

Wheat Flour Proteins as Affected by Transglutaminase and Glucose Oxidase

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ABSTRACT

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Enzymes are good tool to modify wheat proteins by creating new bonds between the protein chains. In this study, the effect of the addition of glucose oxidase (GO) and transglutaminase (TG) on the wheat flour proteins is presented. The modification of wheat proteins was determined by analyzing the changes in gluten quality, alveograph parameters, and protein modifications. The amount of wet gluten increased with the addition of GO and TG, but the gluten quality was not improved in any

case. Regarding the alveograph parameters, the effect of GO was readily evident obtaining wheat dough with higher tenacity and lower extensibility than the control, while TG led to doughs with lower tenacity and that were also less extensible. The protein modifications were characterized by free-zone capillary electrophoresis (FZCE). FZCE data indicated that TG polymerizes mainly glutenins and, of those, the high molecular weight glutenin subunits were the most affected.

Cereal storage proteins of wheat play a fundamental role in the breadmaking process. They have the ability to form gluten, a necessary network to amalgamate the other wheat components, mainly carbohydrates, and the gas produced during proofing. The gluten characteristics are one of the main parameters that govern flour quality and subsequent breadmaking quality of the wheat (MacRitchie 1987). Several factors affect the final gluten quality such as cultivar, environmental conditions, insect infestation level, and postharvest conditions. Variability in any of them could result in a reduced capacity of the storage proteins to form gluten (Zhu and Khan 2001). Numerous breeding programs have attempted to improve breadmaking quality of wheat, however some new cultivars developed are not appropriate for breadmaking and require some protein modifications.

Dough conditioners have been developed to overcome deficiencies in the breadmaking quality of the wheat gluten. The oxidizing agents ascorbic acid, azodicarbonamide, and potassium bromate are the most commonly used (Tsen 1969). However, recent indications that some of them may cause cancer are decreasing their use (Wolf et al 1998).

The use of enzymes is the best alternative to chemical compounds because they are generally recognized as safe (GRAS) and do not remain active after baking. Among the enzymes that can confer strength to the dough are transglutaminases (TG) and glucose oxidases (GO). These enzymes act through different catalytic mechanisms and may induce changes in the polymerized form of the glutenin subunits and maybe transform soluble proteins into insoluble ones. Transglutaminase (EC 2.3.2.13) is an acyl transferase that catalyzes inter- or intramolecular cross-linking through the formation of peptide bonds between glutamine and lysine residues. A number of food applications of TG focus on increasing the functional value of milk, meat, and fish proteins (Zhu et al 1995; Motoki and Seguro 1998).

Gerrard et al (1998) reported an improvement of the breadcrumb strength similar to that produced by oxidizing agents. Glucose oxidase (EC 1.1.3.4) catalyzes the oxidation of glucose, producing a molecule of hydrogen peroxide that can either form disulfide bonds between proteins (Haaralsita and Pullinen 1992), or form tyrosine cross-links, whose role in the gluten structure was recently reported (Tilley et al 2001). The addition of GO improves the loaf volume of bread and the crumb grain (Vemulapalli et al 1998).

The purpose of this study was to modify wheat proteins to improve dough rheological properties and gluten strength through the addition of TG or GO.

MATERIALS AND METHODS

Wheat grain from Bolero cultivar wheats was provided by Porta S.A., Huesca, Spain. Wheat characteristics were 81.3kg/hL test weight, 32.2 g thousand kernel weight, and 12.4% protein (based on 12% moisture content). Chemical reagents were purchased from Sigma (St. Louis, MO) and were of the highest purity. Transglutaminase (100 TG U/g) was a gift from Ajinomoto Co., Japan. Glucose oxidase (500 GO U/g) was kindly provided by Novo Nordisk, Madrid, Spain.

Milling Process

To ensure a uniform distribution, enzymes were added to the tempering solution during the milling process because Haros et al (2002) reported this method was a good alternative for the enzyme addition. After appropriate cleaning, a 600-g sample lot of wheat kernels was tempered to 15.5% moisture in a Chopin conditioner by adding the necessary amount of water (unless otherwise specified). For the tempering, wheat kernels were kept at 20–25°C for 16 hr and then milled in a laboratory Chopin mill. Different enzyme concentrations were added to the tempering water for the enzyme-pretreated wheat. The concentrations of GO were 0.4–4.0 enzyme activity units per gram of kernel, and up to 2.0 enzyme activity units per gram of kernel in the case of TG. Four sets of samples were milled for each treatment.

