# TITLE PAGE

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| **Article Type** | Research article |
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| **Running Title (within 10 words)** | ACEI Peptides in Goat Milk Fermented by Lactic Acid Bacteria |
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**Abstract** In this study, the inhibitory activity of Angiotensin-I-Converting Enzyme (ACEI) was evaluated in fermented goat milk fermented by lactic acid bacteria (LAB) from fermented foods and breast milk. Furthermore, the potential for ACEI peptides was identified of fermented goat milk with the highest ACEI activity. The proteolytic specificity of LAB was also evaluated. The 2% isolate was inoculated into reconstituted goat milk (11% w/v), then incubated at 37 ℃ until pH 4.6 was reached. The supernatant produced by centrifugation was analyzed for ACEI activity and total peptide. Viable counts of LAB and titratable acidity were also evaluated after fermentation. Peptide identification was carried out using Nano LC/MS/MS, and potential as an ACEI peptide was carried out based on a literature review. The result revealed that ACEI activity was produced in all samples (20.44-60.33%). Fermented goat milk by *Lc. lactis* ssp *lactis* BD17 produced the highest ACEI activity (60.33%; $IC_{50}$ 0.297 ± 0.10 mg/mL) after 48 h incubation, viable counts >8 Log CFU/mL, and peptide content 4.037 ± 0.27/mL. A total of 261 peptides were released, predominantly casein (93%). The proteolytic specificity of *Lc. lactis* ssp *lactis* BD17 through cleavage on the amino acid tyrosine, leucine, glutamic acid, and proline. A total of 21 peptides were identified as ACEI peptides. This study showed that one of the isolates from fermented food, namely *Lc. lactis* ssp *lactis* BD17, has the potential as a starter culture for the production of fermented goat milk which has functional properties as a source of antihypertensive peptides.

**Keywords** ACE inhibitory activity, antihypertensive peptides, goat milk fermented, proteolytic specificity, *Lc. lactis* ssp *lactis* BD17

## Introduction

Hypertension is a primary risk factor for cardiovascular diseases (CVDs), including stroke, heart attack, heart failure, and other complications related to structural damage to the cardiovascular system. In 2021, the World Health Organization (WHO) reported that CVDs are the leading cause of death globally. People who died from CVDs in 2019 were estimated at 17.9 million, representing 31% of all deaths worldwide. Heart attacks and strokes are the main causes of these deaths (85%) (WHO, 2021) . Human blood pressure is regulated by a system called the "Renin Angiotensin Aldosterone system", in which Angiotensin-I-Converting Enzyme inhibitory (ACE) plays an important role. ACE could catalyze the conversion of the decapeptide angiotensin I to the potent vasoconstrictor angiotensin II. Furthermore, this enzyme hydrolyzes bradykinin and stimulates the release of aldosterone which causes vasoconstriction and fluid retention which increases blood pressure (Rai et al., 2017). Therefore, the treatment of clinical hypertension could be done by controlling ACE activity. ACE inhibitors such as captopril, enalapril, alacepril, lisinopril and ramipril are widely used in the clinical treatment of hypertension. However, the use of these synthetic drugs in some cases causes side effects such as coughing, increased blood calcium levels, decreased kidney function, angioedema, and skin rashes (Zeng et al., 2013). Several researchers through in vivo studies on rats with spontaneous hypertension (SHR) and humans with hypertension showed that ACE inhibitors without side effects could be obtained from food protein (Bravo et al., 2019; Chen et al., 2014; Seppo et al., 2003)

Food protein from milk and dairy products such as fermented milk is a source of ACE inhibitory (ACEI) peptides (Begunova et al., 2021;Wu et al., 2019). Among them have been reported from fermented goat milk. The presence of ACE inhibitors in fermented milk is associated with the

presence of lactic acid bacteria (LAB). Lactic acid bacteria are the dominant group of bacteria involved in fermenting milk such as yogurt and kefir. Kefir is a fermented goat milk that has been consumed for hundreds of years and is believed not only as a source of antihypertensive peptides but also as a source of antioxidants and immunological agents (Ibrahim et al., 2017; Parmar et al., 2020)

During fermentation, milk protein could be hydrolyzed by LAB into peptides and amino acids. The abundance and characteristics of peptides released from milk proteins by LAB are strain-dependent (Wang et al., 2015). Among these peptides, the presence of bioactive peptides could be identified (Li et al., 2017). Bioactive peptides differ in size and sequence. Bioactive peptides that have functional properties as ACEI peptides have the characteristics that their molecular weight is generally >3 kDa and the presence of the amino acids proline and phenylalanine in the sequence (Gonzalez-Gonzalez et al., 2013; Wu et al., 2006)

The ability of LAB to release bioactive peptides in fermented milk (Ayyash et al., 2020; Kim et al., 2017), and the status of LAB as "generally recognized as safe" (GRAS) for application in food, have increased the utilization of certain strains of LAB for production of fermented milk with certain functional properties. The purpose of this study was to investigate ACEI activity in goat milk fermented using LAB from fermented foods and breast milk. The potential of ACEI peptides was identified in the <3 kDa fraction of fermented goat milk with the highest ACEI activity. The proteolytic specificity of the LAB used was also evaluated. The ten strains used were selected because they effectively released ACEI peptides in fermented cow milk in our previous study (Rubak et al., 2020).

## Materials and methods

### Lactic acid bacteria

The ten LAB isolates from fermented foods and breast milk used in this study were culture collections from the Laboratory of Food Microbiology, Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, IPB University (Bogor Agricultural University). The isolates were refreshed in de Man Rogosa and Sharpe broth and incubated at 37 ℃ for 24 h, then adapted in fresh skimmed milk for 2 rounds (24 h, 37 ℃) before being used as a starter culture in the experiment.

### Fermentation of goat milk

Goat skimmed milk 11% (w/v) was pasteurized at 95 ℃ for 10 min. After cooling (45 ℃), LAB starter culture (2%) was inoculated followed by incubation at 37 ℃ until pH 4.6 (700 Eutech) was reached. The fermentation process was stopped by heating (75 ℃ for 1 min) followed by centrifugation (Hettich, Zentrifugen, Mikro 22R) at 6000 x g for 10 min, 4℃. The supernatant was collected for analysis of peptide content and ACE inhibitory activity (Chusman, 1971). Viable counts of LAB and titratable acidity were also analyzed from unheated samples.

### Determination of ACE Inhibitory Activity

Hippuryl-L-Histidyl-L-Leucine (HHL, Sigma, USA) was used as an enzyme-substrate. A total of 50 μL of the substrate (50 mM HHL in 0.1 M sodium borate buffer containing 0.3 M NaCl at pH 8.3) was added into a 50 μL sample and incubated at 37 °C for 5 min. To initiate the reaction, 50 μL of 0.1 U/mL ACE (Rabbit lung, Sigma, USA) solution was added, and the mixture was incubated at 37 °C for 5 min. The reaction was stopped by adding 250 μL 1 M HCl. The resulted hippuric acid (HA) was extracted with 1.5 mL ethyl acetate and centrifuged at 2000 x g for 5 min. An aliquot (0.8 mL) of the ethyl acetate layer was transferred to a clean tube and evaporated at 85 ℃ for 60 min. Distilled water (4 mL) was then added to dissolve the HA in the tube, and the amount of HA formed was measured by measuring the optical density at 228 nm (UV-2800, Hitachi, JPN). The extent of inhibition was calculated as 100% [(B-A)/B] where A is the optical density in the presence of ACE and ACEI components, and B is the optical density without the ACEI component.

**$IC_{50}$ and inhibitory efficiency ratio (IER) value**

The $IC_{50}$ of the sample having the highest ACEI activity was calculated from the linear regression equation by plotting the ACE inhibition (%) versus the inhibitory concentration for each sample dilution. The percentage of ACEI activity was divided by the peptide concentration to obtain the IER value.

**Ultrafiltration**

The supernatan of fermented goat milk (4 mL) was pipetted into ultrafiltration centrifuge tubes [molecular weight (MW) cut-off of 3 kDa; Merck, 4 mL, IRL], then centrifuged at 4000 x g for 30 min, 4 ℃. The fractions (<3 kDa and >3 kDa) were collected and the volume was adjusted to 4 mL by addition of water. Fractions were analyzed for ACEI activity.

**Identification of peptides by mass spectrometry**

Peptides in <3 kDa fraction were analyzed by using LC Ultimate 3000 series system Tandem Q Exactive Plus Orbitrap HRMS (Thermo scientific, GER). The samples (5 µL) were injected into the LC Nano MS/MS system. The samples were trapped on a trap column (164649, 30 µm x 5 mm; Thermo scientific) and washed for 6 min with a gradient of 98% solvent A [water/acetonitrile (98:2, v/v), 0.1% formic acid] and 2% solvent B [Water/acetonitrile (2:98, v/v), 0.1% formic acid] at a flow rate of 5 µL/min. The eluted peptides were loaded and separated on a capillary column (PepMap RSLC-C18, 75-µm ×150 mm, 3.5 µm particle size, 100 pore size, Thermo Scientific ES800) at a flow rate of 300 nL/min with a gradient at 2% to 35% solvent B over 30 min, then from 35% to 90% over ten min, followed by 90% solvent B for 5 min, and finally 5% solvent B for 15 min. Electrospray was performed at an ion spray voltage of 3500 eV. Automatically, the peptides were analyzed using Proteomic Discoverer 2.2 software. The range of m/z values was 200–2000.

Identification of ACEI peptides was carried out through a literature search. The investigated is a peptide that provides 100% similarity to the ACEI peptide that has been previously reported by the researchers.

## Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA) performed using SPSS version 22. $P \leq 0.05$ was considered significant. Each experiments were repeated three times, and the data were presented as mean ± SD.

