



# Temperature and cysteine addition effect on formation of sunflower hydrolysate Maillard reaction products and corresponding influence on sensory characteristics assessed by partial least square regression

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## ABSTRACT

Maillard reaction products (MRPs) were prepared from sunflower peptide and D-xylose with or without L-cysteine (PXC or PX) by heating over a range of temperatures (80–140 °C) for 2.0 h and a pH of 7.4 and subsequently the products were sensory evaluated. Partial least square regression (PLSR) was performed to analyze the correlation among data of quantitative sensory descriptive analysis, peptides, GC–MS and free amino acid (FAA) data of PXCs and PXs. Results revealed that MRPs formed at 120 °C with cysteine addition (PXC-120) had greater meat-like flavor, mouthfulness and continuity taste compared to other MRPs. Molecular weight distribution showed that the presence of cysteine inhibited the low molecular weight (LMW) peptide cross-linking but accelerated the high molecular weight (HMW) peptide degradation with increasing temperature. Furthermore, results showed that the peptide above 5 kDa has a significant negative contribution to sensory attributes of PXCs, while the peptide between 1 and 5 kDa showed no significant but positive influence on PX sensory attributes. Sulfur containing compounds showed a significant and positive correlation to sensory attributes of PXCs while nitrogen containing compounds and furan were significantly but negatively correlated to sensory attributes of PXCs.

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## 1. Introduction

Proteins of either animal or vegetable origin, including their peptide derivatives, are of great importance in food industries. Defatted sunflower meal, a by-product of the sunflower oil industry constitutes an important source of proteins. Salgado, Molina Ortiz, Petruccielli, and Mauri (2011) demonstrated the value of sunflower meal as a source of proteins with high water solubility and good physicochemical properties. Villanueva et al. (1999) reported that sunflower peptides from sequential hydrolysis with two proteases of different catalytic activities generated high soluble peptides. Meanwhile, the use of

sunflower peptide in the food industry is still at a low level due to its high concentration in phenolic compounds (chlorogenic and caffeic acids) which affect its organoleptic characteristics (Salgado et al., 2011).

Due to the high demand for natural flavors, food industries necessitated the use of animal or vegetable proteins as raw materials to generate natural flavor enhancers through MR. The MR is a non-enzymatic interaction between nucleophilic amino groups of amines and reactive carbonyl groups of reducing sugars that occurs during thermal processing (Hong, Jung, Kim, Lee, & Kim, 2010). Due to its potential to generate various flavors found in breads, roasted coffee, roasted seeds, vegetables, and cooked meats, the MR has been extensively investigated in order to develop processed flavors and flavor enhancers (Mottram, 1998). During the past years, several studies have been conducted using protein or peptides in the generation of processed flavor enhancers. Soybean protein hydrolysate was extensively used to develop such flavors through MR and it was recognized as an important flavor potentiator and precursor of the Maillard reaction (Liu et al., 2012; Song et al., 2013). A variety of other vegetable protein resources have been studied recently and the findings have demonstrated that the proteins or peptides can be utilized to generate the processed flavor enhancers through MR. Peptides with a smaller molecular weight showed a higher reaction degree to produce more volatile compounds through

**Abbreviations:** AU, Anson unit; LAPU, leucine amino peptidase unit; MR, Maillard reaction; MRPs, Maillard reaction products; SFPs, sunflower protein hydrolysates; PXC, sunflower protein with cysteine; PX, sunflower protein without cysteine; ND, not detected; CGA, chlorogenic acid; MWD, molecular weight distribution; MW, molecular weight; SPME, solid phase microextraction; KI, Kovats index; ID, identification; PLSR, partial least square regression; LMW, low molecular weight; HMW, high molecular weight; kDa, kilodalton; GSH-MRPs, glutathione MRPs.

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the Maillard reaction (Su et al., 2011). Similarly, Eric et al. (2013) reported that sunflower MRPs can be used as a flavor enhancer and as a potential alternative to soybean MRPs and in a study by Guo, Tian, and Small (2010) it was found that temperature and pH influenced not only the number but also the amount of volatile products.

In recent years, various studies have been done on sensory characterization of the flavor and taste of MRPs from different peptides and their subsequent correlations between sensory attributes, volatile compounds, and peptides of different molecular weights. A study by Ogasawara, Katsumata, and Egi (2006a) and Ogasawara, Yamada, and Egi (2006b) who investigated the flavor-enhancing properties of the Maillard reaction products from 1000 to 5000 Da peptides found that the Maillard peptide produced an enhanced effect on flavor, including umami, continuity and mouthfulness in the umami solution and in consommé soup. Furthermore, in their subsequent study in which they characterized the mouthfulness and continuity of long-term ripened miso flavor, they found that the Maillard-reacted peptide (1000–5000 Da) was considered to be the key for not only the basic taste but also the enhanced “kokumi”, a sensation of mouthfulness and continuity of long-ripened miso. Song et al. (2013) reported that microbial transglutaminase (MTGase) cross-linking improved the flavor characteristics (mouthfulness and continuity) of soybean protein hydrolysates. In our recent study, we found that MRPs from the sunflower peptide, xylose and cysteine model system have a greater mouthfulness and continuity taste compared to those from soybean (Eric et al., 2013).

