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Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates

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Abstract

The insolubility of gluten in aqueous solutions is one of the major limitations for its more extensive use in food processing. Wheat gluten was enzymatically hydrolyzed by several commercially available proteases (Alcalase 2.4L, PTN 6.0S, Pepsin, Pancreatin, Neutrase and Protamex™) with protein recovery of 81.3%, 42.5%, 53.3%, 61.6%, 46.3% and 43.8%, respectively. The hydrolytic efficiency of these proteases on wheat gluten was also compared. Alcalase served best for the preparation of wheat gluten hydrolysates with the maximum degree of hydrolysis (DH) 15.8%. Subsequently, the solubility of wheat gluten hydrolysates (WGHs) obtained with those enzymes was comparably evaluated. The products had excellent solubility (>60%) over a pH range of 2–12. The molecular weight distribution of WGHs was further determined by SDS-PAGE and size exclusion chromatography on Sephadex G-15. The results showed that with the increasing of DH values, there occurred a large amount of smaller polypeptides.

Keywords: Wheat gluten; Enzymatic hydrolysis; Solubility; SDS-PAGE

1. Introduction

With the expansion of wheat starch production, wheat gluten, a by-product of the wheat starch industry, is available in large amounts and at relatively low cost. Because it is insoluble in near-neutral pH and is viscoelastic when hydrated, wheat gluten is mainly used presently to enhance the strength of flours for breadmaking and to texturize food. However it is possible to enlarge the field of its applications through chemical or enzymatic modifications. The use of proteolytic enzymes is an efficient protein modification method (Adler-Nissen, 1986). By controlling the reaction conditions during the enzymatic hydrolysis, it is possible to obtain hydrolysates having different characteristics. Much research focused on chemical or enzymatic modifications of wheat gluten had resulted in the enhancement of its solubility, foaming and emulsifying properties (Babiker, Fujisawa, Matsudomi, & Kato, 1996; Batey, 1985; Kammoun, Bejar, & Ellouz, 2003; Linarès, Larré, Lemeste, & Popineau, 2000; Mimouni, Raymand, Merle-Desnoyers, Azanza, & Ducastaing, 1994; Mimouni, Azanza, & Raymond, 1999; Popineau, Huchet, Larré, & Bérot, 2002). Different proteases have been tried, such as pepsin, trypsin, papain, bromelain, and subtilisin. Most work on enzymatic hydrolysis of wheat gluten focused on peptic hydrolysis, which is known to have a broad side chain specificity and to produce large polypeptides from gluten (Bietz & Rothfus, 1970; Cornell & Mothes, 1995; Masson, Tomé, & Popineau, 1986). Wheat gluten hydrolysis results in peptide mixtures with high solubilities and altered foaming and emulsifying characteristics, depending on the degree of hydrolysis.

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Besides the improvement of functional properties of protein hydrolysates, some studies dealed with the production of low molecular peptides. These peptides have the advantage of being absorbed in the intestine without any digestion in the stomach and have low allergenic effects (Gonzalez-Tello, Camacho, Jurado, Paez, & Guadex, 1994b). This explains their preferential use in many formulas such as diets for nursing infants or sick adults and as stimulations for persons liable to develop allergy.

The objective of this present research was to examine the use of commercially available proteolytic enzymes for the preparation of wheat gluten hydrolysates (WGHs), to characterize the hydrolysates by determining their solubility and estimating the molecular weight by SDS-PAGE and Sephadex G-15.

2. Materials and methods

2.1. Materials

Wheat gluten was supplied by Lotus Gourmet Powder Group Company (Henan, China).

2.2. Chemicals and reagents

All chemicals and reagents used in this work were food-grade or reagent-grade. Alcalase 2.4L (EC 3.4.21.62, from *Bacillus licheniformis*, 2.4 AU/g), Pancreatin Trypsin Novo 6.0S (PTN 6.0S) (EC 3.4.21.4, from porcine pancreas, 1350 USP u/mg), Neutrase (EC 3.4.24.28, from *Bacillus amyloliquefaciens*, 1.5 AU/g) and Protamex™ (EC 3.4.24.28, from *Bacillus subtilis*, 1.5 AU/g) were donated by Novo Nordisk (Bagsvaerd, Denmark). Porcine pepsin (EC 3.4.23.1, 3140 BP. units/g) and Pancreatin (146 USP u/mg) were supplied by Deyang biochemical products. The Sephadex G-15 column used for analyzing the molecular weight distribution was purchased from Pharmacia (Uppsala, Sweden).

