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Improvement of Emulsifying Properties of Wheat Gluten Hydrolysate/λ-Carrageenan Conjugates

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Summary

Gluten hydrolysate was prepared through limited enzymatic hydrolysis of wheat gluten resulting from the byproducts of wheat starch. The enzyme applied in the present study was Protamex. Response surface methodology was used to investigate the effects of pH, gluten hydrolysate (GHP)/λ-carrageenan (λC) ratio and reaction time on emulsifying properties of the GHP-λC conjugate. The regression model for emulsion activity index (EAI) was significant at p=0.001, while reaction time had a significant effect on EAI of the conjugate with regression coefficient of 4.25. The interactions of pH and GHP/λC ratio, and GHP/λC ratio and reaction time significantly affected the EAI of the conjugate. Both the emulsifying property and nitrogen solubility index (NSI) of GHP-λC conjugate prepared under the optimal conditions increased more remarkably, compared to the control. The denaturation temperature of GHP-λC conjugate obviously increased compared to wheat gluten. The addition of GHP-λC conjugate had different effects on dough characteristics. Moreover, this conjugate can delay the increase in the bread crumb firmness during storage. It demonstrated that this conjugate could improve the dough characteristics and had anti-staling properties of bread.

Key words: wheat gluten, λ-carrageenan, limited enzymatic hydrolysis, protein-polysaccharide, response surface methodology, emulsifying properties

Introduction

Wheat gluten, the byproduct of the wheat starch industry, is a typical water-insoluble protein consisting of more than 60 different molecular mass polymorphic polypeptides. Of the gluten proteins, gliadin has a relative molecular mass between 30 000 and 80 000 Da whereas glutenin is multi-chained with relative molecular mass up to several million Da (*1,2*). The low solubility of wheat gluten limits its utilization in food industry. The improvement of the functional properties of the proteins by enzymatic or chemical modification has been widely conducted (*3,4*). Finley (*5*) reported that mild acid treatment caused the increase in solubility of wheat gluten. Kato *et al.* (*6*) found that deamidation of gluten by proteolytic enzymes was effective in increasing its functional properties. Limited enzymatic hydrolysis using proteases resulted in improved solubility and properties of emulsion and foam of wheat gluten (*7–11*). Subjected to limited hydrolysis by chymotrypsin, the obtained peptides could be separated (*12,13*). The emulsifying and foaming properties of gluten treated with protease (chymotrypsin, papain, pronase, and pepsin) or using mild acid, followed by microbial transglutaminase cross-linking, were improved greatly (*14*).

The effects of polysaccharides on the functional properties of wheat bread have been investigated. Polysaccharides improved dough stability, bread performance

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and bread shelf life (*15*). Interactions of protein and polysaccharide in the bulk solution or at the interface depended on the characteristics of protein and polysaccharide structure as well as on the pH value (*16*,*17*). Aqueous solutions of proteins and polysaccharides exhibited complex coacervation (complexation), miscibility and segregation.

Only a few studies in the literature are available on interactions between polysaccharides and gluten protein. Associative interactions exist among microbial polysaccharides, carrageenan and alginate with purified gluten protein (*18*). The increase in viscosity of the mixture from deamidated gluten and sodium alginate was observed by Howell *et al*. (*19*), who discussed two possible explanations: phase separation and electrostatic interactions between carboxyl groups and amide groups. In addition, León *et al*. (*20*) found that a fraction of hydrophobic gluten protein could interact with λ-carrageenan, and suggested that sulphate groups of hydrocolloids and the amino groups of glutamines present in the gluten protein were associated with the interaction. Ribotta *et al*. (*21*) demonstrated that carrageenan isoforms and pectin could form hydrophilic complexes with gluten proteins and the capacity of the complexation appeared to be related to the density of the anionic group in the hydrocolloid. However, their assumption is doubtful because glutamine has an amide side chain, which is not ionizable. Electrostatic interactions can involve lysine and terminal NH_2 in gluten protein.

Polysaccharides have a strong effect on the functional properties of intact food proteins (*22*), but, this behaviour was quite different from that observed for functional properties of protein hydrolysates used (*23*). The potential effects of interactions between polysaccharides and gluten protein, particularly in gluten hydrolysates obtained after limited enzymatic hydrolysis, on the functional properties have not been investigated.

In this present study, the objective was to determine the effect of λ-carrageenan addition on emulsifying properties of gluten and gluten hydrolysate obtained after Protamex hydrolysis, and to evaluate potential interactions between these different variables. In addition, response surface methodology was used to investigate the interactions of pH, GHP/λC ratio and reaction time in the emulsifying properties of the conjugate.