## Result and Discussion

### Characteristics of goat milk fermented by LAB from fermented food and breast milk

Milk has been known as a suitable growth medium for LAB. The population of the ten LAB in fermented goat milk reached 9 log CFU/mL. Viable counts of the LAB ranged from 9.18 ± 0.46 to 9.79 ± 0.39 log CFU/mL. When the pH reached 4.6, there was no difference in population (Table 1), which is in accordance with previous results obtained by Elkhtab et al. (2017) and from fermented cow milk using similar cultures (Rubak et al., 2020). However, the fermentation time to reach pH 4.6 was different between isolates that ranged from 18 to 48 h with titratable acidity ranging from 0.77 ± 0.06 to 0.94 ± 0.04%. A short fermentation time (18 h) was observed in the fermentation by *Lb. rhamnosus* R2, while the longest fermentation time (48 h) occurred in the fermentation by *Lb. fermentum* S206, *Lb. delbrueckii* BD7, and *Lc. lactis* ssp *lactis* BD17. In our research the fermentation was ended at pH 4.6 to obtain high production of peptides. The release of a bioactive peptide from the protein matrix by culture could decrease when the pH value falls below 4.5 (Gonzalez-Gonzalez et al., 2013). Increase in coagulation could inhibit bacterial cell

diffusion to protein tissue, thus inhibiting access of CEP to milk protein for hydrolysis. Further acidification could be avoided by stopping fermentation when it reaches pH 4.6, or the pH must be controlled by adding alkaline solutions such as sodium hydroxide (Chen et al., 2015).

Insert Table 1

**ACE inhibitory activity**

ACE inhibitory activity was detected in all supernatants of goat milk fermented in the range of 20.44 ± 2.33 to 60.79 ± 8.78% (Table 2). The highest percentage of ACEI activity (>50%) was obtained in goat milk fermented by *Lb. delbrueckii* BD7 and *Lc. lactis* ssp *lactis* BD17, but it was not significantly different (>0.05) from that of *Lb. kefiri* YK4 and *Lb. kefiri* JK17. It has been reported that goat milk could be used as a potential precursor for the production of ACE inhibitors through the fermentation process (Izquierdo-González et al., 2019). Starter cultures of LAB, growth conditions, and substrate are factors that influence ACEI production in fermented milk (Wang et al., 2015; Shu et al., 2015; Li et al., 2017). *Lactobacillus* species are known to produce high ACEI activity (>50%) in fermented milk (Hati et al., 2018; Wu et al., 2019). The variation of ACEI activity between LAB in milk fermented is related to its proteolytic activity. The proteolytic activity of LAB is determined by the specificity of its proteolytic components (Chen et al., 2015).

The proteolytic activity of LAB in goat milk was measured by the OPA method, with results ranging from 3.55 ± 0.26 to 5.69 ± 0.21 mg/mL (Table 2). Goat milk fermented by *P. pentasaceus* 1 W2SR04 and *Lb. kefiri* YK4 (>5 mg/mL) showed high peptide content. In a study by Toe et al. (2019), *P. pentasaceus* species also showed high proteolytic activity. However, high peptide

content was not always associated with high ACEI activity in samples. This is also seen in the results of our study. ACE inhibitory activity is more related to the abundance of ACEI peptides that could be released during fermentation.

The IER values evaluated in ten samples showed that the highest IER values were obtained in fermented goat milk of *Lc. lactis* ssp *lactis* BD17. The $IC_{50}$ value was also determined in this sample and the result was 0.297 ± 0.10 mg/mL. The $IC_{50}$ value reflects the peptide concentration required to inhibit 50% ACE. The $IC_{50}$ value in fermented milk <1 mg/mL (Gútiez et al., 2013). Our results show that the obtained $IC_{50}$ value is lower than that of fermented milk of other *Lactobacillus* species, as reported by Qian et al. (2011) in fermented milk by *Lb. delbrueckii* ($IC_{50}$ 67.71 ± 7.62 mg/mL), by Moslehishad et al. (2013) in fermented milk by *Lb. rhamnosus* ($IC_{50}$: 3.947 ± 0.029 mg/mL) and by Chen et al. (2007) in fermented milk using several isolates ($IC_{50}$ : 0.65 mg/mL) and in koumiss (52.47 ± 2.87 mg/mL) (Chen et al., 2010). Barla et al. (2016) have also reported from fermented milk by *Lb. brevis, Lb. buchnery* and *W. hellenica* ($IC_{50}$: 0.28-0.83 mg/mL).

Insert Table 2

**ACE inhibitory activity of > 3 kDa and <3 kDa fraction**

Filtration using a 3 kDa MW cut-off showed that the ACEI activity was concentrated in the MW fraction <3 kDa (Table 3). There was no significant difference (>0.05) in the ACEI activity of the <3 kDa fraction compared to the supernatant (without filtration). Bioactive peptides with

ACEI activity have been reported as peptides with MW of < 3 kDa (Gonzalez-Gonzalez et al., 2013).

Insert Table 3

**Characteristics of peptides in the <3 kDa fraction of fermented goat milk of *Lc. lactis* ssp *lactis* BD17**

A total of 261 peptides were released in fermented goat milk by *Lc. lactis* ssp *lactis* BD17 (Table 4 and Supplementary Data). Most of the peptides were hydrolyzed from casein (97%) and whey (3%). The main fraction of goat milk protein is casein, which is 80% of the total milk protein (Jandal, 1996). This explains the abundance of peptide hydrolyzed from casein in our results. Another thing that casein has a very flexible and open structure so it is very sensitive to proteolysis. While whey protein is more resistant which is explained by the presence of a globular structure (Swaisgood, 1993). According to the results, β-casein (54.02%) was the most accessible to the proteolytic system of *Lc. lactis* ssp *lactis* BD17 to release a number of peptides. The cleavage site's dominance on the β-casein was also shown by *Lb. rhamnosus* CGMCC11055 (Guo et al., 2016) and *L. delbrueckii* subsp. *lactis* ACA-DC 178 (Hebert et al., 2008).

Insert Table 4

The spectrum of MS analysis revealed a large number of peaks with retention times of 8 to 65 min (Figure 1), representing an abundance of released peptides with peptide mass/molecular

charge (m/z) ranging from 310.1 to 1146.0. The diversity of detected ions indicates that the nine peaks with retention time (RT) of 11.29 to 30.97 corresponded to thirteen peptides identified in goat milk fermented by *Lc. lactis* ssp *lactis* BD17 (Table 5). ARHPHPHLSFM (κ-casein; RT 11.29,11.89; m/z 665.33, 673.33) was a peptide that exhibited a prominent peak according to its abundance in the sample. This peptide was also identified as being present in goat milk kefir (Izquierdo-Gonzales et al., 2019) and goat milk (Ibrahim et al., 2017). Another signal was associated with the abundance of peptides in goat milk fermented by *Lc. lactis* ssp *lactis* BD17 from the parent protein β-casein, namely MPFPKYPVEPF (RT 20.54; m/z 676.34), QEPVLGPVRGPFPI (RT 29.61; m/z 753.43) and RDMPIQAFLL (RT 23.59; m/z 602.33). This peptide has also been identified in goat milk kefir (Izquierdo-Gonzales et al., 2019) and bovine kefir (Ebner et al., 2015).

Insert Fig 1

Insert Table 5

The release of peptides from the protein matrix is initiated by *Cell Envelope Proteinase* (CEP), one of the essential enzymes in the LAB proteolytic system (Griffiths & Tellez, 2013) which cleaves the proteins resulting in peptides with 4 to 30 amino acid. The CEP of LAB is classified into three types based on the hydrolysis of casein (Kunji, 1996): (1) CEP type PI which specifically hydrolyzes β-casein, (2) CEP type PIII which hydrolyzes αS1-casein and κ-casein, (3) CEP intermediate type PI/PIII which hydrolyzes β-casein and αS1-casein. Based on this classification, our results indicate that the CEP of *Lc. lactis* ssp *lactis* BD17 represents CEP types I and III. These

types of CEP were also reported from *Lb. casei* PRA205 and *Lb. rhamnosus* PRA331(Solieri et al., 2018), and *Lb. paracasei* ssp. *Paracasei* (Nikolić et al., 2009).

Types and domains in the CEP region of each LAB provide variations in the specificity of the hydrolyzed substrate which have implications for the diversity of molecular weights and amino acid sequences of the released peptides (Raveschot et al., 2020). The molecular weight of the peptide released by *Lc. lactis* ssp *lactis* BD17 ranged from 659 to 2201.1 Dalton with amino acid residues ranging from 6 to 20. An investigation was carried out to determine the specificity of cleavage of CEP *Lc. lactis* ssp *lactis* BD17 in goat milk parent protein during fermentation to release a number of peptides. The results are presented in Figure 2. It appears that sites of *Lc. lactis* ssp *lactis* BD17 dispersed throughout the parent protein, although certain amino acids are favorite for cleavage by CEP of *Lc. lactis* ssp *lactis* BD17. In the αS1-casein region, the cleavage sites were frequently at serine, aspartate, and phenylalanine amino acid (f183-f184, f186-f187, and f184-f185), in αS2-casein region the sites were at amino acids tyrosine, leucine, and glutamine (f116-f117, f114-f115, f112-f113), and in the β-casein region the sites were at amino acids tyrosine, leucine, glutamic acid and proline (f208-f209, f207-f208, f123-f124, f125-f126). Moreover, in a κ-casein region the cleavage sites were at amino acids leucine, tyrosine, and alanine (f53-f54, f51-f52, f44-f45). These results indicate that the cleavage sites of casein by *Lc. lactis* ssp *lactis* BD17 occur mostly in hydrophobic and aromatic amino acids. It seems that hydrophobic and aromatic amino acids are more easily accessed and released from parent protein. Similar results have been reported by Lozo et al. (2011) on *Lb*. subsp. *paracasei* BGHN14 (prtp), *Lb. rhamnosus* BGT10 (prtR), and *Lb. helveticus* BGRA43 (prtP), and Hebert et al. (2008) reported on *Lb. delbrueckii* subsp. *lactis* CRL 581.

