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Enzymatic susceptibility of wheat gluten after subcritical water treatment

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Abstract Subcritical water (SCW) hydrolysis is an alternative to traditional methods of protein hydrolysis that uses water as a reaction medium. In this study, the effect of SCW treatment on heat-induced conformational changes in wheat gluten and its relation to enzymatic susceptibility were investigated. The degree of deamidation increased rapidly from 12.5 to 47.4% with increase in the temperature range of 160–220 °C. Protein solubility increased in a similar pattern with degree of deamidation and almost all protein was solubilized after treatment with SCW at 200 °C. SCW treatment in a particular time–temperature combination results in a significant decrease in enzymatic susceptibility. After SCW treatment at 220 °C for 20 min, enzymatic susceptibility of gluten protein was exceedingly decreased to nearly complete loss. Because of excess degradation and deamidation and small molecular size (less than 6500 Da) many hydrolysis sites disappear and are difficult to access by protease.

Keywords Subcritical water · Wheat gluten · Enzyme susceptibility · Hydrothermal process · Enzyme hydrolysis

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Introduction

Wheat gluten (WG) is a byproduct of wheat starch, which has attracted increasing attention owing to its unique characteristic and low cost [1]. Nevertheless, its low solubility obstructs enzymatic hydrolysis and restricts its application to the food industry. WG has a large portion of uncharged amino acid residues, which leads to the formation of extensive intermolecular associations through hydrogen bonding, and decreases the solubility in water due to the association of protein molecules as large aggregates rather than individual molecules at neutral pH [2].

Hence, it is very important to facilitate the enzymatic hydrolysis of WG by modification or pretreatment before hydrolysis. To efficiently modify WG properties, acid pretreatment and/or enzymatic hydrolysis is generally uses [3, 4]. Acid deamidation can modify the proteins in several ways such as increasing the electrostatic repulsion, stretching proteins, changing charge density, and breaking hydrogen bonds by transforming amides of Gln and Asn into carboxyl groups [5]. These treatments could unfold protein conformation and strengthen protein–water interactions [5, 6].

Recently, thermal protein hydrolysis is gaining importance in economical as well as ecological aspects [7]. Subcritical water (SCW) hydrolysis is an alternative to traditional methods of protein hydrolysis that uses water as a reaction medium. The advantages of SCW hydrolysis in comparison to conventional techniques are cost-effectiveness, harmlessness of the solvent water, and short residence times in the range of minutes [8].

SCW refers to water above its normal boiling temperature (100 °C at 0.10 MPa) but below its critical temperature (374 °C at 22 MPa), which is kept in liquid state by

applying pressure. As the temperature and pressure approach the critical point, the ion product of water increases because water molecules dissociate into hydroxonium ions (H_3O^+) and hydroxide ions (OH^-) [9]. The dissociation constant of water at 25 °C is 10^{-14} $(mol/L)^2$. However, at temperatures around 250 °C, it increases by three orders of magnitude [10^{-11} $(mol/L)^2$], which leads to a significant increase in the power of the hydrolysis reaction [9]. Moreover, lower dielectric constant, viscosity, and surface tension of SCW enhance the solubilization of treated raw material, which accelerates the reaction rate [10]. SCW has been applied to plant protein process for modifying their functional properties and generating protein hydrolysates and AAs [5–13].

Combined SCW pretreatment and enzyme hydrolysis can cause changes in the physico-chemical properties and functionalities of WG hydrolysates. However, to date, limited research is focused on the impact of structural properties on the enzymatic susceptibility of the SCW-treated WG for hydrolysis [14]. The objective of the present work was to elucidate the changes in the enzyme susceptibility of WG after SCW treatment. Here SCW-treated WG was subjected to protease hydrolysis. The changes in amino nitrogen (AN), soluble nitrogen, degree of deamidation, molecular weight distribution, and amino acid composition of the hydrolysates were analyzed. On the basis of these results, the relation between the structural changes in WG after SCW treatment and protease susceptibility was elucidated.

Materials and methods

Materials and chemicals

Commercial WG was purchased from Cargill Ltd. (made in Netherland). The crude protein content of WG determined by the micro-Kjeldahl method (N × 5.7) was 68.9% (w/w); the moisture content was 8.0% (w/w). Alcalase 2.4 L, Flavourzyme 1000 L, Neutrase, and Protamex were purchased from Novozymes (Bagsvaerd, Denmark). All chemicals were purchased from Sigma (St. Louis, MO, USA).

