



Exploration of potential angiotensin converting enzyme inhibitory peptides generated from enzymatic hydrolysis of goat milk proteins



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ABSTRACT

Goat milk is seemed to be a perfect food as similar to the human's milk in nature. Goat milk proteins are potential sources for the production of bioactive peptides with various biological activities. Bioactive peptides can be used as functional ingredients in the formulation of health-enhancing nutraceuticals, and as potent drugs with positive impact on body functions. The objective of this study was to explore the potential of goat milk derived proteins as a precursor of bioactive peptides with especially reference to angiotensin converting enzyme (ACE) inhibitory peptides. The various computational approaches (BLAST, BIOPEP, and PeptideRanker) were used to predict the potential of goat milk derived proteins for releasing bioactive ACE inhibitory peptides. Pepsin and chymotrypsin A proteases were used for *in silico* proteolysis and pepsin majorly released the higher number of ACE inhibitory peptides as compared with chymotrypsin A. PeptideRanker tool was used to screen the most potent theoretically release bioactive ACE inhibitory peptides. Furthermore, the sensory characteristics and toxicity profile of selected most potential ACE inhibitory peptides were also studied using BIOPEP and ToxinPred tools, respectively. Bioactive peptides or hydrolysates released from the goat milk proteins can be considered for the development of nutraceuticals and pharmaceuticals products. The result of this study also provides a theoretical basis for the development of goat milk derived proteins as a suitable source for the generation of bio-functional ingredients for prevention and treatment of hypertension. This study also demonstrates the usefulness of computational approaches.

1. Introduction

Milk is a fluid that secreted from the mammary gland of female mammalian species, and its primary function is to meet the complete nutritional requirements of neonates. Milk protein is consisting of whey proteins (20%) and caseins (80%). It is an excellent wholesome source of nourishment and also exhibits a range of biological activities that influence digestion, resistance to diseases, metabolic responses to absorb nutrients, growth and specific organs development. Milk and milk derived proteins are considerably recognized as a major source for peptides with a wide range of physiological functionalities include immunoglobulins, enzymes and enzyme inhibitors, growth stimulating, binding or carrier proteins, antihypertensive, antithrombotic, antioxidative, and antibacterial activities (El-Salam and El-Shibiny, 2013).

Worldwide the production rate (15% of global milk production) for non-bovine animal milk increases simultaneously its cultural, economic, and ecological interest has also been growing (El-Salam and El-Shibiny, 2013). Most of the non-bovine milk and milk products have

been claimed to have special nutritional and therapeutic characteristics among such, goat milk proteins and peptides found to be antibacterial, antioxidant, angiotensin converting enzyme (ACE) inhibitory properties (De Gobba et al., 2014). Goat milk seems to be a perfect food as it contains all the essential amino acids without high amount of fat and mucus producing materials (Atanasova and Ivanova, 2010). The importance of goat milk is intensifying consistently because utilization of bovine milk had become a common cause of food allergy in infants. However, goat milk proteins are more digestible and being recommended for babies when human milk is lacking (Ibrahim et al., 2017).

Bioactive peptides can be released upon enzymatic hydrolysis, food processing, during microbial fermentation or gastrointestinal digestion (Pal and Suresh, 2016). The bioactive peptides derived from milk proteins found to provide a health-promoting effect in humans when fortified into food products. From the last decades, a voluminous research work has been focussed on the exploration of bovine's milk protein derived bioactive peptides. However, in most dairy species

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(other than bovine) the repertoire of potential milk bioactive proteins or their derived peptides remains to be unravelled. It is a great challenge for milk industries and researchers to provide the basis for health-promoting properties of milk proteins and peptides of dairy species other than bovine. The angiotensin converting enzyme (ACE) inhibitory peptides are of particularly interesting because they can potentially contribute to protecting against oxidative stress and the associated diseases such as cardiovascular disorders (Pal and Suresh, 2016). The major risk factor for cardiovascular disease is hypertension. In this regard, the angiotensin-I-converting enzyme play the major role and its inhibitors are present naturally in milk (Kamau et al., 2010). Angiotensin-I-converting enzyme is a key enzyme in regulation of blood pressure. Many synthetic ACE inhibitors (captopril, enalapril, fosinopril, lisinopril, and ramipril) were identified and used for the hypertension treatment. However, these synthetic inhibitors have side effects (Kamau et al., 2010). Hence, milk derived bioactive peptides can be served as alternative for synthetic ACE inhibitors.

Therefore, the objectives of this investigation was to explore the potential ACE inhibitory activities of hydrolysates and peptides from goat milk proteins liberated upon cleavage with commercially available enzymes using various computational approaches. Moreover, the sensory and toxicity characteristics potential of selected most potent bioactive ACE inhibitory peptides were also evaluated.

