CHARACTERISTICS OF WHEY PROTEIN HYDROLYSATES FROM CHEESE WHEY, FAVORS ON VARIOUS FOOD APPLICATION

Article Highlights

- HMF showed no specific pattern with the increment of hydrolysis time of WPH-35. Only alcalase samples showed a gradual increment.
- All hydrolysates in alkaline media exhibited more than 50% solubility. pH 4 showed the lowest solubility of whey protein hydrolysate.
- The highest degree of hydrolysis was exhibited by protease M hydrolysates while alcalase hydrolysates exhibited the least response.
- A remarkable increase of NPN level within the first 30 min hydrolysis in each enzyme type is reported.
- The highest overall acceptability resulted from protease M and alcalase by sensory evaluation.

Abstract

This study was conducted to investigate the production of whey protein hydrolysates, examining the physiochemical properties with five enzyme types named alcalase, protease S, protease M, trypsin, and pepsin. Whey protein concentrate was adjusted by ultrafiltration, increasing the whey content to 135% that of initial levels. The hydrolysates have been shown to improve the characteristics of a number of food products, and the type of enzyme has a considerable influence on the end result of hydrolysates production. Bulk density, solubility, NPN, foaming capacity, and the degree of hydrolysis were increased with hydrolysis time. Maximum bulk density was shown by protease S. Pepsin and alcalase gradually increased the foaming capacity, resulting in a comparatively lower pH and a lower degree of hydrolysis. The highest degree of hydrolysis was shown by protease M. The highest NPN value was provided by pepsin, which was much greater than that of other enzymes. There was no significant difference in NPN according to the enzyme type applied. All hydrolysates in alkaline media were shown more than 50% solubility. HMF contents were also shown an obvious difference with the enzyme type.

Keywords: whey protein, enzyme hydrolysates, functionality.

Whey is regarded as a valuable by-product of cheese making. It contains proteins that have a high nutritional value and are an important source of bioactive sequences [1]. Whey protein technology has been developed for strengthening the functional protein in the food industry [2]. Whey contains approximately 20% milk proteins, and about 50% β-lactoglobulin (β-Lg), 22% α-lactalbumin (α-La), and numerous other proteins [3].

Modification of proteins based on enzymatic hydrolysis has a broad potential for designing protein functionality for specific applications [4]. The properties of the hydrolysates are dependent on the type of enzyme used, the degree of hydrolysis, and the substrate pretreatment [5]. Enzymes from different origins may vary in their capacity to hydrolyze whey proteins, and thus, may influence the physical/chemical characteristics of the hydrolysates [6].

Whey protein concentrates (WPC) provide an excellent food resource because of their relatively high protein concentration, excellent nutritional quality
and exceptional functional characteristics. The functional characteristics of whey proteins are highly valued in the food industry by food formulators, due to their wide range of functional benefits. Generally, whey protein hydrolysates for application to the food industry should have high solubility, low viscosity, as well as suitable foaming, gelling and emulsifying properties over those of native proteins, depending on the application [7].

This experiment is based on the proven features of WPC hydrolysates and their functionalities which can be utilized in various industries. The industry continues to innovate, while at the same time focusing on finding new uses and new markets for these value-added ingredients.

MATERIAL AND METHODS

Sample preparation

Fresh cheddar cheese whey was clarified and subjected to heat treatment in a vat pasteurizer to 70 °C/5 min after adjusting the pH value to 7, followed by cooling to 50 °C. Ultrafiltration was carried out at 50 °C at 6 and 2 bar inlet and outlet pressures, respectively. The membrane module (supplied by Danish Separation Systems AS, Denmark) was equipped with flat sheet ultrafiltration membranes of GR-60-PP made of polysulphone. The molecular weight cut-off range was 10,000 Da. The experiment was carried out in a spiral wound type module (DSS LabUnit M20, Alfa Laval Nakskov, Denmark). The total effective surface area of membranes was 0.036 m². After the first filtration, the permeate was removed and the retentate was further subjected to spray drying using a Mini spray dryer B-191 (BUCHI, Switzerland) to obtain dried WPC resulting in a protein content of approximately 35%. Inlet and outlet air temperatures were 175 and 75 °C, respectively [8].

