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CONTENTS

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Review

Application of Antioxidants in Food Processing Industry: Options to Improve the Extraction Yields and Market Value of Natural Products

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ABSTRACT

Antioxidants are substances that are capable of slowing down the autoxidation process of other compounds or neutralize free radicals. They have been used in food processing industries as a means to hinder oxidation, enhance flavor, aroma and color. Antioxidants have also been used and valued for treatment of various diseases such as cancer and coronary heart disease. Even though, synthetic antioxidants including butylated hydroxytoluene (BHT) may cause side effects to human health and presumed unsafe to be used, they are the ones in a great use in the area of food processing industries as most food and pharmaceutical products contain them. The objective of this review work is therefore to provide an overview of the findings related to the presence of antioxidants in plant sources particularly those that have not been extensively studied and evaluated such as fruits and vegetables by-products. To minimize their effects, researches have been conducted aiming to substitute them with antioxidants from natural sources. Recent studies show that synthesis of natural antioxidants from fruit and vegetable waste has gained great attention. Further research has to be performed on plant phenols and processing of agricultural and industrial by-products as a potential source for extraction of antioxidants. In order to increase the affirmative effect and usefulness of antioxidants to human health, it is recommended to follow a balanced and varieties of diets instead of taking antioxidant supplements on a regular basis. Therefore, we should consume a diet high in antioxidant rich fruits and vegetables day by day. Furthermore, nutritional importance, promotion of health and prevention against damages caused by free radicals can lead to the potential applications of antioxidants in food industries in more intensified approaches. In a nutshell, antioxidant foods and ingredients are an important component of the food industry and thus reconsidering the health implications of adding antioxidants to foods require unfathomable investigations.

Keywords

Antioxidants; Free radical; Food processing; Plant phenols; By-products; phytochemicals.

Abbreviations

BHT: Butylated hydroxytoluene; BHA: Butylated hydroxyanisole; PG: Propyl gallate; DG: Dodecyl gallate; ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species.

INTRODUCTION

Antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property.[1] These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol, and uric acid, are produced during normal metabolism in the body.[2] Other lighter antioxidants are found in the diet. Although there are several enzymes system within the body that scavenges free radicals, the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid),

and ß-carotene.[3] The body cannot manufacture these micronutrients, so they must be supplied in the diet.

In the 20th century, antioxidants entered in the widely emerging food industry as an important means to limit the degradation of stored foods as a result of oxidation process.[2] Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radicals are any chemical species capable of independent existence with one or more unpaired electrons in their outermost shell, which seek out and capture electrons from other substances to achieve neutrality.[3,4] An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules or neutralize free radicals.[5]

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can be damaging to cells since they can produce free radicals which initiate chain reactions, leading to membrane and other lipid per oxidation, DNA damage, etc,.[6] Antioxidants ends these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.[7] Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin E, α-carotene, selenium and polyphenol as well as enzymes such as catalase, superoxide dismutase and various peroxidases.[1,8] Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains, coffee, tea, wine, herbs, spices and some meats, poultry and fish.[9,10]

In the recent years, considerable research has been carried out evaluating natural substances as antioxidative additives in food products, leading to novel combinations of antioxidants and the development of novel food products. In addition to their antioxidative capacity, these natural additives have positive effects on the human body with documented health benefits.

CLSSSIFICAION OF ANTIOXIDANTS

In nature, various antioxidants are commonly found in food products. Literature suggests the availability of a wide range of antioxidants and their classifications based on where they perform their activities, their mode of action (preventive and scavenging), their background and their biochemical characteristics.[11] Natural antioxidants, synthetic antioxidants, dietary antioxidant and endogenous antioxidant are identified as the most common antioxidants and play an important role in preservation of food.[8] Antioxidant also can be mostly classified into enzymatic (Superoxide dismutase, catalase, glutathione systems) and non-enzymatic (ascorbic acid, glutathione, melatonin, tocopherols and tocotrienols (vitamin E), uric acid).

