 248	8 193	4 147
albumins + globulins	22.	23 089	21 652	22 175	9 585	20 171	8 850	20 257	7 989

TH1/TR1[a,w] mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-threonine (18.75% w/w)/ L-tryptophan (18.75% w/w)
TH2/TR2[b,x] mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-threonine (27.78% w/w)/ L-tryptophan (27.78% w/w)
TH3/TR3[c,y] mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-threonine (35.00% w/w)/ L-tryptophan (35.00% w/w)
TH4/TR4[d,z] mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-threonine (40.91% w/w)/ L-tryptophan (40.91% w/w)

Table VII illustrates that, in comparison with the control dough, larger areas of proteinaceous fractions were detected in the lowest amount of the mixture of flour + L-ascorbic acid + L-tryptophan (TR1). The peak areas of gliadins (in comparation with TR1; TR2 58%, TR3 63%, TR4 64%), albumins and globulins (in comparation with TR1; TR2 56%, TR3 59%, TR4 63%) were decreasing rapidly with the increasing amount of this mixture. Nevertheless, the amount of albumins and globulins was higher (about 96%) than in the control sample.

Tables VI and VII show that mixtures of flour + L-ascorbic acid + L-tryptophan (TR1-TR4) had stronger oxidising effects on the gluten network than mixtures of flour + L-ascorbic acid + L-threonine (TH1-TH4). The possibility to detect glutenin fractions was gradually worsen with the increasing amount of L-tryptophan in mixture.

Table VIII illustrates the differences between proteinaceous fractions for the same amount of the mixtures with different kind of oxidising agent added.

Table VIII illustrates that the samples (TH1-TH4) show a higher ability to extract glutenin, gliadin, albumin and globulin fractions. Higher amount of the mixture of flour + L-ascorbic acid + L-tryptophan (TR3-TR4) led to more intensive gluten networking and thus the extraction of glutenins from the solution was impossible.

CONCLUSION AND PRACTICAL APPLICATION

As it is clear from the results, mixtures of flour + L-ascorbic acid + L-cysteine hydrochloride monohydrate had a stronger reducing effect and thus a more significant impact on the viscosity and extensibility of dough. Increased compactness and thus better rheological properties (elasticity and extensibility of dough) is expected in doughs with mixtures of flour + L-ascorbic acid + inactivated dry yeast. Evaluation of the differences in the areas of proteinaceous fractions presents in the samples with the same amount of combinations of the oxidising and reducing agents plus flour observed showed that the proportion of peak areas of glutenins and gliadins is always higher in the samples (IDY1-IDY3). It means that these doughs will be of better quality than those with mixtures of flour + L-ascorbic acid + L-cysteine hydrochloride monohydrate. On the other hand, the proportion of the peak areas of albumins and globulins is higher in the samples (C1-C3). Thus, these doughs will have a decreased volume in comparison with doughs with the mixtures of flour + L-ascorbic acid + inactivated dry yeast.

It was detected that, within the range of combinations of oxidising and reducing agents tested, the mixture of flour + L-ascorbic acid + L-tryptophan had stronger oxidising effects on the gluten network. With the increasing amount of L-tryptophan in mixtures, it was gradually impossible to detect the individual proteinaceous fractions. Furthermore, while observing the differences in the areas of proteinaceous fractions with the same amount and combinations of oxidising and reducing agents, it was detected that the samples with the mixtures of flour + L-ascorbic acid + L-threonine show a better ability to extract glutenin, gliadin, albumin and globulin subunits. Thus, it can be said that the mixtures of flour + L-ascorbic acid + L-tryptophan will have better oxidising effects on dough. Higher concentrations of L-tryptophan in mixture lead to more intensive gluten networking and thus the extraction and further detection of proteinaceous fractions was impossible. Baker products from these mixtures will have a greater volume and better porosity than doughs with the mixtures of flour + L-ascorbic acid + L-threonine. However, because of good oxidising abilities of both combinations of the mixtures, both alternatives can be recommended depending on the quality of the flour required.