SCW treatment of WG

SCW treatment was performed using the 2 L batch reactor (Chem-I Engineering Inc. Anyang, Korea). In all experiments, the pressure corresponded to or slightly exceeded the vapor pressure curve at the given temperature. WG hydrothermolysis was performed at reaction temperatures ranging from 160 to 260 °C for 20 min or at a constant temperature (T = 200 °C) for 0–40 min. WG suspension (10%, w/v) of 1.0 L was transferred into the reaction vessel, which had been preheated to approximately 80 °C. The time when the reactor temperature reached the set point was taken as the starting time of the reaction (t = 0 min). The reaction mixture was maintained at the predetermined temperature with an accuracy of ± 1 °C for a given time. The hydrolysate of the WG sample was removed from the receiving tank and immediately stored in a refrigerator. Then, the insoluble residue was separated from the liquid phase by centrifugation at 10,000×*g* for 20 min. The supernatant was collected and filtered through a PTFE membrane filter (0.45 μm, Sartourius, Gottingen, Germany).

Turbidity

The WG samples were removed and immediately cooled to room temperature in an ice water bath. The turbidity of each solution was estimated by measuring the absorbance of the solutions at 600 nm using a T6 Vis–UV spectrophotometer (Genesys 10S UV–Vis, Thermo Scientific, Shanghai, China). The absorbance was used as an indicator for turbidity measurement [8].

Enzyme hydrolysis

The SCW-treated samples (50 mL) were adjusted to pH 7.0 with 4 M NaOH and kept at 50 °C in a shaking water bath (Model 83R, Vision Scientific Co., Ltd., Daejeon, Korea) at 200 rpm. The enzyme hydrolysis reaction was initiated by the addition of the enzyme at an enzyme-to-substrate ratio of 1:100 (w/w) and continued for 24 h. During enzyme hydrolysis, samples were periodically obtained at the reaction times of 0, 3, 6, 18, and 24 h for analysis, and the pH was kept constant by adding 0.1 N NaOH After enzyme hydrolysis, samples were heated at 95 °C for 20 min to inactivate the enzyme. The resulting hydrolysates were then rapidly cooled to room temperature and centrifuged at 10,000×*g* for 10 min at 4 °C.

Degree of deamidation (DD)

The DD of samples was determined as the ratio of the amount of ammonia released from the samples to the total ammonia of WG suspension. Untreated WG (0.5 g) was dissolved in 5 mL of 3 M HCl, then sealed in a 10 mL glass ampoule and heated at 121 °C for 3 h to deamidate completely. The amount of ammonia obtained in this step was the total ammonia in WG. The amount of ammonia released from the deamidated samples was determined with the Megazyme ammonia kit.

Degree of hydrolysis (DH)

The DH was calculated using the relation between AN and total nitrogen (TN) according to Equation: DH (%) = (AN/TN) × 100. The Sorensen method was used for quantifying the AN [15]. The TN was determined by the auto-Kjeldahl apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland).

Protein solubility (PS)

SCW-treated WG suspensions and their enzymatic hydrolysates were centrifuged at 1580×*g* for 20 min at 4 °C. The protein content in the supernatants was determined by the micro-Kjeldahl method using the nitrogen–protein conversion factor of 5.7 [16]. PS was calculated as nitrogen soluble index (%) = (the protein content in the supernatants/the total protein content of suspension in the reaction) × 100.

Determination of molecular weight distribution

Size exclusive chromatography analysis was performed using an ÄKTA purifier LC system (Amersham International plc, Buckinghamshire, UK) with a Superdex Peptide HR 10/30 column. For the ÄKTA purifier system, the mobile phase was 0.02 M sodium phosphate buffer containing 0.25 M NaCl (pH 7.2) at a flow rate of 0.5 mL/min. Absorbance at 214 nm was recorded [17]). Six protein and peptide standards, conalbumin (75,000 Da), ovalbumin (43,000 Da), cytochrome C (12,384 Da), aprotinin (6512 Da), bacitracin (1422 Da), and Gly–Gly–Gly (189 Da), were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Amino acid composition

