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Peptide profiling and the bioactivity character of yogurt in the simulated gastrointestinal digestion

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ABSTRACT

This study investigated the relationship between peptide profiles and the bioactivity character of yogurt in simulated gastrointestinal trials. A total of 250, 434 and 466 peptides were identified by LC-MS/MS analyses of yogurt, gastric digest and pancreatic digest. Forty peptides of yogurt survived in gastrointestinal digestion. κ-CN and β-CN contributed the diversity of peptides during the fermentation process and gastrointestinal digestion, respectively. The favorite of κ-CN by lactic acid bacteria complemented gut digestion by hydrolyzing κ-CN, the low abundance milk proteins. The potential bioactivities were evaluated by *in vitro* ACE and DPP-IV inhibition assays. The ACE inhibition rate of the pancreatic digests was ~4 - and ~2 - fold greater than that of yogurt and the gastric digests. The ACE inhibitory peptides generated during gastrointestinal digestion improved the ACE inhibitory activity of the gastric and pancreatic digests. The DPP-IV inhibition rate of the pancreatic digest was ~6 - and ~3 - fold greater than that of yogurt and the gastric digest. The numbers of potential DPP-IV inhibitory peptides were positively correlated to the DPP-IV inhibitory activity of the gastric and pancreatic digests.

Biological significance: The present study describes the characters and bioactivities of peptides from yogurt in a simulated gastrointestinal digestion. The number of peptides identified from yogurt and gastrointestinal digests by LC-MS/MS increased in the simulated gastrointestinal trials. The *in vitro* ACE and DPP-IV inhibition bioactivities revealed that the bioactivity of yogurt was enhanced during gastrointestinal digestion. The correlation between peptides and bioactivity *in vitro* indicated that not only the peptides amount but also the proportion of peptides with high bioactivities contributed to increased bioactivity during gastrointestinal digestion. The study of peptides identified from yogurt and digests revealed that the number of released peptides was not determined by the abundance of the parent proteins but by whether the enzymes favored the protein.

In summary, peptide profiling and bioactivities of yogurt in simulated gastrointestinal digestion helped to elucidate the health benefits of yogurt peptides. The results further revealed that pre-digestion of milk by lactic acid bacteria are complementary to generate bioactive peptides and to provide particular yogurt functions.

1. Introduction

Milk is considered an excellent source of high quality protein in the human diet [1]. Apart from its high nutritional value, milk proteins release peptides with multiple health-promoting and disease-preventing activities during food processing and gastrointestinal digestion. These peptides provide health benefits for the cardiovascular system, intestinal health, body defenses and nervous system [2,3]. Bioactive peptides are encrypted within the amino acid sequences of milk proteins and are released and activated by enzymatic hydrolysis, microbial hydrolysis and gut digestion. Fermentation is an attractive approach to generate bioactive peptides from milk proteins using microbes. Yogurt is a fermented dairy product that releases bioactive peptides from milk proteins by lactic acid bacteria during fermentation [4]. In addition to the effects of probiotic strains, peptides in yogurt exert powerful effects on weight control [3,5] and other actions compared with other dairy products. Angiotensin-converting enzyme (ACE) inhibitory, antioxidant, and immunomodulatory peptides were identified from yogurt [2,4,6–12]. A recent meta-analysis of cohort studies showed that a high intake of yogurt and other dairy foods is associated with a decreased risk of type 2 diabetes (T2D) [13]. Clinical studies have revealed positive effects of probiotic strains, vitamin D and calcium from yogurt on adults with T2D, but that the role of proteins is not clear [14].

Pre-released bioactive peptides and inactive fragments in yogurt were digested by enzymes in gastrointestinal trials. The outcomes and bioactivities of bioactive peptides in gastrointestinal trials are two important factors that need to be considered. With the development of "omic" science, proteomics and peptidomics have been used to monitor the outcomes of dietary proteins in gastrointestinal digestion [15,16]. These "omic" techniques have been applied to study the survival of

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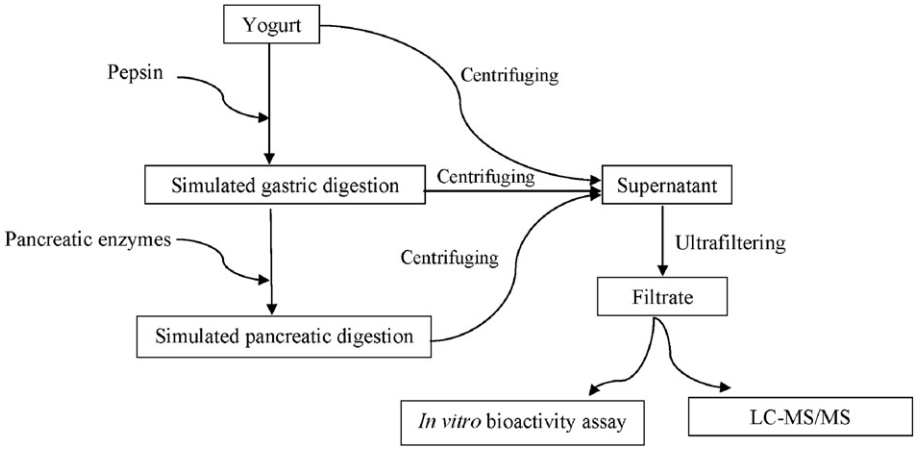