Insert Fig 2

**ACE inhibitory peptides**

Twenty-one of the 261 peptides released by *Lc. lactis* ssp *lactis* BD17 were identified as ACEI peptides (Table 5), most of which were released from β-casein. One of peptides namely ARHPHPHLSFM from parent protein κ-casein; 116-f117 was reported as an ACEI peptide (Ibrahim et al., 2017), and VLNENLR (αS1-casein; f39-f40) (Swaisgood, 1993). ACE inhibitory peptides could be identified based on their amino acid sequence (Lunow et al., 2015).

Insert Table 6

The three amino acids located at the C-terminus could determine whether a peptide could act as an ACEI peptide (Wu et al., 2006). Amino acids from aliphatic groups (proline, isoleucine, valine) and aromatic amino acids (phenylalanine) are the dominant amino acids found in the ACEI peptide. Our investigation of other peptide released by *Lc. lactis* ssp *lactis* BD17 which has not been identified as an ACEI peptide according to a literature search, demonstrated its potential as an ACEI peptide. A total of 36% of these peptides had a proline amino acid residue and 21% a phenylalanine amino acid residue at the C-terminus.

The characteristics of the ACEI peptide were not only observed in the presence of amino acids at the C-terminus. By other researchers, the presence of amino acids at the N-terminal was also evaluated. Aslam et al. (2018) showed that three identified ACEI peptides were released in goat milk fermented by *Lb. helveticus* cicc22171 has hydrophobic/aliphatic amino acids not only at the

C-terminus but also at the N-terminus (valine and proline). Daliri et al. (2018) also presented their research results that four peptides identified as ACEI peptides were associated with the presence of negative amino acids (glutamate) and uncharged amino acids (glutamine) at their N-terminus. The presence of this amino acid in the *Lc. lactis* ssp *lactis* BD17 peptide was also identified, namely in the peptide released from the parent protein β-casein and α-S1-casein (Table 4).

In addition to the presence of certain amino acids in the ACEI peptide sequence. Another characteristic of ACEI peptides is their molecular weight. ACE inhibitory peptides are generally short peptides with molecular weight < 3 kDa, may consist of 6 to 16 amino acids (Ibrahim et al., 2017). However, ACEI peptides with 20 amino acid residues have also been reported (Elkhtab et al., 2017). The ACEI peptide identified in our study has a molecular weight of <2 kDa (657-1994 Da), consisting of 6 to 18 amino acids. Short peptides are known to easily bind to the active site of ACE (Aslam et al., 2018). ACE inhibitory peptide binding to the active of ACE is facilitated by hydrogen bonding, hydrophobic interactions, and disrupting the stability of the $Zn^{+2}$ ion. The presence of ACEI peptide in fermented milk is highly dependent on the type of LAB used for the fermentation process. it is therefore very important to use isolates that have been shown to have the ability to release ACEI peptides. *Lc. lactis* ssp *lactis* BD17 used in this study was an isolate isolated from kefir. Although the ability of this strain has only been explored in this study, as a comparison, the results of studies using kefir grain could be presented. Ebner et al. (2015) stated that kefir microbes were able to release 12 ACEI peptides in fermented milk. The same thing was also conveyed by Dallas et al. (2016) that kefir microbes release 29 bioactive peptides, including ACEI peptides in fermented cow milk. A recent study by Izquierdo-González et al. (2019) showed that in goat milk using kefir grains, five ACEI peptides were identified.

## Conclusion

ACE inhibitory activity was detected in all fermented goat milk using isolates from fermented food and breast milk. Goat milk fermented using *Lc. lactis* ssp *lactis* BD17 produced the highest ACE inhibitory activity ($IC_{50}$: 0.297 ± 0.10 mg/mL) after 48 h of incubation. A total of 261 peptides were hydrolyzed by *Lc. lactis* ssp *lactis* BD17 during fermentation, most of which were released from casein (β-casein). The peptide has a molecular weight of 659 to 2201.1 Dalton, consisting of 6-20 amino acid residues. The CEP specificity of *Lc. lactis* ssp *lactis* BD17 in goat milk parent protein, dominant with cleavage on the amino acids tyrosine, leucine, glutamic acid, and proline. A total of 21 peptides were identified as ACEI peptides, having 100% homology to the reported ACEI peptides. Several characteristics of ACEI peptides are present in peptides hydrolyzed by *Lc. lactis* ssp *lactis* BD17. These peptides mostly have hydrophobic and aromatic amino acids at the C-terminus. The results of this study add to the information that *Lc. lactis* ssp *lactis* BD17 is a candidate that could be considered as a starter culture to obtain fermented milk that has functional properties as a source of ACE inhibitory peptides.



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## Conflicts of interest

The authors declare no conflicts of interest

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533 **Tables and Figures**

534

535 **Table 1**. Profile of goat milk fermented by LAB from kefir grains and breast milk

| Culture | Fermentation time to reach pH 4.6 (h) | Titratable acidity (%) | Viable count (Log CFU/mL) |
|---|---|---|---|
| *Lactobacillus rhamnosus* R2 | 18 | 0.90 ± 0.01 | 9.69 ± 0.11 |
| *Pediococcus pentasaceus* 1 W2SR04 | 24 | 0.94 ± 0.04 | 9.18 ± 0.46 |
| *Lactobacillus kefiri* YK4 | 24 | 0.80 ± 0.03 | 9.79 ± 0.39 |
| *Lactobacillus kefiri* JK17 | 24 | 0.79 ± 0.02 | 9.67 ± 0.07 |
| *Lactobacillus fermentum* R6 | 24 | 0.84 ± 0.01 | 9.58 ± 0.03 |
| *Lactobacillus plantarum* 1W22408 | 32 | 0.77 ± 0.06 | 9.25 ± 0.39 |
| *Lactobacillus* R7F | 36 | 0.81 ± 0.02 | 9.46 ± 0.52 |
| *Lactobacillus fermentum* S206 | 48 | 0.70 ± 0.06 | 9.53 ± 0.17 |
| *Lactobacillus delbrueckii* BD7 | 48 | 0.75 ± 0.05 | 9.73 ± 0.36 |
| *Lactococcus lactis* ssp *lactis* BD17 | 48 | 0.75 ± 0.05 | 9.55 ± 0.38 |

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**Table 2.** ACE inhibitory activity, peptide content, and IER of goat milk fermented by LAB after reaching pH 4.6

| Culture | Inhibition of ACE (%) | Peptide content (mg/mL) | IER (% per mg/mL) |
|---|---|---|---|
| *Pediococcus pentasaceus* 1 W2SR04 | 20.44 ± 2.33$^{c}$ | 5.696 ± 0.21$^{a}$ | 3.60 ± 0.49$^{c}$ |
| *Lactobacillus kefiri* YK4 | 58.65 ± 8.87$^{a}$ | 5.691 ± 0.25$^{a}$ | 10.31 ± 1.55$^{b}$ |
| *Lactobacillus fermentum* S206 | 26.64 ± 3.90$^{c}$ | 4.809 ± 0.23$^{b}$ | 5.59 ± 1.04$^{c}$ |
| *Lactobacillus delbrueckii* BD7 | 60.79 ± 8.78$^{a}$ | 4.767 ± 0.27$^{b}$ | 12.81 ± 2.12$^{ab}$ |
| *Lactobacillus kefiri* JK17 | 56.94 ± 2.81$^{a}$ | 4.750 ± 0.30$^{b}$ | 12.06 ± 1.23$^{b}$ |
| *Lactobacillus fermentum* R6 | 48.50 ± 6.92$^{ab}$ | 4.089 ± 0.10$^{c}$ | 11.83 ± 1.42$^{b}$ |
| *Lactobacillus* R7F | 42.85 ± 5.10$^{b}$ | 4.007 ± 0.23$^{c}$ | 10.66 ± 0.73$^{b}$ |
| *Lactococcus lactis* ssp *lactis* BD17 | 60.33 ± 4.73$^{a}$ | 4.037 ± 0.27$^{c}$ | 14.99 ± 1.26 $^{a}$ |
| *Lactobacillus rhamnosus* R2 | 40.51 ± 6.15$^{b}$ | 3.658 ± 0.19$^{c}$ | 11.03 ± 1.10$^{b}$ |
| *Lactobacillus plantarum* I W22408 | 39.69 ± 4.70$^{b}$ | 3.548 ± 0.26$^{c}$ | 11.15 ± 0.53$^{b}$ |

$^{a\text{-}c}$ Different superscript in the same column indicated significant ($p$ <0.05)

547 **Table 3.** ACE inhibitor activity of supernatant (without ultrafiltration), fractions of >3 kDa and

548 <3 kDa of goat milk fermented by *Lc. lactis* ssp *lactis* BD17

549

| ACE inhibitory activity (%) | | |
|---|---|---|
| Supernatant (Without ultrafiltration) | >3 kDa | <3 kDa |
| 60.33 ± 4.73[a] | 24.57± 2.36[b] | 57.31 ± 2.41[a] |

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553 **Table 4.** Characteristics of peptides (<3 kDa) of fermented goat milk of *Lc. lactis* ssp *lactis*

