Functional Fermented Milk Products

O. Brunser, M. Gotteland, S. Cruchet

Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile

Abstract

Fermented foods have been used since prehistoric times. Their number, variety and geographic origin are considerable, and different substrates and agents including bacteria, yeasts and moulds have been used in their preparation. In the last few decades the scientific approach to the study of the participating microorganisms and the resulting products have provided a better understanding of their biological importance. Among the many health-related properties of fermented foods, effects on blood pressure have been described after casein hydrolysis by lactic acid bacteria. Peptides with antimicrobial activity, mainly against Gram-negative bacteria, and derived from casein have also been identified. This could explain, at least in part, the antidiarrheal effects of fermented products including those on traveler’s diarrhea and against colonization by Helicobacter pylori. One of the best known advantages of fermented milk products is their capacity to improve lactose tolerance in hypolactasic subjects. With the growing prevalence of allergies and inflammatory bowel diseases, considerable interest has been focused on the effects of lactic acid bacteria in these conditions; there is evidence that these agents are associated with improvements in allergy; no such evidence exists for Crohn’s disease or ulcerative colitis. A cholesterol-lowering capacity has also been described for some microorganisms. Not all the fermenting microorganisms have probiotic capacities as the latter are strain-specific.

Milk and milk-derived products constitute a significant part of the diet of all ethnic groups at all ages. Because it is difficult to preserve foodstuffs, all cultures have resorted to fermentation as one of the procedures used to preserve foods for longer periods of time. As these are consumed routinely by the population, children also consume them as part of their diet from weaning. Fermented products derived from milk have become predominant; some of these, such as kefir, koumiss and shubat from goat, mare and camel milks, respectively, contain a wide range of nonpathogenic microorganisms including bacteria, moulds and yeasts which generate complex ecosystems. The
preparation of fermented foodstuffs was initially an empirical process; however interest has increased about the participating microorganisms, the chemical reactions implicated in the fermentation process, the compounds formed and their health benefits. Improvements in systems of food preservation and the massive industrialization of food elaboration during the second part of the last century decreased the consumption of fermented foodstuffs and, consequently of live microorganisms. It is tempting to speculate that this may have resulted in changes in the human resident microbiota and may be related to the increased prevalence of allergies and chronic inflammatory and autoimmune diseases [1].

**Milk Fermentation and Metabolite Production**

Fermented products derived from milk result from metabolism of lactose by different bacteria. The Codex Alimentarius of 1992 defines yogurt as agglomerated milk resulting from fermentation of lactose by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Other lactic acid bacteria (LAB), such as other species of *Lactobacillus*, *Streptococcus* and *Bifidobacterium*, can be added to starter strains to produce fermented milks with specific textural, organoleptic or functional (in the case of probiotic strains) characteristics.

The elaboration of fermented milks results from an intense fermentation process by the LAB, sometimes in association with yeast, acetic bacteria or moulds. A permease transports lactose into the bacterial cell where the disaccharide is hydrolyzed by a β-galactosidase into glucose and galactose; the latter is exported out of the cell while glucose is phosphorylated and converted first to pyruvic acid by an aldolase and then to lactic acid by lactate dehydrogenase [2]. There is a synergistic relation between *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* throughout the process: the former uses for its growth the amino acids and peptides produced by *L. bulgaricus* from the milk proteins while the growth of *L. bulgaricus* is stimulated by the carbon dioxide and short chain fatty acids produced by *S. thermophilus* [3]. Twenty to forty percent of the lactose in milk is transformed into lactic acid during yogurt elaboration such that in the final product the lactose concentration is 3.8–4.0 g/dl. The lactic acid concentration in yogurt ranges between 0.7 and 1.2 g/dl and the pH between 3.9 and 4.2. Bacterial lactase improves the intestinal hydrolysis of the residual lactose by hypolactasic individuals. In addition, the orocecal transit of fermented milk products is slower compared with the unfermented milk, allowing a more efficient action of both the bacterial lactase and any residual intestinal lactase. These processes explain why yogurt improves the intestinal digestion of lactose and at the same time the symptomatology in hypolactasic individuals [4].

