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The book is structured to give students a solid foundation in biology, highlighting the basic unit of life, the cell, and exploring how life functions at various levels of organization. It emphasizes the interconnections between all forms of life and the environment, showcasing the intricate balance that sustains life on Earth.

The text is typically well-illustrated, with diagrams and figures that help explain complex scientific concepts. It often incorporates real-world examples to demonstrate how biology relates to everyday life, making the subject matter more accessible and engaging.

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The Living World: Fundamentals of Life Sciences

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THE LIVING WORLD

Fundamentals of Life Sciences

Dr. Somudra Pal

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The Living World: Fundamentals of Life Sciences

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Microbial Volatile Compounds: A Sneak Peek into Their Sources, Mechanism of Action & Applications

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Chapter - 1

Microbial Volatile Compounds: A Sneak Peek into Their Sources, Mechanism of Action & Applications

Madiha Perween, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

Microbial Volatile Organic Compounds (VOCs), generated by bacteria and fungi, are tiny signaling molecules with low molecular masses (<300 Da), high vapor pressure, and low boiling point. They are classified into many categories, such as ketones, alcohols, terpenoids, sulfur- containing chemicals, alkenes, and so on. These chemicals are recognized to play a key function in enhancing plant growth and tolerance to abiotic stress. They are also efficient in acting as a natural pesticide inducing the activation of plant defence mechanisms and inhibiting the growth of other bacteria on the plant. In this review paper we have primarily focused on addressing the different effects of VOCs on plants. Microbial VOCs are highlighted because of their biodegradable structure, absence of any toxic substances, and even the regulation of several physiological functions of plants. These also effect the different processes of plant like photosynthesis, it's metabolism, phytohormone pathway, etc. In this paper we have summarized the different effects of VOCs in plants, and different physiological mechanism of plants activated by VOCs, some challenges intervening these applications of VOCs, and the future prospects highlighting the further applications in agriculture.

Keywords: Volatile organic compounds, pesticides, fertilizers, agriculture, physiological mechanisms of plants

1. Introduction

Chemical substances having saturated vapor pressures greater than 102 kPa at 25 °C are referred to be volatile organic compounds (VOCs). VOCs are produced by both microbes and plants and enable intra-and interspecific communication. Plants create volatile organic compounds (VOCs) to defend themselves against herbivores and diseases, warn their neighbors of assault, compete with other plants, and/or feed microbes (Poveda *et al*., 2021).

Microorganisms use VOCs to communicate or attack one another. VOCs are released by microorganisms as a means of defence or communication. Because of their capacity to prevent plant diseases from growing and developing, trigger the activation of plant defences, or encourage plant growth and development, microbial volatile organic compounds, or MVOCs, can be very beneficial to plants and their usage in agriculture. Growing knowledge of the significance of microbial volatilome has made MVOCs a valuable biotechnological resource in plant production systems in recent years (Poveda *et al*., 2021). Bacterial VOCs are classified into many chemical categories, such as ketones, alcohols, terpenoids, sulfur-containing chemicals, and alkenes. Certain chemicals are shared by all microbes, whereas others are unique to a single strain. A single bacterium can produce up to 100 distinct VOCs. Microbial VOC production is a complex, multivariate process. VOC emissions can vary depending on nutrition medium composition, pH, aeration, and culture growth stage (Sidovora *et al*., 2021). Microorganisms can influence inter-kingdom communicate through chemicals, leading to either antagonistic or favourable interactions. VOCs can influence microbes, plants, insects, nematodes, and other creatures by inhibiting or stimulating their growth and development, causing plant resistance, and affecting their overall health. VOC production may have a role in microbial competition within an ecological niche (e.g., in the rhizosphere of plants), antagonistic relationships between plant-pathogenic and plant-associated bacteria, and microorganisms in human and animal microflora (Sidorova *et al*., 2021). Microorganisms live on plant surfaces, including the rhizosphere, phyllosphere, and anthosphere. The unique characteristics of these sphere can impact microorganisms' capacity to thrive and establish themselves there. This article explores the impact of plant-emitted volatile organic compounds (VOCs) on microbial colonizers, specifically bacteria. Plants interact with specific microbial species, rather than a random variety (Junker *et al*., 2013). Bacterial communities on leaf surfaces in a Brazilian tropical forest vary significantly between tree species and, to a lesser extent, within them. Bacteria can impact plant fitness and plant-animal interactions, both positively and negatively (Junker *et al*., 2013). Bacteria and microbes can select for VOCs, allowing plants to manage microbial colonizers on their surfaces. This promotes mutualist growth and prevents harmful germs from establishing themselves (Junker *et al*., 2013). Microbial VOCs boost plant development by regulating nutrient acquisition, photosynthesis, phytohormones and metabolic processes (Fincheira *et al.*, 2021). Microbial Volatile Organic Compounds (VOC) have a significant role in mutualism and antagonism. Microbial volatiles influence

cellular and developmental processes both within and between species.Microbialvolatiles'exactmethodofactionremainsunknown.Discoverin gthemechanism of action of microbial volatiles as an interphase between plant health and microbes can reveal unique mechanisms for governing biological processes critical to plant fitness and propose solutionstoagriculturalandenvironmentalissues(Panpatteetal,2017).Microbiali nteractionsvia info chemicals are critical for the evolution of spatial distribution and activity changes in ecosystems. Microorganisms produce a diverse spectrum of info chemicals, many of which are secondary metabolites, with the majority being soluble and many being volatile. Volatile Organic Compounds (VOCs) have been detected in soil atmosphere sand linked to community structure and function (Wheatley *et al*., 2002). These effects are usually transient, e.g. removal of antagonist is followed by complete recovery. Up-and down-regulation of gene expression, by mRNA and protein profiling has been demonstrated. VOCs have played an important role during the evolution of microorganisms in the context of their communities (Wheatley *et al*., 2002).

Volatile Organic Compounds: Microbial VOCs are signal molecules with a low molecular weight (<300g/mol), low boiling point, high vapor pressure (0.01k Paat 20 °C), and lipophilic moiety (Fincheira *et al*., 2021). The volatility of VOCs is primarily determined by their physical-chemical properties. Microbial VOCs can have various chemical structures, including alkanes, alkenes, ketones, alcohols, sulfides, and terpenes (Fincheira *et al*., 2021). The VOC profile includes a range of chemicals with varying quantities, emitted as metabolic products based on growth circumstances. Environmental parameters such as growth substrate, temperature, oxygen, pH, and moisture might impact VOC emission profiles. Bacillus species cultivated in low-oxygen environments can create 2,3-butanediol and 2-hydroxy-2-butanone (Fincheira *et al*., 2021). Bacteria, fungus, and actinomycetes produce significant amount of volatile chemicals in soil, with bacteria having the highest concentration (1011 cells/g) (Tahir *et al*., 2017). Bacterial volatiles promote plant development by controlling phytohormone synthesis and metabolism. *In vitro* and growth chamber tests were carried out to evaluate the influence of volatile organic compounds (VOCs) produced by the plant growth boosting rhizobacterium *Bacillus subtilis* strain SYST2 on hormone regulation and growth promotion in tomato plants (Tahir *et al*., 2017). Furthermore, MVOCs affect plant development and systemic plant resistance, while also serving as attractants or repellents for insects and other plant-threatening stressors (Srikamwang *et al*., 2023). VOCs are used for their food and flavor characteristics, as indirect indicators of microbial

development, to encourage plant growth, and to attract insect pests. Because these compounds may travel great distances from their source, they are effective chemical signaling molecules (semiochemicals) in non-aqueous environments, allowing bacteria to engage in "chemical conversations". (Bennett *et al*., 2012). So far, 94 distinct organic and five inorganic compounds have been examined; the majority of them impact plant and fungal growth, physiology, and defense responses, but little is known about their precise molecular and cellular targets. Most bioactive VOCs are unlikely to interact with specific volatile organic compounds due to large overlaps in their emission spectra (Piechulla *et al*., 2017).

2. Physiological Mechanism of Plants Activated by VOCs

Volatiles are crucial for the survival and establishment of bacterial populations in their ecological niches, as well as the formation of diverse communities. Volatiles can spread aqueous solutions, travel long distances in the atmosphere, and act both above and below earth (Panpatte *et al*., 2017). Recent research shows that microbial volatile organic compounds (VOCs) can induce physiological changes that promote plant growth. Several research have been undertaken to better understand the dynamic interplay between bacteria and plants through VOC emissions Bacteria influence plant growth by numerous processes (Santoro *et al*., 2015). A recently revealed process involves the creation of microbial volatile organic compounds (mVOCs), which are gaseous molecules that can interact with plants in soil. mVOCs can boost plant growth directly through induced systemic resistance (ISR) or indirectly by suppressing phytopathogens (biocontrol) (Santoro *et al*., 2015).

Hence the role of VOCs in modulating the various physiological mechanisms of plants are listed below:

2.1 Moderation of Photosynthesis: Photosynthesis is procedure in which plants use solar energy to convert Carbon dioxide into carbohydrates and release oxygen as a byproduct in the chloroplast (Fincheira *et al*., 2021). Photosynthesis produces sugar, which serves as the primary energy source for plant growth. *B. subtilis* GB03 produced unknown VOCs that significantly impacted photosynthesis in *A. thaliana* plants (Fincheira *et al*., 2021). Other *Bacillus* strains have been shown to affect photosynthesis. Gene ontology (GO) analysis reveals differential expression of photosynthesis-related genes in *A. thaliana* seedling sex posed to *B. amyloliquefaciens* FZB42-released VOCs. More recently, VOC

submitted by *B. subtilis* SYST2increasedthephotosynthesis rate and stomatal conductance on *S. lycopersicum* plants after 14, 21, and 28 days of exposure (Fincheira *et al*., 2021).

2.2 Nutrients Balance: Plants can grow and develop to their full potential when they receive enough nutrients. It mentions that sufficient nutrient content has a major impact on plant performance. According to certain findings, microbial volatile organic compounds (VOCs) are essential for controlling the uptake of nutrients (Fincheira *et al*., 2021). While sulfur, iron, and copper content increased on *A. thaliana*'s root, *B. subtilis* GB03's VOCs boosted the amount of copper on the leaves. The concentration of macro-and micronutrients in the shoot and root of maize was found to be affected by VOC blends generated by *S. plymuthica* and *F. culmorum*, which suggests that soil microorganisms can regulate the nutrient status (Fincheira *et al*., 2021). Bacteria interact with plants in many ways. In recent years, bacterial volatile synthesis has arisen as a unique way for bacteria to influence plant growth. Exposure to volatile produced by specific bacterial strains has been proven to enhance plant biomass by to five times or cause plant mortality (Bailly *et al*., 2012). Despite these dramatic growth changes, the elucidation of the chemicals responsible, the process of perception by the plant, and the specific metabolic changes caused in plant is still in its infancy (Bailly *et al*., 2012).

2.3 The Improvement of Abiotic Stress on Plants with the use of VOCs: Plant growth-promoting rhizobacteria (PGPR) are beneficial soil microbes that can help plants grow or adaptor stress (Liu *et al*., 2015) Some PGPR were utilized in agriculture, resulting in higher seedling emergence, plant weight, crop yield, and disease resistance. PGPR stimulate plant growth by releasing nonvolatile chemicals, such as the hormones auxin and cytokinin, as well as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces plant ethylene levels, and siderophores, which facilitate root uptake of metal nutrients (Liu *et al*., 2015) Microbial VOCs may promote plant development while also induce disease resistance and abiotic stress tolerance, however the latter phenomenon has only been examined a few times. Recent reviews have described the chemical composition of microbial VOCs, as well as the effects of microbial VOCs on plant biomass production and disease resistance (Liu *et al*., 2015).

3. Challenges in Applying VOCs in Agriculture

According to research conducted since 2003, (VOCs) have demonstrated an effective ability to enhance plant health by inducing growth and increasing plant tolerance to abiotic stress.

Understanding the chemical makeup of microbial volatile organic compounds (VOCs) is crucial for creating an efficient application system (Fincheira *et al*., 2021). Because of their length carbon chains, certain volatile organic compounds (VOCs) have a high degree of lipophilicity, making it challenging to use them because of their poor water solubility. Additionally, the high volatility caused by the VOCs low boiling temperatures and high vapor pressure might result in significant loss of VOCs in field settings (Fincheira *et al*., 2021). Volatile organic compounds (VOCs) produced by plant growth-promoting rhizobacteria (PGPR) have important functions in regulating plant growth and inducing systemic resistance to diseases (Park *et al*., 2015). Despite their significance, the physiological roles of the particular VOCs generated by Pseudomonas fluorescens SS101 (Pf. SS101) have not been fully understood. The effects of Pf. SS101 and its VOCs on plant growth promotion were studied both *in vitro* and in planta. Plants exposed to Pf. SS101 under both conditions shown considerable growth promotion, indicating that its VOCs play an important role in encouraging plant growth (Park *et al*., 2015). Using microorganisms or their metabolites as biological control agents (BCAs) is a potential strategy for managing RKNs. Among the metabolites, volatile organic compounds (VOCs) have garnered growing attention because of their ability to control not only RKNs but al-soother plant diseases, such as insects, fungi, and bacteria (Diyapoglu *et al*., 2022).

4. Application as Pesticides

The production of volatile organic compounds (VOCs) by microorganisms is a significant factor in their selection as biocontrol agents against plant diseases. VOCs produced by the biocontrol strain *Bacillus amyloliquefaciens* T-5 and assessed their impact on the growth and virulence features of tomato bacterial wilt pathogen *Ralstonia solanacearum*. (Raza *et al*., 2016). The adoption of synthetic pesticides and artificial fertilizers help must increase agricultural production, butat a significant environmental cost. The use of beneficial microorganism's plant interaction has been advocated as an environmentally benign way to improve plant tolerance to stress and raise productivity indefinitely. We present an overview of scientific evidence demonstrating that this favorable interaction is frequently mediated by the

emission of microbial Volatile Organic Compounds (Russo *et al*., 2022). Under regulated conditions, VOCs have been shown to exhibit strong antibacterial, nematicidal, pesticidal, plant defense, tolerance-induced, and plant-growth-promoting properties. Antifungal VOCs include dimethyl trisulfide, dimethyl disulfide, benzothiazole, nonane, decanone, and 1-butanol (Rani *et al.*, 2023). *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, and *Burkholderia* bacteria create plant growth-promoting VOCs such as acetoin and 2,3-butenediol. These VOCs influence the expression of genes involved in plant defense and development (including Arabidopsis, tobacco, tomato, potato, millet, and maize). VOCs are also known to affect pathogenesis-related genes, induce systemic resistance, modulate plant metabolic pathways, and acquire nutrients (Rani *et al*., 2023). Volatile organic compounds (VOCs) have various plant-friendly features. In this study, we assessed the ability of *Bacillus javensis 4's* VOCs to prevent the formation of phytopathogens and stimulate plant growth (Ghazala *et al*., 2022). We investigated the antifungal and plant growth-promoting (PGP) properties of diffusible and volatile organic compounds (VOCs) generated by different bacterial isolates. The bacterial strains reviewed as *Pseudomonas fluorescens* UM16, UM240, UM256, and UM270 based on their complete 16S ribosomal gene sequencing (Hernández *et al*., 2015).

5. Future Prospects

Microbial volatile organic compounds (VOCs) have surfaced as a novel approach to enhance agricultural yield and alleviate ecological issues associated with chemical fertilization. To achieve microbial VOC application in a greenhouse or in the field, more research needs to be done. In- depth research on plants should be done to ascertain all the effects of applying microbial volatiles, or stress (Panpatte *et al*., 2017). Therefore, in order to give a full picture of plant response to microbial VOC and its ramifications, investigation at the genomic, proteomic, metabolomic, and other "omics" levels should be taken into consideration. Few studies have been conducted on carrier design that enables regulated release of volatile organic compounds (VOCs) in environmental settings, despite the fact that numerous studies assess the impact of VOCs in plants. One Of the primary obstacle to its use in agricultural systems is the development of carriers with sustainable qualities for the controlled release of volatile organic compounds (VOCs). Because of their low toxicity and biocompatibility, the current encapsulation systems of chemicals connected to the food and pharmaceutical field may be a valuable search method. Therefore, in order to enhance plant growth, tests should be designed to assess the impact and

effectiveness of VOCs produced from carriers. (Panpatte *et al*., 2017). Microbes in the soil produce a wide range of volatile organic compounds (VOCs), which can dramatically alter plant growth and health. Each bacterium produces a unique blend of VOCs that play crucial roles not only in the microbe's life cycle, but also in interactions with other species such as bacteria and plants. In this chapter, we described how VOCs promote plant growth and defend against abiotic and biotic stress.

Conflict of Interest

The authors have no conflict of interest.

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Chapter - 2
An Overview of Cost-Effective Plant Based Diet in Mitigating Oxidative Stress

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Chapter - 2

An Overview of Cost-Effective Plant Based Diet in Mitigating Oxidative Stress

Ishani Das, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

A cost-effective plant-based diet has the potential to significantly mitigate oxidative stress, a condition marked by an imbalance between free radicals and antioxidants in the body, which contributes to the development of chronic diseases such as cardiovascular disorders, diabetes, and cancer. This dietary approach is rich in naturally occurring antioxidants, including vitamins Cand E, polyphenols, flavonoids, and carotenoids, found in fruits, vegetables, legumes, whole grains, and nuts. These nutrients help neutralize free radicals, reducing oxidative damage to cells and tissues. The affordability of a plant-based diet is enhanced by focusing on locally available, minimally processed foods such as lentils, beans, oats, and seasonal produce, which are often more cost-effective than animal-based products. The emphasis on fiber-rich foods not only supports antioxidant defense but also promotes gut health, aiding in the reduction of inflammation and oxidative stress. Moreover, the avoidance of expensive processed vegan products and supplements further enhances the economic feasibility of this diet. Research indicates that diets abundant in plant-based nutrients may reduce oxidative stress markers, contributing to overall health and longevity. The cost-effectiveness and accessibility of plant- based foods make this dietary approach a viable solution for reducing oxidative stress, particularly in populations at risk of chronic disease. This sustainable, nutrient-dense diet supports both individual health and environmental sustainability. In this review, we will focus how cost-effective and stable plant-based diets can prevent the oxidative stress in body.

