Conventional and Novel Technologies in the Production of Dairy Bioactive Peptides

Mian Anjum Murtaza, Shafeeqa Irfan1, Iram Hafiz2, Muhammad Modassar A. N. Ranjha1, Abdul Rahaman4, Mian Shamas Murtaza4, Salam A. Ibrahim5* and Shahida Anusha Siddiqui6,7

1 Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan, 2 Institute of Chemistry, University of Sargodha, Sargodha, Pakistan, 3 School of Food Science and Engineering, South China University of Technology, Guangzhou, China, 4 Department of Food Science and Technology, Muhammad Nawaz Sharif (MNS) University of Agriculture, Multan, Pakistan, 5 Food Microbiology and Biotechnology Laboratory, North Carolina Agricultural and Technical State University; Greensboro, NC, United States, 6 Campus Straubing for Biotechnology and Sustainability, Technical University of Munich, Straubing, Germany, 7 German Institute of Food Technologies (DIL e.V.), Quakenbrück, Germany

Background: In recent years, researchers have focused on functional ingredients, functional foods, and nutraceuticals due to the rapidly increasing interest in bioactive components, especially in bioactive peptides. Dairy proteins are a rich and balanced source of amino acids and their derived bioactive peptides, which possess biological and physiological properties. In the dairy industry, microbial fermentation and enzymatic hydrolysis are promising methods for producing bioactive peptides because of their rapid efficiency, and mild reaction conditions. However, these methods utilize less raw material, take long reaction time, result in low yields, and low activity products when used alone, which pose industry to seek for novel methods as pretreatments to increase the yield of bioactive peptides.

Scope and Approach: This review emphasizes the production of peptides from the dairy proteins and discusses the potential use of novel technologies as pretreatments to conventional methods of bioactive peptides production from dairy proteins, including the mechanisms of novel technologies along with respective examples of use, advantages, limitations, and challenges to each technology.

Key Findings and Conclusion: Noteworthily, hydrolysis of dairy proteins liberate wide-range of peptides that possess remarkable biological functions to maintain human health. Novel technologies in the dairy industry such as ultrasound-assisted processing (UAP), microwave-assisted processing (MAP), and high pressure processing (HPP) are innovative and environmentally friendly. Generally, novel technologies are less effectual compared to conventional methods, therefore used in combination with fermentation and enzymatic hydrolysis, and are promising pretreatments to modify peptides’ profile, improve the yields, and high liberation of bioactive peptides as compared to conventional technologies. UAP is an innovative and most efficient technology as its mechanical effects and cavitation change the protein conformation, increase the biological activities of enzymes, and enhance enzymatic hydrolysis reaction rate.

Keywords: dairy proteins, bioactive peptides production, green technologies, ultrasound-assisted extraction, fermentation, enzymatic hydrolysis
**HIGHLIGHTS**

- Novel technologies are innovative, environmentally friendly, and promising pretreatments.
- Mechanisms and applications of novel technologies as pretreatments have been discussed.
- Novel technologies coupled with conventional methods are energy efficient and result in high extraction yield and rate have been reviewed.
- Potential bioactivity and functions of dairy proteins have been discussed.
- Ultrasound assisted processing showed the most efficient applications in dairy industry have been outlined.

**INTRODUCTION**

Bioactive peptides are specific peptide motifs of 2–20 amino acids embedded in parent proteins that possess the ability to alter or influence metabolic activities in the human body because of their particular fragments in proteins (1, 2). Bioactive peptides offer several biological functionalities such as free radical inhibition, thrombosis inhibition, and immunity improvement (3). There are two main methods of bioactive peptides production by microbial fermentation and enzymatic hydrolysis of proteins. A wide range of bioactive peptides can be produced using different cleavage specificities of the proteolytic enzymes (4). Usually, bioactive peptides consist of less than 20 amino acids and 10 kDa molecular weight. Their functionalities also depend upon the sequence of amino acids, their compositions, and molecular weights (5). Milk proteins contain several peptides that exert strong biological properties and are widely studied as a source of bioactive peptides (6, 7). Many studies have reported the availability of bioactive peptides in milk, fermented dairy products, and various types of cheese (8–10). Milk derived bioactive peptides are associated with many health beneficial effects, including immunomodulation, antithrombotic activity, antihypertension, antimicrobial activity, and opiate activity (11, 12).

The bioactive peptides are separated, identified, and purified by employing high-performance liquid chromatography (HPLC) (5). However, characterization of peptides is carried out by the protein hydrolyzed fractionation method (13) and functional properties of peptides are assessed by the amino acid composition of the bioactive peptide (14). For fractionation, the ultrafiltration membrane system is the preliminary step to separate the required molecular weight fractions from hydrolyzates (15). **Figure 1** illustrates and summarizes the process of bioactive peptides production from dairy proteins, including source preparation, extraction, and hydrolysis of protein (denaturation), fractionation of desired peptides through gel-filtration chromatography (GFC), their purification by HPLC, and identification through liquid chromatography-mass spectrometry (LC-MS/MS).

The dairy industry relies on microbial fermentation and enzymatic hydrolysis to produce bioactive peptides, which are evolving, coupled with conventional methods to generate high yields of bioactive peptides from dairy proteins quickly and at a low cost. **Figure 2** illustrates and summarizes the conventional and green novel technologies employed in the dairy industry to produce bioactive peptides. Ultrasound, microwave (16) and high-pressure processing (17) are the efficient, novel, green technologies, but these are emerging technologies with attention to dairy industry, and their promising effects have been entirely understood when employed as pretreatments. Ultrasound waves break, weaken, or clean the electrostatic and hydrophobic interactions of milk proteins through shear forces and cavitation and bring conformational changes in proteins (18, 19). Microwave heating has many benefits like easy operation, less processing, and high efficient energy, making it suitable in continuous food processing (20). High-pressure processing (HPP) is a potential technique used as a pre-treatment method to release bioactive peptides by enhancing the enzymatic digestibility of proteins due to conformational changes in proteins that influence their functional properties boosting their digestibility. It has also been applied to milk and milk products (21, 22).

This review emphasizes the production of peptides from dairy proteins and discusses the potential use of novel technologies in context to conventional methods of bioactive peptides production from dairy proteins, including the mechanisms and their respective examples of use, advantages, limitations, and challenges to each technology.

**MILK AND FERMENTED DAIRY PRODUCTS: SOURCE OF BIOACTIVE PEPTIDES**

Milk and dairy products comprise various essential nutrients such as bioactive agents (antioxidants), minerals, omega-3 fatty acids, linoleic acid, oleic acid, and vitamins, making them nutritious foodstuff (23). Oxidative stress and damage to the body can be prevented by consuming antioxidant-rich foods (24). Milk and its products are a well-known source of antioxidants as they contain: significant amounts of daidzein polyphenolic metabolites, antioxidative enzymes, i.e., glutathione peroxidase, catalase, superoxide dismutase, and sulfur-containing amino acids, i.e., carotenoids, vitamins A and E, cysteine, and methionine (25). Generally, bovine milk protein is comprised of lactoferrin, caseins, immunoglobulins, beta-lactoglobulin (β-LG), alpha-lactalbumin (α-LA), fractions of protease-peptide, and some whey proteins (transferrin and serum albumin) as main fractions (26). **Figure 3** shows the major bioactive components of milk with biological properties.

Milk contains various useful molecules encompassing bioactive peptides (27, 28). Dietary proteins contain bioactive peptides in them, which are naturally found inactive in parent protein sequences and liberated only during food processing or gastrointestinal digestion. Peptides work as regulatory compounds with hormone-like activity after liberation. In dairy, milk proteins are the potent source of bioactive peptides which exert various biological functions, i.e., antioxidant,
**FIGURE 1** | Schematic diagram of the production of bioactive peptides from dairy products.

**FIGURE 2** | Novel and conventional methods of bioactive peptides production from dairy proteins.
antimicrobial, anticancer, and anti-hypertensive factors (29, 30). As cited in Table 1, many researchers have assessed the biological activities of bioactive peptides from various milk sources, including camel and bovine casein hydrolysates (31), buffalo casein (32), camel whey protein hydrolyzate (33), camel milk lactoferrin (34), goat milk (35), yak milk (36), goat milk (37), buffalo milk (38), skim milk (6) camel milk (39), whey protein hydrolyzate (40), UHT treated milk (41), and milk and dairy products (42) by using various microorganisms and microbial enzymes for proteolysis. The bioactive peptides are liberated during gastrointestinal digestion (in vivo), milk products’ manufacturing, and proteolysis (in vitro).

Reportedly, fermented milk products contain phosphopeptides, ACE-inhibitory peptides, and casomorphins (43). Bovine α-lactalbumin and β-casein have shown bioactive peptide sequences like LDQW, INYW, and NSLP, FP, HQP, respectively (44, 45). Another in vitro study revealed two antioxidative peptide sequences KVLPVPEK and AVPYPQR, by following milk casein hydrolysis (46). However, digestion and fermentation of goat milk can also release antioxidative peptide sequences like EALEKFDK and EALEKFDK (47, 48).