2.3. Proteolysis with different enzymes

Wheat gluten was hydrolyzed with six different enzymes, under the conditions given in Table 1, based on optimum hydrolysis conditions. Wheat gluten was dissolved as a 5% (w/v) solution, the suspension was adjusted to appropriate temperature and pH, depending on the used enzyme, and incubated for 30 min by stirring continuously. The reaction was initiated by the addition of the enzyme to give a final enzyme-to-substrate ratio of 1:100 (w/w). For Pepsin, the mixture was incubated at 37 °C for 24 h and no significant change of the pH was found during the reaction. But for other enzymes, the reaction was continued for 6 h and the pH of the mixture was kept constant by continuously adding a 4 M NaOH solution to the reaction mixture. After the reaction period, the mixture was cooled, adjusted to pH 7.0 with 0.5 M and 4 M NaOH and heated at 95 °C for 10 min to inactivate the enzyme. Then the mixture was centrifuged at 10,000 g for 20 min at 4 °C in a ZOPR-52D refrigerating centrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan). The supernatant was freeze-dried and stored at −20 °C.

2.4. Degree of hydrolysis

2.4.1. pH-stat assay

The degree of hydrolysis (DH%), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (h_{tot}), in each case, was calculated from the amount of base consumed (Adler-Nissen, 1986), as given below:

$$\mathrm{DH}\% = \frac{h \times 100}{h_{\mathrm{tot}}} = \frac{B \times N_{\mathrm{b}} \times 100}{\alpha \times M_{\mathrm{p}} \times h_{\mathrm{tot}}}$$

where B is base consumption in ml; N_b is normality of the base; α is average degree of dissociation of the α-NH_2 groups; MP is mass of protein ($N \times 5.7$) in g; h is the hydrolysis equivalents in meqv/g protein and h_{tot} is total number of peptide bonds in the protein substrate (8.38 meqv/g gluten protein).

2.4.2. Trichloroacetic acid (TCA) assay

For peptic hydrolysis, DH was determined by the ratio of the percentage of 10% TCA-soluble nitrogen to total nitrogen in the sample (Drago & González, 2001). Aliquots were removed at the final time required and mixed with 20% TCA to create 10% TCA-soluble and TCA-insoluble fractions. After 30 min, the mixture was centrifuged at 3000g and the supernatants were analysed for nitrogen by the semimicro-Kjeldahl method (AOAC, 1990).

2.5. Determination of protein recovery of WGHs from wheat gluten

Protein recovery was calculated as the ratio of the amount of protein (% $N \times 5.7$) present in the hydrolysates to the initial amount of protein present in the reaction mixture.

Table 1
Conditions for the hydrolysis of wheat gluten with different proteases

Reaction conditions	Proteases					
	Alcalase 2.4L	PTN 6.0S	Pepsin	Pancreatin	Protamex	Neutrase
pH	8.5	8.5	2.0	8.5	6.5	7.0
T (°C)	60	47	37	37	50	50

2.6. Solubility of WGHs

The modified nitrogen solubility index (NSI) procedure of Morr et al. (1985) was used to measure the solubility of gluten and WGHs at a pH range of 2–12. The solubility of the hydrolysate was expressed as the fraction of soluble *N* to total *N*.

2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on all samples by 2-ME according to the discontinuous electrophoresis method of Laemmli (1970) using a 4% stacking gel and 13% acrylamide gel. Reduction of disulfide bonds was performed by 2-mercaptoethanol (5% v/v) at 100 °C for 3 min. All samples reduced were centrifuged at 4000*g* for 10 min, and the supernatants were used to load the gels. The separating gel was run at a constant current of 20 mA for about 3 h. The gels were stained in Coomassie brilliant blue R-250. Molecular weights of protein hydrolyates were estimated by using the following markers including phosphorylase (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43.0 kDa), bovine carbonic anhydrase (31.0 kDa), trypsin inhibitor (20.1 kDa), and hen egg white lysozyme (14.4 kDa).

2.8. Size exclusion chromatography

Peptide fractions of the hydrolysates were separated using column chromatography as described by Chen, Muramoto, and Yamaguchi (1995). The lyophilized hydrolysate (50 mg) was dissolved with 1 ml of deionized water. The resulting solution was fractioned by gel filtration on Sephadex G-15 column (1.6 × 100 cm), and eluted with deionized water. Each 5 ml fraction was collected at a flow rate of 30 ml/h, and monitored at 280 nm the absorbance to separate peptide fractions. Bovine serum albumin (Mr 66.0 kDa), Vitamin B (Mr 1355), and tyrosine (Mr 181) were used as the comparable standards of molecular weight.