Keywords: Antioxidants, meal planning, oxidative stress, phytochemicals, plant-based diet

1. Introduction

The emergence of human diseases is significantly influenced by oxidative stress. Reactive oxygen species (ROS) have a role in the

development, differentiation, progression, and death of cells. These species include hydrogen peroxide, hypochlorite, superoxide anion, singlet oxygen, and lipid peroxides. Membrane lipids, nucleic acids, proteins, enzymes, andothertiny molecules can all react with them. Reduced levels of reactive oxygen species (ROS) are essential for intracellular signaling and pathogen defense, whereas increased ROS levels are link to a variety of human illnesses, including as diabetes, cancer, atherosclerosis, ischemia, immune system dysfunction, and endocrine disorders (Rajendran *et al*. 2014). The mitochondrial respiratory chain is one of the primary source of cellular ROS, which are created during the synthesis of ATP in normal oxygen metabolism. ROS are therefore commonly regarded as by products if the energy source for cellular functions. Over production of RO can cause oxidative damage to DNA, lipids, proteins, and other macromolecules. This can accelerate aging and the onset of a number of diseases, including cancer, respiratory disorders, cardiovascular diseases, neurological disorders, and digestive disorders. Cell death has been connected to oxidative stress (OS), has been connected to oxidative stress (Yang *et* al 2020). The body uses multiple methods to offset the negative effects of OS. Whether produced internally are obtained externally, antioxidants are essential for eliminating ROS and reducing oxidative stress (Gilgun-Sherki *et al*. 2001).

The Antioxidant Hypothesis states that having more reducing agents, or antioxidants, will also reduce the risk of developing chronic illnesses because they may prevent oxidative damage. Dietary ingredients are likely to be advantageous because of their capacity to act as chemical antioxidants, which will fortify cellular defence and protect cells components from oxidative harm. (Astley *et* al 2003). To combat OS, a variety of phytochemicals with antioxidant qualities are required. Phytochemicals are separated into main and secondary metabolites based on their roles in plant metabolism. Proteins, carbohydrates, amino acids, lipids, purines, and nucleic acid pyrimidines are examples of primary metabolites. Vegetative life depends on them. Conversely, secondary metabolites are the leftover chemicals generated by metabolic pathways that deviate from the primary metabolic pathways. (Reshi *et al*. 2023).

Consuming little processed food, oil, and animal products (dairy and eggs) as possible is the aim of a nutrient-dense plant-based diet. It encourages a diet rich in fruits, vegetables (raw or cooked), beans, peas, lentils, soybeans, seeds, and nuts (in lower amounts) and is frequently low in fat. (Blaney *et al*. 2011). Although often regarded as non-nutritive, fruits and vegetables are thought to contain certain phytochemicals that may help reduce the symptoms of chronic illnesses. The chemical components

antiviral, antifungal, and antibiotic qualities have been discovered; they serve as a defense mechanism against pathogens and protect plants from UV light-induced severe leaf damage (Bourgaud *et al.* 2001). Pharmaceuticals and synthetic antioxidants are commonly used to combat oxidative stress, but they may not be sustainable due to cost, limited efficacy, and potential side effects. As an alternative, plant-based diets rich in natural antioxidants offer a viable and cost-effective solution for mitigating oxidative damage.

By consuming foods high in antioxidants, this review seeks to assess how well plant-based diets may reduce oxidative stress. In order to highlight how dietary methods can be used as preventive or supplementary treatments in controlling disorders associated to oxidative stress, the cost- effectiveness of the dietary strategy in comparison to pharmaceutical therapies will be evaluated.

2. Antioxidants in Plant-based Diets and their Role in Reducing Oxidative Stress

The most significant compounds that aid in inhibiting the oxidation process are antioxidants. Since oxidation is partially described as a chemical process that might produce free radicals, chain reactions may develop, which could seriously harm an organism's cells (Mititelu *et al*. 2020). Foods high in naturally occurring antioxidants, including fruits, vegetables, and other plant-based diets, are beneficial for preventing disease (Quispe *et al*. 2022).

2.1 Polyphenols as Antioxidants

Polyphenols limit the autocatalytic degradation of the enzyme or hemeprotein to non-active forms by aiding in the catalytic breakdown of hydrogen peroxide and hydroperoxides to non-radical forms. The primary function of polyphenols is that of chain-breaking free radicals. The easiest way to assess the antioxidant activity of phenolic compounds is to figure out the rate constants for H-atom abstraction or electron donation from the phenolic groups by a radical like the peroxyl radical. Because they are metal chelators, polyphenols have the ability to function as pro-or antioxidants. Since polyphenols form hydrogen bonds, they interact more readily with a wide range of molecules, particularly proteins, which include enzymes, substrates, receptors, and several others active molecules that alters the activity. Singlet oxygen is effectively quenched by polyphenols (Wright *et* al 2001). Berries, grapes, and dark leafy greens are some of the most polyphenol-rich foods.

2.2 Flavonoids as Antioxidants

Flavonoids, known for their neuroprotective properties under a variety of pathophysiological conditions, may promote synaptogenesis and neurogenesis by preventing oxidative stress and inflammation of the nervous system (Cichon *et al*. 2020). Flavonoids, a subclass of polyphenols, are present in foods like apples, onions, tea, and citrus fruits. Flavonoids exhibit strong free-radical scavenging abilities, which help reduce cellular damage caused by oxidative stress.

2.3 Vitamins and Minerals as Antioxidants

Oranges, strawberries and bell peppers are just a few of the fruits and vegetables that contain the water-soluble vitamin C. It is a strong antioxidant that helps shield cells from oxidative harm. Moreover, vitamin C supports the production of collagen, the immune system, and the absorption of iron. Vitamin E is a fat-soluble vitamin that can be found in leafy greens, almonds, and seeds. It functions as a lipid-soluble antioxidant to shield cell membranes from oxidative damage (Madhab *et* al 2023).

Antioxidant	Plant source	Role in body
Vitamin C	Citrus fruits (oranges, lemons), berries (strawberries, blueberries), bell peppers, broccoli	Neutralizes free radicals, regenerates other antioxidants, supports immune function, promotes skin health
Vitamin E	Nuts (almonds, hazelnuts), seeds, spinach, avocados, vegetable oils (sunflower, olive)	Protects cell membranes from oxidative damage, supports skin health, has anti-inflammatory properties
Beta-Carotene	Carrots, sweet potatoes, butternut squash, dark leafy greens (kale, spinach)	Converts to vitamin A for-vision health, supports immune function, protects against skin damage
Flavonoids	Apples, berries, onions, dark chocolate, tea (green and black)	Reduces oxidative stress, has anti-inflammatory effects, supports cardiovascular health

3. Phytochemicals Derived from Plants

Fruits, vegetables, cereal grains, edible macro fungi, microalgae, and medicinal plants are rich sources of antioxidant phytochemicals (Deng *et* al 2012). Phytochemicals that act as antioxidants are abundant in common fruits such berries, grapes, Chinese dates, pomegranates, guava, sweet potatoes, persimmons, Chinese wampee, and plums (Zhang *et al*. 2011).

Additionally, fruit wastes (seed and peel) include high concentrations of antioxidant phytochemicals such as epicatechin, gallic acid, kaempferol,

catechin, and cyanidin 3-glucoside. It has been found that some plants contain significant levels of antioxidant capability and total phenolic content. Chinese toon bud, loosestrife, penile leaf, cowpea, caraway, lotus root, sweet potato leaf, green soybean, pepper leaf, ginseng leaf, broccoli, and chives are some of these veggies. (Deng *et al*. 2013).

3.1 Polyphenolics and Carotenoids

Polyphenols and carotenoids are the two main families of antioxidant phytochemicals that primarily give foods and plants their antioxidant properties. For instance, β-carotene, quercetin, myricetin, and kaempferol are the main antioxidant phytochemicals found in Cape gooseberries (Zhang *et al*. 2013). The primary antioxidant compounds among the phytochemicals present in strawberries are ellagitannins and anthocyanins. (Giampieri *et al*. 2011).

The five classes of dietary polyphenols include tannins, coumarins, stilbenes, phenolic acids, and flavonoids. Anthocyanidins, isoflavonoids, flavonols, flavones, flavanols, and flavanones are further classifications for flavonoids. There may be a direct correlation between the total phenolic content and the total antioxidant activity in phytochemical extracts of various fruits. Fruits with higher levels of total phenolic content have more potent antioxidant properties (Yang *et al*. 2009).

The phytochemicals known as carotenoids are what give food its yellow, orange, and red hues. Fruits and vegetables are the primary sources of carotenoids in the human diet, with α-carotene, β-carotene, lycopene, lutein, and cryptoxanthin being the principal carotenoids found in diets and the human body. For instance, the red color of tomatoes is attributed to their high lycopene content.

3.2 Flavonoids

Moreover, the ability of the flavonoids isolated from Euterpe oleracea pulp to absorb radicals indicates that they have strong antioxidant activity. Natural polyphenols, which can scavenge radicals by replacing hydroxyl groups in the aromatic rings of phenolic compounds, are the most common antioxidants found in human diets. (Rokayya *et al*. 2013).

4. Mechanisms of Action of Phytochemicals in Oxidative Stress Mitigation

Phytochemicals exert their antioxidant effects through several mechanisms. These compounds play a crucial role in protecting cells from damage caused by reactive oxygen species (ROS) and contribute to the overall health benefits of a plant-based diet.

4.1 Defense Against free Radicals

The antioxidant system is the most significant defence system category because it directly combats free radicals by scavenging, converting, or degrading them into less reactive forms. There are two types of antioxidants sindhis defence mechanism: enzymatic and nonenzymatic. (Engwa *et al*. 2018).

4.2 Metal Chelation

Because they are rich in phyto antioxidants and strong defences against heavy metal toxicity, several edible plants and foods are widely known for their antioxidant behaviour. Tocopherols, lutein, lycopene, carotenoids, phenolic compounds, flavonoids and isoflavones are some examples of these plant-based antioxidants. They can scavenge free radicals, inhibit oxidation, and function as chelators and reductants. (Ceramella *et al*. 2024)

4.3 Anti-inflammatory Effects

Therapeutic possibilities for a variety of inflammatory illnesses may include phytochemicals that demonstrate anti-inflammatory mechanisms that lessen chronic inflammation (Zhu *et al*. 2018).

4.4 Inhibition of ROS-Producing Enzymes

Some of the layers of antioxidant defence that have been suggested as preventive measures against nucleic acid oxidation include the oxidative DNA lesion-causing DNA repair system, chelation of metals that catalyse ROS formation, inducible enzymatic removal of ROS, and nonenzymatic removal of ROS by scavenger molecules. Cellular molecules that can scavenge radicals are the first line of defence in controlling ROS levels. (Sies, H. *et al*. 1993).

5. Role of Antioxidants in the Treatment of Diseases

Antioxidants in plant-based foods play a vital role in protecting human health by neutralizing harmful free radicals, reducing oxidative stress, and preventing damage to cells and tissues. This protective action can help lower the risk of many chronic diseases, such as cardiovascular disease, cancer, neurodegenerative disorders, and diabetes.

5.1 Prevention of Cardiovascular Diseases

Flavonoids have been associated with lower incidence or mortality from CVD in adults in Europe and the US, according to epidemiological research. One of the primary pathogenic elements of CVD is the overproduction of oxidants, which has complex etiology. Endothelial cell damage and negative

vasodilator effects might result from oxidative damage. Antioxidant polyphenols have been demonstrated to have the ability to alter molecular processes that lead to improvement in endothelial function, and as a result, they are crucial in the prevention of cardiovascular disease. *In vitro*, flavonoids from pulp from *Euterpe oleracea* shown atheroprotective properties (Costa *et al*. 2013).

Because polyphenols also have other physiological benefits, such lowering blood pressure and inflammation, they may help shield the cardiovascular they may help shield the cardiovascular harm in addition to oxidative stress (Prahalathan *et al*. 2012).

5.2 Cancer Prevention

Many plant-based foods include antioxidant phytochemicals, which are believed to contribute to their potential to prevent cancer. Numerous studies have attempted to identify particular phytochemicals like carotenoids, flavonoids, and phenolic acids as well as antioxidants like vitamins C and E. (Wang *et al*. 2011).

Example can be cited that of curcumin's significant sensitivity to peroxyl radicals, along with its proven anti-inflammatory and antioxidant properties, make it a free radical scavenger. In this case, the curcumin extract from turmeric may act as a chemotherapeutic agent and offer protection against intestinal, skin, colon, and oral cancers. (Rozzo *et al*. 2014). Oxidative stress activates a variety of transcription factors, including NF-κB, AP-1, p53 (the gene encoding a protein that regulates the cell cycle and thus acts as a tumour suppressor), hypoxia-inducible factor1-alpha (HIF-1α), peroxisome proliferator-activated receptor gamma (PPAR-γ), β-catenin/Wnt (which regulates stem cell pluripotency and cell fate decisions during development), and Nrf2 (basic leucine zipper (bZIP) protein that regulates the expression of antioxidant proteins that protect damage caused by oxidation and inflammation (Reuter *et al*. 2010).

- **Carotenoids** (in carrots, sweet potatoes, and tomatoes).
- **Sulforaphane** (in broccoli, Brussels sprouts, and kale).
- **Polyphenols** (in green tea, dark chocolate, and berries).
- **Resveratrol** (in grapes, berries, and peanuts).

5.3 Diabetes Management and Prevention

A plant-based, whole-foods diet that is high in fiber often consists of whole grains, legumes, fruits, vegetables, and nuts. It has been discovered

that each of these components protects against diabetes (Spiegelman *et al*. 2010). Studies on the use of high-carb, low-fat diet to treat hyperglycemia date back to the 1950s, and they show that a diet high in vegetables is an excellent way to treat diabetes. The first significant randomized clinical trial was conducted by Barnard *et al*. on diabetes patients receiving only plant-based (vegan) diet therapy. The study compared the vegan diet to a conventional diet based on the 2003 American Diabetes Association (ADA) guidelines (Jenkins *et al*. 2006).

One potentially crippling microvascular consequence of diabetes is diabetic neuropathy. Plant-based diets have been demonstrated to reduce diabetic neuropathic pain in at least two minor studies. In a 25-day residential lifestyle program with plant-based meals, 81% of participants showed a remarkable remission of burning neuropathy; those who followed the diet after returning home experienced a sustained response. (Crane *et* al 1994). A recent randomized controlled pilot trial also showed that a plant-based diet can effectively treat diabetic neuropathy: after 20 weeks on a plant-based diet, individuals with painful diabetic neuropathy who were living in the community showed substantial improvement in pain levels when compared to those on a control diet (Bunner *et al*. 2015). Eliminating animal products, refined grains, and added sugar from whole-foods, plant-based diet promotes insulin sensitivity by helping people lose extra weight and maintain a healthy body weight. But as previously mentioned, metabolic and epidemiologic research supports the idea that plant-based diets improve insulin resistance even in the absence of weight loss and/or after controlling for body weight statistically (Tobias *et al*. 2014).

6. Cost-Effectiveness of Plant-based Diets

6.1 Affordability of Plant-based Food

Individual, cultural, societal, and environmental factors all play a part in food choices, but affordability is a major one. Unhealthy eating patterns are more prevalent in low socioeconomic level subcategories than in affluent ones. (Giskes *et al*. 2010).

The cost of the diet change can be calculate number of ways. While some studies evaluate the costs of various quantities of v "healthier" or "less healthy" (e.g., more vs. less healthy grains), other studies examine the price differences of "healthier" versus "less healthy" total dietary patterns. Additionally, food prices can be estimated using the cost of unit portion each day (cost/average portion) or per calorie (cost/kcal). To make matters more complicated, the affordability of different food comparison is determined by

the link between household food budgets and local food pricing. The cost of the diet change can be calculated in a number of ways. Some studies compare the costs of "healthier" and "less healthy" general dietary options. (Darmon *et al*. 2008). In order to ensure that everyone has access to a nutritious, environmentally sustainable and reasonably priced diet, agricultural food systems must develop low-cost, nutrient-dense and environmentally friendly food products.

6.2 Comparison with Pharmaceutical Antioxidants

A plant-based diet can be cost-effective, especially when centered on affordable whole foods such as legumes, grains, and seasonal fruits and vegetables. Overtime, a plant-based diet may also lower healthcare costs due to its disease-preventive properties**.**

6.3 Long-Term Health and Economic Benefits

Plant-based diets are generally considered acceptable in law-and low-middle income countries because of cultural, financial, ethical and/or religious reasons, while in high-income countries people often consider meat an important part of a meal and the attitude towards vegetarian/vegan diets are to be inconvenient, difficult to prepare and less enjoyable than omnivorous diets and also give long-term health (Moreno *et al*. 2022).

Conclusion and Future Aspects

A plant-based diet is an effective and cost- efficient strategy for mitigating oxidative stress and preventing oxidative stress-related diseases. Rich in natural antioxidants, plant-based foods provide a safer, more sustainable, and more accessible alternative to pharmaceutical antioxidants. Additionally, plant-based diets offer long-term health benefits and economic savings by reducing the risk of chronic diseases and lowering healthcare costs. Increased awareness and accessibility of plant-based foods can play a critical role in promoting public health and well-being.

Further research is needed to optimize plant-based dietary patterns for specific populations, focusing on maximizing antioxidant intake while ensuring overall nutritional adequacy. Public health strategies that promote the adoption of cost-effective plant-based diets could have a substantial impact on reducing the global burden of oxidative stress-related diseases.

Conflict of Interest

The authors have no conflict of interest.

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Chapter - 3
Microbial Remediation of Waterbody that Polluted by Heavy Metal Releases from Textile Companies

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Chapter - 3

Microbial Remediation of Waterbody that Polluted by Heavy Metal Releases from Textile Companies

Anuska Laha and Debjit De

Abstract

The primary danger to human health is posed by heavy metals, which have been well investigated and whose effects are routinely assessed by international organisations like the World Health Organization (WHO). Several reports suggest that extreme lead exposure can cause cognitive disorder, such as ADHD, Insomnia in children and kidney diseases in adult. Consumption of heavy metals such as arsenic (As) can cause skin harm, colour change and spot on hands and feet and also may cause skin cancer and affects lungs and kidneys. This is the reason we should find biological remedy which can remove and reduce the amount of heavy metals that release in waterbody from textile companies. A creative and promising technique for removing and decreasing heavy metals from water is bioremediation. In bioremediation of heavy metals, microorganisms are crucial. Genetic engineering can be used to create genetically engineered organisms, which have the potential to produce fewer polycyclic hydrocarbons (PAHs). Several microbial organisms aid in the bioremediation of heavy metals, including *Aspergillus niger*, *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Azotobacter*, *Alcaligenes*, *Phormidium valderianum*, *Ganoderma applanatum*, *Methylosinus*, *Rhodococcus*, *Mycobacterium*, *Stereum hirsutum*, *Nocardia*, *Methanogens*, and *Flavobacterium*. We will go over several facets of microbial remediation in this study, along with some of its limitations.

