

Sensory and antigenic properties of enzymatic wheat gluten hydrolysates produced in an enzyme membrane reactor in comparison with batch

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Abstract The aim of this study was to compare the sensory and antigenic properties of enzymatic wheat gluten hydrolysates produced with Flavourzyme and glutaminase in batch and continuous process, respectively. The hydrolysate samples were obtained by a standard batch process with microfiltration (16 µm) and ultrafiltration (10 kDa) and an optimized continuous enzyme membrane reactor (EMR) process with an ultrafiltration membrane (10 kDa). Two drying methods were applied: oven and freeze drying. Sensory evaluation showed that the EMR hydrolysates were significantly different and had an improved taste impact for the attributes “overall intensity,” “salty,” “umami” and “mushroom.” The antigenic potential of the EMR hydrolysates was reduced (222 ± 4 ppm gliadin) compared to batch microfiltered hydrolysate (9030 ± 89 ppm) and batch ultrafiltered hydrolysate (843 ± 49 ppm). The superior quality of wheat gluten hydrolysate from the EMR process compared to batch process was proven since sensory properties of wheat gluten hydrolysate were improved and the antigenic potential was reduced.

Keywords Wheat gluten hydrolysis · Savory hydrolysates · Enzyme membrane reactor · Antigenicity · Sensory

Abbreviations

AN	Amino nitrogen
CSTR	Continuously stirred reactor
EMR	Enzyme membrane reactor
FAA	Free amino groups
GTU	Glutamine transforming units
<i>p</i> -NA	<i>para</i> -Nitroaniline
MF	Microfiltration
PPM	Parts per million
TN	Total nitrogen
UF	Ultrafiltration

Introduction

The main reason to produce wheat gluten hydrolysates is the taste benefit that can be obtained using natural and known ingredients: wheat gluten, water and salt [1]. Hydrolyzed plant proteins are used widely as ingredients in culinary products for their umami taste and for improving the overall flavor quality. In addition to the umami-enhancing properties of L-glutamic acid, several other amino acids and peptides contribute to the umami taste [1–3]. The contribution of peptides to umami taste has been debated controversially, since peptides tested in pure form with a trained human sensory panel resulted in neutral, sour or bitter taste perception, as reported previously [4]. Reaction products, such as glutamate glycoconjugates and pyroglutamyl peptides that probably occur during the downstream processing of hydrolysate products, are known to contribute to flavor perception as well [1, 5, 6]. Thus, the sensory complexity of protein hydrolysates goes beyond the recognition of the five basic tastes: salty, sweet, sour, bitter and umami. Therefore, product discrimination tests and assessment of retronasal flavor perception of wheat gluten hydrolysates

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in this study were performed with a trained human sensory panel in order to understand the effect of processing on the taste and flavor perception of protein hydrolysates.

In addition to the taste benefit that protein hydrolysates can deliver, consumers and producers are concerned about the allergenic potential of food protein products. Since wheat gluten is known to be of concern for patients with food allergies, the antigenic potential of hydrolysates produced in batch and continuous process has been compared. Celiac disease, for example, is the inflammation of the small intestine which adversely affects the absorption of water and nutrients. The assessment of allergenic potential of wheat gluten hydrolysates is a key for the producers and consumers of such products. Previous studies have shown that microbial fermentation and enzymatic treatment of wheat gluten can lower its allergenic potential [7–9]. Some hydrolysates have shown to be effective in treating and controlling food allergy reactions [10]. In this study, obtaining a hydrolysate which, according to food legislation, can be claimed to be “gluten-free” (gluten <20 ppm), as has been shown before [9], was not the target. The aim was to investigate and confirm the difference in the reduction in allergenic potential using batch and enzyme membrane reactor (EMR) processing.

Hydrolysis is conventionally performed in a batch process due to process simplicity, because Flavourzyme™ is product-inhibited [11–14]. However, a continuous EMR process can have promising economic and sustainability advantages over the batch process, provided that enzyme stability is established and the process can run stably [12, 13, 15–18]. In this study, the effects of EMR technology on the wheat gluten hydrolysate product quality: product composition, sensorial attributes and antigenic properties, in comparison with a batch process were investigated. The aim was to obtain a product with comparable or improved sensory or lowered antigenic properties.