Fig. 1. Schematic overview of the analytical strategy to identify peptides from yogurt in the simulated gastrointestinal digestion.

peptides from milk proteins in a gastrointestinal trial [17] and peptidome changes of cheese in simulated gastrointestinal digestion [18]. The bioactivities and stabilities of known bioactive peptides derived from diary proteins have been investigated in stimulated gut digestion [19], in animals [20,21] and in humans [22,23]. Whether in simulated gastrointestinal digestion or *in vivo* digestion, known bioactive peptides have been targets to evaluate the bioactivity of peptides in gut digestion [21–23]. However, a few known bioactive peptides cannot reflect the bioactivity profiles of the amount of peptides released during digestion. No studies and methods have determined the bioactivities characters of peptides identified by LC-MS/MS from dietary proteins that were digested in gastrointestinal trials. The outcomes and bioactivity profiles of peptides from yogurt proteins in gastrointestinal trials are still not clear.

Yogurt is a traditional health food. The special function of yogurt [13] and bioactive peptides pre-released in yogurt processes [2] cause yogurt to be a good model to study peptide profiling and character in gastrointestinal trials. The aim of the present work was to investigate the relationship between yogurt peptide profiles and their bioactive characters in simulated gastrointestinal trials. The peptidome of yogurt in stimulated gastrointestinal trials was analyzed by LC-MS/MS. The potential bioactivity of the yogurt peptides were tested by *in vitro* assays. The correlation between yogurt peptide profiles and bioactive characters in simulated gastrointestinal digestion was also investigated.

2. Materials and methods

2.1. Materials and reagents

Yogurt was purchased from Liaoning Huishan Dairy Group Company (Liaoning, China). The product name is Feng Wei Suan Ru and the strains used are *Streptococcus thermophellolus* and *Lactobacilus bulgaricus* according to the product information. Pepsin (from porcine gastric mucosa, ≥0.6 U/mg) , trypsin (from bovine pancreas, ≥10,000 U/mg), α-chymotrypsin (from bovine pancreas, ≥40 U/mg), elastase (from porcine pancreas, ≥4 U/mg), carboxypeptidase A (from bovine pancreas, ≥50 U/mg), bovine casein and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Acetonitrile (ACN, HPLC grade) was from Merck (Darmstadt, Germany). All of the water used in the experiments was purified using a Milli-Q system from Millipore Company (Bedford, MA, USA). Protein marker, Bis-tris polyacrylamide gels, and lane marker reducing sample

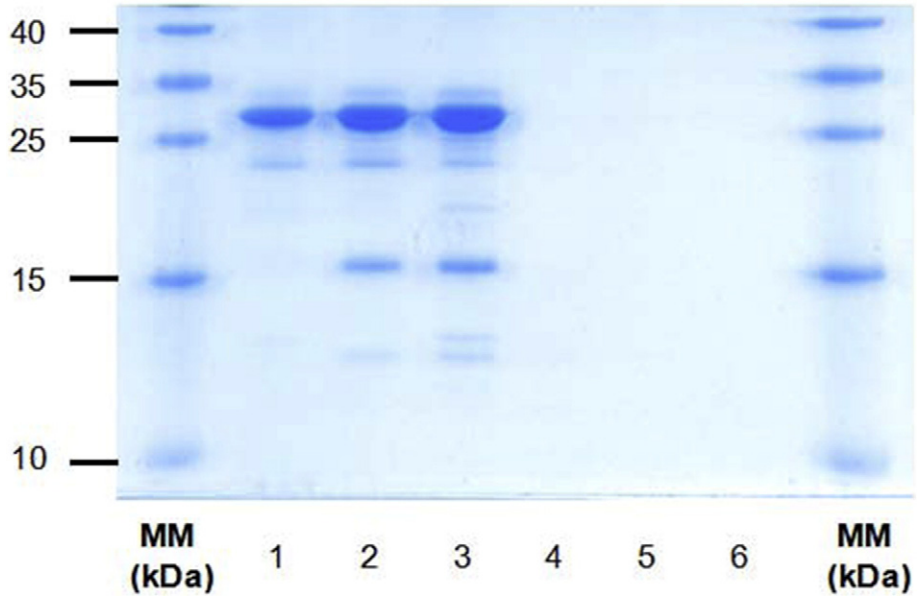

Fig. 2. SDS-PAGE of commercial casein (lane 1), yogurt (lane 2), gastric digest (lane 3), pancreatic digest (lane 4), pepsin solution (lane 5), pancreatic enzymes solution (lane 6), MM = molecular marker.