Materials and Methods

Raw materials

Commercial wheat gluten was obtained from Lianhua Co., China. Gluten contained 71.5 % (mass fraction, on dry basis) protein and 6.8 % moisture. Protamex ($1.0 \cdot 10^5$ units/g) was provided by Novo Nordisk Co. (Beijing, China). The other chemicals were of analytical grade. λ-carrageenan (λC) was purchased from JAYI Food Science and Technology Institute in Shanghai.

Preparation of gluten hydrolysate (GHP) by Protamex

The aqueous dispersion (8 %) of wheat gluten was heated for 30 min in a water bath at 90 °C prior to enzymatic hydrolysis. The gluten hydrolysis by Protamex was carried out for 3.5 h at 48 °C (heated magnetic stirrer, Model DF-1, Jintan, China) and pH=6.8, with a ratio of enzyme to substrate (E/S) of 2000 unit/g. The enzyme was inactivated for 10 min by heat treatment at 100 °C. The resulting hydrolysate was rapidly cooled to 25 °C in the ice bath, then freeze-dried and finally stored until use at –20 °C.

Preparation of GHP-λC conjugates

GHP-λC conjugate was prepared according to the method by Martinez *et al*. (*23*). Solutions of λC were made by dissolving the λC in 0.005 mol/L of KH_2PO_4/Na_2HPO_4 buffer at 70 °C. To avoid bacterial growth, 0.2 g/L of sodium azide was added to the solutions. GHP was added to the solutions of λC and then pH was adjusted to a given value. The reaction was performed at 70 °C for different durations of time. The resulting conjugates were freeze-dried prior to further use.

SDS-PAGE analysis

Gel electrophoresis was conducted according to the method of Laemmli (*24*) using V/m=10 % acrylamide separating gel and 2.5 % acrylamide stacking gel. Samples were prepared in Tris-glycine buffer of pH=8.8, containing 1.5 % SDS and the gel sheets were stained for protein with Coomassie brilliant blue R-250.

Measurement of emulsifying properties

The emulsifying properties were determined by the method of Pearce and Kinsella (*25*), with a few modifications. To prepare emulsions, 15 mL of pure peanut oil and 45 mL of 0.1 % protein solution in 0.2 mol/L of phosphate buffer (pH=7.0) were mixed, shaken and homogenized for 1 min at 10 000 rpm with a FA25 lab high shear dispersing machine (Fluko Equipment Shanghai Co. Ltd.) at 25 °C. A 200-μL sample of each emulsion was taken from the bottom of the container after different intervals and diluted with 20 mL of 0.1 % sodium dodecyl sulphate solution. The absorbance of the diluted emulsion was measured at 500 nm. The emulsifying activity (EA) was determined from the absorbance measured immediately after emulsion formation.

Solubility determination

Sample solution (1 %) was adjusted to pH values from 2 to 10. After being left standing for 5 min, the samples were centrifuged for 20 min at 3800 rpm. Protein was determined in the supernatant by the Lowry method with a modification (*26*). The standard curve was made with bovine serum albumin (BSA). Nitrogen solubility index (NSI %) was calculated from the protein content in the sample relative to the protein content in the solution.

Measurement of thermal property

Thermal property was determined with differential scanning calorimetry (DSC, DT-40, Japan) according to the method of Molina and Añón (*27*). The range of scanning temperature was 30-200 °C, the increase rate of temperature was 10 °C/min, and the flow rate of nitrogen was 30 mL/min. Sample used in this study was about 10 mg.

Baking procedure

Baking was carried out according to our previous method with a modification (*28*). The dough formulation comprised wheat flour 100 g, compressed yeast 1.6 g, sodium chloride 1.5 g and shortening 3.0 g. The addition of water depended on a farinograph test using the 500 BU line. The resulting dough was allowed to rest for 15 min in a cabinet at 30 °C and 70 % relative humidity (RH). The bulk dough was then sheeted in a roller having two rolls of 50×12.6 cm^2. The dough was divided into pieces of 80 g, hand-moulded, proofed at 30 °C and 96 % RH up to its maximum volume, and then baked for 18 min at 200 °C.

Bread loaf specific volume was determined by rapeseed displacement. Bread was stored at 20 °C for different time.

Measurement of firmness of bread crumb

The firmness of bread was determined with TPA model of Texture Analyzer (TA-XT2i, Stable Micro Systems, England). Bread slices (15-mm thickness) were compressed using a 50-mm diameter aluminium plunger with 5 kg of load cells. The rates of pretest, test and post-test were 3.0, 1.7 and 1.7 mm/s, respectively. The compression curves of the bread crumbs (distance *vs.* force) were plotted, and the force at 25 % compression was recorded as a measure of bread firmness in accordance with the AACC International method 74–09 (*29*). Six slices from each loaf were analyzed. The mean coefficient of variation for the determination of bread crumb firmness on different days was calculated on the 5 % level.