Cheese is a widely used fermented milk product. Many studies have reported that cheese is a vital source of a wide range of biologically active substances such as proteins and all essential amino acids (except cysteine and methionine), minerals, vitamins, and short-chain fatty acids (49, 50). Cheese contains bioactive compounds with biological activities such as peptides, conjugated linoleic acid (CLA), exopolysaccharides, γ-aminobutyric acid (GABA), vitamins, and organic acids, and fatty acids. According to in vitro and in vivo studies, these bioactive compounds may have antiproliferative, antimicrobial, and antioxidant activities and inhibit ACE (angiotensin-converting enzyme) (51, 52). As shown in Table 2, many bioactive peptides have been identified in different fermented dairy products, such as Iranian ultrafiltered white cheese (53), fermented milk (54), cultured dairy product (55), Hard cow milk cheese (56), fermented casein (57), Prato cheese (58), fermented whey proteins (59), commercial fermented milk (60), goat milk Tulum cheese and cow milk Tulum cheese (61), cow and buffalo cheddar cheeses (62), fermented milk (Lassi) (63), yogurt (64), symbiotic yogurt (65), and curd and whey (66).

CONVENTIONAL METHODS OF PRODUCTION

In the dairy industry, the conventional methods for producing bioactive peptides are microbial fermentation and enzymatic hydrolysis, summarized in Table 1.

Microbial Fermentation

Fermentation is a primeval preservation method that utilizes lactic acid bacteria proteolytic systems as an efficient approach to produce bioactive peptides from food. Generally, lactic acid bacteria fermentation is carried out both naturally and under controlled conditions, which improve technological and nutritional properties of food and ultimately develops texture and flavor in them (67, 68). Usually, the milk fermentation is carried out by Lactobacillus strains; till now, most known bioactive peptides have been isolated through milk cultures (27). Because different milk sources (cow, buffalo, goat, yak, camel, or mare) have distinctive proteins, different bioactive peptides are produced on hydrolysis of casein and whey proteins usage of the same Lactobacillus strain (69). Lactic acid bacteria fulfill their need for essential and growth-promoting amino
| References              | Microorganisms/Microbial enzyme | Protein fragment | Amino acid sequence                                           | Bioactivity                                      |
|-------------------------|---------------------------------|------------------|---------------------------------------------------------------|--------------------------------------------------|
| Mudgil et al. (31)      | Alcalase and pronase E          | NR               | FLWPEYGAL, LPTGWLM, MFE, GPAHCLL                               | Anti-diabetic (inhibition of α-amylase (AA), α-glucosidase (AG), and dipeptidyl peptidase IV (DPP-IV)) |
| Shannugam et al. (32)   | Pepsin, trypsin, chymotrypsin and their combination | α S1 Casein     | HIKKDVPSER, EDVPSER                                           | ACE inhibitory                                   |
|                         |                                 | α S2 Casein     | EQLSTSEENSK, NPWDQVK, YGVPVLNPWDQVK, RNASLPTPLNR, NAVPIPTPL    |                                                  |
|                         |                                 | β  Casein        | IHPPACTQGS, YQEPVLGCPVR, VLPVPQK, YVPEPFTESQSL                |                                                  |
|                         |                                 | κ  Casein        | YPIQOYVR, YPSYLGVYYQKPVAL, HPHPLSF                            |                                                  |
| Baba et al. (33)        | Pepsin                          | NR               | PAGNFMGLMGHMR, PAIACCLPPCHVM, MLPLMLPFTMGOY, PAGNFLPPAAPVYM    | α-amylase and α-glucosidase inhibitory            |
| Khajeh et al. (34)      | S. aureus, P. aeruginosa, and A. baumannii | NR             | IAGKCGPLPV, AASKSRSVNW, CTTPSAESSKCAQ, ECOIASTEKADAVT, LRPAEAV, GTENPQTH, KOSCHGL, . . . , RRCSTSP | Antimicrobial                                    |
| Parmal et al. (35)      | Lactobacillus fermentum (M2)    | NR               | SQCDQPTTLAR, TIDMESTEVFTK, YIQKEDVPSER                        | ACE inhibitory                                   |
| Liu et al. (36)         | Alcalase Trypsin                | Yak-CN          | REEEL, GKEKEVNL, LPVPQ, HPHPLHL, VPVPV, VYPPQ                 | Antioxidative                                    |
| Parmar et al. (37)      | L. fermentum (M5) (KU366365) L. paracasei (M16) (KU366368) L. rhamnosus (NK2) (KR080695) L. casei (NK9) (KR732325) L. fermentum TDS030603 (MTCC 25067) | CASA1_CAPHI Alpha-S1-casein OS | LARPKHPIHROGLESPE, ENSGKTITMPLW                  | Antioxidative                                    |
| Zhao et al. (38)        | Dregea sinensis Hems. protease. | αS1 - CN (110-117) | TEEENRLNFLKISQY, PEEIKTVDDKHYQKALNEI                        | Antimicrobial                                    |
| Guzmán-Rodríguez et al. (6) | Lactobacillus casei SHIROTA | β-CN κ-CN       | NR                                                             | Iron binding                                     |
| Wali et al. (39)        | Trypsin Pepsin Alcalase Papain  | NR               | RLDQGQSSPRRTVLGTRPDNDWLGASPOVQVR                              | Antioxidative                                    |
| Jiang et al. (40)       | Trypsin                         | α-La (113-117), (115-123), (109-122), (94-108), (99-114), (63-79), (60-80) | KILDK, LDQWLCCKL, ALCSHEKLDWLCEK, KILDVGNYLWALHK, VGINYLWALKALCSEK, NDQPHSSNICSIDEK, FDLDDLDTDIDCMVKKILDLK | Antioxidative                                    |
|                         |                                 | β-Lg (149-162), (61-75), (125-141), (102-124) | LSNFQQLEEQQHI WENGECAQQKIAEK                                 |                                                  |
|                         |                                 |                 | TPEVDDEALEKFDKALK                                             |                                                  |
|                         |                                 |                 | YLLFCMENSAEPEQSLACQCLVR                                       |                                                  |
| Özturk and Akin (60)    | Lactobacillus casei Shirot A1 Lactobacillus johnsonii LA1 | α-La β-Lg       | NR                                                             | Antithrombotic                                   |
| Elkhtab et al. (41)     | Lactic acid bacteria strains    | κ-CN             | LVESPPLENTVO, VLESPPELN, RSYPYGIN                             | ACE inhibitory                                   |
|                         |                                 | β-CN             | DQIHHPAQTK                                                   | Antihypertensive                                 |
|                         | Kombucha culture                | αS1-CN           | AVPOEVLVNLRLR, FVAPEPVFGKEK                                    |                                                  |
|                         |                                 | αS2-CN           | KFKGFVPEPFAVE, VAPFPEVGK                                      |                                                  |
|                         |                                 | β-CN             | LVYPFPGPLH, LVYPPGLPAAPVLPQ                                    |                                                  |
| Capriotti et al. (42)   | Lactobacillus helveticus        | β-CN (205-209)   | FPIV                                                          | ACE inhibitory                                   |

NR, not reported; αS1-CN, alpha-S1-casein; α-La, alpha lactalbumin; κ-CN, kappa-casein; β-CN, beta-casein; β-lg, beta lactoglobulin; CASA1_CAPHI OS, Capra hircus alpha-S1-casein; CASA2_CAPHI Alpha-S2-casein OS, Capra hircus alpha-S2-casein.
| References | Product | Protein fragment | Amino acid sequence | Bioactivity |
|------------|---------|------------------|---------------------|------------|
| Yousefi et al. (53) | Iranian ultrafiltered white cheese | α\_S1\_CN (1–6) α\_S1\_CN (102–108) | RPKHPI, KKYNVQP | ACE inhibitory |
| | | β\_CN (f205–209), (f126–133), (f114–121), (f57–68), (f193–209) | FPIIV, FKPVPVER, YPVEFTE, SLVYFPFGPIHIN, YQEPVLGPRGPFPIII | |
| Kim et al. (54) | Fermented milk | NR | ATISAG | Lipase Inhibitory |
| Mullaiselvan et al. (55) | Cultured dairy product | α\_S1\_CN α\_S2\_CN β\_CN | NR | Casein phosphopeptide Immunomodulatory |
| Timón et al. (56) | Hard cow milk cheese | α\_S1\_CN β\_CN | EIVPN, DKIHFP, VAPFPQ | Antioxidative |
| Fan et al. (57) | Fermented casein | α\_S1\_CN (f10–23), (f10–22), (f1–23), (f14–23), (f10–21), (f24–34), (f24–38), (f80–98) | KILDK, LDQVLCIKEK, ALCSEK, KILDKVINGNYLAIK, VGNYLAHKALCSEK, NQDOPHSSICNISCDDK, FDODLTDIMCVKULLDK | ACE inhibitory, Antioxidative |
| Baptista et al. (58) | Prato cheese | β\_CN (f194–209) | NR | ACE inhibitory |
| Daliri et al. (59) | Fermented whey proteins | α\_S1\_CN (f10–23), (f1–23), (f14–23), (f10–21), (f24–34), (f24–38), (f80–98) | GLPQEVLNENLLRF, GLPQEVLNENLLRF, EIVPN, DKIHFP, VAPFPQ | ACE inhibitory, Antihypertensive |
| | | β\_CN (f1–27), (f1–23), (f192–209), (f193–209), (f193–208), (f194–209), (f195–209), (f80–98) | RELEELNPGEIVESL, RELEELNPGEIVE, RELEELNPGE, RELEELNPGEIV, LYQEPVLGPRGPFPIII, YQEPVLGPRGPFPIII, GEPVLGPRGPFPIII, EPVLGPRGPFPIII, VPPPLGPEVMGV | |
| | | | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |
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| | | | κ\_CN (f161–169), (f155–169), (f149–169), (f151–169), (f159–169), (f152–169), (f150–169), (f157–169), (f151–169), (f151–165), (f151–163), (f149–162), (f149–163), (f151–162), (f116–141), (f110–151), (f106–149) | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |
| | | | β\_g (f130–149), (f130–146), (f130–145), (f1–11), (f153–162), (f147–156), (f1–11), (f112–153), (f153–162), (f147–156), (f110–13) | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |
| | | | Lactophorin (PP3) (f1–18), (f1–17), (f57–67), (f54–67) | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |
| | | | PIGR (f383–404) | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |
| | | | UP (GP2) (f455–473) | TVQVTSTAV, SPPTEINTVQVTSTAV, SPEIESPEINTVQVTSTAV, EIVESPPEINTVQVTSTAV, INTVQVTSTAV, VIESPEINTVQVTSTAV, PEVPTEINTVQVTSTAV, PEINTVQVTSTAV, EVIESPEINTVQVTSTAV, EVIESPEINTVQVTSTAV, SPEIESPEIN, SPEIESPEINTVQ, EVIESPEIN | |

