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REVIEW ARTICLE

RECENT ADVANCEMENTS IN FERMENTED MILK AND MILK PRODUCTS

KHAN MOHD SARIM[1*], RUCHI SRIVASTAVA[2], SANJAY KUMAR VISHWAKARMA[3]

[1]National Bureau of Agriculturally Important Microorganisms (NBAIM), India, 275103
[2]Department of Biological Sciences, Sam Higginbottom University of Agriculture and Technology, Allahabad, India
[3]Mohd. Hassan PG College, Jaunpur, Uttar Pradesh, India, 222001
*Corresponding author: khanmohdsarim@gmail.com

Abstract: Milk, a fascinating physiological liquid loaded with nutrients and bioactive compounds offer the health benefit to consumers of all age group. Milk derived products such as cheese, kefir, curd, kumis, yogurt etc. with added nutrient values also enriching nutraceutical properties of this valuable commodity. Starter organism in any dairy fermentation is one of the most important components and need special attention for regular improvements. Biotechnological and nanotechnological implementation to produce genetically modified starter organism and added values respectively are the optimistic concepts for milk products manufacturers. Moreover, *In-silico* tool is a new age computational methodology that allows us to study, modulate, design and predict the fermentation process on the computer prior to large-scale fermentation. Optimistically, the simultaneous use of milk biology, computational methods and obviously the intellectual inputs will soon provide a better solution for desired quality and nutritionally enriched healthier and safer milk-derived products for civil society.

Keywords: Foodomics, fortification, milk, Response Surface Methodology, starter culture, yoghurt

Introduction

Milk: Nature's Gift to Mankind

Milk, a reservoir of varieties of important nutrients and health promoting components is definitely a precious nature's gift. Milk from different sources has different composition and properties and used for production of various milk derived products. Interestingly it is a source of water, energy, vitamins, minerals and other biologically important peptides. Milk constitute

~85% water, although is not a source of nutrients but definitely useful for various metabolic processes and maintains total blood volume, nutrients transport and release waste product out of body discharged from various tissues and organ (Blumberg, 2004), it regulates body temperature through sweat and dehydration, a cause of several metabolic disorders (Blumberg, 2004). After that, high carbohydrate content specifically lactose is prime attraction of consuming milk, glucose serves as stored energy in the form of glycogen and this carbohydrate also play a critical role in hormone metabolism. In addition to this, milk loaded with essential fatty acid such as linoleic (18:2) and linolenic (18:3) acids, used to synthesize long chain fatty acid i.e. arachidonic acid (AA, 20:4o-6), docopentaenoic acid (DPA, 22:4o-6), eicosapentaenoic acid (EPA, 20:5o-3) and docohexaenoic acid (DHA, 22:6o-3) as described by Much *et al*. (2013) and Calder (2016). These fatty acids are responsible for the production of various hormones such as thromboxanes, prostaglandins and leukotrienes which perform diverse functions in our body like coagulation of blood, muscular contraction etc. Beside all these whey proteins in milk provides large quantity of immunoglobins. Moreover, milk is a rich source of vitamins too i.e. vitamin A, C, D, E, K, Vitamin B1 (thiamin) Vitamin B2 (riboflavin) Vitamin B3 (niacin) Vitamin B5 (pantothenic acid) Vitamin B6 (pyridoxine) Vitamin B12 (cyanocobolamine) and folate (Fox *et al*., 2015). Table 1 represents the nutritional richness of human milk as compared to milk from different animal sources (i.e. goat, cow and buffalo), their total estimated content of protein, carbohydrate, trace element, vitamins and enzymes. The buffalo milk is rich in protein, total fat, calcium, sodium, phosphorous, zinc, vitamin A, vitamin E and niacin as compared to milk from goat, cow and human. Human milk is rich in lactose a rapid energy source and copper micronutrient which is an essential trace element incorporates in metalloenzymes for essential metabolic process.

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Table 1. Nutritional composition of various type of milk

Components	**Cow**	**Buffalo**	**Goat**	**Human**	**Health effect**	**References**
Protein (g)	3.3	4.0	3.4	1.0	Provides bioactive compounds, essential amino acids and peptides	Medhammar *et al*. (2011)
Lactose (g)	4.7	4.4	4.4	6.9	Rapid energy source	Jenness, 1980 Khan *et al*. (2019)
Total fat (g)	3.3	7.5	3.9	4.4	Reserve energy source	Park, 2006
Calcium (mg)	112	191	118	32	Healthy bone and teeth prevent hypertension and decrease colon and breast cancer.	Posati and Orr, 1976 Soliman (2005)
Magnesium (mg)	11	12	14	3	Participate in several reaction and prevent asthma	Jenness, 1980 Medhammar *et al*. (2011)
Phosphorus (mg)	91	185	100.4	14	Active role in bone formation	Park, 2006
Sodium (mg)	42	47	44	17	Membrane transport	Medhammar *et al*. (2011)
Potassium (mg)	145	112	102	51	Important electrolyte of cellular and electrical function	Jenness, 1980 FAO and WHO, 2002
Manganese (µg)	8	-	18	-	Function as co-factor in many enzymes which catalyze reactions of energy metabolism, protein, RNA and DNA	Medhammar *et* al. (2011)

					synthesis, It also regulate electrical potential of nervous tissues and cell membranes.	
Iron (mg)	0.1	0.2	0.3	-	Carry oxygen from blood to tissues as important component of hemoglobin, electron transfer in cell and important element of many enzymes	FAO and WHO, 2002
Zinc (mg)	0.4	0.5	0.3	0.2	Essential component of many enzymes, gene expression and lipid metabolism	Watson *et al*. (2017)
Copper (mg)	-	-	-	0.1	Essential trace element incorporates in metalloenzymes	Kapadiya *et al*. (2016)
Selenium (µg)	1.8		1.1	1.8	Immune and antioxidant system, mediate growth and developed	Watson *et al*. (2017)
Vitamin A (µg)	35	69	45	61	Important for vision, epithelial cellular integrity, and immune function.	Kapadiya *et al*. (2016) Nanda and Nakao, 2003
Vitamin D (µg)	0.2		0.1	0.05	Maintain blood calcium phosphate level, bone muscle contraction and nerve conduction	FAO, 2013 Park *et al*. (2007)
Vitamin E (µg)	0.08	0.19	0.05	0.08	Function as anti-oxidant	FAO, 2013