Enzyme Activity Determination

The enzyme activity was measured in the resulting flour from each tempering conditions. Transglutaminase activity was spectrophotometrically measured as described in Folk and Cole (1966) by using the hydroxamate method. One unit of TG is defined as the amount of enzyme that releases 1 μmol of hydroxamic acid in 1 min at 37°C. The GO activity was measured by using glucose as substrate, and coupling that reaction to the *o*-dianisidine oxidation in the presence of peroxidase. One unit of GO is defined as the amount of enzyme that oxidizes 1 μmol of *o*-dianisidine/min at 25°C. Two extractions were made from each sample and four replicates per extract.

Physical Measurements

Wet gluten and gluten index were determined according to the Approved Method (AACC 2000). The alveograph test (Chopin, Tripette et Renaud, Paris, France) followed the Approved Method. The parameters registered were tenacity (*P*), extensibility (*L*), the ratio of work input to deformation or energy and the deformation curve (*P/L*) (Rosell et al 2001).

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Sample Preparation for Protein Characterization

A sequential extraction was obtained for each class of proteins. Albumins and globulins were preextracted from the flours as reported by Bean et al (1998). Gliadins were then extracted from that precipitate by vortexing 200 mg of the preextracted wheat flour with 1.0 mL of 1-propanol and water (50:50, v/v) for 5 min and then centrifuging at $15,700 \times g$ for 2 min as reported in Bean and Lookhart (1998). The precipitates were extracted twice more with the same solution and centrifuged at $15,700 \times g$ for 2 min; the last two supernatants were discarded. Glutenins were obtained by mixing the gliadin-free pellet with 1.0 mL of 1-propanol and water (50:50, v/v), containing 65 mM dithiothreitol and then centrifuging at $15,700 \times g$ for 2 min. High molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) were prepared by acetone precipitation as described by Bean and Lookhart (2000). Four repetitions of each sample were made for each determination.

Capillary Electrophoresis Analysis

Separations were made using a Beckman PACE 5510 instrument. Uncoated fused silica capillaries (Polymicro, Phoenix, AZ) of 50 μm i.d. \times 27 cm (20 cm L/D) were used for all separations.

Free-zone capillary electrophoresis (FZCE) was performed with 50mM iminodiacetic acid (IDA) in acetonitrile and hydroxypropyl methyl cellulose (HPMC) and water (20:0.05:79.95, v/v) at 45°C and 30 kV, the optimum separation conditions described by Bean and Lookhart (2000).

RESULTS AND DISCUSSION

Physical Properties of Enzyme-Treated Flours

The effect of the enzyme treatment on the gluten properties was assessed by determining both the wet gluten and the gluten index values. Enzyme treatment led to flours with modified gluten characteristics as in Table I. The amount of wet gluten slightly increased with a high dosage of GO and TG. In GO, the wheat treat-

ment with 2.0 and 4.0 U/g led to high wet gluten content; the same result was obtained with the TG treatment at dosage >1.0 U/g. These results could be attributed to the presence of some compounds bonded to gluten due to the enzyme activity. The gluten quality determined by the gluten index values was not modified by the addition of increasing concentrations of GO. Conversely, the addition of TG decreased the gluten index. To determine whether these enzymes have a synergistic effect, a sample was tempered using the lowest concentration of GO and TG. The final effect was similar to that observed by adding GO. It should be expected that the gluten index would increase with the enzyme treatment because the enzymes used have the ability to form new covalent bonds. No modification was observed with GO; lower gluten index values were obtained when kernels were treated with high TG concentrations, although the values (with the exception of the treatment with 1.0 and 1.5 U/g of kernel) were still in the optimum gluten index range for breadmaking (60–90) as reported by Perten (1990). These results differ from Larre et al (2000), who found a decrease of gluten solubility after TG treatment of gluten due to the polymerization reaction. The difference could be due to the lower amount of enzyme used in this study (at least 150 times lower). Consequently, the polymerization promoted by the enzyme treatment might not be sufficient to produce a size change detectable by a gross mechanical method like the gluten index.