(Continued)
TABLE 2 | Continued

| References | Product | Protein fragment | Amino acid sequence | Bioactivity |
|------------|---------|-----------------|---------------------|-------------|
| Murtaza et al. (39) | Production of Dairy Bioactive Peptides | | | |
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and papain to hydrolyze the Bactrian camel milk and isolated three novel antioxidant peptides.

Enzymatic hydrolysis has certain shortcomings, such as higher cost to produce pure bioactive peptides, casein coagulation on heating, and bitterness, therefore, choice of enzymatic hydrolysis conditions must be taken into account before application (88, 89).

**NOVEL PROCESSING TECHNOLOGIES**

The novel processing technologies such as ultrasound-assisted processing (UAP), microwave-assisted processing (MAP), high-pressure processing (HPP), pulsed electric field processing (PEF), subcritical water processing (SWP), and ohmic heating relies on physical processes to improve the degree of hydrolysis during bioactive peptides production (90, 91). However, following applications of UAP, MAP, and HPP as pretreatments have been found in the dairy industry to prepare bioactive peptides.

**Ultrasound-Assisted Processing**

Ultrasound-assisted processing is a novel, eco-friendly, and non-thermal physical technology that involves >20 kHz frequency of sound waves to produce peptides (91, 92). In ultrasound treatment, acoustic cavitation, acoustic streaming, and mechanical vibrations are produced on the passage of ultrasound waves through a medium. Acoustic streaming can allow and improve the transfer of mass through a medium. The mechanical vibrations can change solid particle size and structure (93). In a liquid medium, ultrasound treatment follows the cavitation process in which pre-existing micro-bubbles expand and contract. However, during these oscillations, bubbles keep growing until they reach their resonance size range and then collapse violently in case of transient/inertial cavitation (94, 95). In transient cavitation, physical shearing, high-pressure and extreme localized temperatures (2,000–5,000 K) are produced on collapsing of increased sized bubbles (within few acoustic cycles) into fragments at low ultrasound frequency. However, stable cavitation results in relatively mild streaming effects on collapsing of the little increased bubbles (over a large number of acoustic cycles) at higher frequencies. Cavitation also owns the ability to induce chemical changes along with physical effects. When cavitation is applied to an aqueous medium, a highly reactive radical is formed inside the bubble (on reaction of gas molecules and water vapor reaction) due to the availability of generated localized high temperature. The ultrasound cavitation chemical effects are visible at 300–500 kHz frequencies and physical effects are visible at 20 kHz frequency (94). Protein structures undergo conformational changes by ultrasound processing, such as acoustic cavitation, forces of chemical and physical effects (96, 97).

As presented in Table 3, recently UAP has been employed as pretreatment for various milk proteins hydrolysis, including whey proteins (98), caprine milk protein (99), fresh milk (100), cheddar cheese (101), whey protein isolate (102), whey protein (103), and milk protein concentrate (104, 105). UAP in combination with enzymatic hydrolysis has been employed for various proteins, i.e., eggshell membrane (106), egg white (107), and isolated oat protein (108). Ulug et al. (91) reported that the application of UAP is carried out in combination with enzymatic hydrolysis, to increase the production of bioactive peptide, as UAP alone cannot break the peptide bond. Ultrasound pretreatment enhance the enzymes accessibility into the peptide bonds of foods that results in the increased release of bioactive peptides. Basically, ultrasound processing generates the acoustic forces that increase the available surface area for enzyme protein interactions by reducing the size of the fat globules that get covered with whey proteins and casein micelles, ultimately, increases the access of proteolytic enzymes to the proteins (109, 110). Wu et al. (111) in their study on the thermodynamic properties of whey protein hydrolyzed by alcalase with ultrasonics pretreatment reported that the hydrolyzates showed significantly increased ACE inhibitory and immunomodulatory activities when the whey protein enzymatic hydrolysis was assisted by the ultrasound. Sonication pretreatment induces the whey protein unfolding, increased free sulphhydryl content, and conformational changes with increased β-sheets and β-turns formation (111). Similar study exhibited that ultrasound-assisted pretreatment combined with low purity enzymes show the increased hydrolysis rate that may be due to changes in free sulphhydryl clusters and disulfide bond (112), hydrophobic protein content, and surface hydrophobicity (113). Lorenzetti et al. (102) reported that ultrasound pretreatment before hydrolysis of whey protein isolate could help to develop the economic ingredients for the dairy industry.

UAP application is beneficial to reduce the disadvantages resulting from hydrolysis by conventional enzymes, i.e., long-time hydrolysis and low conversion rate (114). Generally, UAP equipment requires fewer installations, low maintenance, around 85% energy efficiency, and cost between €10,000 and 200,000 (115). Undeniably, UAP is one of the novel and most preferable techniques for producing bioactive peptides due to numerous advantages such as faster start-up, extraction selectivity, high process control, reduced temperature and time, and faster mass and energy transfer (116).

**Microwave-Assisted Processing**

Microwaves encompass electromagnetic radiation of 300 MHz–300 GHz range (117). Microwaves energy follows molecular interactions (ionic conduction and dipolar rotation mechanisms) as a medium transportation mode. On applying electromagnetic field, charged colloidal molecules migrate and flow through a stationary medium in ionic conduction and led to resistance in the solution, which produces thermal energy. On the other hand, dipole rearrangement occurs on electromagnetic fields in dipolar rotation (118).

Microwave treatment is carried out in the food processing ovens in which an alternating electric field is used to generate the microwaves having 2.45 GHz frequency and <1 cm wavelength typically. These microwaves do not cause breakage of covalent
TABLE 3 | Applications of ultrasound-assisted processing for the production of bioactive peptides.