Free and bound amino acid composition of WG hydrolysates after hydrothermolysis were analyzed using an VDSpher 100 C18-E column (4.6 mm × 150 mm, 3.5 µm/VDS optilab, Germany)-equipped HPLC system comprising pump, auto sampler, oven, UV (Thermo dionex, USA), and fluorescence detector 1260FLD (Agilent, USA). The mobile phase A was 40 mM sodium phosphate dibasic (pH 7.0), whereas the mobile phase B was 3DW/acetonitrile/methanol (10%:45%:45%, v/v). Bound amino acids were analyzed after hydrolysis with 6 M HCl at 130 °C for 24 h in sealed glass tubes under N_2 atmosphere. These hydrolyzed samples were then diluted with 3DW and filtered using a 0.45-µm syringe filter followed by HPLC analysis [18].

Abbreviated word index

WG-SCW (T): wheat gluten treated with subcritical water at various temperatures (T)

WG-SCW (t): wheat gluten treated with subcritical water for different time (t)

H-WG-SCW: enzymatic hydrolyzed wheat gluten treated with subcritical water

Results and discussion

Hydrothermal treatment of WG with SCW

A WG suspension (10%, w/v) was treated with SCW at temperatures ranging from 160 to 260 °C for 20 min or at a constant temperature (T = 200 °C) for 0–40 min as a batch process. Under the experimental conditions, SCW acted as a solvent, reagent, and catalyst [19]. The appearance of these gluten suspensions is illustrated in Fig. 1. After treatment with SCW, WG was converted into little granules and aggregated at 160 °C [Fig. 1(A)]. The samples comprised two parts: particulate aggregates and aqueous solution. At 180 °C, owing to the disaggregation and degradation of protein, the particle size decreased [9].

As shown in Fig. 1(B), at 200 °C, the protein was aggregated to produce large particulate flocculants even at 0 min (the time when the reactor temperature reached 200 °C), but as the holding time increased, the large particulate matter decreased. In addition, the color of the protein suspension became darker due to the Maillard-like reaction that occurred between the produced amino acids and the small amount of residual sugar in the commercial WG [20].

The turbidity (absorbance value at 600 nm) of SCW-treated WG at various temperatures for 20 min (WG-SCW) increased almost linearly as the treatment temperature increased in the range of 160–200 °C. The further increase of temperature beyond 200 °C induced solubilization of protein, which resulted in decrease of turbidity [16].

The pH of the supernatant of the hydrothermolysate was dramatically increased from the treatment temperature of 200 °C and reached 9.32 at 260 °C (data not shown). This drastic increase in pH may be attributable to the deamidation and thermal decomposition of amino acids, of heat-labile amino acids [21]. In contrast to the effect of treatment temperature described above, the pH of WG-SCWs at various treatment times was constantly 5.2.

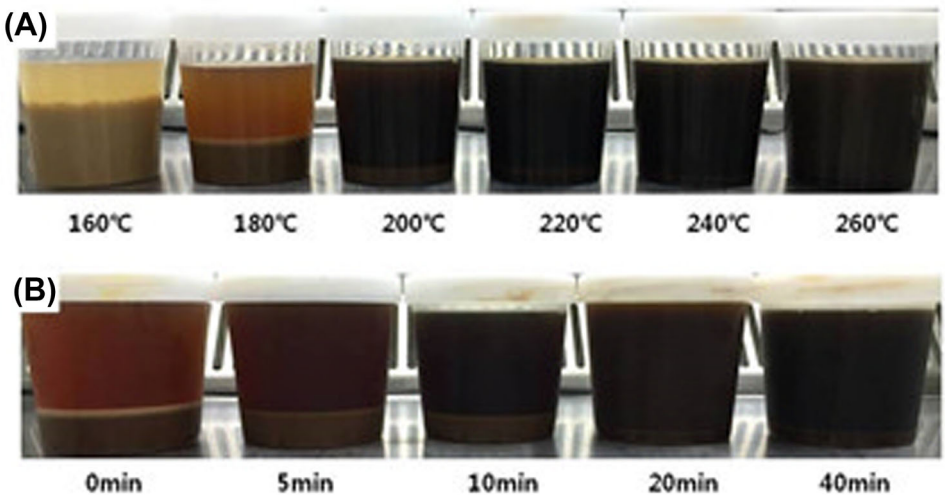

Fig. 1 Photographs of the wheat gluten (WG) suspension (10%) after treatment with subcritical water (SCW) at various temperatures for 20 min (**A**) and for various holding times at 200 °C (**B**)