Materials and methods

Materials

The materials used for hydrolysis were of food grade. Wheat gluten (protein 85 ± 2 % w/w) was obtained from Loryma GmbH (Zwingenberg, Germany). Flavourzyme™ (1000 L) was purchased from Novozymes (Bagsværd, Denmark). Glutaminase (Daiwa SD-C100S, 100 GTU/g) was obtained from Amano (Konan, Shiga, Japan). Sodium chloride was purchased from Südsalz GmbH (Heilbronn, Germany). The chemicals used for analysis were of analytical grade. Silver nitrate solution (0.1 mol/L), nitric acid, sodium chloride solution (0.1 mol/L), boric acid, sulfuric acid, Missouri tablets, sodium hydroxide (32 % w/v),

hydrochloric acid solution (0.1 mol/L), sodium hydroxide solution (0.1 mol/L), trisodium citrate, citric acid monohydrate, barium chloride, norvaline, formic acid, acetonitrile and sodium phosphate were purchased from Merck KGaA (Darmstadt, Germany). Ninhydrin was purchased from Fluka AG (St. Gallen, Switzerland). AccQ-Tag™ Ultra reagent was obtained from the Waters Corporation (Milford, USA).

Determination of enzyme activity

The Flavourzyme™ leucine aminopeptidase I activity was determined with Leu-*p*NA as a substrate [19–22]. Enzyme solution (19 µL) was mixed with 200 µL assay buffer (100 mmol/L Tris/HCl, pH 8). The Leu-*p*NA substrate solution (19 µL, 40 mmol/L in ethanol) was added, and the increase in absorption over time was measured in a spectrophotometer (Ultrospec 5300 Pro, GE Healthcare, Freiburg, Germany) at 405 nm for 10 min at 23 °C.

The glutaminase activity was determined with L- γ -glutamyl-*p*-nitroanilide as the substrate. Enzyme solution (19 µL) was mixed with 200 µL assay buffer (100 mmol/L sodium phosphate buffer, pH 6). The substrate solution (19 µL, 20 mmol/L in 100 mmol/L sodium phosphate buffer, pH 6) was added, and the increase in absorption over time was measured in a spectrophotometer, as described above.

One katal of enzyme activity was defined as the release of 1 mol *p*-nitroaniline per s.

Production of hydrolysates

A graphical representation of the processes applied for the production of hydrolysate powders is given in Fig. 1. The processes were: batch hydrolysis in a continuously stirred reactor (CSTR; 1), followed by microfiltration (MF; 1a) and ultrafiltration (UF; 1b) and continuous hydrolysis in an EMR (2) incorporating a UF membrane. The batch hydrolysis followed by MF was performed as the reference process. The batch hydrolysis followed by UF was performed for direct comparison of batch and EMR process without the effect of different means of filtration. Glutaminase enzyme was included in the batch process to convert the amino acid glutamine to glutamic acid. This was not the case in the EMR process. A glutaminase posttreatment step was included in the latter for a direct comparison (see below).

Batch hydrolysis was performed in a CSTR (DISTEK Symphony 7100, Riggttek GmbH, München, Germany). Tap water was preheated to $T = 50$ °C, and Flavourzyme™ (50 nkat_{Leu-*p*NA}/mL), wheat gluten (220 g/L), glutaminase (3.5 nkat/mL) and sodium chloride (80 g/L) were added. Hydrolysis was performed for 16 h at $T = 50$ °C, pH

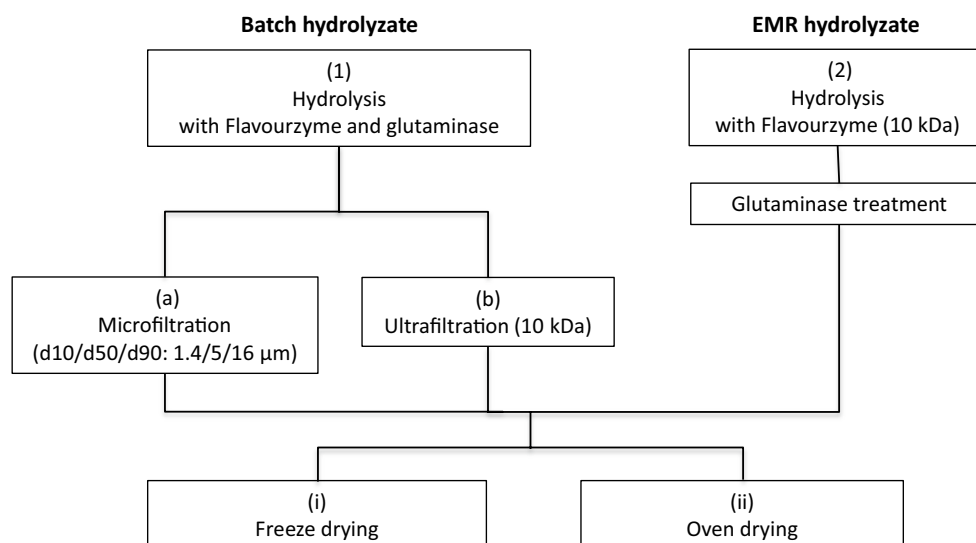


Fig. 1 Process flow diagrams of the batch (1) microfiltered (1a) and ultrafiltered (1b), and EMR (2) processes followed by freeze (i) and oven drying (ii)

5.8 ± 0.2 , and the enzymes were inactivated at $T = 90^\circ\text{C}$ for 10 min. The MF for the standard batch process (see below) was conducted within 8 h, resulting in a total batch processing time of 24 h.