| References | Protein source | Equipment | Type of treatment | Treatment conditions | Peptides/ hydrolyzate size | Major findings |
|------------|----------------|-----------|-------------------|---------------------|---------------------------|---------------|
| Abadía-García et al. (98) | Whey proteins | Probe ultrasound homogenizer | The high intensity ultrasound (HIUS) pretreatment before enzymatic hydrolysis (bromelain) | The ultrasonic pretreatment at 500 W, 20 kHz, 25 and 50% amplitude, 10 min | Higher concentration of peptides with a molecular weight below 5 kDa was found when ultrasound pretreatment was applied. | In comparison to control, both HIUS pretreatments resulted in lower IC50 values in hydrolyzates, small size fractions (1 and 3 kDa) showed highest ACE inhibition activity, and significant changes were observed in structure of whey protein. |
| Koirala et al. (99) | Caprine milk protein | Probe sonicator | The ultrasonic pretreatment before enzymatic hydrolysis (pepsin and neutral protease) | 200 W power, 24 kHz frequency and a fixed cycle of 0.5 | Ultrasonic pre-treated caprine milk proteins had a higher degree of hydrolysis with neutral protease at 360 min and with pepsin at 300 min. The molecular weight of peptides after sonication was not measured. | The ultrasonication pretreatment increased the soluble protein concentration in caprine milk, enhanced peptides and protein hydrolyzates production, and accelerated unfolding of complex insoluble protein structure into a simpler soluble matrix, and increased bioactive antioxidant and ACE-inhibitory activities. |
| Cui et al. (100) | Milk protein | Multi-mode ultrasonic | The ultrasonic pretreatment before enzymatic hydrolysis (neutral protease) | Single frequency 28 kHz, various times ranging 10–60 min, different levels of ultrasound density between 10 and 50 W/L at initial temperature 30°C. | The highest degree of hydrolysis reported from pepsin. The molecular weight of peptides after sonication was not measured. | Compared with control and non-ultrasonic samples, the ultrasonic pretreatment showed significantly increased ACE inhibitory activity of milk protein (28 kHz, 20 W, and 40 min). Also, secondary structure studies showed reduced content of α-helix and β-corner, increased content of β-folding, and random coil in ultrasonic treated milk proteins. And, increased surface hydrophobicity and the content of free sulfhydryl, reduced content of disulfide bond in ultrasonic treated milk protein. |
| Munir et al. (101) | Cheddar cheese | Probe sonicator | The ultrasonic pretreatment of milk before cheddar cheese manufacturing and compared with control and other processing techniques. | 80% amplitude 20 kHz frequency at <40°C. Applied in two levels: US-1 (21 J/g calorimetric power) & US-2 (41 J/g) | The molecular weight of peptides after sonication was not measured. | In comparison to control, both levels of ultrasonic treatments increased the proteolysis process of cheese as well as fat content, ACE-inhibition activity, total phenolics, total flavonoids, antioxidant and DPPH scavenging activities of the cheddar cheese during ripening. |
| Lorenzetti et al. (102) | Whey protein isolate | Ultrasonic tip sonicator | The ultrasonic pretreatment before enzymatic hydrolysis (low purity enzymes: pepsin and papain) | 20 kHz frequency, pepsin (4 min at 400 W), papain (2 min at 300 W) | The highest degree of hydrolysis reported from pepsin. The molecular weight of peptides after sonication was not measured. | The ultrasonic pretreatment reduced the 6 h in the process. The highest degree of hydrolysis occurred with the use of pepsin (10 h, 37°C, and pH 2.5). After partial enzymatic hydrolysis and ultrasound pretreatment a higher proportion of low molar mass peptides were observed at 1,000–2,000 g.mol⁻¹. |

(Continued)
bonds because of their non-ionizing radiation nature (119, 120) but, these can either induce thermal or non-thermal changes in the milk. Microwaves generate heat by friction that results from the oscillation of molecules as dipoles of water try to align their arrangements under the influence of microwave field. So, thermal effects are resulted from the generation of localized heat due to friction of molecules, on the other hand, non-thermal effects (accelerated protein unfolding rate) alone arise from the rearrangement of molecules in milk (120).

Microwave-assisted processing has been employed for various milk proteins hydrolysis including cheddar cheese (101), bovine whey proteins (121), milk protein concentrate (105), bovine serum albumin (122), and bovine whey protein concentrate (123) as cited in Table 4. MAP is one of the most preferred alternative technologies to conventional heat processing methods as it enhances functional properties, extends shelf life, and improves microbial safety of food products (124, 125). In their study, Izquierdo and coworkers found that MAP could make proteins specific sites potentially available to proteolytic enzymes by continuous protein molecules unfolding and rearrangement (123). In a study, the surface plasmon resonance sensing method was used to investigate the unfolding of protein by employing MAP at 2.45 GHz. The results showed that at the same temperature, MAP heating has a higher impact on the unfolding and denaturation enthalpy (ΔH), reduction of reactive thiol groups and changes in secondary structure suggest protein rearrangements and aggregate formation.

According to response surface analysis, the highest ACE inhibitory activity (IC50 = 0.044 mg mL−1) could be achieved by 4.11 min, 2.32 h and 2.33% for ultrasound pretreatment time, hydrolysis time and E/S ratio, respectively. Also, the ultrasound pretreatment has a significant effect on ACE inhibition of enzyme hydrolyzates from MPC during enzymatic hydrolysis with digestive enzymes.

TABLE 3 (Continued)

| References            | Protein source | Equipment          | Type of treatment          | Treatment conditions | Peptides/ hydrolyzate size | Major findings |
|-----------------------|----------------|--------------------|----------------------------|----------------------|---------------------------|---------------|
| Abadía-García et al. (103) | Whey protein concentrate (MPC) | Ultrasound homogenizer | The ultrasonic pretreatment before enzymatic hydrolysis (vegetable proteases) | 20 kHz frequency, 750 W nominal power, amplitude between 30 and 60%. | The molecular weight of peptides after sonication was not measured. | The results showed that ultrasound density exerted a significant effect on proteolysis increased the ACE inhibition by 15% and a 95% reduction of hydrolysis time in bromelain hydrolyzates. Also, changes in denaturation enthalpy (ΔH), reduction of reactive thiol groups and changes in secondary structure suggest protein rearrangements and aggregate formation. |
| Uluko et al. (104)    | Milk protein concentrate (MPC) | Ultrasound homogenizer | The ultrasonic pretreatment before enzymatic hydrolysis (neutrase) | Different combinations of independent variables were set. The ultrasonic pretreatment at 90°C, US at 800 W and 20 kHz for 10 min. Samples were jacketed with ice during treatment. Control received no pretreatment. | The molecular weight of peptides after sonication was not measured. | Compared with the control and other treatments, US pretreated samples showed the highest radical scavenging activity (EC50 = 0.283 mg mL−1) and had the highest number of hydrophobic peptides. |
| Uluko et al. (105)    | Milk protein concentrate (MPC) | Cell disruptor      | The ultrasonic pretreatment before enzymatic hydrolysis with digestive enzymes (papain and trypsin) and compared with thermal and microwave pre-treatments. | Different combination of pretreatments were set. | The molecular weight of peptides after sonication was not measured. | |

TABLE 4

| Major findings | Type of treatment | Treatment conditions | Peptides/ hydrolyzate size |
|----------------|-------------------|----------------------|---------------------------|
|                |                   |                      |                           |

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TABLE 4 | Applications of microwave-assisted processing for the production of bioactive peptides.

| References       | Protein source          | Equipment                        | Type of treatment                                                                 | Treatment conditions                                                                 | Peptides/hydrolyzate size | Major findings                                                                                                                                                                                                 |
|------------------|-------------------------|----------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Munir et al. (101) | Cheddar cheese          | Microwave oven                   | The microwave pretreatment of milk before cheddar cheese manufacturing and compared with control and other processing techniques. | Temperature <40°C, specific energy 86.5 J/g                                              | The molecular weight of peptides after microwave treatment was not measured. In comparison to control, MA showed increased antioxidant activity and ACE-inhibitory potential of cheese. However, ultrasound was the most effective pre-treatment to improve the antioxidant capacity of cheddar cheese during ripening. |
| El Mecherfi et al. (121) | Bovine whey proteins | Microwave device consisted of a solid-state microwave generator | Microwave pre-treatment followed by proteolysis (pepsin), and compared with conventional heating. | Different microwave temperatures conditions at 37, 50, 65, and 70°C for 30 min and microwave power was not reported | The highest degree of hydrolysis reported from pepsin compared to conventional heating. Whey proteins showed two major bands with molecular weights: 18 kDa bovine beta-lactoglobulin and 14 kDa alpha-lactalbumin. The microwave heating process in concomitance with enzymatic proteolysis improved the susceptibility of resistant proteins (BLG) to pepsinolysis. Also, hydrolyzed whey protein hydrolysates were obtained by MA only at 65°C and in a shorter time compared with the conventional thermal treatment. |
| Uluko et al. (105) | Milk protein concentrate (MPC) | Microwave                         | Microwave pre-treatment followed by enzymatic hydrolysis with digestive enzymes (pepsin and trypsin) and compared with thermal and ultrasound pre-treatments. | Samples were microwaved for 10 min and microwave power was not reported | The peptides have been concentrated in the filtrates of 5 kDa molecular weight | Microwave pretreated filtrates (<5 kDa) improved the radical scavenging activity compared to control; however, when microwave pretreatment was used in combination with other treatments, the samples showed lower radical scavenging activity than the control. Ultrasound was the most effective pre-treatment to improve the antioxidant capacity of milk protein concentrate. |
| Chen et al. (122) | Bovine serum albumin (BSA) | MAS-II Smart Microwave Digestion System | Continuous microwave-assisted protein digestion with an immobilized enzyme (trypsin) | Continuous microwave power at 100–700 W for 5–20 min for BSA digestion. | The molecular weight of the BSA-derived peptides ranged from 3 to 14 kDa (at 300, 500, and 700 W) | The bioactivity of peptides was not measured. Continuous microwave-assisted enzymatic digestion with immobilized enzyme was a fast and efficient digestion method for protein. Different levels of microwave power significantly affected the number of peptides obtained from the BSA. |
| Izquierdo et al. (123) | Bovine whey protein concentrate (WPC) | Oven MDS-2000                     | Microwave pre-treatment followed by proteolysis (pronase, chymotrypsin, papain, corolases 7089 and PN-L 100, alcalase and, neutrase) | 532 W, 40 or 50°C during 5 min | The molecular weight of peptides after microwave treatment was not measured. | Microwave irradiation (MWI) treatment enhanced the enzymatic hydrolysis of bovine WPC. Pronase and Papan showed the highest proteolysis under MWI followed by Alcalase. |

with proteolytic enzymes by accelerating the rapid hydrolysis of protein into peptides and producing more coverage of sequence (129). Before proteolytic hydrolysis, cleavage sites of proteins are probably exposed by microwave radiations that cause a change in protease cleavage sites (130).