Experimental design and statistical analysis

Response surface methodology (RSM) was used to study the simultaneous effect of the three experimental variables. Three levels of each variable were chosen on the basis of the central composite design principle (*30*). The levels of pH, GHP/λC ratio and reaction time for each experiment in the central composite design are presented in Table 1. Statistical analysis was performed using Minitab 12.5 for Windows (Minitab Inc., State College, PA).

Table 1. Levels of variables established according to the central composite rotatable design

Treatment	pH	GHP/λC ratio	reaction time/day	EAI/(m^2/g)
1	9.00	7.00	20	31.6
2	9.00	7.00	20	33.3
3	9.00	6.00	20	27.4
4	10.00	6.00	22	29.5
5	8.00	6.00	18	30.6
6	9.00	7.00	18	36.7
7	8.00	7.00	20	32.4
8	10.00	7.00	20	33.6
9	9.00	7.00	20	33.6
10	9.00	7.00	20	32.8
11	9.00	7.00	20	32.8
12	8.00	8.00	22	37.1
13	10.00	8.00	18	28.3
14	9.00	8.00	20	29.4
15	9.00	7.00	22	28.2

For each experimental factor the variance was partitioned into three components (linear, quadratic and interaction) to assess the adequacy of the second order polynomial function and relative importance of these components. The significance of the equation parameters for each response variable was assessed by F test. These three-dimensional representations showed the effect of two independent variables on the response at a constant value of the third independent variable set at the center point.

Results and Discussion

Basic indices of GHP

The degree of hydrolysis (DH) of gluten protein was 6.6 %, while the gluten hydrolysate showed the highest solubility at DH=6.6 % (data not shown). The EAI of GHP was 22.24 m^2/g, while the ESI was 6.4 min. The emulsifying properties of GHP improved markedly, compared to the EAI (4.32 m^2/g) of the original gluten.

Interaction of GHP and λC by response surface analysis

In this study, response surface methodology was used to study the interaction between GHP and λC and optimize the variables such as pH, GHP/λC ratio, reaction time, temperature and concentration of wheat hydrolysate by Protamex (GHP). The reaction was performed at 70 °C, with the concentration of GHP being 10 %. Furthermore, pH, GHP/λC ratio and reaction time were independent variables, and EAI was regarded as estimated response. The results are presented in Table 1.

The following second-order polynomial equation of function X_i was fitted for each variable assessed:

$$Y=\alpha_0+\alpha_1X_1+\alpha_2X_2+\alpha_3X_3+\alpha_{11}X_1^2+\alpha_{22}X_2^2+\alpha_{33}X_3^2+\alpha_{12}X_1X_2+ \\ +\alpha_{13}X_1X_3+\alpha_{23}X_2X_3$$

where Y is the estimated response, EAI; X_1, X_2, X_3, are pH, WHP/λC ratio and reaction time, respectively; α_0, α_1, α_2, α_3, α_{11}, α_{12}, α_{13}, α_{22}, α_{23}, α_{33} are constant and regression coefficients of the models.

The regression equation and regression coefficients were obtained by SAS-RSR (response surface regression) procedure with the following equation:

$$Y_{EAI} = 32.31+0.59X_1+0.88X_2-4.25X_3+1.28X_1^2-3.19X_2^2+ \\ +0.71X_3^2-6.23X_1X_2-0.51X_1X_3+3.12X_2X_3.$$

Analysis of variances indicated that the regression model for EAI was significant at p=0.0091, while the lack of error was not significant at p<0.05 (Table 2). Obviously, reaction time had a significant effect on EAI of conjugate (regression coefficient of 4.25). Regression coefficients between GHP/λC ratio, pH, and EAI were 0.88 and 0.59, respectively.

Table 2. Regression coefficients and analysis of variance of the regression model for EAI[a]

Terms	α	Sum of square	Mean square	F	P
Constant	32.31	114.31	12.70	10.58	0.0000
pH	0.59	0.70	0.70	0.58	0.4794
GHP/λC	0.88	1.53	1.53	1.28	0.3099
RT	–4.25	36.17	36.17	30.12	0.0027
pH×pH	1.28	4.28	4.28	3.57	0.1175
GHP/λC×GHP/λC	–3.19	26.58	26.58	22.14	0.0053
pH×RT	0.71	1.33	1.33	1.10	0.3415
pH×GHP/λC	–6.23	51.81	51.81	43.15	0.0012
pH×RT	–0.51	0.34	0.34	0.29	0.6160
GHP/λC×RT	3.21	13.02	13.02	10.84	0.0216
Residual	6.00	1.20			
Lack of error	3.75	3.75	6.66		0.0613
Correlation coefficient			$r^2 = 0.982$		