The EMR experiments were performed in a 2-L membrane bioreactor (MMS AG, Urdorf, Switzerland) under optimal conditions: $T = 50 \pm 1^\circ\text{C}$, FlavourzymeTM ($116 \pm 4 \text{ nkat}_{\text{Leu-pNA}}/\text{mL}$), wheat gluten initial feed rate 19.2 g/L/h , sodium chloride 80 g/L and permeate initial flux $57 \text{ L/m}^2/\text{h}$, as described previously [18]. The EMR was conducted for 24 h. The permeate stream was collected from the start and pooled before downstream processing.

Downstream processing

The MF (filter cloth: SF2014, pore size distribution d10/d50/d90: $1.4/5/16 \mu\text{m}$, Strassburger Filter GmbH, Westhofen, Germany) of the standard batch product was performed with a depth filter (EF 14/2CW, KHS Prozesstechnik GmbH, Bad Kreuznach, Germany). Cellulose was applied as a filter aid (30 g/L). The UF (MWCO 10 kDa) of the batch product was performed at $T = 50^\circ\text{C}$ with a transmembrane pressure of 1–3 bar.

The EMR process included an additional glutaminase treatment step to convert free L-glutamine to L-glutamic acid. This was carried out with the EMR permeate batchwise in a Schott flask (2 L) with a magnetic stirrer (200 rpm, 2mag AG, München, Germany) in a water bath (Memmert GmbH and Co. KG, Schwabach, Germany) at $T = 60^\circ\text{C}$ for 16 h. The pH was 5.8 ± 0.2 .

The concentration of all hydrolysate samples was obtained by evaporation (R215, Büchi Labortechnik AG, Flawil, Switzerland) at $T = 60^\circ\text{C}$ and $P = 0.05 \text{ mPa}$.

The drying of all three hydrolysis products (1a, 1 b, and 2; see Fig. 1) was performed both in a freeze dryer (i) (Alpha 2-4, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and in a standard vacuum oven (ii) at $T = 60^\circ\text{C}$, $P = 50 \text{ mbar}$, $t = 8 \text{ h}$ (Memmert GmbH & Co. KG, Schwabach, Germany).

Sodium chloride

The sodium chloride level was determined with potentiometric titration using a silver nitrate solution (0.1 mol/L). Samples were dissolved in distilled water ($2 \text{ g}/100 \text{ g}$). The sample solution was adjusted to pH 1.5 with nitric acid before the start of the titration with silver nitrate solution. The sodium chloride level was a function of the silver nitrate consumed during titration.

Total nitrogen

The total nitrogen (TN) content was determined using the Kjeldahl method [23]. Bound organic nitrogen in the hydrolysate (500 mg) was mineralized to ammonium sulfate at $T = 420^\circ\text{C}$, for 120 min with sulfuric acid ($95\text{--}97 \text{ \%v/v}$) and a Missouri catalyzer ($49.8 \text{ g}/100 \text{ g Na}_2\text{SO}_4$, $49.8 \text{ g}/100 \text{ g K}_2\text{SO}_4$, $0.3 \text{ g}/100 \text{ g CuSO}_4$) in a mineralization system (K-438, Büchi Labortechnik AG, Flawil, Switzerland). Ammonia was released with sodium hydroxide ($32 \text{ \%w/v}$) and collected by condensation in boric acid (60 mL) in a distillation unit (Büchi339, Büchi

Labortechnik AG, Flawil, Switzerland). The TN content was correlated directly with the amount of hydrochloric acid solution (0.1 mol/L) consumed with titration to pH 4.65 in comparison with a blank. Protein content was calculated by multiplication of the TN determined by 6.25.

Amino nitrogen

The amino nitrogen (AN) was determined by incubation of a 0.5-g sample in 50 mL ninhydrin solution (5 % w/v) in citrate buffer (0.1 mol/L, pH 2.5) for 15 min at $T = 100\text{ }^{\circ}\text{C}$. The carbon dioxide released was flushed with nitrogen (30 mL/min) and reacted with barium (barium chloride solution 2 % w/v, pH 11.2 set with NaOH) to barium carbonate and led to a pH shift. Titration with NaOH solution (0.1 mol/L) back to pH 11.2 correlated with the amount of AN. The free amino groups (FAA) were calculated by multiplication of the AN determined by 6.25 [24].

The degree of hydrolysis was calculated by dividing the AN by the TN and multiplying the product by 100 %.