Vitamin B_{12} (µg)	0.50	0.40	0.07	0.05	Essential component of folate metabolism	FAO, 2013
Folic acid (µg)	8.5	0.6	1.0	5.0	Nucleotide synthesis	FAO, 2013
Riboflavin (µg)	0.20	0.11	0.13	0.04	Functions as co-enzyme in various oxido-reduction reactions	FAO, 2013
Thiamin (mg)	0.04	0.05	0.06	0.01	Function as co-enzyme in carbohydrate and amino acid metabolism	Kapadiya *et al*. (2016) Nanda and Nakao, 2003
Niacin (mg)	0.13	0.17	0.24	0.18	Function as co-enzyme in proton transfer with number of dehydrogenases	FAO, 2013 Esperance *et al*. (2009)
Biotin (µg)	2.0	13.0	2.5	-	Function as co-enzyme carboxylation	FAO, 2013

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Fermented Milk and Milk Products: Traditional and Modern View

Fermented milk consumption for regular health and nutritional uptake is an old practice but consistent efforts for the production of derived milk products enriched them with many necessary nutritional ingredients lucratively revolutionizing the milk products manufacturing. Common dairy products are as follow:

a. *Kefir:* acid alcoholic nature, fermented milk product originated in Balkan province of east Europe (Fontán *et al*., 2006), generally produced from fermented milk and also with kefir grains that is left over of kefir fermentation (Bensmira *et al*., 2010). Kefir grains are white, gelatinous, white yellow substance of variable diameter composed of lactic acid bacteria, yeast and acetic acid bacteria of 10^8 CFU/g, 10^{6-7} CFU/g and 10^5 CFU/g respectively. After each fermentation, these grains break up and make new generation grains which exhibit same characteristics like old grains (Gao *et al*., 2012). The microbial composition that makes "kefir" is slightly complex classified into lactic acid bacteria, acetic acid bacteria, yeast and fungi also, and characterized in lactose fermentative, non-lactose fermentative and homo fermentative and hetero fermentative, that include *Lactobacillus acidophilus, L. paracasei* ssp. *paracasei, L. delbrueckii* ssp. *bulgaricus, L. kefiranofaciens and L. plantarum.* Predominant acetic acid bacteria are *Acetobacter* sp., *Glucanobacter* sp., while common yeast strains isolated from Kefir granules includes *Kluyveromyces marxianus, Saccharomyces cerevisia*, *Candida kefyr* etc. Beside this a plethora of dairy cultures are involved in kefir manufacturing of different origin and different type. Kefir provides a number of health benefits such as antitumor activity (Liu et al., 2002) reduction of high blood pressure (Maeda *et al*., 2004), antimicrobial activity (Rodrigues *et al*., 2005) and modulation of immune system (Piermaria *et al*., 2010).

b. *Cheese:* One of the most important and most loving milk products among all is not only highly rich in nutrients but also has better taste and smooth texture. It is also the most diverse milk product with a number of variants which are as follow.

- *Roquefort*: It is originated in France, made up of sheep milk and appears hidewith blue pockets that are actually the colonies of fermenting agent *Penicillium roqueforti*. Roquefort cheese also contains valuable nutraceutical compounds as evidenced by the study of Petyaev *et al*. (2013), where they demonstrated that a protein extract from

Roquefort cheese effectively inhibited the *Chlamydia pneumoniae* propagation in human cell line. In addition to this, as fungi is involved in cheese manufacturing, so aflatoxin is also a serious issue, and constant efforts have been put forward to reduce the risk of mycotoxin. Dall'asta *et al.* (2007) reported Ochratoxin A first time in Roquefort cheese which raised a new health concern in blue cheese manufacturing.

- *Camembert:* It is also originated in France but made up of cow milk with an aging period of three months. Fermenting agent is *Penicillium candidum* that appear as tufted head of a dandelion. Hence, cheese also known as a "bloomy rind" cheese, but different attributes can influence the quality of ripened cheese as evident by the following study. Beside mould, few lactic acid bacteria also contribute in sensory characteristics of end product. In cheese industry these are distributed in two groups i.e. mesophilic and thermophilic. *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* forms mesophilic group bacteria and frequently use for the production of closed or without eye cheese as there is no gas formation and termed O type bacteria. Lactic acid is chiral, consisting of two enantiomers. One is known as l-(+)-lactic acid or (S)-lactic acid and the other, its mirror image, is d-(−)-lactic acid or (R)-lactic acid. A mixture of the two in equal amounts is called DL-lactic acid, or racemic lactic acid. Three different attributes i.e. type of lactic acid bacteria, type of cheese ripening moulds and the inoculation method, were used to determine the effect on cheese production and study demonstrated that after changes in O or DL (Dextro-Levo) type lactic bacteria, spray or direct mixture of mould in milk followed by sensory evaluation using Quantitative Descriptive Analysis (QDA) influence of these attributes were clearly observed (Beltrán-Barrientos *et al*., 2019). O type mesophilic starter culture and *Penicilium candidum* maturation mould given more typical camembert cheese (Galli *et al*., 2016). Beside all these there are following recent advancement in camembert cheese production. (i) Development of mathematical model for growth kinetic of *S. aureus* on camembert cheese (Lee *et al*., 2015); (ii) Modulation of mycelial growth and appearance of cheese by ripening culture (Lessard *et al*., 2014); and (iii) Effect of relative humidity and temperature on the physiochemical and microbiological attributes during ripening of camembert cheese with

Kluyveromyce smarxianus, Geotrichum candidum, Penicillium camemberti, and *Brevibacterium aurantiacum*as model organisms (Leclercq-Perlat *et al*., 2012).

