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Short communication

In vitro stability of bioactive peptides derived from fermented soy milk against heat treatment, pH and gastrointestinal enzymes

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ABSTRACT

In this study, bioactive peptides (antioxidative and ACE-inhibitory), which had previously been identified in soy milk fermented by a *Lactobacillus plantarum* strain C2, were examined for stability during processing and after *in vitro* digestion. Results indicate that, antioxidative and ACE-inhibitory activity of peptides was either retained or enhanced after applying diverse heating conditions (from 25 to 121 °C). Conversely, the peptides retained almost the same ACE-inhibitory activity before and after treatment with pH from 2 to 7, but their ABTS radical scavenging activity was slightly decreased. Moreover, the significant increment (11–35%) in antioxidant activity was documented with different concentrations of trypsin, pepsin and pancretin, whereas ACE-inhibitory peptides showed some resistance to gastrointestinal digestion. This study indicates the high stability of ACE-inhibitory peptides against temperature of processing and gastrointestinal digestion. The ability of soy peptides to resist in various thermal and *in vitro* gastrointestinal conditions may be useful for their application as an ingredients for the development of functional foods.

1. Introduction

Several *in vitro* and *in vivo* studies have demonstrated that bioactive dietary peptides positively influence human health via a wide range of biological functions including antioxidative, antihypertensive, antimicrobial, antiobesity, hypocholesterolemic, anticancer, immunomodulatory *etc.* (Nongonierma & FitzGerald, 2017; Singh, Vij, & Hati, 2014). However, it is necessary that bioactive peptides remain intact during food processing and gastrointestinal (GI) digestion before reaching to their target sites to accomplish physiological effects. Therefore, delivery and bioavailability of peptides offers many challenges to the food technologist, pharmacist and clinicians, because a bioactive peptide does not always imply a physiological effect *in vivo* after oral administration (Vermeirssen, Van Camp, & Verstraete, 2004).

Biologically active peptides can be hydrolyzed during different stages of the GI digestion before being transferred and absorbed to the intestinal epithelium (Picariello et al., 2010). The GI tract is known to be one of the major barriers in the human body. The conditions in the GI tract, such as digestive enzymes and pH values in the stomach might influence the structures and functions of the peptides (Ao & Li, 2013).

Similarly, peptides can be hydrolyzed by high temperature treatment applied during food processing (Wang et al., 2017). Therefore, resistance of bioactive peptides to GI barriers and processing temperatures must be evaluated along with their health promoting properties. In this context, several studies have proved that *in vitro* digestion methods are useful tools to analyze structural changes, bioavailability and digestibility of bioactive compounds (Chen & Li, 2012; Hur, Lim, Decker, & McClements, 2011).

Previously, we have characterized 51 peptides from 10 kDa fraction of soy milk fermented by *Lactobacillus plantarum* strain C2. Among them, 17 peptides were showing both antioxidant and ACE-inhibitory activities (Singh & Vij, 2017). This study was designed to assess the effect of temperature and simulated GI conditions on the integrity of this 10 kDa bioactive peptides fraction. A two-stage *in vitro* GI environment (pH and digestive enzymes) was used to assess the potential changes in the biofunctional activities (antioxidative and ACE-inhibitory) of peptides.

2. Materials and methods

2.1. Preparation of peptide fraction

Lyophilized Peptides fraction (10 kDa), which had previously been prepared from the cell free supernatant of fermented soy milk using

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molecular weight cut-off (MWCO) membrane, was reconstituted in sterile double distilled water. The peptide content of the reconstituted peptide fraction was 0.628 ± 0.02 mg/ml, determined by OPA method and mentioned in our previous article (Singh & Vij, 2017).

2.2. Treatments of peptides

Thermal stability of peptide fraction was analyzed by treatment with different temperatures (25, 37, 55, 75, 100, 121 °C) using water bath for 2 h. pH stability was analyzed at pH 2, 3, 5, 7 and 10. Trypsin, pepsin and pancreatin (Sigma-Aldrich Corporation, St. Louis, MO, USA) were used for the digestive stability of peptides at different concentration (0.25, 0.50, 1.0 mg/ml). pH and enzyme treatments were given to separate peptide samples for 3 h.

2.3. Bioactivities analysis

The antioxidant activity of treated peptide samples was analyzed by two separate methods ABTS and DPPH radical scavenging assay (Singh & Vij, 2017). For ABTS method, an aliquot of 10 µl of sample was added to 990 µl ABTS solution and decrease in absorbance was recorded over the period of 10 min at 734 nm (Re et al., 1999). DPPH radical scavenging activity was analyzed by adding 250 µl peptide sample to equal amount of DPPH solution, the absorbance of the solution was measured (at 515 nm) against blank followed by 1 h incubation at 37 °C (Brand-Williams, Cuvelier, & Berset, 1995). ACE inhibitory activity was evaluated by mixing 50 µl peptide with 50 µl ACE and 150 µl of HHL solution before incubation at 37 °C for 30 min. Then the hippuric acid liberated by the ACE was measured at 228 nm against blank (Cushman & Cheung, 1971; Singh & Vij, 2017).

2.4. Statistical analysis

Data was analyzed by GraphPad Prism (La Jolla, CA, USA) (version 5.01). One way ANOVA was used for statistical significance between the means. Experiments were performed in triplicate, and values presented are means ± SEM. The level of significance was preset $P < 0.05$.