Effect of SCW treatment on the susceptibility of WG to enzyme hydrolysis

Effect of treatment temperature and time

We investigated the effect of SCW treatment on the susceptibility of WG to proteolysis by Alcalase, Flavourzyme, Neutrase, and Protamex and found that the four proteases showed similar hydrolysis profiles. Furthermore, the DH of all hydrolysates gradually increased with increasing treatment temperature and finally converged on 21.5% at 220 °C (Fig. 2). This result suggested that SCW-treated WG might bind less to the active sites of the proteases as a result of the less favorable changes of substrates in terms of molecule size, conformation, and charge density [22]. At higher temperature above 220 °C, the susceptibility to the proteases became negligible, and thereafter, the DH reached 37.6% at 260 °C only by hydrothermolysis.

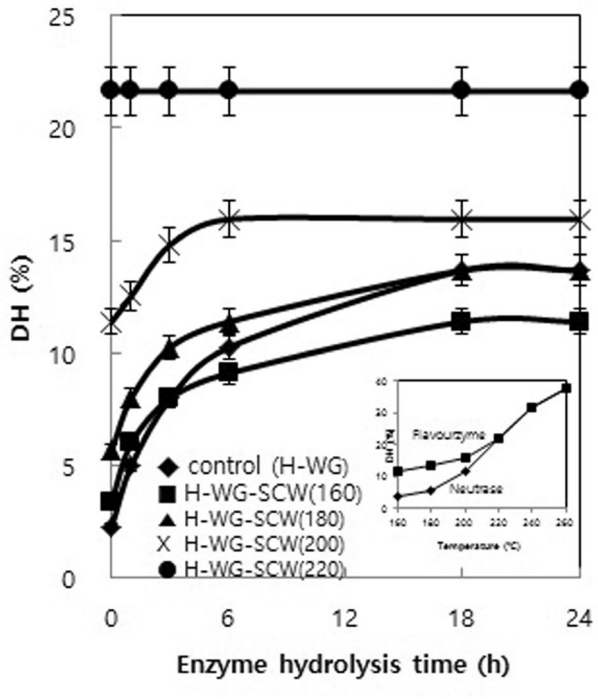

Fig. 2 Enzyme hydrolysis of WG treated with SCW at different temperatures for 20 min [H-WG-SCW (°C)]

Among the proteases, the highest hydrolysis activity was exhibited by Flavourzyme, which is sold as an industrial peptidase preparation (two aminopeptidases, two dipeptidyl peptidases, and three endopeptidases), derived from *Aspergillusoryzae*, and used for protein hydrolysis in various industrial and research applications [23]. Therefore, Flavourzyme was selected as the working enzyme in this study.

The hydrolysis profile of WG-SCW (T) using Flavourzyme was monitored by following DH over 24 h (Fig. 2). The DH at reaction time 0 h, which is the DH of hydrothermolysates after SCW treatment, increased with treatment temperature from 160 to 220 °C due to the increase in the ion product of water. The profile of H-WG-SCW (160 °C) and H-WG-SCW (180 °C) was similar to that of the control. In other words, the hydrolysis by the enzyme was relatively rapid in the first 6 h and then continued slowly until the samples showed a similar DH value of 11.4–13.7% after 24 h. In particular, for H-WG-SCW (160 °C), which included a considerable amount of aggregated protein, as shown in Fig. 1(A), the initial rate of hydrolysis was slightly lower than that of the control. However, most of the aggregated proteins in H-WG-SCW (180 °C) became solubilized, showing a profile similar to that of the control. On the other hand, the DH of H-WG-SCW (200 °C) was not further increased after the first 6 h, showing a plateau interval. No enzyme reaction occurred from the beginning in the case of H-WG-SCW (220 °C).

The comparison of DHs at the enzyme hydrolysis time of 0 and 24 h at each treatment temperature clearly shows that the net increase of DH by the enzyme hydrolysis decreased as the treatment temperature increased and that the two DH values are equal at a treatment temperature higher than 220 °C. This result suggests that the enzyme susceptibility is gradually decreased, owing to the structural deformation of SCW-WG as the treatment temperature is increased, until the thermally stable structures or active sites to which an enzyme may access are all lost at a temperature higher than 220 °C.

