

Bioactive Peptides From Fermented Dairy Products: Production Methods and Functional Claims

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ABSTRACT

Fermented milks and cheeses are often described as sources of bioactive peptides, short protein fragments that, in laboratory tests, can inhibit angiotensin-converting enzyme, bind minerals, scavenge radicals or modulate immune cells. Such statements are attractive to producers and consumers, and they raise a practical question: how much of the claimed function is established for the products actually sold? This narrative mini review outlines how peptides arise during fermentation, ripening and enzymatic treatment, and how the choice of starter strain, substrate and process shapes what is released. It then examines the chain of inference that links a peptide sequence to a functional claim on a label. Much of the supporting evidence comes from in vitro assays and animal models, which say little about survival in the gut, absorption or effect in people. Human trials exist for a few casein-derived peptides and fermented milks, but they are generally modest in size, differ in product and population, and are hard to compare. Claims are only as strong as the weakest link in that chain. The review closes with the evidence a producer or regulator should expect before wording a claim, and it offers no advice to individuals.

Keywords: Bioactive peptides; Fermented milk; Lactic acid bacteria; ACE inhibition; Bioavailability; Functional claims; Narrative review

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Article Class: Mini Review

1. Introduction

Milk protein is, among other things, a store of hidden sequences. Casein and whey proteins are long chains whose short internal stretches are inactive while the protein is intact, yet some of them show measurable activity once they are cut free [1, 2]. Dairy science has called these fragments bioactive peptides for several decades, and fermented milks and cheeses are among the foods in which they are most often sought, because

the microbes that sour milk also break down its protein [3, 4].

The interest is easy to understand. A peptide that inhibits angiotensin-converting enzyme in a laboratory assay, or binds calcium, or scavenges a radical, invites the thought that the food carrying it might do something similar in the body [5, 6]. Producers have built products and marketing around that thought, and a substantial literature now reviews the subject [7, 8, 9]. What the

literature also shows, if read closely, is how many separate steps lie between a sequence on a chromatogram and a sentence on a label.

This mini review does not try to catalogue every peptide ever reported. It asks two narrower questions. First, how do production choices (strain, substrate, enzymes, time) influence which peptides appear in a fermented dairy product? Second, how strong is the chain of inference that carries a laboratory finding to a functional claim, and where is it weakest? The treatment is deliberately cautious. Much of what is said about these peptides is repeated more often than it is tested, and we try to separate the two.

The article is a narrative synthesis of published work. It reports no new data, it quotes no numerical results that we could not check against the primary papers, and it gives no advice to individual consumers or patients. Where a topic is thin, we say so.

2. Literature Review

2.1 What Counts as a Bioactive Peptide

Bioactive peptides are usually described as short fragments of food protein, typically a few to a few dozen amino acids long, that exert an effect beyond basic nutrition [1, 7]. Milk-derived examples are reported to inhibit angiotensin-converting enzyme, bind minerals, act as antioxidants or antimicrobials, interact with opioid receptors, or modulate immune responses [3, 4, 10]. The definition is functional, not structural, and that has consequences: a sequence qualifies because of what it does in an assay, so the list of such peptides grows with the number and kind of assays people choose to run.

Databases now collect thousands of reported sequences and their assay results, and they allow a new peptide list to be searched against known activities by computer [11]. That is useful for screening. It also makes it very easy to label a product as containing bioactive peptides on the strength of a match to a database entry, which is a prediction about a sequence and not a measurement of the food. The distinction between a peptide that could be active and one that has been shown to be active in the product matters throughout this review.

2.2 Proteolysis in Fermented Dairy Foods

Lactic acid bacteria are nutritionally demanding and grow poorly in milk unless they can obtain amino acids from its protein. They manage this with a coordinated proteolytic system: a proteinase on the cell envelope that cuts casein

into larger fragments, transport systems that carry oligopeptides into the cell, and a battery of intracellular peptidases that finish the job [12, 13]. Seen from the dairy side, that machinery is a peptide factory that also happens to be a peptide shredder.

Whether a given peptide survives in the product depends on the balance between release and further degradation. Peptidases and amino acid catabolism in these bacteria can remove the very fragments that an assay would flag as active [14]. This is one reason that different strains, and even the same strain at different stages of growth, can produce fermented milks with quite different peptide profiles, and why reports on one starter culture cannot be assumed to apply to another [15, 16]. Reviews of the subject stress that activity is often latent in the parent protein and is released, modified or destroyed by processing [2].