3. Results and discussion

3.1. Thermal stability

Thermal stability of bioactive peptides are important because food products underwent several heat treatments before reach to the market. Usually, thermal treatment can cause protein denaturation, association, and aggregation. The significant improvement ($P < 0.01$) was observed in antioxidant activity (ABTS) at 25 °C (12.67%), 75 °C (13.21%) and 100 °C (12.58%) (Fig. 1A). Similarly, the DPPH radical scavenging activity was also increased ($P < 0.01$) at 75 °C (17.09%) and 100 °C (17.52%) (Fig. 1B). These two methods are based on transfer of electron to free radicals and neutralize them (Gallego, Mora, Reig & Toldrá, 2017), therefore the enhancement in activity in both the assay may be due to further hydrolysis of peptides, which are able to stabilize more free radicals. Reports also suggest that temperatures more than 60 °C could affect the secondary structure of the peptides (Wang et al., 2017). Similar to our results, Gallego et al. (2017) also reported heat stability of a SNAAC peptide after exposure to 50, 72, and 90 °C temperatures. On the other hand, significant ($P < 0.05$) increment was observed in ACE-inhibitory activity at 37 °C (12.73%) and 75 °C (12.55%) (Fig. 1C). Results reflects that soy bioactive peptides can tolerate temperature upto 121 °C and become active, which is interesting in preparing foods with these functional ingredients. Our results in accordance with Wu and Ding (2002), who also studied stability of soy protein derived peptides at different temperature such as 20, 40, 60, 80, and 100 °C, they observed that these peptides retained ACE inhibitory activity

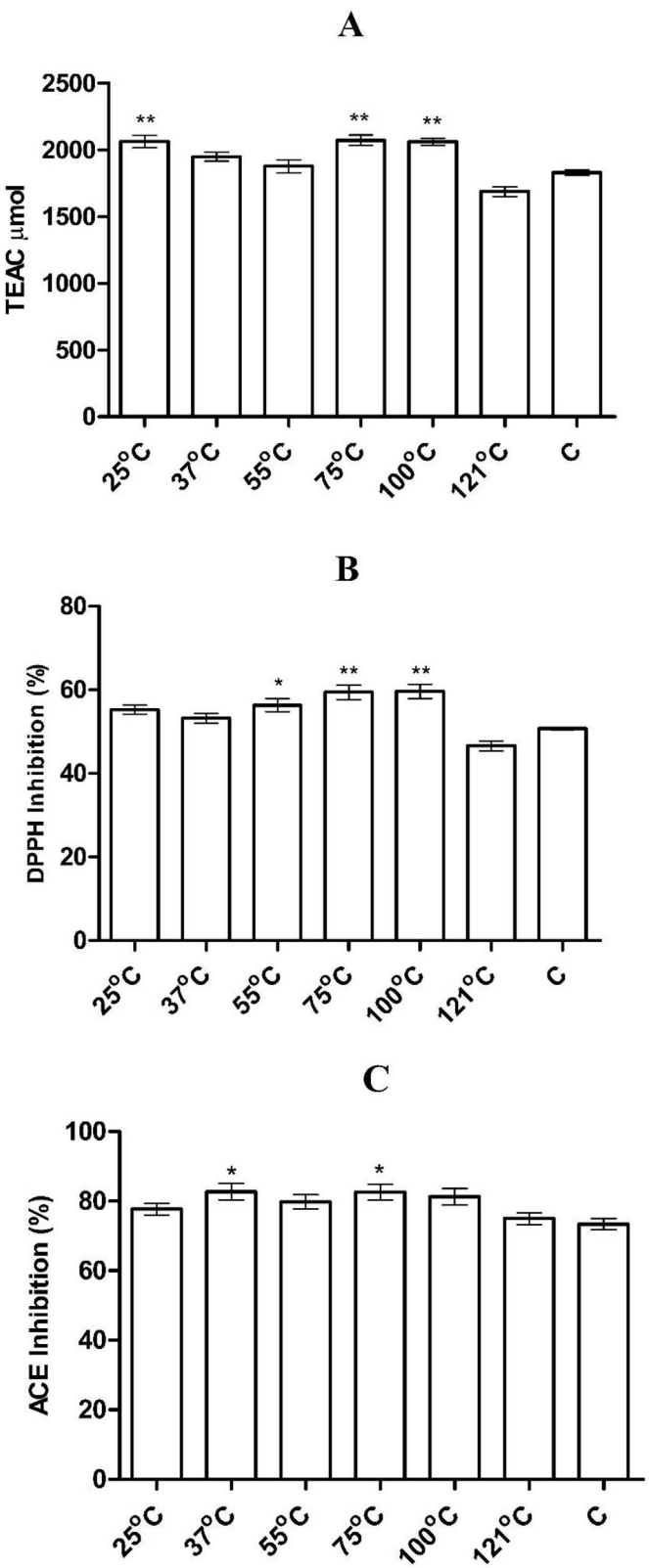

Fig. 1. Stability of soy bioactive peptides against thermal treatments. Panel "A" ABTS radical scavenging activity; panel "B" DPPH radical scavenging activity "C" ACE-inhibitory activity. Data was analyzed by one way ANOVA using Dunnett's Multiple Comparison Test. Graphs represents the mean ± SEM of each experiment performed in triplicate. Columns bearing with '**' and '*' represent $P < 0.01$ and $P < 0.05$ statistically significant difference, respectively from control (C).