Figure 3 shows the time profile of enzyme hydrolysis of WG-SCW samples, which were treated for different holding times (min) at 200 °C. The DHs at enzyme reaction time 0 h increased from 5.7 to 14.8% with increasing holding time from 0 to 40 min. Moreover, DHs after enzyme hydrolysis for 24 h increased from 14.8 to 18.2%. Similar to our results, many researchers [7, 11, 19, 24] confirmed the significant influence of treatment temperature and holding time on hydrothermolysis and found that the effect of holding time on DH was less than that of treatment temperature.

The DH profiles of H-WG-SCW (5 min) and H-WG-SCW (10 min) were characterized by an initial fast rate of increase for the first 6 h, followed by a leveling off with prolonging of the enzyme hydrolysis time (Fig. 3). However, in the case of H-WG-SCW (20 min), enzyme hydrolysis occurred in the first 6 h but was not continued thereafter. The H-WG-SCW (40 min) showed the same pattern, but the DH was shifted up. This up-shift of the DH curve is presumed to occur because the stable conformation at 200 °C formed completely until a holding time of 20 min; thus, the enzyme susceptibility was not changed further even after 40 min. The increase in DH could be due to hydrothermolysis. Sanphorka et al. [20] suggested a simplified reaction scheme for protein aggregation/degradation and their hydrolysis. Protein concentrations decreased within 5 min as the proteins aggregated and became solid particles so that only a small amount of protein remained in the liquid solution. After protein aggregations, the protein/polypeptide concentrations were increased as the aggregated protein disaggregated and was partially hydrolyzed by SCW to produce polypeptides and amino acids, with both product yields being strongly affected by the temperature and reaction time.

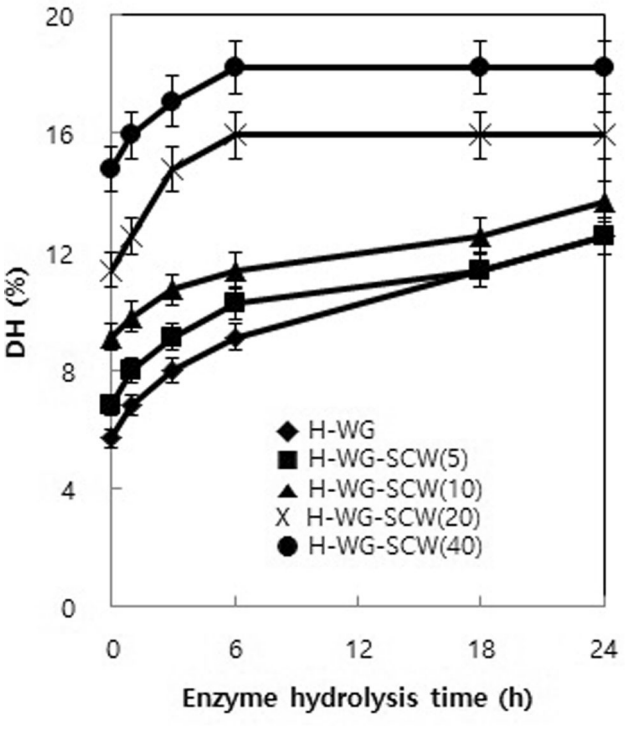

Fig. 3 Enzyme hydrolysis of WG treated with SCW for different holding times at 200 °C [H-WG-SCW (min)]

PS and DD

Figure 4 shows the effects of SCW treatment (160–260 °C) on the DD and PS of WG. The DD increased rapidly from 12.5 to 42.8% with increase in temperature up to 200 °C, followed by a slow increase with an increase in the treatment temperature above 200 °C. The PS increased in a similar pattern as the DD, and almost all protein was solubilized after treatment with SCW at 200 °C [25]. Considering that the DH was only around 2.3–11.5% in the temperature range of 160–200 °C as mentioned before, it is