Generally, in contrast to conventional methods, MAP offers several benefits: reproducibility, reduced processing time, hydrolysis efficiency, cost-effectiveness, convenience, and simple handling, making it one of the most preferred methods (131).
### TABLE 5 | Applications of high-pressure processing for the production of bioactive peptides.

| References        | Protein source            | Equipment                  | Type of treatment                        | Treatment conditions                                                                 | Peptides/ hydrolyzate size                        | Major findings                                                                                                                                                                                                                                                                                                                                 |
|-------------------|---------------------------|----------------------------|------------------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Landim et al. (139) | Whey protein concentrate (WPC) | High hydrostatic pressure equipment | The HPP pretreatment of WPC | Different pressure (100, 250, and 400 MPa) and time (5, 20, and 35 min) levels for each treatment | The molecular weight of peptides after HPP treatment was not measured. | As compared to conventional hydrolysis, the HPP pretreatment increased antioxidant activity, less soluble protein hydrolyzates, and decreased allergenicity.                                                                                       |
| Paula et al. (140) | Whey protein concentrate   | High hydrostatic pressure equipment | The HPP assisted hydrolysis and pretreatment of whey protein | Different pressure (100, 250, and 400 MPa) and time (5, 20, and 35 min) levels for each treatment | The molecular weight of peptides after HPP treatment was not measured. | In comparison to conventional hydrolysis, HPP assisted hydrolysis resulted in 35% protein reduction at 100 MPa after 35 min, and HPP pretreatment resulted that about 98% peptic hydrolysis of β-lactoglobulin and increased antioxidant capacity of hydrolyzates. In comparison to control, MA and US-1, HPP showed increased antioxidant activity and ACE-inhibitory potential of cheese. However, ultrasound was the most effective pre-treatment to improve the antioxidant capacity of cheddar cheese during ripening. |
| Munir et al. (101) | Cheddar cheese             | High-pressure vessel        | The HPP pretreatment of milk before cheddar cheese manufacturing and compared with control and other processing techniques. | The high-pressure processing at 400 MPa for 15 min, at temperature <40°C | The molecular weight of peptides after HPP treatment was not measured. | In comparison to control, MA and US-1, HPP showed increased antioxidant activity and ACE-inhibitory potential of cheese. However, ultrasound was the most effective pre-treatment to improve the antioxidant capacity of cheddar cheese during ripening. |
| Boukil et al. (141) | Bovine whey protein beta-lactoglobulin (β-LG) | Discontinuous hydrostatic pressurization unit | HHP pre-treatment followed by tryptic hydrolysis | Three different pressures at 0.1 (control), 400, and 600 MPa for 10 min at room temperature | Tryptic hydrolysis of pre-pressurized β-LG at 400 MPa generated two new peptides, (QEAKDAFLGSF and WENGECAQKK), and their relative abundance decreased at 600 MPa. | HHP pre-treatment at 400 MPa improved the generation of bioactive peptides compared to the control and 600 MPa. The relative proportions of the bioactive peptides in hydrolyzates were 38.64% at 400 MPa, higher than the control, and 600 MPa (26.7 and 20.5%, respectively). Whey protein hydrolyzates with HHP treatment could reduce inflammation and oxidative stress in intestinal cells. A significant reduction of H2O2-induced IL-8 secretion was observed for the HHP treated hydrolyzates (50%) compared to the control (30%). Increased proteolysis and levels of free fatty acids were found in cheese manufactured from milk HP-treated at 600 MPa. |
| Piccolomini et al. (142) | Whey protein isolate (WPI) | Avure High-pressure Processing System | HHP pre-treatment followed by proteolysis (pepsin, trypsin, and chymotrypsin) | Pressure levels at 550 MPa and control | High molecular weight peptides were removed with a membrane with a molecular weight cut-off 10 kDa. | High molecular weight peptides with HHP treatment could reduce inflammation and oxidative stress in intestinal cells. A significant reduction of H2O2-induced IL-8 secretion was observed for the HHP treated hydrolyzates (50%) compared to the control (30%). |
| Voigt et al. (22) | Cheddar cheese             | Equipment type not mentioned | Raw and HP-treated milk and their impact on cheddar cheese during ripening | 400 or 600 MPa for 10 min at 20°C | The molecular weight of peptides after HPP treatment was not measured. | Increased proteolysis and levels of free fatty acids were found in cheese manufactured from milk HP-treated at 600 MPa. Proteolysis during or after high-pressure treatment showed longer and more hydrophobic peptides than proteolysis at atmospheric pressure. |
| Chicón et al. (143) | β-Lactoglobulin            | 900 HP apparatus            | HHP pre-treatment followed by proteolysis (chymotrypsin) | Pressure levels at 400 MPa | The molecular weight of peptides after HPP treatment was not measured. | Proteolysis during or after high-pressure treatment showed longer and more hydrophobic peptides than proteolysis at atmospheric pressure. |

(Continued)
High Hydrostatic Pressure Processing

High-pressure processing (HPP) is a green, novel, and non-thermal technology that encompasses the application of 100–1000 MPa pressure, with or without treatment of heat primarily for the deactivation of pathogenic microorganisms along with molds, yeast, and vegetative bacteria, enhancing nutritional and functional properties of food products in the food industry. Depending on the food type, HPP treatment duration varies between 0 and 30 min (132, 133). Also, both treatment duration and pressure-transmitting fluid, and adiabatic heating result in a 3–9°C increase of temperature per 100 MPa (134). This technology has advantages over other technologies due to low to moderate temperature and causing the least damage to the bioactive compounds. HPP involves the combination of pressure and heat, resulting in conformational changes of protein and biological, chemical, and physical changes in food compounds (135).

High-pressure processing can be carried out in three different modes like semi-continuous, continuous, and batch. Batch HPP is an efficient and simple mode. The pressure chamber is filled with a prepacked sample and sealed, the air in the pressure chamber is replaced by pouring water, and then pressure is built until the desired point is achieved. After a particular time the chamber is depressurized. Finally, processed food is taken out. On the other hand, continuous/dynamic HPP (136) involves utilizing a moving piston to push the food through a narrow gap (137). While in the case of semi-continuous HPP, the flow of liquid is introduced and contained in the same chamber at constant pressure for a specific time, after that, processed liquid food is stored in sterile tanks (138).

High-pressure processing has been employed for various milk proteins hydrolysis including whey protein concentrate (139, 140), cheddar cheese (101), bovine whey protein beta-lactoglobulin (141), whey protein isolate (142), cheddar cheese (22), beta-lactoglobulin (143), and bovine whey proteins (144) as cited in Table 5. Relatively, HPP is a well-developed technology that has many applications to milk and cheese (21, 22, 145). Munir et al. (101) reported the increased ACE Inhibitory activity HPP treated milk cheese and indicated that HPP results in efficient bioactive peptides liberation and proteolysis by imparting change in indigenous milk enzymes structures by subjecting more active sites for protein reaction (22, 146). Various studies have been reported in which the patterns of native and pressure-treated proteins have been compared. Indeed, Maynard and coworkers found that under pressurization tryptic β-LG hydrolysis generated a low concentration of intermediate hydrolysis peptides (147). On the other hand, Knudsen and coworkers reported the application of HPP at the beginning step of trypic β-LG hydrolysis that generated an increased amount of high molecular weight peptides and hydrophobic peptides (148).

In the food industry, high-pressure processing is well known as a clean method compared to conventional methods as it offers numerous advantages such as homogeneous and constant pressurization at ambient temperatures, utilize less energy due to maintenance of constant pressure when reached absolute pressure, quick pressurization, and de-pressurization, reduced processing time, and it’s throughout applications irrespective of shape or size in the food system (149–151). However, the applications of this technology have certain limitations such as batch operation and costly infrastructure around 0.6–4 M US dollars accounting for 75–80% of the investment as the initial investment (152, 153). HPP has limited effects on covalent bond cleavage and production of bioactive peptides alone, therefore, it’s employed in combination with enzymatic hydrolysis to denature protein and improved access to sites of enzyme cleavage to get efficient and increased production process of bioactive peptides (91).

**FUTURE OUTLOOK**

Numerous studies on the identification and evaluation of *in vitro* bioactivity of peptides from protein hydrolyzates of
several sources of protein suggest that novel technologies should be employed to isolate novel ingredients to prepare novel functional foods. But, the application of novel technologies is an emerging field of rising significance in the dairy industry as, till now, there are minimal studies on the improvement of fermentation/enzymatic hydrolysis using UA, MA, and HPP as pretreatments to produce bioactive peptides while fermentation/enzymatic hydrolysis are promising conventional methods to generate peptides at industrial level. Thus, fermentation/enzymatic hydrolysis of dairy proteins treated with ultrasound, microwave, and high-pressure is possible to generate improved bioactive peptides at a lower cost and short time compared to only conventional applications methods.