Alveographic parameters were also determined because it is an extended method used to assess breadmaking quality of the flours. Table II shows that GO addition increased the tenacity, and the effect increased with the enzyme concentration. Conversely, the extensibility (L) was largely reduced with treatment at 2.0 U/g of kernel, showing no further decrease at the highest GO concentration tested (4.0 U/g of kernel). As a consequence, the deformation curve ratio (P/L) when adding 2.0 U/g of kernel, was twice that of the control flour (without enzyme treatment). Therefore, some types of deficiencies in breadmaking quality of wheat flour could be overcome by GO treatment. Studies conducted with an extensigraph showed that the addition of GO resulted in dough that was less

TABLE I
Effect of Enzyme Treatment During Milling on Gluten Properties Determined by Wet Gluten Content and Gluten Index

Enzyme	Dosage (U/g of kernel)	Enzyme Activity ^a (U/g of flour)	Wet Gluten (%) ^b	Gluten Index (%) ^b
Control	0.0	–	33.0 \pm 0.3	81.2 \pm 1.1
Glucose oxidase (GO)	0.4	0.10 \pm 0.01	32.9 \pm 0.9	80.3 \pm 7.9
	2.0	0.59 \pm 0.03	35.9 \pm 0.5	82.1 \pm 2.3
	4.0	0.94 \pm 0.04	35.5 \pm 1.5	83.1 \pm 4.3
	0.01	nd	32.8 \pm 0.7	79.4 \pm 5.3
Transglutaminase (TG)	0.05	0.01 \pm 0.00	33.7 \pm 0.1	80.8 \pm 0.4
	0.1	0.02 \pm 0.00	31.4 \pm 0.5	88.6 \pm 0.0
	1.0	0.22 \pm 0.03	36.2 \pm 1.3	65.9 \pm 0.7
	1.5	0.32 \pm 0.02	34.3 \pm 0.0	66.5 \pm 0.2
	2.0	0.52 \pm 0.03	35.0 \pm 0.2	67.1 \pm 0.1
	TG + GO	0.01 + 0.4	nd	33.9 \pm 0.4

^a Remaining enzyme activities in resulting flour.

^b Mean \pm standard deviation obtained from four assays per wheat sample.

TABLE II
Effect of Enzyme Treatments on Alveographic Parameters of Flour^a

Enzyme	Dosage (U/g of kernel)	P (mm)	L (mm)	P/L	W (10^{-4} J)
Control	0.0	41.0 \pm 1.7	164.0 \pm 6.7	0.25 \pm 0.00	143 \pm 0
Glucose oxidase (GO)	0.4	38.0 \pm 0.0	168.0 \pm 14.0	0.23 \pm 0.01	145 \pm 8
	2.0	50.0 \pm 1.3	99.0 \pm 3.3	0.51 \pm 0.01	138 \pm 11
	4.0	57.0 \pm 0.3	97.0 \pm 2.7	0.59 \pm 0.01	130 \pm 7
	0.01	41.0 \pm 0.0	162.0 \pm 0.0	0.25 \pm 0.00	148 \pm 0
Transglutaminase (TG)	0.05	37.0 \pm 0.0	159.0 \pm 0.0	0.24 \pm 0.00	144 \pm 1
	0.1	38.0 \pm 0.7	160.0 \pm 0.0	0.24 \pm 0.00	144 \pm 2
	1.0	34.0 \pm 0.0	156.0 \pm 3.8	0.22 \pm 0.03	125 \pm 11
	1.5	34.0 \pm 0.7	158.0 \pm 1.3	0.22 \pm 0.00	116 \pm 1
	2.0	31.0 \pm 1.3	137.0 \pm 2.0	0.23 \pm 0.01	109 \pm 5
	TG + GO	0.01 + 0.4	41.0 \pm 2.0	142.0 \pm 11.0	0.29 \pm 0.01

^a Mean \pm standard deviation obtained from four assays per wheat sample.

extensible and more resistant than the control (Poulsen and Bak Hostrup 1998).

The addition of TG also modified the alveographic parameters. However, in this case, the TG treatment yielded a steady decrease of tenacity (*P*) with the increase of enzyme concentration. The effect on the extensibility was not so clear, only a slight decrease could be observed. In addition, a decrease of deformation energy (*W*) was observed. A synergistic effect of the TG and GO, in terms of decrease in extensibility, was also obtained.