- *Feta and Cotija*: It is introduced to the world by ancient Greece prepared from at least 70% of sheep and rest of goat milk, has also a three month short period of aging. Usually served as sandwich, salad or boiled with olive oil. Mexico the origin of cottija cheese, it is prepared by cow milk with short aging period have varying taste form bitter in younger cheese to tangier flavour during aging served as soup or with fresh beans.

- *Mozzarella:* One of the trendiest cheeses, mozzarella is a gift from a descent food loving country Italy. Mozzarella cheese usually prepare from the buffalo or cow's milk water and curd is heated in warm water and extended by hand before rolling into moist balls. Balls of cheese may either be sold fresh, or in packed. Despite the organoleptic property and great test of mozzarella and other dairy products, scope of improvement in product quality always attracted researchers, and constant efforts have been made in improving mozzarella cheese. Sela *et al.* (2014) nicely described the relationship between yield, time, milk composition and the whey recovery during mozzarella production in their study.
- *Emmental:* Emmental is considered as "Mountain" cheese, originated in Switzerland, it is prepared from cow milks. The holes in cheese are actually the carbon dioxide gas bubbles created by the bacterium *Propionibacterium freudenreichii* when consumes lactic acid. Emmental has distinct sweetness with a spiciness that hits the tongue when it finished.
- *Cheddar*: Developed from cow's milk and originated in Somerset, England, cheddar refers to a cheese preparing process in which curds cooked and minced into short pieces. These pieces are pressed into big blocks and stacked one on top of another block which helps to push out any remaining moisture. Cheddar cheese is known for its taste and consumers generally prefer mild flavour cheddar that is not aged, and Draked's study about the variation and degree of preference of cheddar cheese by consumers definitely helped manufacturers about the consumers' preference of mild cheddar cheese (Drake *et al*., 2008).

- *Gouda and Manchego*: Cheese common in Netherlands, is Gouda a semi-hard or hard cheese prepared from cow's milk. Its flavour and quantity vary from mild to creamy wax-coated, and hard, crumbly. Aged Gouda cheese has a crunchy texture either because of calcium lactate crystals or the tyrosine. Spain is the land that introduced cheese lovers to Manchego, an ivory to straw yellow milk product derived from sheep milk has buttery rich texture at young stage and produced crunchy crystalline texture with salty flavour on aging.

c. *Yoghurt:* Originated from Middle Eastern land is a fermented milk product sometime added with variety of natural milk derivative e.g. skim milk powder, or whey. *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are the chief microbial agents in yoghurt making. However, its starter cultures may contain several other microorganisms as well, i.e. *L. casei, L. acidophilus, L. lactis, L. jugurti, L. helveticus, B. longum, B. bifidus and B. infantis.* As the fermentation proceed it causes increase in the growth of starter microbial culture from 10^8 to 10^{10} CFU/ g and subsequently produce various carbonyl compounds such as acetaldehyde, 2,3-butanedione, 3-methyl-2-butenal, 2,3-pentanedione, dimethyl sulfide, lactic acid, 3-methylbutyric acid, benzothiozole, caproic acid, 2-methyl tetrahydrothiophen-3-one, 1-octen-3-one, 1-nonen-3-one, dimethyl trisulfide, methional, acetic acid and guaiacol (Sfakianakis *et al*., 2014). All these compounds impart to the characteristic flavour of yogurt.

Probiotic: an optimistic concept for public health

Probiotics are live microbes, when consume orally, they alter the gastrointestinal microflora and promote health benefits (Figure 1). Many bacterial and fungal species are regularly being used as probiotics in variety of dairy products developments. Consumption of fermented milk is an era old practice, and modern probiotic containing food commodities are the derivatives of fermented milk (Perles, 1977).

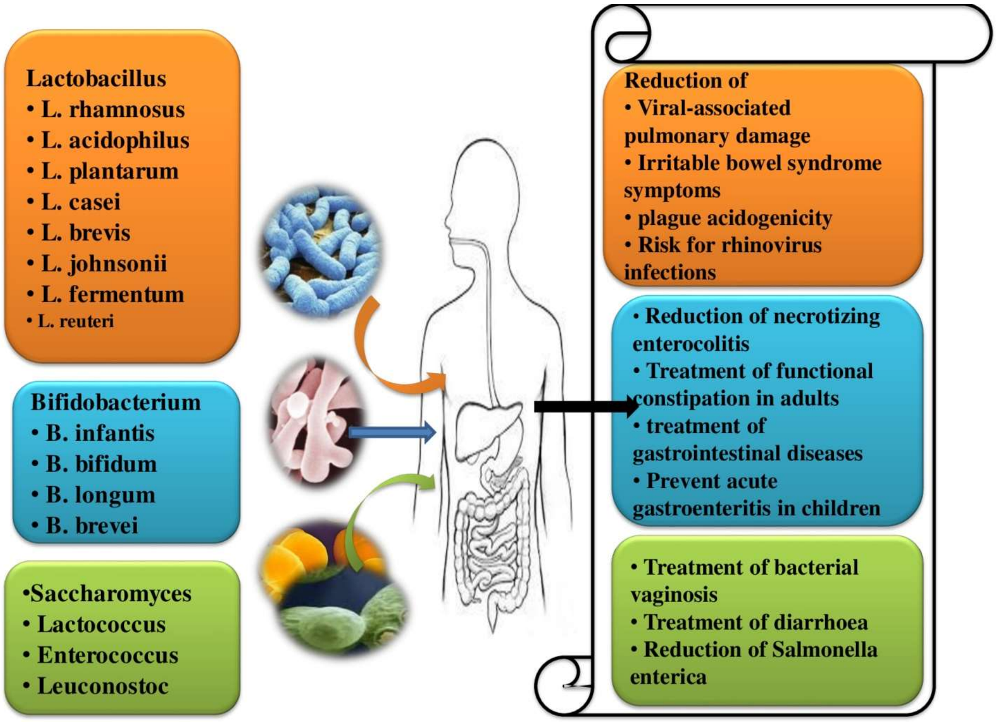

Fig. 1. Potential microbes with probiotic characteristics and their health effects