554 BD17

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| Parent protein* | Total Peptides | Range of precursor m/z | Range of precussor MW | Amino acid residue | Dominant peptide |
|---|---|---|---|---|---|
| αS1-casein | 41 | 330.1-1146.0 | 659.3-2291.1 | 6 - 20 | FSDIPNPIGSE, FSDIPNPIGSENSGKTTMP, NSGKTTMPLW |
| αS2-casein | 37 | 310.1- 906.9 | 772.4-1812.9 | 6 - 15 | QGPIVLNPWDQVKR |
| β-casein | 141 | 326.7-1072.5 | 652.40-2201.1 | 6 - 18 | QEPVLGPVRGPFPII, QEPVLGPVRGPFPI, QEPVLGPVRGPFPIIV, VLGPVRGPFPIIV, TQTPVVVPPFLQPE |
| κ-casein | 36 | 376.5-750.8 | 761.45-1500.7 | 6 - 10 | ARHPHPHLSFM |
| ά-lactalbumin | 3 | 497.7-563.7 | 994.4-1126.4 | 8 - 10 | AFHTSGYDTQ |
| β-lactoglobulin | 3 | 420.5-482.2 | 903.57-1259.7 | 8 - 11 | |

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557 *Protein access code at https://www.uniprot.org/. A: Alanine, D: Aspartic Acid, E: Glutamic Acid, F:

558 Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P:

559 Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y: Tyrosine,

560 MW: Molecular Weight.

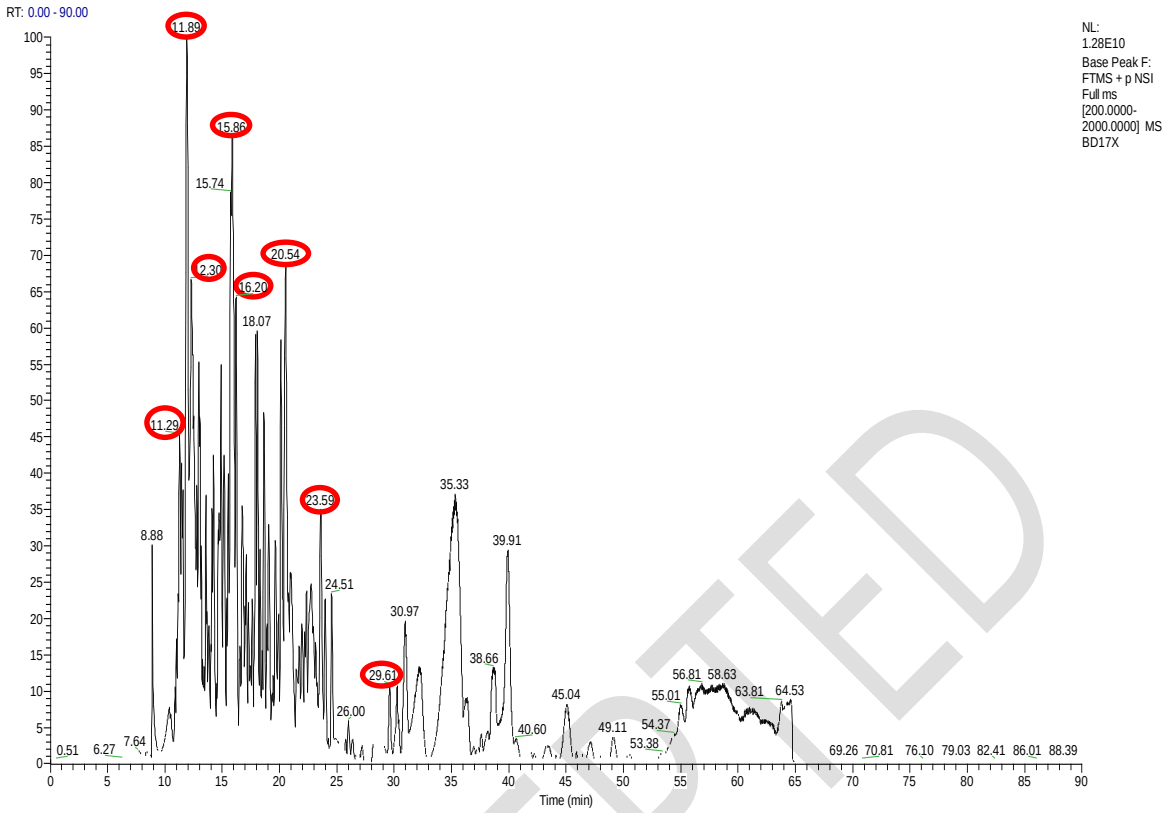


**Fig. 1.** Nano LC-MS/MS chromatogram of goat milk fermented by *Lc. lactis* ssp *lactis* BD17

577 **Table 5.** Peptides identified of goat milk fermented by *Lc. lactis* ssp *lactis* BD17 with Retention

578 Time (RT) detected on Nano LC-MS/MS. Peptides in bold letters indicate a match with RT.

579

| **Retention Time (min)** | **Retention time (min)** | **m/z [Da]** | **MH+ [Da]** | **Peptide identified** | **Parent Protein** |
|---|---|---|---|---|---|
| 11.29 | 11.29 | 665.33 | 1329.66 | **ARHPHPHLSFM** | κ-casein (A0A140T8A9*) |
| | 11.22 | 495.75 | 990.49 | ITVDDKHY | αs2-casein (P02663*) |
| 11.89 | 11.81 | 563.74 | 1126.48 | AFHTSGYDTQ | ά- lactalbumin (B6V3I5*) |
| | 11.87 | 581.34 | 1161.67 | EKNRLNFLK | αs2-casein (P02663*) |
| | 11.89 | 673.33 | 1345.66 | **ARHPHPHLSFM** | κ-casein (A0A140T8A9*) |
| 12.30 | 12.30 | 559.75 | 1118.49 | **QQQTEDELQ** | β-casein (P02666*) |
| | 12.30 | 546.79 | 1092.57 | **YQKALNEIN** | αs2-casein (P02663*) |
| 15.86 | 15.86 | 470.74 | 940.47 | **IPNPIGSEN** | αs1-casein (P02662*) |
| | 15.86 | 521.27 | 1041.54 | **RYLGYLEQ** | αs1-casein (P02662*) |
| 16.20 | 16.20 | 413.72 | 826.43 | **IPNPIGSE** | αs1-casein (P02662*) |
| | 16.20 | 560.78 | 1120.55 | **FYQKFPQY** | αs2-casein (P02663*) |
| | 16.24 | 621.31 | 1241.61 | DELQDKIHPF | β-casein (P02666*) |
| | 16.26 | 457.24 | 913.48 | RYLGYLE | αs1-casein (P02662*) |
| | 16.26 | 610.29 | 1219.57 | SRYPSYGLNY | κ-casein (A0A140T8A9*) |
| 20.54 | 20.54 | 676.34 | 1351.68 | **MPFPKYPVEPF** | β-casein (P02666*) |
| | 20.59 | 329.20 | 657.40 | VVVPPF | β-casein (P02666*) |
| 23.59 | 23.50 | 683.86 | 1366.72 | RDMPIQAFLLY | β-casein (P02666*) |

| Retention Time (min) | Retention time (min) | m/z [Da] | MH+ [Da] | Peptide identified | Parent Protein |
|---|---|---|---|---|---|
| | 23.51 | 559.80 | 1118.59 | MFPPQSVLSL | β-casein (P02666*) |
| | 23.53 | 385.74 | 770.48 | VVVPPFL | β-casein (P02666*) |
| | 23.54 | 948.04 | 1895.07 | LYQEPVLGPVRGPFPII | β-casein (P02666*) |
| | 23.56 | 795.47 | 1589.94 | EPVLGPVRGPFPIIV | β-casein (P02666*) |
| | 23.59 | 602.33 | 1203.66 | **RDMPIQAFLL** | β-casein (P02666*) |
| 24.51 | 24.50 | 859.50 | 1718.00 | QEPVLGPVRGPFPIIV | β-casein (P02666*) |
| | 24.55 | 484.79 | 968.58 | TPVVVPPFL | β-casein (P02666*) |
| 26.00 | 26.07 | 548.30 | 1095.59 | MPIQAFLLY | β-casein (P02666*) |
| | 26.08 | 809.97 | 1618.93 | QEPVLGPVRGPFPII | β-casein (P02666*) |
| 29.61 | 29.61 | 753.42 | 1505.84 | **QEPVLGPVRGPFPI** | β-casein (P02666*) |
| 30.97 | 30.98 | 776.42 | 1551.83 | TQTPVVVPPFLQPE | β-casein (P02666*) |

*Protein access code at https://www.uniprot.org/.A: Alanine, D: Aspartic Acid, E: Glutamic Acid, F: Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y: Tyrosine