3. Results and discussion

3.1. Enzymatic hydrolysis of wheat gluten

The control of enzymatic hydrolysis was related to the mechanism of proteolytic reactions involving a soluble enzyme and an insoluble substrate (wheat gluten). The hydrolysis of wheat gluten with Alcalase, Trypsin, Pancreatin, Neutrase and Protamex proceeded at a rapid rate during the initial 30 min and then slowed down thereafter (Fig. 1), indicating that maximum cleavage of peptides occurred within 30 min of hydrolysis. This was typical for hydrolysis curves published for fish protein and whey protein (Mutilangi, Panyam, & Kilara, 1995). DH values varied from 0% to 15.8% after 360 min of incubation, depending upon the enzymes used. Alcalase gave the highest DH values and was more efficient for gluten hydrolysis than the others. For peptic hydrolysis of wheat gluten, DH was determined by the TCA assay. The hydrolytic curve (Fig. 2) was similar to Fig. 1, with DH values varied from 0% to 27.2% during 24 h of incubation.

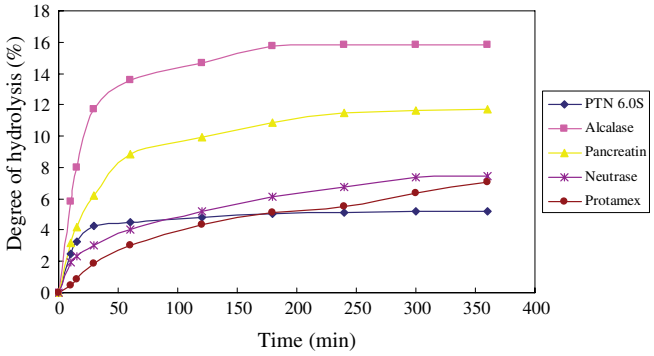

Fig. 1. Enzymatic hydrolysis of wheat gluten with different proteases at 1:100 (w/w) enzyme/substrate and wheat gluten concentrations at 5%.

The correlation between the results of the two determination methods of degree of hydrolysis in wheat gluten hydrolysis with Alcalase was also studied. The results obtained by the TCA assay were highly correlated with those obtained by the pH-stat assay ($y = 4.1422x - 3.52$, correlation coefficient of 0.9843). The DH varied from 0% to 16.0% and 0% to 63.7% determined respectively by pH-stat and TCA assay. This indicated that the hydrolytic efficiency of Alcalase was much higher than that of pepsin.

3.2. Protein recovery of WGHs from wheat gluten

The yields of protein hydrolysates with different proteolytic enzymes were given in Table 2. This is an important measurement because a maximum recovery is desired in the production of hydrolyzed food proteins. Although many factors affected the yield of hydrolysis, the type of enzyme used had a marked effect on the yield and properties of the final product. High protein recovery by Alcalase (81.3 ± 0.1%) and its low cost may provide an incentive for using it in commercial operations.

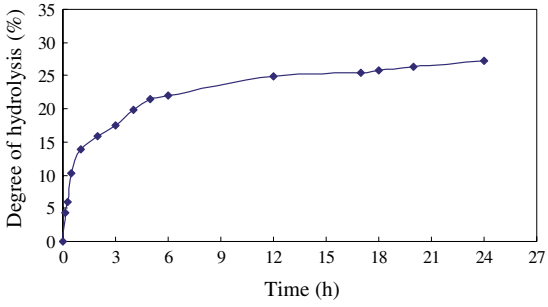

Fig. 2. Time course of peptic hydrolysis of wheat gluten obtained by the TCA assay.

Table 2
Protein recovery from wheat gluten and protein content for different WGHs

WGHs	Protein content (%)	Protein recovery (%)
Alcalase 2.4L	79.7 ± 0.73	81.3 ± 0.13
PTN 6.0S	80.9 ± 0.28	42.5 ± 0.68
Pepsin	78.3 ± 0.51	53.3 ± 0.42
Pancreatin	80.2 ± 0.16	61.6 ± 0.37
Protamex	80.8 ± 0.35	43.8 ± 0.28
Neutrase	80.5 ± 0.43	46.3 ± 0.39

Values are shown as mean ± SD of three analyses.

3.3. Solubility of wheat gluten hydrolysates

All WGHs presented a similar pattern of NSI values, and the solubilities of those hydrolysates were pH-independent over the studied range. The solubility increased from 14% for wheat gluten to over 60% for all WGHs, as shown in Fig. 3. Such behavior could be explained by the fact that smaller, more hydrophilic and more solvated polypeptide units were produced as a consequence of enzymatic hydrolysis; hence, aggregates were no longer formed, even at the isoelectric pH.