Keywords: Heavy metals, environmental toxicity, bioremediation, water pollution, microbial remediation

Introduction

Pollution from heavy metals is one of the worst effects of industrialisation. Heavy metals are poisonous, non-biodegradable, xenobiotic metals that are stable. When heavy metals enter the body by a pollutant

(polluted air, soil, or water), they stay there for a longer amount of time [1]. Worldwide, heavy metal contamination from textile effluent has grown to be a serious environmental issue. The bathochromic shift caused by the incorporation of metal ions in dye molecules results in deeper but dull hues, which enhances coloration. The textile industry uses a lot of water, and because of this, during the dyeing process all these chemicals cannot completely combine with fibres. As a result, a large amount of wastewater containing metal-containing dyes and heavy metal ions such as Cr(VI), Pb(II), Cd(II), and Zn(II) is left behind. These high levels of chemical concentrations in effluents have a deleterious effect on human health, the environment, and natural water supplies [2].

High content of heavy metals can disrupt body immune system. Several reports suggest that exposure to heavy metals may cause any neurological disorder and diseases such as ADHD, arthritis, insomnia, autoimmune diseases. A widespread childhood behavioural problem is ADHD. It is the most prevalent neurogenetic behaviour in children and is caused by a variety of causes, including exposure to heavy metals like lead and interactions between genes and environment. There is a connection between ADHD and heavy metal toxicity [3]. Millions of people are affected by the most prevalent type of sleep disorder: insomnia. One of the studies found that high lead levels in blood may cause sleep problems from the age 3 to 5 in early adolescence [4]. A metal that may cause osteoarthritis is leads which has effect on bones as well as cartilage [5].

We need to develop cost-effective ways to reduce the amount of heavy metals in the water sources in heavy metal pollution has negative impact on both the environment and human health. One of the more environmentally friendly, practical, safer, and clean technologies for cleaning up polluted environments is bioremediation. The technique of using biological components to eliminate harmful waste from the environment is now referred to as "bioremediation". The most efficient technique for managing pollution and assisting in the removal of heavy metals from soil and water is bioremediation. Numerous biological organisms, including fungi, bacteria, algae, and higher plants, are employed in the bioremediation process as key instruments to reduce the concentration of heavy metals in the environment. Microbes are highly helpful in cleaning up environments contaminated with heavy metals [6].

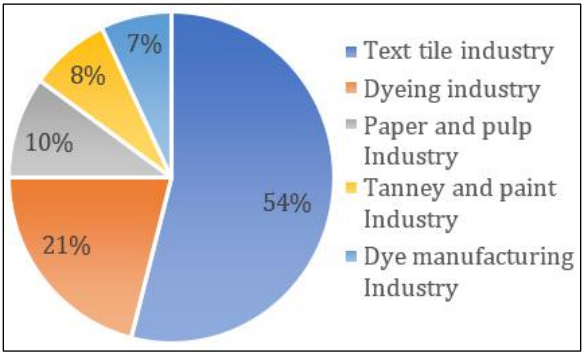

Fig 1: Comparison of the wastewater discharged by several industries with eyes [7]

Heavy Metal Pollution-Textile Industry

Heavy metal residues, bacteria, and chemicals that are highly concentrated can have a range of detrimental impact on health when present in textile effluent. Heavy metals may occasionally be naturally present in the fibre structure. Furthermore, primary colours including metal complex acid dyes, chromic acid dyes, reactive metal-containing dyes, and direct metal-containing dyes can contain heavy metals. Additionally, fibres can be chemically treated to strengthen their resistance to light and moisture, and metals can be used as oxidant catalyst in fabric bleaching methods to acquire heavy metals. Heavy metal pollution from industrial waste isa significant environmental issue on global scale. Heavy metals are family of metals and metalloids with atomic weights ranging from 63.5 to 200.6, atomic density greater than 4000 kg. m-3, antigravity greater than 5.0. Because of their high solubility in aquatic environments, the heavy metals lead, cadmium, copper, arsenic, nickel, chromium, zinc, and mercury have been classified as dangerous. Moreover, heavy metals can be harmful to living beings even at low concentrations and can be absorbed by them [8].

In order to produce textile fabric of the proper quality, a wide variety of primary and secondary chemicals are used during the textile processing process. The effluent from textile wastewater includes a variety of chemicals that are used at different stages of the textile manufacturing process, such as soils, waste fabrics saturated in oil and grease, hydrocarbon and chlorinated solvents, oxygenated solvents, and pigments and dyes. The four primary industries that produce wastewater textile share pre-treatment, dyeing, printing, and finishing. In the production and finishing of textiles, a wide variety of heavy metals and salts are used; these are briefly displayed in Tables 1 and 2.

Table 1: Illustrate Metal Contamination from Different Dyeing Methods [10]

Different dyes	Metal-contaminated dyeing technique
Vat dyes	Due to the technique of production, vat dyes contain heavy metals (Cu, Fe, Mn, Ba, and Pb).
Reactive dyes	Heavy metals can be found in chromophores as essential components or as byproducts of the synthesis process. The majority of colours created using phthalocyanine reactive dyes are blue and turquoise.
Chrome dyes	The molecule does not contain the chromium that is present in the final colourant. Rather, it is used as dichromate of chromate salt in the dyeing procedure to facilitate dye fixing when printing paste chromium (III) salts, like chromium acetate, are infrequently utilised.
Metal Complex Dyes	Cr(III) is a necessary component of chromophores, and unfixed dye can lead to metals in the effluent. As an illustration, consider the colours 1:2 cobalt complex, 1:2 chromium complex, 1:1 chromium complex, and 1:2 chrome azo dye.

Table 2: Displays the metal content from several textile processing stages [10]

Textile Processing Stage	Used Chemicals	Origin of the pollutant	Heavy metal derivative
Fibre production	Lead and Lead Acetate	Dyeing auxiliary/Mordant	Pb(II)
	Cadmium	Pigment stage	
			Cd(II)
	Zinc Salt	Catalyst	
			Zn(II)
	Mercury	Metal impurities	
			Hg(II)
	Tin	Metal impurities	
			Sn(II)
	Chromium Compound	Oxidizing agent	
			Cr(VI)
Colouring, dyeing and printing	Lead Acetate	Oxidising/reducing agent	
			Pb(II)
	Copper Acetate	Oxidising/reducing agent	
		Oxidising/reducing agent	Cu(II)
	Copper Nitrate		
		Fixing Agent	
			Cu(II)
	Sodium dichromate		

		Fixing agent	
	Potassium Dichromate		Cr(VI)
	Chromium compound	Fixation(dye)	
			Cr(VI)
	Zinc compound		
		Fixation	
	Zinc		Cr(VI)
		Fixation and biocide	
			Zn(II)
			Zn(II)

The World Health Organization (WHO) and the Environmental Protection Agency (EPA)have set a maximum acceptable discharge level of 6.0×10−3 mg L−1.70 for wastewater and consumable water. A major environmental problems resulting from air intake and anthropogenic activity groundwater contamination [11, 12, 13]. The main cause of heavy metal pollution is industrial effluent. The production of batteries, textile dyes, hydrometallurgy, tanning, smelting of ores, metal plating, motor, electrochemical, house paint, fuel additives and plumbing pipes are among the industries that generate wastewater [14]. These industries transport heavy metals such as Cr (III,VI), As(III,V), Cd(II), Pb(II), Cu(II), Zn(II), and Hg(II) that are detrimental to the environment and living things. Of these heavy metals, lead (Pb) is one of the toxic heavy metals that can cause neurological disorders, high blood pressure, digestive issues, kidney dysfunction, muscle and joint pain, and problems with fertility in adults. Lead exposure can also cause delays in the development of the brain in infants and young children [9].

Bioremediation and its Process

Three essential components are needed for bioremediation. These three essential components are food, nourishment, and microorganisms. The bioremediation triangle is made up of these three essential components. Everywhere on Earth has microorganisms, with the exception of active volcanoes. Ineffective bioremediation is typically caused by a shortage of food and nutrients. Water and dirt provide food for microorganisms. Two advantageous purposes are served by the contamination for the bacterium. The pollution provides a source of carbon, which is necessary for growth, to start with. Second, the microbe can break chemical bonds and extract electrons from the contaminants in order to produce energy. We refer to this kind of reaction as an oxidation and reduction process. Electron acceptors

are substances that gain electrons; on the other hand, oxidized contaminants are those that lose electrons. To make more cells, some electrons and carbon are mixed with the energy from the electron transfer. Microbes can regularly use nitrate, sulphate, iron, and CO^2, although oxygen is the most common electron acceptor they employ [6]. The majority of bioremediation systems operate in an aerobic environment, although in an anaerobic environment [15], microbial organisms maybe able to break down compounds that would otherwise be resistant.

Nutrient chains, which are essential to maintaining the biological balance of life on Earth, depend heavily on microorganisms. They are required for the completion of several nutritional and geochemical cycles, including the sulphur, phosphorus, nitrogen, and carbon cycles [16-19]. The process of extracting contaminated material to be treated elsewhere is called bioremediation. Technologies for bioremediation include landfarming, bioreactor composting, biostimulation, phytoremediation, bioventing, and bioleaching [20-25]. It has been found that microorganisms that are capable of biodegrading in diverse environments are actively involved in microbial consortium. *Xanthobacter*, *Penicillium*, *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Mycobacterium*, and *Nitrosomonas* are some of these microorganisms [26]. Microbes employed in bioremediation include aerobic and anaerobic bacteria.

Pseudomonas, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* are examples of **aerobic bacteria** with degradative capacity. The breakdown of pesticides and hydrocarbons, such as alkanes and polyaromatic compounds, has repeatedly been demonstrated by these bacteria. Many bacteria rely solely on contaminants for carbon and energy [6].

Anaerobic are less frequently used bacteria than aerobic bacteria. River sediments containing polychlorinated biphenyls can be bioremediated by aerobic bacteria, and solvents such as trichloroethylene and chloroform can be dechlorinated [6].

Microbial Remediation of Textile Waste Water

Since they can maintain metal toxicity in a variety of ways, microorganisms are essential biological agents in the cleanup of environments contaminated by heavy metals. A lot of research has been done on the use of microbes to sequester, precipitate, or alter the oxidation state of large amounts of heavy metals [27, 28]. Rather than using one single type of strain culture, a consortia of bacterial strains can be used for successful bioremediation of heavy metals [29]. Certain process employed by

microorganisms enable the microbial remediation of textile waste water. Microbial bioremediation employs the subsequent mechanisms:

1) Cell wall components or intracellular metal-binding proteins and peptides, such as phytochelatins and metallothioneins (MT), as well as some substances like bacterial siderophores, which are primarily catecholates, can sequester the harmful metal.
2) Metabolic pathways being changes to prevent the absorption of metals.
3) Enzymes convert metals into forms that are safe.
4) Reducing the amount of metal with in cells with accurate efflux mechanism [30].

Siderophores, which are iron-chelating substances produced by bacteria, improve the mobility and decrease the bioavailability of metals, facilitating their removal from waste water [29].

Scientists have discovered that certain naturally occurring bacteria, such as *Azotobacter chroococcum*, *Paenibacillus jamilae* and Sporosarcina pasteurii, are able to remediate lead contamination. Through adsorption and accumulation, bacteria mostly remove lead (II) from the environment. Pb(II) can be efficiently absorbed by bacterial biomass, both living and non-living. According to Li *et al*. [31], *Pseudomonas* sp. I3, a *Psychrotrophic bacteria* resistant to lead, had peak Pb(II) biosorption capacities of 42.37 mg/g and 49.48 mg/g, respectively, in conditions involving living and dead biomass [32].

Conclusion

In conclusion, microbial remediation is promising and environmental-friendly way to remove heavy metals from contaminated water. Bacteria use different mechanisms to remove heavy metals, such as bio-absorption, bioaccumulation, biotransformation. However, there have different so many chemical and physical methods that help to remove heavy metals, those processes are expensive. Microbial remediation is actually effective approach to reduce the heavy metal by transforming it into useful minerals and it is also cost-effective method. Microbes are also capable of persisting high concentration of heavy metals. Though, we cannot remove heavy metals completely from environment through microbial remediation but we can use microbes in wastewater treatment and heavy metals management from soil and water. We should find more microbes that are capable of heavy metal removal and work on implication to reduce heavy metal pollution.

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Chapter - 4
Saffron-Does it have any Contribution in Fighting against Cancer?

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Chapter - 4

Saffron-Does it have any Contribution in Fighting against Cancer?

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Abstract

Saffron is the dried stigma of *Crocus sativus L.*, an ancient spice and culinary colouring. It belongs to the Iridaceae family and has been regarded as the most exotic spice marketed globally. Saffron has been used as an herbal remedy for a number of conditions, such as menstrual problems, asthma, liver illnesses, cramping etc. Saffron has two primary natural carotenoids: crocin and crocetin. Crocetin, which is produced when crocin undergoes hydrolysis, is another ingredient found in saffron. Cancer has a significant negative influence life expectancy and quality of life. Natural ingredients found in vegetables, herbs, and spices may help prevent or treat a number of malignancies. Preclinical research has demonstrated the strong anti-tumorous effects of some carotenoids in both vitro and vivo settings, indicating their potential for therapeutic and preventive applications across a range of tissues. The anti- cancer properties of saffron and its compounds have been well studied, and a number of malignancies have been proposed as prospective treatments and preventative measures using saffron as a dietary supplement. The most frequent cancer cures are radiation therapy, chemotherapy, and surgery. However, radiation therapy and chemotherapy have a number of adverse effects, including pain, exhaustion, fertility problems, sexual dysfunction, and mental distress. Surgical tumor removal is also a very intrusive procedure. By preventing the production of nucleic acids, boosting the antioxidant defenses, and triggering apoptosis and differentiation processes, crocetin slows the growth of cancer cells.

Keywords: Ancient spice, carotenoids, cancer, anti-tumorous, dietary supplement

Introduction

In nations that are both industrialized and developing, cancer is the leading cause of death and a major global public health concern due to its

high incidence and fatality rates. Globally, there were 9.6 million cancer-related deaths and 18.1 million new cases of cancer in 2018. Certain malignancies can be avoided by adopting the right dietary, lifestyle, and physical activity adjustments (Shakeri *et al*. 2020). A combination approach, involving the administration of artificial or natural medicines to prevent the development of cancer, could serve as an appropriate method for either treating or preventing cancer (Zhang *et al*. 2013). Many researchers have recently started considering the use of herbal substances for cancer prevention or treatment, due to the negative effects of conventional cancer treatment medicine and drug resistance (Shakeri *et al*. 2020). Natural compounds have strong efficacy and minimal toxicity, making them particularly interesting as chemo-preventive treatments. They've been used for a very long time to treat and prevent a number of illnesses, including cancer (Bolhassani *et al*. 2013). Selective toxicity to cancer cells is exhibited by saffron extract and its primary carotenoids, crocin and crocetin. Their unique qualities, such as their bioavailability and bio-accessibility, are what render them particularly suited for investigation as health- promoting foods with possible therapeutic applications. They additionally happen to be nontoxic and non-mutagenic to normal cells (Arzi *et al*. 2021). Saffron, the member of the Iridaceae family has been originated in the middle east but now these are cultivated in several nations with vastly diverse climates.

Fig 1: Saffron (dried stigmas)

The ideal weather consists of pleasant summers, mild winters, and rainy autumns. Despite reports that saffron can withstand temperatures as low as -18 °C and as high as 40 °C. This spice requires two or three weeks to flower; during that time, the dark red stigmas must be carefully detached from the flowers by hand. To obtain 1 kilogram of pure dried saffron, 150,000 to 200,000 flowers' stigmas must be extracted (Khorasanchi *et al*. 2018). Research has revealed that the anti-cancer properties of crocin are linked to the induction of apoptosis through the enhancement of the Bax/Bcl-2 ratio and the inhibition of the G2/M and G1 stages of the cell cycle. Research has

shown that crocetin inhibits DNA synthesis and RNA polymerase II activity, therefore decreasing proliferation by impacting EGFR, phospho Cdc-2, Cdc25c, and cyclin B1, while accelerating apoptosis through an increased Bax/Bcl-2 ratio (Arzi *et al*. 2021). Saffron has shown anti-metastatic properties against different types of cancers, like breast cancer, cervical cancer, liver cancer, lung cancer, skin cancer, prostate cancer etc.

Discussion

Chemo preventive Effect of Saffron

An inhibitory effect of saffron on cellular DNA and RNA synthesis, an inhibitory effect on free radical chain reactions, and the ability of saffron extract to metabolically convert naturally occurring carotenoids into retinoids were some of the early theories regarding the antitumor activities of saffron and its constituents. Nevertheless, a different study found that the anticancer effect did not need the conversion of carotenoids to vitamin A (Zhang *et al*. 2013). Crocin has been demonstrated in numerous studies to have strong antiproliferative properties on a variety of cell lines generated from the majority of human cancer types. It's interesting to note that crocin had no noticeable effect on the proliferation of non-cancerous cells. Furthermore, crocin has been demonstrated to have an anticancer effect on a number of cancers, including stomach and breast tum ours in animal models, in a number of *in vivo* investigations (Hoshyar *et al*. 2017).

The Commencement of Apoptosis

It is also known as programmed cell death. It’s an important mechanism that helps to reduce cell development of cancerous cells. Saffron's anti-inflammatory and anti-oxidant features have given rise to studies into its anti-cancerous effects. Saffron exhibits cytotoxicity, suppresses cancer cell proliferation, and inhibits resistance to anti-cancer therapies in a concentration-and time-dependent manner (Poursamimi *et al*. 2020). Saffron selectively target cancer cells and induces apoptosis (Shakeri *et al*. 2020). This process involves two key pathways: intrinsic and extrinsic. The intrinsic pathway starts with intracellular signals and alters the inner membrane of mitochondria. These alterations trigger cytochrome C release, the development of an apoptosome complex, and the activation of the caspase cascade. The extrinsic pathway transmits death signals from the cell surface to intracellular signalling pathways via trans-membrane receptor interactions, primarily involving TNF receptors. Intrinsic signals activate the caspase cascade, leading to cell death through apoptosis (Hoshyar *et al*. 2017). Saffron has been shown in tests to stimulate p53-associated apoptosis

in colon cancer cells. Two p53 isogenic cell lines were treated with *C. sativus* L., which inhibited proliferation by generating DNA damage and apoptosis (Naeimi *et al*. 2018). DNA analysis showed apoptosis-related sequences as early as 12 hours post-treatment. Crocin has been shown to induce apoptosis in human gastric cancer cells through enhancing the Bax/Bcl-2 ratio and activating caspases (Hoshyar *et al*. 2017). Crocetin treatment of p53-expressing cancer cells induced cell death through apoptosis-related pathways, including elevated BAX activation (Naeimi *et al*. 2018). In order to facilitate its use in clinical trials, the precise molecular pathways underlying saffron's tumoricidal and anti-cancer properties must be determined (Premkumar *et al*. 2010).