Amino acid analysis

Amino acid analysis was performed with an Acquity® ultraperformance liquid chromatography (UPLC) system using an ultraviolet detector (Waters Corporation, Milford, USA). Derivatization was carried out by the addition of 20 μL AccQ·Tag™ Ultra reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) to 80 μL of conveniently diluted sample in borate buffer. The mixture was thoroughly mixed and incubated for 1 min at room temperature and then for 10 min at $55\text{ }^{\circ}\text{C}$. The internal standard was 25 $\mu\text{mol/L}$ norvaline (1:100 stock solution 2.5 mmol/L in 0.1 N HCl). Mobile phases were solvent A: AccQ·Tag™ Ultra eluent A concentrate 100 % v/v, solvent B: Milli-Q water 90 % v/v and AccQ·Tag™ Ultra eluent B 10 % v/v, solvent C: Milli-Q water 100 % v/v and solvent D: AccQ·Tag™ Ultra eluent B 100 % v/v. The conditions of the elution gradient were as follows: 0 min (A: 2 %; B: 0 %; C: 98 %; D: 0 %), 0.29 min (A: 2 %; B: 0 %; C: 98 %; D: 0 %), 5.49 min (A: 9 %; B: 80 %; C: 11 %; D: 0 %), 7.10 min (A: 8 %; B: 15.6 %; C: 57.9 %; D: 18.5 %), 7.30 min (A: 8 %; B: 15.6 %; C: 57.9 %; D: 18.5 %), 7.69 min (A: 7.8 %; B: 0 %; C: 70.9 %; D: 21.3 %), 7.99 min (A: 4 %; B: 0 %; C: 36.3 %; D: 59.7 %), 8.59 min (A: 4 %; B: 0 %; C: 36.3 %; D: 59.7 %), 8.68 min (A: 2 %; B: 0 %; C: 98 %; D: 0 %) and 10.2 min (A: 2 %; B: 0 %; C: 98 %; D: 0 %). The elution gradient, detection wavelength (260 nm) and flowrate (0.7 mL/min) applied were according to the recommendation by Waters [25].

Mass spectrometric detection of peptides

Nano-LC–ESI–MS/MS measurements were taken on an ACQUITY nano-UPLC system (Waters, USA) coupled to a LTQ-Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Germany). Samples were concentrated and desalted on a precolumn (2 cm $\times$ 180 μm , symmetry C₁₈, 5- μm particle size, Waters, USA) and separated on a 25 cm $\times$ 75 μm BEH 130 C18 reversed-phase column (1.7- μm particle size, Waters, USA). Gradient elution was performed from 1 to 50 % acetonitrile in 0.1 % FA within 90 min. The LTQ-Orbitrap was operated under the control of Xcalibur 2.1.03 software. Survey spectra ($m/z = 250\text{--}1800$) were detected in the Orbitrap at a resolution of 60,000 at $m/z = 400$. Data-dependent tandem mass spectra were generated for the five most abundant peptide precursors in the linear ion trap. Internal calibration was performed using lock mass ions from ambient air for all measurements using the Orbitrap detector [26].

The mass spectrometry (MS) data analysis—Mascot 2.3 (Matrix Science, UK) was used as a search engine for protein identification. Spectra were searched against the green plant subset of the National Center for Biotechnology Information protein sequence database downloaded as FASTA-formatted sequences from <ftp://ftp.ncbi.nih.gov/blast/db/FASTA/nr.gz>. Search parameters specified no specific cleaving enzyme, a 5-ppm mass tolerance for peptide precursors and a tolerance of 0.6 Da for fragment ions. Carbamidomethylation of cysteine residues was set as a fixed modification. Methionine oxidation was allowed as a variable modification.

Sensorial product characterization—discrimination method (triangle test)

A sensory triangle test was performed to determine whether both the oven- and freeze-dried EMR products were significantly different ($p < 0.05$) from the oven- and freeze-dried batch products, respectively. The panel consisted of 12 participants conducting two repetitions resulting in $n = 24$ for each test. Two of the same products were compared with one other product. Products were served at the same temperature ($T = 65 \pm 1\text{ }^{\circ}\text{C}$) in a randomized design. Products were labeled with three-digit codes and served in dark cups to ensure an objective tasting environment. An individual questionnaire was used for documentation. The products were first tasted with and then without nose clips to distinguish whether a possible difference was perceived by tongue (taste) or retronasal (flavor). The nose clip ensured the blocking of retronasal perception.