Cheese adds time. During ripening, proteolysis continues slowly under the action of residual enzymes and the microbiota, so the peptide pool of a young cheese differs from that of an aged one of the same variety [17]. Kefir, made with a community of bacteria and yeasts instead of a defined starter, produces a very complex mixture, and peptide profiling of it has shown how many distinct fragments can arise from caseins even in a single product type [18]. Whey proteins, which are more resistant to the proteinases of many starters, form a separate substrate with its own literature [19].

2.3 Enzymes, Strains and Process Design

Producers who want more control rarely rely on the starter alone. A common strategy is to treat milk protein with a defined proteinase, before or alongside fermentation, and then to let the culture continue the work [20]. Casein digested with the extracellular proteinase of a *Lactobacillus helveticus* strain was one of the early routes to antihypertensive peptides in animal tests [21, 22], and later human work used casein hydrolysate in a similar spirit [23]. Table 1 summarises these routes and the main uncertainty that attaches to each.

The practical difficulty is that process variables interact. Temperature, pH, the stage at which the culture is harvested, the heat treatment of the milk, the dose of enzyme, the fat and solids content and the storage period can all shift the peptide profile, and each is a decision a producer may change for reasons that have nothing to do with peptides. A product that is reformulated for taste or shelf life may therefore change its peptide

content without anyone intending it to. Such differences are small in any one batch, but they matter when a claim is meant to hold for every pack on the shelf. Strain choice is the most visible variable, yet it is far from the only one [1, 15].

Table 1. Routes by which peptides arise in fermented dairy products.

Route	How peptides arise	Main uncertainty
Starter proteolysis	Cell-envelope proteinase and peptidases release and trim fragments [12, 13]	Strain-specific; fragments may be broken down again [14]
Enzyme treatment plus culture	Added proteinases act on milk protein before or during fermentation [20, 23]	Product no longer purely fermented?
Cheese ripening	Slow proteolysis over the ripening period [17]	Profile shifts with age and variety
Kefir and mixed cultures	Mixed microbiota and grains act together [18]	Hard to standardise batch to batch
Whey-based systems	Whey proteins serve as substrate [19]	Fewer human data

Note: Qualitative summary. Peptide content differs with strain, substrate and time; no quantities are given because they are not transferable between products.

2.4 Activities Reported for Dairy Peptides

The activity most often cited for fermented milk is inhibition of angiotensin-converting enzyme, the enzyme that converts angiotensin I to the vasoconstrictor angiotensin II. Peptides with this property were purified from a cultured sour milk in the 1990s [24] and tested in spontaneously hypertensive rats [25]. Short sequences rich in proline, which are released from beta- and kappa-casein, feature prominently, and structure-activity work on di- and tripeptides suggests that the amino acids at the carboxyl end of the chain carry much of the influence on potency [5, 26]. Cheese has also yielded peptides with this activity [17].

Other activities are reported with less fanfare. Casein phosphopeptides bind minerals, certain fragments show antioxidant behaviour in chemical assays, some are antimicrobial in culture, and others have been linked to immune or opioid-type signalling [1, 4, 10]. Each of these has its own assay, its own caveats and its own distance to travel before it becomes a statement about people. We concentrate on ACE inhibition because it has the longest record of human testing, and because it illustrates the difficulties clearly.

2.5 Digestion, Absorption and Bioavailability

A fermented milk is swallowed and digested, and the peptides in the jar are not the peptides that

reach the intestinal wall. Gastric acid, pepsin, pancreatic proteinases and brush-border peptidases rework everything, and peptides made in the product may be destroyed, trimmed or newly created on the way [27, 28]. Standardised protocols for static digestion in the laboratory now exist so that studies can be compared [29, 30], but they are simplifications; the real tract has food matrix effects, variable transit, and individual differences in enzymes that a tube cannot reproduce. A digestive tract that has been given a real meal behaves differently from a tube of buffer.