In the dairy industry, mostly milk is used as a medium in novel technologies. So, there is a gap in understanding that either the treatment of novel technologies enhances or alters the fermentation/enzymatic hydrolysis in whole milk, fermented milk, yogurt, cheese, and other dairy products. The synergistic effect of possible novel technologies can be investigated to understand the liberation of bioactive peptides at a low cost and short time. For instance, microwave heating and ultrasound waves/HPP pressure combination could be tested to explore the effect of heat treatment and high frequency/pressure on the release of bioactive peptides from dairy proteins. As several studies reported that the applications of these novel technologies could generate lower-cost ingredients with a higher content of available amino acids for the dairy industry.

Future studies are expected to establish the actual applications of novel technologies by investigating the maximum potential of these processing technologies to comprehend their possible specificities in the proteins’ cleavage, generate novel, and known bioactive peptides, effects on specificity, and modification of amino acids in dairy proteins.

CONCLUSION

It is noteworthy that milk protein hydrolysis liberates a wide variety of bioactive peptides that possess remarkable biological functions to maintain human health. The knowledge of bioactive peptides from milk and other dairy proteins and their health benefits increases with each passing day. It’s also opening new doors to exciting offers such as novel functional foods that can help manage and prevent several chronic diseases including cardiovascular diseases, diabetes, hypertension, cancer, etc.

Although the dairy industry is slow in embracing novel technologies but reported studies to depict that UAP, MAP, and HPP are innovative, environmentally friendly, and promising pretreatments to modify the profile of peptides, improve the yields of peptides, and higher liberation of bioactive peptides as compared to conventional processing technologies. Novel technologies require sustainable, environment-friendly, and highly specialized cost equipment and workers to operate the equipment. These novel processing technologies are coupled with conventional methods for unfolding, denaturing, or aggregating the milk proteins by breaking down weak molecular interactions with less or no effect on covalent bonds. The influence of pretreatments is intensified by fermentation/enzymatic hydrolysis, which results in a higher amount of liberated low molecular weight bioactive peptides, enhanced hydrolysis, and increased proteolysis of dairy proteins which ultimately increases their bioactivity. Many studies have reported the isolation of novel bioactive peptides from dairy proteins after employing novel technologies as pretreatments.

Ultrasound-assisted processing is an innovative and most efficient technology as it offers easy control, simple operation, mild operating conditions, the ability to achieve industrial amplification and production, and effective influence of auxiliary enzymatic hydrolysis. Its mechanical effects and cavitation change the protein conformation, increase the biological functionalities of enzymes, and enhance the reaction rate of enzymatic hydrolysis. Though novel technologies are innovative, environmentally friendly, and promising pretreatments, their trend is increasing and acquisitioning momentum to produce bioactive peptides.

AUTHOR CONTRIBUTIONS

MAM and SI: writing original draft. IH, MMANR, AR, and MSM: reviewing and editing. SI and MMANR: conceptualization and methodology. MAM, MMANR, and SAI: supervision and project administration. SAS: visualization and data curation. SAI: funding acquisition. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Nagpal R, Behare P, Rana R, Kumar A, Kumar M, Arora S, et al. Bioactive peptides derived from milk proteins and their health beneficial potentials: an update. Food Funct. (2011) 2:18ñ27. doi: 10.1039/c0fo00016g

2. Phelan M, Kerins D. The potential role of milk-derived peptides in cardiovascular disease. Food Funct. (2011) 2:153ñ67. doi: 10.1039/ c1fo10017c

3. Shazly AB, He Z, El-Aziz MA, Zeng M, Zhang S, Qin F , et al. Fractionation and identification of novel antioxidant peptides from buffalo and bovine...
casein hydrolysates. *Food Chem.* (2017) 232:753–62. doi: 10.1016/j.foodchem.2017.04.071

4. Zhang Y, Zhang H, Wang L, Guo X, Qi X, Qian H. Influence of the degree of hydrolysis (DH) on antioxidant properties and radical-scavenging activities of peanut peptides prepared from fermented peanut meal. *Eur Food Res Technol.* (2011) 233:41–50. doi: 10.1007/s00217-011-1466-0

5. Singh BP, Vij S, Hati S. Functional significance of bioactive peptides derived from soybean. *Peptides*. (2014) 54:171–9. doi: 10.1016/j.peptides.2014.01.022

6. Guzmán-Rodríguez F, Gómez-Ruizy L, Rodriguez-Serrano G, Alatorre-Santamaria S, García-Garibay M, Cruz-Guerrero WA. Iron binding and antimicrobial peptides released during the fermentation of milk by *Lactobacillus casei shirota*. *Rev Mex Ing Quim.* (2019) 18:1161–6. doi: 10.24275/urn:dc:revmxingquim/2019/v18/3/Guzman

7. Toldrá F, Reig M, Aristoy MC, Mora L. Generation of bioactive peptides during food processing. *Food Chem.* (2018) 267:395–404. doi: 10.1016/j.foodchem.2017.06.119

8. Soleymanzadeh N, Mirdamadi S, Mirzaei M, Kianirad M. Novel β-casein derived antioxidant and ACE-inhibitory active peptide from camel milk fermented by *Lecanostoc lactis* PTCC1899: identification and molecular docking. *Int Dairy J.* (2019) 97:201–8. doi: 10.1016/j.idairyj.2019.05.012

9. Sultan S, Huma N, Butt MS, Aleem M, Abbas M. Therapeutic potential of dairy bioactive peptides: a contemporary perspective. *Crit Rev Food Sci Nutr.* (2018) 58:105–15. doi: 10.1080/10408398.2015.1136590

10. Taha S, El Abd M, De Gobba C, Abdel-Hamid M, Khalil E, Hassan D. Antioxidant and antibacterial activities of bioactive peptides in buffaloes' yoghurt fermented with different starter cultures. *Food Sci Biotechnol.* (2017) 26:1325–32. doi: 10.1007/s10068-017-1160-9

11. Pescuma M, Hébert EM, Rasesona H, Drouet M, Choiset Y, Haertlé T, et al. Proteolytic action of *Lactobacillus delbrueckii* subsp. bulgaricus CRL 656 reduces antigenic response to bovine β-lactoglobulin. *Food Chem.* (2011) 127:487–92. doi: 10.1016/j.foodchem.2011.01.029

12. Stelwagen K, Carpenter E, Haigh B, Hodgkinson A, Wheeler TT. Immune components of bovine colostrum and milk1. *J Anim Sci.* (2009) 87:3–9. doi: 10.2527/jas.2008-1377

13. Mahdi C, Untari H, Padaga MC. Identification and characterization of bioactive peptides of fermented goat milk as A sources of antioxidant as a therapeutic natural product. *IOP Conf Ser.)* (2018) 299:012014. doi: 10.1088/1757-899X/299/1/012014

14. Kitts D, Weiler K. Bioactive proteins and peptides from food sources. *Crit Rev Food Sci Nutr.* (2014) 13:5ñ25. doi: 10.17306/j.afs.2014.1.1

15. Kitts D, Weiler K. Bioactive proteins and peptides from food sources. *Food Qual Saf.* (2013) 9:1309ñ23. doi: 10.2174/138161203454883

16. Roblet C, Amiot J, Lavigne C, Marette A, Lessard M, Jean J, et al. Screening remarks about agro-foods. *Innov Food Sci Emerg Technol.* (2003) 4:318ñ39. doi: 10.1016/S1466-9583(02)00004-0

17. Voigt DD, Chevalier F, Donaghy JA, Patterson MF, Qian MC, Kelly AL. Effect of high-pressure treatment of milk for cheese manufacture on proteolysis, lipolysis, texture and functionality of cheddar cheese during ripening. *Innov Food Sci Emerg Technol.* (2012) 13:23ñ30. doi: 10.1016/j.ifset.2011.10.004

18. Wang YC, Yu RC, Chou CC. Antioxidative activities of soy milk with lactic acid bacteria and bifidobacteria. *Food Microbiol.* (2006) 23:128ñ35. doi: 10.1016/j.fm.2005.01.020

19. Usá B, Yilmaz-Ersan L. Antioxidant enzymes of milk and their biological effects. *Ziınat Fakültesi Derg Uludag ? Üniversitesi.* (2013): 129–30.