Table 1 Analytical values of hydrolysate powders from batch with microfiltration (MF) and ultrafiltration (UF) and continuous EMR processes followed by freeze and oven drying after normalization of salt content (Fig. 1)

	Freeze-dried			Oven-dried		
	Batch MF (1.a.i)	Batch UF (1.b.i)	EMR (2.i)	Batch MF (1.a.ii)	Batch UF (1.b.ii)	EMR (2.ii)
Sodium chloride [%-w/w]			75.8 ± 1.5			
Protein (TN × 6.25) [%-w/w]	17.7 ± 0.2	17.6 ± 1.2	18.2 ± 0.2	18.2 ± 0.2	17.7 ± 0.2	17.6 ± 1.2
FAA equivalents (AN × 6.25) [%-w/w]	5.4 ± 0.1	6.2 ± 0.1	6.4 ± 0.1	5.3 ± 0.1	5.9 ± 0.1	6.9 ± 0.1
Glutamic acid [%-w/w]	2.5 ± 0.4	2.9 ± 0.2	3.8 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	3.8 ± 0.04
Degree of hydrolysis [%]	31	35	35	29	33	39

Sensorial product characterization—descriptive method (monadic profiling)

Sensory monadic profiling was performed to specify and quantify the differences in taste attributes. Eight sensitive assessors were trained to rate a specific range of attributes on a ten-point hedonic scale. Therefore, they tasted the products sequentially, without direct comparison, and judged them within their intensity toward the attribute. Analysis of variance (ANOVA) was used in order to decide whether there was any significant difference ($p < 0.05$) between products in terms of the attribute. A Duncan's multiple comparison test was used to decide whether there was a significant difference in the intensity of an attribute. Intensities were judged significantly different if the scale difference between them was greater than the least significant difference (LSD) value of the attribute.

Antigenicity with enzyme-linked immunosorbent assay

Antigenicity of the hydrolysates was investigated by quantitative determination of gliadin epitopes, combining two commercial testing kits: a sandwich enzyme immunoassay (Ridascreen® R7001, R-Biopharm AG, Darmstadt, Germany) and a competitive enzyme immunoassay (Ridascreen® R7021, R-Biopharm AG, Darmstadt, Germany). The procedures described by the manufacturer were followed. Sample extraction for the sandwich enzyme immunoassay was carried out using RIDA® extraction solution (R7099, R-Biopharm AG, Darmstadt, Germany) and 2-propanol solution (68 %-v/v). Sample extraction for the competitive enzyme immunoassay was carried out with 1 g of product in 10 mL of ethanol (60 %-v/v) in distilled water, following the manual. This methodology was recommended by Codex Alimentarius and AOAC-OMA. Products can be declared gluten-free when they contain less than 20 mg/kg gluten. Absorbance was measured at 450 nm in a microplate photometer (Multiskan FC, Thermo Fischer Scientific™, Dreieich, Germany).

Statistical evaluation

If not stated otherwise, the result values represented the average value of the measurements ± the standard error of the mean. The same was applied for the error bars in the figures below.

Results and discussion

Product qualities of batch and continuously produced wheat gluten hydrolysates were compared by analysis of the product composition, sensorial attributes and antigenic properties. The aim was to obtain an EMR product with comparable or improved sensory and comparable or reduced antigenic properties compared to the reference batch process.

Product composition analyses

The sodium chloride content of the six hydrolysate powders was normalized to 75.8 ± 1.5 g/100 g (Table 1). The resulting protein content after normalizing was 17.6 ± 1.2 g/100 g. The remaining fraction was composed of fat and starch (data not shown). The amount of FAA in the EMR product increased compared to the batch MF product: from 5.3 ± 0.1 to 6.9 ± 0.1 g/100 g for oven-dried and from 5.4 ± 0.1 to 6.4 ± 0.1 g/100 g for freeze-dried. The amount of FAA in the batch UF product was 5.9 ± 0.1 g/100 g for the oven-dried and 6.2 ± 0.1 g/100 g for the freeze-dried. Consequently, the degree of hydrolysis improved from 31 ± 1 % in the batch MF process to 38 ± 1 % in the EMR process. All amino acids increased in the EMR product compared to the batch MF and batch UF products, but not in equal amounts (Fig. 2). L-Histidine, L-asparagine, L-serine, L-arginine, L-glutamine, L-threonine, L-alanine, L-tyrosine, L-methionine, L-valine, L-isoleucine, L-leucine and L-phenylalanine levels increased 40–60 %-w/w (average freeze-dried and oven-dried). L-Glutamic acid, glycine, L-aspartic acid, L-proline, L-cysteine, L-lysine and