During milk fermentation some vitamins such as pantothenic acid and vitamin B12 decrease while folic acid and niacin increase. Cow’s milk proteins represent a complex mixture of which about 80% are caseins with four main fractions
(\(\alpha_{s1}\), \(\alpha_{s2}\), \(\beta\)- and \(\kappa\)-caseins) in an approximate proportion of 38:11:38:13. One to two percent of the casein is hydrolyzed by LAB proteases releasing amino acids and peptides which are metabolized by the bacteria or accumulate in the product. Milk triglycerides are not modified during fermentation due to the absence of lipases in LAB. Fermented milks also contain growth factors, hormones and immune-stimulating molecules such as peptidoglycans, polysaccharides and teichoic acid. Some bioactive peptides derived from milk protein hydrolysis modulate the immune system, inhibit pathogen growth, or exert anti-inflammatory activities [5]. Some peptides, such as the casein macropeptide, also stimulate the growth of colonic \textit{Bifidobacterium} populations [6].

**Fermentation and Production of Peptides with Hypotensive Properties**

Milk protein fermentation by some LAB results in the release of tripeptides with blood pressure-lowering activities. Two of these, Isoleucyl-Prolyl-Proline (Ile-Pro-Pro) and Valyl-Prolyl-Proline (Val-Pro-Pro), isolated from casein digests by \textit{L. helveticus}, lower blood pressure in spontaneously hypertensive rats and in humans with mild hypertension [7]. Although hypertension is considered a disease of mature and old age, the precursor conditions are often present at a young age; furthermore, hypertension secondary to a number of conditions (kidney, endocrine, neurological diseases, etc.) are frequent in childhood and it is important to consider all useful preventive and therapeutic possibilities, including those associated with the effect of LAB upon food constituents [8].

In vivo and in vitro angiotensin-converting enzyme (ACE)-inhibitory activity originating from casein fractions has been detected during milk fermentation by different LAB strains [9]. These peptides are also of low molecular weight but their activities become apparent only after further proteolysis by pepsin and by trypsin. ACE is one of the main molecules that regulate blood pressure through its effects on the synthesis of angiotensin II, a potent vasoconstrictor which, also induces the degradation of bradykinin, a powerful vasodilator. The net result is that ACE inhibition causes the lowering of the blood pressure. In a study carried out with kefir prepared from goat’s milk, ACE-inhibitory peptides were detected in 16 sequences of amino acids that showed anti-hypertensive activity. Further hydrolysis of the original peptides with gastric and pancreatic enzymes resulted in new molecules with vasomotor effects [10].

In another study, middle-aged individuals with moderate hypertension received twice daily 150 ml of a milk fermented with \textit{L. helveticus} LBK-16H for 10 weeks with 7.5 mg/100 g of Ile-Pro-Pro and 10 mg/100 g of Val-Pro-Pro. The control group received the same product without the added active peptides. During a 4-week run in and in the course of a follow-up period of equal
duration, patients and controls received either a product fermented by a different probiotic or the control product. There was a significant decrease in the systolic and diastolic pressures in the groups receiving the milk fermented by the *Lactobacillus* or peptides [7]. Such reductions in blood pressure are of epidemiologic significance from the point of view of public health. Although both Ile-Pro-Pro and Val-Pro-Pro have been reported to be powerful inhibitors of ACE, no changes in its activity were observed in any of these participants, suggesting that another mechanism may be operating.

**Antimicrobial Activity in Fermented Foodstuffs**

A number of bioactive antimicrobial polypeptides encrypted in milk proteins have been identified; these are released during fermentation and/or digestion in the gastrointestinal tract, where they are released either as propeptides or as mature C- or N-terminal peptides. The antimicrobial peptides from casein are very potent and include caseicidins, isracidins, casocidin-I, kappacin and lactoferricin [11]. They exert lytic activities on bacteria by becoming inserted in the bacterial membrane to form channels which disrupt the microorganisms, allowing the income of water and the outward diffusion of electrolytes and small molecules. These antibacterial peptides have specificity for prokaryotic membranes. Fermentation of casein by *L. acidophilus* DPC6026 produces three peptides that represent fragments of isracidin, two of them with potent activity mostly against Gram-positive but also against Gram-negative bacteria such as *Streptococcus mutans*, *Escherichia coli* O157:H7 and *Enterobacter zakazakii*. The latter has been recognized as an etiological agent for meningitis in neonates, and infant formulae have served as a reservoir. The possibility of incorporating caseicins A or B may protect against *E. zakazakii* and should increase interest in these ingredients for protective purposes [12, 13].