Saffron's anti-metastatic effects: in *in vitro*

Saffron and its compounds have been shown in numerous studies to have anticancer impact on a variety of malignant cells *in-vitro*. The methods used to determine cytotoxicity or the unique characteristics of the cells can be blamed for the reported variations in sensitivity. It has been demonstrated that saffron and its extracts suppressed the synthesis of nucleic acids in tumor cells, but had no significant influence on protein synthesis (Premkumar *et al*. 2010). Moreover, saffron may influence transcription and directly target DNA sequences.

Fig 2: Effects of Crocin & crocin on causing cell death in many cell lines (Hoshyar *et al*. 2013, Moradzadeh *et al*. 2017)

In gastric adenocarcinoma tumor cell line, saffron extract at 20, 40, and 100 µg/ml administered for 24, 48, and 72 hours decreased the expression of self-renewal genes like OCT4, KLF, SOX2, NANOG, and nucleostemin (Shakeri *et al*. 2020). In most cases the mechanism of action area are as follows: induction of apoptosis via caspase pathway, increasing of apoptosis, reduction of DNA and RNA synthesis, inhibition of cell proliferation, induction of cytotoxicity (Moradzadeh *et al*. 2017), antioxidant action, tumor metabolism regulation, immunological modulation and cell-to-cell communication stimulation, radical scavenging, stopping the advancement of the cell cycle, and reduction of matrix metalloproteinase expression (Shakeri *et al*. 2020).

Effects in *In vivo*

The majority of *in-vivo* research has concentrated on the constituents of saffron rather than the plant itself, despite the following studies showing the positive anti- cancer properties of saffron. An extract from saffron has chemoprotective properties because it regulates lipid peroxidation, antioxidant activity, and detoxification. For a period of up to 12 weeks, oral administration of 100 mg/kg body weight (bw) of saffron extract postponed the development and spread of papillomavirus-induced skin cancers in rats. Additionally, the rat model of soft tissue sarcoma showed that the same saffron extract treatment inhibited the growth of new tumors. Albino rat with implanted sarcoma cells live longer when given 200 mg/kg bw of methanolic saffron extracts orally (Shakeri *et al*. 2020). Following crocetin administration, ELISA and fluorescence microscopy were used to evaluate the levels of plasma MDA, PMN, L-1β, TNF-α, and iNOS, respectively. The findings demonstrated that in the MCA mice, crocetin lowers the levels of MDA, PMN, IL-1β, TNF-α, and nitrates. The results presented here demonstrate that crocetin may have anti-inflammatory or chemo preventive effects on cervical cancer (Moradzadeh *et al*. 2017). Other mechanisms were activation of the body's antioxidant defenses, inhibition of tumor growth, suppressing the expression of MMP and urokinase to inhibit metastasis.

Colorectal Cancer

Using three colon cancer cell lines, researchers examined the antiproliferative and anticancer properties of C. sativus extract and crocin. They discovered that both substances markedly slowed the growth cells while having no negative effects on normal cells and additionally demonstrated how dietary crocin (0, 50, 100, and 200 ppm) inhibited the development of colon cancer linked to colitis (Naeimi *et al*. 2018). In an

investigation, it was shown that in SKOV3 and HeLa cells, as well as in HCT-116 colorectal cancer cells, crocetin raises Nrf2 and lowers LDHA. Numerous studies are looking at the stimulatory effects ofsaffrononp53-associated apoptosis in relation to colon cancer. And one scientist had demonstrated that C. sativus L. treatment of two p53 isogenic cell lines decreased proliferation by inducing apoptosis and DNA damage. After noting a complete halt in the G2/M phase in HCT116 p53 wild-type cells and a delay in the S/G2 phase in HCT116 p53−/− cells, the scientists came to the conclusion that p53 is required for the saffron extract's impact on cell cycle distribution (Moradzadeh *et al*. 2017).

Gastric Cancer

Globally, gastric cancer ranks second in terms of cancer-related mortality for both sexes. The mainstay of treatment for stomach cancer is systemic chemotherapy; unfortunately, less than 30% of patients survive for five years after starting this regimen. The scientists have examined the potential therapeutic benefit of crocetin for gastric cancer using both *in vivo* (chemically produced gastric cancer in rats) and *in vitro* (gastric adenocarcinoma [AGS] cells). The *in vitro* findings revealed that crocetin caused apoptosis in AGS cells, and the *in vivo* investigation showed that crocetin at doses of 50 to 100 mg/kg protected against tissue degradation generated by 1-methyl-3-nitro-1-nitrosoguanidine (Naeimi *et al*. 2018). Crocin stops AGS and HGC-27, the two types of gastric cancer cells, from migrating and invading. Furthermore, EMT is inhibited by crocin-treated gastric cancer cells by upregulating the expression of E-cadherin mRNA and protein and downregulating the expression of Snail and N-cadherin mRNA and protein. Krueppel-like factor 5 (KLF5) and Hypoxia Inducible-Factor 1A(HIF-1a) have been shown to be expressed more human gastric cancer tissues removed surgically from individuals with gastric cancer, as well as in AGS and HGC-27 cells (in comparison to normal cells and tissue). Crocin had the power to significantly lower the high expressions. A transcription factor called KLF5 is known to encourage certain cancers, including stomach cancer (Arzi *et al*. 2021).

Breast Cancer

Analogs of crocetin that have been isolated from various Crocus species stop the growth of breast cancer cells. It has been determined that crocetin, in a manner that is dose-dependent, can inhibit the invasion of MDA-MB-231, a kind of metastatic breast cancer cell. Crocetin (1 and 10 lM) reduces pro-MMP2 and MMP2 expressions, according to western blot experiments

(Arzi *et al*. 2021). The findings demonstrated that crocetin greatly reduces MMP expression, which in turn prevents cell invasion as well as proliferation. Another study showed that SE (200-2000μg/ml) enhance Bax protein expression MCF-7 cells and apoptosis via a caspase-dependent route. Another group used MTT and PI assays to measure the cytotoxicity and apoptosis of crocetin PLGA nanoparticles (100-800 μM) vs doxorubicin (DOX) nanoparticles (0.5-10 μM) on the MCF-7 cell line (Moradzadeh *et al*. 2017).

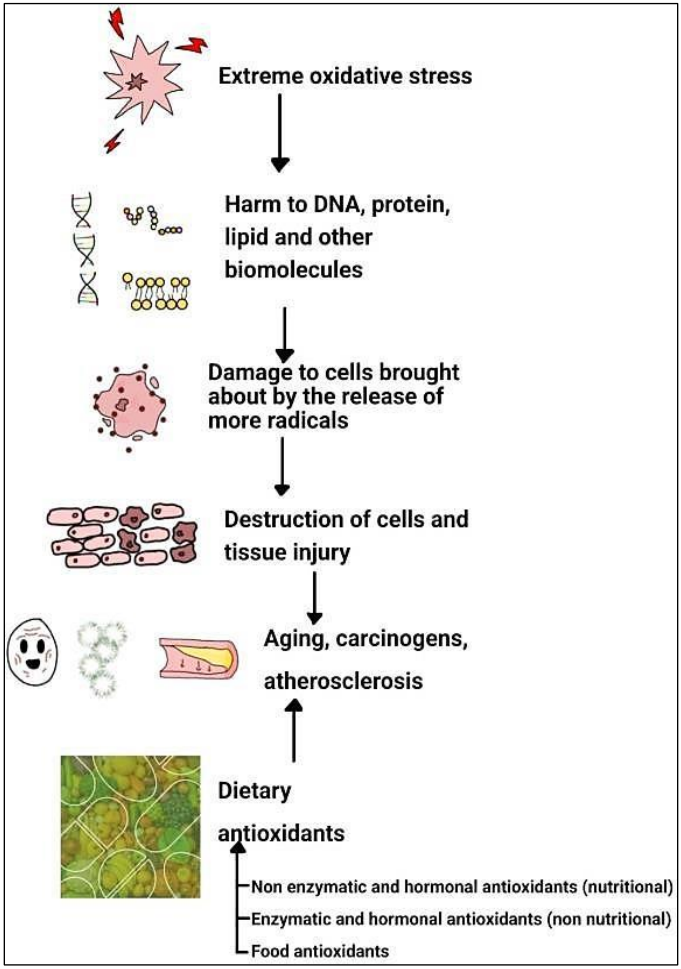

Fig 3: Dietary antioxidants: the antioxidants decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen on normal physiological function in humans. They delay or prevent the oxidation of substrate (Bolhassani *et al*. 2013)

Conclusion

A decreased incidence of sickness and mortality is correlated with a higher dietary consumption of antioxidants, according to epidemiological studies. Saffron is a naturally occurring plant with anti-tumor and anti-carcinogenic properties. Its constituents, particularly its carotenoids, do not have a deleterious effect on non-malignant cells. The purpose of this study was to determine whether saffron may be used as a natural medicine, namely as an anti- cancer medication. Through a variety of mechanisms, such as apoptosis, stopping the progression of the cell cycle, modulating the metabolism of tumor cells, inhibiting RNA and DNA synthesis, DNA fragmentation, LDH activity, antioxidant activity, and a decrease in the expression of self-renewal genes, saffron selective toxicity is applied to cancerous cells. Furthermore, it inhibits the development of tumors by interacting with and eliminating free radicals.

Futures Cope

It is necessary to more research on saffron's extract as it has effect on numerous types of cancer. It doesn't focus on one type. Research using cell and animal models has validated the anti-primary tumor and antimetastatic properties of saffron carotenoids, as well as their nontoxicity on normal cells and tissues. These findings confer considerable therapeutic benefit on saffron and its carotenoids, in conjunction with sufficient bio accessibility and bioavailability. Because of this, it is advised that they be regarded as food constituents with advantageous antimetastatic qualities and/or prospective all-purpose anticancer capabilities. At the present time, it's important to figure out how much carotenoids the general public may consume without being cytotoxic.

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Chapter - 5
The use of Adverse Outcome Pathways in the Safety Evaluation of Food Additives

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Chapter - 5

The use of Adverse Outcome Pathways in the Safety Evaluation of Food Additives

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Abstract

In the past 10 years, adverse outcome pathways useful instruments with wide-ranging application potential have been created in the domains of toxicology and chemical risk assessment. Their usage in the food business has remained largely untapped, despite their will-documented use in the pharmaceutical and cosmetics sectors. To this end, the application of adverseoutcomepathwaysinthesafetyassessmentoffoodadditiveswasrecentlyin vestigated by an expert panel of the International Life Sciences Institute Europe. One of the main goals of the workshop was to discuss the potentials and difficulties of using adverse outcome pathways in the safety assessment of food additives by bringing together representatives from the regulatory, industrial, and academic sectors. A new guidance document from the European Food Safety Authority (EFSA) outlines the parameters for the risk assessment of food additives. This recommendation applies to the risk assessment of food additives and calls for a tiered approach that weighs the need for toxicity data against the associated risks. The methodology was developed to assess the following fundamental domains: carcinogenicity, sub chronic, chronic, and reproductive and developmental toxicity, genotoxicity, and toxicokinetics. The expert group triggered in a depth discussion on potentials and challenges related to the application in the adverse outcome pathway in the food industry. The present manuscript experiment follows and describes the major outcomes and recommendation of the experiment and the expert group characterized the coverage of critical effects relevant in the safety evaluation of food additives by adverse outcome pathways.

Keywords: adverse outcome pathway, toxicokinetics, risk assessment, EFSA, genotoxicity, food additives

Introduction

Toxicology and chemical safety evaluation practices have undergone significant transformation in recent decades. This field is currently evolving

from traditional toxicology, which emphasizes the assessment of apical toxicity endpoints in animal models, to a more human-relevant, animal-free toxicology that is based on molecular insights. The introduction of adverse outcome pathways (AOPs) (Ankley *et al*. 2010) has facilitated this paradigm change, which is gaining global traction. This is especially applicable to the cosmetics industry, as European legislation has enacted a comprehensive prohibition on animal testing several years ago (Desprez *et al*. 2018). In this context, graded animal-free methodologies for the safety assessment of cosmetic components, informed by exposure and mechanistic data, have been established (Dent *et al*. 2018). This has prompted other businesses to investigate the implementation of similar methodologies for chemical risk assessment, thereby adhering to the 3Rs approach (i.e., replacement, reduction, and refinement of animal experimentation) (Russell *et al*. 1959). The food additive sector predominantly depends on animal model testing for hazard identification. The task force on "New Approaches to Chemical Risk Assessment for Foods and Food Ingredients" at the International Life Sciences Institute (ILSI) Europe rigorously scrutinizes and assesses non-animal methodologies for integration into existing chemical risk assessment practices. In 2017, this ILSI Europe task force formed an expert group to investigate the application of AOPs in the safety assessment of food additives. A primary activity of this expert group was the facilitation of a workshop designed to stimulate a comprehensive dialogue regarding the opportunities and obstacles associated with the implementation of AOPs in the food industry (Dent *et al*. 2018). The Scientific Committee on Food assessed food additives and provided recommendations to the European Commission. During the reorganisation of scientific committees, the SCF was rebranded in 1997 as the Scientific Committee on Food (SCF). The SCF remained operational until the establishment of the European Food Safety Authority (EFSA) in 2003. Initially, food additives are assessed by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel), which was divided in 2008 into the Panel on Food Additives and Nutrient Sources Added to Food (ANS Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel). Currently, the EFSA ANS Panel is tasked with the risk assessment of food additives inside the European Union (Hayder *et al*. 2011).

Toxicokinetics

The objective of studies on systemic availability is to determine if the chemical or its metabolites are absorbed from the gastrointestinal tract. In this regard, the physicochemical parameters (e.g., molecular weight,

hydrophilicity, and lipophilicity) of the substance must be evaluated alongside models for bioavailability derived from *in vitro* and *in vivo* investigations. Evidence of minimal absorption, whether derived from experimental investigations or theoretical analysis, may serve as a scientific rationale for foregoing advanced toxicological studies, contingent upon unequivocally negative genotoxicity test results and the absence of toxicity in sub-chronic toxicity assessments. Absorption data for structurally analogous drugs may provide valuable insights. Nevertheless, the requisite sensitivity to ascertain minimal absorption levels will typically need *in vivo* investigations utilising labelled chemicals (Wilson *et al*. 2005). If absorption is not negligible, additional data on absorption, distribution, metabolism, and excretion (ADME), including the identification and quantification of metabolites, is necessary. Fundamental single-dose toxicokinetic parameters, such as the area under the plasma concentration-time curve following oral administration, peak concentration, time to peak concentration, elimination half-life, and bioavailability, must be established through *in vivo* studies in accordance with the organisation for Economic Cooperation and Development (OECD) Technical Guidance (TG) (Gurtler 2010). A variety of dosage levels should be utilised to assess the linearity of kinetic parameters and potential saturation. The impetus for studies would be restricted or sluggish excretion or any other mechanism leading to bioaccumulation. In this scenario, research involving repeated dosages in experimental animals or human kinetic data (Wilson *et al*. 2005) is pertinent.

Genotoxicity

The Tier 1 *in vitro* assessment of genotoxicity should encompass gene mutations, as well as structural and numerical chromosomal abnormalities, as advised by the EFSA Scientific Committee (Wilson *et al*. 2005). According to this suggestion, a bacterial reverse mutation assay and an in vitro mammalian cell micronucleus test are mandated for all food additives. Any inconclusive, ambiguous, or affirmative results obtained from *in vitro* tests at Tier 1 necessitate further investigation. EFSA's guidance document on genotoxicity testing methodologies (Hayder *et al*. 211) indicates that inconclusive and equivocal test results may be elucidated through additional *in vitro* testing, while *in vivo* research may also be required. A favourable outcome in a Tier 1 research necessitates subsequent examination at Tier 2 to see if the dangers manifested *in vivo*. In the event of negative *in vivo* genotoxicity assays, it is essential to establish, through cytotoxicity or kinetic data, that the target tissue was subjected to exposure (Desprez *et al*. 2018).

Critical Considerations and Perspectives

The workshop's results unequivocally underscored the significance of AOPs and its potential application in the safety evaluation of food additives. The workshop highlighted that the application of AOPs in the safety evaluation process is now predominantly at the hazard identification stage. Consequently, AOPs may assist in delineating essential effects, target organs, and the significance of bad outcomes for human risk assessment (Ehrlich *et al*. 2015). The use of AOPs into a testing approach for hazard characterization (i.e., dose-response assessment) of new chemical entities to define points of departure seamless easy. The USE has recently introduced a risk-based tiered testing methodology that integrates computer modelling, high-throughput tests, and AOPs to determine points-of-departure for chemical safety evaluation (Thomas *et al*. 2019).

The existing EFSA guidance paper for food additive evaluation offers a flexible framework that recognises the incorporation of integrated testing techniques and other methodologies to supplement the data mandated by this guidance. In certain instances, such as the assessment of aspartame, physiologically based pharmacokinetic modelling was utilised, and it is anticipated that this modelling will gain significance in the evaluation of additives in the future. In silico quantitative structure-activity relationship (QSAR) approaches mi assistant assessing impurities and metabolites. Specialised research may be employed to examine the mechanism of action, and "Omics" methodologies could be beneficial in this context.

Compliance with Ethical Standards

The opinions articulated and the conclusion drawn in this publication are solely those of the authors and do not necessarily reflect the perspectives of ILSI Europe or its member firms. Experts do not receive compensation for the time dedicated to this activity. Non-industry members of the expert group were provided with financial assistance for travel and accommodation expenses by the “New Approaches to Chemical Risk Assessment for Foods and Food Ingredients” task force to attend meetings regarding the manuscript, along with a modest compensatory payment that could be declined (Russel *et al*. 1959).

Conclusions

Participants in the workshop also came to the conclusion that AOPs could be particularly helpful in determining the potential dangers of food additives by pinpointing the specific organ or system at risk, as well as the relevant model organism or type of unfavorable human effects. In addition,

the workshop attendees agreed that AIOPs can help lessen the need for animal testing when determining which food additives provide an unnecessary risk. In fact, AOPs have the potential to pave the way for anew way of looking at safety assessment, one that relies on human-based mechanistic methods instead of predicting or extrapolating evidence from animal toxicity. Establishing health-based advice values requires hazard characterisation, which in turn requires additional research into how AOPs might aid in the definition of a dose-response curve. This might be achieved by first creating quantitative AOPs that represent the concentration-response curves for the rate-limiting stage in causing the detrimental effect; these curves could then be used to create in vivo dose-response curves using PBK models.