Human sampling studies are valuable for exactly this reason. Work in healthy volunteers who consumed milk protein showed that peptides appear progressively along the small intestine, which gives a direct look at what is actually present at the site of absorption [31]. For the lactotripeptides from casein, a study of a lactotripeptide-enriched milk beverage reported that fragments were absorbed intact into the circulation [32]. These are important results, but they concern particular peptides in particular products, and the field has few such studies compared with the number of peptides that have been proposed.

2.6 From Product to Claim

A functional claim joins four statements: the product contains the peptide; the peptide survives and is absorbed; the peptide acts on a relevant target; and that action changes an outcome people care about. Each statement requires a different kind of evidence, and an assay can only speak to the first, and to the third in a limited way [33, 34]. The wording on a label is a fifth step, in which a cautious scientific finding is condensed into a sentence that a shopper can read in seconds.

A further confusion arises from vocabulary. A fermented food is defined by desired microbial growth and enzymatic conversion of its components, and the term says nothing about live organisms at the point of consumption or about health effects [35]. A probiotic is a different thing, defined by live microorganisms shown to confer a health benefit [36]. A product can be fermented without being probiotic, probiotic without being rich in peptides, and rich in peptides without any of the effects its marketing implies. The terms are often blurred on packaging, and Table 3 sets out some common wordings and the evidence each would reasonably require. Readers who keep the three terms apart will find most of the confusion disappears.

Table 3. Common claim wordings and the evidence a reader might reasonably expect.

Wording	Evidence expected	Typical gap
Contains ACE-inhibitory peptides	Assay plus characterised product [11, 34]	Tube activity, not human effect
Supports healthy blood pressure	Controlled trials with this product [37, 41]	Product and population specific
Made with live cultures	Process description [35]	Silent on peptide content
Probiotic	Strain-level evidence [36]	A different question from peptides
Clinically proven	Independent replication [39, 44]	Rarely defined or repeated

Note: Examples of wording are illustrative, not quotations from any product or regulator.

3. Materials and Methods

3.1 Scope and Question

The review asks a two-part question. How do production methods for fermented dairy products determine which peptides are present? And how well does the published evidence support the functional claims commonly made for those peptides? We divided the second part into the links of the inference chain described above (content, survival, absorption, action, outcome, wording) and asked which links have been tested in humans. None of these steps is difficult, but each one is easy to skip.

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the proteolytic systems of lactic acid bacteria and the production of peptides in fermented milks and cheeses; the structure and testing of angiotensin-converting enzyme inhibitors derived from milk; digestion, absorption and bioavailability; and the methodological and regulatory-style literature on how claims are judged. Sources were chosen for relevance, standing in the field and clarity of method. Studies on other foods are used only for what they teach about method.

We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. The consequence is that the review is a better guide to the structure of the evidence than to the exact size of any reported effect, and it avoids quoting figures from individual trials that could not be checked against the primary papers for this article.

3.3 Appraisal Framework

To decide how much a report can carry, we asked four questions of each study type. Does it show activity, survival, absorption or an outcome? Was the product the same as, or comparable to, the one being claimed for? Were the participants or animals of a kind to whom the claim would apply, and was there a control [37, 38]? And can the result be repeated by an independent group, or by pooling the available trials [39, 40]? Table 2 sets out the study types and what each can and cannot show.

Table 2. Kinds of evidence behind a peptide claim and what each can show.

Evidence type	What it can show	What it cannot show
Enzyme assay (in vitro)	Activity of a sequence in a tube [26, 32]	Survival, absorption, effect
Digestion model	Fate of peptides under standard conditions [29, 30]	Individual variation in people
Animal study	Biological plausibility in a living system [21, 25]	Transfer to people at dietary doses
Absorption study	Whether fragments reach the blood [27, 32]	Whether they act once there
Small human trial	Response in a defined group [41, 42]	General effect; long-term outcome
Pooled analysis	Average direction across trials [39, 40]	Reason for differences between trials

Note: Tiers are the authors' simplification of the study types discussed in the cited literature.

4. Results

4.1 What Production Choices Determine

The first finding is that production choices matter a great deal and that their effect is rarely transferable. Starter strains differ in their proteolytic equipment, so fermented milks started with different strains of the same species can differ in the peptides they contain [15, 16]. Early work comparing starters showed that peptides with ACE-inhibitory activity could be produced in fermented milk by particular strains, with the activity depending on the strain used [16]. Enzyme treatment adds a second layer of control, and ripening or mixed-culture fermentation add others (Figure 1) [18, 20]. This is why the same species of starter can look excellent in one report and unremarkable in the next.