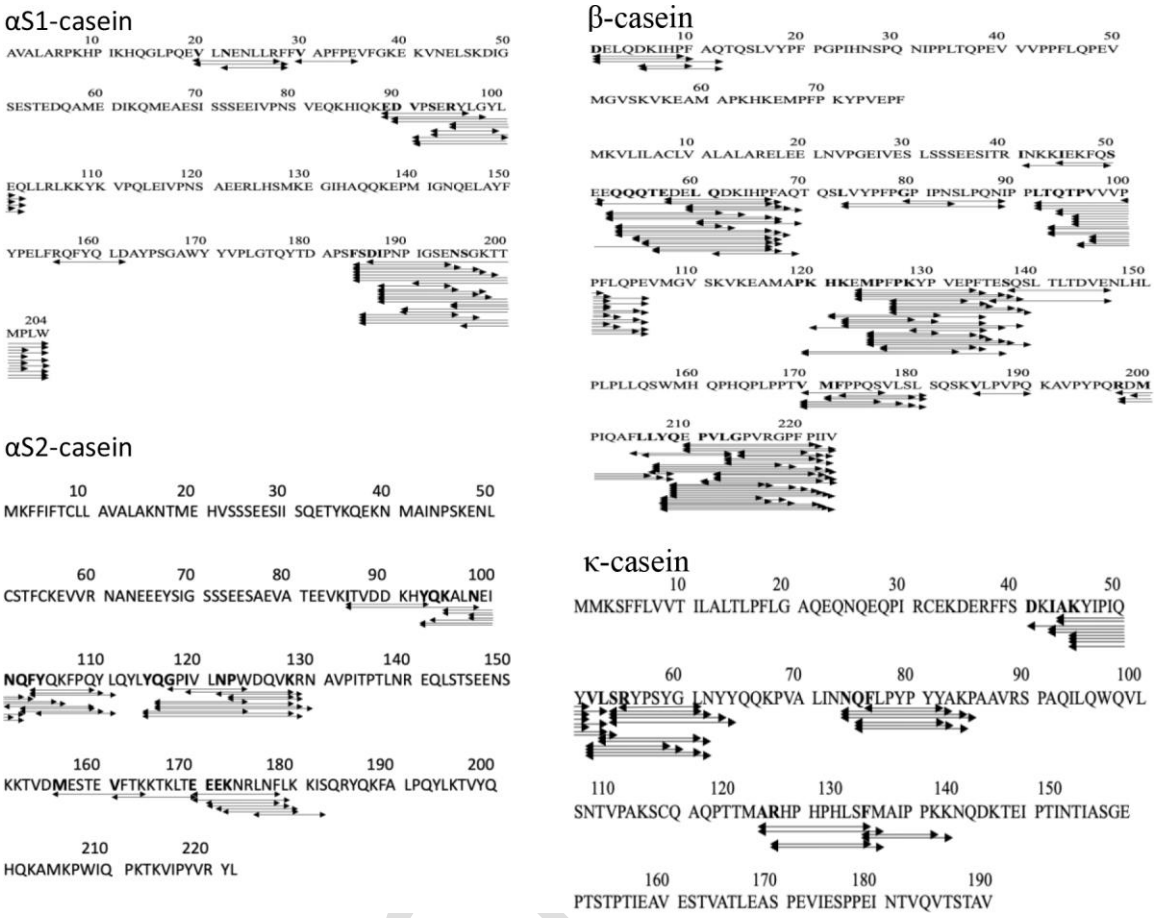


**Fig. 2**. Cleavage specifics of the peptide of the parent protein αs1-casein, αs2- casein, β- casein, and κ- casein

596 **Table 6**. ACE inhibitory peptides in fermented goat milk of *Lc. lactis* ssp *lactis* BD17 as

597 reported in the literature

| Parent Protein* | Sequence | m/z [Da] | MH+ [Da] | Charge | References |
|---|---|---|---|---|---|
| κ-cas. | ARHPHPHLSFM | 665.33 | 1329.66 | 3 | Ibrahim et al. (2017) |
| β-cas. | DELQDKIHPF | 621.30 | 1241.61 | 2 | Rodríguez-Figueroa et al. (2012) |
| β-cas. | DKIHPF | 378.71 | 756.41 | 2 | Fan et al. (2018) |
| β-cas. | DKIHPFAQ | 478.25 | 955.50 | 2 | Gobbetti et al. (2000) |
| β-cas. | EMPFPKYPVEPF | 740.86 | 1480.71 | 2 | Papadimitriou et al. (2007) |
| β-cas. | ELQDKIHPF | 563.80 | 1126.59 | 2 | Fan et al. (2018) |
| β-cas. | GPVRGPFPI | 470.27 | 939.54 | 2 | Amorim et al. (2019) |
| β-cas. | LGPVRGPFP | 470.27 | 939.54 | 2 | Hernandes-Ledesma et al. (2004) |
| β-cas. | LTQTPVVVPPF | 599.34 | 1197.68 | 2 | Villegas et al. (2014) |
| β-cas. | LVYPFPGPIHNSLPQN | 896.96 | 1792.93 | 2 | Quirós et al. (2009) |
| β-cas. | LYQEPVLGPVRGPFPIIV | 997.58 | 1994.14 | 2 | Pihlanto et al. (2010) |
| β- cas. | MPFPKYPVEP | 602.80 | 1204.60 | 2 | Contreras et al. (2009) |
| β-cas. | MPFPKYPVEPF | 676.34 | 1351.67 | 2 | Hayes et al. (2007) |
| β-cas. | QEPVLGPVRGPFP | 696.88 | 1392.76 | 2 | Hernandes-Ledesma et al. (2004) |
| β-cas. | QEPVLGPVRGPFPIIV | 859.50 | 1718.00 | 2 | Perpetuo et al. (2003) |

| Parent Protein* | Sequence | m/z [Da] | MH+ [Da] | Charge | References |
|---|---|---|---|---|---|
| αs1-cas. | VLNENLR | 485.78 | 970.56 | 2 | Zhao et al. (2019) |
| β-cas. | VLGPVRGPFP | 519.81 | 1038.61 | 2 | Gútiez et al. (2013) |
| β-cas. | VVVPPF | 329.20 | 657.39 | 2 | Torres-Llanez et al. (2011) |
| β-cas. | YQEPVLGPVRGPFPI | 834.95 | 1668.90 | 2 | Zhao et al. (2019) |
| β-cas. | YQEPVLGPVRGPFPIIV | 627.69 | 1881.06 | 3 | Zhao et al. (2019) |
| β-cas. | YQEPVLGPVR | 579.31 | 1157.63 | 2 | Kalyankar et al. (2013) |

*Protein access code at https://www.uniprot.org/.A:Alanine, D:Aspartic Acid, E:Glutamic Acid, F: Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y: Tyrosine, cas: casein

603 **Supplementari Dataset 1.** Peptide profile (<3 kDa) released from goat milk proteins during