[a]GHP/λC, gluten hydrolysate/λ-carrageenan ratio; RT, reaction time (day); α, constant and partial regression coefficient

Moreover, analysis of variance could explain the influence of interaction of variables on the estimated EAI by the quadratic model. Reaction time had linear effects ($p<0.05$), whereas GHP/λC ratio had quadratic effects ($p<0.05$) on the EAI of the conjugate. In addition, pH and GHP/λC ratio, GHP/λC ratio and reaction time had significant interactions ($p<0.05$) on the EAI. As shown in Table 2, reaction time was the most important variable affecting the EAI of conjugates, with the regression coefficient of reaction time for EAI being 4.25.

The three-dimensional representations show the effect of two independent variables on the response at a constant value of the third independent variable, which was set at the center point (Figs. 1–3). In addition, interactions of two variables can be observed. Varying pH levels caused an increase in the EAI of the conjugate when GHP/λC ratio was 6:1, but the EAI of the conjugate decreased drastically when GHP/λC ratio was 8:1 (Fig. 1a). Significant interaction of pH and GHP/λC ratio was detected (Fig. 1b).

When GHP/λC ratio was 7:1, the EAI of the conjugates decreased at first and then increased gradually with the increase of pH values studied. There was no significant interaction between pH and reaction time (Figs. 2a and 2b). According to Figs. 3a and 3b, at constant pH, the EAI of the conjugates increased at first and then decreased rapidly with the increase of GHP/λC ratio. The highest EAI of the conjugate was observed when GHP/λC ratio ranged from 6.5–7.0 and reaction time was 18 days. In addition, when reaction time exceeded 22 days, the EAI of the conjugate increased visibly and then declined gently.

According to RSA software and validation, the optimal reaction parameters were determined as follows: pH=10.0, GHP/λC ratio of 6:1 and reaction time of 18 days. Thus, the EAI of the conjugate obtained at the optimal conditions was 44.22 m^2/g. The difference between the real result and the estimated result was only 1.6 %.

SDS-PAGE of GHP-λC conjugate

After being hydrolyzed by Protamex, the fractions of high molecular mass (HMM) in gluten hydrolysate decreased greatly compared with the original gluten (Fig. 4). The two new bands occurred in HMM zone in SDS-PAGE pattern of the conjugate (lane 3). Otherwise, some of the bands disappeared. This indicated that the conjugate of GHP and λC was produced.

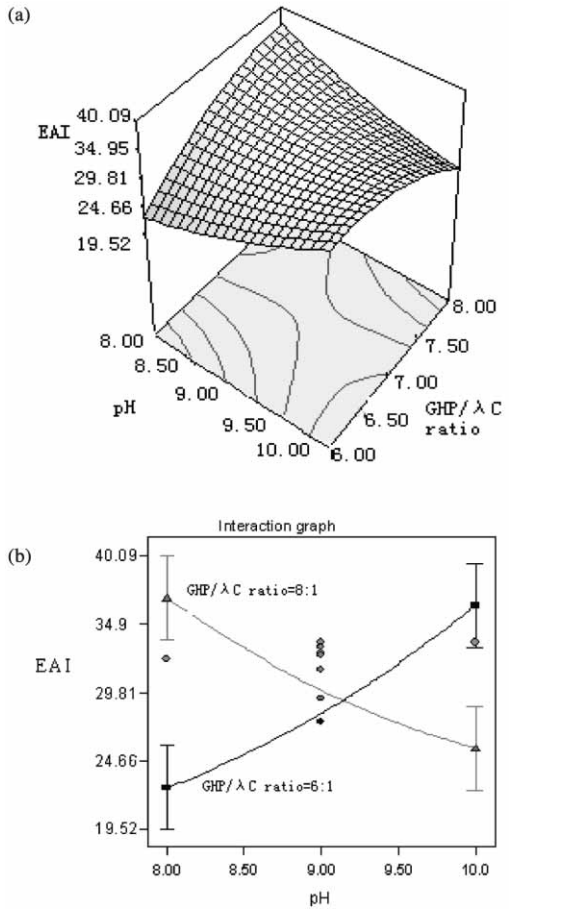

Fig. 1. Effects of pH and GHP/λC ratio on conjugate EAI. Actual factor: reaction time=20 days. EAI: emulsion activity index; GHP/λC ratio: gluten hydrolysate by Protamex/λ-carrageenan ratio