The word 'probiotics' was originated from Greek word pro bios or 'for life'. The term probiotic was first given by Lilly and Stillwell, (1965). Prebiotics are nondigestible oligosaccharides compounds that promote the growth of beneficial lactic acid bacteria in the colon and exert antagonism to *Salmonella* sp. or *Escherichia coli*, limiting their proliferation. Inulin, galactooligosaccharides (GOS), fructooligosaccharides (FOS), polydextose and lactulose are recognized as established prebiotics, whereas iso-maltooligosaccharides (IMO), xylooligosaccahrides (XOS), and lactitol are known as new prebiotics that have great applications in processing (Xu *et al*., 2009; Femia *et al*., 2010).

Modern Approaches for Selection of Desired Starters and Improved Shelf Life

Starter cultures

Starter cultures are microorganisms that are added to milk in order to get a desired outcome in the final product such as cheese, yogurt, kefir etc, most often through their growth and "fermentation" processes. The natural microflora of the milk is inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. Starter organisms also influence flavour and texture of cultured products through the breakdown of proteins, fats and other milk constituent's along with the pH effect. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of starter cultures is for the production of lactic acid from lactose (milk sugar), lactic acid assists in the coagulation of milk protein by lowering its pH. Bacteria that produce lactic acid are generally referred to as "lactic acid bacteria" (LAB) (Robinson, 1991)

Next step after optimized application of starter culture for milk derived production is the selection and genetic modification in dairy starter culture in order to get the desired product with great efficiency. The primary target of biotechnologist for the above said purpose is metabolic engineering, as primary criteria for selection of starter culture are metabolic properties includes lactose utilization, proteolysis, and flavour production. Lactic acid production from lactose is the elementary function of starter cultures and the gene (lac+) responsible for this conversion is located onto plasmid. During many subculture this piece of genetic material (i.e. plasmid) may lost and here the biotechnologist must be interested to stabilize this important lac+ gene on the chromosome. The very first effort to stabilize this was performed by MacKay and Baldwin (1978) through prophage transduction method, where they induced sixty lactose positive transductants using UV irradiation. Milk casein is a suitable target for genetic improvement. Increasing proteolytic or amino acid conversion ability of an organism can enhance the efficiency of the fermentation process and the overall quality of food as well. Integration of target gene in chromosome or overexpression of endopeptideases and exopeptidases are the major concern in genetic modification for proteolytic activity. The most noticeable factor in today's competitive market environment is flavour production. The key substrate for this function is citrate and *Lactobacillus lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* species frequently being use for flavour production. The key gene for this function is also plasmid linked and again the stability of function property is utmost necessary. Swindell *et al.* (1996) devised a way to fix this issue by inactivating α-acetolactate decarboxylase (*aldB*) gene which convert acetolactate to acetoin and decrease availability of flavour compound precursor. Diacetyl is an acetoin compound that adds a buttery flavour and characteristic odour in various kind of cheese produced during citrate metabolism by lactic acid bacteria.

Beside conventional starter cultures many other nonstarter dairy cultures also have great input in food quality development. Recently O'Brian *et al*. (2017) evaluated a non-starter culture *Lactobacillus nodensis* in Gouda cheese manufacturing. During ripening cheese were assayed for various quality parameters and surprisingly it has been noticed that *L. nodensis* induced proteolysis which added flavour to cheese, and this was determined by the presence of sulphur containing compounds in higher concentration. In a similar way *Lactobacillus plantarum* I91 and *Lactobacillus casei* I90 non-starter cultures isolated from cheese were studied during ripening and milk containing above two stains as adjunct with starter culture *Streptococcus thermophilus*, showed higher concentration of free amino acid, an indication of increased proteolysis. In addition to this another confirmation of flavour production was increased presence of aspartate followed by diacetyl (Milesi *et al*., 2010). A US patent (US6413765B1) was granted to Walfridsson and coworker for a genetically modified *Leuconostoc pseudomesenteroides* strains DSM 12099 and DSM 12465 with enhanced diacetyl reductase activity, beside the flavour development dairy starter cultures are also very sensitive to phage infection.