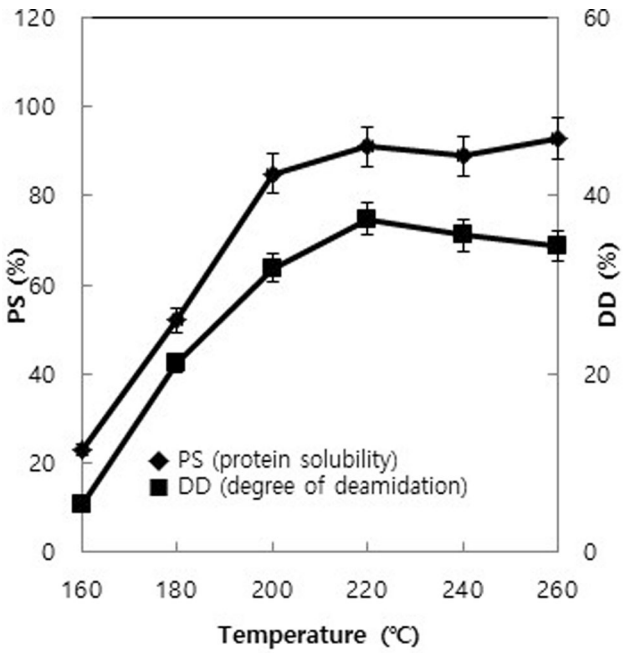

Fig. 4 Protein solubility and degree of deamidation of WG

presumed that the solubilization of the proteins in the temperature range occurs mainly by the dissociation of aggregated proteins and polypeptides due to deamidation and decomposition rather than the hydrolysis of peptide bonds [26, 27]. In general, the characteristics of water as a reactive solvent can be evaluated with two parameters: its ion product (dissociation constant, K_w) and dielectric constant. Increasing the value of K_w increases the concentration of both hydrogen and hydroxide ions (equal to the square-root of the K_w), which leads to a significant increase in the power of the hydrolysis reaction. Under high ion-product values, water possesses the properties of an acid catalyst [13]. Therefore, peptide bonds are broken down into smaller molecules of soluble proteins or amino acids. Delete In the case of hydrolysis of WG using a weak acid, the conformational change is induced by not changing the peptide bond of gluten molecules if the DD is 40% or lower. The measurement of the helix content of deamidated gluten showed that the helix content had decreased to 10% in the deamidated gluten and remained at this value even when the DD had increased to 40% or higher, whereas the helix content of native gluten was about 30 to 40%. This result supports the premise that a partially stable structure exists in gluten molecules, as described above [27].

Changes in molecular weight distribution

The enzymatic hydrolysate of untreated WG and the hydrothermolysates after SCW treatment at 180, 200, and 220 °C for 20 min were submitted to molecular weight distribution analysis by gel permeation chromatography (Fig. 5). The changes in the gel filtration patterns are similar to those of the electrophoretic patterns. The SCW treatment led to total degradation of high MW (molecular weight, glutenin subunits. The hydrothermolysates of the SCW-treated WG at 180 °C seemed to contain most of the molecules under 43,000 Da and mainly comprise low MW glutenin and gliadin monomers. The gel filtration pattern of WG-SCW (200) was characterized by the highest percentage of peptides with MW 10,000 Da and similar to that of the hydrolysate of untreated WG with Flavourzyme for 24 h [Fig. 5D)]. Hong et al. [28] reported the similar MW distribution pattern after 24 h hydrolysis of WG with Alcalase and Flavourzyme. However, after SCW treatment at 220 °C, MW of almost of them decreased to less than 6500 Da.

