Supplementary Materials

Understanding the molecular weight distribution of proteins and peptides

All chemicals required for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were procured from Bio-Rad (Bio-Rad, Hercules, USA). A vertical electrophoresis system (Bio-Rad Mini Protean 3 Cell along with power PAC1000) was used in experiment. Electrophoretic separation of proteins and peptides was performed by constant 200 V, 54 mA and 11W. Dilution of samples was performed with laemmli sample buffer (2X) and 10% of 2-mercaptoethanol. In individual well within stacking gel, 10 μL of appropriate diluted sample was loaded and running time was 60 min at RT. For gel staining purpose, 0.2% of Coomassie Brillant Blue R250 in 9% of acetic acid (96%) - 45% of ethanol was used. Gel staining was performed for 30 min at RT. Subsequently, de-staining of gel was performed with 50% (volume basis) of methanol-water and 10% (volume basis) of acetic acid at RT. Gel Doc System 2000 (Bio-Rad, CA, USA) was used to capture the gel image.

Immunoblotting

Clinically proved cow's milk positive human sera and polyclonal antibodies against casein, α-lactalbumin, β-lactoglobulin produced in rabbit were received from the Food Science Research Group, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary. Peroxidase-labelled goat anti-human Immunoglobulin E (IgE) secondary antibody and peroxidase-labelled goat anti-rabbit (Rb) Immunoglobulin G (IgG) secondary antibody were purchased from Sigma-Aldrich (Sigma-Aldrich, Schnelldorf, Germany). Prior to experiment, human sera were pooled, and IgG fraction of antibodies were purified from the polyclonal rabbit serum. Proteins and peptides from SDS-PAGE gels were transferred onto a polyvinylidene difluoride (PVDF) membrane with pore size 0.45 μm (Merck Millipore, MA, USA) according to the manufacturer's instruction manual. For that purpose, trans blot semi-dry transfer cell (Bio-Rad, CA, USA) was adopted and it was operated with 0.25 V and 0.08 mA/cm^2 for 1 h. Clinically proved cow's milk positive human sera (pooled) together with peroxidase-labelled goat anti-human IgE secondary antibody was used to identify the milk protein allergens. Furthermore, polyclonal antibodies, such as anti-casein Rb IgG, anti-α-lactalbumin Rb IgG and anti-β-lactoglobulin Rb IgG together with peroxidase-labelled goat anti-Rb IgG secondary antibody were used to identify the antigenicity of the milk proteins in samples. The binding patterns of antibody were visualised using a substrate solution, containing 4-chloronaphtol, hydrogen peroxide and ethanol in 16 mM phosphate buffered saline solution, pH 7.2. Image analysis of blot was carried out by Gel Doc 2000 system (Bio-Rad, CA, USA).

Determination of protein concentration

Appropriately diluted 100 μL of supernatant and 3 mL of Bradford reagent (Sigma-Aldrich, Schnelldorf, Germany) were mixed and vortexed. Subsequently, mixture was incubated at RT for 30 minutes in an incubator (HACH, Düsseldorf, Germany). Spectrophotometric analysis was performed with wavelength 580 nm in a UV-Vis spectrophotometer (EvolutionTM 300; Thermo ScientificTM, Waltham, MA, USA) at RT. Bovine serum albumin (Sigma-Aldrich, Schnelldorf, Germany) was used as a standard in assay. During colorimetric determination, a blank sample, prepared with 100 μL of DI water and 3 mL of Bradford reagent was used.

Ferric Reducing Ability of Plasma (FRAP) assay

2,4,6-Tris(2-pyridyl)-s-triazine was procured from the Sigma-Aldrich (Sigma-Aldrich, Schnelldorf, Germany). Appropriate diluted 100 μL of supernatant was mixed with 2.9 mL of reagent (5 mL of 20 mM of ferric chloride + 5 mL of 10 mM of 2,4,6-Tris(2-pyridyl)-s-triazine with 40 mM of hydrochloric

acid + 50 mL of 300 mM of sodium acetate buffer, pH 3.6) and incubated at temperature 35 °C for 30 min in an incubator (HACH, Düsseldorf, Germany). In this method, reduction of the Fe^{3+}-TPTZ (ferrictripyridyltriazine) complex to the Fe^{2+}-TPTZ at low pH in the presence of antioxidant peptides/ proteins produced blue color, which was measured with wavelength 593 nm in a UV-Vis spectrophotometer (EvolutionTM 300; Thermo ScientificTM, Waltham MA, USA) in RT. Ascorbic acid (99.7%, Merck, Darmstadt, Germany) was considered as a reference of antioxidant capacity of proteins and peptides. A blank sample was prepared with 100 μL of DI water and 2.9 mL of reagent for spectrophotometric analysis.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical-Scavenging assay