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Chapter - 6
Microbial Contamination of Dairy Products

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Chapter - 6

Microbial Contamination of Dairy Products

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Abstract

The diverse range of dairy products on the market puts pressure on microbiologist, engineers, and technologists to devise the most effective strategies for blocking microbe entry, eliminating those that do make it in along with their enzymes, and stopping the spread and activity of those that make it past processing controls. Spore-forming bacteria, yeasts, molds, heterofermentative lactobacilli and aerobic psychotropic Gram-negative bacteria are among the problematic spoilage microorganisms. The degree of contamination of pasteurized fluid milk products with extracellular hydrolytic enzymes produced by psychrotrophic bacteria is a significant factor in determining how long the goods will last on the shelf. In addition to obvious color or texture changes, broad range of metabolic by- products are produced during the fungal deterioration of dairy products, which results in strange tastes and aromas. In cheeses, gassing faults can be caused by bacteria or spore-forming microorganisms. The application of one or more of these slows the rate at which various dairy products deteriorate. The subsequent procedures: lactic acid fermentation to lower pH; the addition of acids or other permitted preservatives; the introduction of a beneficial microbiome that prevents unwanted bacteria from growing; adding sugar or salt to minimize packing to reduce the amount of available oxygen, eliminating water, measuring the water activity and chilling. The types of bacteria responsible for food deterioration in dairy products vary greatly. Due to the selective effects of production and formulation process, handling, distribution, packaging, and storage.

Keywords: Dairy products, spore-forming bacteria, psychrotrophic Gram-negative bacteria, lactic acid fermentation, formulation processes

Introduction

Dairy products are used in many different ways within and between populations. The malleability of populations is mostly determined by

individual preferences (such as avoiding lactose intolerance, sensitivity to milk, and veganism) and rationality. Population differences are influenced by number of factors, including culture, religion, availability, cost, and genetic diversity in lactose tolerance. Consuming large amounts of dairy products on a daily basis is often associated with good eating routine quality wealthy nations and is positively correlated in ecological terms with tall stature. When unfermented milk is gathered in vessels that have not been adequately clean and maybe contaminated with an inadvertent starter culture from the previous day's milking, it will "turn" relatively quickly in temperate or hot areas. This means that long before humans started milking animals, spoiled milk would have been developed. While it is impossible to pinpoint the exact historical context in which certain lactose-processing bacterial societies were initially employed and consciously dispersed, deposits from ancient potsherds sections-clearly meant for unction as sifters-have been dated back a tremendous amount of time (Salque *et al*. 2013).

Such "trained" fermentative organic entities fulfill the extremely valuable twofold needs of fractional lactose processing and arrangement of β-galactosidase (lactase), which continues to separate lactose after utilization, as illustrated in a follow-up article by Savaiano in this supplement issue. The high quantities of lactose in milk, which would have ordinarily had extremely detrimental effects on the gastrointestinal tract, would have been simpler for early humans to be if they had possessed both the qualities. These were the first move toward letting humans fully utilize their domesticated milk-producing animals (Savaiano *et al*. 2014). The only two items that are specifically made to satisfy every nutrient need of a complex organism that people eat are milk and eggs. It follows that diet plans that include a significant amount of these food types are supplement-rich and, for the most part, demonstrate a proper balance of the essential supplements for healthy growth and development, especially with regard to amino acid corrosive equilibrium. As seen in, dairy products significantly contribute to the daily nutritional requirements of Americans (Rice *et al*. 2013).

Milk and milk products seem to be low cost and high density in cost-versus-nutrient-density matrix and thus they are good additions to a balanced diet in wealthy country settings. All food pyramid and food plate diet plan reference them. However, in low-income environments, the situation is different because milk products are expensive (in comparison to neighbouring wage levels) and viewed as luxury foods. Consequently, milk consumption is skewed toward the urban elite seven herding households will often sell their milk rather than consume it at home.

Furthermore, even though there is no refrigeration, there is a demand for fermented dairy products (Drewnowski *et al*. 2011).

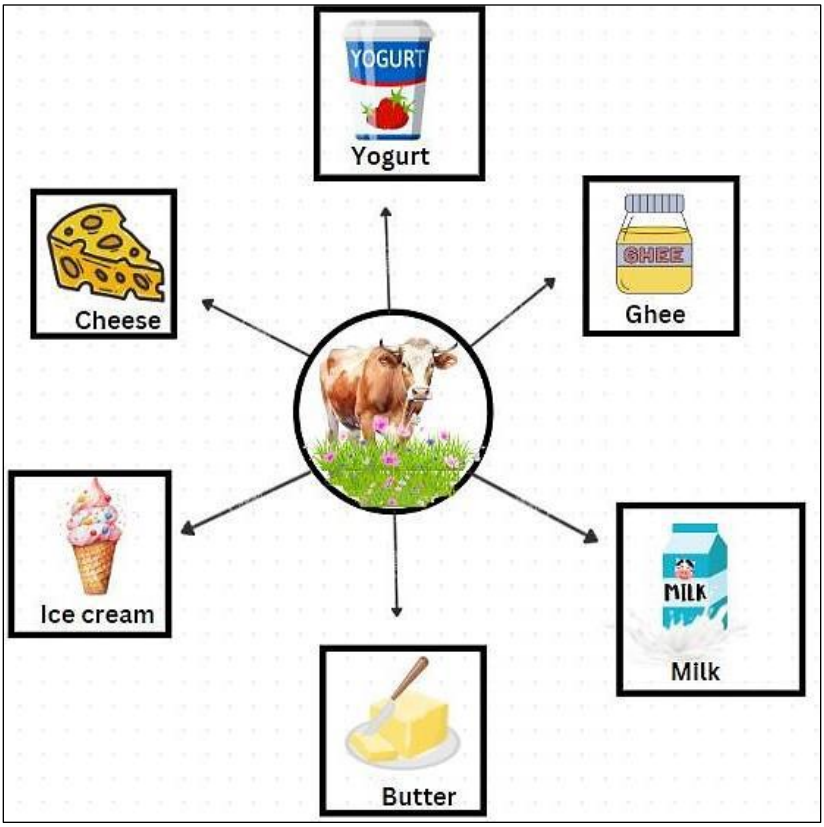

Fig 1: Dairy products

Types of Products made from Milk

Global dairy production is very outstanding industry. A total of 644 million tons of milk were produced worldwide in 2005, of which 541 million tons were from cows. In terms of milk production, the Y came in at 80 million tons (20.9 billion gallons), Russia at 31 million tons, India at 88 million tons, and the European Union at 142 million tons. The total amount of cheddar produced was 8.6 million tons in Western Europe and 4.8 million tons in the US and Europe (Kutzemeier *et al*. 2006). The wide range of milk-based products available globally leads to an equally astounding quantity of spoilage germs. 6% of US consumers said they would eat more dairy products if they were kept fresher for longer, according to a review of their usage (Lempert *et al*. 2004). Products can vary widely in terms of how

quickly they spoil and how long they last. A few examples of the variables that can affect the rate of waste include the handling limitations, pH, moisture content, and temperature of capacity. **Table 1** lists the common dairy products and the common decay microorganisms associated with them in brief.

Table 1: Dairy products and typical types of spoilage microorganisms' microbial activity

Food Products	Microbial Activity of Spoilage Microbes
Pasteurized milk	Microbial Enzymatic, spore formers, and psychograph's degradation
Dried Milk	Enzymatic breakdown by microbes
Raw Milk	Diverse range of microorganisms
Concentrated Milk	Bacteria that form spores and fungi that are oxygen-loving
Cultured Buttermilk, sour cream	Coliforms, yeasts, lactic acid bacteria, and psychograph's
Yogurt, yogurt-based drinks	Yeasts
Butter	Enzymatic degradation and psychograph's
Other fermented dairy foods	Coliforms and fungus
Cottage cheese	Microorganisms, yeasts, molds, coliforms, and psychograph's enzymatic breakdown
Soft, fresh cheeses	Coliforms, fungus, lactic acid bacteria, psychograph's, enzymatic degradation by microbes
Ripened cheeses	Spore-forming bacteria, lactic acid bacteria, fungi, enzymatic breakdown by microbes
Cream cheese, processed cheese	Spore-forming bacteria and fungi

Types of Microorganisms that Cause Spoilage

Lactic Acid Fermenting Bacteria

Lactococcus, which generate encapsulated slime, can multiply to the point that buttermilk and sour cream become extraordinarily viscous and diacetyl can be reduced as well. These products get their yogurt-like flavor from lactococcus growing at 7 °C and generating diacetyl reductase (Hogarty *et al*. 1982). Cheeses that have ripened might produce off odors and gas due to the presence of heterofermentative lactic corrosive microscopic organisms such *lactobacilli* and Leuconostoc. These microorganisms break down lactose to produce lactate, acetic acid, ethanol, and CO_2 at roughly equimolar concentrations (Hutkins *et al*. 2001). They grow more readily than homofermentative starter culture bacteria when ripening takes place at 15 °C as opposed to 8 °C (Cromie *et al*. 1987).

The Heterofermentative bacteria, which are commonly found in cheese, finish the fermentation by generating off-flavors and gas if the home of fermentative lactic acid bacteria in the cheese do not completely metabolize the fermentable sugar (Johnson *et al*. 2001). The remaining galactose in cheese is one type of substrate that many heterofermentative bacteria may utilize to create gas. Also capable of co-metabolizing lactic and citrus acids to create CO_2, are facultative lactobacilli (Fryer *et al*. 1970). Little gas can be produced in cheese through the amino acid breakdown process when using nonstarter culture, propionibacteria, naturally occurring lactobacilli, and *Lactococcus lactis* sub sp. Lactis (Martley *et al*. 1993). When too much gas is created, cheeses can break. By certain strains of *Streptococcus thermophilus* and *Lactobacillus helveticus*, which decarboxylate glutamic corrosive to produce CO_2 and 4 aminobutyric corrosive (Zoon *et al*. 1996).

Coliforms

Coliforms, like psychopaths, have the ability to reduce the diacetyl content of sour cream and buttermilk, giving them yogurt-like flavor (Wang *et al*. 1981). Due to the shortage duration of coliforms in such conditions, the gradual, lactic, corrosive manufacture of cheese starting societies tend to favor the development and production of gas these microscopic organisms. Soft, mold-ripened cheeses have a higher pH during ripening, which promotes the growth of coliform bacteria (Frank *et al*. 2007).

Psychrotrophs

Psychotrophic microorganisms, such as rod-shaped, aerobic, Gram-negative pseudomonads and similar species, make up a substantial fraction of the bacteria found in raw milk. Pseudomonas species typically accounted for 65-70% of the psychrotrophs removed from crude milk (Griffiths *et al*. 1987). The ability of pseudomonas to hydrolyze and use big amounts of lipids and proteins for development at temperatures as low as 3-7 °C makes them remarkable. The Enterobacteriaceae family and its members from the genera Aerococcus, Lactococcus, Micrococcus, and Bacilli are further significant psychrotrophs linked to raw milk. Sour cream and buttermilk's diacetyl content can be decreased by pseudomonas (Wang *et al*. 1981).

Resulting in imbalance in the ratio of diacetyl to acetaldehyde, which gives rise to a "green" or yogurt-like taste. Gram-negative psychrotrophic bacteria can thrive in cottage cheese due to its generally slightly acidic pH (Cousin *et al*. 1982). In contrast, the pH of creamed curd is within the more positive range of 5.0-5.3, while that of cottage cheese curd is between 4.5 and 4.7. Curd usual salt content is inadequate in preventing the growth of

contaminants in bacteria, which is why psychrotrophic bacteria usually have a lower shelf life. Psychrotroph have the ability to reduce the yield and quality of cheddar curd when present in crude milk at cell counts of greater than 106 CFU/ml (Fairbairn *et al*. 1986).

Fungi

Yeasts are able to flourish at the low pH found in cultured goods like buttermilk. Sour cream and the development of yeasty or fermented off-flavors may ensue. Furthermore, yeasts are able to digest the diacetyl included in these compounds (18). Resulting in a yogurt-like flavor.

The level of diacetyl in the product is often reduced when curds are contaminated with the common yeast *Geotrichum candidum*. *Geotrichum candidum* decrease the amount of diacetyl in low-fat cottage cheese 52% to 56% when it was stored for 15 to 19 days at 4 to 7 degrees Celsius (Antinone *et al*. 1993).

Yogurt and mature milk waste is mostly caused by yeast, whose growth is inhibited by the law pH. A reasonable shelf life of yogurt produced using acceptable assembly procedure should be three times am on that 5 °C and should include about the yeast cells. However, yogurts whose starting counts exceed 100 CFU/g may frequently spoil quickly. Giudici, Masini, and Caggia (1996) focused on the role of galactose in the break down of yogurt by yeast and reasoned that galactose, which is the result of lactose hydrolysis by the lactic starter societies, was matured by galactose-positive types of yeasts, such as *Saccharomyces cerevisiae* and *Hansenula anomala*. When yeasts reach 105-106 CFU/g, gassy appearance, yeasty and fermented off-flavors (Fleet *et al*. 1990).

Because of their high nutritional content and low pH, most cheeses are considered healthy. growth of yeast that ruin food. Surface moisture, often rich in peptides, lactic acid, and amino acids, which promotes quick development. Many yeast produce both CO_2 and alcohol. creating cheddar that has a yeasty preference (Horwood *et al*. 1987). When cheese is packed under modified or vacuum conditions, it can bulge because yeast produces a lot of CO_2 (Vivier *et al*. 1994).

Spore-forming bacteria

Spore-forming bacteria usually come from raw milk and end up in processed dairy products. They were rarely seen in amount higher than 5,000/ml prior to pasteurization (Mikolajcik *et al*. 1978). But they can also contaminate milk after processing (Griffiths *et al*. 1990). *Bacillus cereus, B.*

subtilis, B. mycoides, and *B. licheniformis, B. megaterium* are the bacteria that yield spores in dairy products most commonly. In more than 80% of the raw milk samples in one review, psychrotrophic *B. cereus* was not present (Meer *et al*. 1991). In order for a significant portion of the surviving spores to be ready to develop at a positive development temperature, the intensity of purification initiates (heat shock) (Cromie *et al*. 1989). Milk casein is coagulated at a pH that is typically high by proteases that resemble chymosins and are generated by many of these bacilli (Choudhery *et al*. 1970).

Process cheeses may also infrequently have gassy flaws due to *C. butyricum* or other microorganisms. Sporogenesis the *C. certain* spores are not entirely destroyed by the typical heating process cheese production. They could therefore germinate and release gas unless there numbers are low, the pH is below 5.8, the salt content is least 6% of the serum, and the cheese is stored at or below 20 °C (Kosikowski *et al*. 1977). The fermentation byproducts in these cheeses are acetic and butyric acids, hydrogen and carbon dioxide. An overview of the recognized reasons why cheese causes bloating goods are displayed in **Table 2**.

Table 2: Causes of gas in various cheese varieties

Organisms	Cheese-related effects	Defect time
Yeast	Uncooked milk Egyptian Domiati, blue-veined, Camembert and Feta	Quick blowing
Propionibacteria	Sbrinz, an Argentinean	Late blowing
Eubacterium sp.	Cheddar	Late blowing
Heterofermentative Lactobacilli	Saint Paulin, Oka, Cheddar, and Gouda	Late blowing
Clostridium tyrobutyricum	Cheddar, Grana, Emmental, Gouda and Swiss	Late blowing
Coliforms	Filata Cheese and raw milk pasta	Quick blowing
Lactobacillus fermentum	Mozzarella and Provolone	Late blowing

Other Microorganisms

A facultative anaerobe called *Eubacterium* sp. can grow at pH 5.0-5.5 when exposed to 9.5% salt may make cheddar cheese gassy (Myhr *et al*. 1982). A thermoduric *Enterococcus faecalis* sub sp. liquefaciens is responsible for the unusual white-spot defect found in Swiss cheese. The suppression of Lactobacillus fermentum and propionibacteria by this bacterium results in poor eye development. Furthermore, the cheddar lacked flavor as well (Nath *et al*. 1986).

Origins of Microorganisms that Cause Spoilage

Dairy Products Contamination

Cocci forms prefer cheeses with washed curds in particular, so great care should be taken to screen the type of water used in these cycles (Frank *et al*. 1978). To filter the type of water used in these cycles, great care should be given. Because of their presence in the brackish waters, yeasts frequently contaminate salted cheeses in saline solutions (Kaminarides *et al*. 1992). Since most mold species have adapted so successfully to the conditions of cheese production, it might be difficult to completely eliminate them from a manufacturing plant. owing to fungus cheese manufacturing equipment, the air, the curd and whey, and the surroundings of the cheese plant were all linked to the "thread mold" flaw in cheddar cheese (Hocking *et al*. 1992). In a study, *Penicillium commune* was discovered in Danish cheese manufacturing facilities. in the cheese coating and unpacking departments during a seven-years pan (Lund *et al*. 2003). Additionally, ascospores of *B. nivea* and other species that have been shown to be resistant to intense purification-such as *Talaromyces avellaneus*, *Neosartorya fischeri var. spinosa*, and *Eupenicillium brefeldianum* have been found in raw milk (Pitt *et al*. 2022).

One of the main causes of the processing and packaging systems' failure is development. of biofilms on the surfaces of gears. These particular microbes proliferate when nutrients and water linger on surfaces that have been cleaned and repurposed. Bacteria in sessile forms called biofilms are more resistant to chemical sanitizers. are the identical microorganisms found in suspension in plankton. Compound sanitizers may not be able to penetrate milk products due to biofilms that transfer useful microorganisms (Frank *et al*. 1990).

Contamination of Raw Milk

Dairy products have a high nutritional content, which makes them good media. for the development of the microbes. Milk has a pH that is almost neutral and is high in water and nutrients. Many enzymes that are required to break down the proteins and lipids in bacteria in order for them to continue growing do not need lactose, which is the main sugar. It is essential to know where many of the spoilages originate from. dairy products' microbiota, it isappropriatetodiscusshowtheproductionandhandlingprocessescaninitiallycontaminate milk. A lot of very young calves have aseptic mammary gland production lacking in germs. collected milk tests, but the likelihood of releasing microscopic organisms in milk extracted aseptically from the

nipples increases with the number of milking. The vast volumes of milk that the cow's mammary glands and teats produce, the actions of the draining mechanism, and the passage of time cause the nipple channels to become more open, all contribute to the anxiety that the cow feels. The canal in the teat that allows germs to enter the glands may become more perforated as a result of these tensions. High levels of decomposition microorganisms are addressed by ecological contaminants. They permeate every part of the place they inhabit. the milkers' hands, the equipment, the cow, and water. As a result of the 38cm (15in.) of vacuum that milking machine create on the nipples during draining, bacteria that are present on the cow's surfaces in the water leftover from the preparation procedure can enter the system and contaminate the milk. Additionally, bacteria that can contaminate milk are transferred to the floor when inflated cluster descend. Milk is strained or fermented, which breaks microbial chains and clusters while providing the oxygen needed for aerobic growth. The possibility of faster augmentation exists when there are fewer cells in competition with one another than in communities. Bacteria water and air in the filling equipment for immediate surroundings are the primary sources of contamination pasteurized milk, and they can remain for extended periods of time. Again, microbes that contaminate purified milk originates primarily from water and air in the filling mechanism or from transient environmental conditions, and they can remain present for extended periods of time (Eneroth *et al*. 2000).