**Fermented Foodstuffs in the Prevention and Treatment of Acute Diarrhea**

Fermented milks and cheeses have been used since Biblical times for their medicinal properties, particularly in the management of gastrointestinal diseases. The origin of yogurt is unknown and it has been prepared with the milk of buffaloes, cows, donkeys, sheep, camels and goats. Marriott and Davidson [14] postulated that acidified milks would be easier to digest by children and would prevent diarrhea. This was confirmed by in vitro studies showing that they inhibit the growth of enteropathogens. Early studies supported the idea that the administration of some bacteria exerted positive effects on bacterial diarrhea; it was suggested that this resulted from the modulation of immunity [15].
Effects on Bacterial Diarrhea

Few studies relate the administration of fermented milks to diarrhea associated with enteropathogenic bacteria. A study in the Karelian Republic showed that *L. casei rhamnosus* GG (LGG) did not shorten the duration of diarrhea in infants and children [16]. Studies on the preventive capacity of lactobacilli on diarrhea associated with enterotoxigenic *E. coli* in adults were also negative, even when high doses of LAB were administered at frequent intervals [17]. This was confirmed by other studies showing that the duration of the episodes was not shortened, although stool volumes decreased significantly.

Fermented products not derived from milk have also been evaluated for the management of diarrhea, mostly in Africa and Asia and, again, the number of studies is small. The effectiveness of a fermented maize gruel was compared with the unfermented preparation and with the WHO/UNICEF oral rehydration solution in children. No differences in stool frequency and duration of diarrhea were observed but the fermented product was better accepted [18]. Children who regularly consumed other fermented gruels had a 40% lower frequency of diarrhea during a 9-month follow-up compared to a control group. Some fermented cereals have been shown to exert antibacterial activity in vitro [19]. A study in Ghana evaluated the effect of a fermented millet product in 13-month-old children whose diarrhea had lasted 48h; 24.4% of them were dehydrated and 90.2% had malaria, and the mean number of episodes of diarrhea in the preceding year was 2.7. Neither the enteropathogens, the LAB in the product nor their possible variations along time were characterized [20]. The product improved neither the cure rate nor the duration of the episodes compared with the heat-inactivated control; furthermore, many patients were receiving antibiotics and anti-malarial drugs concomitantly and it is not known to what extent this affected the results.

In Chile, 82 weaned infants less than 12 months of age received an acidified milk with *L. helveticus* and *S. thermophilus* for 6 months; the control group received a comparable, nonfermented milk. Patients were contacted twice weekly for detection of diarrhea. The acidified product exerted a preventive effect on the incidence of diarrhea, the number of days during which the children were affected and the duration of the episodes. No differences were observed in the enteropathogens detected [21] (table 1).

Effects on Viral Diarrhea

Different species and strains of *Lactobacillus* have been evaluated for their capacity to modify viral diarrhea; LGG has been the most extensively tested, especially in rotavirus infection in children. When administered with a fermented milk, a shorter duration of the disease was observed (table 2) [22]. A recent study in children from day care centers who received *L. reuteri* or *Bifidobacterium lactis* Bb12 revealed lower numbers of episodes of fever of shorter duration when *L. reuteri* was administered [23]. This suggests that there is specificity in these effects, and is in agreement with earlier results.
obtained in Finland comparing the effect of LGG, or a combination of \textit{S. thermophilus} and \textit{L. delbruekii} in children with diarrhea. LGG significantly shortened the duration of diarrhea. The number of immunoglobulin-secreting cells stimulated by these bacteria was comparable, but during convalescence LGG was associated with enhanced numbers of IgA specific antibody-secreting cells and higher levels of serum IgA antibodies to rotavirus [24].

In addition to the specific mechanisms activated by LAB as part of the defensive responses against pathogens, other benefits probably relate to the activation of innate immunity, the modulation of the resident colonic microbiota, the stimulation of mucus secretion, and protection of the gastrointestinal barrier function [15, 25].

