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Glucosamine-induced glycation of hydrolysed meat proteins in the presence or absence of transglutaminase: Chemical modifications and taste-enhancing activity

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ABSTRACT

Salt reduction in food is a challenging task. The food processing sector has adopted taste enhancers to replace salt partially. In this study, a flavour enhancer formulation (liquid seasoning) was produced using enzymatically hydrolysed poultry proteins isolate (PPI). The PPI obtained through the isoelectric solubilisation precipitation process (ISP) was hydrolysed with Alcalase and glycated with glucosamine (GlcN) at moderate temperatures (37/50 °C) in the presence or absence of transglutaminase (TGase). The glycated hydrolysates showed reduced fluorescence advanced glycated end-products (AGE) and a reduced amount of alpha-dicarbonyl compounds (α-DC). An untrained consumer panel ranked the meat protein hydrolysate seasoning saltier than the salty standard seasoning solution ($p < 0.05$) regardless of GlcN glycation (both tested at 0.3 M Na^+). GlcN treatments showed a tendency ($p = 0.0593$) to increase savouriness. Free glutamic acid and free aspartic acid found in the PPI hydrolysate likely increased the salty perception.

1. Introduction

The global trend to reduce sodium in food is due to health concerns of hypertension and cardiovascular disease. One of the strategies to reduce salt intake is through the addition of flavour enhancers in processed foods. Unlike food flavours, flavour enhancers do not possess flavour or taste themselves, but rather intensify the flavours of other compounds. Common commercial flavour enhancers are inosinates, gluanylates and glutamates. Since consumers are demanding "natural" food ingredients, several studies explored alternatives such as the deamidation of wheat gluten protein (Liao et al., 2010; Schlichtherle-Cerny & Amado, 2002), enzymatic hydrolysis of shrimp protein (Cheung & Li-Chan, 2014), and chicken muscle protein (Maehashi, Matsuzaki, Yamamoto, & Udaka, 1999). These peptides and proteins were reported to enhance the umami (savoury) and salty taste. For instance, Maehashi et al. (1999) isolated an umami fraction from chicken proteins hydrolysed with bromelain; within this fraction several di- and tri-peptides containing glutamic acid (i.e. Glu–Glu and Ala-Glu-Asp, respectively) were identified. These peptides demonstrated an increase to the umami taste when used in combination with 5′-inosine monophosphate (IMP), a commercial flavour enhancer. Also, meat proteins contain high levels of glutamic and aspartic acid that can be released with chemical or enzymatic hydrolysis to increase the umami taste. The recent interest in the valorisation of meat and fish processing by-products has led to the production of muscle protein isolates recovered through the isoelectric solubilisation and precipitation process (ISP). This can be a suitable and an economic protein-based biomass to hydrolyse and unleash the potential of glutamic acid in amplifying the deliciousness of food. For instance, Hrynets, Omana, Xu, and Betti

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(2011) found that glutamic acid significantly increased after the ISP of mechanically separated poultry meat (MSPM) compared to the starting material. Even without hydrolysis, poultry protein isolate (PPI) was successfully incorporated in chicken patties with positive consumer sensory acceptability (Khiari, Pietrasik, Gaudette, & Betti, 2014).

The Maillard reaction, also known as glycation, is a common process to generate food flavours. This reaction involves the condensation of the carbonyl group of a reducing sugar with an amino compound, followed by the degradation of the condensation products to alpha-dicarbonyl (α-DCs). Subsequently, α-DCs react with other compounds such as amines, amino acids, aldehydes, hydrogen sulphide and ammonia, leading to many important classes of flavour compounds, including furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and other heterocyclic compounds. Several studies have been conducted in a simple model system consisting of an amino acid/peptide and a reducing sugar to generate specific flavour compounds (Lee, Jo, & Kim, 2010; Xu et al., 2013). Recently, some compounds generated through the Maillard reaction, specifically the so called "Maillard reacted peptides" (MRP), possess flavour-enhancing properties. For instance, soy protein isolate glycated with various reducing sugars (Katsumata et al., 2008; Lan et al., 2010; Ogasawara, Katsumata, & Egi, 2006; Song et al., 2013) as well as xylose conjugated with sunflower protein hydrolysate (Eric et al., 2013) yielded a mixture of MRP (modified peptides + glycopeptides), which not only enhanced the savoury taste, but also increased the intensity of the mouthfulness and continuity sensations known as "kokumi". On the other hand, despite the usefulness of the Maillard reaction to generate both flavours and MRP, one of the negative effects is the production of browning compounds. These compounds are usually associated with toxic substances, such as acrylamide and advanced glycation end-products (AGEs). This becomes particularly relevant when the Maillard reaction is conducted at elevated temperatures (i.e. >100 °C), especially during cooking (Mottram, Wedzicha, & Dodson, 2002; Poulsen et al., 2013; Stadler et al., 2002). Consumers demand the production of "clean" and "natural" label ingredients. Therefore, alternative approaches which minimise the formation of browning compounds whilst increasing the production of the flavour enhancing MRP are of interest. In this aspect, Hong, Gottardi, Ndagijimana, and Betti (2014) and Hrynets, Ndagijimana, and Betti (2013) had successfully glycated fish gelatin hydrolysate and myofibrillar proteins at moderate temperature (37 °C) to generate modified peptides and proteins with enhanced bioactivity (i.e. antioxidant capacity) and functionality (i.e. solubility), respectively. This was likely due to the use of the highly reactive amino sugar glucosamine (GlcN) in concert with the enzyme transglutaminase (TGase). Here, novel glycoconjugates may be produced due to the ability of TGase to catalyse the transfer of an acyl group from a glutamine amino acid in a protein or peptide sequence to the amino group of GlcN, forming a stable isopeptide bond. The effectiveness of using GlcN as a way to modify peptides has been already exploited by Katsumata et al. (2008). In this study a fraction of soy protein hydrolysate was glycated at 95 °C for 4.5 h generating MRPs that modulate the salty taste. However, at 95 °C, browning and heterocyclic compounds may be generated. It is our intention in this current research to generate MRP using GlcN at significantly lower temperatures that still amplify saltiness and increase savouriness.

