

INPLASY

INPLASY2026100036

doi: 10.37766/inplasy2026.10.0036

Received: 8 October 2026

Published: 8 October 2026

Corresponding author:

Saule Iskakova

saule-62@mail.ru

Author Affiliation:

S. Seifullin Kazakh Agrotechnical Research University.

Enzymatic hydrolysis of goat (*Capra hircus*) muscle proteins: from hydrolysates and peptides to digestibility and functional food ingredients - protocol for a systematic review

Mukhanbetova, N; Makangali, K; Iskakova, SA.

ADMINISTRATIVE INFORMATION

Support - This review received no specific grant from any funding agency.

Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY2026100036

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 8 October 2026 and was last updated on 8 October 2026.

INTRODUCTION

Review question / Objective Primary question. In studies of goat (*Capra hircus*) skeletal-muscle proteins, how do the type of protease and the hydrolysis conditions (pH, temperature, time, enzyme-to-substrate ratio, thermal pretreatment), as well as simulated gastrointestinal digestion, affect the degree of hydrolysis (DH), molecular weight distribution, peptide and amino-acid profile, digestibility, techno-functional properties and bioactivity of the resulting hydrolysates and peptides?

Framework (PICOS adapted for food technology). P (substrate): skeletal muscle of *Capra hircus* - meat, primal cuts, myofibrillar or sarcoplasmic protein fractions, meat extracts, raw or heat-treated. I (process): hydrolysis with exogenous proteases (commercial, plant, animal or microbial), single or sequential, and simulated gastrointestinal digestion. C (comparator): unhydrolysed protein; other enzymes, doses, times or temperatures within the same study; where available, the same

process applied to meat of other species in the same experiment. O (outcomes): DH, molecular weight distribution, peptide sequences, amino-acid profile, in vitro/in vivo digestibility, techno-functional properties, bioactivity (ACE, DPP-IV, antioxidant, antimicrobial, anti-inflammatory) and performance in food matrices. S (study design): in vitro, cell-culture, animal and pilot technological studies.

Secondary objectives. (i) To appraise the methodological quality and reporting of the evidence; (ii) to grade the level of evidence for bioactivity from crude hydrolysate to in vivo; (iii) to judge whether quantitative synthesis is feasible; (iv) to identify research gaps relevant to the development of goat-meat hydrolysates as ingredients of specialised (including gerodietetic) meat products.

Rationale Goat meat is a lean red meat with a high protein content and a favourable fatty-acid profile, and Kazakhstan has one of the largest small-ruminant populations in Central Asia, much of it kept in households. Yet goat meat is rarely

processed industrially and is hardly used as a source of functional food ingredients.

Enzymatic hydrolysis is a mild, controllable way of converting muscle proteins into hydrolysates with improved digestibility and into peptides with antihypertensive (ACE-inhibitory), antidiabetic (DPP-IV-inhibitory), antioxidant and other activities. Such ingredients are of particular interest for specialised foods, including products for older adults, in whom adequate, easily digestible protein and cardiometabolic health are priorities.

Most of the evidence on meat-derived hydrolysates concerns beef, pork, poultry and fish, and research on goats has focused on milk proteins. Studies on goat meat are few and are scattered across national journals, conference proceedings and reports on slaughter by-products. They use different enzymes, processing conditions and analytical methods (for example, DH determined by OPA, TNBS, pH-stat or amino-nitrogen titration), which makes their results difficult to compare. To our knowledge, no systematic review has examined goat skeletal-muscle proteins along the whole chain from raw material to hydrolysate, peptide, digestibility and food application.

A systematic review is therefore needed to collate this dispersed evidence, appraise its quality, establish which processes and outcomes have been studied, and define methodological standards for future work. The results will directly inform the authors' experimental programme on thermally pretreated goat meat hydrolysed with pepsin and on meat products for specialised nutrition.

Condition being studied The review does not concern a disease. The "condition" is the enzymatic proteolysis of goat (*Capra hircus*) skeletal-muscle proteins and the technological and nutritional properties of the resulting hydrolysates and peptides.

Goat skeletal muscle consists mainly of myofibrillar proteins (myosin heavy chain $\approx$ 200–220 kDa, actin $\approx$ 42 kDa), sarcoplasmic proteins and connective-tissue collagen. During hydrolysis, endo- and exopeptidases cleave these proteins into polypeptides, oligopeptides and free amino acids. The extent of cleavage is expressed as the degree of hydrolysis, and the product is characterised by its molecular weight distribution and peptide composition. Both depend on enzyme specificity and on process conditions, and also on the state of the substrate, which is altered by heat treatment (denaturation, oxidation, aggregation).