\textbf{Table 1.} Incidence of episodes of acute diarrhea in relation to age in children who received an acidified formula or a control non-acidified formula

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Acidified formula</th>
<th>Control formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>children/episode</td>
<td>incidence</td>
</tr>
<tr>
<td></td>
<td>of diarrhea</td>
<td></td>
</tr>
<tr>
<td>3–5</td>
<td>7</td>
<td>9.9</td>
</tr>
<tr>
<td>6–8</td>
<td>81</td>
<td>12.6</td>
</tr>
<tr>
<td>9–11</td>
<td>150</td>
<td>7.3</td>
</tr>
<tr>
<td>12–15</td>
<td>165</td>
<td>9.7</td>
</tr>
<tr>
<td>Total</td>
<td>403</td>
<td>9.7</td>
</tr>
</tbody>
</table>

\textit{From Brunser et al. [21].}

\textbf{Table 2.} Effect of \textit{Lactobacillus casei rhamnosus} GG (LGG) on the recovery from acute diarrhea in infants and preschool children (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of diarrhea, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Fermented milk with LGG</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>(2) Freeze-dried LGG</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>(3) Pasteurized yogurt</td>
<td>2.4 ± 1.1</td>
</tr>
</tbody>
</table>

The study was carried out on 71 well-nourished children 4–45 months of age; 82% of the cases were associated with rotavirus. The amounts of LGG provided were: $10^{10–11}$ CFU in 125 g of fermented milk twice daily; freeze-dried LGG $10^{10–11}$ CFU once daily; pasteurized preparation 125 g twice daily.

\textit{Modified from Isolauri et al. [22].}
Table 3. Serum cholesterol changes in individuals randomly allocated to receive a fermented milk (FM) containing Lactobacillus acidophilus L1 (L1 FM) of human origin or Lactobacillus acidophilus ATCC 43211 (ATCC)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Baseline average</th>
<th>Week 2</th>
<th>Week 2 minus baseline</th>
<th>% change</th>
<th>P vs. baseline</th>
<th>P vs. placebo</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 FM</td>
<td>14</td>
<td>6.27 ± 0.19</td>
<td>6.06 ± 0.17</td>
<td>−0.21 ± 0.08</td>
<td>3.2 ± 1.2</td>
<td>0.0015</td>
<td>0.0008</td>
<td>0.03</td>
</tr>
<tr>
<td>ATCC FM</td>
<td>15</td>
<td>6.38 ± 0.23</td>
<td>6.30 ± 0.23</td>
<td>−0.08 ± 0.09</td>
<td>1.2 ± 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 FM</td>
<td>21</td>
<td>6.53 ± 0.17</td>
<td>6.27 ± 0.18</td>
<td>−0.26 ± 0.10</td>
<td>3.8 ± 1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>19</td>
<td>6.30 ± 0.14</td>
<td>6.40 ± 0.13</td>
<td>−0.10 ± 0.10</td>
<td>1.9 ± 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 FM</td>
<td>35</td>
<td>6.42 ± 0.13</td>
<td>6.18 ± 0.13</td>
<td>−0.24 ± 0.07</td>
<td>−3.6 ± 1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Placebo = Fermented milk without active bacteria. Values are expressed as mmol/l (means ± SEM). Modified from Anderson and Gilliland [45].

Fermented Foods and H. pylori Colonization

H. pylori is a highly prevalent bacterial agent that colonizes the human gastric mucosa; it is considered as an etiological factor for gastroduodenal ulcers and a risk factor for gastric cancer. In developing countries the colonization begins early in life and affects a high proportion of the pediatric population; most individuals remain asymptomatic. Antibiotic treatments have a high cost and are not 100% effective because of resistance and difficulties with patient compliance due to gastrointestinal intolerance. Furthermore, children are rapidly recolonized after treatment. Products containing LAB or probiotics have been proposed to manage H. pylori colonization in at-risk populations. A multicenter, prospective, randomized, double-blind, controlled study was carried out in symptomatic H. pylori-positive children to compare 7 days of treatment with antibiotics and omeprazole with the same regime supplemented with a fermented milk containing L. casei DN-114 001 for 14 days [26]. The fermented product significantly increased the eradication rate from 57.5 to 84.6% (p = 0.0045). In another study, 65 children received the 7-day standard triple therapy supplemented with 250 ml of a commercial yogurt containing B. animalis and L. casei, or milk during 3 months. No differences in the rate of eradication were observed between the groups [27]. Cruchet et al. [28] used a commercial product containing either L. johnsonii La1 or L. paracasei ST11 or the respective heat-inactivated controls in 326 asymptomatic H. pylori-positive children during 4 weeks. No eradication
was observed but a significant decrease in the values of the $^{13}$C-urea breath test ($^{13}$C-UBT) was detected only in children receiving live La1. The decrease in the UBT values induced by La1 correlated directly with the basal values before treatment [28]. In another study, a yogurt containing *L. gasseri* OLL 2716 (LG21) was administered daily to 12 children colonized with *H. pylori* for 8 weeks. The $^{13}$C-UBT and the serum levels of pepsinogen I and II were measured after 4 and 10 weeks; while no differences in $^{13}$C-UBT values at 4 and 10 weeks were observed, the pepsinogen I/II ratio at 4 weeks was significantly higher, suggesting a decrease in *H. pylori*-associated gastric mucosal inflammation [29]. These results suggest that fermented milk products and probiotics may be used to maintain low densities of *H. pylori* in colonized subjects.