604 fermentation using *Lc. lactis* ssp *lactis* BD17

605

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| αs1- | DIPNPIGSENSGKTTMPLW | 2057.00 | 1029.00 | 2057.00 | 1029.00 | 2 | 21.97 |
| casein | DVPSERYLGYLE | 1440.70 | 720.85 | 1440.70 | 720.85 | 2 | 19.95 |
| | DVPSERYLGYLEQ | 1568.76 | 784.88 | 1568.76 | 784.88 | 2 | 19.81 |
| | EDVPSERY | 994.45 | 497.73 | 994.45 | 497.73 | 2 | 12.21 |
| | EDVPSERYLG | 1164.55 | 582.78 | 1164.55 | 582.78 | 2 | 14.95 |
| | FSDIPNPIGSE | 1175.56 | 588.28 | 1175.56 | 588.28 | 2 | 19.45 |
| | FSDIPNPIGSEN | 1289.60 | 645.30 | 1289.60 | 645.30 | 2 | 19.16 |
| | FSDIPNPIGSENSG | 1433.65 | 717.33 | 1433.65 | 717.33 | 2 | 19.04 |
| | FSDIPNPIGSENSGK | 1561.75 | 781.38 | 1561.75 | 781.38 | 2 | 16.64 |
| | FSDIPNPIGSENSGKT | 1662.79 | 831.90 | 1662.80 | 831.90 | 2 | 16.62 |
| | FSDIPNPIGSENSGKTTMP | 1991.93 | 996.47 | 1991.94 | 996.47 | 2 | 17.96 |
| | FSDIPNPIGSENSGKTTMPLW | 2291.10 | 1146.05 | 2291.10 | 1146.05 | 2 | 21.96 |
| | IGSENSGKTTMPLW | 1520.74 | 760.87 | 1520.74 | 760.87 | 2 | 18.41 |
| | IPNPIGSE | 826.43 | 413.72 | 826.43 | 413.72 | 2 | 16.20 |
| | IPNPIGSEN | 940.47 | 470.74 | 940.47 | 470.74 | 2 | 15.86 |
| | IPNPIGSENSEKT | 1385.69 | 693.35 | 1385.69 | 693.35 | 2 | 14.46 |
| | IPNPIGSENSG | 1084.52 | 542.77 | 1084.53 | 542.77 | 2 | 15.65 |
| | IPNPIGSENSGK | 1212.62 | 606.81 | 1212.62 | 606.81 | 2 | 13.16 |
| | IPNPIGSENSGKTTMP | 1642.81 | 821.91 | 1642.81 | 821.91 | 2 | 15.69 |
| | IPNPIGSENSGKTTMPLW | 1941.97 | 971.49 | 1941.97 | 971.49 | 2 | 20.38 |
| | NENLLRF | 905.48 | 453.24 | 905.48 | 453.25 | 2 | 17.8 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | NSGKTTMPL | 948.48 | 474.74 | 948.48 | 474.74 | 2 | 12.03 |
| | NSGKTTMPLW | 1134.56 | 567.78 | 1134.56 | 567.78 | 2 | 17.5 |
| | PIGSENSGKTTMP | 1318.63 | 659.82 | 1318.63 | 659.82 | 2 | 12.1 |
| | PIGSENSGKTTMPLW | 1617.79 | 809.40 | 1617.79 | 809.40 | 2 | 18.7 |
| | RQFYQLD | 969.48 | 485.24 | 969.48 | 485.24 | 2 | 16.04 |
| | RYLGYLE | 913.48 | 457.24 | 913.48 | 457.24 | 2 | 16.26 |
| | RYLGYLEQ | 1041.54 | 521.27 | 1041.54 | 521.27 | 2 | 15.86 |
| | SDIPNPIGSEN | 1142.53 | 571.77 | 1142.53 | 571.77 | 2 | 17.19 |
| | SDIPNPIGSENSG | 1286.58 | 643.79 | 1286.59 | 643.80 | 2 | 17.07 |
| | SDIPNPIGSENSGKTTMP | 1844.87 | 922.94 | 1844.87 | 922.94 | 2 | 16.72 |
| | SDIPNPIGSENSGKTTMPLW | 2144.03 | 1072.52 | 2144.03 | 1072.52 | 2 | 21.19 |
| | SERYLGY | 887.42 | 444.22 | 887.43 | 444.22 | 2 | 14.12 |
| | SERYLGYLE | 1129.55 | 565.28 | 1129.55 | 565.28 | 2 | 17.28 |
| | SGKTTMPLW | 1020.52 | 510.76 | 1020.52 | 510.76 | 2 | 17.59 |
| | VAPFPE | 659.34 | 330.17 | 659.34 | 330.17 | 2 | 17.23 |
| | VLNENLLR | 970.57 | 485.79 | 970.57 | 485.79 | 2 | 15.29 |
| | VLNENLLRF | 1117.64 | 559.32 | 1117.64 | 559.32 | 2 | 19.78 |
| | VPSERYLGYL | 1196.63 | 598.82 | 1196.63 | 598.82 | 2 | 18.44 |
| | VPSERYLGYLE | 1325.67 | 663.34 | 1325.67 | 663.34 | 2 | 18.13 |
| | VPSERYLGYLEQ | 1453.73 | 727.37 | 1453.73 | 727.37 | 2 | 17.95 |
| αs2-casein | EEEKNRLNF | 1178.58 | 589.79 | 1178.58 | 589.79 | 2 | 12.64 |
| | EEEKNRLNFL | 1291.66 | 646.33 | 1291.66 | 646.34 | 2 | 15.88 |
| | EEKNRLNF | 1049.54 | 525.27 | 1049.54 | 525.27 | 2 | 11.90 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | EEKNRLNFL | 1162.62 | 581.81 | 1162.62 | 581.81 | 2 | 15.54 |
| | EKNRLNFLK | 1161.67 | 581.34 | 1161.67 | 581.34 | 2 | 11.87 |
| | FYQKFPQ | 957.48 | 479.24 | 957.48 | 479.25 | 2 | 14.06 |
| | FYQKFPQY | 1120.55 | 560.78 | 1120.55 | 560.78 | 2 | 16.20 |
| | FYQKFPQYL | 1233.63 | 617.32 | 1233.63 | 617.32 | 2 | 18.63 |
| | GPIVLNPW | 895.50 | 448.25 | 895.50 | 448.26 | 2 | 22.37 |
| | ITVDDKHY | 990.49 | 495.75 | 990.49 | 495.75 | 2 | 11.22 |
| | ITVDDKHYQKALN | 1544.80 | 515.61 | 1544.81 | 515.61 | 3 | 12.22 |
| | IVLNPWDQVK | 1211.68 | 606.34 | 1211.68 | 606.34 | 2 | 18.66 |
| | KALNEINQ | 929.51 | 465.26 | 929.51 | 465.26 | 2 | 8.98 |
| | KALNEINQF | 1076.57 | 538.79 | 1076.57 | 538.79 | 2 | 15.44 |
| | KNRLNFLK | 1032.63 | 344.88 | 1032.63 | 344.88 | 3 | 10.93 |
| | LNFLKKIS | 962.60 | 481.80 | 962.60 | 481.81 | 2 | 15.05 |
| | MESTEVFTK | 1071.50 | 536.25 | 1071.50 | 536.25 | 2 | 13.62 |
| | NEINQFYQ | 1055.48 | 528.24 | 1055.48 | 528.24 | 2 | 17.52 |
| | NEINQFYQK | 1183.57 | 592.29 | 1183.57 | 592.29 | 2 | 14.66 |
| | NPWDQVKR | 1042.54 | 348.19 | 1042.54 | 348.19 | 3 | 12.96 |
| | NQFYQKFPQ | 1199.58 | 600.30 | 1199.58 | 600.30 | 2 | 15.36 |
| | PWDQVK | 772.40 | 386.70 | 772.40 | 386.70 | 2 | 12.47 |
| | PWDQVKR | 928.50 | 310.17 | 928.50 | 310.17 | 3 | 10.59 |
| | QFYQKFPQ | 1085.54 | 543.27 | 1085.54 | 543.27 | 2 | 14.89 |
| | QFYQKFPQY | 1248.60 | 624.80 | 1248.60 | 624.81 | 2 | 16.83 |
| | QGPIVLNPW | 1023.56 | 512.28 | 1023.56 | 512.28 | 2 | 22.27 |
| | QGPIVLNPWDQVK | 1493.81 | 747.41 | 1493.81 | 747.41 | 2 | 19.51 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | QGPIVLNPWDQVKR | 1649.90 | 825.46 | 1649.91 | 825.46 | 2 | 17.90 |
| | QGPIVLNPWDQVKRN | 1763.95 | 882.48 | 1763.96 | 882.48 | 2 | 17.75 |
| | QKALNEINQ | 1057.56 | 529.28 | 1057.56 | 529.29 | 2 | 10.95 |
| | VFTKKTKLTE | 1194.71 | 597.86 | 1194.71 | 597.86 | 2 | 9.81 |
| | YQGPIVLNPW | 1186.62 | 593.82 | 1186.63 | 593.82 | 2 | 23.21 |
| | YQGPIVLNPWDQVK | 1656.87 | 828.94 | 1656.87 | 828.94 | 2 | 20.32 |
| | YQGPIVLNPWDQVKR | 1812.97 | 906.99 | 1812.98 | 906.99 | 2 | 18.51 |
| | YQKALNEIN | 1092.57 | 546.79 | 1092.57 | 546.79 | 2 | 12.30 |
| | YQKALNEINQ | 1220.62 | 610.82 | 1220.63 | 610.82 | 2 | 12.46 |
| | YQKFPQYL | 1086.56 | 543.78 | 1086.56 | 543.78 | 2 | 17.42 |
| β-casein | DELQDKIHP | 1094.55 | 547.78 | 1094.55 | 547.78 | 2 | 12.66 |
| | DELQDKIHPF | 1241.61 | 621.31 | 1241.62 | 621.31 | 2 | 16.24 |
| | DELQDKIHPFAQ | 1440.71 | 480.91 | 1440.71 | 480.91 | 3 | 16.44 |
| | DKIHPF | 756.40 | 378.70 | 756.40 | 378.71 | 2 | 20.37 |
| | DKIHPFAQ | 955.50 | 478.25 | 955.50 | 478.25 | 2 | 12.10 |
| | EDELQDKIHP | 1223.59 | 408.53 | 1223.59 | 408.53 | 3 | 13.45 |
| | EDELQDKIHPF | 1370.66 | 685.83 | 1370.66 | 685.83 | 2 | 16.69 |
| | EEQQQTEDELQDKIHP | 1966.90 | 656.30 | 1966.90 | 656.30 | 3 | 14.60 |
| | ELQDKIHPF | 1126.59 | 563.80 | 1126.59 | 563.80 | 2 | 14.92 |
| | ELQDKIHPFA | 1197.62 | 599.32 | 1197.63 | 599.32 | 2 | 15.04 |
| | ELQDKIHPFAQ | 1325.68 | 663.35 | 1325.68 | 663.35 | 2 | 14.89 |
| | EMPFPKYPVEP | 1333.65 | 667.33 | 1333.65 | 667.33 | 2 | 18.71 |
| | EMPFPKYPVEPF | 1480.72 | 740.86 | 1480.72 | 740.86 | 2 | 21.40 |
| | EMPFPKYPVEPFTE | 1726.80 | 863.90 | 1726.80 | 863.91 | 2 | 19.59 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | EMPFPKYPVEPFTES | 1813.83 | 907.42 | 1813.84 | 907.42 | 2 | 20.89 |
| | EMPFPKYPVEPFTESQ | 1941.89 | 971.45 | 1941.89 | 971.45 | 2 | 20.65 |
| | EPVLGPVRGPFP | 1264.70 | 632.86 | 1264.70 | 632.86 | 2 | 18.78 |
| | EPVLGPVRGPFPI | 1377.79 | 689.40 | 1377.79 | 689.40 | 2 | 20.98 |
| | EPVLGPVRGPFPII | 1490.87 | 745.94 | 1490.87 | 745.94 | 2 | 22.40 |
| | EPVLGPVRGPFPIIV | 1589.95 | 795.48 | 1589.94 | 795.47 | 2 | 23.56 |
| | EQQQTEDELQ | 1247.54 | 624.27 | 1247.54 | 624.27 | 2 | 12.66 |
| | EQQQTEDELQDK | 1490.66 | 745.83 | 1490.66 | 745.83 | 2 | 10.45 |
| | EQQQTEDELQDKIHP | 1837.85 | 613.29 | 1837.86 | 613.29 | 3 | 13.82 |
| | EQQQTEDELQDKIHPF | 1984.92 | 992.96 | 1984.92 | 992.97 | 2 | 16.78 |
| | FLLYQEPVL | 1121.62 | 561.32 | 1121.62 | 561.32 | 2 | 24.24 |
| | FPKYPVE | 879.46 | 440.23 | 879.46 | 440.23 | 2 | 14.87 |
| | FPKYPVEPF | 1123.58 | 562.29 | 1123.58 | 562.29 | 2 | 19.12 |
| | FPKYPVEPFT | 1224.63 | 612.82 | 1224.63 | 612.82 | 2 | 18.77 |
| | FPKYPVEPFTES | 1440.70 | 720.85 | 1440.70 | 720.86 | 2 | 18.66 |
| | FPKYPVEPFTESQ | 1568.76 | 784.88 | 1568.76 | 784.89 | 2 | 18.65 |
| | FPKYPVEPFTESQS | 1655.79 | 828.40 | 1655.80 | 828.40 | 2 | 18.47 |
| | FPPQSVLSL | 987.55 | 494.28 | 987.55 | 494.28 | 2 | 22.89 |
| | GPIPNSLPQN | 1036.54 | 518.77 | 1036.54 | 518.77 | 2 | 16.11 |
| | GPVRGPFP | 826.46 | 413.73 | 826.46 | 413.73 | 2 | 14.30 |
| | GPVRGPFPI | 939.54 | 470.27 | 939.54 | 470.27 | 2 | 17.48 |
| | GPVRGPFPII | 1052.62 | 526.82 | 1052.63 | 526.82 | 2 | 20.18 |
| | HKEMPFPKYP | 1273.64 | 637.32 | 1273.64 | 637.32 | 2 | 13.54 |
| | HKEMPFPKYPVEPF | 1745.87 | 873.44 | 1745.87 | 873.44 | 2 | 17.82 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | IEKFQSE | 880.44 | 440.72 | 880.44 | 440.72 | 2 | 10.32 |