General compositional analysis of WPC-35

Protein content was measured by the Kjeldahl method and fat by the Röse-Gottlieb method [9-10]. Ash and lactose contents were also determined according to the method of AOAC [11-12].

WPC-35 hydrolysates preparation

WPC-35 hydrolysates were produced according to the method of Abubakar [13]. The enzymes were added to the WPC 10% solution at a mass ratio of 1:25 at 37 °C in a shaking incubator (VS-8480S, Vision). It was separated into hydrolyzed periods for 0.5, 1, 2, 3, 4 and 5 h, while shaking in response to 180RPM, using each enzyme. It was then put in a 95 °C water bath for 10 min to inactivate the enzymes and was cooled to room temperature. The mixture was then centrifuged at 3000 rpm (Combi-S14R, Hanil, Korea) for 30 min, and the upper solution was dried completely using a freeze dryer (FDU-1200, EYELA) approximately for 24 h. The dried powder was then sealed and refrigerated.

Commercially available enzymes were used: alcalase 2.4 LFG (Novozymes, Denmark, EC. 3.4.21.62), derived from Bacillus licheniformis, protease M “Amano” G (Amano Enzyme, Japan, lot no. PRG0152301-MG), protease S “Amano” 2G (Amano Enzyme, Japan, lot no. PRG0250850SG), derived from Aspergillus oryzae and Bacillus stearothermophilus, respectively. Also, trypsin (Novozymes, Denmark, EC3.4.21.4), was derived from bovine pancreases, and pepsin was obtained from porcine stomach mucus (Wako Pure Chemical Industries. Ltd, Japan, EC 3.4.23.1).

Functional characteristics analysis

Bulk density was measured from 5 g weight samples. Bulk density was determined by measuring the volume of 5 g of powder in a 15 ml disposable conical tube after exposure to compaction by 100 times at roughly 50 taps/min hand tapping [14]. Foam expansion was determined by a slight modification of Lawhon method [15]. 5 g sample and 50 ml distilled water were blended at room temperature for 5 min in an 80 ml beaker using an agitator (NZ-1000, EYELA) at 2000 rpm speed, and the pH was adjusted to 7. The foam expansion ratio was calculated based on the initial volume and the final volume. Solubility was measured in the pH range of 2 to 10 using the method of Butler [16]. The samples were centrifuged at 3000 rpm (MICRO-12, Hanil, Korea) and solubility was determined based on absorbance, using a spectrophotometer (Optizen 2120UV, Mecasys) at 280 nm. Maximum values were compared and calculate the solubility. According to method of Keeney [17] 5-hydroxymethyl-2-furfural (HMF) was calculated. Degree of protein hydrolysis was measured according to the method of Adler-Nissen [18]. For non-protein nitrogen (NPN), the method of Lowry [19] was followed.

Sensory test

Overall acceptability, taste and odor were evaluated using a five-point hedonic scale ranging from “dislike extremely” to “like extremely”. And also bitterness was evaluated by using a seven-point scale of bitterness as 1 - threshold, 2 - very slight, 3 - slight, 4 - slight moderate, 5 - moderate, 6 - moderate strong and 7 - strong. Sensory testing was performed by ten trained personnel at Konkuk University [20].
Statistical analysis

All experiments were performed at least three times under each experimental condition and mean values were determined. The experimental results were analyzed using Statistical Analysis System [21]. Duncan’s multiple range test was used to determine differences between treatment means.

RESULTS AND DISCUSSION

General components of WPC-35

The following nutritional parameters were determined for the general composition of the whey protein. The contents were resulted as protein 34.94%, lactose 53.32%, fat 3.50% and ash 3.24%.

Characteristic of WPC-35 hydrolysates

Bulk density

Bulk density is a considerable factor for whey when used as a seasoning and in textural applications of food. In the case of drying methods, such as processed, after hydrolysis, the density becomes different. The high bulk density of the spray dried WPC after enzyme hydrolysis can be due to smaller particles fill the spaces between the larger particles.