Such protective effects may be due to the inhibition of *H. pylori* growth through the release by some LAB strains of bacteriocins or of organic acids, and to the decrease in the adhesion of the pathogen to gastric epithelial cells. In addition, probiotics and fermented milk products may stabilize the gastric barrier function, decrease mucosal inflammation, and stimulate healing; the antioxidant and anti-inflammatory properties of LAB may also participate.

**Gluten Digestion by Microorganisms in the Management of Celiac Disease**

The celiac patients should remain on a strict gluten-free diet for life; however, this is complicated and many abandon this diet. Another problem with the gluten-free diet is that wheat flour and gluten are used to thicken, increase the consistency, palatability and ‘mouth’ of a considerable number of foodstuffs and culinary preparations. The possibility of using microorganisms to neutralize the pathogenic capacity of gluten and gliadin for celiac patients is currently being explored in many laboratories. The rationale of this is that some LAB, yeasts and molds express proteases which should hydrolyze the deleterious peptide sequences in gluten. The resulting products should be harmless for the patients and acceptable from the culinary point of view [30]. There have been a few preliminary assays in celiac patients but the results have not been clear-cut due to methodological problems.

**Fermentation of Carbohydrates: Solving the Problem of Lactose Maldigestion and Intolerance**

Congenital deficiency of lactase activity is exceptional. Lactose malabsorption may develop after damage to the intestinal mucosa by pathogens or, more frequently, due to the genetically programmed disappearance of lactase
activity in adolescents and adults. Hypolactasic individuals may experience symptoms when the disaccharide load is high enough (lactose intolerance). The symptoms are bloating, flatulence, cramping pain and liquid stools, sometimes expelled explosively. Affected individuals learn instinctively how much lactose and lactose-containing products they can tolerate. The relationship between the amount of lactose and the magnitude of the symptoms is not linear as there is considerable variation in the responses to the same dose.

Because many LAB have lactase activity and survive their passage through the gastrointestinal tract, their intake improves the symptoms of intolerance and the efficiency of lactose digestion. Their β-galactosidase is released into the intestinal lumen by the bile salts and remains functional. *L. acidophilus* and *L. delbrueckii ssp bulgaricus*, the species most frequently used for production of yogurts and fermented milk products, have high levels of lactase activity [7]. Some probiotics have low lactase activity and do not contribute significantly to lactose digestion.

Lactose fermentation by LAB results in the production and absorption of D(-)-lactate; as the human body lacks a D(-)-lactate dehydrogenase, the question arises as to whether this may cause acidosis when feeding yogurt and fermented products to infants. Studies carried out in Santiago on 6-month-old infants demonstrated that this does not occur [31], a finding corroborated elsewhere [32].

**Probiotics and Fermented Foods for Control of Inflammatory Bowel Disease**

It has been postulated that ulcerative colitis and Crohn’s disease are associated with alterations in the resident colonic microbiota and in the responses this elicits from the local and systemic immunity. Considerable interest has been awakened about the effects of probiotics and LAB on their severity, remission and relapse-free interval. A preliminary study in Crohn’s disease by Guandalini [33] showed that it was possible to taper corticosteroids. This has been contradicted by subsequent randomized, controlled studies [34]. The question whether LAB or probiotics exert beneficial effects remains so far unanswered.

