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Effects Of Adding Cow Milk To Curd On Microbes and Peptides, and their Effects on Gut Microbiota; Short Chain Fatty Acids and Peptides. A Double Blind, Randomized, Placebo controlled Comparison.

Background. Milk, curd and yogurt are important elements of the human diet, due to their high nutritional value and their appealing sensory properties. During milk processing (homogenization, pasteurization) and further yogurt manufacture (fermentation) physicochemical changes occur that affect the flavor and texture of these products while the development of standardized processes contributes to the development of desirable textural and flavor characteristics. Yogurt is defined as the product being manufactured from milk, with or without the addition of some natural derivative of milk, such as skim milk powder, whey concentrates, caseinates or cream, with a gel structure. The gel like texture results from the coagulation of the milk proteins, due to the lactic acid secreted by defined species of bacteria cultures. Furthermore, these bacteria must be "viable and abundant" at the time of consumption. In the making of the curd, we do not add any thing accept few ml of older curd in the milk, for fermentation and conversion of milk to curd.

Lactobacillus converts milk to curd by producing lactic acid and making the taste of the curd sour. Lactobacillus is the microorganism present in curd. This bacterium converts milk into curd at 30 to 40oC.Yogurt is a popular fermented dairy product produced by lactic acid bacteria, including Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. During yogurt production, these bacteria **produce lactic acid, decreasing pH and causing milk protein to coagulate**. **Lactobacillus Bacteria** in Milk that changes Milk to Curd. When milk is heated to a temperature of 30-40 degrees centigrade and a small amount of old curd added to it, the lactobacillus in that curd sample gets activated and multiplies. These convert the lactose into lactic acid. When milk gets converted into curd, the bacteria improves the nutritional quality by increasing **vitamin-B12**. In our stomach, the LAB plays a beneficial role in preventing disease causing microbes. The growth of bacterial cells within dairy foods is heavily influenced by parameters such as **pH, water activity and salt-in-moisture levels as well as temperature**. The use of starter bacteria is needed in order to acidify the cheese milk before and during dairy food production

Milk is converted into curd or yogurt by the process of fermentation. Milk consists of globular proteins called casein. Here curd forms because of the chemical reaction between the lactic acid bacteria and casein. During fermentation, the bacteria use enzymes to produce energy (ATP) from lactose. The pasteurization process kills any pathogens that can spoil milk as well as to eliminate potential competitors of the active cultures. After milk pasteurization, the milk is cooled down to 108 degrees Fahrenheit, the temperature for optimal growth of yogurt starter cultures. Instead, the two bacteria used in yogurt production, Lactobacillus delbruekii sp. bulgaricus and Streptococcus thermophilus, help each other grow until they reach a stable balance. Together, they transform the lactose naturally present in milk into lactic acid, creating yogurt.

Our hypothesis is that adding milk to the curd in equal amount may cause formation of new lactobaccilus strains or increase the number and potency of exiting strains and produce peptides or the mixture may develop increased activity of existing peptides. Our preliminary observations found that adding milk to curd is associated with increased density of lactobaccilus in the product after 12-24 hours, which needs confirmation. There is no study in the literature to find out the effects of adding milk to curd or with basil leaves with reference to its protective constituents. Basil leave has potential antioxidant flavonoids with antiinflammatory effects. There are gaps in the knowledge about what happens in the final product of yogurt in relation to the development or the occurrence of concentration of existing microbes, or new microbes or making of peptides in the final yogurt ready for consumption. The effects of curd versus mixture of curd and milk in equal amount on gut microbiota are not yet known.

Objectives.

In vitro:

1.To find out the lactobaccilus and other microbes in the mix, after 5 min, 1,hr, 6hr and 12hr and 24 hr of mix preparation.

2. To find out the contents of peptides, fatty acids, quantity and activity, after, 5 min, 1, hr, 6 hr, 12 hr and 24 hr of mixed preparation.

In Vivo:

1.To find out the effects of commonly available yogurt on microbiota.