3.4. Electrophoretic characterization

To study the effect of different enzymes on protein breakdown in wheat gluten, SDS-PAGE of WGHs was performed to obtain information on their molecular weight. The result is shown in Fig. 4, where lane s shows molecular weight marker, and lanes 1–6 shows WGHs obtained with Alcalase, Pepsin, PTN 6.0S, Pancreatin, Neutrase and Protamex™. The molecular weight of all the WGHs was drastically decreased below 40 kDa compared with raw gluten, which includes HMW subunits of glutenin (65–90 kDa), ω-gliadins (40–75 kDa), LMW subunits of glutenin, α-gliadins and γ-gliadins (30–45 kDa) (Shewry & Tatham, 1997). Most of WGHs had an obvious band near 36.5 kDa, this is similar with the results of Bietz and Wall (1972), but WGH with pepsin is an exception, which had lots of large polypeptides below 30 kDa. It could also be seen that with the increasing of DH values

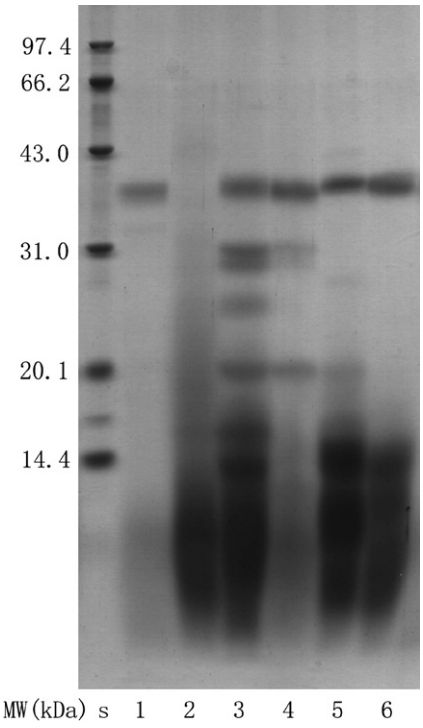

Fig. 4. SDS-PAGE profile of WGHs obtained with different enzymes. Lanes 1–6 are referred to WGHs by Alcalase, Pepsin, PTN 6.0S, Pancreatin, Neutrase and Protamex™ respectively. And lane s is molecular weight marker.

amounts of smaller polypeptides below 14.4 kDa occurred, especially for WGH obtained with Alcalase which indicated more polypeptides below 10 kDa were present.

3.5. Distribution of molecular weight of WGHs

Molecular weight distributions of wheat gluten hydrolysates obtained with Alcalase (AWGH) and Neutrase (NWGH) were shown in Fig. 5 by size exclusion chromatography on Sephadex G-15. According to Freitas et al. (1993), the eluate analysis by absorption at 280 nm detects the presence of peptides and amino acids, with aromatic acid chains. Albumin (66 kDa) used to standardize the column, was

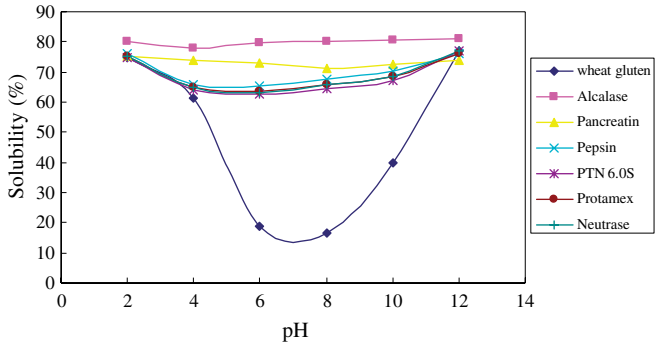

Fig. 3. Effect of pH on solubility profiles of WGHs obtained with different enzymes.

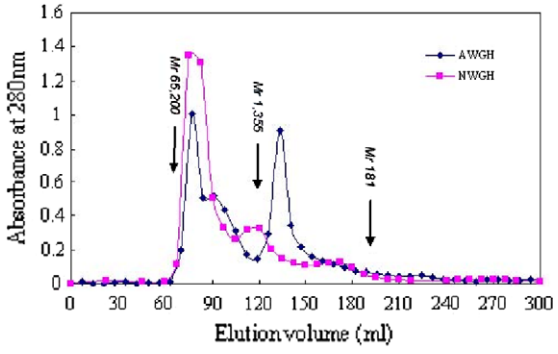

Fig. 5. Elution profiles of WGHs obtained with Alcalase and Neutrase separated by Sephadex G-15 chromatography.

eluted in the exclusion volume, as expected. Vitamin B (Mr 1355) showed an elution volume of 120 ml, and tyrosine (Mr 181) was eluted at about 192 ml. AWGH was much more hydrolyzed than NWGH with more smaller peptides occurring (molecular weight below 1355). The hydrolysates were separated into pools for further study after gel filtration according to the profile characteristics obtained by absorbance at 280 nm.