20. Punja H, Tokas J, Malik A, Sangwan S, Baloda S, Singh N, et al. Identification and detection of bioactive peptides in milk and dairy products: remarks about agro-foods. *Molecules.* (2020) 25:3328. doi: 10.3390/molecules25153328

21. Dziva B, Dziva M. Milk proteins-derived bioactive peptides in dairy products: molecular, biological and methodological aspects. *Acta Sci Pol Technol. Aliment.* (2019) 27:318ñ39. doi: 10.1016/j.jaf.jsfa.2017.04.071

22. Shiouda S, Nakamachi T. PAcAP as a neuroprotective factor in ischemic neuronal injuries. *Peptides.* (2015) 72:202–7. doi: 10.1016/j.peptides.2015.08.006

23. Egger L, Ménard O. Update on bioactive peptides after milk and cheese digestion. *Curr Opin Food Sci.* (2017) 14:116ñ21. doi: 10.1016/j.cofs.2017.03.003

24. Sánchez A, Vázquez A. Bioactive peptides: a review. *Food Qual Saf.* (2017) 1:29–46. doi: 10.1016/j.fqsafe.2016.07.006

25. Shangmugam VP, Kapila S, Kapila R, Muthukumar S, et al. Isolation and characterization of angiotensin converting enzyme inhibitory peptide derived from buffalo casein. *Int J Pept Res Ther.* (2017) 27:1481ñ487. doi: 10.3168/JDS.2020-19271

26. Khajeh E, Jamshidian-Mojaver M, Naeemipour M, Farzin H. Simulated gastrointestinal digestion of camel and bovine casein hydrolysates: identification and characterization of novel anti-diabetic bioactive peptides. *Food Chem.* (2021) 353:129374. doi: 10.1016/j.foodchem.2021.129374

27. Panchal GK, Das S, Sarkure A, Singh BP, Hati S. Production and characterization of novel angiotensin converting enzyme inhibitory peptide derived from camel whey proteins. *J Dairy Sci.* (2021) 104:1366ñ74. doi: 10.3168/jds.2020-19271

28. Khajeh E, Jamshidian-Mojaver M, Naeemipour M, Farzin H. The identification of a novel peptide derived from lactoferrin isolated from camel milk with potential antimicrobial activity. *Iran J Med Microbiol.* (2021) 15:302ñ16. doi: 10.30699/IJMM.15.3.302

29. Panchal GK, Das S, Sarkure A, Singh BP, Hati S. Production and characterization of antioxidant peptides during lactic fermentation of goat milk. *J Food Process Preserv.* (2021) 45:e15992. doi: 10.1111/jfpp.15992

30. Liu Q, Yang M, Zhao B, Yang F. Isolation of antioxidant peptides from yak casein hydrolysate. *RSC Adv.* (2019) 9:10844ñ51. doi: 10.1039/d9ra02644a

31. Parmar H, Hati S, Panchal G, Sarkure AA. Purification and production of novel angiotensin i-converting enzyme (ACE) inhibitory bioactive peptides derived from fermented goat milk. *Int J Pept Res Ther.* (2020) 26:997ñ1011. doi: 10.3109/109899-019-09902-7

32. Zhao Q, Shi Y, Wang X, and Huang A. Characterization of a novel antimicrobial peptide from buffalo casein hydrolysate based on live bacteria adsorption. *J Dairy Sci.* (2020) 103:11116ñ11128.

33. Wali A, Yanhua G, Ishimou U, Yili A, Aisa HA, Salikhov S. Identification and identification of three novel antioxidant peptides from the bacterium camel milk hydrolysates. *Int J Pept Res Ther.* (2019) 26:1–10.
40. Jiang B, Na J, Wang L, Li D, Liu C, Feng Z. Separation and enrichment of antioxidant peptides from whey protein isolate hydrolysate by aqueous two-phase extraction and aqueous two-phase flotation. *Food Sci.* (2019) 8:34. doi: 10.3390/foods8010034

41. Elkahhat E, El-Alfi M, Shenana M, Mohamed A, Yousef AE. New potentially hypotensive peptides liberated in milk during fermentation with selected lactic acid bacteria and kombucha cultures. *J Dairy Sci.* (2017) 100:9508–20. doi: 10.3168/jds.2017-13150

42. Caprriott A, Cavaile C, Piovesana S, Samperi R, Laganà A. Recent trends in the analysis of bioactive peptides in milk and dairy products. *Anal Bioanal Chem.* (2016) 408:2677–85. doi: 10.1007/s00216-016-9303-8

43. Korhonen H. Milk-derived bioactive peptides: from science to applications. *J Funct Foods.* (2009) 1:177–87. doi: 10.1016/j.jff.2009.01.007

44. O’Keeffe MB, Fitzgerald RJ. Identification of short peptide sequences in complex milk protein hydrolysates. *Food Chem.* (2015) 184:140–6. doi: 10.1016/j.foodchem.2015.03.077

45. Sadat L, Cakir-Kiefer C, N’Negue MA, Gaillard JL, Girardet JM, Miclo L. Isoalation and identification of antioxidative peptides from bovine α-lactalbumin. *Int Dairy J.* (2011) 21:214–21. doi: 10.1016/j.idairyj.2010.11.011

46. Tonolo F, Foda A, Cesaro L, Scalvon C, Marin O, Ferro S, et al. Milk-derived bioactive peptides exhibit antioxidant activity through the keap1-Nrf2 signaling pathway. *J Funct Foods.* (2020) 64:103696. doi: 10.1016/j.jff.2019.103696

47. Ahmed AS, El-Bassiony T, Elmalt LM, Ibrahim HR. Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Res. Int.* (2015) 74:80–8. doi: 10.1016/j.foodres.2015.04.032

48. Zhang Y, Shen Y, Zhang H, Wang L, Zhang H, Qian H, et al. Isolation, purification and identification of two antioxidative peptides from water hyacinth leaf protein hydrolysates (WHILPH). *Eur Food Res Technol.* (2018) 244:83–96. doi: 10.1007/s00217-017-2941-z

49. Ahmed AS, El-Bassiony T, Elmalt LM, Ibrahim HR. Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Res. Int.* (2015) 74:80–8. doi: 10.1016/j.foodres.2015.04.032

50. Walther B, Schmid A, Sieber R, Wehrmüller K. Cheese in nutrition and health. *Dairy Sci. Technol.* (2017) 101:68–111. doi: 10.1007/s10601-015-0016-7

51. Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Laganà A. Recent trends in the analysis of bioactive peptides in milk and dairy products. *Anal Bioanal Chem.* (2016) 408:2677–85. doi: 10.1007/s00216-016-9303-8

52. O’Keeffe MB, Fitzgerald RJ. Identification of short peptide sequences in complex milk protein hydrolysates. *Food Chem.* (2015) 184:140–6. doi: 10.1016/j.foodchem.2015.03.077

53. Sadat L, Cakir-Kiefer C, N’Negue MA, Gaillard JL, Girardet JM, Miclo L. Isolation and identification of antioxidative peptides from bovine α-lactalbumin. *Int Dairy J.* (2011) 21:214–21. doi: 10.1016/j.idairyj.2010.11.011

54. Kim S, Lim SD. Separation and enrichment of lipase inhibitory peptide from fermented milk by Lactobacillus delbrueckii ssp. bulgaricus CM4. *Int J Food Sci Technol.* (2020) 56:14891. doi: 10.1111/ijf.14891

55. Kim S, Lim SD. Separation and purification of lipase inhibitory peptide from fermented milk by Lactobacillus delbrueckii ssp. bulgaricus CM4. *Int J Food Sci Technol.* (2020) 56:14891. doi: 10.1111/ijf.14891

56. Timón ML, Andrés AI, Otte J, Petrón MJ. Antioxidant peptides (≤3 kDa) identified on hard cow milk cheese with rennet from different origin. *Food Res Int.* (2019) 120:643–9. doi: 10.1016/j.foodres.2018.11.019

57. Fan M, Guo T, Li W, Chen J, Li F, Wang C, et al. Isolation and identification of novel casein-derived bioactive peptides and potential functions in fermented casein with Lactobacillus helveticus. *J Bacteriol.* (1995) 173:3472–8. doi: 10.1128/jb.173.11.3472-3478.1995

58. Daliri EBM, Lee BH, Park BJ, Kim SH, Oh DH. Antihypertensive peptides from whey proteins fermented by lactic acid bacteria. *Food Sci Biotechnol.* (2018) 27:1781–9. doi: 10.1007/s10068-018-0423-0

59. Daliri EBM, Lee BH, Park BJ, Kim SH, Oh DH. Antihypertensive peptides from whey proteins fermented by lactic acid bacteria. *Food Sci Biotechnol.* (2018) 27:1781–9. doi: 10.1007/s10068-018-0423-0
93. Tho P, Manasseh R, Ooi A. Cavitation microstreaming patterns in single and multibubble sonoluminescence. J Acoust Soc Am. (2002) 112:1405–13. doi: 10.1121/1.1502898

94. Chandrasekaran S, Ramanathan S, Basak T. Microwave food processing: A review. Food Res Int. (2013) 52:243–61. doi: 10.1016/j.foodres.2013.02.033

95. Dabbour M, He R, Mintah B, Golly MK, Ma H. Ultrasound pretreatment of sunflower protein: impact on enzymolysis, ACE-inhibition activity, and structure characterization. J Food Process Preserv. (2020) 44:e14398. doi: 10.1111/jfpp.14398

96. Abadía-García L, Castaño-Tostado E, Cardador-Martínez A, Martín-Delcampo Ste, Amaya-Llanos SL. Production of ACE inhibitory peptides from whey proteins modified by high intensity ultrasound using bromelain. Foods. (2021) 10:102099. doi: 10.3390/FOODS10092099