The second finding is that the peptide pool is dynamic. Release and degradation proceed together, so a measurement taken at one time describes one moment in a changing system [12, 14]. A peptide count at the end of fermentation

may differ from a count at the end of shelf life, and neither is a count at the point of digestion. Claims that fail to say when and in which sample the peptides were measured are hard to interpret.

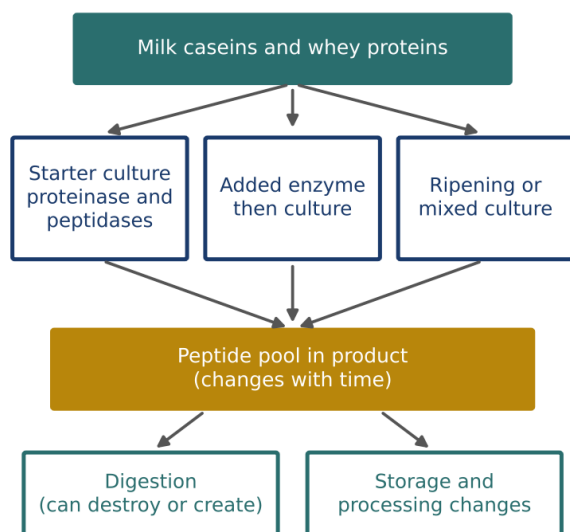


Figure 1. Three production routes converge on a peptide pool that later steps can change (schematic, not data).

4.2 Evidence From Laboratory and Animal Work

Three kinds of preclinical evidence dominate the literature (Figure 2 shows how far each kind answers the questions that matter). Enzyme assays measure how strongly a sequence inhibits a target enzyme, and they are cheap, quick and reproducible [26]. Animal studies, most often in spontaneously hypertensive rats, test whether a preparation lowers blood pressure in a living system [21, 25]. Digestion models estimate what is left of a peptide after passage through simulated gastric and intestinal fluids [29, 30].

Each has a well-known limitation. An assay result describes a peptide in a buffer; it does not tell us what remains after digestion or whether the peptide reaches its target [27]. Animal doses are frequently far above what a person would obtain from a serving of fermented milk, and the animal model, being a hypertensive strain, does not stand in for the general population. Digestion models are useful for ranking but cannot mimic every feature of a human meal. The sequence from assay to animal to human is therefore a series of steps, any of which may fail, and it is a pattern in the field that a candidate which looks excellent at the first step loses its edge by the third.

An important practical point follows. Potency in an assay is not a good predictor of effect in a body, because a very potent peptide that is destroyed in the gut is of no use, while a modest one that

survives may matter more [27, 28]. This is why reviews of the area recommend that candidate peptides be validated through absorption and in vivo tests, not selected on assay potency alone [32, 34].

	Activity	Survives digestion	Reaches blood	Changes outcome
Enzyme assay	direct	no	no	no
Digestion model	partly	direct	no	no
Animal study	direct	partly	partly	partly
Absorption study	no	partly	direct	no
Small human trial	no	no	partly	direct

Qualitative grid for illustration, not data

Figure 2. How far each kind of evidence answers each question (qualitative grid, not data).

4.3 Human Studies

Human trials of fermented milk and related casein preparations began in the 1990s. A placebo-controlled study of sour milk in hypertensive subjects [41], a study of a fermented milk high in bioactive peptides [42], work with a powdered *Lactobacillus helveticus*-fermented milk in people with high-normal blood pressure or mild hypertension [43], and a study of casein hydrolysate [23] are among the best-known examples. Pooled analyses of trials of the casein-derived lactotripeptides have reported a favourable average difference in blood pressure [39], and analyses of probiotic fermented milks and of probiotics more broadly have also reported small average differences [40, 44].

These findings are interesting, but they have to be read with the design in mind. The trials differ in product (a fermented milk, a powder, a hydrolysate, an enriched beverage), in dose, in length, in the population studied and in the way blood pressure was measured. Critical reviews have pointed out that results are not uniform across studies and populations, and that the size of the effect appears to depend on the context in which the product is tested [37, 38]. Studies are generally of modest size, and pooling them cannot remove differences in the products that went into them. The point is not that a study is poor, only that its result travels less far than its abstract suggests.