Bulk density was significantly increased after hydrolysis (Figure 1), and increased with hydrolysis time. Bulk density either remained unchanged or just slightly changed in 1 to 3 h. But in alcalase, it gradually increased from 0.36 to 0.43 g/ml until 4 h. Pepsin hydrolysates showed a lesser value of bulk density increase from 0.32 to 0.38 g/ml during 4 h. Protease M and protease S resulted in increase from 0.42 to 0.47 g/ml, and 0.37 to 0.48 g/ml, respectively, for 30 min to 5 h hydrolysis. There was a significant difference with different enzymes reported in literature as well. Bulk density of whey proteins was shown to range from 0.2 to 0.4 g/ml [22], but in WPH it ranged from 0.38 to 0.46 g/ml for 30 min to 5 h. During protein hydrolysis, amide bonds are cleaved and, after addition of a water molecule, peptides and/or free amino acids are released. The newly formed peptides can be new substrates for the enzyme [23]. The proteolytic activity of the enzymes on β-Lg and α-La proteins basically influences of the bulk density differences on each hydrolysates.

Foam expansion

All the samples exhibited increasing foam expansion capacity with hydrolyzed time regardless the enzyme type (Figure 2). However, protease M was unchanged in terms of their foam expansion capacity 150% for 30 min to 2 h. Protease S also unchanged its foaming expansion capacity 162.5% for 1 to 3 h. Comparatively, pepsin hydrolysates increased rapidly with the hydrolysis time. Alcalase resulted in the highest foaming expansion capacity of 287.5% after 5 h.
Hydrolysis of proteins results in a reduction of molecular weight, which might promote foam formation due to the faster diffusion of molecules to the interface. On the other hand, peptides formed during hydrolysis might destabilize protein foams by displacement of proteins, or by disturbing protein interactions [24]. Furthermore, hydrolysis leads to increased charge density, which might negatively influence foam stability, because foam stability has been shown to improve when the electrostatic repulsion of proteins is minimal [25]. Foam formation is influenced by the ability of the foaming agents to quickly migrate to, and adsorb on, the air/water interface, as well as their ability to reduce the surface tension of the solution. The flexibility of proteins is an important factor in the reduction of surface tension [26]. Under certain conditions, it is possible to obtain peptides with the same structure and distribution of polar and hydrophobic residues, which result in better emulsifying properties than intact protein [27]. The same results were applicable in WPC-35.

**HMF (5-hydroxymethyl-2-furfural)**

HMF has been identified in a wide variety of heat-processed foods, including milk. It is created during storage [28]. The reaction occurs in the presence of amino acids and reducing sugars together in a long-term storage. In addition, the higher the temperature, moisture content, pH, alkaline, lysine, arginine, and the same basic amino acids, the faster the reaction.

HMF contents of the samples of the trypsin and pepsin hydrolysates decreased from 117.69 to 93.15, and 91.2 µmol/l, respectively, after 30 min hydrolysis, while that of the other enzyme samples increased (Table 1). The pepsin hydrolysates were lower than the intact HMF contents and the maximum was shown at 3 h hydrolysis, as 114.92 µmol/l. A gradual increment was recognized only in alcalase samples. In Pepsin and Trypsin hydrolysates, the HMF content was decreased and then increased within the first 30 min. However, in the other enzymes, it was increased with hydrolysis time without a gradual pattern. The highest values of HMF content were determined after 2 to 4 h hydrolysis samples. There was significant difference according to the type of enzyme used. Excessive production of HMF can lead to changes in taste.

**Solubility**

It can be identified, according to the results, that the samples that maintained a pH of 4, have the lowest solubility level of each hydrolysates, and this is not dependent on the type of the enzyme used. The solubility at the isoelectric point of proteins increases with hydrolysis, which is mainly the result of a reduction in molecular, weight and an increase in the number of polar groups [29].

Solubility in the isoelectric range increased from 75 to 86.1% for WPC and its hydrolysates, respectively. The solubility was gradually increased along with pH level in enzyme hydrolysates. In a previous study, the solubility of the WPC varied significantly according to the exposed hydrophobic amino acids used [30]. The maximum solubility was laid determined between pH 6-10. Also, the duration of hydrolysis increases the solubility level. Samples hydrolyzed for 5 h showed higher solubility levels for every enzyme. More extensive hydrolysis has been shown to increase solubility [31] which is in agreement with the results of this experiment.