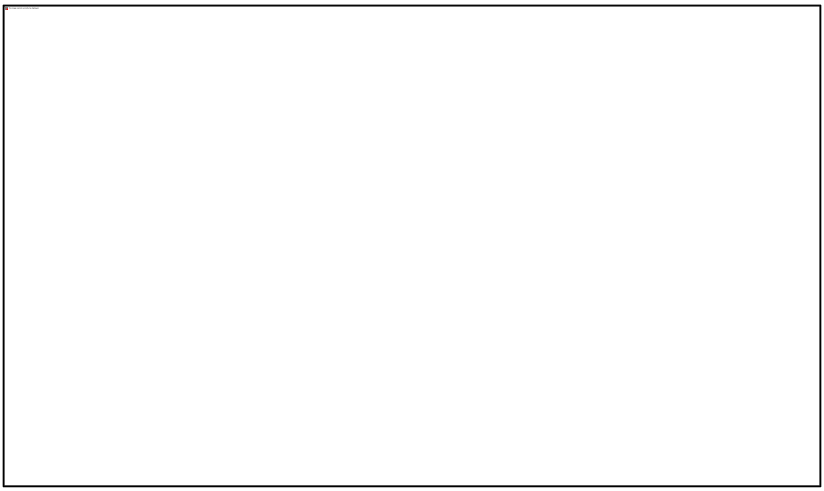

Fig 2: Sources of raw milk contamination

Spoilage Related Factors

Cheese Spoilage

Salt, pH, and water activity are a few of the variables that affect how quickly cheese deteriorates. Regarding temperature, moisture ratio, kinds and features of lactic starter cultures moreover, the suitability of sullying bacteria, as well as the characteristics and concentrations of residual enzymes the deteriorating reactions of cheeses can be influenced by so many factors that it is not surprising that their waste qualities alter with time. The cheeses that go bad the fastest are the delicate or unripened varieties, which usually have the highest pH values and the lowest salt content but the highest proportion of moisture. It is interesting to note that because of their yearly low pH, low redox potential, and low water activity, matured, aged cheeses retain their beneficial eating qualities for extended period of time.

Melilli *et al*. (2004) found that for newly created, filata cheeses made from crude milk, Because of the first salt and the greater burning temperature (18 °C), the coliform grow more rapidly, which caused the cheese to generate gas. Ent Doctor Agglomerans and *Pseudomonas* spp., two bacteria that cause spoilage, grew more quickly in cottage cheese due to the cheese's increased pH and storage temperature (Brocklehurst *et al*. 1988).

Liquid Milk Products' Spoilage

Pasteurized milk might lose its shelf life due to a variety of somatic illnesses. cells in milk that hasn't been boiled. Centralization of Plasmin, astable protease with strong intensity, and lipoprotein lipase in freshly produced milk are question ably correlated with increased cell counts (Barbano *et al*. 2006).

The key factors that determine the amount of these enzymes in the milk supply are the starting cell counts of psychrotrophic bacteria, their generation times, their capacity to manufacture certain enzymes, and the length of time and temperature at which the milk is held prior to processing. The duration till spoiling can be shortened by combining the actions of these enzymes with those of bacterial hydrolases. It takes a number of factors, including a high number of lipase producers (>106 CFU/ml), enzyme stability during the heat process, long-term storage, and ideal pH, temperature, and water activity conditions, for residual lipases in processed dairy products to create lipolyzed taste (Stead *et al*. 1986).

Preventing Spoilage in Dairy Products

The high salt concentration of butter restricts its serum-in-lipid emulsion. The tiny quantity of nutrients that are present in the

microorganisms' droplets cause the contamination germs to thrive. On the off chance that the moisture and salt are not evenly distributed, psychrotrophic microorganisms can nonetheless grow and produce lipase in chilled salted spread (Deeth *et al*. 2020).

When utilized in large amounts Until it is utilized, condensed milk in its concentrated state needs to be kept chilled. By adding about 44% sucrose or glucose, the water content can be lowered and the product preserved. activity below the point of law 0.95, at which viable spores will start to germinate. The added component of the brought down water flow is lactose, which makes up around 53% of the nonfat milk solids. These products are economically cleaned in the jars at the point of canning as better consolidated milk or dissipated milk, and waste only rarely occurs. Freezing prevents the growth of microorganisms and chemical reactions. Thus, the only place where frozen good night become contaminated by microbes are in the combinations or ingredients used before freezing (Jay *et al*. 1995).

Detection and Isolation

Microbiological norms for pointer microorganisms have long been used as a gauge for the safety and quality of dairy products, and many of these microbes are governed by national laws or policies. Boor, Carey, Murphy, and Zadoks (2005) published the findings of audit of pasteurized milk quality collected from 23 plants in New York State over a ten-year period. These tests may not always be correlated with the shelf life of the products, but they can be helpful as general indicator of how clean the dairy processing facility. The percentage of samples that satisfied the Grade A Pasteurized Milk Ordinance Standard Plate Count requirement of 20,000 CFU/ml after 14 days of storage at 6.1 °C varied from 12 to 32% annually. When testing for coliform bacteria began, 5-15% of the samples tested positive; after 14 days of capacity, the percentage rose to 34%.

33-59% of the milks were still deemed acceptable after fourteen days of capacity, according to tactile tests. Thes pore-shaping species Paenibacillus (39%) and Bacillus (32%), as well as the heat-tolerant *Microbacterium lacticum* (14%), were home to the majority of trash bacteria after about 17 days of growth. As a result of initiatives to increase the safety and quality of milk products in the early 20th century, the American Public Health Association produced the Standard Methods for the Examination of Dairy Products, which standardized the procedures for identifying spoiling indications (Wehr *et al*. 2004).

Conclusion

Pasteurization has reduced the risk of microbial deterioration in dairy products, but the process of preventing it in cheddar has progressed more slowly. A good place to start in lowering or getting rid of a lot of spoilage germs would be to implement standardized pasteurization processes globally. Even after processing, it is still challenging to stop surviving organisms from growing and to stop spoiling microbes from contaminating the product. Innovative technologies and preservatives are needed to increase the shelf life of dairy products and stop the growth of spoiling bacteria. Due to the limited use of currently approved antimycotics like natamycin and sorbic acid, there is a good opportunity to increase the amount of additives that the dairy processor can use in their arsenal. It is also necessary todoresearchtodeterminethewaysinwhichspoilingmicroorganismsinteractwith present preservation technology. It may be possible to identify the specific handling conditions that cause postprocess tainting by using more advanced techniques for identifying waste organisms, especially the slowly growing psychrotrophs and growths. In the next century, dairy processors will face numerous challenges, but maintaining the quality and timeliness of this incredibly nourishing meal should not be one of them.

Author Contributions

Acquisition and interpretation of data is done by Diptika Dey. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Chapter - 7
Uses of Honey as a Supplemental Treatment

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Chapter - 7

Uses of Honey as a Supplemental Treatment

Diptika Dey, Akram Gazi and Rupesh Dutta Banik

Abstract

It is well known that honey has several health benefits for people. Now a days, the mechanisms underlying many of those beneficial impacts have been studied. Here, the most studied aspects of honey are succinctly summarized to demonstrate its potency as an alternative treatment. As to this study, honey has several health benefits like controlling human infections, antiviral, anticancer, and benefits for the metabolism, cardiovascular system, and metabolism. It is also anti-inflammatory, immunomodulatory, and antioxidant. Furthermore, this study confirm that the honey's biological activity is essentially determined by its floral and antioxidant. Further research is needed to fully understand the promising synergies that have been demonstrated between honey and antibiotics, as well as their potential antiviral effects. When these studies are considered collectively, they show that honey has nutritional value. The use of natural food products with functional properties has gained popularity as a means of improving human health. Honey has antibacterial, anti-inflammatory, and antioxidant biological activities that may improve health. Honey can be an important source of antioxidants in human nutrition and can therefore have positive effects on human health, including anti-mutagenic, antitumor, and cardiovascular protection. Honey's natural antioxidants are primarily phenolic compounds. Various honey have also been demonstrated to reduce inflammatory responses, making them potentially helpful for anti-inflammatory therapy. In place of more costly and sophisticated wound care products, honey has been widely used in wound therapy recently.

Keywords: Antiviral, anticancer, antioxidant, antibacterial, cardiovascular protection, wound therapy

Introduction

Apis bees obtain their nectar from plants. Use the nectar that has been collected from plants to make money once it has been digested and

regurgitated. This process results in the production of honey (Alvarez-Suarez *et al*. 2014). Itisa sweet, flavorful, naturally occurring products that is supersaturation in sugar sand has high nutritional content. Honey contains variety of minor constituents in addition to sugars, including minerals, organic acids, vitamins, carotenoids, polyphenols, amino-acids, proteins, and enzymes like catalase and glucose oxidase. In addition to the previously mentioned prebiotic benefits of oligosaccharides (Viuda-Martos *et al*. 2008). These small ingredients may be responsible for the majority of honey's biological qualities (such as antibacterial, antiviral, anti-inflammatory, anti-ulcerous, immunomodulatory, vasodilative, hypotensive, anti-hypercholesterolemic, anti-browning, disinfectant, and antitumor), as well as many of its uses. But each honey's unique composition varies greatly depending on the type of flowers used, the location, the time of year, and the post-harvest processing (Da Silva *et al*. 2016).

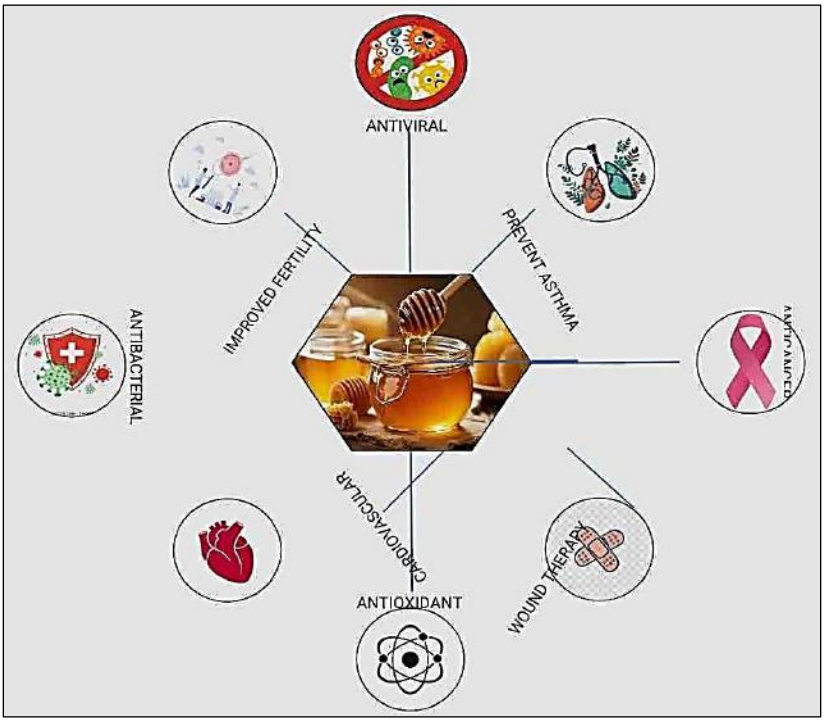

Fig 1: Benefits of Honey

Medicinal History of Honey

Evidence from stone age paintings demonstrates that the use of bee products, such honey, for medicinal purposes dates back 8,000 years. Honey

has been used as medication for long time, as evidenced by ancient scrolls, tablets, and books such as the Sumerian Clay Tablets (6200 BC), Egyptian papyri (1900-1250 BC), Veda (Hindu literature) dating back 5000 years, Holy Quran, Bible, and Hippocrates (460-357BC). (Mijanur Rahman M *et al*. 2014) (Newman TG 1983) (Molan PC 2001).

Formulation of Chemicals

The macro and micronutrient content of honey is primarily determined by three factors:

1) Species of bee.
2) Source of flowers.
3) Processing and ambient conditions.

The primary components of honey are sugars; amino acids, proteins, enzymes, organic acids, vitamins, minerals, volatile compounds, and polyphenols are present in much smaller amounts. Also, Vitamins A (retinol), E (tocopherol), K (anti-haemorrhagic vitamin), B1 (thiamine), B2 (riboflavin), B6, Niacin, C (ascorbic acid), Panthothenic acid, flavonoids, ethyl ester, hydroxybenzoic acid, octadecanoic acid, cinnamon and fatty acids are among the essential biological bioactive compounds found in honey (Bogdanov *et al*. 2008) (Muhammad *et al*. 2015).

Around three-quarters of the sugars in honey are monosaccharides, namely glucose and fructose. The remaining 10 to 15% of the carbohydrates in honey are made up of various oligosaccharides, including trisaccharides (maltotriose and melezitose) and disaccharides (sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, and kojibiose) (Bogdanov *et al*. 2008).

The main sugar in honey is typically fructose, and one of the plants that produces the most fructose is acacia (Escuredo *et al*. 2014). Except in certain cases, such as rape (*Brassica napus*) and dandelion (*Taraxacum officinale*) honey, which has higher glucose content than fructose (Oddo *et al*. 2004).

Function of Antioxidants

According to a recent review, honey has antioxidant properties both in vivo and in vitro, regardless of where it comes from or what kind off lower it comes from (Ferreira *et al*. 2009). Tests conducted *in vitro* demonstrate that honey has the ability to scavenge free radicals (such as1,1-diphenyl-2-picrylhydrazyl, peroxyl radicals [oxygen radical absorbance capacity], 2,2′-azino-bis [3-ethylbenzothiazoline-6-sulphonicacid], and nitric oxide [NO]),

as well as reduce ferric cations, chelate metal ions, inhibit β-carotene bleaching, and inhibit lipid peroxidation as determined by thiobarbituric acid reactive substances (Alvarez-Suarez *et al*. 2009).

Honey's Immunomodulatory effect on the Healing of Wounds

Haemostasis (seconds to minutes), inflammation (3-5 days), proliferation (4-14 days), and remodelling (8 days to 1 year) are the four broad overlapping stages of wound healing. Debris and bacteria are eliminated during the inflammatory phase that follows haemostasis. The proliferative stage is typified by blood vessel invasion, connective tissue and epithelium regeneration, hand wound contraction. Collagen is reorganized, an extra tissue is eliminated by apoptosis during the remodelling stage (Song *et al*. 2011).

As a protective reaction to trauma and/or any pathogenic agents, tissue regeneration during wound healing begins with an immunostimulatory or inflammatory action after the Haemostasis stage. Nevertheless, a complete recovery is not possible when there is an uncontrolled inflammatory response, especially if this stage lasts, which results in a chronic wound (Hadagali *et al*. 2014).

Anti-tumor Effects

Honey may help prevent cancer and may also have an impact on the growth and spread of tumors, according to research. *In vitro* research has been done for most these studies. Honeys from various floral sources (*Abies cephalonica*, Acacia, Citrus, *Erica arborea*, forest, multifloral, *Pinus* sp., rosemary, thyme and tualang tree) have shown potential anticancer activity when tested on several types of human cancer cell lines (breast, prostate, endometrial, cervical, lung, skin, kidney, bladder, liver, and oral squamous cell carcinoma and osteosarcoma). (Fernandez-Cabezudo *et al*., 2013; Spilioti *et al*., 2014; Ghashm *et al*., 2010; Tsiapara *et al*., 2009)

Impact on Metabolism and Cardiovascular Systems

Certain metabolic changes, such as insulin resistance, glucose intolerance, hypertension, atherogenic dyslipidemia, and abdominal obesity, may raise the risk of cardiovascular diseases (Bel-Serrat *et al*. 2013). In addition, moderate honey consumption European children flowers the likelihood of having higher concentrations of high-sensitivity C-reactive protein (CRP), according to González-Gil *et al*. Consequently, it appears that the type of diet has some bearing on inflammation (González-Gil *et al*. 2016).

Honey and Asthma

In folk medicine, honey is frequently used to alleviate fever, coughing, and inflammation. It has been demonstrated that honey has the power to lessen asthma-related symptoms or to function as a preventive measure to stop asthma from starting. In animal models, oral honey ingestion was used as a treatment for both bronchial asthma and chronic bronchitis (Ghashm AA *et al*. 2010).

Honey and Fertility

In the past, Egyptian offered honey as a fertility food. Furthermore, honey has long been taken by many civilizations to increase male vigor. There are a number of reasons why infertility occurs as well as possible treatments. Give its abundance of vitamins, iron, calcium, and other minerals, as well as its immune-boosting qualities, honey bee pollen is believed to enhance egg quality and overall fertility and fecundity, according to a number of reports. It has been recommended that men with impotence issues and women with infertility issues, such as irregular ovulation, take honey. It is thought that adding honey to warm milk might significantly increase sperm count in men who are infertile or unfertile. It is well known that honey contains a lot of vitamin B, which is necessary for the synthesis of testosterone. There is a favorable association between testosterone concentration and honey ingestion, according to certain literature. Given honey's high nitric oxide level, a molecule involved in vasodilatation, it has been suggested that honey can induce and enhance erection in men who suffer from impotence or dysfunctional erection. Research indicates that consuming 100 grams of honey can elevate blood levels of nitric oxide by as much as 50%. According to teachings in complementary and alternative medicine, honey boosts women's ovaries and uteri and enhances the quality of sperm in males. According to authors of anew study, adding honey to a cryoprotectant solution improves the quality of the sperm overall (Fakhrildin *et al*. 2014). Other researchers have shown that feeding bee pollen to rabbits improves their fertility and semen quality using male rabbits. In addition, young rabbits given bee pollen had a greater survival rate and gain more body weight (Attia *et al*. 2011). Another team of researchers has demonstrated that couples who were not able to conceive naturally experienced increased fertility after applying royal jelly and bee honey vaginally around the time of sexual activity (Abdelhafiz *et al*. 2008).