4. Conclusion

Enzymatic hydrolysis is a suitable route to improve the solubility of wheat gluten. All the WGHs, obtained with Alcalase 2.4L, PTN 6.0S, Pepsin, Pancreatin, Neutrase and Protamex^{TM}, had a high protein recovery varying from 42.5% to 81.3%. Alcalase served best for the preparation of wheat gluten hydrolysates with the maximum degree of hydrolysis of 15.8%. The solubility of all WGHs was high at all DH over a wide pH range compared to control gluten. The molecular weight distribution of WGHs showed there occurred lots of smaller polypeptides. Further study on the bioactivities of WGHs is in process.

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References

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*. New York: Elsevier Applied Science Publisher.

AOAC (1990). *Official methods of analysis* (15th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Babiker, E. F., Fujisawa, N., Matsudomi, N., & Kato, A. (1996). Improvement in the functional properties of gluten by protease digestion or acid hydrolysis followed by microbial transglutaminase treatment. *Journal of Agricultural and Food Chemistry, 44*, 3746–3750.

Batey, I. L. (1985). Enzymatic solubilization of wheat gluten. *Journal of Applied Biochemistry, 7*, 423–429.

Bietz, J. A., & Rothfus, J. A. (1970). Comparison of peptides from wheat gliadin and glutenin. *Cereal Chemistry, 47*, 381–392.

Bietz, J. A., & Wall, J. S. (1972). Wheat gluten subunits: molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Cereal Chemistry, 49*, 416–430.

Chen, H. M., Muramoto, K., & Yamaguchi, F. (1995). Structural analysis of antioxidative peptides from soybean β-conglycinin. *Journal of Agricultural and Food Chemistry, 43*, 574–578.

Cornell, H. J., & Mothes, T. (1995). Further studies of the in vitro activity of synthetic gliadin peptides in celiac disease. *Biochimica et Biophysica Acta, 1270*, 168–172.

Drago, S. R., & González, R. J. (2001). Foaming properties of enzymatically hydrolysed wheat gluten. *Innovative Food Science and Emerging Technologies, 1*, 269–273.

Freitas, O., Padovan, G. P., Vilela, L., Santos, J. E., Dutra de Oliveira, J. E., & Greene, L. J. (1993). Characterization of protein hydrolysates prepared for enteral nutrition. *Journal of Agricultural and Food Chemistry, 41*, 1432–1438.

Gonzalez-Tello, P., Camacho, F., Jurado, E., Paez, M. P., & Guadex, E. M. (1994b). Enzymatic hydrolysis of whey protein: II. *Molecular-Weight Range. Biotechnology Bioengineering, 44*, 529–534.

Kammoun, R., Bejar, S., & Ellouz, R. (2003). Protein size distribution and inhibitory effect of wheat hydrolysates on Neutrase. *Bioresource Technology, 90*, 249–254.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Linarès, E., Larré, C., Lemeste, M., & Popineau, Y. (2000). Emulsifying and foaming properties of gluten hydrolysates with an increasing degree of hydrolysis: role of soluble and insoluble fractions. *Cereal Chemistry, 77*(4), 414–420.

Masson, P., Tomé, D., & Popineau, Y. (1986). Peptic hydrolysis of gluten, glutenin and gliadin from wheat grain: kinetics and characterisation of peptides. *Journal of the Science of Food and Agriculture, 37*, 1223–1235.

Mimouni, B., Azanza, J. L., & Raymond, J. (1999). Influence of double enzymic hydrolyses on gluten functionality. *Journal of the Science of Food and Agriculture, 79*, 1048–1053.

Mimouni, B., Raymand, J., Merle-Desnoyers, A. M., Azanza, J. L., & Ducastaing, A. (1994). Combined acid deamidation and enzymic hydrolysis for improvement of the functional properties of wheat gluten. *Journal of Cereal Science, 21*, 153–165.

Morr, V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., et al. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science, 50*, 1715–1718.

Mutilangi, W. A. M., Panyam, D., & Kilara, A. (1995). Hydrolysates from proteolysis of heat-denatured whey proteins. *Journal of Food Science, 60*(5), 1104–1109.

Popineau, Y., Huchet, B., Larré, C., & Bérot, S. (2002). Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science, 35*, 327–335.

Shewry, P. R., & Tatham, A. S. (1997). Biotechnology of wheat quality. *Journal of the Science and Food Agriculture, 73*, 397–406.