2. To find out the effects of mix of curd and milk in equal amount on microbiota, compared to placebo and yoghurt.

Methods

Boiled milk 200ml then cool it.
Make curd out of 100 ml milk
Washed basil leaves
Take samples from each and mix

Group 1: either with milk (5 samples) or
Group 2: placebo (5 sample).
Group 3: with Basil leave extract, 1 gm each sample (5 samples)
Mix both milk and curd to examine the content for Lactobaccilus strains, Bifido, GABA and peptides. immediately within 5 min, 1 hour, 6 hr, 12 hr and 24 hr.
The experts doing the experiment and examining the content should be blind to group.

Contents to be Examined.

Streptococcus thermophilus, Lactobacillus casei, and Bifidobacterium bifidum etc
SCFA like butyrate,acetate
Antimicrobial peptides
Antihypertensive peptides
Other peptides.
GABA

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Abstract: Previous studies found variations in the health-promoting effects of consuming different dairy products. This study aims at investigating the chemical composition of microbial fermented yogurt, chemically acidified yogurt and whole milk to understand the differences in the effects these products exert on human health. For this purpose, peptides and small compounds present in the products were examined using a combination of liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopic techniques. Results revealed that each product had its own characteristic peptide, free amino acid and small compound profile, and database search for bioactivity

disclosed that fermented yogurt manufactured using a starter culture is associated with a higher bioactivity potential than chemically acidified yogurt or whole milk. Additional cold storage (14 days) further enhances the bioactivity potential of fermented yogurt while heat-inactivation, ensuring long shelf-life, modulates the proteins available for proteolysis and thereby the peptide profile generated.

Keywords: Metabolites; Bioactive; Proteolysis; Starter cultures; Mass spectrometry; Extended shelf-life

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To analyse the effect of cell-associated peptidases in yogurt starter culture strains Lactobacillus delbrueckii ssp. bulgaricus (LB) and Streptococcus thermophilus (ST) on milk-protein-based antimicrobial and hypotensive peptides in order to determine their survival in yogurt-type dairy foods. The 11mer antimicrobial and 12mer hypotensive milk-protein-derived peptides were incubated with mid-log cells of LB and ST, which are required for yogurt production. Incubations were performed at pH 4.5 and 7.0, and samples removed at various time points were analysed by reversed-phase high-performance liquid chromatography (RP-HPLC). The peptides remained mostly intact at pH 4.5 in the presence of ST strains and moderately digested by exposure to LB cells. Peptide loss occurred more rapidly and was more extensive after incubation at pH 7.0. The 11mer and 12mer bioactive peptides may be added at the end of the yogurt-making process when the pH level has dropped to 4.5, limiting the overall extent of proteolysis. The results show the feasibility of using milk-protein-based antimicrobial and hypotensive peptides as food supplements to improve the health-promoting qualities of liquid and semi-solid dairy foods prepared by the yogurt fermentation process.

Mirzapour-Kouhdasht, A.; Garcia-Vaquero, M. Cardioprotective Peptides from Milk Processing and Dairy Products: From Bioactivity to Final Products including Commercialization and Legislation. Foods 2022, 11, 1270. https:// doi.org/10.3390/foods11091270

Recent research has revealed the potential of peptides derived from dairy products preventing cardiovascular disorders, one of the main causes of death worldwide. This review provides an overview of the main cardioprotective effects (assayed in vitro, in vivo, and ex vivo) of bioactive peptides derived from different dairy processing methods (fermentation and enzymatic hydrolysis) and dairy products (yogurt, cheese, and kefir), as well as the beneficial or detrimental effects of the process of gastrointestinal digestion following oral consumption on the biological activities of dairy-derived peptides. The main literature available on the structure–function relationship of dairy bioactive peptides, such as molecular docking and quantitative structure–activity relationships, and their allergenicity and toxicity will also be covered together with the main legislative frameworks governing the commercialization of these compounds. The current products and companies currently commercializing their products as a source of bioactive peptides will also be summarized, emphasizing the main challenges and opportunities for the industrial exploitation of dairy bioactive peptides in the market of functional food and nutraceuticals.