97. Koirala S, Prathumpai W, Anal AK. Effect of ultrasound pretreatment followed by enzymatic hydrolysis of caprine milk proteins and on antioxidant and angiotensin converting enzyme (ACE) inhibitory activity of peptides thus produced. Int Dairy J. (2021) 118:105026. doi: 10.1016/j.idairyj.2021.105026

98. Cui P, Yang X, Liang Q, Huang S, Lu F, owusu J, et al. Ultrasound-assisted preparation of ACE inhibitory peptide from milk protein and establishment of its in-situ real-time infrared monitoring model. Ultrason Sonochem. (2020) 62:104859. doi: 10.1016/j.ultsonch.2019.104859

99. Munir M, Nadeem M, Mahmood Qureshi T, Gamalh CJ, Martin GJO, Hemar Y, et al. Effect of sonication, microwaves and high-pressure processing on ACE-inhibitory activity and antioxidant potential of cheddar cheese during ripening. Ultrason Sonochem. (2020) 67:105140. doi: 10.1016/j.ultsonch.2020.105140

100. Lorenzetti A, Penha FM, Cunha Petrus JC, Rezzadori K. Low purity enzymes and ultrasound pretreatment applied to partially hydrolyze whey protein. Food Biophys. (2016) 11:3549–62. doi: 10.1007/s11483-016-9244-x

101. Munir M, Nadeem M, Mahmood Qureshi T, Gamalh CJ, Martin GJO, Hemar Y, et al. Effect of ultrasound pretreatment on antioxidative capacity and antioxidant potential of cheese during ripening. Int Dairy J. (2017) 67:84–90. doi: 10.1016/j.idairyj.2016.08.010

102. Jovanović JR, Stefanović AB, Šekuljica NV, Tanasković SMJ, Dojčinović MB, Bugarski BM, et al. Ultrasound pretreatment as an useful tool to enhance egg white protein hydrolysis: kinetics, reaction model, and thermodynamics. J Food Sci. (2016) 81:C2664–75. doi: 10.1111/1750-3841.13503

103. Wang B, Atungulu GG, Khir R, Geng J, Ma H, Li Y, et al. Ultrasonic treatment effect on enzymolysis kinetics and activities of ACE-inhibitory peptides from oat-isolated protein. Food Biophys. (2015) 10:244–52. doi: 10.1007/s11483-014-9375-y

104. Bermúdez-Aguirre D, Mawson R, Barbosa-Cánovas GV. Microstructure of fat globules in whole milk after sonication treatment. J Food Sci. (2008) 73:E325–32. doi: 10.1111/j.1750-3841.2008.00875.X

105. Leong TSH, Walter V, Gamalh CJ, Yang M, Martin GJO, Ashokkumar M. Functionalised dairy streams: tailoring protein functionality using sonication and heating. Ultrason Sonochem. (2018) 48:499–508. doi: 10.1016/j.ultsonch.2018.07.010

106. Wu Q, Zhang X, Jia J, Kuang C, Yang H. Effect of ultrasound pretreatment on whey protein hydrolysis by alcalase: thermodynamic parameters, physicochemical properties and bioactivities. Process Biochem. (2018) 67:46–54. doi: 10.1016/j.procbio.2018.02.007

107. Zhou C, Ma H, Yu X, Liu B, Yagoub AEGA, Pan Z. Pretreatment of defatted wheat germ proteins (by-products of flour mill industry) using ultrasonic horn and bath reactors: Effect on structure and preparation of ACE-inhibitory peptides. Ultrason Sonochem. (2013) 20:1390–400. doi: 10.1016/j.ultsonch.2013.04.005

108. Yang X, Li Y, Li S, Oladejo AO, Wang Y, Huang S, et al. Effects of multi-frequency ultrasound pretreatment under low power density on the enzymolysis and the structure characterization of defatted wheat germ
131. Sparr Eskilsson C, Björklund E. Analytical-scale microwave-assisted protein hydrolysis with immobilized enzymes. J Sci Food Agric. (2017) 97:599–607. doi: 10.1002/jsfa.9660

132. Gomaa A. Microbial inactivation and chemical properties of bovine milk during microwave heating. J Food Sci Technol. (2015) 50:2105–12. doi: 10.1016/j.jfist.2004.04.001

133. Naderi N, House JD, Pouliot Y, Doyen A. Effects of high hydrostatic pressure processing on hen egg compounds and egg products. Compr Rev Food Sci Food Saf. (2017) 16:707–20. doi: 10.1111/1541-4337.12273

134. Balasubramaniam VM, Ting EY, Stewart CM, Robbins JA. Recommended laboratory practices for conducting high pressure microbial inactivation experiments. Innov Food Sci Emerg Technol. (2004) 5:299–306. doi: 10.1016/j.ifset.2004.04.001

135. Messens W, Van Camp J, Huyghebaert A. The use of high pressure to modify the functionality of food proteins. Trends Food Sci Technol. (1997) 8:107–12. doi: 10.1016/S0924-2244(97)01015-7

136. Oliveira MMD, Augusto PED, Cruz AG, Da, Cristianini M. Effect of dynamic high pressure on milk fermentation kinetics and rheological properties of probiotic fermented milk. Innov Food Sci Emerg Technol. (2014) 26:67–75. doi: 10.1016/j.ifset.2014.05.013

137. Singh RP, Yousef AE. Technical Elements of New and Emerging Non-Thermal Food Technologies. Rome: FAO (2001).

138. Balasubramaniam V, Barbosa-Canovas GV, Lelieveld H. High Pressure Processing of Food. Food Engineering Series. New York, NY: Springer (2016). p. 39–65.

139. Landim AP, Matsubara NK, da Silva-Santos JE, Mellinger-Silva C, Rosenthal A. Application of preliminary high pressure processing for improving bioactive characteristics and reducing antigenicity of whey protein hydrolysates. J Food Sci Technol. (2021) 1:1082032112210221. doi: 10.1177/108203212122102216

140. Paula A, Landim M, Hidalgo Chávez DW, Santos Da Rosa J, Mellinger-Silva C, Rosenthal A. Effect of high hydrostatic pressure on the antioxidant capacity and peptic hydrolysis of whey proteins. Ciênc Rural. (2021) 51:2021. doi: 10.1590/0103-8478CR20200560

141. Boukil A, Suwal S, Chamberland J, Pouliot Y, Doyen A. Ultrafiltration performance and recovery of bioactive peptides after fractionation of trypsin hydrolysate generated from pressure-treated B-lactoglobulin. J Membr Sci. (2018) 565:42–53. doi: 10.1016/j.memsci.2018.03.079

142. Piccolomini A, Iskandar M, Lands L, Kubow S. Enzymatic treatments on the hydrolysis and immunoreactivity of dairy whey proteins. Int Dairy J. (2005) 15:471–81. doi: 10.1016/j.idairyj.2005.08.009

143. Huppertz T, Fox PF, Kelly AL. Properties of casein micelles in high pressure-treated bovine milk. Food Chem. (2004) 87:103–10. doi: 10.1016/j.foodchem.2003.10.025

144. Juan B, Barron LJR, Ferragut V, Trujillo AJ. Effects of high pressure treatment on the tryptic hydrolysis of bovine β-lactoglobulin A induced by high hydrostatic pressure. J Agric Food Chem. (2006) 54:2333–41. doi: 10.1021/jf051983s

145. Peñas E, Préstamo G, Luisa Baeza M, Martínez-Molero MI, Gomez R. Effects of combined high pressure and enzymatic treatments on the hydrolysis and immunoreactivity of dairy whey proteins. Int Dairy J. (2016) 68:331–9. doi: 10.1016/j.idairyj.2005.08.009

146. Vaidyanath R, Jayas DS. Non-uniform temperature distribution during microwave heating of food materials-A review. Food and Bioprocess Technology. (2010) 3:161–71. doi: 10.1007/s11947-008-0136-0

147. Zhong H, Marcus SL, Li L. Microwave-assisted acid hydrolysis of proteins combined with liquid chromatography MALDI MS/MS for protein identification. J Am Soc Mass Spectrom. (2005) 16:471–81. doi: 10.1016/j.jasms.2004.12.017

148. Lo Y, Li J, Lin SJ, Yang ZS, Jin HX. Preparation of antioxidant peptide by microwave-assisted hydrolysis of collagen and its protective effect against H2O2-induced damage of RAW264.7 cells. Mar Drugs. (2019) 17:642. doi: 10.3390/md17110642

149. Sparr Eskilsson C, Björklund E. Analytical-scale microwave-assisted extraction. J Chromatogr. (2000) 902:227–50. doi: 10.1016/S0021-9673(00)00923-1

150. Chassekhati N, Akbarian M, Morshed A, Poursharif Z, Aghamohammadmi B, Moayedi F. Microbiological effects of high pressure processing on foodnet Microbiological effects of high pressure processing on food. J Bio. (2014) 4:133–45.
153. Yamamoto K. Food processing by high hydrostatic pressure. Biosci Biotechnol Biochem. (2017) 81:672–9. doi: 10.1080/09168451.2017.1281723

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