ISOLATION AND CHARACRTERIZATION OF MICROBIAL CONTAMINANT IN PASTEURIZED AND UN PASTEURIZED MILK

A Dissertation submitted by

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In partial fulfilment of the requirement of the degree in

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In

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CEPCI-Cashew Export Promote Council of India

Kollam

JULY–2022

DECLARATION

I, MOONISHA THUDDATHAGE. (VM20FPT010), hereby declare that this project entitled "**ISOLATION AND CHARACRTERIZATION OF MICROBIAL CONTAMINANT IN PASTEURIZED AND UN PASTEURIZED MILK** "

submitted Mahatma Gandhi University, Kottayam in partial fulfilment of the requirements for the award of the degree of Master of Vocational studies in Food Processing Technology is an authentic record of the original research work carried out by me during the period of March to July 2022 under the supervision of Dr. Prabhakumari C, Dy. Principal Scientist, Department of biotechnology , CEPCI, KOLLAM**.** I also declare that this project has not previously formed the basis for the award to us for any master degree, diploma, fellowship or other title of any other university or society.

MOONISHA THUDDATHAGE

Place: Ernakulam

Date:

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MOONISHA THUDDATHAGE

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CERTIFICATE

This is to certify that this dissertation entitled **"Isolation and characterization of microbial contaminant in pasteurized and unpasteurized milk"** is a bonafide record of independent work carried out by **Ms. Moonisha Thuddathage (Reg No: VM20FPT010)** M.Voc Food processing technology, St. Teresa's College, Ernakulam during three month project work under the guidance and supervision of **Dr. Prabhakumari.C** of this Institute. It is further certified that she has independently reviewed the literature, performed all the tests, analyzed the results and critically discussed the findings in presented data.

This is in partial fulfillment of the requirements for the M.Voc Food processing technology of M.G University.

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ABBREVIATIONS

Ach-	Acetylcholine
ATP -	Adenosine Triphosphate
E coli -	*Escherichia coli*
EDTA	Ethylene diamine tetra acetic acid
g/l-	Grams Per Litre
GRAS	Generally Recognized as Safe
H_2O_2-	Hydrogen Peroxide
HCl	Hydrogen chloride
L-	Liter
LAB-	Lactic Acid Bacteria
MHA	Mueller Hinton Agar
mL-	Milliliter
MRS-	De Man, Rogosa and Sharp Agar
S. aureus-	Staphylococcus aureus

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ABSTRACT

The study was carried out with aim to isolate and characterize the microbial contamination in pasteurized and unpasteurized milk. Total five samples were taken and from that two pasteurized and three unpasteurized samples. The milk is serial diluted and pour it into the MRS agar and kept in the incubator for the observation. Lactobacillus cultures were isolated and identified by biochemical tests. Biochemical tests such as Gram staining, catalase, oxidize, indole test, and various sugar fermentation tests are done.

INTRODUCTION

1. **INTRODUCTION**

Dairy products or milk products, also known as lacticinia, are food products made from (or containing) milk. The most common dairy animals are cow, water buffalo, nanny goat, and ewe. Dairy products include common grocery store food items in the Western world such as yogurt, cheese and butter. A facility that produces dairy products is known as a *dairy*. Dairy products are consumed worldwide to varying degrees Some people avoid some or all dairy products either because of lactose intolerance, veganism, or other health reasons or beliefs.

Types of dairy products

Milk

Milk is produced after optional homogenization or pasteurization, in several grades after standardization of the fat level, and possible addition of the bacteria *Streptococcus lactis* and *Leuconostoc citrovorum*. Milk can be broken down into several different categories based on type of product produced, including cream, butter, cheese, infant formula, and yogurt.

Milk varies in fat content. Skim milk is milk with zero fat, while whole milk products contain fat.

Milk is an ingredient in many confectioneries. Milk can be added to chocolate to produce milk chocolate.

Cream

Cream is a dairy product composed of the higher-fat layer skimmed from the top of milk before homogenization. In un-homogenized milk, the fat, which is less dense, eventually rises to the top. In the industrial production of cream, this process is accelerated by using centrifuges called "separators". In many countries, it is sold in several grades depending on the total butterfat content. It can be dried to a powder for shipment to distant markets, and contains high levels of saturated fat.

Butter

Butter is a dairy product made from the fat and protein components of churned cream. It is a semi-solid emulsion at room temperature, consisting of approximately 80% butterfat. It is used at room temperature as a spread, melted as a condiment, and used as an ingredient in baking, sauce making, pan frying, and other cooking procedures.

Fermented

Fermented milk products or fermented dairy products, also known as cultured dairy foods, cultured dairy products, or cultured milk products, are dairy foods that have been fermented with lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc*.

The fermentation process increases the shelf life of the product while enhancing its taste and improving the digestibility of its milk. There is evidence that fermented milk products have been produced since around 10,000 BC.[1] A range of different Lactobacilli strains has been grown in laboratories allowing for many cultured milk products with different flavors and characteristics.

Yogurt

Yogurt also spelled yoghurt, yogourt or yoghourt, is a food produced by bacterial fermentation of milk.The bacteria used to make yogurt are known as *yogurt cultures*. Fermentation of sugars in the milk by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tart flavor. Cow's milk is the milk most commonly used to make yogurt. Milk from water buffalo, goats, ewes, mares, camels, and yaks are also used to produce yogurt. The milk used may be homogenized or not. It may be pasteurized or raw. Each type of milk produces substantially different results.

Cheese

Cheese is a dairy product produced in wide ranges of flavors, textures, and forms by coagulation of the milk protein casein. It comprises proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. During production, the milk is usually acidified and the enzymes of either rennet or bacterial enzymes with similar activity are added to cause the casein to coagulate. The solid curds are then separated from the liquid whey and pressed into finished cheese.[1] Some cheeses have aromatic molds on the rind, the outer layer, or throughout.

CONTAMINANTS OF MILK AND DAIRY PRODUCTS: CONTAMINATION RESULTING FROM FARM AND DAIRY PRACTICES

Pesticides

Sources and Occurrence

The employment of commercial synthetic chemicals to combat insects as disease vectors and pests in the agricultural sector was particularly intensified on a global scale after World War II. These 'pioneer' chemicals were organochlorines (OCs) such as the insecticide 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), endrin (insecticide/rodenticide), and hexachlorobenzene (HCB) (fungicide), all showing persistence in the environment and thus prolonged efficacy. However, due to their lipophilicity and resistance to biodegradation, these compounds accumulate in the biosphere and detectable levels may be found essentially on a global scale in many foods including milk and dairy products. Owing to this concern, more labile pesticides of the organophosphate (OP) class rapidly replaced the stable OC compounds (which were banned in many countries in Europe and in the United States already in the 1970s), which in turn has led to a steady decline of OC residues such as DDT (and its metabolites) and hexachlorocyclohexane (HCH) isomers in the environment and consequently also in dairy products. This tendency is reflected in the levels of major OCs in dairy products reported in national contaminant surveys conducted over the past decades. Such trends are evident in European countries. In Germany, for example, levels of OCs (total DDT, HCB, lindane, aldrin, dieldrin, heptachlor/heptachlor epoxide) in bovine milk declined considerably (>50%) from 1974 to 1981, mainly as a consequence of the OC ban. However, in some developing countries, recent surveys show regular elevation in the concentrations of certain OC residues as these may not be totally banned but restricted in their use, as a result of which some animals may still be exposed to the compounds (e.g., lindane, DDT). A seemingly contradictory picture is drawn when comparing the surveys of the United States Department of Agriculture (USDA) Pesticide Data Program (PDP) for the years 1998 and 2004 (or 2005). In the 2004 survey, the major isomer of 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDE p,p0) was detected in 96% of the milk samples tested versus close to 14% in 1998 (Table 1). Similarly, diphenylamine was found in 98%, and the OC insecticide dieldrin in 41% of the samples. This large increase in the number and frequency of pesticides detected in the more recent surveys can be explained by much more sensitive analytical methods being employed. Essentially all the limits of detection were significantly lower for the milk samples tested in 2004 and beyond. A more recent pesticide residue survey conducted by the USDA in 2011 showed that of the 743 whole milk samples analyzed, only 5 revealed the presence of pesticide residues, albeit all well below the US Environmental Protection Agency (EPA)-established tolerance levels for those compounds. The residues detected in milk were iprodione, trans- and cis-permethrin, dicofol p,p0 , and piperonyl butoxide. However, surveillance of OC pesticide residues remains important as they may still find their way into milk either through intensive insecticide use on field crops or from an earlier contamination of the environment. An example of the impact of previous intensive usage of DDT in the environment is New Zealand, where relatively high levels of DDT breakdown products have been found in butter, for example, DDE, albeit at levels below the Codex EMRL