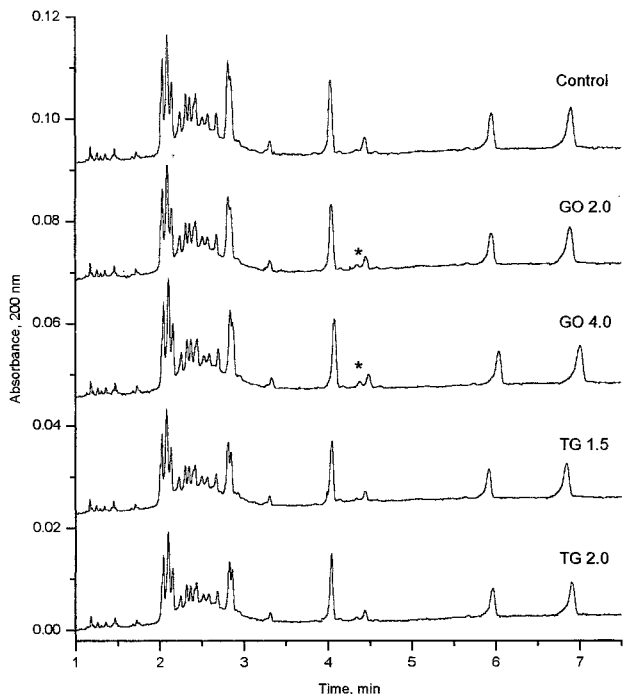


Fig. 1. Effect of enzyme treatments with glucose oxidase (GO) and transglutaminase (TG) on electrophoregrams of gliadin fraction. Numbers refer to enzyme concentration used in the treatment (U/g of kernel). Asterisks indicate differences among samples. Separations were in an uncoated capillary 50 μ m i.d. x 27 cm long (20 cm L/D) at 45°C and 30 kV. Samples were pressure-injected (0.5 psi) for 4 sec.

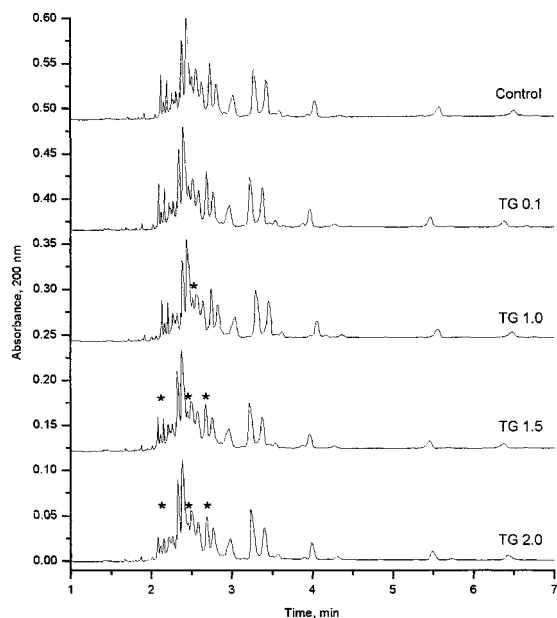


Fig. 2. FZCE profile of glutenins from flours treated at different transglutaminase (TG) concentrations. Numbers refer to enzyme concentration (U/g of kernel) during milling. Asterisks indicate differences among samples. FZCE conditions as described in Fig. 1.

The results obtained with both techniques revealed that the enzyme treatment modified the properties of the proteins of wheat flour. Comparison of the effects of both enzymes (GO and TG) on the gluten quality and alveographic parameters indicates that higher effect is promoted by GO treatment than by TG.

Protein Modification by FZCE

Separation of the wheat proteins extracted from flours treated with and without enzyme solutions were performed by SE-HPLC. No differences in the total areas beneath the chromatogram curves were detected either in the alcohol-soluble proteins or the glutenins (results not shown). Vemulapalli and Hosney (1998) also found

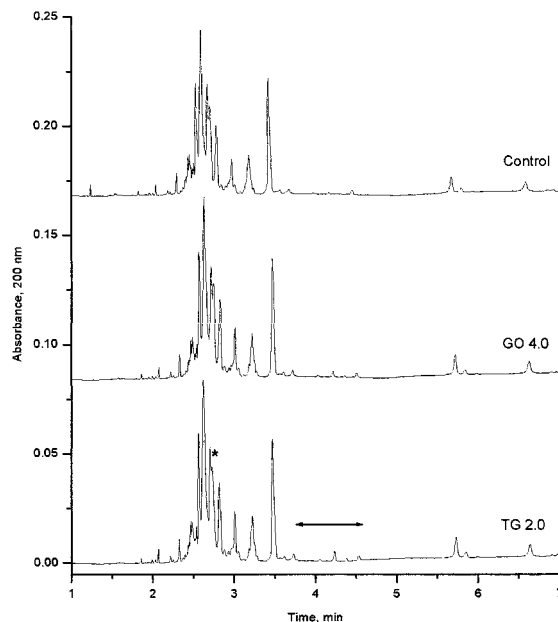


Fig. 3. Electrophoregram profiles of LMW-GS from flours subjected to enzyme treatments with glucose oxidase (GO) and transglutaminase (TG). Numbers refer to enzyme concentration used in the treatment (U/g of kernel). Asterisks indicate differences among samples. Arrow indicates range where differences were detected. FZCE conditions are the same as in Fig. 1.