**Fermented Milk and Probiotics in the Management of Allergy**

Atopic diseases such as eczema, allergic rhinitis and asthma are chronic allergic disorders whose prevalence has increased considerably during the past 20 years. According to the hygiene hypothesis, there is an inverse association between infections early in life and atopy. The higher frequency of allergies may be related to changes in food consumption patterns, industrial treatments resulting in the disappearance of naturally occurring LAB, alterations
in the intestinal microbiota and decreases in intestinal infections. It is estimated that food allergies affect 3.5% of adults and 8–10% of children; the allergens most frequently involved are egg, peanut, milk, fish, nuts, shellfish, wheat, kiwi and mustard.

Various studies have shown a relationship between allergic conditions and the composition of the gut microbiota. The fecal counts of bifidobacteria in allergic infants, particularly those with atopic eczema, are significantly lower than in healthy peers [35]. Clostridium, Bacteroides and Staphylococcus may also be altered and their numbers may correlate with the IgE serum levels. The colonic population of Bifidobacterium of allergic infants shows higher counts of B. adolescentis and B. longum and lower counts of B. bifidum, in contrast to their healthy peers [36]; such a microbiota is associated with the synthesis of TNF-α and IL-12 by macrophage-like cells in vitro [37]. The gut microbiota participates in the establishment of immune oral tolerance by reorienting the Th2 responder phenotype of newborns towards the Th1 cell-mediated immune response and through the secretion of TGF-β and IgA. The microbiota also participates in the regulation of the gut barrier, which blocks the transfer of food antigens and microorganisms across the epithelium, processes which are implicated in the altered immune responses of atopic children.

It has been proposed that fermented food products containing LAB could modulate the homeostasis of the gut microbiota and decrease the risk and symptoms of allergy. Randomized, double-blind, placebo-controlled clinical trials have evaluated whether probiotic intake alleviates atopic eczema in children. LGG, and sometimes Bifidobacterium Bb12, decrease SCORAD as well as the fecal α1-antitrypsin and TNF-α, plasmatic sCD4 and the eosinophil protein X in urine [38]. A decrease in atopic eczema in infants from atopic families was observed when mothers were given LGG prepartum and during lactation (23 vs. 46% in the probiotic and placebo groups, respectively) [39]; TGF-β2 levels were increased in their milk [40]. The protective effect persisted until 4 years of age [41]. In atopic dermatitis, the daily administration of LGG for 4 weeks decreased SCORAD in those children with high IgE levels [42]. on the contrary, no improvement in SCORAD and inflammatory parameters was observed in infants less than 5 months of age receiving a hydrolyzed, whey-based formula alone or supplemented with LGG or with L. rhamnosus for 3 months [43].

**Effect of Fermented Foodstuffs on Blood Lipids**

The effect of fermented foodstuffs and LAB on lipid metabolism, especially the triglyceride and cholesterol blood levels, has aroused considerable interest. Studies in the Massai of Kenya reported decreases of up to 18% in blood cholesterol, but with the daily intake of large volumes of fermented milk and consequent weight gain. In other studies, fermented milk products have shown
cholesterol-lowering effects, especially for total cholesterol and LDL-cholesterol, with some of these even inducing modest increases of HDL-cholesterol in healthy and in moderately hypercholesterolemic adults. Similar effects have also been observed with fermented soy products [44]. These could be due to the incorporation of cholesterol by the bacteria, synthesis of conjugated linoleic acid, deconjugation of bile salts, production of propionic acid which inhibits the expression of hepatic enzymes implicated in the de novo synthesis of lipids [45]. These studies have been conducted in adults, and little is known about the possible responses of children and the long-term repercussions later in life.

In summary, fermented foods have a long history as components of the human diet; the recent application of modern research methodologies is demonstrating that LAB and their products participate in a variety of metabolic processes that are beneficial for health.

References

Discussion

Dr. Ribeiro: I just want to return to a comment that was made during the discussion about probiotics in diarrhea. With all respect to the data of Isolauri and Guandalini, our experience definitely does not confirm what has been stressed. But the point is when we really look for severe dehydrating diarrhea, in the methodological design we all include really dehydrated patients with less than 3 days of diarrhea, meaning that we have a very uniform group with an average of 3–6 ml/kg/h of stool output, and we use strong variables to control the study. At least in our common vulnerable population, we don’t see the effect of LGG or several other probiotics. We are still not convinced that probiotics could be useful in the treatment, not the prevention, of acute diarrhea in settings like here in Brazil or countries where the conditions are similar.