Traditional and Advance Production Technologies for Fermented Milk Product

Foodomics

Foodomics can be defined as 'studies in food and nutrition domains through the application of advanced OMICS technologies in order to improve consumer's well-being, health, and confidence (Cifuentes, 2009; Herrero *et al*., 2010). Foodomics starts with the identification of any microorganism related to food in any aspect. For this species identification using either 16S rDNA or ITS (Internal transcribed sequences) region analysis is common widespread method (Jo Handelsman, 2004). On the other hand next-generation sequencing (NGS) of hypervariable regions of ITS or 16S rRNA such as V4-V5 offers the real analysis of whole microbial composition of a given sample, and enable researchers to identify many strains that may possibly be not studied by any other method. Metagenomics has contributed significantly in this context. Here, nucleic acid from whole community or directly from provided material is purified and sequenced under the Illumina platform (MiSeq, HiSeq2500, and NovaSeq 6000), Ion Torrent, PacBio, and OxfordNanoPore. Operational taxonomic units (OTUs) generated through, are analysed statistically for the richness or evenness of a particular type of species. Since each strain has unique OTUs, therefore, its richness level in community can be easily understood. Therefore, next generation sequencing or Illumina sequencing provides more fine architectures of microbial communities. Several online databases have generated for the analysis of whole genome of plant associated microbes that alternatively give the real picture of associated metabolic pathways. Plant Associated Microbe Gene Ontology (PAMGO) developed defined GO term useful to describe numerous processes in microbes associated with plants and animals. Pathogen host database i.e. PHI-base, and ghostkoala, a commonly used tool for deciphering molecular architecture of different metabolic pathways available in eukaryotic microbiome. In addition to this, database available for human gut microbe's interaction are GMrepo (Wu *et al*., 2020), and gutMDisorder (Chenge *et al*., 2020). In comparison to classical methods which were completely based on conventional cultural methods for microbial identification, using 16S rRNA gene sequencing and phylogenetic tree preparation, NGS and illumina sequencing provides more accurate information about the whole community through sequencing the metagenome. NGS is slight costly, however, affordable. Data generated through NGS can be easily simulated using computational tools, by OTUs generation and subsequent classification by tools e.g. MOTHUR, and QIIME for 16S rRNA, and PIPITS for ITS region analysis. Few other databases used for this purpose are SINA (Pruesse *et al*., 2012) IDTAXA (Murali *et al*., 2018), CREST et al. (Lanzen *et al*., 2012) and placer (Metson *et al*., 2010). Beside this, ENA, MG-RAST, and IGM are frequently being used platforms to reveals the function by automatically annotating nucleotide sequences similarity to those genes that have already been annotated and available in different databases (Escobar-Zepeda *et al*., 2016; Hoque *et al*., 2019).

In addition to this, microbiome analysis is an advance way to understand the complete microbiota exists in/on the object and also helpful to observe changes in microbiome during various cattle diseases, in this context mastitis is an important inflammatory bacterial disease of mammary glands in cattle liable to economic loss. Falentin *et al.* (2016) in their study using pyrosequencing of V3-V4 region 16S rDNA analysis revealed that microbiota of healthy bovine mammary gland are quite different form diseased bovine mammary gland, and divided them into two clusters. These taxonomic markers can be very useful tools to develop diagnostic methods. Nutrigenomics deals with the association of nutrients and molecular responses, and other high throughput technology can add up understanding of biotic and abiotic constituents' interaction for improved quality milk. Sela and Mills (2014) described how combining the two high throughput approaches i.e. nutrigenomics and microbiome analysis can enable us to understand the host gene expression and understanding of level of interconnectedness of nutrients and microbiota as well. This integrated approach has potential to recognize the genetic predictors for diet and disease response (Turnbaugh *et al*., 2009; Dimitrov, 2011).

Modern OMICS approaches are also providing fascinating insight in the development of varieties of milk products. Cheese and other milk products development composite of many microbial communities that appears and go under succession during product maturation and ripening etc. here, metatranscriptomic a high throughput technology able to decode the gene expression of an organism in a set of environmental condition. De Filippis *et al.* (2016) successfully used this technique to uncover microbial community succession and gene expression during Italian cheese ripening. They modulated ripening conditions and observed the alteration in microbial community structure and provided evidences that nonstarter lactic acid bacteria were contributed significantly in cheese maturation and concluded that increased temperature modulated the expression of genes related to proteolysis, lipolysis and amino acid metabolism. Modern OMICS techniques have been frequently used to assess the quality and product development of milk and milk products such as, community functioning in surface ripened cheese by meta-OMICS technology (Dugat-Bony *et al*., 2015); sensory properties developments of ripened Camembert-type cheese (Lessard *et al*., 2012); differences in microbial composition in continent type cheese (O'Sullivan *et al.,* 2015); comparative genomics of proteolytic system of bacteria (Liu *et al*., 2010).

Fortification of milk and derived products to enhanced nutritional quality

Addition of necessary and important components such as iodine, iron, vitamins, essential metal etc. in staple food to enhance the nutritional quality of that food is called as fortification. It is a safe practice that cut the nutritional break in any population. It provides a nutritional benefit without requiring consumers to change eating habits or procuring patterns. The consumption of dairy products is increasing day by day, the fortified products with mineral and other important nutrient can be helpful to reduce nutrient deficiency in mass population. Food safety is an important unavoidable aspect related to any food. Hence, the incorporation of biologically safe product in required concentration through fortification also conferring the compliance of dairy products quality as per health agencies guidelines towards public health (Grunert, 2005). Vitamin D is a crucial component that synthesize readily in the skin from its precursor 7-dehydrocholesterol when body exposes to direct sunlight or alternatively it can be achieved through adding this component in food supplements. The recommended dietary allowance (RDA) of vitamin D, by the Food and Nutrition Board of the Institute of Medicine, is between 600 and 800 IU/d with the assumption that there is no vitamin D synthesis from sunlight exposure, estimated average requirement is 400 IU (Institute of Medicine, 2011), and the tolerable upper limit (UL) intake is 4,000 IU/d for all ages (National Institute of Health, 2011). Therefore, many research groups have been studying on the incorporation of this vitamin in fermented dairy products. In this context, Tippetts *et al.* (2012) carried out fortification of cheese with vitamin D_3, using protein emulsion and evaluated the retention time. Emulsion was prepared using different dairy proteins and concluded that vitamin retention increases many fold in this emulsion cheese model system. In addition to this, other mineral such as calcium has also been incorporated in dairy products. Vitamin D plays a crucial role in the translocation of calcium and facilitates mineralization of bone matrix and deficiency leads to hypocalcaemia. Exclusion of rickets in developed countries attributed to the incorporation of vitamin D in milk and milk products, this initiative practiced in Canada and USA in early 1930s, however, few cases reported several time but regular monitoring and precautions in respect to public health are sufficient to control this problem in these countries (Welch *et al*., 2000). Fortification of milk with Vitamin D also reduces osteoporosis in the aged people specifically those live at higher latitude regions (Kinyamu *et al*., 1998). Table 2 depicts the micronutrients and elements fortified milk and milk products lactose intolerance is a common problem associated with inability of an individual to breakdown lactose sugar present in milk due to lack of β-glucosidase activity. This enzyme is responsible for cleavage of β-glucoside bond between glucose and galactose moieties of lactose sugar. A total of Fourteen (14) *lac Z* sequences were retrieved from NCBI (www.ncbi.nlm.nih.gov) gene search.