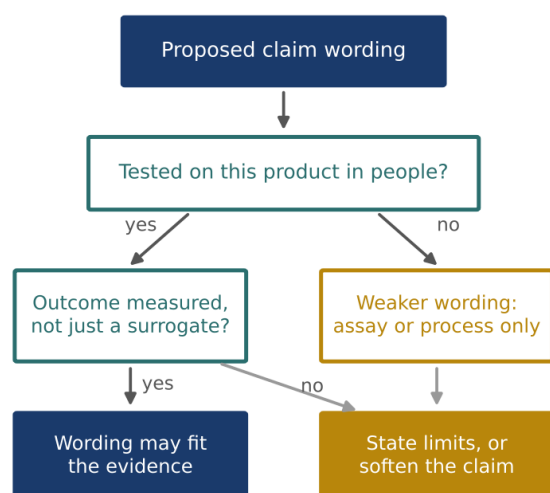
There is also the question of what a blood-pressure difference measured in a trial means for outcomes that matter to people. A surrogate measure can

move without any change in later disease, and no review we have cited claims otherwise. For that reason we regard the human evidence as suggestive for a handful of products, not as established for fermented dairy foods as a class. The same caution applies to other proposed activities, for which the human record is thinner still [10, 33].

4.4 Reading the Gap Between Evidence and Wording

Placed side by side, the pieces make an uneven picture. Content in the product is the best-studied link for a few products and the least studied for most; survival and absorption have been examined for a few peptides; clinical action has been examined for fewer; and independent replication is rarer again [9, 33]. A claim worded as though every link were secure can therefore be technically derived from real studies and still overstate what they show.

Wording problems are not always dishonest. They often arise from compression. A study of one fermented milk in one population becomes “fermented milk lowers blood pressure” in a summary, and that sentence becomes “contains peptides that support healthy blood pressure” on a pack. Each step is small, and the end result is a statement that none of the original investigators made. Figure 3 offers a decision sketch for testing a given wording against the chain.



Schematic decision sketch; not a regulatory test

Figure 3. Decision sketch for testing whether a claim wording is supported (schematic).

Put plainly, the literature supports a cautious statement. Fermented dairy foods can contain bioactive peptides, production choices influence which ones, a few casein-derived peptides have been studied through absorption and in trials, and the human evidence for an effect is suggestive but

limited and product-specific. A generalised claim for fermented dairy products as a class is not supported by what we can cite with confidence. That is a modest statement, and it is the one the evidence will currently bear.

4.5 Analytical and Labelling Questions

Two further issues deserve attention. The first is analytical. Peptides in a complex food are measured by mass spectrometry and related methods, and the number reported depends heavily on the method and the threshold for identification; the detailed profiling of kefir illustrates how many fragments can be seen, and also how hard it is to say which of them matter [11, 18]. Quantifying a specific active peptide against a standard is a stronger approach than counting sequences, but it is done less often.

The second concerns labelling. A claim attached to a food presupposes that the product on the shelf is the product that was tested. Batch variation in fermentation, differences between brands using nominally the same culture, and changes in recipe over time all weaken that presupposition [15, 20]. A claim supported by trials of one named product cannot be borrowed by another, and a label that cites the literature on peptides in general, without data on the product itself, is making a weaker claim than it may appear. A reader of a product claim needs to know which of these links the claim actually rests on.

5. Discussion

5.1 Interpretation

The main finding of this review is a conditional one. Fermented dairy products are a plausible source of bioactive peptides, and for a few well-studied casein-derived sequences there is a respectable chain of evidence from assay through animal work to small human trials. Beyond that, the chain thins quickly. For most reported peptides and most products, the evidence stops at the assay, and the step from tube to person has not been taken [27, 33].

The second conditional concerns generalisation. Because production choices change the peptide profile, a result from one named product belongs to that product. Evidence on one starter does not transfer to another strain of the same species, nor evidence on a hydrolysate to a ripened cheese [2, 15]. Reviews that group fermented dairy foods together are therefore describing a category whose members behave differently.