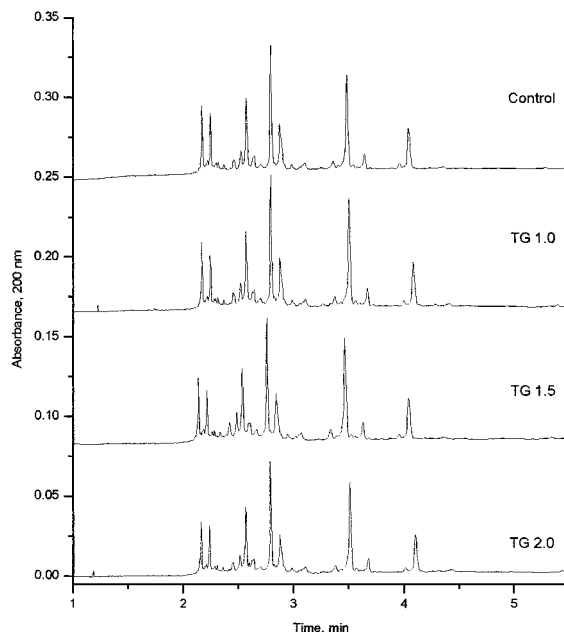


Fig. 4. Effect of transglutaminase (TG) treatment on HMW-GS electrophoregram patterns. Numbers refer to enzyme concentration used (U/g of kernel). FZCE conditions are the same as in Fig. 1.

that GO did not act directly on gluten proteins. Only the treatment with the highest concentration of TG (2.0 U/g of kernel) produced a slight decrease in the glutenin fraction (results not shown). Therefore, no noticeable change in the size of the proteins was produced by the enzyme treatment, with the exception of the above-mentioned TG treatment. Larre et al (2000) described a decrease of the gluten solubility with the polymerization reaction promoted by TG. In addition, they reported that the extent of polymerization and, in consequence, the amount of polymerized products was largely related to the quantity of enzyme. This would explain why this study found a decrease in the glutenins only at the highest TG concentration, which still was very low compared with Larre et al (2000).

The flour proteins were fractionated and analyzed by FZCE to better understand the effect of various enzyme treatments. Comparison of the gliadin electrophoregram from GO-treated flours revealed the increase of a peak at the low mobility region, which increased with the enzyme concentration (Fig. 1). The profile of the gliadins from TG-treated samples showed a decrease in the amount of all gliadin types. That effect was more pronounced at the highest enzyme concentration (2.0 U/g of kernel). There was a decrease in the intensity of the SDS-PAGE bands of gluten proteins that had been treated with TG; the LMW-GS and gliadins, and within the latter, γ gliadins were the most affected (Larre et al 2000). However, in the present study, a reduction of all the gliadin peaks was observed.

No differences were detected in the glutenin electrophoregrams from GO-treated samples. These findings agree with previous results of Vemulapalli and Hosney (1998), that GO did not affect gluten solubility and viscosity properties, and the effect on dough might be due to its oxidizing action on the water-soluble fraction.

Conversely, TG treatment induced several changes in the glutenin FZCE. In Fig. 2, there is a progressive decrease of some peaks at the higher mobility region. To better separate the glutenins, the LMW and HMW glutenin fractions were analyzed by FZCE. Only at the highest TG treatment were differences found in the LMW-GS pattern obtained from the protein extracts from flours treated with GO and TG (Fig. 3). Those differences were small, a decrease in one peak and also an increase in the peaks located at low electrophoretic mobilities. As previously indicated, Larre et al (2000) reported a decrease in the SDS-PAGE bands corresponding to LMW-GS when gluten was modified by TG. In this study, we were able to detect the specific peaks modified by the TG reaction by using FZCE.

Electrophoregrams of the HMW-GS from GO-treated samples, again did not reveal changes in the protein profile (results not shown). On the contrary, increasing the TG concentration produced a progressive decrease in the height of some HMW-GS peaks (Fig. 4), at 2.1, 2.2, 2.7, and 2.8 min of retention time. These results were similar to the findings of Larre et al (2000), where a reduction of the SDS-PAGE band intensity of the HMW-GS, along with the presence of new bands corresponding to higher molecular weight molecules, were described.