(Extraneous Maximum Residue Limit) of 0.02 mg kg1 for the sum of DDT and metabolites. In pasture-based economies in particular, environmental contamination with OCs in animal products may be difficult to predict and control, particularly during drought or marginal malnutrition, where animal fat reserves are mobilized to maintain milk output, periodically leading to higher incorporation of OC residues into milk. Other potential routes of contamination are the application of sewage sludge to land, or dairy animal feedstuffs imported from certain countries where agricultural practices and/or malaria control still include the use of OCs such as lindane and DDT. Notably, OCs and their metabolites (e.g., DDT/DDE) are also classified as environmental contaminants belonging to the socalled 'persistent organic pollutants' (POPs). In 2004, the United Nations Stockholm Convention on POPs came into force and

became international law. About 100 nations agreed to sign the treaty to control the release of or to ban the 12 initial POPs (i.e., aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene, HCB, PCBs, dioxins, and furans). Pesticide residues in milk may have a number of potential sources, including environmental (water, soil, air) contamination of the animal feed (fodder), or treating dairy animals or their direct living environment to protect against disease vectors (mites, ticks, insects). Direct contamination of milk may ensue via uncontrolled sanitary measures of the immediate surroundings of the animal, or treatment of the dairy utensils that are employed during milking or storage. However, more important routes of contamination are indirect, one of which may be through the application and administration of pesticides to the milk producing animal, orally, cutaneous, or via inhalation when animals are housed in a closed environment during treatment. In all cases, the active ingredient may be absorbed, subsequently metabolized, and eventually excreted into the milk of the lactating animal. This potential route may be a consequence of treatment of the animal with ectoparasiticides and endoparasiticides, but only if this usage is uncontrolled and fails to respect the prescribed withholding time before milking. Carryover into milk – in both cases – may originate from absorption of the active ingredient passing the blood–milk barrier in the mammary gland, the efficacy of the process being dependent on the chemical/physical properties of the molecule (e.g., lipo- or hydrophilicity, molecular weight, and preference to carrier proteins). Endoparasiticides, usually administered to animals orally, as feed additives, by injection or in the form of pour-on preparations, are employed mainly against helminths, which include the tapeworms, roundworms, and flukes. The most widely used compounds in the past were levamisole and the benzimidazoles (e.g., thiabendazole), which are largely replaced today by the highly effective fungal metabolite ivermectin. Studies on the excretion rate of anthelminthics show that prevalence of measurable residues in milk is – in most cases – shorter than 5 days after therapeutic treatment. Cutaneous treatment of animals against ectoparasites (insecticides, acaricides) includes compounds mainly of the OP class, carbamates, pyrethroids, organotin compounds, and certain organonitrogen compounds such as the acaricide amitraz. Studies have shown that many residues are below the detection limit in the milk already 2 days after application (e.g., coumaphos, at a dose of 6 mg kg1 body weight (bw)), but some may persist for up to 5 days at levels >0.01 mg kg1 (fenchlorphos, permethrin). Thus, hygienic treatment of dairy animal presents no real risk of residues in milk if the treatment is carried out as directed. However, contamination of utensils or factory premises may result in the occasional presence of insecticides (e.g., chlorpyrifos, diazinon, malathion), used to control, for example, cockroaches

Health Impact

The applications of modern pesticides in agriculture, on food and forage plants, practically bear no risk of significant residues in milk, a premise being strict adherence to good agricultural practice. Levels of OC pesticides, potentially contaminating milk via the environment, have been decreasing over the past decade(s) and international efforts are under way to further reduce environmental contamination. Current residue levels in milk fat do, however, reflect both past and current usage patterns. The results of surveys do not raise health concerns in developed countries.

Analytical Aspects

The range of pesticides and pesticide formulations registered for use in agriculture represents a large diversity of chemical structures characterized by different physicochemical properties. Today, approximately 800 compounds are used in pesticide formulations, and laboratory analysts developing detection methods also need to consider several metabolites, degradation products, and 'phased out' as well as 'old' persistent chemicals. Residue analysis in food is probably one of the most demanding areas of analytical chemistry, requiring in many cases the determination of compounds at the microgram or nanogram per kilogram level. Without doubt, the most efficient approach to pesticide residue analysis involves the use of multiclass, multiresidue methods. The basic steps of pesticide residue analysis can be defined as

- sampling
- extraction
- clean-up (e.g., gel permeation chromatography, silica gel, florisil columns)
- (separation) and identification
- quantification

The procedure chosen for the isolation, clean-up, and analysis of the food sample is dictated by the composition of the food matrix, especially the fat content. The polarity ranges of the different pesticide families in water are an important consideration in the development of a universal residue method, which should have the widest scope possible. An approach used widely today in laboratories to extract residues is the QuEChERS method (quick, easy, cheap, effective, rugged, and safe), which entails extraction with acetonitrile and salting out with, for example, anhydrous magnesium sulfate and sodium acetate. The QuEChERS approach has been evaluated for pesticides in fatty foods including milk, coupled with a clean-up by dispersive solid-phase extraction to remove matrix coextractives. Good recovery was achieved with semipolar and polar pesticides. Today, a plethora of residue methods that are based on mass spectrometry as the choice of technology for detection are described in the literature and have essentially replaced the classical capillary gas chromatography (GC) coupled to selective detectors

Antimicrobial Drugs

Sources and Occurrence

Antimicrobial drugs are administered to treat bacterial infections or employed prophylactically to prevent spread of disease, or to augment growth and yield in animals and animal products. All antimicrobial drugs administered to dairy animals enter the milk to a certain degree, and each drug is given a certain withdrawal (waiting) period, during which time the concentration in the tissues declines and the drug is eliminated from the animal. The most frequently and commonly used antimicrobial drugs are antibiotics, used to combat mastitis-causing pathogens. Mastitis is a disease that inflicts significant economic losses on the industry on a global scale, estimated in the United States alone at approximately US $1.8–2 billion per annum. Other infectious diseases such as laminitis, respiratory diseases, and metritis are also treated with antimicrobial agents, but are of minor comparative importance. Mastitis is an inflammation of the mammary gland and is characterized by an increase in somatic cell count in the milk and by pathological change in the mammary tissue. Mastitis-causing pathogens include bacteria, mycoplasmas, and fungi, and can be broadly categorized into specific udder pathogens (contagious pathogens) and environmental pathogens. Numerous products are available for therapeutic and prophylactic treatment of the udder and are usually administered by intramammary (intracisternal) injection of the infected quarter(s), and in severe cases systemically. One can distinguish between lactating-cow and dry-cow therapies, the latter characterized by slow-release preparations that are active over several weeks during a dry period, and which should be taken into account in the case of early parturition. Each registered antimicrobial preparation has a recommended withdrawal time, which must be adhered to in order to avoid illegal levels of residues in the milk. It is rare that antimicrobials will be retained for longer than the normal withdrawal times, but could be extended in certain cases of low milk yield, for example, due to milk fever. The antimicrobials administered in mastitis treatment span a wide range of compounds, including the b-lactams (penicillins, cephalosporins), tetracyclines, macrolides, aminoglycosides, quinolones, and polymyxins. Of these, the b-lactams are the most widely used for lactating cows, and >170 formulations are currently available on the market (taking into account Switzerland, United Kingdom, France, United States of America, and Ireland). Of these, more than 50% contain either penicillin G or cloxacillin (semisynthetic isoxazolyl penicillin), with roughly equal distribution of the two antibiotics in the preparations. Moreover, to improve the efficacy and to increase the spectrum of activity of the preparations, many of the compounds are used as formulations that contain two or even more different families of antimicrobials in the same preparation. National surveys in developed countries show that contamination of bovine milk (residue-positive samples using a bacterial inhibition screening test) at tanker level is generally low (low single-digit percentage). Such findings may not necessarily be equated with the failure rate since it could also encompass the so-called ‘false positives’ (i.e., still within legislative limits but positive in the rapid test), thus depending on the sensitivity of the test toward certain individual antibiotics. In 2003, the US National Milk Drug Residue Database, for example, reported the results of over 4 million samples analyzed by using rapid test kits (milk from pickup tanks, pasteurized fluid milk, and milk products). Many of these tests were for b-lactam residues and the most violations, approximately 3000