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was purchased from the Sigma-Aldrich (Sigma-Aldrich, Schnelldorf, Germany). Assay was performed with 100 μL of supernatant and 3.9 mL of 6×10^{-5} M of DPPH-methanol solution. Control sample was prepared with 100 μL of methanol and 3.9 mL of 6×10^{-5} M of DPPH-methanol solution. The mixtures were vortexed and incubated at RT in dark condition for 30 min. Subsequently, absorbance was measured at 517 nm with a UV-Vis spectrophotometer (EvolutionTM 300; Thermo ScientificTM, Waltham MA, USA). DPPH – radical scavenging activity was calculated based on percentage change of absorbance from control.

Estimation of angiotensin-converting-enzyme inhibitory activity

Recombinant angiotensin converting enzyme was received from Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Hungary. Enzymatic reaction mixture (final volume 200 μL in each well), consisted of 100 mM of TRIS HCl (pH 7), 50 mM of sodium chloride, 10 μM of zinc chloride, 15 μM of substrate Abz-FRK(Dnp)-P (Sigma-Aldrich, Schnelldorf, Germany), recombinant angiotensin converting enzyme (amount of the recombinant angiotensin converting enzyme was chosen to result in about 10-fold activity than that in human serum), and supernatants of milks and corresponding fermentation broth (in a dilution range of 10-fold to 10^6-fold) was used to determine IC_{50} value of samples. Activity of angiotensin-converting enzyme in the absence of the supernatants of samples was used to define uninhibited activity. Reaction was initiated by the addition of substrate. The level of inhibition was calculated as % of uninhibited activity in each plate. The values of K_M and V_{max} were determined by measuring the activity of 160 – 4860-fold diluted samples in the presence of 15 μM, 7.5 μM, 3.75 μM and 1.625 μM of substrate. Measurements were performed in a fluorescent plate reader (BMG Novostar, BMG Labtech, Germany) at temperature 37 °C. Changes in optical density were measured with excitation wavelength of 340 nm and emission wavelength of 405 nm for at least 20 cycles.

Microbiological Assay

Bacteriological agar powder, peptone, sodium chloride and soybean casein digestive medium were procured from Merck (Merck, Darmstadt, Germany). *Bacillus cereus*, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes CCM 4699* were obtained from the Strain collection unit of Hungarian University of Agriculture and Life Sciences, Budapest, Hungary. Overnight grown (freshly prepared) each culture from soybean casein digest agar plate was diluted with maximum recovery diluent (8.5 g of sodium chloride + 1 g of peptone in 1 L of de-ionized water) to reach the bacterial concentration 10^6 colony-forming units·mL^{-1} in individual tube. Freshly prepared bacterial cultures with 8×10^6 colony-forming units·mL^{-1} were spread on soybean casein digestive agar medium and agar well with diameter 5 mm was prepared. Agar wells were filled with 100 μL of supernatants. Microbial plates were incubated at temperature 37 °C for 48 h in an incubator (HACH, Düsseldorf, Germany). After 24 h of incubation, diameter of zone of inhibition in plates was measured by excluding the diameter of wells (5

mm) using a digital Vernier caliper (UEMATSU SHOKAI CO., LTD., Sendai, Japan).

Near Infrared (NIR) Spectroscopy and Aquaphotomics analysis

During tryptic hydrolysis of LMPC and subsequently proteolysis with lactic acid bacteria scanning was performed with wavelength range 900 - 1700 nm by diffuse reflectance mode considering 1.0 cm path length in cuvette at every minute. The spectral data was pre-treated to reduce spectral noise and baseline variations, occurred due to the scattering. The Savitzky-Golay (SG) smoothing with 15 points and second-order polynomial were used to reduce the noise and minimize their impact to the signal tendency. Standard normal variate (SNV) pre-treatment was also applied to correct the multiplicative interferences by light scattering. For the aquaphotomics analysis, the spectral data was truncated specifically in the wavelength range 1300-1600 nm (first overtone of O-H bond). Principal Component Analysis (PCA) was performed to observe the underlying hidden pattern in spectral results as well as to identify outliers. An aquagram was made from the spectral data to visualize the spectral pattern changes, occurred during tryptic hydrolysis of LMPC and subsequently proteolysis with lactic acid bacteria. Aquagram is represented by a star-shaped diagram, which showed normalized and averaged absorbance at the water matrix coordinates (WAMACS), corresponding to different water molecule species.