Dietary Antioxidants

Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties.[12-14] Vitamin C, vitamin E, ß-carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein are among the most widely studied dietary antioxidants.[8]

In extracellular fluids vitamin C is considered as the most important water-soluble antioxidant. It is capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid per oxidation is initiated. Vitamin E, a major lipid soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid per oxidation. It has been cited that vitamin C is capable of regenerating vitamin E.[15]

ß-carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests that ß-carotene may work synergistically with other vitamins.[16] In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers". Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity.[8]

Endogenous Antioxidants

In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes-glutathione peroxidase, catalase, and superoxide dismutase (SOD)-metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary in take of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms.[16] Glutathione, an important water-soluble antioxidant, is synthesized from the aminoacids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism.[17]

Lipoic acid, yet another important endogenous antioxidant, categorized as a "thiol" or "biothiol," is a sulfur-containing molecule that is known for its involvement in the reaction that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha ketoglutarate, in the Krebs cycle.[8]

Exogenous Antioxidants

Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds).[16] There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs.[8]

Synthetic Antioxidants

Synthetic antioxidants are those antioxidants do not occur in na-

ture but chemically synthesized and added to food products as preservatives to help prevent lipid oxidation.[18] In order to have a standard antioxidant activity measurement system to compare with natural antioxidants and to be incorporated into food, synthetic antioxidants have been developed. These pure compounds are added to food so it can withstand various treatments and conditions as well as to prolong shelf life. Today, almost all processed foods have synthetic antioxidants incorporated, which are reported to be safe.[19] Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the most widely used chemical antioxidants.[11] Inconsistent data's have been published regarding the allowable daily intake and exposure to some synthetic antioxidants. Furthermore, contradictory data are available relating to the effect of synthetic antioxidants on human health. Therefore, further research has to be performed in this regard. Some of the synthetic antioxidants currently permitted for use in foods include BHT, BHA, propyl gallate (PG), dodecyl gallate (DG) and tertiary butylhydroquinone (TBHQ).[18,20]

Natural Antioxidants

Natural antioxidants are those oxidants that are found in natural sources, such as fruits, vegetables and meats.[21] Natural antioxidants can be found in all plant parts such as fruits, vegetables, nuts, seeds, leaves, roots and barks.[21,22] There are several common natural antioxidants which are found in everyday foods, the most common of which being vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin A (carotenoids), various polyphenols including flavonoids, anthocyanins, lycopene (a type of carotenoid), and coenzyme Q_{10}, also known as Ubiquitin, which is a type of protein.[8] Natural antioxidants are synthesized by plants (e.g. vitamins and other naturally-occurring chemicals in our food). Natural antioxidants are found in most fresh foods.[23]

Table 1. *Sources of Some Natural Antioxidants*[22,25]

Compounds	Natural Source
Carotenoids	Dark leafy vegetables, carrots, sweet potatoes, yams, tomatoes, apricots, citrus fruits, kale, papaya
Catechins	Green tea, berries, certain oilseeds
Flavonoids (polyphenols)	oilseeds, lettuce, berries, eggplants, peppers, citrus fruits, cruciferous vegetables, onions, black tea
Lycopene	Tomatoes, papaya, watermelon, guava,
Phenolic acids	Oilseeds and certain oils, cereals, grains
Vitamin C	Fruits and vegetables, berries, citrus fruits, green peppers, potatoes.
VitaminE(tocopherols)	Oilseed, palm oil, nuts, eggs, dairy products, whole grains, vegetables, cereals, margarine, etc.
Extracts	Extract from green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran

MAIN SOURCES OF NATURAL ANTIOXIDANTS FROM FOODS

Plants provide rich natural antioxidants. Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish.[9] Natural antioxidants are present in plants (Table 1), and this is why the basic source of these compounds for humans is plant-derived products.[22] Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds such as vitamins A, C and E, ß-carotene and important minerals.[24] There are wide variations between the total phenolic contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different authors.[20] The human antioxidant system is divided into two major groups, enzymatic antioxidants and non-enzymatic oxidants.[25,26]

Enzymatic Antioxidants

Enzymatic antioxidants further divided into primary and secondary enzymatic defenses. With regard to the primary defense, it is composed of three important enzymes that prevent the formation or neutralize free radicals: glutathione peroxidase, catalase and superoxide dismutase.[26] The secondary enzymatic defense includes glutathione reductase and glucose-6-phosphate dehydrogenase.[27,28] These two enzymes do not neutralize free radicals directly, however, they may contribute to the activity of other endogenous antioxidants.