Dr. Brunser: That is a good point. If you are dealing mostly with diarrhea of bacterial origin probably the effect of probiotics will not be as evident. However, there are studies showing that the addition of probiotics to oral rehydration solutions results in better outcomes. With regard to viral diarrhea we have little personal experience but the situation in Finland is different, of course, from the situation here in Brazil. I would like to make a comment on the Finnish studies; they used a special formula which is whey predominant and with partially hydrolyzed protein to which LGG are added. I wonder what effect this substrate has because in general people just add a probiotic to regular milk or a regular formula.

Dr. Barclay: I would just like to come back to the very interesting results you showed on the effect of certain peptides on blood pressure. The Dietary Approach to Stop Hypertension (DASH) [1] studies in the United States in hypertensive adults showed that increased dairy product intake and also fruit and vegetable intakes resulted in a remarkable lowering of blood pressure. One hypothesis from the DASH studies is that it could be the ratio of dietary calcium, magnesium and potassium versus sodium that could be having an effect on blood pressure. I am just wondering whether there are any data in children on this; whether dietary intake in this period could have an effect on blood pressure in adult life?

Dr. Brunser: Not as far as I know, but pediatric nephrologists regularly check blood pressure in the children. There are studies in the Afro-American population which show that about 25% of the children, when they are 12–15 years old, are mildly hypertensive. The intake of milk is also associated with lower systolic blood pressure levels in adults.
**Dr. Silveira:** I would like your opinion on the relation between the process of fermentation and celiac disease.

**Dr. Brunser:** There are a few studies on the effect of gluten fermentation by different combinations of bacteria to destroy or reduce the epitopes that damage the mucosa in celiac disease. The general idea is that through fermentation you decrease the complexity of the gluten, and you probably decrease the number and concentration of intact epitopes; I wonder if it can be reduced to zero because that is what is needed. There are no studies that have tested these results in celiac patients. I also think that it is an ethical problem. One study was done in Italy in which they incubated ex vivo biopsies of intestinal mucosa with hydrolysates of gluten, and the authors stated that there was no increase in the infiltration of the lamina propria or outflows of cytokines from the biopsies. The Italian study produced pasta with this fermented gluten, and it tastes good and it could be positive for the patients; however, in celiac disease there should probably be no gluten in the diet whatsoever.

**Dr. Uy:** I have two questions. In your opinion is there any deleterious effect from an excess intake of probiotics? As the probiotics, the bacteria, are not made equally, they have different effects. Can all the identified bacteria be incorporated in a capsule and then one capsule taken per day?

**Dr. Brunser:** As more and more probiotics in yogurt products are being consumed in the Western world, there have been increases in the reports of individuals having bacteremia due to some kind of *Lactobacillus*, a few of these apparently originated from the food that was ingested. But there are cases of people who ingest enormous amounts of yogurt and nothing has happened: individuals with AIDS eat yogurt and they are advised to eat yogurt for its effects in stabilizing the resident microbiota of the gastrointestinal tract, and there are apparently no deleterious effects in these patients. So the conclusion is that yes, they are safe, if anything can be absolutely safe in this world. As for the combinations, that is an interesting field because you may combine different capacities, for example for the stimulation of the immune system and to obtain better results, but it requires very careful clinical evaluation.

**Dr. Fisberg:** In many countries and many societies there is a common habit of introducing yogurts and fermented dairy products very early. In the last years pediatric societies are trying to postpone the introduction of these products until probably 1 year of age. What is your opinion on this?

**Dr. Brunser:** If you eat yogurt you provide a load of D(-)-lactic acid and lactic acid in general. With Dr. Haschke-Becher we showed that the amount of D(-)-lactate excreted in 12-hour collections of urine is not excessive. Recently Connolly et al. [2] measured the blood concentration of D(-)-lactic acid in children eating yogurt and again levels were normal. Giving or not giving fermented products depends on many things, many of which escape us pediatricians. In Chile, mothers give yogurt without asking their pediatrician. On the other hand if you take into account the potential positive effects, it is difficult to say no. Then again, when children don’t like milk, yogurt becomes a good source of proteins and minerals. So there is no straight answer to that.

**Dr. Solomons:** The overlap is that the starter cultures for yogurts are lactic acid bacteria and that fermented milk products, yogurt and acidified milks, are fed as complementary food. It would be nice to have a review of that experience. But what confuses me is that we tend to conflate discussion of the simple fermented products with probiotics – which don’t ferment. I have always had a problem with the co-discussion of fermented milk products and lactic acid bacteria, that we are interested in being probiotics, and the fact that sometimes we combine the two, one is a vehicle and the other is a probiotic.