Difference of thermal characteristics of GHP-λC conjugates

Most of food ingredients need to be heated to some degree in the course of processing, but heat treatment can lead to the denaturation of or influence the functional properties of food proteins. Currently, differential scanning calorimetry (DSC) is being used widely to study the thermal properties of proteins. The DSC patterns of original wheat gluten, gluten hydrolysate obtained after hydrolysis by Protamex, and GHP-λC conjugate are presented in Table 3. The denaturation temperatures of the original wheat gluten, gluten hydrolysate, a blend of gluten hydrolysate and λC, and GHP-λC conjugate were 83.5, 89.9, 89.4 and 93.9 °C, respectively. The DSC pattern of λ-carrageenan alone had no denaturation peak in

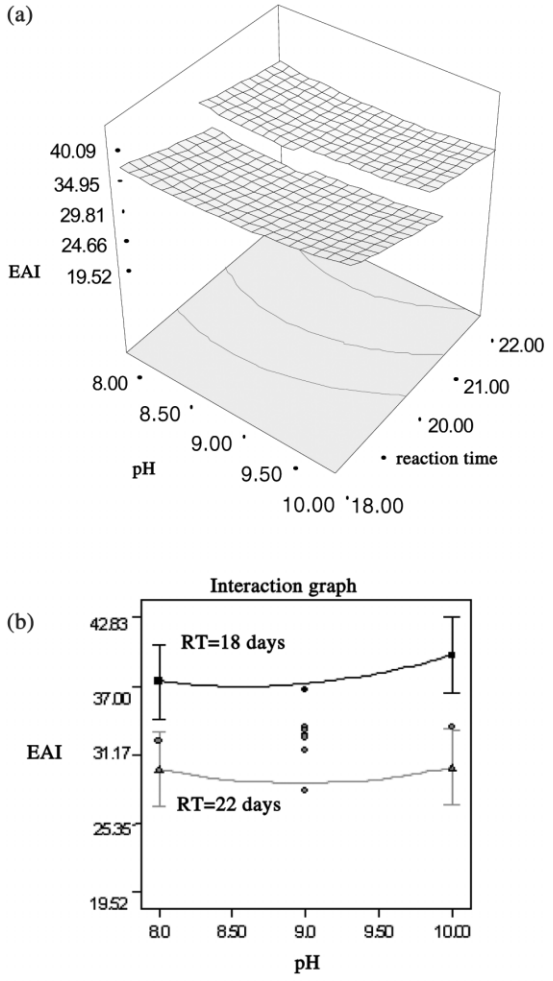

Fig. 2. Effects of pH and reaction time on conjugate EAI. Actual factor: GHP/λC ratio=7:1. EAI: emulsion activity index; RT: reaction time

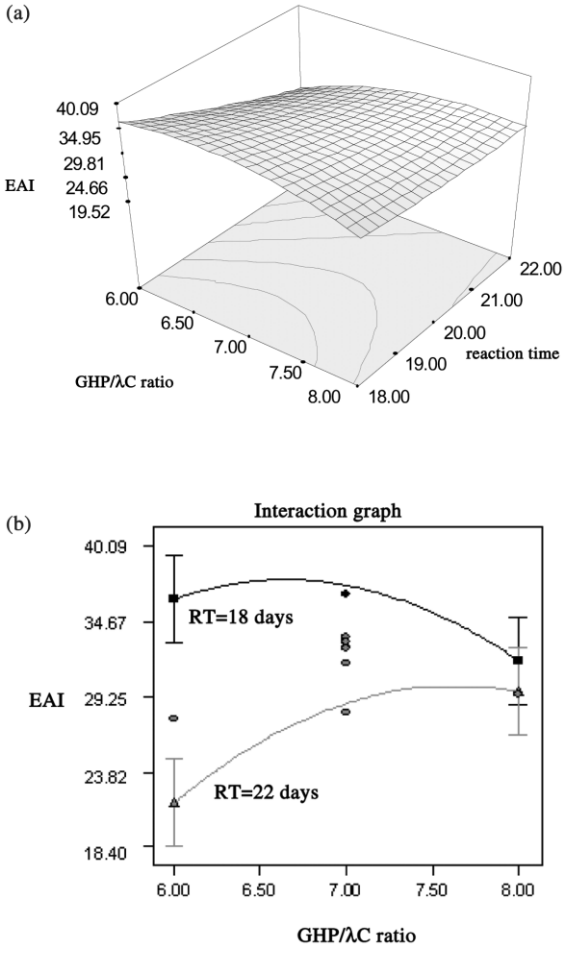

Fig. 3. Effects of reaction time and GHP/λC ratio on conjugate EAI. Actual factor: pH=9.0. EAI: emulsion activity index; GHP/λC ratio: gluten hydrolysate by Protamex/λ-carrageenan ratio; RT: reaction time

the temperature range tested. There was no difference between the denaturation temperatures of the gluten hydrolysate, and the blend of gluten hydrolysate and λC. This indicated that the denaturation peak was mostly caused by the conjugate in the DSC pattern of the conjugate. Denaturation temperature of wheat gluten modified by Protamex and λC increased significantly. The GHP-λC conjugate had a strong heat stability and resistance to the denaturation of protein.