**Degree of protein hydrolysis**

The degree of protein hydrolysis is defined as the percentage of the total number of peptide bonds in a protein that have been cleaved during hydrolysis [32]. According to the TNBS level, the degree of protein hydrolysis is shown in Figure 3, and it can be seen that it increased with hydrolysis time. The values were sharply increased within the first 30 min, and then more gradually increased. The best response was shown by protease M hydrolysates, which increased from 9.22 to 11.78%, and there was no significant difference according to the enzyme type which was used. The degree of hydrolysis was increased twice from the intact value of 4.65% within the first 30 min, when the enzymes protease M and protease S were applied, and the values were 9.22 and 9.96%.

### Table 1. The change of HMF (µmol/l) in WPC-35 during hydrolysis by proteases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Hydrolyzed time, h</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcalase</td>
<td></td>
<td>117.69±10.89</td>
<td>127.58±0.99</td>
<td>136.94±3.59</td>
<td>198.41±0.18</td>
<td>212.58±0.68</td>
<td>178.14±10.38</td>
<td>152.2±10.83</td>
</tr>
<tr>
<td>Protease M</td>
<td></td>
<td>117.69±10.89</td>
<td>179.00±11.96</td>
<td>245.14±0.93</td>
<td>177.89±2.35</td>
<td>194.52±11.14</td>
<td>210.79±2.72</td>
<td>182.32±10.51</td>
</tr>
<tr>
<td>Protease S</td>
<td></td>
<td>117.69±10.89</td>
<td>177.27±3.98</td>
<td>183.54±15.87</td>
<td>211.40±49.6</td>
<td>200.79±12.64</td>
<td>250.92±9.58</td>
<td>228.29±5.4</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
<td>117.69±10.89</td>
<td>93.15±7.32</td>
<td>112.02±10.51</td>
<td>126.66±11.04</td>
<td>158.54±5.9</td>
<td>139.82±13.63</td>
<td>125.1±4.61</td>
</tr>
<tr>
<td>Pepsin</td>
<td></td>
<td>117.69±10.89</td>
<td>91.2±6.77</td>
<td>98.48±5.14</td>
<td>114.92±9.27</td>
<td>112.2±9.92</td>
<td>104.67±3.59</td>
<td>94.21±5.25</td>
</tr>
</tbody>
</table>
respectively. For alcalase 4 h was needed to increase two-fold the degree of hydrolysis from the starting point, and alcalase showed the least response among the selected enzymes comparatively which the values were appeared from 7.94 to 9.48%, from 30 min to 4 h. The changes in functional properties of whey proteins are related to peptides produced by enzymatic hydrolysis, which are mainly characterized by a lower molecular weight, exposure of hydrophobic groups, and by an increased number of ionic groups [33].

Non-protein nitrogen (NPN)

NPN contains many effective components having a physiological activity, such as a variety of watersoluble amino acids and low-molecular peptides. Suido et al. mentioned that the NPN contained in whey is physiologically nutritional, but most of it is cationic and is therefore removed by desalting together with minerals; hence, it could not be utilized at all [34]. On the other hand, the NPN content is a factor responsible for bitterness due to its hydrophobic characteristics [35].

According to the analysis (Figure 4), the NPN level increased with hydrolysis time. The highest values were given by trypsin, which was at its maximum (1.643) at 3 h and pepsin (1.923) at 3 h hydrolysates. Similarly, WPC hydrolysates in different protein concentrations in previous studies also reported higher NPN contents [36]. There was a huge increase of NPN level within the first 30 min in each enzyme type. Protease M had a lower response compared with other enzymes. The values were 1.168 to 1.434.

CONCLUSIONS

The protein content of the first retentate from the ultrafiltration of cheese whey is easily adjustable in to 35% WP. These results indicate that structural changes of the production of hydrolysates in whey proteins, and the selection of specific enzyme and proper hydrolysis conditions, are important factors for specific
food applications. These characteristics of hydrol-
ysates can be beneficially utilized for the development
of various food products, and can be developed using
first filtration retentate of ultrafiltration, after spray dry-
ing and treating with the preferable enzyme. Out of
the experimental enzymes, Protease M is the most
suitable enzyme to use in WPC hydrolysis in food
applications due to the best performances in bulk
density, degree of hydrolysis and sensory preferences
and also in comparatively better functional character-
istics.

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KARAKTERISTIKE HIDROLIZATA PROTEINA IZ SURUTKE: UTICAJ NA RAZLIČITE PRIME NE U PREHRAMBENIM PROIZVODIMA


Ključne reči: protein surutke, enzimski hidrolizati, funkcionalnost.