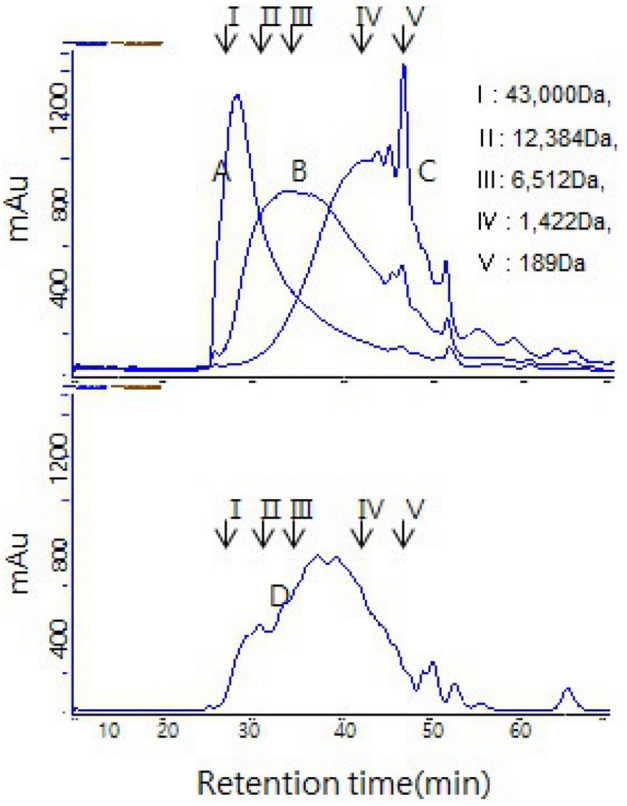

Fig. 5 Size exclusive chromatography profiles of the SCW-treated WG at 180 °C (**A**), 200 °C (**B**), and 220 °C (**C**) for 20 min, and the enzymatic hydrolysate of untreated WG (**D**)

Amino acids analysis

Total AAs of WG-SCW (T)

Table 1 shows the total and free AAs composition of the SCW-treated WG at different temperatures and their enzymatic hydrolysates. Glutamic acid (Glu) and proline (Pro) predominates, followed by leucine (Leu), phenylalanine (Phe), and serine (Ser). The composition and content of total AAs in WG-SCW (180) remained nearly unchanged compared with untreated WG (control) whereas the 220 °C hydrolysis resulted in 10% reduction of the sum of the total AAs. The amount of aspartic acid (Asp), serine (Ser), threonine (Thr), and arginine (Arg) exhibited significantly decreasing trends at a higher treatment temperature above 200 °C. It was reported that the cleavage of Ser and Thr residues is faster than that of other peptide residue [29].

Asp is considered the most important AA that showed a particularly significant change among the AAs in WG-SCW treated at various temperatures. The content of Aspin, the original gluten suspension, was 3.05% (2140 mg/kg) of the total AAs content, but it was drastically decreased to 0.4% (240 mg/kg) in the WG-SCW

Table 1 Amino acids composition of the untreated WG, enzymatic hydrolyzed WG (H-WG), SCW-treated WG at different temperatures [WG-SCW (T)], and enzymatic hydrolysates of WG-SCW (T) [H-WG-SCW (T)]

Amino acid	WG		H-WG	WG-SCW (180)		WG-SCW (200)		WG-SCW (220)		H-WG-SCW (180)		H-WG-SCW (200)		H-WG-SCW (220)	
	Total (mg/kg)	Free (mg/L)	Free (mg/L)	Total (mg/kg)	Free (mg/L)	Total (mg/kg)	Free (mg/L)	Total (mg/kg)	Free (mg/L)	Total (mg/kg)	Free (mg/L)	Total (mg/kg)	Free (mg/L)	Total (mg/kg)	Free (mg/L)
Aspartic acid	2140	14	59	1659	185	806	151	240	46	1664	116	767	136	244	42
Glutamic acid	28,802	6	312	29,008	5	297,598	18	29,851	51	29,930	74	29,054	42	29,751	58
Asparagin	0	11	84	0	18	0	17	0	4	0	29	0	30	0	7
Serine	3408	2	9	3122	28	2659	82	1353	95	3069	308	2555	240	1330	126
Glutamine	0	0	4380	0	0	0	1	0	3	0	1748	0	496	0	98
Histidine	1234	1	434	1128	7	1033	24	797	59	1192	192	1016	95	817	73
Glycine	2195	2	155	2176	24	2291	100	2206	259	2231	78	2230	118	2184	236
Threonine	1784	1	366	1159	8	1290	19	606	19	1630	261	1254	207	618	99
Arginine	2487	9	30	1832	15	1366	25	968	34	1572	29	1320	206	946	98
Alanine	1774	8	412	1676	40	1896	138	2171	406	1737	245	1854	235	2191	424
GABA	5	6	3	11	4	15	2	30	3	11	4	17	2	34	3
Tyrosine	1947	2	409	1953	9	2110	29	2003	59	2012	273	2049	204	1968	137
Valine	2670	2	611	2560	14	2599	47	2376	80	2689	392	2631	355	2480	234
Methonine	1028	1	181	936	6	977	13	845	18	975	134	944	48	847	8
Phenylalanine	3569	3	563	3472	7	3537	21	3297	58	3572	466	3447	382	3279	205
Isoleucine	2485	1	574	2404	9	2459	17	2098	28	2585	330	2536	288	2405	178
Leucine	4653	4	1611	4429	14	4584	40	4155	94	4583	1265	4449	1042	4174	516
Lysine	981	1	111	638	4	604	6	488	10	731	41	552	23	464	17
Proline	9277	4	0	8739	31	9032	93	8282	375	8922	79	8772	126	8084	362
Total	70,441	78	10,304	67,302	427	67,017	842	61,766	1700	69,104	6061	65,446	4274	61,815	2920