Practical application

Properties of wheat flour are able to improve on required quality by using of combination of individual reducing and oxidising agents as additives chosen by us (L-cysteine hydrochloride monohydrate, inactivated dry yeast, L-ascorbic acid, L-threonine, L-tryptophan) and it is reflecting in quality of prepared doughs and bakery products.

SOUHRN

Změny zastoupení bílkovinných frakcí v pšeničné mouce za přídavku odlišných kombinací oxidačních a redukčních činidel

Byl zkoumán vliv různých kombinací redukčních a oxidačních činidel (L-cysteinu hydrochloridu monohydrátu + L-askorbové kyseliny, inaktivovaného droždí + L-askorbové kyseliny, L-threoninu + L-askorbové kyseliny, L-tryptofanu + L-askorbové kyseliny) na změny v zastoupení gluteninů, gliadinů, albuminů, globulinů v pšeničné mouce. Do pšeničné mouky byly přidány různé kombinace aminokyselin v odlišných koncentracích. Změny proteinových frakcí způsobené jednotlivými kombinacemi aminokyselin v odlišných koncentracích oproti kontrolnímu vzorku (čistá pšeničná mouka) byly vyhodnoceny pomocí Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC). Bylo zjištěno, že směs mouky + L-askorbové kyseliny + L-cysteinu hydrochloridu monohydrátu měla silnější redukční účinek na těsto než směs mouky + L-askorbové kyseliny + inaktivovaného droždí. Směs mouky + L-askorbové kyseliny + L-tryptofanu však měla silnější oxidační účinek na těsto než směs mouky + L-askorbové kyseliny + L-threoninu.

aminokyselina, oxidační činidlo, redukční činidlo, mouka, směs

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APPENDIX

An alphabetical list of abbreviations

C1	mixture of flour (75.19% w/w + L-ascorbic acid (22.56% w/w) + L-cysteine hydrochloride monohydrate (2.26% w/w)
C2	mixture of flour (73.53% w/w + L-ascorbic acid (22.06% w/w) + L-cysteine hydrochloride monohydrate (4.41% w/w)
C3	mixture of flour (72.46% w/w + L-ascorbic acid (21.74% w/w) + L-cysteine hydrochloride monohydrate (5.80% w/w)
D250	the drop in pressure at 250 seconds from PrMax minus 20%
D450	the drop in pressure at 450 seconds from PrMax minus 20%
FN	Falling number
GC	gluten content in dry matter
HMW-GS	high molecular weight glutenin subunits
IDY1	mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + inactivated dry yeast (18.75% w/w)
IDY2	mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + inactivated dry yeast (27.78% w/w)
IDY3	mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + inactivated dry yeast (35.00% w/w)
L	extensibility
LMW-GS	low molecular weight glutenin subunits
mb	millibar
MC	moisture content
min.	minute
mm	millimetre
P	tenacity
pH	pH
P/L	configuration ratio
PrMax	maximum pressure
RT	retention time
s	second
SDS	Sedimentation test according to Axford
SE-HPLC	Size Exclusion High performance liquid chromatography
SH	sulfhydryl group
SS	disulfide bond
TH1	mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-threonine (18.75% w/w)
TH2	mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-threonine (27.78% w/w)
TH3	mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-threonine (35.00% w/w)
TH4	mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-threonine (40.91% w/w)
Tol	time that pressure is higher than PrMax minus 20%
TPrMax	time to reach maximum pressure
TR1	mixture of flour (62.50% w/w + L-ascorbic acid (18.75% w/w) + L-tryptophan (18.75% w/w)

TR2 mixture of flour (55.56% w/w + L-ascorbic acid (16.67% w/w) + L-tryptophan (27.78% w/w)
TR3 mixture of flour (50.00% w/w + L-ascorbic acid (15.00% w/w) + L-tryptophan (35.00% w/w)
TR4 mixture of flour (45.45% w/w + L-ascorbic acid (13.64% w/w) + L-tryptophan (40.91% w/w)
W deformation energy

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