Values of the enthalpy changes of the original wheat gluten, gluten hydrolysate, the blend of gluten hydrolysate and λC, and λC-GHP conjugate were calculated according to the area of the peaks in DSC, and they were 22.42, 8.17, 8.06 and 9.46 J/g, respectively. The values of the enthalpy changes of the gluten hydrolysate and the conjugate were lower than the original gluten. Possibly due to the cross-linking of the wheat gluten proteins and λC, the protein structure enabled an easy entry of the energy and its contact with the internal structure of the gluten proteins.

In addition, the half peak length ($\Delta T_{1/2}$) in the DSC pattern of the GHP-λC conjugate increased compared to the original gluten and gluten hydrolysate (Table 3).

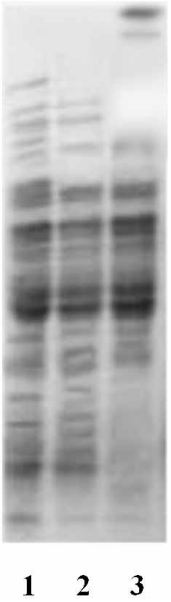

Fig. 4. SDS-PAGE of GHP-λC conjugate. Lane 1, original wheat gluten; lane 2, gluten hydrolysate; lane 3, GHP-λC conjugate

Table 3. Thermal properties of GHP-λC conjugate[a]

Sample	DT[b]/°C	ΔH/(J/g)	$\Delta T_{1/2}$
Original gluten	83.5	22.42	8.45
Gluten hydrolysate	89.9	8.17	11.36
λC alone	–	–	–
Blend of GHP and λC	89.4	8.06	10.96
GHP-λC conjugate	93.9	9.46	12.51

[a]Preparation condition of the conjugate: pH=10.0, GHP/λC ratio=6:1, reaction time=10 days, reaction temperature=70 °C. [b]DT, denaturation temperature; ΔH, enthalpy change; $\Delta T_{1/2}$, half peak length

$\Delta T_{1/2}$ is generally considered as an important indicator of denaturation harmony. The $\Delta T_{1/2}$ values of the original wheat gluten, gluten hydrolysate, the blend of gluten hydrolysate and λC, and GHP-λC conjugate were 8.45, 11.36, 10.96 and 12.51, respectively. This indicated that the harmony of the modified glutens decreased during the thermal denaturation.

Change in solubility

Solubility is one of the most important physicochemical and functional properties of a protein. Low solubility may cause an unattractive appearance and a sandy mouthfeel of the final product (*31*). The limited solubility of wheat gluten in aqueous solvent has generally been attributed to its large molecular size and intermolecular aggregation, arising from strong non-covalent interactions, involving hydrogen bonds and hydrophobic interactions (*32*). The pH dependence of the solubility of the wheat gluten, gluten hydrolysate and GHP-λC conjugate is shown in Fig. 5. The solubility was significantly improved by a limited enzymatic hydrolysis and linkage of WHP and λC, which may be explained by the cleavable peptide bonds. The pH-solubility profile of the original wheat gluten sample exhibited a typical bell-shaped curve, with minimum solubility of 3.7 % at the isoelectric point (pI). Meanwhile, the GHP-λC conjugate had higher solubility than the original wheat gluten and gluten hydrolysate at the levels of all pH values. In addition, the isoelectric point of the conjugate shifted to about pH=8.0.

Effect of GHP-λC conjugate on dough characteristics

The effects of the GHP-λC conjugate on the dough rheology during mixing were determined with the farinograph and extensograph. The parameters determined were water absorption or percentage of the water required to yield dough consistency of 500 BU (Brabender units) regarding dough development time (DDT, the time to reach the maximum consistency), stability (time the dough consistency remains at 500 BU) and mixing tolerance index (MTI, consistency difference between the peak height and 5 min after the peak, BU).