Table 2. List of milk and milk products fortified with various essential minerals

Mineral/nutrient		**Host milk**	**mg/Kg**	**Reference**
ferrous bisglycinate ferrous lactate and ferrous sulfate	Fe	Cow's milk	63 mg/kg, (79 mg/kg, 83 mg/kg	Nkhata *et al*. (2015)
Zinc sulphate and zinc acetate	Zn	Buffalo's Milk	40 mg/l	Gamal El- Din *et al.* (2012)
Ammonium ferric sulphate, ammonium ferrous sulphate	Fe	Cheese	20 mg iron /Kg	El-Kholy *et al*. (2011)
Fe-casein, Fe-polyphosphate-whey protein, Fe-whey protein, or $FeCl_3$	Fe	Cheese	40 mg/Kg	Zhang and Mahoney, (1990)
Ferrous Gluconate and Zinc Oxide	Fe and Zn	Cow's milk	14.9 mg/l	Villalpando *et al*. (2006)
zinc, iron, selenium, copper, vitamins A, C and E	Fe, Zn, Se, Cu and vitamins	Milk	7.8 mg Zn, 9.6 mg Fe, 4.2 mg Se, 0.27 mg Cu, 156 mg vitamin A, 40.2 mg vitamin C, and 7.5 mg/l	Sazawal *et al*. (2010)
calcium chloride, calcium lactate and calcium gluconate	Calcium	Cow milk	50 mg/100 m	Singh *et al*. (2007)
tocopheryl acetate/d	Vitamin D		100 mg/l	Welch *et al*., (2000)

Sequences from lactic acid bacteria, *E.coli* and a eukaryotic sequence accession are as follow: NC_002662, NC_008531, NC_002695, NC_004307, NC_006814, NC_007929, NC_006814, NZ_AYYW01000002, NC_014319, NZ_AP012326, NZ_LN774769, NZ_CP009709, NC_001136, and NC_010471. Sequences were aligned and compared using ClustalW through International Union of Biochemistry IUB substitution matrix, with 15 matrix gap penalty and 6.66 gap extension penalties. Phylogenetic tree was reconstructed by Neighbor-joining method with pairwise distance and thousand bootstraps using MEGA 7.0 (Tamura *et al*., 2016). Evolutionary divergence matrix of *lac Z* gene among 14 lactic acid and non-lactic acid bacteria depicted maximum distance between sequence X and Y. Average distance is a divergence index within and among species which means higher the value of XY far apart gene it is. In Table 3 among lactic acid bacteria maximum distance was observed in *Bifidobacterium_longum*_NCC2705 and *Lb. acidophilus* and *Lactobacillus_salivarius*_UCC11. Values below the diagonal represent standard error estimate(s) while those above the diagonal are the average genetic distances between species. In addition to this there is very less difference between *lac Z* of *Lactococcus* and *Lactobacillus* species. Moreover, the lac Z gene of *Lb. salivarius_UCC118* and *E coli* O157:H7 are more divergent and showed maximum distance of 45.8. The genetic variation among lac Z gene of different starter organisms is possibly due to either insertion or deletion of genetic element/s in gene sequence. Callanan *et al*. (2005) in their study of lac Z gene diversity found some novel insertion sequences (ISL*he1* and ISL*he15*) in *Lactobacillus helveticus*, where ISL*he1* reduced the β-glucosidase activity and ISL*he15* exhibited lactose independent enzymatic activity. Later, they reported an Insertion sequence (IS) ISLasa12 fixed in bacterial population.

Advance tools assistance for milk quality and product development

Milk quality analysis is a prerequisite part for further processing. Physical appearance and microbiological analysis are the traditional methods for determining milk quality, but these are not sufficient for rapid milk quality analysis. Culture dependent methods require sample dilution, spreading on agar plates and enumeration of resultant colonies, whereas culture independent methods reduce time duration require for time taking incubation procedures.

Table 3. Evolutionary divergence of *lac Z* gene between lactic acid bacteria and other species

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
(1) *Lc. lactis_subsp._lactis*_Il1403		0.3	32.3	78.2	0.0	1.3	0.0	6.2	0.0	2.9	48.7	5.6	14.0	2.0
(2) *L. mesenteroides_subsp._mesenteroides*_ATCC_8293	0.1		28.2	70.2	0.0	1.6	0.0	4.4	0.0	1.9	42.3	4.7	10.3	0.9
(3) *E. coli*_O157:H7_str._Sakai_NC_002695.1	0.0	0.0		10.1	29.8	45.8	29.8	12.3	32.0	15.2	1.7	10.4	4.6	32.5
(4) *B. longum*_NCC2705_chromosome	0.0	0.0	0.0		74.0	97.2	74.0	41.5	76.2	49.3	3.3	41.4	27.6	73.7
(5) *Lb. acidophilus*_NCFM_chromosome_NC_006814.3	1.0	1.0	0.0	0.0		1.1	0.0	5.2	0.0	1.9	45.3	4.8	12.1	1.4
(6) *Lb. salivarius*_UCC118_chromosome_NC_007929.1	0.0	0.0	0.0	0.0	0.0		1.1	12.8	1.0	8.1	63.7	13.3	21.7	2.9
(7) *Lb. acidophilus*_NCFM_NC_006814.3	1.0	1.0	0.0	0.0	1.0	0.0		5.2	0.0	1.9	45.3	4.8	12.1	1.4
(8) *Lb. animalis*_KCTC_3501_DSM_20602_NODE_	0.0	0.0	0.0	0.0	0.0	0.0	0.0		5.4	0.5	21.7	0.8	4.3	4.5
(9) *L. gasicomitatum*_LMG_18811_NC_014319	1.0	1.0	0.0	0.0	1.0	0.0	1.0	0.0		2.7	47.2	5.9	13.3	0.6
(10) *L. citreum*_KM20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0		26.7	0.1	4.2	3.5
(11) *B. dentium*_JCM_1195_NZ_AP012326	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		21.3	11.1	45.9
(12) *L. piscium*_MKFS47_NZ_LN774769	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0		3.1	7.2
(13) *B. coagulans* 1_ATCC_7050_NZ_CP009709	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		14.8
(14) *S. cerevisiae*_S288C_NC_001136	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	