2. Bioactive Peptides Derived from Milk Bioactive peptides from milk are normally sequences of 2–50 amino acids [18] generated by in vivo and/or in vitro digestion processes, displaying cardioprotective effects upon their release from casein [4]. These peptides may

contribute to lower the risk of CVD through several pathways as previously described in Figure 1. These routes for CVD prevention include: (i) inhibiting the renin secreted from the kidney; (ii) inhibiting the conversion of angiotensin I into angiotensin II; and (iii) inhibiting the conversion of bradykinin to inactive components (non-vasodilator agents) [10,19]. Milk production worldwide has continued to grow over the last decade: 497 million metric tons of cow milk were produced in 2015, rising to levels of up to 544 million metric tons by 2021 [20]. The main proteinaceous constituents of milk that could be used for the generation of cardioprotective peptides include caseins (αS1-casein, αS2-casein, β-casein, and k-casein), α-lactalbumin (α-LA), β-lactoglobulin (β-LG), immunoglobulins, lactoferrin,

protease–peptide fractions, serum albumin, and transferrin [21]. These proteins could be used for either in vivo or in vitro digestion to generate bioactive peptides and then purify them by different separation techniques to assess their cardioprotective effects. Different factors affect the cardioprotective effects of bioactive peptides from milk, including the protein source, peptide sequence, and proteolytic activity. The hydrolysis process of milk by proteolytic enzymes is currently the preferred approach to obtain cardioprotective bioactive peptides from dairy proteins. The advantages of this method include high reproducibility, target specificity and controllable conditions during the hydrolysis [22], resulting in a cost-efficient process really suitable for the generation of these compounds at industrial level. There are several studies researching the cardioprotective effects of bioactive peptides derived from dairy proteins following a hydrolysis process [23–27]. The hydrolysis conditions can affect the bioactive peptides' production efficiency as well as their cardioprotective activity. Four major factors of the enzymatic hydrolysis process have been introduced to control the cardioprotective effects of generated bioactive peptides derived from dairy products including hydrolysis temperature, time, pH, and enzyme to substrate ratio. In a research performed by Guo et al. [28], the optimum hydrolysis conditions for ACE inhibitory of whey protein concentrate hydrolysates (obtained by crude proteinases from Lactobacillus helveticus LB13) were determined by response surface methodology (RSM). The results of this study indicated that the optimum conditions for achieving ACE inhibitory activity of 92.2% were an enzyme to substrate ratio of 0.60, 8 h, pH of 9.18, and 38.9 ◦C of temperature. The degree of hydrolysis is a factor that is positively related to the cardioprotective activity of the bioactive peptides [29]. This degree of hydrolysis is normally increased by increasing the processing time, temperature, and pH until certain levels specific for each protein in which no further degree of hydrolysis is appreciated due to the denaturation of the hydrolytic enzymes under unfavorable conditions [30]. The enzyme to substrate ratio is not linearly related to the degree of hydrolysis and cardioprotective effects of the peptides [31]. This effect could be due to an enzymatic steric effect that does not allow contact between the protein and the catalytic sites in the enzymes, and the reduction of substrate diffusion and saturation reaction rates [30]. In a study performed by Mazorra-Manzano et al. [32], whey protein was hydrolyzed to generate ACE inhibitory peptides using plant proteases. Whey protein hydrolysates (especially those from β-lactoglobulin) revealed the highest ACE inhibitory of 75–90%. However, the authors did not determine the amino acid sequences of the bioactive peptides from these hydrolysates responsible for that effect. The peptide sequences discovered from different milk proteins using proteolytic enzymes, as well as the cardioprotective activities reported in the scientific literature, are summarized in Table 1. Lin et al. [33] used qula casein from yak milk and hydrolyzed it using different enzymes (alcalase, α-chymotrypsin, thermolysin, proteinase K, trypsin, and papain). The authors identified 3 bioactive peptides with ACE inhibitory activity in vitro, PFPGPIPN, KYIPIQ, and LPLPLL, with IC50 of 12.79, 7.28, and 10.46 μM, respectively [33]. Lin et al. [34], indicated that qula casein hydrolysed by two approaches (combination of thermolysin + alcalase and thermolysin + proteinase K) could be a source of ACE inhibitory peptides. The identified bioactive peptides (KFPQY, MPFPKYP, MFPPQ, and QWQVL) were chemically synthesized, among.