Non-Enzymatic Antioxidants

The non-enzymatic antioxidants are actually the scavengers of ROS and reactive nitrogen species (RNS); these involve peptides (glutathione); vitamin E and C (inhibits oxidation of membrane lipid); nitrogen compounds such as uric acid, which is a natural scavenger of peroxynitrite in plasma; albumin; bilirubin; N-Acetylcysteine (NAC); melatonin which directly reacts with ROS and form disulfides.[19,29,30]

ANTIOXIDANTS MECHANISM OF ACTION

The possible mechanisms of action of antioxidants were first explored when it was recognized that substance with anti-oxidative activity is likely to be the one that itself readily oxidized. An antioxidant can be defined as: "*any substance that, when present in low concentrations compared to that of an oxidizable substrate, delays or inhibits the oxidation of that substrate*".[31,32] A free radical can be defined as, "*any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital and capture electrons from other substances in order to neutralize themselves*".[33] The existence of an unpaired electron results in certain common properties shared by most of the radicals.[23]

Two principal mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system, example lipid radicals.[34] Chain-breaking antioxidants act by scavenging free radicals and donating hydrogen atoms.[35] The second mechanism involves removal of ROS and RNS initiator by quenching chain initiator catalyst.[36] Preventative antioxidants are generally metal chelators and reductants capable of sparing other antioxidants *in vivo*.[35] These reactive species are capable of causing damage to the vital biological molecules such as deoxyribonucleic acid (DNA), proteins, carbo-

hydrates, and lipids[37] and resulted in a homeostatic disruption.[23]

Chain Reactions of Free Radicals

The mechanism of chain reactions can be divided into the three stages: initiation, propagation and termination.[32,38]

On the first stage of oxidation reaction from biological systems RH are formed radicals $R^{\cdot}$ as a resultof abstraction of a hydrogen atom $H^{\cdot}$:

Initiation Stage:

(1) $RH \rightarrow R^{\cdot}+H^{\cdot}$
(2) $R^{\cdot} \rightarrow R^{\cdot}+O_2 \rightarrow ROO^{\cdot}$
(3) $2ROOH \rightarrow ROO^{\cdot}+RO^{\cdot}+H_2O$

After initiation, propagation of the free radical chain occurs, in which molecule of oxygen from environment react with reactive radical species, resulting in formation of peroxides and peroxyl radical $ROO^{\cdot}$. These intermediates may further propagate free radical reactions:

Propagation Stage:

(1) $R^{\cdot}+O_2 \rightarrow ROO^{\cdot}$
(2) $ROO^{\cdot}+RH \rightarrow ROOH+R^{\cdot}$
(3) $RO^{\cdot}+RH \rightarrow ROH+R^{\cdot}$

In the last stages, interaction of two radicals may lead to formation of non-radical adduct and termination of free radical chain:

Termination Stage:

(1) $R^{\cdot}+R^{\cdot} \rightarrow R–R$
(2) $R^{\cdot}+ROO^{\cdot} \rightarrow ROOR$
(3) $ROO^{\cdot}+ROO^{\cdot} \rightarrow ROOR+O_2$
(4) Antioxidants$+O_2 \rightarrow$ Oxidized antioxidants[38,39]

Antioxidants can slow lipid oxidation by inactivating or scavenging free radicals, thus inhibiting initiation and propagation reactions.[32] The antioxidants function by the very simple and effective method of donating hydrogen atom to free radicals and thus terminating their life.[9]