2. Materials and methods

2.1. Protein sequences of goat milk proteins

On the basis of the literature, the primary protein sequences of three representative goat milk protein were used in present study. The major contributors of goat milk protein sequences are alpha S1 casein (Accession number # CAD45345.1), alpha-S2-casein (Accession number # CAA46278.1), and beta-casein (Accession number # CAB39200.1), obtained from national centre for biotechnology information (NCBI) database (<https://www.ncbi.nlm.nih.gov>).

2.2. BLAST analysis of goat milk protein sequences

The selected goat milk protein sequences were used for the homology analysis, and their protein sequences were also aligned with each other using BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). BLAST analysis was used to calculate the significant areas of commonly present amino acid sequences. The representative protein sequences were accessed from NCBI database in the FASTA format. A pair wise sequence alignment will usually be displayed while a series of informative data can be generated such as scores, identities, positives, and gaps in percentage (Altschul et al., 2005, 1997). The protein sequences alpha S1 casein, alpha-S2-casein, and beta-casein were aligned against each other using BLAST analysis tool.

2.3. Amino acid compositions

The amino acid compositions of the selected goat milk protein sequences were determined using ProtParam tool (<http://web.expasy.org/protparam/>). ProtParam is an *in silico* analysis program that computes the physico-chemical properties of a protein or peptide from its amino acid sequences (Gasteiger et al., 2005). The total number of the amino acids, molecular weight, and theoretical pI of the selected goat milk proteins were also evaluated.

2.4. BIOPEP analysis

2.4.1. Profile of potential biological activity and their occurrence frequency

The protein sequences of alpha S1 casein, alpha-S2-casein, and beta-casein were obtained from NCBI database and analyzed for the profiles of potential biological activity using BIOPEP tool (<http://www.uwm.edu.pl/biochemia/index.php/en/biopep>) (Minkiewicz et al., 2008).

The potential goat milk protein derived ACE inhibitory peptide sequences were screened and their occurrence frequencies in the selected goat milk protein sequences were also calculated. The occurrence frequency of ACE inhibitory peptides derived from the selected goat milk protein has been computed using the following equation

$$A = a/N \quad (\text{A.1})$$

Where, A = occurrence frequency of the bioactive peptides, a = number of bioactive peptides, and N = total number of amino acid residues in selected goat milk protein sequences.

2.4.2. *In silico* proteolysis

The protein sequences of goat milk protein were subjected for *in silico* proteolysis for the prediction of theoretically released peptide sequences using enzymatic action program available in the BIOPEP tool (Minkiewicz et al., 2008). The pepsin pH 2.0, and chymotrypsin A were selected for the *in silico* proteolysis. Furthermore, theoretically released goat milk protein derived peptides obtained using various proteases were submitted to search for active fragments option of the BIOPEP tool. The ACE inhibitory peptides were screened from the list of potential bioactive peptides released from selected goat milk protein sequences. The goat milk protein derived peptides containing potential ACE inhibitory activity were selected for further analysis. The possibilities of the release of ACE inhibitory peptides by proteases (pepsin and chymotrypsin A) were also evaluated using BIOPEP tool. The following equations were used to calculate the release frequency of fragments with ACE inhibitory activity by selected protease (A_E) and the relative frequency of the release of fragments with ACE inhibitory activity by selected enzymes (W) (Minkiewicz et al., 2011).

$$A_E = d/N \quad (\text{B.1})$$

Where, d = the number of fragments released by enzymes with ACE inhibitory activity in the protein sequence, N = number of amino acid residues in protein chain.

$$W = A_E/A \quad (\text{B.2})$$

The values of these parameters were calculated based on Eqs. (A.1) and (B.1), respectively (Minkiewicz et al., 2008).

2.4.3. Sensory characteristics

Peptides and amino acids have the capacity to alter the taste of food commodities and products (Iwaniak et al., 2016a). Traditionally, the sensory evaluation of peptides and peptides containing foods and beverages production has been conducted by a trained panel. The most of the traditional sensory panel has the several drawbacks such as it is a time consuming and expensive process as well as panellists need to be compensated for their time and effort. Hence, applications of alternatives methodology for sensory assessment have been increased (Newman et al., 2014). Therefore, sensory characteristics of the selected ACE inhibitory peptides were predicted using BIOPEP tool (Iwaniak et al., 2016b, 2016a).

2.5. Peptide ranking

The potential of the goat milk proteins derived ACE inhibitory peptides were predicted using PeptideRanker (<http://bioware.ucd.ie/compass/biowareweb/>) tool (Mooney et al., 2012). It is a web-based server to predict the probability of biological activity of peptide sequences. PeptideRanker tool gives the peptide score in the range of 0–1. The maximum score represents the most potentially active peptides, and least score denotes the least active peptides. The selected most potent ACE inhibitory peptides derived from goat milk protein sequences were subjected to further analysis.

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