Short peptides, usually below 3 kDa, may inhibit angiotensin I-converting enzyme or dipeptidyl peptidase-IV, scavenge radicals or chelate metals,

and hydrolysis may change solubility, emulsifying, foaming and water-binding properties and the digestibility of the protein. Whether these effects persist after gastrointestinal digestion, absorption and incorporation into foods determines their practical value. The review examines this whole chain for goat muscle proteins.

METHODS

Search strategy Databases: Scopus; Web of Science Core Collection (SCI-EXPANDED, ESCI, CPCI-S); PubMed/MEDLINE; CAB Abstracts; FSTA; AGRIS; CAS SciFinder-n (peptide sequences). Other sources: Google Scholar (first 200–300 records per query); eLIBRARY.ru (RSCI) and CyberLeninka (Russian- and Kazakh-language literature); Garuda/SINTA (Indonesia); KCI (Korea); CNKI (China); ProQuest Dissertations, BASE, CORE; backward and forward citation searching; contact with authors. No language or date limits. Search dates and record counts will be reported per PRISMA-S.

Scopus: (TITLE-ABS-KEY((goat* OR caprine OR chevon OR cabrito OR "Capra hircus" OR "goat kid*") W/5 (meat* OR muscle* OR myofibril* OR sarcoplasm* OR loin OR longissimus OR carcass* OR flesh)) OR TITLE-ABS-KEY(chevon OR cabrito)) AND TITLE-ABS-KEY(hydrolys* OR hydrolyz* OR proteoly* OR protease* OR peptidase* OR alcalase OR pepsin OR trypsin OR chymotrypsin OR pancreatin OR papain OR bromelain OR flavourzyme OR protamex OR neutrase OR "in vitro digest*" OR "gastrointestinal digest*" OR infogest OR digestib*) AND TITLE-ABS-KEY(peptid* OR hydrolysate* OR "degree of hydrolysis" OR "molecular weight" OR "amino acid*" OR bioactiv* OR antioxid* OR "ACE inhibit*" OR angiotensin* OR "DPP-IV" OR antimicrob* OR emulsif* OR foam* OR solubilit* OR "functional propert*" OR bioavailab* OR bitter*) AND NOT TITLE(milk OR casein* OR whey OR cheese* OR dairy OR colostrum) AND NOT TITLE-ABS-KEY(goatfish* OR goatgrass)

Web of Science: the same blocks with TS= and NEAR/5 instead of W/5; dairy terms excluded with TI=.

PubMed: ("Goats"[Mesh] OR goat*[tiab] OR caprine[tiab] OR chevon[tiab] OR "Capra hircus"[tiab]) AND ("Meat"[Mesh] OR "Meat Proteins"[Mesh] OR "Muscle Proteins"[Mesh] OR meat*[tiab] OR muscle*[tiab] OR myofibril*[tiab]) AND ("Protein Hydrolysates"[Mesh] OR "Hydrolysis"[Mesh] OR "Proteolysis"[Mesh] OR "Peptides"[Mesh] OR "Digestion"[Mesh] OR hydrolys*[tiab] OR proteoly*[tiab] OR peptide*[tiab] OR pepsin[tiab] OR alcalase[tiab] OR infogest[tiab]

OR digestib*[tiab]) NOT (milk[ti] OR casein*[ti] OR whey[ti] OR cheese*[ti])

CAB Abstracts / FSTA (Ovid or EBSCO): thesaurus terms (goat meat; goats; protein hydrolysates; proteolysis; peptides; digestibility) combined with free-text terms as above, using adj5 (Ovid) or N5 (EBSCO).

Russian/Kazakh: козлятина OR «мясо коз» AND (гидролиз OR гидролизат OR пептид* OR переваримость OR атакуемость); ешкі еті AND (гидролиз OR пептид). Indonesian: hidrolisat protein daging kambing; peptida bioaktif kambing. Dairy exclusions applied to the title only will be audited by screening a random sample of up to 200 removed records; if any is relevant, the exclusion will be relaxed.

Participant or population Not applicable to human participants. The “population” is the substrate: skeletal muscle of domestic goats (*Capra hircus*) of any breed, age or sex - meat, primal cuts, myofibrillar or sarcoplasmic protein fractions, or meat extracts, raw or heat-treated. Mixed meat-and-bone material will be included but analysed separately. Excluded: goat milk and dairy proteins; slaughter by-products without skeletal muscle (viscera, blood, bone, skin, placenta), which will be listed but not synthesised; meat of other species unless compared with goat meat within the same experiment.

Intervention Hydrolysis of goat muscle proteins with exogenous proteases – commercial preparations (e.g., Alcalase, Protamex, Flavourzyme, Neutrase), animal enzymes (pepsin, trypsin, chymotrypsin, pancreatin), plant enzymes (papain, bromelain, ficin) or microbial enzymes - used singly or sequentially, with or without thermal pretreatment, under any pH, temperature, time and enzyme-to-substrate ratio. Simulated gastrointestinal digestion (e.g., INFOGEST) of goat meat or of its hydrolysates is also included. Heat, acid or alkaline treatment without proteases, ordinary ageing and fermentation with starter cultures are excluded.