**Dr. Brunser:** Fermented milks and yogurt are produced by specific starters. You may add probiotics to these fermented products and yogurt. Some probiotics are able
to ferment lactose, but most are not. So you have a baseline product which is the fermented milk and you add a strain of bacteria, or whatever, that has specific functional capacities, and this is the whole problem: to distinguish what is produced by the basic product and what by the probiotics. We are beginning to understand that these bacteria are more complex and more plastic in their capacities to adapt to the environment and to acquire new genes by mechanisms such as conjugation and to synthesize all kinds of molecules. Let’s not forget that, for example, antibiotics originated initially from the activity of bacteria, and that desferoxamine, used to combat iron poisoning, is the product of a bacterium. We have used bacteria for many years, sometimes in empirical ways. We are beginning to understand bacteria as very complex organisms whose synthetic capabilities we are harnessing for our purposes.

Dr. Solomons: I would assert that the benefits historically ascribed to fermented yogurts and fermentated and acidified milks have nothing to do with what is currently the molecular biology of probiotics. If they did, it would be very casual and coincidental – not systemic. On the other hand, the fermented byproducts have a potential danger for the consumption of fermentated milks that has never really been raised, and that is the bacterial metabolism in lactose. The simple sugar, galactose which persists in the system along with lactic acid, can represent a danger. As ‘free’ galactose in the circulation, this sugar damages the lens of the eye. Normally, an epimerase enzyme in the liver immediately converts absorbed galactose to glucose. But the enzyme is inhibited by alcohol in very low concentrations. So that by having alcohol and a fermentated beverage at the same time, one can get very high levels of galactose in the blood which accumulate in the lens of the eye, as in a galactosemic child. There are all sorts of drinks which are combined milk or fermentated milk and alcohol (White Russian, Sombrero, etc.). By drinking several of those in an evening, you can get blind drunk because over time it will lead to an accumulation of galactose. Thus a combination of two kinds of fermentation has the potential for adverse consequences.

Dr. Brunser: Your point is very interesting. When you introduce galactose in the small intestine, it will be absorbed very efficiently by Sglut1. Now there are fermentated products that naturally contain alcohol, for example kumiss and kefir contain alcohol. I haven’t seen any reports on individuals from areas where they consume kumiss or kefir exhibiting signs of excessive alcohol intake. I don’t think the amount of ethanol is that big.

Dr. Agostoni: I go back to my own experience regarding Dr. Solomons’ fundamental question. This has been largely debated over the last 3 years within the Committee on Nutrition of ESPGHAN. Just to make clear there are 3 different types of products: formula with probiotics; formula with prebiotics, and fermentated formulas without live bacteria. These are 3 totally different conceptual products, and there are trials investigating their effects. In my opinion, the black hole of the literature is represented by the role of yogurt, a simple fermentated milk product in the first part of the complementary feeding period.

Dr. Brunser: I think in the use of fermentated milk and yogurts and similar products in infants and children, there is the problem of empirism. If we want to transform empirism into scientifically proven truth, carefully planned clinical observations will have to be made over time. Humankind has carried out empirical observations for centuries in very large numbers of individuals to validate some ideas, and in this way a large body of knowledge about foodstuffs and their properties has been acquired. I am sure people have been giving all kinds of fermentated milks and other products to their children for hundreds or thousands of years and they probably never observed any effects that they considered deleterious. What we want now is to have a statistical analysis for every variable to assure that it is scientifically valid.

Dr. Fisberg: Certainly one of the empirical situations that we have is based on allergy, especially because pediatricians appear to give these products based on
allergy. The data that we have show that there are no allergies with these kinds of products.

*Dr. Michaelsen:* It is just a comment on fermented cereal products. When we talk about complementary feeding in developing countries, fermented cereal products might or might not have probiotic effects. Apart from that, there are some very interesting benefits: they have very good food safety; a low pH and with a low pH minerals are better absorbed; there is also a decrease in phytate content and thereby a better absorption, and because of the decrease in phytate and fiber there is better energy density. So there are a lot of good reasons to try to promote fermented cereal foods, especially in developing countries.

**References**