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In this aspect, milk whey used for production of Ricotta cheese was analyzed by both culture dependent and independent techniques. Here, Sattin *et al.* (2016) used NGS method for determining microbiological quality of milk and GC-MS for evaluation of volatile compounds present in milk. Beside technological advancements application of co-culture of different potential probiotic strain in a process is an effective strategy of dairy product formulation. In a study, *Bacillus* sp. with other potential dairy starter cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were combined for the production of soy curd which improved the rheological and antioxidant properties of curd (Shobharani *et al*., 2015). Although, modern technologies gearing up this kind of methods, traditional but effective strategies and assisting modern concepts of product developments. *Bouhezza* is an Algerian ripened cheese manufactured specifically in a goatskin bag with "*Lben*", salt and raw milk for several weeks. Zitoun-Hamama *et al.* (2016) in her study evaluated Algerian cheese volatile compounds by using smart electronic nose, SPME and GC-MS analysis. Smart nose is an electronic devise made up of SMart Nose® (SMart Nose 1.51, LDZ, CH-2074 Marin-Epagnier, Switzerland) system, based on mass spectrometry, which allows the analysis of headspace volatile compounds without chromatographic separation. Extraction of volatile compounds was made using a gastight syringe, performing a headspace injection. Smart Nose system incorporates the Combi Pal autosampler CTC Analytics AG (CTC Combi Pal) with the Cycle Composer software; SMart Nose 1.52, statistical software delivered with SMart Nose system, a high-sensitivity quadrupole mass spectrometer (Infion AG) with an ionic mass detection ranging from 1 to 200 amu and which is equipped with a specific statistical software (SMart Nose 1.51) allowing application of a multivariate analysis on data acquisition. The cheese aroma profile was rich and mainly characterized by esters, aldehydes and ketones. SMart Nose results showed that farmhouse *Bouhezza* volatile profile depended on both the cheese-making process and the ripening time. Gas Chromatography/MS identified volatile esters, aldehydes and ketones. Crystals in cheese may be considered defects or positive features, depending on the variety and mode of production (industrial, artisanal). Powder x-ray diffractometry (PXRD) offers a simple means to identify and resolve complex combinations of crystals that contribute to cheese characteristics (Tansman *et al*., 2015; Purohit *et al*., 2019; Tansman, 2017).

Methods for preventing food adulteration

Milk and milk products quality analysis is a serious issue, as milk is manipulated by addition of whey, recently research work focused to identify milk adulteration by detecting glycomacropeptide (GMP). C-terminal part (106–169) of Kappa casein contains Glycomacropeptide (GMP) that discharge into whey during cheese preparation due to action of chymosin. GMP is a biologically active compound and has gained much attention due to its unique set of amino acids beneficial for the detection of milk adulteration. These properties of GMP have given new dimension for the profitable utilization of cheese whey to the dairy industry. Many studies successfully demonstrated the use GMP associated tools for the detection of milk adulteration. The non-immunological methods to detect such alteration are quite expensive and recently Chávez *et al*. (2012) developed a sandwich ELISA of high sensitivity for detection of glycomacropeptide in raw milk by using polyclonal rabbit anti-GMP antibody. Calibration curve with known concentration of whey demonstrated the detection ability of assay was 0.047% (v/v) and quantification limit 0.14%. High specificity, no cross reactivity with the milk components, recovery ratio of 95.62% and 113.88% strengthened the potential of new developed assay. Beside GMP added alteration in milk, incorporation of plant oil in cheese is also comes under fraudulent action in food processing. Traditional chemical methods are time consuming therefore, application of modern technologies have been used to gear up the plant oil detection in cheese manufacturing (Figure 2). Dankowska *et al*. (2015) used the potential of fluorescence spectroscopy for detection of plant oil and found the lowest detection limits of oil, 3.0 and 4.4% respectively.

Computational tools to understand microbial interactions designing desired product

Introduction of computer and computer added tools in the biological field revolutionized the understanding and working in biological sciences. Variety of mathematical model has also been implemented for optimization of process parameters. A new statistical tool, response surface methodology (RSM) being frequently used to design experimental model, measuring the effects of factors, most suitable broth condition for highest possible compound production and even for the new product formulation.