On the other hand, the correlation between sensory attributes, volatile compounds, peptides of different molecular weights and instrumental data (e-nose) has also been studied using the partial least square regression (PLSR) (Song, Zhang, Hayat, Huang, et al., 2010). Song et al. (2012) found the correlation of molecular weight (MW) of peptides, odor-active compounds and sensory attributes, electronic nose measurements, sensory evaluation and characteristic compounds through PLSR and concluded that the beef base with a DH of 29.13% and the moderate oxidized tallow were the desirable precursors for imparting aroma characteristics of beef-like process flavor and beef flavor respectively. Lee, Kwon, Kim, and Kim (2011), studied the relationship between volatile compounds and the sensory attributes of glutathione-Maillard reaction products (GSH-MRPs) prepared under different reaction conditions and concluded that volatile compounds such as 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol, 2-propylpyrazine, 1-furan-2-ylpropan-2-one, 1H-pyrrole, 2-methylthiophene, and 2-(furan-2-ylmethyl)disulfanylmethyl furan could be identified as possible key contributors to the beef-related attributes of GSH-MRPs.

In order to enhance the flavor and sensory characteristics of MRPs, different studies have been carried out to determine favorable parameters and flavor precursors (Jousse, Jongen, Agterof, Russell, & Braat, 2002). Van Boekel (2006) reported that the flavor formation depends on the type of sugars and amino acids involved, and on reaction temperature, time, pH and water content. However, the type of sugar and amino acid factors determines the type of flavor compounds formed, while the other factors influence the kinetics. However, temperature was recognized as the most important parameter that affects the reaction rates and flavor characteristics of foods (Labuza & Baisier, 1992; Lan et al., 2010; You, Luo, Shen, & Song, 2011). On the other side, the influence of cysteine on flavor and sensory characteristics has been studied by the addition of cysteine to different MR systems (Huang et al., 2011). Huang et al. (2012) reported that cysteine significantly contributes in the color inhibition and enhances the sensory characteristics (mouthfulness and continuity) of MRPs from the soybean protein hydrolysate and xylose system than thiamine. Many other studies presented cysteine as a flavor precursor due to its contribution in the production of the meaty flavor through pyrolysis or Strecker degradation with dicarbonyl compounds (Cerny, 2007; Madruga & Mottram, 1998; Mottram, 1998; Yu & Zhang, 2010).

Though the effect of temperature and cysteine addition on MRP flavor formation and peptide molecular weight modification has been

investigated in recent years (Eric et al., 2013; Lan et al., 2010), no documented studies have been carried out to determine the combined effect of temperature and cysteine addition on MRP properties, especially given that the correlation of corresponding MRPs to the sensory characteristics was not quantitative. Therefore, the objective of this study was to determine the effect of different temperature ranges and cysteine addition on physico-chemical changes and sensory characteristics of sunflower protein hydrolysate MRPs and to clarify the subsequent influence of volatile compounds, free amino acid and peptides of different molecular weights on the sensory attributes through the partial least square regression (PLSR) analysis.

## 2. Materials and methods

### 2.1. Chemicals

Sunflower meal was obtained from Suntime International Seed Co. Ltd. (Xinjiang, China). Alcalase 2.4 L FG and flavourzyme 500 MG were purchased from Novo Co., Ltd. (Novozyme Nordisk, Bagsvaerd, Denmark). Chlorogenic acid standard ( $\geq 98\%$ ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other solvents/chemicals used were of analytical grade and obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

### 2.2. Preparation of sunflower isolates and hydrolysates

Sunflower protein isolates were prepared according to the method of Eric et al. (2013). Twenty grams of SFPI were dissolved in distilled water at final concentrations of 8% (w/v). The sample was pre-treated at 85 °C for 30 min in order to destroy the tertiary structure of the protein (Huang et al., 2011). After cooling, SFPI was hydrolyzed at pH 8.0 and 55 °C, for 4 h with alcalase enzyme/substrate ratio (E/S) of 0.014 AU/g. After the first step hydrolysis, the sample was subjected to a second hydrolysis for 3 h with pHs of 6.5 and 50 °C with flavourzyme enzyme/substrate ratio (E/S) of 2.0 LAPU/g. The hydrolysate was incubated at 95 °C for 10 min to inactivate the enzymes and the precipitate was then removed by centrifugation (Hitachi, RX II series, Japan) at 10,000 rpm for 30 min and 4 °C. The final supernatant was collected and then freeze-dried to get sunflower protein hydrolysates (SFPHs).

### 2.3. Preparation of Maillard reaction products

The weighed 1.0854 g SFPH (equivalent to 1 g peptide), and appropriate amounts of D-xylose (0.5 g) and L-cysteine (0.375 g) were dissolved into the beaker with distilled water to a final concentration of 10% (w/v). The pH of the solution was adjusted to 7.4 with 2 mol/L NaOH or 2 mol/L HCl. The solution was placed in a Pyrex vial (50 mL), which was then sealed with a silicon/Teflon septum. The samples were heated at different temperatures of 80 °C, 100 °C, 120 °C and 140 °C and underwent a magnetic stirring for 120 min in an oil bath placed in a fume hood. The heated mixture was termed “Maillard reaction products” (PXC). MRPs were immediately cooled in ice-water after heating. Samples without added cysteine were prepared in similar conditions and were termed “control” peptide/xylose (PX). Samples were stored at –20 °C till further use.

### 2.4. Measurement of pH

A pH meter (model SP-71, Mettler Toledo, Inc., Shanghai, China) was used for the determination of pH values before and after the Maillard reaction.

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