Changes in the characteristics of dough after the addition of GHP-λC conjugate are shown in Table 4. Apparently, the addition of 1 or 2 % of wheat gluten increased significantly ($p<0.05$) the water absorption of the dough, but no significant ($p<0.05$) changes in water absorption were found when 0.5 or 1 % GHP-λC conjugate was incorporated. There was no obvious change in

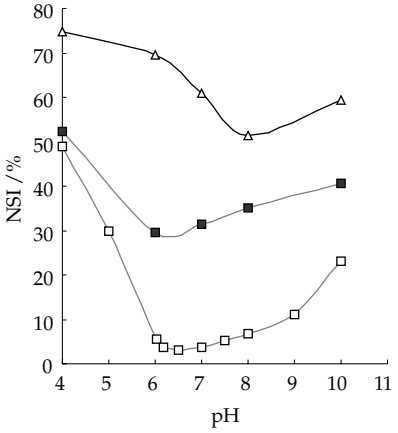

Fig. 5. Nitrogen soluble index of GHP-λC conjugate -□, control; -■-, gluten hydrolysate; -△-, GHP-λC conjugate. The preparation condition of the conjugate: pH=10.0, GHP/λC ratio=6:1, reaction time=10 days, reaction temperature=70 °C

Table 4. Rheological properties of wheat dough containing the gluten hydrolysate/λ-carrageenan conjugate*

Terms	Control	*w*(WG) = 1 %	*w*(WG) = 2 %	*w*(GHP-λC) = 0.5 %	*w*(GHP-λC) = 1 %	GHP alone	λC alone	blend of GHP and λC
WA/%	60.8^{ac}	62.2^{b}	63.4^{b}	61.1^{c}	60.8^{cd}	62.5^{ce}	65.4^{f}	64.5^{df}
DDT/min	3.5^{a}	4.3^{c}	3.5^{ab}	3.5^{a}	3.7^{a}	3.9^{ac}	3.6^{a}	3.5^{ab}
S/min	5.5^{b}	6.2^{ab}	6.1^{bc}	4.0^{ac}	3.9^{a}	4.3^{ac}	5.0^{b}	4.8^{ad}
MTI/BU	90^{bc}	89^{b}	80^{ab}	128^{a}	134^{ad}	96^{b}	100^{ab}	95^{ac}
Area/cm^2	70^{b}	76^{ab}	86^{ac}	75^{ab}	71^{bc}	64^{b}	73^{bc}	71^{ab}
R/BU	209^{bc}	208^{b}	232^{a}	213^{ab}	216^{ab}	189^{bd}	195^{c}	190^{c}
E/mm	176^{c}	187^{ac}	184^{ac}	165^{cd}	168^{bc}	182^{ac}	169^{bc}	180^{cd}
R_{max}/BU	272^{bd}	288^{ab}	332^{a}	285^{bc}	270^{ab}	254^{b}	273^{cd}	259^{b}
R/E/(BU/mm)	1.2^{cd}	1.1^{c}	1.3^{bc}	1.2^{ac}	1.2^{d}	1.0^{c}	1.1^{bc}	1.0^{ac}

*Control, dough without added gluten; GHP alone, 1 % GHP; λC alone, 1 % λC; blend of GHP and λC, 0.5 % GHP and 0.5 % λC; WA, water absorption; DDT, dough development time; S, stability; MTI, mixing tolerance index; R, resistance at 5 min; E, extensibility; R_{max}, maximum resistance; R/E, resistance/extensibility. Extensograph properties are determined using the dough rested for 90 min. Values followed by the same letter in the same line are not significantly different ($p<0.05$) according to Duncan's multiple range test

DDT. Stability of dough decreased after the addition of the conjugate. In addition, MTI had an opposite trend, indicating that the strength of the dough after the addition of the conjugate decreased.

The resistance, extensibility and curve area of the dough after the addition of the conjugate from the extensograph results exhibited no significant change compared to the original dough or after the addition of the original gluten (Table 4).

Effect of GHP-λC conjugate on breadmaking quality

The change in breadmaking quality of wheat flour after the addition of GHP-λC conjugate is shown in Table 5. Increases in loaf volume, specific loaf volume (SLV) and bread score were noticed after the addition of the wheat gluten and GHP-λC conjugate. Particularly the addition of 0.5 % GHP-λC conjugate improved significantly ($p<0.05$) the loaf volume, SLV and score, and, thus, greatly improved the breadmaking quality of wheat flour. These results agree with the observation of Guarda *et al.* (*15*), who thought that the improvement in breadmaking quality was probably due to the increase in water absorption of dough and texture characteristics of bread.