Technological Impact

The occurrence of residues of antimicrobials in milk besides being of interest in the context of consumer health and the development of antibiotic resistance has both an economical and technological impact on the dairy industry. Antimicrobial residues at or below MRL (Maximum Residue Limit) levels can influence bacterial fermentation processes involved in the production of some dairy products, such as cheese or yogurt. In model trials with yogurt and mesophilic cultures, b-lactam antibiotics (penicillin G, cloxacillin), as well as oxytetracycline and some macrolides (spiramycin and tylosin), for example, significantly impaired lactic acid production by the bacterial cultures at EU-MRL levels. Differences are reported between cultures as well as between the various antibiotics tested. Yogurt cultures in most cases were shown to be more susceptible to antibiotic residues than mesophilic starter cultures. Within the tests employing yogurt cultures, L(þ)-lactate formation was particularly inhibited by penicillin, whereas spiramycin impaired mostly D()-lactate formation. Besides lactic acid formation, production of aroma compounds (e.g., diacetyl in butter), formation of carbon dioxide for eye formation in cheeses (e.g., Emmental cheese), and polysaccharide formation to thicken fermented milks (e.g., yogurt) are important characteristics of bacterial cultures used in the production of dairy products. Along with the inhibition of acid formation by lactic acid bacteria, antimicrobial residues have been reported to be able to result in inadequate ripening, off-flavors or lower flavor intensity, and uneven texture such as uneven eye development, as well as brown spot defects in propionic acid-fermented cheeses. Ultimately, residues of antimicrobials may lead to a deterioration of quality and to monetary losses in the dairy industry by inhibiting starter cultures in dairy technological processes

Health Impact

A general concern linked to the widespread usage of antimicrobials at the farm level is the potential development of antibioticresistant pathogens, particularly if treatment is not diagnostically targeted. This may complicate human treatment and possibly cause selection of antibiotic-resistant strains in the gut. Further concern was raised that sensitive individuals may exhibit allergic reactions to residues of antibiotics and/or their metabolites, mainly b-lactam antibiotics. However, the allergenic risk is very low. Only the individuals sensitized through previous therapeutic exposure can react with mild and transient symptoms around the tolerance levels (Codex MRL for penicillin G is 4 mg l1). National surveys on residues in milk in developed countries only very seldom reveal positive samples exceeding these levels. Thus, regular monitoring is the only pragmatic approach to residue problems faced by the dairy industry in many countries, particularly in the manufacture of fermented milk products such as yogurt and cheese. The available data and global trends reflected by national surveys indicate that a direct health hazard for humans can be virtually excluded

Other Drug Classes

Nonsteroidal Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory agents such as phenylbutazone, flunixin, or dipyrone may be used to treat bovine mastitis. However, a wider range of these drugs such as, for example, ibuprofen, meloxicam, metamizole, and ketoprofen, may be administered to dairy cattle in farms where cows are confined (limited walking space versus cows in a pasture), mainly to relieve joint inflammation and pain. Currently, there is a paucity of data on the residues of such substances in milk. b-Adrenergic Agonists The b-agonists are hormonal-type growth promoters licensed in most EU member states for therapeutic use as bronchospasmolytics (horses, calves) and tocolytic agents (cows). The illegal usage of these compounds to improve the efficiency of feed utilization and/ or to enhance carcass leanness in meat-producing animals has been reported in the literature. Thus, they are also referred to as repartitioning agents, because their effect on carcass composition is to increase the deposition of protein while reducing fat accretion. The administration of b-agonists, such as clenbuterol, in feedlots and consequently their presence in the edible tissues and milk of the animals can constitute a real health risk, with potential exposure of consumers to pharmacologically active levels that have in the past led to poisoning cases after ingestion of liver or meat. Depending on their structures, b-agonists can have relatively long plasma half-lives, slow rates of elimination, and high oral potencies. For example, clenbuterol administered (10 mg kg1 bw) to dairy cows is secreted into the milk resulting in levels directly related to those in the blood plasma, and range from 5.5 to 22.5 mg kg1 . However, it is not known whether metabolites of the drugs are also excreted into the milk. Overall, the risk of physiologically active levels of residues in milk and thus a potential health impact on the consumer is minimal, because the illegal usage is in most cases limited to meat-producing animals.

Hormones

The employment of hormones in animal husbandry serves a number of purposes, which include increased food production, medical treatment, or improved reproductive performance. Hormones that have an impact on food production are classified as growth promoters, anabolics, or performance enhancers, with the prime goal of enhancing economic competitiveness. However, this use is acceptable only if no potential threats are known to the health of consumers and the animals involved. The use of hormones as growth promoters is approved in some countries; for example, in the United States and Canada, the natural steroid hormones estradiol, testosterone, and progesterone and the (semi)synthetic hormones melengestrol acetate, trenbolone acetate, and zeranol are approved for use only in meat-producing animals. Safety concerns have been raised by other countries, for example, EU member states, regarding hormone residues in meat. However, elevated levels of residues in milk are not expected if hormones are applied appropriately

Sources and Occurrence

Natural Hormones (Steroids, Peptide/Protein)

The endogenous steroids 17b-estradiol, progesterone, and testosterone and their derivatives are the main sex hormones present in all mammals. They can be used for anabolic purposes, and the two female sex hormones 17b-estradiol and progesterone are also used to induce lactation, control/improve fertility, and synchronize estrous cycle. The natural steroid hormone content of milk will fluctuate depending on the physiological and nutritional status of the animal and probably other factors. Hormone levels, mainly estrogens and progesterone, are also used for diagnostic purpose (estrus, pregnancy). Reported levels in the literature for whole milk are, for example, for total estrogens, 50–70 ng l1 and for progesterone, 10– 13 mg l1 . Steroids are soluble in lipids; therefore, dairy products with lower fat content contain comparatively lower concentrations of steroids. Steroid treatment, for most purposes at low levels, will not lead to a detectable increase of residues in milk. Only higher dose treatments, for example, for fertility treatment or other medical purposes, may lead to a short-term enhancement of steroids in milk. Oxytocin is a naturally occurring peptide hormone (9 amino acids), excreted by all mammals for induction and maintenance of labor and promotion of milk ejection. It has an important pharmaceutical use in veterinary and human medicine. Treatment is via injection, intravenously, intramuscularly, or subcutaneously. Since the peptide has a short half-life in animals, treatment can usually not be detected via increased oxytocin levels in milk. Oxytocin use has been reported in buffaloes to facilitate milking, as they seem to be more difficult to milk than cows. The use of oxytocin for the treatment of mastitis has also been reported in some countries, the mode of action being the stimulation of milk ejection, which is correlated with increased pathogen removal from the udder. There is a paucity of data in the scientific literature on the levels of oxytocin in milk. For whole milk, approximately 50 microunits per milliliter has been reported, and skim milk apparently contains lower levels (15–20 microunits per milliliter). The legal status of the oxytocin applications may vary in different countries, and concerns have been raised with regard to animal welfare. Bovine somatotropin (BST), also termed bovine growth hormone, is a polypeptide hormone (190–191 amino acids) produced by the pituitary gland in all cattle. It promotes growth and regulates fat, protein, and carbohydrate metabolism. Many of the physiological effects of BST may be mediated via increased blood levels of insulin-like growth factor 1 (IGF-1), also called somatomedin, and produced mainly in the liver in response to somatotropin. Recently, a virtually identical BST has been produced by recombinant DNA technology via genetically engineered bacteria (recombinant BST (rBST)). When injected into dairy cattle, rBST improves milk production efficiency by up to 20% under optimal management conditions. This rBST is commercially available, and the first product was marketed in the United States under the trade name Posilac after approval by the Food and Drug Administration (FDA) in 1993. The use of rBST is considered safe and currently approved in many countries. However, clinical trials with rBST have indicated an increase in the incidence and severity of mastitis, and other concerns have been raised with regard to animal welfare, which include increased food disorders, reproductive disorders, and localized swellings at injection sites (injections are repeated at fortnightly intervals). Treatment of cows with rBST does not lead to an increase in BST in milk. Since rBST and natural BST are basically identical, analytical differentiation by mass spectrometry is possible only at amounts above 0.001 mg kg1 . BST levels in milk are generally less than 3 ng ml1 , but may occasionally increase up to 10 ng ml1 . Furthermore, both rBST and BST are denatured during pasteurization and heat treatment. The overall nutrient composition of milk is not altered by rBST treatment.