The Prebiotic Qualities

Prebiotics are indigestible food ingredients, mainly oligosaccharides, that have a functional effect on the gastrointestinal tract. They Are Typically

Short-chain carbohydrates. They are selectively fermented by lactobacilli and bifidobacteria, which increase their population in the colon. Overall, these processes improve the host's health raising the synthesis of short-chain fatty acids, lowering pH, decreasing fat absorption, and reducing ammonia production. The degree of polymerization and types of oligomer, which vary from food to food, are the primary factors influencing the efficiency of prebiotics such as fructooligosaccharides, galacto-oligosaccharides, and inulin, which are found in some natural foods (Roberfroid M.B. 2000). Due to its about 0.75% fructooligosaccharide content, honey may have prebiotic properties. Moreover, fructooligosaccharides and their monomeric derivatives have the ability to counteract the deleterious effects of bile salts on *Bifidobacterium* spp., a common resident of the human digestive tract (Perrin S *et al*. 2001). Many monofloral honeys, such as sourwood, alfalfa and sage, honeydew, chestnut and acacia, clover, and eucalyptus, have been shown to have prebiotic properties. The potency of each honey varies greatly depending on the flowers from which it derived. Furthermore, a few investigators noted that honey's prebiotic activity was much inferior to that of inulin or fructooligosaccharides, operating primarily on Bifidobacterium, or in some other way (Macedo LN *et al*. 2008; Kajiwara S *et al*. 2002).

Utilizing Honey to Manage Human Pathogens

Honey has been utilized as an antimicrobial since ancient times. Because antibiotic-resistant bacterial pathogens are becoming more common and raise the risk of even basic medical interventions, there is currently a renewed interest in this practice. Honey's antibacterial properties have been associated with a number of variables, such as its hydrogen peroxide (H_2O_2) content, low pH, high osmolarity, phenolic and aromatic acid content, honey glycoproteins, and Maillard Reaction Products (a non-enzymatic browning reaction that occurs when reducing carbohydrates interact with amino acids or proteins (Wasfi R *et al*. 2016) (Brudzynski K *et al*. 2015).

Action of Anti Virulence

AVAs, or anti-virulence agents, are an additional or different strategy to fight the virulence factors (VFs) that are becoming resistant to infection be adversely influence their production or activity. This action is linked to the fact that AVAs do not impede bacterial growth, which prevents them from applying the same environmental pressure as traditional antimicrobials. This pressure causes positive selection of genetic mutations that result in resistance cells that are able to survive. One potential natural anti-VF effect of honey is its ability to act. Certain infection black iron within the host, and they rely on siderophores to get iron to compensate.

This makes preventing the synthesis of siderophores an additional strategy for managing infections. Manuka honey was found to reduce *P. aeruginosa'* virulence in the study conducted by Kronda *et al*. (2013).

Using Honey in Conjunction with Other Antimicrobial Agents

There have been studies conducted on the use of honey in conjunction with other antimicrobial agents. Honey reduces the likelihood of pathogen survival after exposure, which in turn lowers the chance of resistant bacteria emerging when combined with other antimicrobial agents. Manuka honey showed synergistic or additive effects against the two strains of each bacterial species when used in conjunction with the antibiotic's tobramycin and colistin to treat *P. aeruginosa* and *Burkholderia cepacia* infections (Jenkins *et al*. 2015).

Antiviral Properties of Honey

There is a lack of research on honey's antiviral properties. In vitro tests using Madin-Darby canine kidney cells have demonstrated the efficacy of manuka honey, one of the most thoroughly researched types of honey, against influenza viruses (Watanabe *et al*. 2014). Moreover, manuka and clover honey shown efficacy against the varicella zoster virus in vitro when tested in human malignant melanoma cells (MeWo) (Shahzad A *et al*. 2012). Additionally, manuka and commercial honey appear to have anti-herpes simplex virus (HSV-1) properties when isolated using Vero Cells, when honey solutions have anti-rubella properties when isolated using monkey kidney cell cultures (Hashemipour MA *et al*. 2014). Applying honey helped to treat vaginal and labial recurrent herpes lesions. According to the paper, honey may have a beneficial effect on herpetic lesion symptoms and signs by inhibiting prostaglandins at the lesion site (Al-Waili NS *et al*. 2004). The antiviral activity in these investigation has not been linked to any specific molecule or combination of chemicals found in honey. Consider that quercetin and rutin exhibit antiviral action against HSV, syncytial virus, poliovirus, and Sindb is Virus, while Golang has been shown to be effective against HSV and coxsackie B virus. This suggests that flavonoids may play a significant role in this activity. Although these flavonoids may be present in honey, a clear link between the substance's chemical makeup and antiviral qualities has not yet been explicitly proven (Viuda-Martos M *et al*. 2008).

Table 1: Health Benefits and the Mechanism of Action of Honey

Health Benefits	Mechanisms of Action	Supporting Research/Findings	Reference
Antibacterial	Antibacterial properties, potential synergy with antibiotics	Honey can control human infections, particularly bacteria; further research needed to explore synergy with antibiotics.	Wasfi R *et al*., 2016
Antiviral	Antiviral potential	Studies suggest honey may have antiviral effects, though more research is necessary.	Kronda *et al*., 2013
Anticancer/Anti-tumor	Antimutagenic properties, anti-inflammatory	Honey shows antitumor properties through its antioxidant and anti-inflammatory activity, particularly due to phenolic compounds.	Spilioti *et al*., 2014
Cardiovascular Protection	Antioxidant, anti-inflammatory	Honey offers cardiovascular protection through antioxidant and anti-inflammatory mechanisms.	González-Gil *et al*., 2016
Antioxidant	Rich in phenolic compounds, reduces oxidative stress	Honey is a significant source of natural antioxidants (phenolic compounds), supporting general health and fighting free radicals.	Alvarez-Suarez *et al*., 2009
Anti-inflammatory	Reduces inflammatory responses	Honey has demonstrated the ability to reduce inflammation, making it a candidate for anti-inflammatory therapy.	Da Silva *et al*., 2016
Wound Healing/Therapy	Antibacterial, anti-inflammatory, cost-effective alternative	Honey is widely used in wound therapy due to its antibacterial and anti-inflammatory properties, serving as an affordable option.	Hadagali *et al*., 2014
Immunomodulatory	Modulates immune responses	Honey has immunomodulatory effects, potentially enhancing immune health.	Da Silva *et al*., 2016
Metabolism Benefits	Improves metabolic processes	Studies suggest honey can positively affect metabolism.	González-Gil *et al*., 2016
Prevents Asthma	Anti-inflammatory, antioxidant properties	The anti-inflammatory and antioxidant actions of honey may help reduce airway inflammation, thus aiding in asthma prevention.	Ghashm AA *et al*., 2010
Improved Fertility	Antioxidant properties, hormone regulation	The high antioxidant content in honey is suggested to improve reproductive health and fertility reducing oxidative stress.	Fakhrildin *et al*., 2014

Conclusions

A thorough analysis of the mechanisms underlying honey health benefits is still being actively studied, despite the abundance of potentially bioactive properties that have already been reported. according to the research done this far, the activity of each honey is highly influenced by the flower or place of origin. Honey has been studied extensively for its potential use as an alternative medicine due to its

1) Antioxidant activity.
2) Anti-inflammatory and immunomodulatory responses.
3) Delaying the development of cancer and cardiovascular diseases.
4) Inhibiting bacterial pathogens by interfering with their pathogenesis potential.
5) Controlling viral infection.
6) Acting as a prebiotic agent to improve gut health.

Author Contributions

Acquisition and interpretation of data is done by Diptika Dey and Akram Gazi. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Chapter - 8
Removal of Synthetic Dye using Green Nanomaterials: A Short Review

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Chapter - 8

Removal of Synthetic Dye using Green Nanomaterials: A Short Review

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Abstract

The primary contaminant, metals, and colours found in textile industry effluents are released into the environment. Dye discharge into waste water streams are common and have a negative impact on the ecosystem. Many methods are emerging to get rid of these dangerous dyes, but nanotechnology is the most dependable and secure. When it comes to solving economic and environmental issues, nanotechnology makes sense. The biosynthesis of nanoparticles offers a number of benefits over traditional techniques and approaches to environmental concerns. It is determined that the biological approach to the creation of nanoparticles is the most effective and promising. An effective method for treating and decolorizing dyes could be to use biosynthesised nanoparticles. This paper includes an analysis of several biosynthesised nanoparticles that are used to break down different colours that are contaminants in wastewater. This review covers bio-synthesised nanoparticles, including iron, silver, zinc, gold, and titanium, that are utilised to break down different colours.

Keywords: Green synthesis, nanomaterials, photocatalysts, dye degradation

Introduction

Water is an essential and valuable resource in our day-to-day life. While water covers 71% of the surface of earth 71%, only 0.007% of all water content is classified as fresh and suitable for consumption (Ruan *et al*., 2019). The rapid technological advancements and global industry advancement is one of the major sources of toxic waste into the natural environment, comprising heavy metals, organic pollutants, and dyes therefore water body become polluted everyday (Agnihotri *et al*., 2018). A substantial quantity of dyes is generated every year by several industries, such as textile, plastic, paper, paint, plastic, cosmetic, leather, hair colouring,

agricultural and pharmaceutical research industries (Pandey *et al*., 2020). These dyes possess an expansion in chemical oxygen demand (COD) and also biochemical oxygen demand (BOD) levels into the aquatic environment, which cause serious health problem in humans (Ruan *et al*., 2019). Beside this, synthetic dyes have carcinogenic effect to human life (Pandey *et al*., 2020).

When it comes to cleaning up dyes from wastewater and soil, several conventional treatment methods are used, such as physical decolorization (using reverse osmosis, adsorption, and filtration), chemical decolorization (using ion exchange chromatography, neutralization, process of recovery, and chemical oxidation), and biological decolorization (using actinomyces and other microorganisms) (Morshedi *et al*., 2013). Therefore, there is an urgent requirement to develop a technique in low-cost, high efficient and environment-friendly for the decolorization of synthetic dyes.

Nanotechnology deals with nanoscale materials that can be produced by various types of methods including physical, biological, and chemical methods. Considering all of these methods, the plant-extract-based green synthesis method covers most if the required criteria in low-cost and eco-friendly techniques (Alizadeh *et al*., 2021). This review contains three major sections. Firstly, the toxicity of synthetic dyes is highlighted. Then, the green synthesis process of nanoparticles is described. And in the third section application of biosynthesized NPs in break down contaminants of waste water are discussed. Finally, the future prospects and a conclusion for an overview of the review has made.

Classification and Toxicity of Synthetic Dyes

Dyes are divided into two major classes natural and synthetic dyes. Further classification of synthetic dyes can be made into three categories:

i) Cationic dyes (represent basic dyes).

ii) Anionic dyes (direct dyes, water-soluble acid dyes, and reactive dyes).

iii) Non-ionic dyes (disperse and vat dyes) (Pereira & Alves, 2012).

The majority of synthetic dyes produce high toxicity in nature and are resistant to deterioration due to their intricate chemical structures (Mehta *et al*., 2021). Table 1 displays the available synthetic dyes that have led to toxicity on aquatic life, human being as well as the overall ecosystem.

Table 1: Several applications and toxic effects of different types of synthetic dyes

Synthetic Dyes	Example	Applications	Toxic effect	Reference
Acid Dyes	Methyl Orange, Congo Red, Acid (yellow, blue, violet, black)	Paper, wool, silk, leather	Mutagenic and carcinogenic to living beings	Jin *et al*., 2018
Direct Dyes	Congo Red, Martins Yellow, Direct Blue, Direct Red 81	Leather, textile	Gastrointestinal infection, skin disease	Khamparia & Jaspal, 2016
Reactive Dyes	Reactive Red 120, Reactive Blue 4,	Wool, cotton, cellulosic fibers	Allergy, conjunctivitis, dermatitis	Ruan *et al*., 2019
	Reactive Yellow 160			
Basic Dyes	Methylene Blue, Crystal Violet, Rhodamine 6G, Aniline Yellow, Malachite Green	Silk, leather, textile, acid-base indicator, printing inks	Carcinogen to human's diarrhea, methemoglobinemia, eye burns	Ozer & Dursun, 2007; Karim *et al*., 2017
Disperse Dyes	Disperse Red, Blue & Orange dyes	Acrylic fibers, polyester nylons	Carcinogenic, allergic for the human body	Ruan *et al*., 2019
Vat Dyes	Vat Orange 28, Vat Orange 15	Cellulose Fiber, fabric	Carcinogenic, increased BOD & COD levels	Berradi *et al*., 2019

Green Synthesis of Nanoparticles by Phytochemicals

Plant-mediated synthesis has many advantages when it comes to produce nanoparticles over traditional approaches like physical, chemical, and biological. These advantages include a simple process, low contamination, less toxicity, eco-friendly, larger scale production of nanoparticles and cost-effectiveness (Roy *et al*., 2023). The primary process for producing green synthesized nanoparticles using plant extracts requires a number of phytochemicals included in the extracts, such as phenolics, quinines, glutathione, alkaloids, flavonoids, terpenoids, carotenoids, serve as capping, stabilizing, and reducing agents (Marslin *et al*. 2018).

At first, the plants are required plant components, following the collection from various sources, are washed (by distilled water) and removed any dust or adhesive, which is dried out at room temperature to turn into a fine powder, and then kept at 25 ℃ to be used later. The necessary quantity of plant material was taken in deionized water, and the mixture was stirred and heated at a specific temperature. The extract is then filtered and the metal solution is added at specific temperature for the synthesis of NPs.

Following the color shift from light to dark, the synthesized NPs are separated using centrifugation at 4 ℃ and 15,000 rpm. Finally, the biosynthesized N Psara washed thrice with deionized water and acetone simultaneously to get rid of contaminants and dried overnight for later use (Nagajyothi *et al*., 2020).

Application of Green-Synthesized NPs in Dye-Degradation

Nanoparticles have a large ratio of surface to volume, resistance for low diffusion, increased adsorption capacity, and quicker adsorption that makes NPs superior adsorbents. By Employing nano-silica powder made with silver nanoparticles, dyes including Eosin yellow, brilliant blue Congo red, and Bromophenol blue are adsorbed at an initial dosage of 50mg/L of NPs, showed 99% removal of dye (Mehta *et al*., 2021).

The cooperation of NPs and H2O2allowed for the decolorization of methyl dye. This mixture resulted in the formation of free hydroxyl (OH·) radicals, which caused the dye solution to decolorize with 73.6% efficiency by cleaving the azo bond(-N=N-) present in methyl orange dye (Kouhbanani *et al*., 2019). Some examples from the literature study of green-synthesized NPs in dye degradation are shown in Table 2.

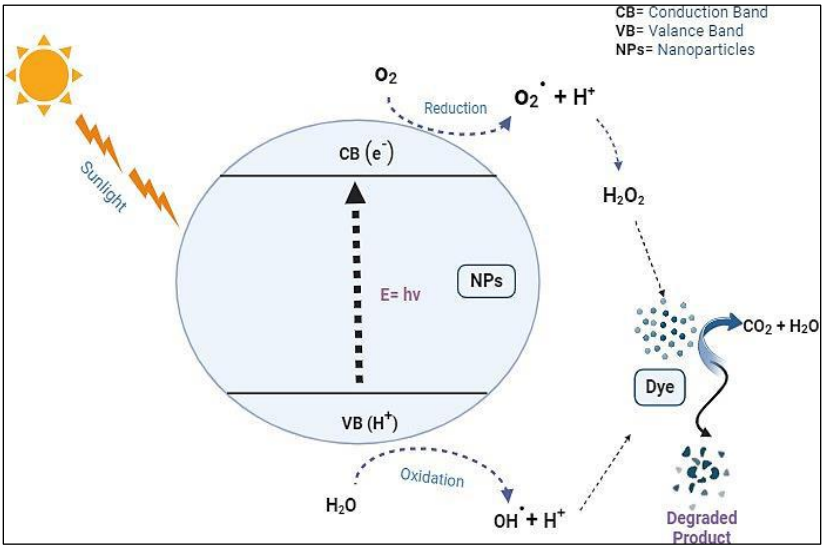

Fig 1: Generalized dye degradation process photocatalytic activity using Phyto-synthesized NPs

Table 2: Applications green-synthesized NPs in synthetic dye degradation

SL. No.	Plant Components	Name of Nanoparticle	Targeted Dye	Method of Degradation	Degradation Efficiency or Rate constant	Reference
1.	Kondagogu (*Cochlospermum gossypium*) leaves	TiO2NPs	Methylene Blue	Photocatalytic activity	96.8%	Saranya *et al*., 2018
2.	*Nephelium lappaceum* L. peel extract	NiONPs	Rhodamine B	Photocatalytic degradation	92.3%	Adinaveen *et al*., 2019
3.	*Ruellia tuberosa* leaves	FeO-NPs	Crystal violet	Photocatalytic activity in sunlight	80%	Vasanthara j *et al*., 2019
4.	*Citrus paradise* peel extract	α-Fe2O3, γ-Fe2O3, Fe3O4NPs	Methylene Blue, Methyl Orange, Methyl Red	Adsorption	80.76%; 89.64%, 96.65%	Kumar *et al*., 2020
5.	*Ficus carica* fruit extract	γ-Fe2O3NPs	4-Nitrophenol	Chemical method using NaBH4	49.97 x 10^{-3} min^{-1}	Kumar *et al*., 2021
6.	*Camellia japonica* leaf extract	AgNPs	Eosin-Y	Photocatalytic activity in visible light	>97%	Karthik *et al*., 2017
7.	Peel extract of Banana	CuONPs	Congo Red	Photocatalysis in sunlight	88%	Aminuzzaman, Kei, and Liang, 2017
8.	*Sansevieria roxburghiana* leaves extract	AuNPs	Acridine Orange, Phenol Red, Bromothymol Blue	Chemical catalysis using NaBH4	40.44%, 85.88%; 88.16%	Kumar *et al*., 2019

Conclusion and Future Perspectives

Pollution of the land and water is primarily caused by synthetic dyes. Numerous techniques, including chemical, biological, and physical ones, have been developed to remove dyes from soil and water; however, all techniques have constraints. This review has emphasized the applications of eco-friendly green synthesized NPs for the degradation of synthetic dyes. Traditional and cutting-edge dye remediation methods were unable to almost completely degrade the dye, hence they were ineffective. Therefore, it would be prudent to concentrate more on synthesizing and applying these environmentally benign NPs for wastewater management and degradation of dyes.

Though NPs have demonstrated the potential to eliminate dyes, certain problems still need to be resolved. Before their widespread use, it is crucial to look into the ecotoxicity of NPs in aquatic environments. Reuse and regeneration procedures for nanoparticles are highly desirable for industrial use. Therefore, further study should be done on the practical and affordable way of NPs regeneration.