Methodologies for the Quantification of Antioxidant Activity

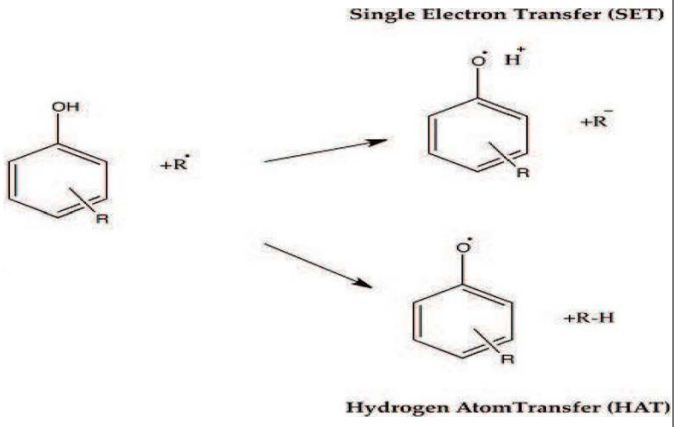
The measurements of the antioxidant activity can be carried out based on the information we want to obtain:

- Direct determination: a radical is used as a quantification factor (since it produces an analytical signal). In this sense, the addition of the antioxidant, before or after the generation of the radical, causes a decreasing in the signal (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) or (2,2-diphenyl-1-picrylhydrazyl (DPPH) methods), which is proportional to the antioxidant activity of the sample.
- Indirect determination: the presence of free radicals causes the loss or appearance of a reagent and therefore, in the presence of an antioxidant, an increasing or decreasing in the signal is caused (oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) methods) proportional to the antioxidant activity of the sample.

In that way, it is necessary to mention the differences between the free radical stabilizing activity or antiradicalaria (indirect methods) and the antioxidant activity (direct methods), the first being completely determined by the reactivity of an antioxidant against free radicals, characterized by reaction speed, while the second measures the ability to retard oxidative processes.[40] In this sense, the results of the antioxidant capacity measurement obtained by each of the methods do not always coincide, even among methods based on the same redox mechanism, there may be variations. Therefore, it is recommended that an assessment of the antioxidant capacity be carried out using more than one analytical technique and comparisons among results only be made when the same method has been used and samples have been obtained with the same solvents.[41,42]

In general, it has been suggested to combine FRAP and ABTS techniques.[43] This is because the use of the FRAP technique in combination with others such as ABTS and DPPH, allows to evaluate different interactions of the antioxidant compounds, expanding the knowledge about them, which is relevant in the exploration of the antioxidant properties of nutraceutical products from natural sources or simply from some products included in the diet, such as fruits and vegetables.[44] When two techniques are used, as mentioned above, is generally sought that through one of them, is possible to determine the antioxidant activity based on transfer reactions of one electron single electron transfer (SET) and on the other, this same property is determined based on a transfer reaction of a hydrogen atom transfer (HAT for its acronym in English) between an antioxidant and a free radical, allowing to evaluate the two mechanisms to extend the spectrum of the results obtained.[45] In Figure 1, the HAT and SET mechanisms are showed.

Figure 1. *Mechanisms of Antioxidant Reacting with Free Radical: Single Electron Transfer (SET) and Hydrogen Atom Transfer (HAT)*



Regarding the expression of results of antioxidant capacity, several methods (FRAP, ABTS and ORAC) express the results in µmol Trolox/g of sample on dry or wet basis (Trolox is a water-soluble analog of vitamin E). Likewise, these results can be expressed in terms of vitamin C and E. In summary, a suitable method for the quantification of antioxidant activity should consider the electron transfer and hydrogen atoms reaction, establish the oxidation substrate, ensure that the substrate and how to induce oxidation, became relevant in terms of oxidative damage, be simple, have a mechanism and a denied endpoint, use available and affordable instrumentation, be reproducible, be adaptable to measure hydrophilic and lipophilic antioxidants, use different sources of free radicals with relevant biological characteristics and be adaptable for routine large-scale analyzes.[46]