Semisynthetic and Synthetic Hormones

In mastitis treatment, synthetic corticosteroids, for example, dexamethasone, prednisolone, and derivatives thereof (flumethasone), are administered systemically or into the mammary gland to relieve inflammatory conditions. The (semi)synthetic hormones, melengestrol acetate, trenbolone acetate, and zeranol, are approved in some countries as growth promoters in meat-producing animals. rBST is virtually identical to the naturally occurring BST (see above)

Analytical Aspects –

Hormones and Antimicrobial Drugs For the analysis of antimicrobials, and hormones in milk and milk products, emphasis is placed on rapid tests that provide an accept/reject answer at the farm or slaughter house. These tests give qualitative or semiquantitative results, their aim being to check legislative compliance at an early stage in the food chain. Many of the tests are performance-validated and certified by the Association of Official Analytical Chemists (AOAC), and the majority are based on the inhibition of growth of microbial test organisms, ligand assays using biological receptors, or antibodies. Inhibition tests can detect a wide range of antimicrobial compounds, whereas receptor or immunological assays are specific for a family or limited range of compounds. In dairy farming, upstream checks avoid bulk tanker rejection caused by potential issues at single farms. At the farm level, simplification of the test is crucial, as is speed and ease of operation. Essentially, a visual read-off with an accept/reject decision (ideally close to or at the MRL) within minutes is required. A diverse range of immunological and protein receptor tests designed for particular classes of antibiotics are now commercially available. For example, the BetaStar (a rapid lateral flow assay for the determination of beta-lactam residues in milk) has been optimized to achieve a result within 2 min (two incubation steps), which is ideal for conducting on-farm checks. However, recent developments in rapid tests are toward multiplexing, with technologies that can cover multiple classes of antibiotics. Technology platforms range from simple lateral flow devices, flow cytometry, optical and electrochemical biosensors, up to more sophisticated 'omics' approaches, such as ambient mass spectrometry and near-infrared spectroscopy. The development of test kits that enable the detection of multiple antimicrobials in a single strip at or close to the MRL has also significantly advanced in recent years. An example is the 4Sensor, a competitive antibody-based assay in a multiplex dipstick format for the detection of betalactams, tetracyclines, dihydrostreptomycin, streptomycin, and chloramphenicol in milk, in a single operation. Many different multiscreening antibody-based kits are available in plate format but these require a reader and are therefore inconvenient for farm use. A further promising technology uses flow cytometric immunoassays with microspheres or beads functionalized by size, color, fluorescence, or magnetism. Flow cytometry is far better suited to multiplexing than dipstick-based tests and, depending on the test format, up to 100 different assay combinations can be handled at high throughput. However, for some drugs, there are currently no reliable rapid tests to detect residues at the legislative levels, or, in certain cases, to detect traces of the drug if no MRL has been set. In such instances, liquid chromatography (LC) or GC based methods must be employed; they also play a major role as quantitative and confirmatory techniques. In fact, over the past decade a plethora of chromatography–tandem mass spectrometry (LC-MS/MS) methods have been developed to detect and quantify residues of antimicrobials down to low parts per billion in all relevant food and feed matrices, including dairy products. To enhance speed and shorten tedious cleanup steps, techniques such as automated turbulent flow online LC-MS/MS look very promising, with reports of the successful analysis of 36 compounds from seven different classes of antimicrobials in a single run-in milk. To better anticipate risks, laboratories are now gradually shifting toward nontargeted screening tools in the analysis of veterinary drugs and growth-promoting agents. The use of full-scan high resolution accurate mass spectrometry (HRMS), such as time-of-flight mass spectrometry (ToF) and Fourier transform Orbitrap mass spectrometry are gradually becoming routine tools of analysis. HRMS instruments show promise for antimicrobial screening in all relevant matrices, characterized by high throughput and fast data acquisition, and also have the potential to be used in quantitative analysis. The main challenge for HRMS is that higher resolving power may be required to achieve adequate sensitivity for the detection of banned compounds. In the case of endogenous hormones, the natural variation in concentrations in tissues and milk of animals makes the detection of legal or illegal use particularly difficult. Furthermore, concentrations in healthy animals are in the same order of magnitude as those observed in animals that have received hormone implants. For this reason, sophisticated analytical techniques based on the detection of abnormal ratios of hormones to precursors or metabolites coupled with stable isotope dilution analysis have to be used to detect potentially illegal use of these compounds

Sanitizers/Disinfectants

Cleaning and disinfection are critical steps of good manufacturing practice in the food production and dairy sector. They are in fact part of the production cycle, and most equipment in a dairy plant is cleaned-in-place (CIP), to ensure removal of bacteria and residual milk (fat, protein) from the surfaces of equipment/piping etc. Residues of detergents and disinfectants/sanitizers can be introduced into milk on the farm and at the dairy plant level, particularly if cleaning, disinfection, draining, and rinsing procedures of milking equipment and containers are improperly conducted. Residues of sanitizers may occur in milk and dairy products at very low concentrations and are present as indirect and incidental food contaminant

Sources and Occurrence

Contamination of milk with disinfectants could potentially ensue via two principal routes, that is, application as teat and skin disinfectants, and treatment of the milk plants. Dipping or spraying of teats with bactericides after milking may help to control mastitis pathogens. Disinfection of the udder after milking is particularly useful against pathogens from the infected mammary gland, whereas a premilking treatment is more effective in controlling pathogens involved in environmental mastitis. Furthermore, contamination can occur during contact of milk with cleaned and disinfected surfaces in milking equipment and dairy plants. The most commonly used disinfectants in the dairy industry are iodine-liberating agents (iodophores) and chlorine-containing compounds, such as chlorhexidine and hypochlorite, as well as quaternary ammonium compounds (QACs) and peroxy compounds (Table 2). Iodophores are organic compounds containing iodine in a micellar cage of polyvinylpyrrolidone or nonoxynol complex. When diluted, iodine is liberated and can exert its bactericidal properties. Commercial preparations contain 0.3–1.75% iodine, of which 80–90% is released upon dilution. Chloramine T (N-chloro-p-toluenesulfonamide) usage as a teat disinfectant has been severely restricted over the past 30 years, due mainly to the risk of residues of p-toluenesulfonamide (p-TSA) and p-sulfamoylbenzoic acid, traces of which are undesirable in dairy products due to potentially toxic properties of these compounds. Iodine ingested via feed is secreted in milk and presents an additional contribution to the total iodine in dairy products. Due to feeding practices, the iodine content in cow's milk increased by 300–500% from 1965 to 1980. High variations in mean residual iodine levels, reported in several countries, lie between approximately ten and several hundred micrograms per liter. The mean iodine level in a German study was reported at 150 mg kg1 . Hypochlorites are highly effective chemicals; their low cost and ease of use are reflected in the broad use of these sanitizers. While hypochlorites are very reactive, their useful properties are negatively impacted by several factors, such as the presence and formation of undesired by-products (bromate, chlorite, chlorate, and perchlorate), as well as reduced efficacy if surfaces harbor residues of organic materials. Bromate, chlorite, chlorate, and perchlorate are formed in hypochlorite solution during manufacturing steps, transport, and storage. Chlorate and chlorite are degradation products present in all hypochlorite solutions. The hypochlorite ion is unstable and undergoes two independent routes of self-decomposition. In one route, oxygen and chloride are formed and in the other reaction, chlorate and chloride are formed:

The occurrence of residues of chlorate in many different foods, but particularly in fresh fruits, vegetables, and dairy products, has recently highlighted the importance of the purity, handling, storage, and application of chlorine-containing sanitizers. The typical amounts of chlorate found in dairy products and ingredients are most likely not of a food safety concern, but nevertheless warrant

careful attention in terms of controlling the application of sanitizers at farm level and in manufacturing facilities, with focus on following good manufacturing practices. The QACs have also raised much attention in the European Union due to the occurrence of residues in many different foods, including dairy products and dairy ingredients. QACs are mixtures of chemicals characterized by different chain lengths of the alkyl moieties, typically ranging from C8 to C18. They not only act as sanitizers, but due to their properties (they possess both hydrophilic and lipophilic chemical groups), they also function as detergents when present in higher concentration. In addition, QACs are usually odorless, noncorrosive, and relatively nontoxic to users. A survey conducted in Germany by the BfR (German risk assessment authority) showed a mean value of benzalkonium chloride (BAC) in milk at 0.95 mg kg1 (maximum 6.66 mg kg1), and widespread occurrence in cream and ice cream products. Current legislation in the EU has set a temporary MRL for QACs at 0.1 mg kg1

Health Impact

Generally, disinfectants rarely pose serious residue problems. Many sanitizers have defined, specific antimicrobial activities and are consequently likely to have of low mammalian toxicity. Here, emphasis is on iodine, one of the most frequently used teat disinfectants and among the most effective antimicrobial agents, which at high doses can be of potential health concern. However, contamination of milk can largely be avoided by using formulae that do not contain more than 0.5% iodine and by drying of the teats after dipping. Iodine is an essential component of thyroid metabolism and the recommended dietary allowances are 150 mg day1 for adults and 90 mg day1 for children (1–3 years of age). High iodine intake can lead to disturbance of thyroid function. The tolerable upper intake level (UL) is defined as the highest level of intake that is likely to pose no risks of adverse effects in most individuals. The UL for iodine in adults is 1100 mg day1 and for children of 1–3 years of age is 200 mg day1 . Initial adverse effects observed in cases of excessive intake of iodine are characterized by elevated concentrations of thyroid-stimulating hormone. These effects were demonstrated at iodine intake in adults at or just above the UL. At residual levels above 500 mg l1 , exceeding of the UL for iodine by consumption of milk alone may be possible in children. In most people and at 'normal' residual iodine contamination, iodine intake from milk and other common foods is unlikely to exceed the UL and is therefore of no health concern

Melamine and Other Nitrogenous Compounds

Sources and Occurrence

Adulteration of Milk

In 2008, deliberate contamination of milk with the synthetic chemical melamine (2,4,6-triamino-1,3,5-triazine) resulted in a major outbreak of renal disease and associated deaths in infants. In China, close to 52 000 infants and young children were hospitalized for urinary problems, related to the consumption of melamine-contaminated infant formulae and other dairy products. Melamine, a molecule high in nitrogen content, was illegally added to diluted milk to produce a false high reading of protein content in the standard measurement (the Kjeldahl method). Melamine has subsequently been detected in a variety of milk-containing products leading to regulatory measures in many countries, including bans of certain imports. Levels of melamine reported in dairy products (including infant formulae) ranged from 0.09 to 6200 mg kg1 . As in the case of melamine, the deliberate adulteration of milk with specific high nitrogen-content compounds could be carried out to increase the apparent nitrogen, i.e., protein content and thus the value of the milk. Chemical substances, besides melamine and cyanuric acid, that could also significantly increase the nitrogen content of milk are, for example, urea, amidinourea, ammeline, ammelide, cyanamide, dicyandiamide, 3-aminotriazole, 4-aminotriazole, guanidine, biuret, triuret, semicarbazide, and cyromazine. This is not an exhaustive list, but indicates those compounds with a potential likelihood to be found as adulterants to milk.

Other Possible Sources

Transfer of melamine from melamine-containing feed to cow's milk has been reported. Melamine is used in the production of melamine resins, typically by reaction with formaldehyde. It has many industrial uses, including in the production of laminates, glues and adhesives, molding compounds, coatings, and flame retardants. Melamine is a minor metabolite of the pesticide cyromazine and is used in some fertilizers. Some approved uses of melamine in the United States are as an indirect food additive as a component of glues and adhesives, and in Europe as a monomer and as an additive in plastics. Consequently, low levels of melamine can migrate into milk and dairy products from food contact material. These levels are typically below 1 mg kg1 .

Health Impact and Risk Assessment

The primary target for the toxic action of melamine is the kidney and urinary tract. Formation of bladder stones in rodents and kidney stones in humans has been observed as a result of high melamine exposure. In cats and dogs, coexposure to melamine and its structural analogs, mainly cyanuric acid, as well as ammeline and ammelide, was found to induce the formation of crystals in the kidney and consequently acute renal failure

Analytical Methods

Several methods have been published to date, from rapid screening (enzyme-linked immunosorbent assay (ELISA)) to selective quantitative methods, to determine melamine in dairy products and other foods (human and animal). Of the quantitative methods, liquid chromatography/tandem mass spectrometry (LC/MS/MS) meets all performance criteria in terms of sensitivity, specificity, and accuracy, and can be applied to many different matrices. Rapid methods to determine melamine in raw milk are today commercially available, an example being fluorescent dipsticks that can achieve a detection limit at around 0.05 mg kg1 . These dipstick assays coupled to fluorescence detection (small mobile unit) do not require any sample processing, cleaning, or extraction, providing the results in a direct instrumental reading

Nutritional qualities of milk

Milk is an excellent source of vitamins and minerals, particularly calcium. It has an important role in bone health. Nutritionists recommend that people have milk and other dairy products, such as yoghurt and cheese, every day as part of a balanced diet.

The Australian Dietary Guidelines recommend that people over the age of 2 years have mostly reduced fat products to lower the amount of energy (kilojoules) while still getting all of the other nutritional benefits from dairy foods.

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Nutrients in milk

Milk and milk products have a good balance of protein , fat and carbohydrate and are a very important source of essential nutrients, including:

- calcium
- riboflavin
- phosphorous
- vitamins A and B12
- potassium
- magnesium
- zinc
- iodine.

Milk products also have ‘high-quality proteins’ that are well suited to human needs. For example, having milk (or yoghurt) with cereal can provide amino acids that may be lacking in the cereal product.

Milk and health conditions

There are many myths about the negative impacts of milk on health. Changing how much milk you drink because of these myths may mean you are unnecessarily restricting this highly nutritious drink.

Australians often restrict dairy foods when they try to lose weight, believing them to be fattening. While dairy products naturally contain fat, there are many reduced fat products available.

Dairy foods like milk, yoghurt and cheese (particularly reduced-fat products) are not a threat to good health if had as part of a well-balanced nutritious diet.

Research has shown:

- **Cardiovascular health** – there may be a protective effect of milk for stroke risk. The Heart Foundation says that unless you already have coronary heart disease or elevated cholesterol, full fat milk, yoghurt and cheeses are unlikely to increase your risk of heart disease when consumed as part of a healthy eating pattern.
- **Osteoporosis** – if milk and milk products are removed from the diet, it can lead to an inadequate intake of calcium. This is especially a concern for women over the age of 50 and the elderly, who have high calcium needs. Calcium deficiency may lead to conditions like osteoporosis (a disease that results in loss of bone).
- **Colorectal cancer** (also known as bowel cancer) – according to the World Cancer Research Fund, people who regularly eat more than one serve of dairy products each day (particularly milk) have a reduced risk of developing colon cancer.
- **Blood pressure** – having milk and dairy products is associated with lowered blood pressure. And, when low-fat dairy foods are combined with a high intake of fruits and vegetables, blood pressure is lowered more than by just having fruits and vegetables.
- **Type 2 diabetes** – dairy products in general, particularly those that are low-fat, are protective against developing type 2 diabetes.

Many people in Australia believe that nasal stuffiness or increased mucous is related, in part, to how much milk you drink. However, there is no evidence to support this theory. Milk doesn’t encourage extra mucous production.