| | INKKIEKFQ | 1147.68 | 574.34 | 1147.68 | 574.35 | 2 | 9.73 |
| | KEMPFPK | 876.46 | 438.74 | 876.46 | 438.74 | 2 | 11.13 |
| | KEMPFPKYP | 1136.58 | 568.79 | 1136.58 | 568.79 | 2 | 14.79 |
| | KEMPFPKYPVEPF | 1624.81 | 542.27 | 1624.81 | 542.27 | 3 | 17.85 |
| | KEMPFPKYPVEPFTESQ | 2053.99 | 1027.50 | 2053.99 | 1027.50 | 2 | 18.37 |
| | KHKEMPFPKYPVEPLTE | 2070.09 | 518.28 | 2070.07 | 518.27 | 4 | 16.31 |
| | KYPVEPF | 879.46 | 440.23 | 879.46 | 440.23 | 2 | 16.30 |
| | LGPVRGPFP | 939.54 | 470.27 | 939.54 | 470.27 | 2 | 16.86 |
| | LGPVRGPFPI | 1052.62 | 526.82 | 1052.63 | 526.82 | 2 | 19.27 |
| | LGPVRGPFPII | 1165.71 | 583.36 | 1165.71 | 583.36 | 2 | 21.23 |
| | LLYQEPVL | 974.55 | 487.78 | 974.56 | 487.78 | 2 | 20.67 |
| | LQDKIHPF | 997.55 | 499.28 | 997.55 | 499.28 | 2 | 13.46 |
| | LQDKIHPFAQ | 1196.64 | 598.82 | 1196.64 | 598.82 | 2 | 13.32 |
| | LTQTPVVVPP | 1050.62 | 525.81 | 1050.62 | 525.81 | 2 | 17.35 |
| | LTQTPVVVPPF | 1197.69 | 599.35 | 1197.69 | 599.35 | 2 | 22.14 |
| | LTQTPVVVPPFLQPE | 1664.93 | 832.97 | 1664.93 | 832.97 | 2 | 24.04 |
| | LVYPFPGPIHN | 1253.67 | 627.34 | 1253.67 | 627.34 | 2 | 19.39 |
| | LVYPFPGPIHNSLPQN | 1792.93 | 896.97 | 1792.94 | 896.97 | 2 | 19.59 |
| | LYQEPVLGPVR | 1270.72 | 635.86 | 1270.72 | 635.86 | 2 | 16.42 |
| | LYQEPVLGPVRGP | 1424.79 | 712.90 | 1424.79 | 712.90 | 2 | 17.40 |
| | LYQEPVLGPVRGPFPI | 1782.00 | 891.50 | 1781.99 | 891.50 | 2 | 21.94 |
| | LYQEPVLGPVRGPFPII | 1895.07 | 948.04 | 1895.08 | 948.04 | 2 | 23.54 |
| | LYQEPVLGPVRGPFPIIV | 1994.14 | 997.58 | 1994.15 | 997.58 | 2 | 24.37 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | MFPPQSVLS | 1005.51 | 503.26 | 1005.51 | 503.26 | 2 | 19.98 |
| | MFPPQSVLSL | 1118.59 | 559.80 | 1118.59 | 559.80 | 2 | 23.51 |
| | MPFPKYP | 879.44 | 440.22 | 879.44 | 440.23 | 2 | 19.47 |
| | MPFPKYPVEP | 1204.61 | 602.81 | 1204.61 | 602.81 | 2 | 16.50 |
| | MPFPKYPVEPF | 1351.68 | 676.34 | 1351.68 | 676.34 | 2 | 20.54 |
| | MPFPKYPVEPFT | 1452.72 | 726.86 | 1452.72 | 726.87 | 2 | 20.24 |
| | MPFPKYPVEPFTE | 1597.76 | 799.38 | 1597.76 | 799.38 | 2 | 19.13 |
| | MPFPKYPVEPFTES | 1668.80 | 834.90 | 1668.80 | 834.90 | 2 | 20.16 |
| | MPFPKYPVEPFTESQS | 1883.89 | 942.45 | 1883.89 | 942.45 | 2 | 19.98 |
| | MPIQAFLLY | 1095.59 | 548.30 | 1095.59 | 548.30 | 2 | 26.07 |
| | PFPKYPVEP | 1073.57 | 537.29 | 1073.57 | 537.29 | 2 | 16.40 |
| | PFPKYPVEPF | 1220.63 | 610.82 | 1220.64 | 610.82 | 2 | 19.42 |
| | PKHKEMPFPKYPV | 1597.85 | 533.29 | 1597.86 | 533.29 | 3 | 12.70 |
| | PKHKEMPFPKYPVEPFTE | 2201.13 | 734.38 | 2201.11 | 734.37 | 3 | 16.73 |
| | PKYPVEPFTE | 1206.60 | 603.80 | 1206.60 | 603.81 | 2 | 16.75 |
| | PKYPVEPFTES | 1293.63 | 647.32 | 1293.64 | 647.32 | 2 | 16.52 |
| | PVLGPVRGPFPI | 1248.74 | 624.88 | 1248.75 | 624.88 | 2 | 20.30 |
| | PVLGPVRGPFPIIV | 1460.90 | 730.95 | 1460.90 | 730.95 | 2 | 23.12 |
| | PVVVPPFLQP | 1092.64 | 546.83 | 1092.65 | 546.83 | 2 | 23.69 |
| | PVVVPPFLQPE | 1221.69 | 611.35 | 1221.69 | 611.35 | 2 | 23.43 |
| | QDKIHPFAQ | 1083.56 | 542.28 | 1083.56 | 542.28 | 2 | 11.59 |
| | QEPVLGPV | 838.47 | 419.74 | 838.47 | 419.74 | 2 | 17.73 |
| | QEPVLGPVR | 994.57 | 497.79 | 994.57 | 497.79 | 2 | 13.75 |
| | QEPVLGPVRGP | 1148.64 | 574.82 | 1148.64 | 574.82 | 2 | 15.26 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | QEPVLGPVRGPF | 1295.71 | 648.36 | 1295.71 | 648.36 | 2 | 18.59 |
| | QEPVLGPVRGPFP | 1392.76 | 696.88 | 1392.76 | 696.89 | 2 | 18.86 |
| | QEPVLGPVRGPFPI | 1505.85 | 753.43 | 1505.85 | 753.43 | 2 | 26.61 |
| | QEPVLGPVRGPFPII | 1618.93 | 809.97 | 1618.93 | 809.97 | 2 | 26.08 |
| | QEPVLGPVRGPFPIIV | 1718.00 | 859.50 | 1718.00 | 859.50 | 2 | 24.50 |
| | QQQTEDELQ | 1118.49 | 559.75 | 1118.50 | 559.75 | 2 | 12.30 |
| | QQQTEDELQD | 1233.52 | 617.26 | 1233.52 | 617.27 | 2 | 12.81 |
| | QQQTEDELQDKIHP | 1708.81 | 854.91 | 1708.81 | 854.91 | 2 | 13.18 |
| | QQQTEDELQDKIHPF | 1855.88 | 619.30 | 1855.88 | 619.30 | 3 | 16.44 |
| | QQQTEDELQDKIHPFA | 1926.92 | 642.98 | 1926.92 | 642.98 | 3 | 16.39 |
| | QQTEDELQDKIHP | 1580.75 | 790.88 | 1580.76 | 790.88 | 2 | 13.22 |
| | QQTEDELQDKIHPF | 1727.82 | 864.41 | 1727.82 | 864.42 | 2 | 16.54 |
| | QTEDELQDKIHP | 1452.70 | 726.85 | 1452.70 | 726.85 | 2 | 13.75 |
| | QTEDELQDKIHPF | 1599.76 | 800.39 | 1599.76 | 800.39 | 2 | 16.45 |
| | QTPVVVPPFL | 1096.64 | 548.82 | 1096.64 | 548.82 | 2 | 24.27 |
| | QTPVVVPPFLQPE | 1450.79 | 725.90 | 1450.79 | 725.90 | 2 | 23.43 |
| | RDMPIQAFL | 1090.57 | 545.79 | 1090.57 | 545.79 | 2 | 20.65 |
| | RDMPIQAFLL | 1203.65 | 602.33 | 1203.66 | 602.33 | 2 | 23.59 |
| | RDMPIQAFLLY | 1366.72 | 683.86 | 1366.72 | 683.86 | 2 | 23.50 |
| | SEEQQQTEDELQDKIHP | 2053.92 | 1027.47 | 2053.93 | 1027.47 | 2 | 15.05 |
| | SQSLTLTDVE | 1092.54 | 546.77 | 1092.54 | 546.77 | 2 | 18.13 |
| | TEDELQDKIHP | 1324.64 | 662.82 | 1324.64 | 662.82 | 2 | 13.33 |
| | TEDELQDKIHPF | 1471.70 | 736.36 | 1471.71 | 736.36 | 2 | 16.02 |
| | TESQSLTLTDVE | 1322.63 | 661.82 | 1322.63 | 661.82 | 2 | 18.26 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | TPVVVPPF | 855.50 | 428.25 | 855.50 | 428.25 | 2 | 21.27 |
| | TPVVVPPFL | 968.58 | 484.79 | 968.58 | 484.79 | 2 | 24.55 |
| | TPVVVPPFLQP | 1193.69 | 597.35 | 1193.69 | 597.35 | 2 | 23.75 |
| | TPVVVPPFLQPE | 1322.73 | 661.87 | 1322.74 | 661.87 | 2 | 23.43 |
| | TQTPVVVPPF | 1084.60 | 542.80 | 1084.60 | 542.81 | 2 | 21.60 |
| | TQTPVVVPPFL | 1197.69 | 599.35 | 1197.69 | 599.35 | 2 | 24.43 |
| | TQTPVVVPPFLQP | 1422.80 | 711.90 | 1422.80 | 711.90 | 2 | 23.63 |
| | TQTPVVVPPFLQPE | 1551.84 | 776.43 | 1551.84 | 776.42 | 2 | 30.97 |
| | VLGPVRGPFP | 1038.61 | 519.81 | 1038.61 | 519.81 | 2 | 18.19 |
| | VLGPVRGPFPI | 1151.69 | 576.35 | 1151.69 | 576.35 | 2 | 20.03 |
| | VLGPVRGPFPII | 1264.78 | 632.89 | 1264.78 | 632.89 | 2 | 22.78 |
| | VLGPVRGPFPIIV | 1363.84 | 682.43 | 1363.85 | 682.43 | 2 | 28.17 |
| | VLPVPQ | 652.40 | 326.70 | 652.40 | 326.71 | 2 | 15.32 |
| | VMFPPQS | 805.39 | 403.20 | 805.39 | 403.20 | 2 | 16.30 |
| | VMFPPQSVL | 1017.54 | 509.27 | 1017.54 | 509.28 | 2 | 22.36 |
| | VMFPPQSVLS | 1104.57 | 552.79 | 1104.58 | 552.79 | 2 | 21.62 |
| | VMFPPQSVLS | 1120.57 | 560.79 | 1120.57 | 560.79 | 2 | 19.49 |
| | VMFPPQSVLSL | 1233.65 | 617.33 | 1233.65 | 617.33 | 2 | 23.33 |
| | VMFPPQSVLSL | 1217.66 | 609.33 | 1217.66 | 609.33 | 2 | 24.26 |
| | VVVPPF | 657.40 | 329.20 | 657.40 | 329.20 | 2 | 20.59 |
| | VVVPPFL | 770.48 | 385.74 | 770.48 | 385.74 | 2 | 24.53 |
| | VVVPPFLQPE | 1124.63 | 562.82 | 1124.64 | 562.82 | 2 | 24.08 |
| | YQEPVLGPV | 1001.53 | 501.27 | 1001.53 | 501.27 | 2 | 18.92 |
| | YQEPVLGPVR | 1157.63 | 579.32 | 1157.63 | 579.32 | 2 | 15.35 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | YQEPVLGPVRG | 1214.66 | 607.83 | 1214.65 | 607.83 | 2 | 15.39 |
| | YQEPVLGPVRGP | 1311.70 | 656.36 | 1311.71 | 656.36 | 2 | 16.47 |
| | YQEPVLGPVRGPFP | 1555.82 | 778.42 | 1555.83 | 778.42 | 2 | 19.51 |
| | YQEPVLGPVRGPFPI | 1668.91 | 834.96 | 1668.91 | 834.96 | 2 | 64.61 |
| | YQEPVLGPVRGPFPII | 1781.99 | 594.67 | 1781.99 | 594.67 | 3 | 22.93 |
| | YQEPVLGPVRGPFPIIV | 1881.06 | 627.69 | 1881.06 | 627.69 | 3 | 23.72 |
| κ-casein | AKYIPIQY | 995.56 | 498.28 | 995.56 | 498.28 | 2 | 16.34 |
| | AKYIPIQYVL | 1207.70 | 604.36 | 1207.71 | 604.36 | 2 | 20.15 |
| | ARHPHPHLSF | 1198.62 | 599.81 | 1198.62 | 599.82 | 2 | 10.28 |
| | ARHPHPHLSFM | 1329.66 | 665.33 | 1329.66 | 665.34 | 2 | 11.29 |
| | ARHPHPHLSFM | 1345.65 | 673.33 | 1345.66 | 673.33 | 2 | 11.89 |
| | DKIAKYIPIQY | 1351.76 | 676.38 | 1351.76 | 676.38 | 2 | 17.78 |
| | FLPYPYY | 962.47 | 481.74 | 962.47 | 481.74 | 2 | 21.87 |
| | FMAIPPK | 819.44 | 410.23 | 819.44 | 410.23 | 2 | 11.73 |
| | FMAIPPK | 803.45 | 402.23 | 803.45 | 402.23 | 2 | 16.49 |
| | FMAIPPKK | 931.54 | 466.27 | 931.54 | 466.28 | 2 | 11.40 |
| | IAKYIPIQY | 1108.64 | 554.82 | 1108.64 | 554.82 | 2 | 17.45 |
| | IAKYIPIQYVL | 1320.79 | 660.90 | 1320.79 | 660.90 | 2 | 21.29 |
| | KYIPIQ | 761.45 | 381.23 | 761.46 | 381.23 | 2 | 13.23 |
| | KYIPIQY | 924.52 | 462.76 | 924.52 | 462.76 | 2 | 16.01 |
| | KYIPIQYVL | 1136.67 | 568.84 | 1136.67 | 568.84 | 2 | 20.10 |
| | KYIPIQYVLS | 1223.70 | 612.35 | 1223.70 | 612.36 | 2 | 19.15 |
| | LSRYPSYGL | 1055.55 | 528.28 | 1055.55 | 528.28 | 2 | 15.88 |
| | LSRYPSYGLN | 1169.59 | 585.30 | 1169.59 | 585.30 | 2 | 15.07 |