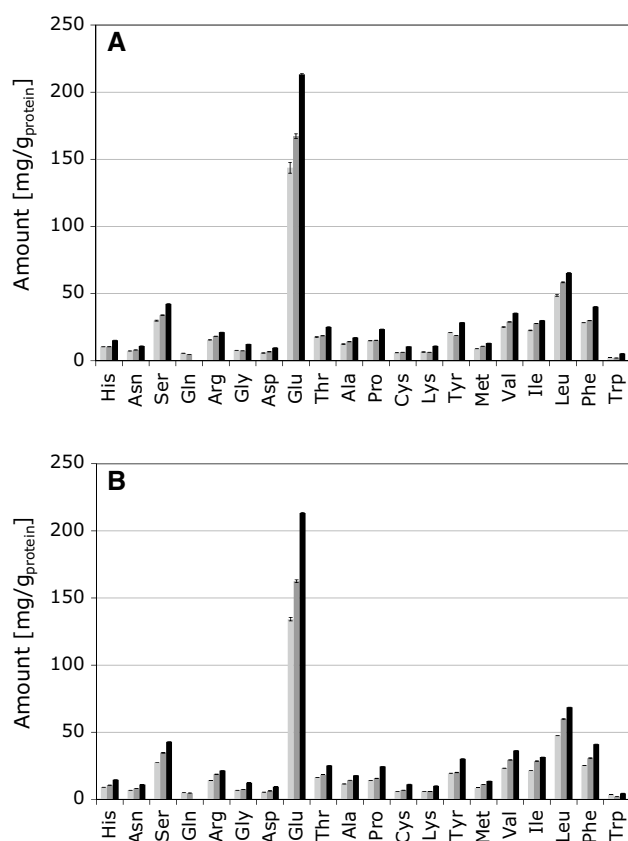


Fig. 2 Increased free amino acid profiles for different hydrolysate products from (light grey square bar) batch with microfiltration (MF), (grey square bar) batch with ultrafiltration (UF) and (black square bar) continuous EMR processes followed by (A) freeze and (B) oven drying

Table 2 Comparison of EMR and standard batch microfiltered hydrolysate in a sensory triangle test

	Response		Significance level
	Total	Correct	(<i>p</i> value)
Freeze-dried			
With nose clip	24	13	0.0284*
Without nose clip	24	15	0.0032**
Oven-dried			
With nose clip	24	13	0.0284*
Without nose clip	24	13	0.0284*

* Indicates significant difference ($p < 0.05$); ** indicates significant difference ($p < 0.01$)

L-tryptophan increased 60–85 %w/w (average freeze-dried and oven-dried). An increased sensory impact on the five basic taste attributes was expected from the increased amount of free amino acids, especially from L-glutamic acid, in the EMR product. The increased amount of free amino acids in the EMR product was an

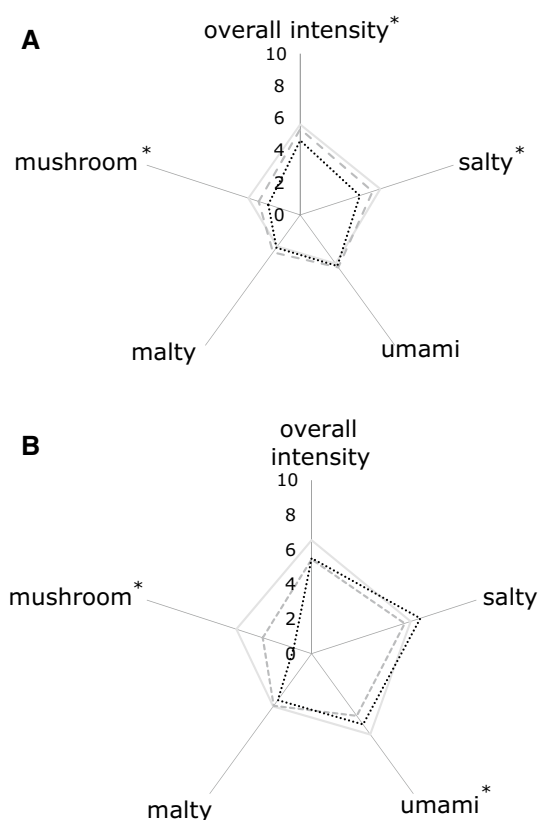


Fig. 3 Identification and scaling of perceived attributes identified with a trained sensory panel in hydrolysates from (dotted line) batch with microfiltration (MF), (dash dotted line) batch with ultrafiltration (UF) and (dash line) continuous EMR processes followed by (A) freeze and (B) oven drying. * Indicates significant difference ($p < 0.05$)

indication of higher exopeptidase activity. In addition, an increased degree of hydrolysis indicated a reduced antigenic potential. Subsequently, sensory product evaluation with a human sensory panel was performed, and the antigenic properties of the wheat gluten hydrolysates were investigated.

Sensorial product evaluation

The triangle test with a human sensory panel ($n = 24$) showed that the freeze-dried batch MF product and EMR product were significantly different in taste (evaluated with nose clip, p value = 0.0284) and in flavor (evaluated without nose clip, p value = 0.0032, Table 2). The EMR product was described to be “more salty, more umami and more intense” in 82 % of the correct answers when tasted with nose clip and in 100 % of the correct answers when tasted without the nose clip. The oven-dried batch MF product and EMR product were significantly different in taste and flavor (evaluated with and without nose clip, p value = 0.0284, Table 2).