PASTEURIZATION

- Pasteurization is a process in which certain packaged and non-packaged foods (such as milk and fruit juice) are treated with mild heat, usually less than 100 °C (212 °F), to eliminate pathogens and extend shelf life.
- The process safeguards foods by destroying or inactivating organisms that contribute to spoilage, including vegetative bacteria but not bacterial spores.
- The process was named after the French scientist Louis Pasteur, whose research in the 1880s demonstrated that thermal processing would inactivate unwanted microorganisms in wine.
- Today, pasteurization is used widely in the dairy industry and other food processing industries to achieve food preservation and food safety.

MILK PASTEURIZATION

- Milk pasteurization is the process of heating milk (or milk product) to a predetermined temperature for a specified period without re-contamination during the entire process.
- The predetermined temperature usually depends on the heat resistance of spoilage microorganisms that the pasteurization program is targeting to destroy.

METHODS USED IN MILK PASTEURIZATION

High-Temperature Short Time (HTST) Pasteurization

- This type of pasteurization is also known as flash pasteurization.
- Flash pasteurization involves heating milk to 71.7°C for 15 seconds to kill *Coxiella burnetii*, which is the most heat-resistant pathogen in raw milk.
- Since it is technically impossible to bring the milk to that exact temperature, it is always safe to work with a range of temperatures. To be safe, you can heat the milk to between 72°C to 74°C for 15 to 20 seconds.
- This will ensure that the milk is heated uniformly to the required temperature.
- This method is most suitable in continuous pasteurization systems.
- Flash pasteurized milk will keep for between 16 and 21 days. For commercial reasons, some manufacturers intentionally reduce the number of days to push the products out of the shelves.

Low-Temperature Long Time (LTLT) pasteurization

- Here, the temperatures used for pasteurization are reduced to 63°C and held for 30 minutes.
- The prolonged holding period alters the structure of the milk proteins making them better suited for making yogurt.
- This method is best for batch pasteurization where the milk is held in a jacketed vat for effective pasteurization.
- There are many designs of batch pasteurizers in the market that are suitable for both domestic and commercial use.

Ultra-High Temperature (UHT) Pasteurization

- This is a completely closed pasteurization method. The product is never exposed even for a fraction of a second during the entire process.
- It involves heating milk or cream to between 135°C to 150°C for one to two seconds, then chilling it immediately and aseptically packaging it in a hermetic (air-tight) container for storage.
- Despite the risk of Millard browning, UHT pasteurization remains the most popular milk preservation method for safe and stable milk.

SIGNIFICANCE OF PASTEURIZATION

Proper pasteurization is necessary for the following reasons:

- The chief objective of milk pasteurization is to destroy pathogenic bacteria that could have a public health concern. By destroying these microorganisms, the product becomes safe for public consumption.
- Secondly, pasteurization eliminates destructive bacteria and enzymes that could cause spoilage of the product. This leads to the prolonged shelf life of the milk.
- There is a need to ensure that the product can keep for longer periods without expensive storage equipment. Pasteurization will eliminate spoilage bacteria and enzymes and extend the shelf life of the product.

IMPORTANCE OF PASTEURIZATION

. Prolonged shelf life

Keeping product fresh long enough for it to make it to market and then on to consumers' pantries is key. Some bacteria and other microorganisms can cause food products to deteriorate faster than it takes for the end consumer to purchase it, so pasteurization is vital to making your food products viable.

2. Preventing disease

Diseases are found in many food products, and removing the organisms that cause those diseases is critical to ensuring your product is safe for general consumption. For example, eggs are known to spread salmonella and avian flu, and pasteurization kills the organisms that cause those diseases.

Some food products are breeding grounds for microbes, which means that as sterile as your processing plant may be, there may be a chance your product would cause disease later on if it's not pasteurized.

3. Quick and safe sanitation

There are many ways to sanitize food products, but few are as quick or as safe as pasteurization. With pasteurization, the temperature of the product is simply raised enough to destroy any microorganisms that may be present. Other methods may involve chemical treatments or radiation, and may not be the safest to use.

Pasteurization is also faster than most methods that rely on filtration or other means.

4. Consistent product quality

By eliminating volatile contaminants, the product becomes more stable, therefore the quality of your product is more consistent. A more consistent product means your customers know what to expect from your production lines, and it's easier to provide reliable results.

5. Potential improvements in flavor and scent

In some cases, the pasteurization process can improve the smell and taste of your product. Often, foods and other products may have bacteria that produce unpleasant smells over time but do not necessarily impact the product's quality besides. Removing those bacteria can create a more consistently pleasant experience for the consumer.

6. Regulatory compliance

Numerous laws are in place that require certain food products to be pasteurized or otherwise treated to remove bacteria and viruses.

For example, the FDA requires that pasteurized eggs or egg products should be used instead of raw eggs in certain products when serving populations such as school children and nursing home patients.

UNPASTEURIZED MILK

Raw milk or **unpasteurized milk** is milk that has not been pasteurized, a process of heating liquid foods to kill pathogens for safe consumption and extending the shelf life.

Proponents of raw milk have asserted numerous supposed benefits to its consumption, including better flavor, better nutrition, and contributions to the building of a healthy immune system. However, no clear benefit to its consumption has been found, and the medical community notes there are considerable dangers, including an increased risk of infection, to its consumption substantial evidence of this increased risk, combined with a lack of any clear benefit, has led countries around the world to either prohibit the sale of raw milk or require warning labels on its packaging when sold.

In countries where it is available for sale, its availability and regulations around its sale vary. In the EU, member states can prohibit or restrict the sale of raw milk, but it is not banned outright; in some member states, the sale of raw milk through vending machines is permitted, though the packaging will typically instruct consumers to boil before consumption. In the US, some dairies have adopted *low-temperature vat pasteurization*, which they say produces a product similar to raw milk.

Contamination in raw milk

How does milk get contaminated?

Milk contamination may occur in these ways:

- Animal feces coming into direct contact with the milk
- Infection of the udder (mastitis)
- Cow diseases (for example, bovine tuberculosis)
- Bacteria that live on the skin of animals
- Environment (for example, feces, dirt, and processing equipment)

AIM AND OBJECTIVE

AIM

isolation and characrterization of microbial contaminant in pasteurized and unpasteurized milk

OBJECTIVE

Isolation of pure cultures of lactobacillus sp from milk samples

To find out the contaminents in pasteurized and un pasteurized milk

Review of Literature

Review of Literature

Health benefits and risks of consuming milk

Milk is a good source of many essential nutrients, including calcium, protein, and vitamin D. Many people see it as a vital part of a balanced diet. Others, however, cite various reasons for choosing not to consume it.

Sources of milk and milk products include cows, sheep, camels, goats, and many others. Milk alternatives include soy milk, almond milk, flax milk, coconut milk, and hemp milk.

This article will focus on the benefits and risks of drinking cow's milk

Milk's healthfulness depends on the individual and the type of milk they consume.

Pasteurized milk that is high in protein, low in fat, and free from unnecessary additives can be healthful for many people.

On the other hand, some flavored milks contain as much sugar as a can of soda. These are not a healthful choice.

Present day cow's milk is not a single product. It can be fresh or long life, fat free, lactose free, fortified with added omega-3s, hormone free, organic, or raw, among other options.

Nutrition

One cup (249 grams) of whole cow's milk with 3.25% fat provides

- **Calories:** 152
- **Water:** 88%
- **Protein:** 8.14 grams
- **Carbs:** 12 grams
- **Sugar:** 12 grams
- **Fiber:** 0 grams
- **Fat:** 8 grams

Milk proteins

Milk is a rich source of protein

proteins in milk can be divided into two groups based on their solubility in water:

- **Insoluble milk proteins** are called casein.
- **Soluble milk proteins** are known as whey proteins.

Both groups of milk proteins are considered to be of excellent quality, with a high proportion of essential amino acids and good digestibility.

Casein

Casein forms the majority — or 80% — of proteins in milk.

It's really a family of different proteins, with alpha-casein being the most abundant.

One important property of casein is its ability to increase the absorption of minerals, such as calcium and phosphorus

It may also promote lower blood pressure

Whey protein

Whey is another family of proteins, accounting for 20% of the protein content in milk.

It's particularly rich in branched-chain amino acids (BCAAs) — such as leucine, isoleucine, and valine.