(220) (Table 1). It is already well known that the dilute acid treatment (in 0.03 N HCl at pH 2.0 and 105 °C) of proteins results in the deamidation of the Gln and Asn residues and a concomitant cleavage of the peptide bond [27]. Han et al. [30] reported that treatment with a dilute acid enabled proteins, including an aspartyl or aspargine residue, to discharge Aspatate at least 100 times faster than other amino acid residues.

On the contrary, the Glu content of WG-SCW (T) was almost constant at about 29,000 mg/kg regardless of the treatment temperature in the range of 160–220 °C.

For AAs in close proximity to Asn or Gln in three-dimensional structures, deamidation may be catalyzed through steric and chemical factors [31].

For example, Asn was deamidated relatively rapidly when followed by glycine (Gly) or Ser in peptide models [32]. In the thermal protein hydrolysis in SCW environment, however, the deamidation/decomposition of Asn seems to have a distinct influence on the structural characteristic of WG substrates. Glu and Asp are the main constituents of β-turn [33]. Matsudomi et al. [27] verified by SDS that deamidation occurs at the early stage of heating and then Asp is discharged to generate low molecular weight fractions from 30 min after the start of heating. As mentioned above, it is also presumed in the present study that the conformational change was completed 20 min after the start of the SCW treatment at 200 °C (Fig. 3).

Free AAs of WG-SCW (T)

The total free AAs in the water soluble fractions of WG-SCW (T) were very low at 427–1700 mg/L, indicating that the AAs were not produced under these SCW conditions (Table 1). Rogalinski et al. [7] estimated the time required for AAs to reach the maximum yield at each temperature from the protein hydrolysis kinetic data under SCW conditions and reported that the temperature at which an AA is substantially produced is 230 °C or higher.

Although the yield was low, the free AA content increased with increase in the treatment temperature. In particular, the content of Gly, alanine (Ala), and proline (Pro) was significantly increased. It has been reported that these amino acids are produced as intermediates from the

decomposition of other amino acids in the amino acid mixture [12].

Free AAs of H-WG-SCW (T)

Table 1 shows the profile of total and free AAs that were liberated after the combined SCW treatment and enzyme hydrolysis. Total free AAs of H-WG-SCW (T) decreased as the treatment temperature increased. In particular, there was a large difference in the concentration of free Gln compared to the control.

The free Gln content of the enzymatic hydrolysate, which is liberated from WG without SCW treatment, was 4380 mg/L.

However, the concentrations in H-WG-SCW (T) decreased significantly with increase in treatment temperature. This trend may be because the enzyme susceptibility was decreased due to the structural change of WG caused by the SCW treatment. Many researchers suggested that enzyme hydrolysis is dependent on the surface area of the substrate for binding sites of the enzyme and the conformational properties of substrates [11, 24, 25].

The effects of SCW (160–260 °C) treatment of WG (10%) on the hydrothermolysis properties and enzyme susceptibility were investigated. When WG was treated with SCW at 200 °C for 20 min, the DD was as high as 42.8% and almost all protein was solubilized. The net increase in DH by enzyme hydrolysis after SCW treatment decreased as the treatment temperature increased in the range of 160–220 °C. At higher temperatures above 220 °C, the susceptibility of the treated WG to the proteases became negligible, and thereafter, the DH reached to 37.6% at 260 °C only by hydrothermolysis. This result suggests that the enzyme susceptibility is gradually decreased due to the structural deformation of WG until the thermally stable structures or active sites to which an enzyme may access are all lost. SCW treatment in a particular time–temperature combination results in a significant decrease in enzymatic susceptibility. Further studies need to take into consideration the influence of processing conditions (time–temperature) of SCW on the functional properties of hydrothermolysates.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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