Comparator Unhydrolysed goat meat or protein (native or heat-treated without enzyme); alternative enzymes, enzyme doses, hydrolysis times, temperatures or pretreatments tested within the same study; and, where reported in the same experiment, the same process applied to meat of other species. Studies without a comparator will be eligible if they report at least one quantitative outcome for a defined hydrolysate.

Study designs to be included Experimental in vitro studies (hydrolysis, simulated digestion, cell

models), in vivo animal studies, human studies and pilot technological studies, published as peer-reviewed articles, full conference papers or theses with complete methods. Reviews, patents, abstracts without full text and purely in silico studies are excluded.

Eligibility criteria Included studies must (i) use goat skeletal-muscle material as defined above; (ii) apply exogenous proteases or simulated gastrointestinal digestion; and (iii) report at least one of DH, molecular weight distribution, peptide sequence, amino-acid profile, digestibility, a techno-functional property, a bioactivity or performance in a food matrix, with an analytical method described. Excluded are studies limited to sensory evaluation, to proximate composition without hydrolysis, or to in silico prediction; duplicate publications (the most complete report will be retained); and studies whose full text cannot be obtained after contacting the authors. No restrictions on publication year, language or country.

Information sources Electronic databases: Scopus; Web of Science Core Collection; PubMed/MEDLINE; CAB Abstracts; FSTA; AGRIS; CAS SciFinder-n. Search engines and regional sources: Google Scholar; eLIBRARY.ru (RSCI); CyberLeninka; Garuda and SINTA-indexed journals (Indonesia); KCI (Republic of Korea); CNKI (China). Grey literature: ProQuest Dissertations & Theses, BASE, CORE, national dissertation repositories. Supplementary methods: backward and forward citation searching of included studies and relevant reviews (Scopus, Web of Science, Google Scholar), and e-mail contact with corresponding authors to obtain missing data or full texts. Publisher platforms (ScienceDirect, SpringerLink, Wiley, Taylor & Francis) will be used only to retrieve full texts. Searches will be updated before final analysis.

Main outcome(s) (1) Degree of hydrolysis (%), with the method (OPA, TNBS, pH-stat, amino-nitrogen/total-nitrogen, TCA-soluble nitrogen) and the time point. (2) Molecular weight distribution (kDa or Da) and its method (SDS-PAGE, Tricine-SDS-PAGE, size-exclusion chromatography, ultrafiltration), including the proportion of peptides below 3 kDa. (3) Bioactivity: ACE inhibition (% at a stated concentration, IC₅₀ in µmol/L or mg/mL), DPP-IV inhibition, and antioxidant activity (DPPH, ABTS, ORAC, FRAP; IC₅₀/EC₅₀ or µmol TE/g). Effect measures: the reported value with SD or SE and n, and within-study differences between hydrolysed and unhydrolysed material or between conditions (mean difference or ratio of means).

Additional outcome(s) Peptide sequences and their validation (synthetic peptides, stability to digestion); amino-acid profile; in vitro digestibility (INFOGEST or other) and intestinal transport (Caco-2); in vivo effects (e.g., blood pressure in spontaneously hypertensive rats) and safety; anti-inflammatory and antimicrobial activity; techno-functional properties (solubility, emulsifying activity and stability, foaming capacity and stability, water- and fat-binding capacity); bitterness and sensory acceptance; yield of hydrolysate; performance and stability in food matrices.

Data management Records from all sources will be exported in RIS format into Zotero, deduplicated automatically and then manually, and uploaded to Rayyan for blinded screening. Data will be extracted into a piloted Excel form (study, substrate, hydrolysis parameters, outcomes, risk of bias, page source of each value). NM will extract all data and SAI will verify every numerical value; discrepancies will be resolved by discussion with KM. Values available only in figures will be digitised and flagged as approximate. Reported values will be recorded unchanged; unit corrections will be made only when confirmed by recalculation and will be flagged. A decision log will document every eligibility and coding decision. All files (search exports, screening decisions, extraction form, risk-of-bias assessments) will be archived and deposited as supplementary material.