Whey proteins have been associated with many beneficial health effects, such as decreased blood pressure and improved mood during periods of stress

Whey protein is excellent for growing and maintaining muscles. As a result, it's a popular supplement among athletes and bodybuilders

Milk fat

Whole milk straight from the cow is around 4% fat.

In many countries, marketing of milk is mainly based on fat content. In the United States, whole milk is 3.25% fat, reduced fat milk 2%, and low fat milk 1%.

Milk fat is one of the most complex of all natural fats, containing about 400 different types of fatty acids

Whole milk is very high in saturated fats, which make up about 70% of its fatty acid content.

Polyunsaturated fats are present in minimal amounts, making up around 2.3% of the total fat content.

Monounsaturated fats make up the rest — about 28% of the total fat content.

In addition, trans fats are naturally found in dairy products.

In contrast to trans fats in processed foods, dairy trans fats — also called ruminant trans fats — are considered beneficial for health.

Milk contains small amounts of trans fats, such as vaccenic acid and conjugated linoleic acid (CLA)

CLA has attracted considerable attention due to its various possible health benefits — though evidence is still limited

Some research suggests that CLA supplements may harm metabolism

Vitamins and minerals

Milk contains all the vitamins and minerals necessary to sustain growth and development in a young calf during its first months of life.

It also provides almost every single nutrient needed by humans — making it one of the most nutritious foods available.

The following vitamins and minerals are found in particularly large amounts in milk:

- **Vitamin B12.** Foods of animal origin are the only rich sources of this essential vitamin. Milk is very high in B12
- **Calcium.** Milk is not only one of the best dietary sources of calcium, but the calcium found in milk is also easily absorbed
- **Riboflavin.** Dairy products are the biggest source of riboflavin — also known as vitamin B2 — in the Western diet
- **Phosphorus.** Dairy products are a good source of phosphorus, a mineral that plays an essential role in many biological processes.

Sometimes fortified with vitamin D

Fortification is the process of adding minerals or vitamins to food products.

As a public health strategy, fortifying milk products with vitamin D is common and even mandatory in some countries

In the United States, 1 cup (240 mL) of vitamin-D-fortified milk may contain 12% of the daily value for this nutrient

Health benefits of milk

Milk is one of the most nutritious foods you can find.

It has been widely studied and seems to have several important health benefits.

In particularly, cow's milk may positively affect your bones and blood pressure.

Bone health and osteoporosis

Osteoporosis — a condition characterized by a decrease in bone density — is the main risk factor for bone fractures among older adults

One of the functions of cow's milk is to promote bone growth and development in the young calf.

Cow's milk seems to have similar effects in people and has been associated with higher bone density The high calcium and protein content of milk are the two main factors believed responsible for this effect However, more recent evidence is conflicting. Some studies have failed to show a connection between dairy intake and osteoporosis

Blood pressure

Abnormally high blood pressure is a major risk factor for heart disease.

Dairy products have been linked to a reduced risk of high blood pressure

It’s thought that the unique combination of calcium, potassium, and magnesium in milk are responsible for this effect

Other factors may also play a part, such as peptides formed during the digestion of casein

Possible adverse effects

The health effects of milk are complex — some components in milk are quite beneficial, while others may have adverse effects.

Lactose intolerance

Lactose, or milk sugar, is the main carbohydrate found in milk.

It’s broken down into its subunits — glucose and galactose — in your digestive system.

However, some people lose the ability to fully digest lactose after childhood — a condition known as lactose intolerance.

An estimated 75% of the world’s population has lactose intolerance, though the proportion of lactose intolerant people varies greatly depending on genetic makeup

Lactose intolerance is most prominent in parts of Asia, Africa, and South America, where its estimated to affect 65–95% of the population

In Europe, the estimated prevalence is 5–15%, with people in Northern Europe being the least affected

In people with lactose intolerance, lactose is not fully absorbed, and some or most of it passes down to the colon, where the residing bacteria start fermenting it.

This fermentation process leads to the formation of short-chain fatty acids (SCFAs) and gas, such as methane and carbon dioxide.

Lactose intolerance is associated with many unpleasant symptoms, including gas, bloating, abdominal cramps, diarrhea, nausea, and vomiting.

Milk allergy

Milk allergy is rare in adults but more frequent in young children

Most often, allergic symptoms are caused by whey proteins called alpha-lactoglobulin and beta-lactoglobulin, but they can also be due to caseins

The main symptoms of milk allergy are skin rash, swelling, breathing problems, vomiting, diarrhea, and blood in stools

Milk and cancer

Many observational studies have looked at the association between milk and cancer risk.

Overall, the evidence is mixed, and very few conclusions can be drawn from the data.

However, a fair number of studies indicate that dairy consumption may increase the risk of prostate cancer in men

Conversely, numerous studies have found a link between dairy consumption and a lower risk of colorectal cancer

As a general recommendation, excessive consumption of milk should be avoided. Moderation is key.

Raw vs. pasteurized milk

Raw milk is a term used for milk that has not been pasteurized or homogenized.

Pasteurization is the process of heating milk to increase shelf life and minimize the risk of illness from harmful microorganisms that may be present in raw milk.

Heating results in a slight decrease in several vitamins, but this loss is not significant from a health perspectiv

Homogenization — the process of breaking the fat globules in milk into smaller units — has no known adverse health effects

Drinking raw milk is associated with a reduced risk of childhood asthma, eczema, and allergies. The reason for this association is still not entirely clear

Although raw milk is more natural than processed milk, its consumption is riskier.

In healthy cows, milk does not contain any bacteria. It's during the milking process, transport, or storage that milk gets contaminated with bacteria — either from the cow itself or the environment.

Most of these bacteria are not harmful — and many may even be beneficial — but occasionally, milk gets contaminated with bacteria that have the potential to cause disease.

Although the risk of getting ill from drinking raw milk is small, a single milk-borne infection may have serious consequences.

People are usually quick to recover, but those with weak immune systems — such as older adults or very young children — are more susceptible to severe illness.

Most public health advocates agree that any potential health benefits of drinking raw milk are outweighed by possible health risks resulting from contamination with harmful bacteria

Staphylococcus aureus contamination in milk

Food-borne diseases (FBD) are defined by the World Health Organization as diseases of infectious or microorganism because of high nutritive value and complex chemical composition toxic nature caused by, or thought to be caused by the Many contaminants find their way to raw milk, consumption of food or water. The pathogenesis of from which they gain access to dairy products . bacteria causing food-borne poisoning depends on Chapaval found production of staphylococcal their capacity to produce toxins after ingestion (in the enterotoxins in milk when milk was stored at temp- digestive tract) or intoxication (ingestion of preformed eratures of 37 ºC to 42 ºC or when exposed to variations toxins in foodstuff). Among the bacteria predominantly in temperature. involved in these diseases, Staphylococcus aureus is a Staphylococcal food poisoning include symptoms leading cause of gastroenteritis resulting from the such as sudden onset of nausea, vomiting, abdominal consumption of contaminated food. Staphylococcal cramps and diarrhea [6]. food poisoning is due to the absorption of Staphylo- On heating at normal cooking temperature, the coccal enterotoxins preformed in the food bacteria may be killed but the toxins remains active Milk and milk products are the prime habitat to . Staphylococcal enterotoxins are highly heat complex microbial ecosystems; these are responsible resistant and are thought to be more heat resistant in for the broad variations in taste, aroma and texture of foodstuffs than in a laboratory culture medium . milk and milk products. Contamination of milk and Besides these, enterotoxins producing S. aureus milk products with pathogenic bacteria is mainly due to are most dangerous and harmful for the human health. processing, handling and unhygienic environment. The About 50 % strain of this organism are able to produce occurrence of these pathogenic bacteria in milk and enterotoxins associated with food poisoning . Illness through S. aureus range from minor skin infection such The selective medium used for isolation of S. as pimples, boils, cellulites, toxic shock syndrome, aureus was Baird Parker Agar (BPA) (HiMedia Pvt. impetigo, and abscesses to life threatening disease such Ltd.). A loopful of inoculum from enrichment were as pneumonia, meningitis, endocarditis, and septicaemia streaked on BP agar and incubated for 48 hours at 37°C. [2]. Especially in India, rate of infection is still higher Characteristic appearance of jet black colonies because of warm and humid climate [10]. For many surrounded by a white halo were considered to be years, S. aureus was the only staphylococcal species presumptive S. aureus The pure cultures were known to produce enterotoxins . streaked on Nutrient agar (HiMedia Pvt. Ltd.) and Thus, the objective of this study was to investigate incubated for 24 hours at 37°C and were further the occurrence of S. aureus in milk and milk products characterized by biochemical tests.