A third point is about the role of the food matrix. Peptides are consumed in a food that also supplies

protein, fat, minerals and live or heat-treated microorganisms, and any of these could contribute to an observed effect, or mask one [35, 40]. A trial of a fermented milk cannot, by itself, assign an effect to the peptides, and trials that compare a peptide-rich product with a closely matched peptide-poor control are what is needed to do so. Nothing in this reading argues against fermented dairy foods; it argues for being precise about what is claimed for them.

5.2 Limitations

This is a narrative mini review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was carried out, and the review does not quote numerical results from individual trials, because these could not be checked against the primary reports for this article. A reader who needs an effect size should consult the original studies directly, or wait for a formal systematic review, which methods exist to support [38, 39].

We have focused on blood pressure because it has the most evidence, and this inevitably narrows the picture. Other proposed activities, such as effects on glucose handling, immunity or mineral absorption, are touched on but not appraised, and some of them rest almost wholly on in vitro work [3, 10]. The conclusions here should not be extended to them without checking. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 What a Convincing Body of Evidence Would Contain

For a producer or regulator who wants to judge a claim, a convincing package would contain a characterised product with the peptide content measured, ideally by a quantitative method, at the point of release and at the end of shelf life. It would add digestion and absorption data for the peptides said to matter, and a controlled human trial of the actual product, with a closely matched control, a defined population, an appropriate length and a clinically meaningful outcome [34, 37]. Independent replication would then complete the picture.

It would also include honest reporting. Trials should say how participants were chosen, which outcomes were prespecified and how many were examined, because a single favourable result among many tested outcomes tells us little. The pooled analyses we have cited are helpful, and they depend on trials that report enough detail to be pooled [39, 44]. These demands are heavy for a small producer, and the practical answer may be

shared evidence on a defined type of product, with transparent limits on which products it covers.

5.4 Directions for Future Work

Three needs stand out. First, studies that follow named peptides from product through digestion and absorption in people, building on the few that exist [31, 32]. Second, controlled trials with matched control products that separate the peptide effect from the effect of the matrix and the culture. Third, clearer definitions on labels, so that “fermented”, “probiotic” and “contains bioactive peptides” cannot be mistaken for one another [35, 36].

Better methods would also help. Agreed protocols for measuring peptides in finished products, reported with the same care as composition data, would let results be compared across laboratories. Databases that record the conditions of each assay, and flag whether activity was ever confirmed beyond the tube, would make predicted activity easier to tell apart from demonstrated activity [11, 30].

5.5 A Practical Reading for Producers and Assessors

For a dairy producer the sensible position is neither to abandon the subject nor to oversell it. A company that wishes to say something about peptides can begin by measuring them in its own product, at more than one point in shelf life, with a method that is described well enough for someone else to repeat [11, 34]. It can then ask which of the links in the chain it can honestly document, and word the claim to match, leaving the rest to be described as under study.

Assessors face the mirror-image task. A dossier that cites the general literature on milk peptides is not a dossier on the product, and a dossier built on a single favourable trial of a different product is not one either. The questions listed earlier, about content, survival, absorption, action and replication, give a simple frame for reading such material, and they apply whether the product is a yoghurt, a ripened cheese or a hydrolysate [9, 28, 37]. None of this is a verdict on any particular food, and nothing here should be read as advice to individuals about their health.

6. Conclusion

Fermented dairy products can contain bioactive peptides, and the way they are made influences which peptides appear. The weakest part of the story is not the chemistry but the inference that follows it. Most evidence comes from assays and animal models, gastrointestinal survival and

absorption are established for only a few peptides, human trials are generally modest and product-specific, and the wording on labels tends to run ahead of all three. Claims for fermented dairy foods as a class are therefore not supported by the evidence we can responsibly summarise.

The practical message is for those who make, assess and regulate such products rather than for individuals. Test the actual product, measure the peptides, follow them through digestion, and judge a claim by the weakest link in its chain. For researchers, the opportunity is a set of well-matched human trials and quantitative product data that would turn a plausible area of food science into one on which claims can be made with confidence.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

M.C. conceived the review and wrote the sections on food composition and claims. S.S. wrote the sections on fermentation and proteolysis. J.P. wrote the sections on digestion, human studies and evidence appraisal. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No animals or human participants were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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