Quality assessment / Risk of bias analysis

Because no validated tool exists for food-protein hydrolysis studies, two reviewers (NM, SAI) will independently apply a ten-domain checklist adapted from the QUIN tool for in vitro studies and, for animal experiments, from SYRCLE. Domains: (1) identification of raw material (species, breed, age, sex, muscle, post-mortem time) - critical; (2) substrate preparation; (3) enzyme identity, supplier and verified activity, basis of E:S - critical; (4) reproducibility of conditions (pH control, temperature, time, substrate concentration); (5) controls (unhydrolysed substrate, enzyme blank, positive control); (6) inactivation and separation; (7) biological versus technical replicates; (8) analytical validity (DH method, calibration, units of IC50, FDR for peptide identification); (9) statistics; (10) selective reporting and conflicts of interest. Each domain is scored 0–2; overall risk is low (> 70%), moderate (50–70%) or high (< 50%), and a score of 0 in a critical domain precludes a low rating. Bioactivity evidence will be graded L0 (in silico) to L8 (human study). Certainty of evidence will be judged with GRADE domains adapted for indirectness of in vitro data.

Strategy of data synthesis Studies will be grouped by stratum (A - goat skeletal muscle; A* - mixed meat and bone), by process (exogenous enzymes versus simulated digestion) and by outcome domain. Results will be presented in structured tables and evidence maps and synthesised narratively according to the SWiM (Synthesis Without Meta-analysis) guideline, using within-study effect directions and magnitudes; vote counting will be based on the direction of effect, not on statistical significance.

Meta-analysis will be performed only if at least three studies report the same outcome with the same analytical method (e.g., DH by OPA for the same enzyme, or ACE IC50 by the same assay in molar units), comparable processes and measures of dispersion. Effect sizes will then be pooled with a random-effects model (REML with Hartung–Knapp adjustment), heterogeneity assessed with τ^2 , I^2 and prediction intervals, and dose– or time–response relationships explored by meta-regression if ten or more data points are available. Publication bias will be explored with funnel plots only if ten or more studies contribute. Scatter plots of DH against time or enzyme dose will be used for visual synthesis. Analyses will be done in R (metafor).

Subgroup analysis If data permit, subgroups will be compared by: enzyme class (endoprotease, exopeptidase preparation, sequential multi-enzyme, gastrointestinal enzymes); process type (exogenous hydrolysis versus simulated digestion); thermal pretreatment (none, $\leq 70^\circ\text{C}$, $71\text{--}100^\circ\text{C}$, $> 100^\circ\text{C}$); substrate (whole meat, myofibrillar fraction, sarcoplasmic fraction, extract) and primal cut; DH method; molecular weight fraction (whole hydrolysate versus $< 3\text{ kDa}$ fraction); and level of bioactivity evidence. If quantitative pooling is not possible, these comparisons will be made descriptively.

Sensitivity analysis Sensitivity analyses will examine the robustness of conclusions by: excluding studies at high risk of bias; excluding mixed meat-and-bone substrates (stratum A*); excluding values digitised from figures; excluding values requiring unit correction; excluding studies in which the DH method was not reported or was inappropriate; and excluding non-peer-reviewed sources (theses, conference papers). If meta-analysis is performed, leave-one-out analysis will also be conducted. Findings that change direction or interpretation in these analyses will be reported as not robust.

Language restriction No language restrictions. Non-English reports will be translated with

machine translation, and all numerical data will be verified by a native speaker or a second reviewer.

Country(ies) involved Kazakhstan (S. Seifullin Kazakh Agrotechnical Research University; NJSC Astana Medical University, Astana).

Other relevant information Goat slaughter by-products (viscera, blood, bone, skin, placenta) and heat-only studies identified by the search will be listed in a supplementary table with reasons for exclusion and discussed qualitatively as complementary evidence for whole-carcass valorisation. Any deviation from this protocol will be documented and justified in the final report. The review will be reported according to PRISMA 2020, PRISMA-S and SWiM.

Keywords goat meat; chevon; enzymatic hydrolysis; protein hydrolysate; bioactive peptides; degree of hydrolysis; ACE inhibition; digestibility; functional food ingredients.

Dissemination plans The completed review will be submitted to a peer-reviewed journal in food science or meat science (e.g., Food Science of Animal Resources) and presented at national and international scientific conferences. Its results will inform the authors' experimental work on goat-meat hydrolysates and meat products for specialised nutrition and will be shared with meat-processing enterprises in Kazakhstan. Extraction forms and search strategies will be made openly available as supplementary material.

Contributions of each author

Author 1 - Nazira Mukhanbetova - Developed the methodology with K. Makangali, performed the literature searches, screening and data extraction, conducted the formal analysis, curated the data, and drafted the manuscript.

Email: mukhanbetova.n@amu.kz

Author 2 - Kadyrzhan Makangali - Conceived the review, designed the methodology with N. Mukhanbetova, resolved disagreements between reviewers, drafted the manuscript, and critically revised it for important intellectual content.

Email: k.makangali@kazatu.edu.kz

Author 3 - Saule Iskakova - Acted as the second independent reviewer in screening, curated and validated the extracted data and risk-of-bias assessments, and critically revised the manuscript. She is the corresponding author.

Email: saule-62@mail.ru