Table 3 Antigenicity of different hydrolysate samples from batch with microfiltration (MF) and ultrafiltration (UF) and continuous EMR processes followed by freeze and oven drying (Fig. 1)

	Process	Sandwich ELISA gluten [ppm]	Competitive ELISA gluten [ppm]
Freeze-dried	Batch MF	9.0 ± 0.8	9030 ± 89
	Batch UF	<5	843 ± 49
	EMR	<5	222 ± 4
Oven-dried	Batch MF	9.2 ± 1.4	5723 ± 856
	Batch UF	<5	557 ± 35
	EMR	<5	224 ± 15

The trained human sensory panel developed their own glossary for the monadic profile, including taste and flavor (retronasal observation) properties. Therefore, the hydrolysate samples were described more accurately. Instead of a single taste difference “umami,” a more complex intensity increase defined as “mushroom” was notified. The monadic profile revealed the sensory attributes in which the samples were significantly different. A trained sensory panel identified five relevant attributes for sensory description of the samples: overall intensity, salty, umami, malty and mushroom. The result showed that the EMR samples were significantly different from the batch MF sample in the attributes: overall intensity, mushroom and salty for the freeze-dried product and mushroom and umami for the oven-dried product (Fig. 3). “Mushroom” was attributed to a mixture of amino acids, small peptides and aroma molecules. Umami taste is mainly delivered from the amino acid L-glutamic acid in the presence of sodium. Other amino acids were previously recognized to have a synergistic effect on the umami taste, and all amino acids released have a bitter, sweet or sour taste of their own [1, 3, 4]. In addition to the amino acids, many other food constituents were responsible for the taste profile of the hydrolysates investigated, such as peptides, glycoconjugates, pyroglutamyl peptides and pyrazines [1, 6, 27].

Antigenic properties of wheat gluten hydrolysates

The antigenic potential of the EMR product decreased significantly in comparison with the batch MF product. The freeze-dried and oven-dried EMR product contained 222 ± 4 and 224 ± 15 ppm gliadin epitopes, respectively (Table 3). This was forty and twenty-five times less than the amount of gliadin epitopes in the freeze-dried and oven-dried batch MF product with 9030 ± 89 and 5723 ± 856 ppm, respectively. The batch UF product showed a reduced amount already ten times smaller at 843 ± 49 and 557 ± 35 ppm gliadin epitopes, respectively, indicating that the gliadin epitopes were mostly larger than 10 kDa. Both hydrolysis processes tested allowed the reduction in the antigenic potential of wheat gluten. Other researchers in our group recently reported that wheat gluten treated with Flavourzyme (25 nkat/mL, determined

with wheat gluten assay; 100 g/L, pH 7.5, $T = 37^\circ\text{C}$, $t = 465$ min) before downstream processing contained only 858 ± 37 ppm gliadin [9]. The lower amount of gliadin level before downstream processing could potentially be explained due to the different hydrolysis process conditions chosen and the subsequently higher degree of hydrolysis reached. The lower substrate concentration was shown to play an important role as well as the different pH and temperature, and the presence of more than eight peptidases with different pH and temperature optima in Flavourzyme could explain the difference in product properties [14]. The centrifugation step contributed especially to a major reduction in antigenicity; therefore, this step seemed to be unavoidable for the production of products with the lowest gliadin content [9]. From a practical point of view, these process steps need careful evaluation, taking into account process economic feasibility and product value. Instead of making a “gluten-free” wheat gluten hydrolysate, it might be much more interesting to investigate the production of a wheat gluten hydrolysate with reduced antigenicity that could be applied potentially to avoid or reduce gluten allergy symptoms, as suggested in the literature [10].

The peptide sequences that were detected in this study using mass spectrometry were analyzed using the peptides search tool in the databases PepBase and SciFinder (Table 4) [28]. Twenty-nine different peptide sequences were found in the batch MF product, seventeen different peptide sequences were found in the batch UF product, and ten generally smaller different peptide sequences were found in the EMR product. The decreased amount of different peptide sequences in the EMR product might be the reason that antigenicity was reduced. Nine peptide sequences could not be correlated with known bioactive or sensory properties. Ten fragments of known celiac bioactive peptides were found (Table 4). These fragments might still exhibit celiac toxic properties. The search revealed the presence of two complete known bioactive peptides RPQQYPQPQPQ and FPPQQYPQPQP in all the samples. These two peptides are known to exhibit celiac toxic properties.