Conflict of Interest

There are no conflicts of interest, declared by the authors.

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Chapter - 9
Eco-Friendly Microbial Strategies for Heavy Metal Detox in Agroecosystem

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Chapter - 9

Eco-Friendly Microbial Strategies for Heavy Metal Detox in Agroecosystem

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Abstract

As our population is increasing the amount of food needed by living being is also increasing. Protein is essential in bodybuilding and other biological processes to lead healthy life. We need a lot of protein to stay health but there aren't enough regular source of protein to give everyone that they need. Especially with more and more increasing population protein deficiency can cause serious health issues like mental retardation, kwashiorkor, malnutrition and even death. Protein sources like meat and dairy products are expensive which makes the problem worse. So, scientists are looking forward into making protein from waste and cheap materials. SCP are proteins extracted from microorganisms using various substrate and can be used as substitute of protein rich foods. Producing SCP from microorganisms using food or other waste is not only a cheaper alternative but also reduces the pollution load of environment. This article discusses about how SCP is made from different waste sources which fulfil protein deficiency in human and other living beings.

Keywords: Microorganisms, Single-cell proteins, waste material, food waste

Introduction

By 2070, world's population will cross over 13 billion people. To keep up with the way we are eating now, we would need to produce about 2250 million tons of meat and dairy products every year. (Abdullahi *et al*. 2021). But the way we are producing food right now won't be enough. We have to find new ways to make sure everyone has enough to eat. One promising alternatives SCP, they are microbial protein used as an alternative for protein rich foods. It is enriched with amino acids like methionine, lysine etc. (Thiviya *et al*. 2022). SCP can be used as food for animal or people ether on its own as supplements. It's becoming more popular because it's easier and faster process to produce it rather than traditional farming or raising animals. India produces a huge amount of waste every year such as fruit and

vegetable waste. (Sekoai *et al*. 2024). These waste materials have potential to ne uses as a substrate for production of SCP using various efficient unicellular microorganisms, they contain carbohydrate, minerals, and vitamins need for the production of substitute protein from microorganisms. (Ahmed *et al*. (2024).

Some microorganisms that are able to produce SCP are

- **Bacteria (Cellulomonas**, *Alcaligene* ssp.) Abdullahi *et al*. (2021).
- **Fungi (Trichoderma**, *Aspergillus Niger*).
- **Algae (Spirulina**, *Chlorella*) *Thiviya et al*. (2022).
- **Yeast (***Candida albicans, Saccharomyces cerevisiae*).

Yeast is a preferred candidate for SCP production because its high nutrition value and ability to grow on low cast materials. They are easy to harvest and has high malic acid and lysine content. Wainaini *et al*. 2020.

Microorganisms Utilized for SCP Production

Fungi

Fungi are key single cell protein in SCP production due to their numerous advantages-easy growth and harvest due to their large size. (5-10 micrometre) and low water activity facilitating simpler recovery. (Sekoai, P *et al*. (2024). Additionally, Fungi are desirable for SCP due to their rich nutritional composition, high protein, and essential amino acid content as well as ample vitamins and lipids. Their ability to thrive at low pH level make production cost effective and rapid. (Abdullahi *et al*. (2021). Progress had been made in selecting optimal fungal strains, substrate and parameters for enhanced yield. Criteria for selecting producer organism for SCP include biomass yield protein and nucleic acid content, substrate diversity utilization and host digestibility. For example, *Candida utilis* has been found as an essential compound for SCP production incorporating *C. utilis* in dairy products. (Abdullahi *et al*. (2021).

Table 1

Example of Fungi	Properties	Reference
Candida utilis	Highly sine and ergosterol content, short harvesting time	Abdullahi *et al*. 2021
Candida intermidia	Good protein content, Efficient grow on lignocellulosic material	Sekoai *et al*. 2024
Geotrichum candidum	Increased protein content, lower recovery costs	Yadav *et al*. 2015

Yarrowia lipolytica	Can utilize hydrophobic compound, fast generation time	Abdullahi *et al*. 2021

Algae

Algae is a great Source of SCP mentioned by many experts. It's rich in protein comparable to sources like soy eggs and meat. Growing algae for SCP is cheap and ecofriendly needing less land and fewer resources (Yadav *et al*. 2015). Algal protein is easy to digest once its cell wall is broken done through methods like drying and boiling. Algae also contain high quality fatty acid minerals and Omega 3 vitamins. Chlorella and spirulina are mostly used in SCP production. (Abdullahi *et al*. 2021). They are also used as supplements and thickeners as well as animal feed. Their use as SCP is increased because they are rich in protein omega 3 fatty acids etc. *Chlorella stigmatophora* has high protein content, lower production and recovery cost, lesser harvesting time make it suitable for production. (Valverde-Pérez B *et al*. 2020) Also other genera such as *Chlorella pyrenoidosa, Arthrospira, Chlorella Sorokina* also used as SCP sources for human feed. (Abdullahi *et al*. 2021).

Some algal genera used for SCP production are

Algal genera	Reference
Chlorella sp.	Abdullahi *et al*. (2021).
Chlorella sorokiniana	Yadav JSS *et al*. 2015
Spirulina sp.	Thiviya *et al.* (2022)
Chlorella pyrenoidosa	Yadav JSS *et al*. 2015
Arthrospira sp.	Abdullahi *et al.* (2021).
Chlorellastigmatophora	Sekoai *et al*. (2024)
Nostoc sp.	Thiviya *et al.* (2022)
Punaleialla sp.	Abdullahi *et al*. (2021).

Bacteria

SCP from bacteria is favoured because it is packed with 50-80% protein, making it easy to grow using cheap materials for food and energy. Bacteria grow faster than algae and fungi making them a top choice for single cell protein. However, using Bacteria as SCP has some challenges (Thiviya *et al*. (2022).

- Bacterial cells have lots of nucleic acid, which can be harmful to both humans and animals. Removing this adds extra costs making production harder. (Sekoai *et al*. 2024)

- Bacterial cells are small and light making them tricky and expensive to collect from the fermentation mixture.
- People are hesitant about eating bacteria-based SCP.
- Some types of bacteria produce toxins that can cause food poisoning.

Some bacteria used for making SCP includes *Aeromonas* sp., *Methylomonas* sp., *Mycobacterium* sp., *Nocardia, Brevibacterium* sp., *Methylophilus* sp. (Yadav *et al*. 2015).

SCP product protein is made from *Methylophilus* by using methanol as substrate to prepare animal feed. (Ahmed *et al*. (2024).

K nio Borealis an SCP product *Methylobacterium extroqueceme* by using methane as substrate to produce fish feed. (Yousef MK. 2012.)

Yeast

Yeast have another source of SCP and has been used for along time. Yeast biomass is mainly used in the pet food industry, providing supplements for dogs, cats, and fish improving their edibility. Torula yeast being commonly used for this purpose. Compared to other sources yeast has several advantages. (Wainaina *et al*. 2020)

- It'easier to harvest compared to large size of bacteria.
- It has higher percentage of malic acid and lysine.
- It can grow and thrive in acidic environment (Ahmed *et al*. (2024) It has long history of traditional use.

Experiment shows that *S. cerevisiae* used efficiently to prepare SCP from food waste material such as banana peels and give satisfactory result. But adding dextrose to its substrate the productivity rate increased by 80% (Sekoai *et al*. (2024).

Traditional Substrate for SCP Production

Traditional substrate used for SCP production are gas oil, methanol, n-alkenes, mostly on those substrate bacteria and yeast are grown. (Ahmed, *et al*. 2024).

Methanol is used to produce Single Cell Protein (SCP) because it has stable composition. It is also non-toxic and easily dissolves in water. However, in the Western world, the ICI Pruteen plant, which was the only facility of its kind, had to shut down because methanol costs were too high, (Sekoai *et al*. 2024). In the Middle East, where methanol is cheaper and fish

meal is expensive, SCP could be a viable option. For SCP intended for human consumption, ethanol is considered better choice. (Salazar-López *et al*. 2022) The future success of ethanol-based SCP will depend on factors like local ethanol production, availability of agricultural carbohydrates, and political decisions about trade and economic policies n-alkanes have been tested for SCP production, but these methods are complex and many have been discontinued due to health risks from potential carcinogens. Recently, Japan and other Eastern countries have focused on developing SCP from alcohol and organic waste. (Ahmed *et al*. 2024).

Alternative Substrate used for Production

Waste materials like straw, citric acid, bagasse, molasses, and food scraps should ideally be recycled into the ecosystem. These materials can significantly pollute waterways if not managed properly. Using them in Single Cell Protein (SCP) production helps reduce pollution and provides edible protein. (Thiviya *et al*. (2022). While efforts have been made to turn animal waste into feed protein, these have often failed due to technological and hygiene issues. Increasingly strict waste management regulations have made waste a costly problem, though it can sometimes be used for SC Pilot's economically feasible. (Salazar-López *et al*. 2022) Large-scale SCP production using waste typically involves yeast and complex bioreactors, with substrates like cheese whey and molasses. New developments, sukha mushroom fermentation of SCP, show promise but are still in the early stages. In developing countries, where high-tech systems are too expensive or where skilled labor is lacking, traditional microbiological methods might benefit from these new biotechnological advances. (Ahmed *et al*. 2024)

Industrial waste

Waste from wood and cotton processing, paper and fuel production, and latex industries often includes polymers like cellulose, lignin, and hemicellulose. These lignocellulose wastes are common but need extensive pre-treatment-mechanical, chemical, or enzymatic-before they can be used by microorganisms to produce Single Cell Protein (SCP). This pre-treatment increases the cost of SCP production. (Sekoai *et al*. (2024)

Paper waste

In 2014, global paper and cardboard production reached 390 million tons. While developed regions like Europe and North America are cycle about 60-70% of their paper, some areas have much lower rates. Paper Production is expected to grow to 490 million tons annually by 2020, leading to more paper waste. Paper waste primarily consists of cellulose. (Thiviya *et*

al., (2022). When hydrolysed, it contains 60-70% sugar, mostly glucose, with some xylose, mannose, arabinose, and galactose, along with lignin, kaolinite clay, and other residues. Contamination by unwanted microbes complicates and increases the cost of SCP production from paper waste *Arthrospira* sp.

Abdullahi *et al*. (2021). Using extremophiles' microorganisms can help avoid contamination and reduce production costs by maintaining selective growth conditions.

Food Waste

Mostly vegetable and food peels are considered as typical food waste for preparing SCP. Carbohydrate- rich waste from fruits like orange, papaya, banana peels, and other agricultural residues such as bagasse of sugarcane, rice husk, and wheat straw are valuable for producing Single Cell Protein (SCP). (Wainaina *et al*. 2020) These materials are abundant, low-cost, and enrich with sugars and nutrients that amicrobial growth. For example, banana peels, which makeup 18-20% of the fruit, are sometimes used as feed for livestock due to their high carbohydrate and nutrient content. (Abdullahi *et al*. 2021). Various microorganisms, including bacteria (Cellulomonas), algae (Spirulina), molds (Trichoderma), and yeast (Candida), are used for SCP production. Yeast is particularly effective because it provides high nutritional value, can efficiently use low-cost raw materials, and is easy to harvest due to its larger size and ability to grow in acidic conditions. (Thiviya *et al*. 2022).

General Process of SCP Production

Steps of these process are:

1) Selection of suitable strain.
2) Fermentation.
3) Harvesting.
4) Post-harvest treatment.
5) SCP processing for food. (Ahmed *et al*. (2024)

These steps are nessecerary for single cell protein synthesis.

Step 1: A suitable strain needs to be selected.

Step 2: Fermentation of the microorganism.

Step 3: Harvesting in proper condition.

Step 4: Post-harvest treatments.

Step 5: Single-cell protein processing to make the biomass edible. (Abdullahi 2021).

Media Preparation

Growth media for food wastes can be prepared by 3 possible ways.

1) By washing the food waste by distilled water after that those are dried, grinded, and sifted and finally blended with distilled water and after filtration growth media is prepared.
2) In the next way peels are washed with 2% H_2SO_4 and rinsed with distilled water. Pieces are cut to reduce residue sizes and pulverized and filtered to prepared media
3) And the last and final what is acid hydrolysis of thermal pretreatment of filtrate, pH adjustments are done to prepare media.

(Abdullahi, N., Dandago, M.A., & Yunusa, A.K. (2021).

Sterilization of Media

Growth media are sterilized at 121 °C for 15 minutes in an autoclave before inoculation (Kapilan *et al*., 2018; Mahan *et al*., 2018). While sterilizing media can enhance the growth of the desired microorganism, it is not always necessary. Gervasi *et al*. (2018) reported successful biomass production in a medium made of food waste mixtures without any thermal or chemical pre-treatments.

Selection of Suitable Strain

In Single Cell Protein (SCP) production, inoculums are often isolated from food and the environment. Oshoma *et al*. (2017) isolated *Aspergillus*

niger from spoiled onion bulb. The commonly used *Saccharomyces cerevisiae* can be isolated from sources like palm wine (Mensah and Twumasi, 2017) and rotten tomatoes (Milala *et al*., 2018). This yeast, when isolated from bread, yields biomass rich in essential amino acids (Ahuja and Kumari, 2019). It has also been grown using bees' fruit peel (Haddish, 2015) and sugarcane bagasse (Samadi *et al*., 2016). *Trichoderma viride* can be sourced from soil (Anichebe *et al*., 2019), along with *Candida lusitaniae* (Shete and Raut, 2018) and *Raoultella ornithinolytica* (Al-Hadithi *et al*., 2018). *Cyberlindnera* sp., a type of Torula yeast, performed well in a banana peel medium with (NH4) $2SO_4$ as nitrogen (Jiruand Melku, 2018). Researchers have also used pure cultures for SCP production, such as *Aspergillus oryzae*, *Trichoderma koningii*, and *Candida tropicalis* from orange waste (Zhou *et al*., 2019), and bakers' yeast from bananas (Kapilan *et al*., 2018). These inoculums are typically grown on general-purpose media like nutrient agar or potato dextrose agar before being introduced to the production medium (Samadi *et al*., 2016; Ahuja and Kumari, 2019). The quality of SCP is influenced by the interaction between different fermentation organisms (Zhou *et al*., 2019), and factors such as inoculum size, age, incubation period, temperature, and moisture to substrate ratio (Yunus *et al*., 2015).

Incubation and Fermentation

To produce biomass, a microbial colony is introduced into the growth medium following autoclaving and, if required, enrichment. Temperatures between 28 and 37°C are ideal for fermentation, and incubation times vary depending on the organism and begin at 48 hours (Rajendran *et al*., 2018; Milala *et al*., 2018; Ahuja and Kumari, 2019). By affecting the lag phase (Robinson *et al*., 2001), enzyme synthesis (Dinarvand *et al*., 2017; Ilgın *et al*., 2020), and detection time (Bidlas *et al*., 2008), inoculum level have an impact on the proliferation of microorganisms (Koutsoumanis and Sofos, 2005; Xu *et al*., 2020). For best development, lower inoculum levels need greater pH and water activity (Skandamis *et al*., 2007). Fermentation and SCP protein concentration are affected by the inoculum's size and age (Reihani and Khosravi-Darani, 2019). Overexposure to inoculation does not raise sizes more than 5%. There was no discernible difference in *Rhodopseudomonas faecalis* biomass at 20% and 30% inoculum levels, according to Patthawaro and Sae Jung (2019). Using a 48-hour-old culture, Yunus *et al*. (2015) found that the highest protein production was obtained from *Candidautilis* and Rhizopus oligosporus biomass cultivated in wheat branat 10% (v/w) inoculum size. Munawar *et al*. (2010) discovered that during submerged fermentation, an inoculum size of 4% (v/v) is ideal for

producing protein in Candida utilis culture in fruit waste extract. At a 7.5% (v/v) inoculum size, Mahat and Mac Rae (1992) produced the most *Rhizopus oligosporus* mycelium with a higher protein content. The ideal inoculum level of acid-tolerant *Methylocaps acidophila* was 20%, according to Xu *et al*. (2020). Enzyme production during solid-state fermentation is likewise strongly impacted by inoculum size (Kosseva, 2013). Alam *et al*. discovered.

Harvesting

The fermented broth is centrifuged for 20 minutes at 4000-6000 rpm following incubation. Following drying, the crude protein content of the collected sediments is examined (Samadi *et al*. 2018). Harvesting and purification are still difficult despite improvements in SCP production (Valverde-Pérez *et al*., 2020). Valverde-Pérez *et al*. (2020) investigated the use of forward osmosis for dewatering methanotrophic biomass. The micro Kjeldahl technique (Milala *et al*., 2018; Jiru and Melku, 2018; Rajendran *et al*., 2018) or Lowry's method (Ahuja and Kumari, 2019) are used to determine the protein content of collected biomass.

Application

1) Provides instant energy.
2) Promotes healthy eyes and skin.
3) Excellent protein supplement for undernourished children.
4) Rich in vitamins, amino acids, minerals, and crude fiber.

Uses in Natural and Therapeutic Medicine

1) Reduces obesity.
2) Lowers diabetic blood sugar levels.
3) Lowers Stress, cholesterol, and body weight.
4) Prevents the buildup of cholesterol.

Cosmetic Uses

1) Maintain healthy hair.
2) Used Herbal Beauty Products like bio lipsticks and face creams.

Poultry Uses

1) Convenient source of protein and nutrients for cattle, birds and fish.

Other Uses

- Microorganisms grow rapidly, doubling biomass in 30 minutes under optimal conditions.

- Produces high-quality protein superior to that of animals and plants.
- Easy genetic manipulation allows for diverse amino acid compositions.
- Uses waste products as substrates, reducing pollution.
- Simple fermentation and culture conditions.
- Unaffected by environmental factors.

Commercial products of SCP

1) Microalgal Flour-used for excess protein and carbohydrates content.
2) Capsule- Chlorella, spirulina, Haematococcus pluvial, are dietary supplements to increase nutrients content.
3) Dairy drinks- Euglena used in fermentation of yogurt as it's excellent stability in low pH beverage.
4) Juice powder-powder enriched with arthrospira aquae improves nutritional content.

Conclusion

As a substitute to animal protein, SCP can offer premium protein at a reasonable price. The most often employed microbe for SCP production is Saccharomyces cerevisiae, which thrives on waste and low-value substrates. Protein can make up more than 60% of this organism's biomass, coupled with other elements including minerals, lipids, and carbs. However, the usage of SCP may be restricted due to its poor digestion and high nucleic acid concentration. Some People May also experience allergic responses. space generation persistent bacteria during biomass drying is a less well-known problem.

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