CONCLUSIONS

The addition of TG or GO modified wheat storage proteins. The changes promoted by GO treatment exhibited very pronounced effects on the physical properties of wheat flour but did not introduce changes in the protein profiles. This might be attributed to the presence of oxygen (involved in the enzyme reaction) in both the gluten index and the alveographic analysis, which was not as available during protein extraction and, in consequence, no oxidative effects were observed in the protein profile. On the other hand, the effects promoted by TG treatments were readily apparent on the protein electrophoretic properties. By using FZCE, the specific protein fraction affected by the enzyme can be detected.

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LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 38-12, 54-30, and 56-81B. The Association: St. Paul, MN.
- Bean, S. R., Bietz, J. A., and Lookhart, G. L. 1998. High-performance capillary electrophoresis of cereal proteins. *J. Chromatogr. A* 814:25-41.
- Bean, S. R., and Lookhart, G. L. 1998. Faster capillary electrophoresis separations of wheat protein through modification to buffer composition and handling properties. *Electrophoresis* 19:3190-3198.
- Bean, S. R., and Lookhart, G. L. 2000. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its application to protein characterization and cultivar differentiation. *J. Agric. Food Chem.* 48:344-353.
- Folk, J. E., and Cole, P. W. 1966. Mechanism of action of guinea pig liver transglutaminase. *J. Biol. Chem.* 241:5518-5525.
- Gerrard, J. A., Fayle, S. E., Wilson, A. J., Newberry, M. P., Ross, M., and Kavale, S. 1998. Dough properties and crumb strength of white pan bread as affected by microbial transglutaminase. *J. Food Sci.* 63:472-475.
- Haarasilta, S., and Pullinen, T. 1992. Novel enzyme combination. A new tool to improve baking results. *Agro Food Industry Hi Technol. (Italy)* 3:12-13.
- Haros, M., Rosell, C. M., and Benedito, C. 2002. Improvement of flour quality through carbohydrases treatment during wheat tempering. *J. Agric. Food Chem.* 50:4126-4130.
- Larre, C., Denery-Papini, S., Popineau, Y., Desserme, C., and Lefebvre, J. 2000. Biochemical analysis and rheological properties of gluten modified by transglutaminase. *Cereal Chem.* 77:32-38.
- MacRitchie, F. 1987. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. *J. Cereal Sci.* 6:259-268.
- Motoki, M., and Seguro, K. 1998. Transglutaminase and its use for food processing. *Trends Food Sci. Technol.* 9:204-210.
- Perten, H. 1990. Rapid measurement of wet gluten quality by the gluten index. *Cereal Foods World* 35:401-402.
- Poulsen, C., and Bak Hostrup, P. 1998. Purification and characterization of a hexose oxidase with excellent strengthening effects in bread. *Cereal Chem.* 75:51-57.
- Rosell, C. M., Rojas, J. A., and Benedito, C. 2001. Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids* 15:75-81.
- Tilley, K. A., Benjamin, R. E., Bagorogoza, K. E., Moses Okot-Kotber, B., Prakash, O., and Kwen, H. 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. *J. Agric. Food Chem.* 49:2627-2632.
- Tsen, C. C. 1969. Effects of oxidizing and reducing agents on changes of flour proteins. *Cereal Chem.* 46:435-442.
- Vemulapalli, V., and Hosney R. C. 1998. Glucose oxidase effects on gluten and water solubles. *Cereal Chem.* 75:859-862.
- Vemulapalli, V., Miller, K. A., and Hosney, R. C. 1998. Glucose oxidase in breadmaking systems. *Cereal Chem.* 75:439-442.
- Wolf, D. C., Crosby, L. M., George, M. H., Kilburn, S. R., Moore, T. M., Miller, R. T., and DeAngelo, A. B. 1998. Time and dose dependent development of potassium bromate induced tumors in male Fischer 344 rats. *Toxicol. Pathol.* 26:724-729.
- Zhu, J., and Khan, K. 2001. Effects of genotype and environment on glutenin polymers and breadmaking quality. *Cereal Chem.* 78:125-130.
- Zhu, Y., Rinzema, A., Tramper, J., and Bol, J. 1995. Microbial transglutaminase. A review of its production and application in food processing. *Appl. Microbial Biotechnol.* 44:277-282.