This is increasingly important, since is known that no single method reflects the total antioxidant capacity of a sample, that is, its ability to act as an antioxidant of lipophilic and hydrophilic compounds through specific mechanisms, in addition to its reactivity against different species.[47] In addition, is known that the antioxidant activity of a sample is not only given by the sum of the antioxidant capacities of the components present in it, but also depends on the synergistic and inhibitory effects that may exist among compounds.[42] Table 2 summarizes the principal methods to quantify the antioxidant activity.[48]

Table 2. *Principles of the Most Common Methods Used to Quantify Invitro Antioxidant Activity*[47]

Principle	Method
Metal reduction	Ferric ion-reducing antioxidant power (FRAP)
Peroxyl radical absorption capacity	Oxygen radical absorbance capacity (ORAC) Total radical-trapping antioxidant parameter (TRAP)
Hydroxyl radical absorption capacity	Deoxyribose assay
Capacity for radicals' absorption generated from certain organic molecules	2,2'-azinobis acid (3-ethylbenzothiazolin)-6-sulfonic (ABTS), radical 2,2 diphenyl-1-picrilhydrazil (DPPH)
Quantification of products generated during the lipid peroxidation	Tiobarbituric acid reactive species (TBARs), oxidation of LDLs

Synergism in Lipid Oxidation

Synergism occurs when a mixture of antioxidants produces a more pronounced activity than the sum of the activities of the individual antioxidants when used separately. Synergism improves the efficiency of antioxidants. Synergist antioxidants can be classified as oxygen scavengers or chelators.[30] To have maximum efficiency, primary antioxidants are often used in combination withother phenolic antioxidants, or with various metal chelating agents.

Kinds of Metal Chelators and Free Radicals

Metal chelators deactivate trace metals that are free or salts of fatty acids by the formation of complex ion or coordination compounds like phosphoric acid, citric acid, ascorbic acid and ethylenediaminetetraacetate (EDTA).[1] Antioxidants normally neutralize the free radicals by being oxidized themselves and act as reducing agents such as thiols, ascorbic acid, or polyphenols.[33]

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system.[49] Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

In a nutshell, free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states (Figure 2). A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases.[29] Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health. Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years.[49]

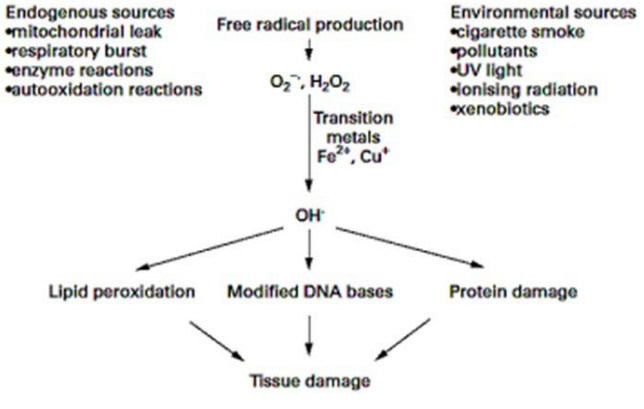


Figure 2. *Sources of Free Radicals and Its Consequences in Human Body*[23]

PROCESSING OF ANTIOXIDANTS

Although antioxidants are abundant in nature, only a limited number of raw materials such as vegetable oils and fats and rosemary leaves are used for manufacturing extracts with antioxidant activity.[50] The demand for natural antioxidants resulted in a reduced use of synthetic antioxidants as they may result in toxicity, carcinogenicity or hepatotoxicity in human body.[51] Since synthetic antioxidants including BHT may cause side effects to human health, it is presumed to be unsafe for consumption and/or for medical purposes.[42] Thus, research's has been conducted in minimizing synthetic antioxidants undesirable health consequences.