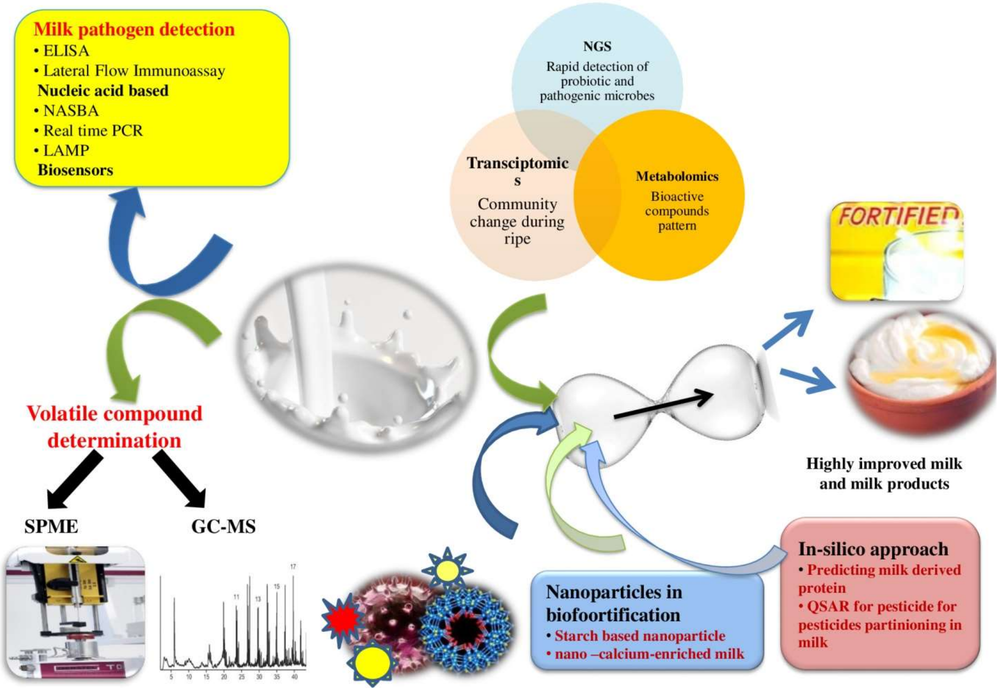

Fig. 2. Advance technologies for quality assessment and nutritional improvement of milk

Suphamityotin (2011) in the study utilized RSM to optimize the condition for maximum extraction of enzyme extraction from soy milk and determined that under optimum condition designed by RSM tool, protein, fat, and the antioxidant activity increased many folds. *Enterococcus faecium* is an efficient probiotic strain frequently used in dairy products manufacturing. Ramakrishnan *et al.* (2012) carried out a study using bacterium *E. faecium* as starter cultures and applied RSM with the following condition cell growth, bacteriocin production, antioxidant property, titratable acidity for probiotic curd formulation. They concluded that under optimized conditions well set bioactive curd was formed that exhibits therapeutic potential against traveler's diarrhea. RSM has also been used by many researchers to optimized media components, food enzyme production (Wang *et al*., 2009) recombinant protein in cheese (Sampiao *et al*., 2010) and also during production of valuable compounds and commodity from milk industry waste (Ghanadzadeh and Ghorbanpour, 2012; Castanha *et al*., 2014).

Bacteriocins are heat stable, ribosomally synthesized antimicrobial peptides produced by bacteria. Evaluation of starter cultures with bacteriocin production potential gives an advantage

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to industry as these cultures are already tagged with GRAS (Generally Regarded as Safe) certification. In addition to this, bacteriocin produced by such bacteria found to resist high temperature and work well in variable pH values (Ahmad *et al.,* 2014). A number of dairy cultures have potential to produce such antimicrobial compounds (Portilla-Vázquez *et al.,* 2016). Bacteriocin have been classified in to three class as follow (i) Short chain of amino acids modified posttranslationally e.g. nisin, (ii) These are heat stable and further divided into pediocin like bacteriocin, unmodified peptide, circular bacteriocin, and unmodified linear bacteriocin, and (iii) Heat sensitive and comparatively large peptides e.g. colicin and helveticin M (Kumariya *et al*., 2019). Synthesis of bacteriocin is governed by inducible genes that might be present on either plasmid or genome and even some time on other mobile genetic element also. These genes are regulated by two component regulatory cascade. The mode of action of bacteriocin in pathogen control is targeting lipid portion of cell envelop which subsequently inhibits peptidoglycan formation. Common mechanism of action of bacteriocin are membrane receptor alteration, substitution of D-alanine to D-alanyl in teichoic acid backbone resulting in cell surface charge neutralization followed by increased permeability of bacterioicin towards periplasmic space, and change in fatty acid composition in cell membrane (Kumariya *et al*., 2015).

In the last few years researchers worked to improve dairy products quality either by incorporating such antimicrobial compound to milk or identify and apply bacteriocin producing starter culture in process. Various studies identified following bacteriocin like compounds in dairy cultures, A heat resistant bacteriocin acidophillin 801 by *Lactobacillus acidophilus* IBB 801 (Zamfir *et al*., 2000; Zamfir *et al*., 2007); Heat resistant and sensitive bacteriocin by lactic acid bacteria group (*Grosu-Tudor et al., 2014*); Nisin like bacteriocin by *Lactococcus lactis* (Zamfir *et* al., 2016), and few low molecular weight bacteriocin was also reported in *Lactobacillus plantarum* and *Lactobacillus helveticus* (Hassan *et al*., 2020). Presence of bacteriocin synthesizing genes in bacterial genomes has also been confirmed. Zamfir *et al.* (2016) identified nisin encoding gene in *Lactococcus lactis* 19.3, a starter culture used in dairy product manufacturing. In a recent study Rafey and co-workers identified the bacteriocin encoding gene in LAB isolated from dairy products. They attempted to identify plnA, plnC, plnEF, and mesY bacteriocin encoding genes in *Lacotococcus mesenteroids* and *Lactobacilus*

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plantarum. Yildrim *et al.* (2016) obtained Lactococcin BZ which was effective against *Listeria monocytogenes* in skim as well as fat mil at 4ºC.

Conclusion

Milk is an essential component of regular diet which is loaded with a number of health beneficial necessary ingredients and regular improvement in milk quality with increase production is today's basic need. Enrichment of deficit or less available components in milk need to be added either through traditional or modern techniques gave a new concept known as fortification. Addition of these nutritional components in milk and derived products increasing their bioavailability which contributes to improved public health. On the other hands, shift from traditional to technological advancements in milk production facilitated large scale production of quality milk products. Information technology in biological sciences given us detailed insight in various life cycle processes and beside this method optimization via statistical tools, available microbial team able to produce captivating diverse milk products that not only providing health benefits but also increasing our understanding of biological, non-biological and informational resource for the wellbeing of human kind.

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