Nutritional benefits, such as reduced antigenic potential by food processing and, in particular, by fermentation, germination and enzymatic hydrolysis, have been

Table 4 Peptide sequences determined by nano-LS-MS/MS and comparison with correlating celiac toxic peptides published previously

Current work			Literature		
Observed mass (M + H) ⁺⁺	Actual mass (M)	Sequence	Sample	Functional sequence	Function (reference)
333.7184	665.4222	VRVPVP	E	VRVPVQLQP	Celiac [35, 36]
710.3549	1418.70	PQQPQQPFPQQP	B	n.d.	
507.2617	1012.51	RPQQPYQPQ	B/BF/E	RPQQPYQPQPQ	Celiac [37–40]
555.7882	1109.56	RPQQPYQPQ	B/BF/E		
619.8171	1237.62	RPQQPYQPQPQ	B/BF/E		
668.3436	1334.67	RPQQPYQPQPQ	B/BF/E		
732.3726	1462.73	RPQQPYQPQPQPQ	B/BF/E		
813.9044	1625.79	RPQQPYQPQPQPQY	B/BF/E		
576.2756	1150.54	QPQNPSQQQP	B/BF	LQPQNPSQQQPQEQ	Celiac [41]
640.3046	1278.59	QPQNPSQQQPQ	BF		
419.7245	837.4344	DVVLQQH	BF	PCMDVVLQQHNI	Celiac [43]
487.2447	972.4748	FPPQQPYP	B/BF/E	FPPQQPYQPQP LGQQQPFPP QQPYQPQPFPSSQQPY	Celiac [42, 43]
599.7977	1197.58	FPPQQPYQPQ	B/BF/E		
712.3573	1422.70	FPPQQPYQPQPQ	B/BF/E		
785.8889	1569.76	FPPQQPYQPQPFP	B/BF		
834.4146	1666.81	FPPQQPYQPQPFP	B/BF		
832.4147	1662.81	FPQPQQPQQPFPQP	B	n.d.	
896.4452	1790.88	FPQPQQPQQPFPQPQ	B	n.d.	
558.7781	1115.54	FPQPQQPFP	B	QQPFPQQPQQPFPQ	Celiac [44]
607.3039	1212.59	FPQPQQPFP	B		
783.8893	1565.76	FPQPQQPFPQQP	B	n.d.	
981.4828	1960.95	GQQQFPFPQQPYQPQP	B	LGQQQFPFPQQPYQPQPFP	Celiac [45]
485.2431	968.4717	PQQFPQQP	B	n.d.	
485.243	968.4715	QQPFPQPQ	B/BF	PQQPFPQPQQPFRQ	Celiac [46]
960.4737	1918.93	QQPQQPFPQQPQQPFP	B	n.d.	
710.3535	1418.69	PQQPFPQQPQQP	B	n.d.	
712.3536	1422.69	QPFPQQPYQPQ	BF	QPFPQQPYQPQPQ	Celiac [47]
549.2733	1096.53	QPFPQQPQQ	B	QQPFPQQPQQPFPQ	Celiac [46]
719.8603	1437.71	QPFPQQPQQPFP	B		
597.799	1193.58	QPQQPFPQPQ	B/BF	QPQQPFPQPQQPFPWQP	Celiac [48]
774.3837	1546.75	QPQQPFPQQPQQP	B	n.d.	
896.4452	1790.88	QPQQPFPQQPQQPFP	B	n.d.	
397.2136	792.4127	QPVQPQP	B	n.d.	
611.8191	1221.62	QPQFPRPQQP	BF	n.d.	

E EMR; *B* batch microfiltered; *BF* batch ultrafiltered; *n.d.* not detected; *celiac* celiac toxic

an interesting field of interest in food research [10, 29, 30]. The present study confirmed that antigenicity can be reduced by enzymatic protein hydrolysis and UF [9, 30]. The present study also confirmed that application of the right enzyme-linked immunosorbent assay (ELISA) method was important. In the case of protein hydrolysates, the competitive ELISA test was suitable. The monoclonal antibodies R5 and IgE recognized potential toxic peptide sequences such as QQPFP and PQQPF [31, 32]. In addition to proper assay-based analytics, clinical trials must be

executed before products are released with claims, indicating that no allergic reactions occur [33, 34].

Conclusions

In this study, enzymatic wheat gluten hydrolysates produced in a standard batch with MF, in a standard batch process with UF and in a continuous EMR process with UF were compared in terms of sensory properties using a

trained panel. Furthermore, the antigenic properties of the hydrolysates were determined. The enzymatic wheat gluten hydrolysate product quality (taste impact and antigenicity) was improved by the application of a continuous EMR process with UF in comparison with standard batch processing with MF and standard batch processing with UF. The taste impact described as “mushroom” by the trained sensory panel correlated with the increased amount of free amino acids and the higher degree of hydrolysis, especially in the oven-dried product, indicating the important role of heat-induced reaction products in wheat gluten hydrolysates. The celiac toxic peptides RPQQYPQPQPQ and FPPQQYPQPQP were identified in all hydrolysate samples, although the antigenicity of the EMR product and batch UF product was reduced.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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