Our main objective was to evaluate the chemical and enzymatic modifications of hydrolysed poultry meat proteins in response to GlcN glycation in the presence or absence of TGase at moderate temperatures (37, 50 °C). We examined how this affected the saltiness and savoury perception of the modified hydrolysates in the seasoning composition. The PPI extracted at a pilot plant facility with the ISP process was hydrolysed with a commercial protease Alcalase and subjected to GlcN glycation treatment in the presence or absence of TGase. Then, it was used to formulate a liquid seasoning composition which was subsequently used for a consumer sensory evaluation. Chemical changes regarding composition and structure due to GlcN treatments were analysed and correlated to the consumer panel results.

2. Materials and methods

2.1. Chemicals

Mechanically separated turkey meat (MSTM) was obtained from Lilydale Inc. (Edmonton, AB, Canada). Food grade Alcalase was a gift from Novozymes (Franklinton, NC). GlcN hydrochloride and reduced glutathione (GSH) were purchased from PureBulk (Roseburg, OR). Food grade microbial TGase (ACTIVA® TI) was manufactured by Ajinomoto (France). Food grade hydrochloric acid, sodium hydroxide and acetic acid were purchased from Fisher Scientific (Fisher Scientific Company, Ottawa, ON). Monosodium glutamate was purchased from Ajinomoto (La Victoria, Peru). Chemicals used for poultry protein isolation and sensory evaluation were of food grade. All other chemicals and solvents used in other analyses and liquid chromatography were of analytical grade or HPLC grade. They were purchased from Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO) and Fisher Scientific (Ottawa, ON, Canada).

2.2. Experimental design

Poultry protein was isolated from commercial MSTM using the acid-aided ISP process. The poultry protein was extracted from 80 kg of MSTM in four batches. Subsequently, they were pooled into one batch, stored at −20 °C and used within 3 months. Extracted protein (6.0–6.5% w/v) was hydrolysed with Alcalase in 11 batches and then pooled. Following enzymatic hydrolysis, the glycation treatment was performed. The hydrolysate (5% protein) was incubated with GlcN either in the presence or absence of TGase at moderate temperatures (37 or 50 °C) and at pH 7.0 ± 0.5, resulting in the following treatments: native hydrolysate 37 °C, native hydrolysate 50 °C, glycated hydrolysate 37 °C in the absence of TGase, glycated hydrolysate 37 °C in the presence of TGase, and glycated hydrolysate 50 °C in the absence of TGase.

For the purpose of chemical analysis, all treatments were acidified to pH 4.9 with food grade HCl at the end point of incubation, pasteurised at 80 °C for 10 min, lyophilised and kept at −18 °C until used. Chemical modification of the hydrolysate in response to GlcN glycation was assessed using spectroscopic techniques (see Section 2.4) and α-dicarbonyl formation (Section 2.5).

On the other hand, for sensory evaluation, the native and glycated hydrolysates were used to formulate a composition (a liquid seasoning) similar to the ones commercially available (i.e. Maggi seasoning). Therefore, acetic acid and HCl (food grade acids) were used to adjust the pH of the seasonings to 4.9 at the end point of incubation and before they were pasteurised (80 °C for 10 min). These liquid seasonings were kept frozen at −18 °C and used within two weeks. Protein, pH and Na^+ content were standardised to 3% w/v, 4.9 and 0.3 M, respectively, prior to sensory evaluation. The taste enhancing properties (saltiness and savouriness) were studied using ranking tests. Seasoning control samples containing glutamate ("savoury seasoning"), glutathione ("kokumi seasoning"), and, a mixture of the two ("kokumi + savoury seasoning") were also produced and standardised at the same levels of Na^+ and pH. For the evaluation of saltiness and savouriness of the seasonings, a randomised complete design was used with sample treatment as experimental unit. Participants were asked to evaluate the intensity of saltiness and savoury tastes perceived in two separate sessions.