Materials and Methods

Collection of bacterial samples: Total five samples, from milk are collected 2 pasteurized milk samples and 3 unpasteurized homemade dairy. Collected from different local markets in Kollam

Mrach 9, 2022.

Serial dilution for bacterial colony

The milk samples were then serially diluted from 0 1 to 0 8 in0.85 % saline solutions.

Further 100 µl of dilutions were transferred into MRS Agar plates and kept for overnight

incubation in the bacteriological incubator maintained at 37ºC.

MRS Agar constituents

Constituents	Weight (g/L)
Peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Tween 80	1mL
Ammonium citrate	2.00

Sodium acetate	5.00
Magnesium sulphate	0.01
Manganese Sulphate	0.05
Dipotassium phosphate	2.00
Agar	12.00
Distilled Water	1L

<u>Isolation and identification of a pure culture</u>

Among the incubated petri plates, 10^{-8} diluted plates with less isolated microbial colony was chosen. Pure culture was isolated after quadrant streaking and the obtained pure culture was sub cultured in both liquid and solid medium.

<u>Gram staining:</u>

Gram staining test was performed for all isolated strains according to the standard procedure. A smear of single colony was prepared on a clean glass slide and the smear was allowed to air-dry and then heat fixed. The heat fixed smear was flooded with crystal violet solution and after one minute, it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally safranin was used as counter stains for 60-80 sec and washed with water, and examined under oil immersion (100X). Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used as positive and negative control, respectively.

<u>Catalase test:</u> A drop of 3% hydrogen peroxide was added to a fresh culture on a sterile glass slide and mixed well. Producing bubble or froth, indicated catalase-positive and no bubble or froth indicated catalase negative. Staphylococcus aureus ATCC 25923 and E. coli ATCC 25922 were used as positive and negative control, respectively.

Mannitol fermentation test: the purpose is to see if the microbe can ferment the carbohydrate (sugar) maltose as a carbon source

If maltose is fermented to produce acid end products, the pH of the medium will drop. A pH indicator in the medium changes color to indicate acid production.

Several media are available for this. Most commonly used is phenol red maltose broth. The medium is a nutrient broth to which 0.5-1.0% maltose is added. The pH indicator phenol red is red at neutral pH but turns yellow at pH <6.8. It also changes to magenta or hot pink at pH >8.4..

An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red maltose broth. The inoculated tube is incubated at 35-37 C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic.

Mannitol Salt Agar for the isolation of Staphylococcus aureus: Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens.
It encourages the growth of a group of certain bacteria while inhibiting the growth of others.
It is a selective medium prepared according to the recommendations of Chapman for the isolation of presumptive pathogenic staphylococci.

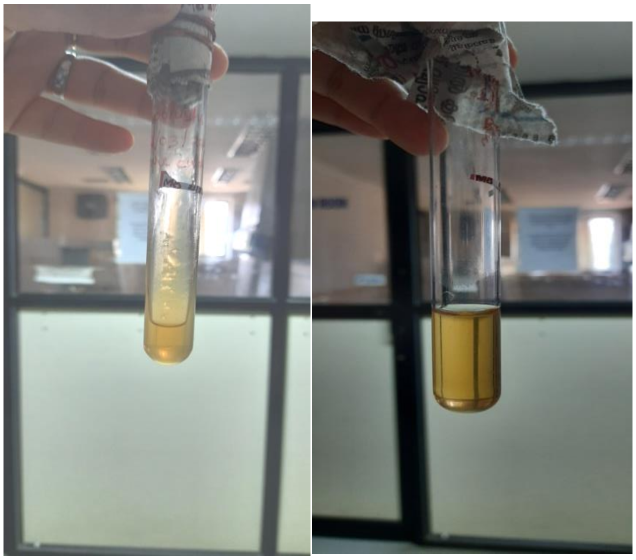
Ingredients	**gram / liter**
Peptone	10g
Beef meat extract	1g
Sodium chloride	75g
Mannitol	10g
Phenol red	0.025g
Agar	15g

RESULTS

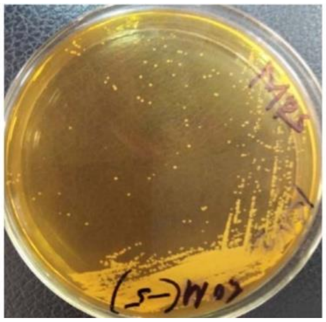
Isolation of pure microbial culture from milk

After performing serial dilution, the cultures obtained were quadrant streaked and later sub cultured and stored for further analysis.

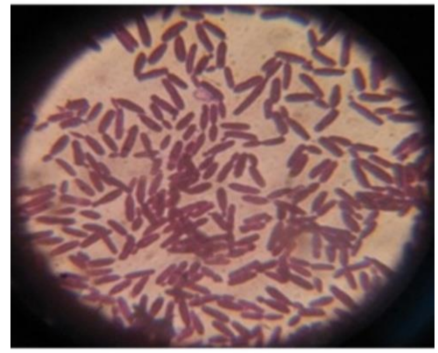
Fig 1a, Culture obtained on Serial Dilution



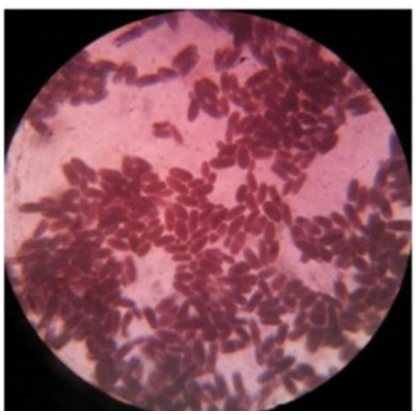
2a Broth and pure culture



Lactobacillus species in MRS agar plate



bacillus



Coccobacilli

s. aureus

Mannitol fermentation test after 48 hrs

DISCUSSION

5. DISCUSSION

Milk is normally sterile in the udder of the cow and the buffalo provided they do not suffer from mastitis (udder infection). If they have mastitis a large number of generally gram positive bacteria such as streptococcus and staphylococcus spp. May present in the milk when it leaves from the udder.

Negligence of hygienic conditions such as improper cleaning of bulk tank, dirty udder, milking equipments, milk handling techniques and improper storage will increase the production of Gram positive and Gram negative bacteria in the bulk tank milk.

Food products serve not only a source of nutrition but also as a substrate for the growth of microorganisms. The growth of microorganisms causes food spoilage, it may result food borne illness, in tropical countries raw milk and milk products are responsible for many outbreaks of gastrointestinal tract. It is also reported that immunocompromised individuals are prone to food borne infections

The findings of present study are

Among 5 milk samples 2 where pasteurized and 3 non pasteurized (home made dairy)

In pasteurized samples we found only lactobacillus sp.

In non pasteurized sample staphylococcus aureus was found.

The differences in the prevalence rate of staphylococcus aureus between milk and milk products may origin from the method of manufacture, storage and handling

Antibiotics resistance development among the bacteria poses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

SUMMARY & CONCLUSION

6. SUMMARY AND CONCLUSION

Staphylococcal food poisoning is of major concern in public health programs worldwide. S. aureus maybe be present in milk and milk products as a result of milk collected from animal suffering from disease condition and excreting S, aureus in milk or due to unhygienic conditions during production, processing, storage and handling of milk products, which are main causes of food borne diseases. Result clearly indicate that milk and milk products available in the market were contaminated with S. aureus posing high risk of food poisoning. Thus more hygienic preventive measures are required to reduce the bacterial contamination, so as to increase the wholesomeness of these milk and milk based products.

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7.BIBLIOGRAPHY

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APPENDICES

- **MRS Agar**

Constituents	**Weight (g/L)**
Peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Tween 80	1mL
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.01
Manganese Sulphate	0.05
Dipotassium phosphate	2.00
Agar	12.00
Distilled Water	1L