Table 5. Changes in quality of bread with gluten hydrolysate/λ-carrageenan conjugate*

Sample	LV/mL	SLV/(mL/g)	Score	Moisture content/%
Control	578^{bc}	3.8^{bc}	59^{a}	32.5^{ab}
1 % WG	620^{d}	4.1^{ae}	64^{ac}	33.8^{b}
2 % WG	633^{de}	4.1^{ad}	66^{ae}	34.1^{ac}
0.5 %GHP-λC	670^{a}	4.7^{d}	$77d^{e}$	35.2^{e}
1% GHP-λC	645^{ad}	4.2^{a}	71^{bde}	33.6^{ac}
1 % GHP	598^{c}	3.9^{ab}	61^{ac}	35.7^{be}
1 % λC	626^{cd}	4.0^{a}	63^{a}	36.4^{d}
Blend (GHP+λC)	619^{d}	3.9^{b}	62^{bc}	35.8^{e}

*Values in this table are the means of triplicate. The values by the same letter in the same column are not significantly different ($p<0.05$) according to Duncan's multiple range test. WG, wheat gluten; GHP-λC, gluten hydrolysate/λ-carrageenan conjugate; LV, loaf volume; SLV, specific loaf volume

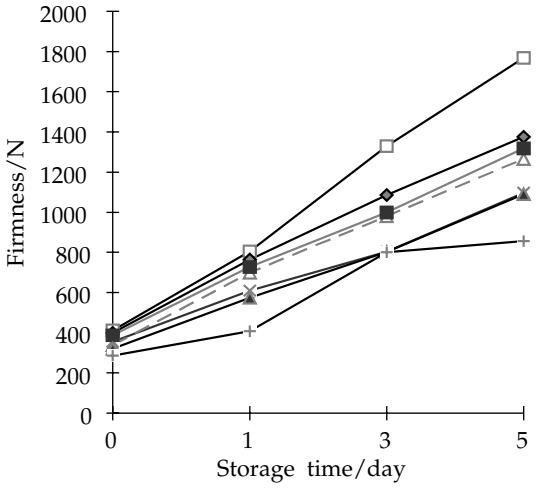

Fig. 6. Change in bread crumb firmness during storage

-□-, control; -△-, 1 % GHP; -×-, 2 % wheat gluten; -◆-, 1 % λC; -■-, blend of GHP and λC; -▲-, 1 % GHP-λC conjugate; -+-, 0.5 % GHP-λC conjugate. The preparation condition of the conjugate: pH=10.0, GHP/λC ratio=6:1, reaction time=10 days, reaction temperature=70 °C

Fig. 6 shows the change in bread crumb firmness during storage at 25 °C. Hardness of bread crumb increased during storage. Application of gluten and GHP-λC conjugate caused a slow drop of hardness. The lowest firmness was observed in the presence of 0.5 % GHP-λC conjugate. Thus, slower increase in bread crumb firmness after the addition of the conjugate shows that the conjugate has anti-staling properties for bread during storage. The influence of the conjugate on hardness might result from the changes occurring in the amorphous part of the starch, perhaps by inhibiting gluten protein-starch interactions or the development of macromolecular entanglement or from the capacity of the gums to retain water even after baking (*33*).

Conclusion

In conclusion, the emulsifying properties of the wheat gluten hydrolysate obtained from the hydrolysis by Protamex were improved significantly through the binding with λC under controlled conditions. The optimal reaction parameters were determined by response surface methodology. The EAI of the conjugates prepared at optimal conditions was 44.22 m^2/g, with the NSI of 61.05 %.

The denaturation temperature of the gluten hydrolysate/λ-carrageenan conjugate increased. The addition of gluten hydrolysate/λ-carrageenan conjugate improved dough characteristics, decreased bread crumb firmness during storage, and showed anti-staling properties of bread. Thus, gluten hydrolysate/λ-carrageenan conjugate exhibited potential application in food and non-food industries.

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Poboljšanje emulgatorskih svojstava konjugata hidrolizata pšeničnoga glutena i λ-karagenana

Sažetak

Hidrolizat glutena pripravljen je ograničenom enzimskom hidrolizom pšeničnoga glutena dobivenog kao nusproizvod pšeničnoga škroba. U radu je upotrijebljen enzim Protamex. Metodom odzivne površine (response surface methodology) ispitivan je utjecaj pH, odnos gluten hidrolizat (GHP)/λ-karagenan (λC) i trajanje reakcije na emulgatorska svojstva GHP-λC konjugata. Regresijski model za indeks aktivnosti emulzije (EAI) bio je značajan pri p=0,001, a vrijeme reakcije bitno je utjecalo na EAI konjugata s koeficijentom regresije od 4,25. Međusobni odnos pH i GHP/λC, te vremena reakcije značajno utječe na EAI konjugata. GHP-λC konjugati, pripravljeni pod optimalnim uvjetima, imali su bitno poboljšana emulgatorska svojstva i indeks topljivosti dušika (NSI) u usporedbi s kontrolnim uzorkom. Temperatura je denaturacije GHP-λC konjugata viša u usporedbi s glutenom. Dodatak GHP-λC konjugata različito je utjecao na osobine tijesta. Konjugat omogućava da se duže očuva svježina krušnih mrvica tijekom skladištenja, poboljšaju svojstva tijesta i uspori starenje kruha.