| Parent protein* | Sequence | Prec MW | Prec m/z | Theor MW | Ther m/z | Charge | RT |
|---|---|---|---|---|---|---|---|
| | NQFLPYPYYA | 1275.60 | 638.30 | 1275.60 | 638.31 | 2 | 22.27 |
| | NQFLPYPYYAK | 1403.70 | 702.35 | 1403.70 | 702.35 | 2 | 18.61 |
| | NQFLPYPYYAKP | 1500.75 | 750.88 | 1500.75 | 750.88 | 2 | 19.05 |
| | QFLPYPYY | 1090.52 | 545.77 | 1090.52 | 545.77 | 2 | 22.46 |
| | QFLPYPYYA | 1161.56 | 581.28 | 1161.56 | 581.28 | 2 | 22.24 |
| | QFLPYPYYAK | 1289.65 | 645.33 | 1289.66 | 645.33 | 2 | 18.58 |
| | RHPHPHLSF | 1127.58 | 376.53 | 1127.59 | 376.53 | 3 | 10.16 |
| | RHPHPHLSFM | 1258.62 | 629.82 | 1258.63 | 629.82 | 2 | 12.78 |
| | RHPHPHLSFm | 1274.62 | 637.81 | 1274.62 | 637.81 | 2 | 10.50 |
| | RYPSYGL | 855.43 | 428.22 | 855.44 | 428.22 | 2 | 14.89 |
| | SRYPSYGL | 942.47 | 471.74 | 942.47 | 471.74 | 2 | 15.20 |
| | SRYPSYGLN | 1056.51 | 528.76 | 1056.51 | 528.76 | 2 | 14.19 |
| | SRYPSYGLNY | 1219.57 | 610.29 | 1219.57 | 610.29 | 2 | 16.26 |
| | SRYPSYGLNYY | 1382.64 | 691.82 | 1382.64 | 691.82 | 2 | 17.51 |
| | VLSRYPSY | 984.51 | 492.76 | 984.51 | 492.76 | 2 | 13.81 |
| | VLSRYPSYG | 1041.53 | 521.27 | 1041.54 | 521.27 | 2 | 13.44 |
| | VLSRYPSYGL | 1154.62 | 577.81 | 1154.62 | 577.81 | 2 | 16.60 |
| | VLSRYPSYGLN | 1268.66 | 634.83 | 1268.66 | 634.84 | 2 | 15.66 |
| ά- lb | AFHTSGYDTQ | 1126.48 | 563.74 | 1126.48 | 563.74 | 2 | 11.81 |
| | DKVGINYW | 994.50 | 497.75 | 994.50 | 497.75 | 2 | 18.44 |
| | FHTSGYDTQ | 1055.44 | 528.22 | 1055.44 | 528.23 | 2 | 11.48 |
| β-lg | KTKIPAVF | 903.57 | 452.29 | 903.57 | 452.29 | 2 | 14.03 |
| | KTKIPAVFKID | 1259.77 | 420.59 | 1259.77 | 420.60 | 3 | 15.07 |
| | LEKFDKAL | 963.55 | 482.28 | 963.55 | 482.28 | 2 | 13.25 |

606

607 *Protein access code at https://www.uniprot.org/.A:Alanine, D: Aspartic Acid, E: Glutamic Acid,

608 F: Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine,

609 P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y:

610 Tyrosine, lb: lactalbumin, lg: lactoglobulin, MW: Molecular Weight, RT: Retention Time.

611

612