Recent study's shows that synthesis of natural antioxidants from fruit and vegetable waste has gained a great attention. Processing of fruits, vegetables, and oilseeds result in high amounts of waste materials such as peels, seeds, stones, and oilseed meals.[43] Disposal of these materials usually represents a problem that is further aggravated by legal restrictions.[20] Moreover, valuable nutrients contained in agro-industrial wastes are lost. Thus, new aspects concerning the use of these wastes as by-products for further exploitation on the production of food additives or supplements with high nutritional value have gained increasing interest because these are high-value products and their recovery may be economically attractive.[43]

Fruit, vegetable, and oilseed processing result in various amounts of by-products depending on the raw material. According to Oreopoulou V et al[43] out of the total fruits and vegetables processed worldwide almost half of it is estimated to be discarded as a waste presented some typical yields of antioxidant content in various fruit and vegetable by-products Table 3.

Table 3. *Antioxidant Content in Various By-Products*[43]

By-product	Phenol content (g/kg dry matter)	References
Apple pomace	2.4	44
Orange peel	1.8 [a]	45
	2.4[b]	46
Lemon peel	13.3[c]	47
	1.9[a]	45
Grapefruit peel	1.6[a]	45
	2.4[d]	46
Grape pomce	13.8[e]	48
Potato peel	7.8[g]	49
Red onion scale	105.5[f]	49
Sunflower hulls	97.5[f]	49
Buckwheat hulls	39.0[f]	49
Durum wheat bran	27.7[g]	50
Oat hulls	0.6	51

a-In fresh peel, expressed as g=kg of fresh fruit; b-Hesperidin (g=kg) in the fresh fruit; c-Flavonoids (eriocitrin and hesperidin); d-Naringin (g=kg) in the fresh fruit; e-Expressed as gallic acid; f-Expressed as ferulic acid; g-Phenolic acids analyzed by HPLC

Agricultural and industrial residues are attractive sources of natural antioxidants.[50] It had been previously reported that these wastes and by-products of fruits and vegetables in food processing industry are an abundant source of antioxidant polyphenols or phenolic compounds. Some studies have already been done on by-products, which could be potential sources of antioxidants.[20] These peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolic compounds which have a wide range of actions including antioxidant, antimutagenic, cardio preventive, antibacterial and antiviral activities.[49] Use of waste as a source of polyphenols and antioxidants may have considerable economic benefit to food processing industries. Therefore a cheap, efficient and environmentally sound utilization of these huge agro-industrial wastes is needed.[52]

Antioxidants are recovered from different plant residuals by extraction process. Using extraction process crude extract of bioactive compounds will be recovered from fruit by-products.[42] There is different extraction process available so far. Solvent extraction, mechanical procedures, molecular distillation, heating in oil at high temperature and supercritical fluid extraction are among the methods used for extraction of antioxidants.[40] Figure 3 shows the utilization of agro-industrial by-products for extraction of antioxidants using organic solvent or water.

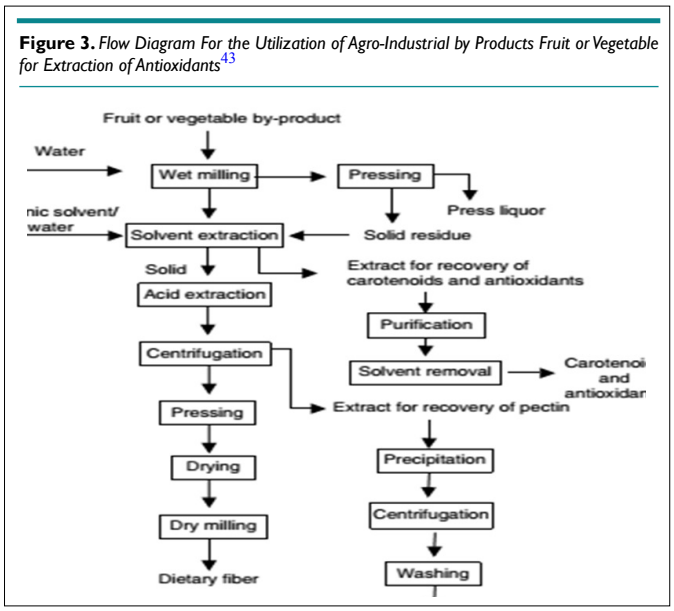


Figure 3. *Flow Diagram For the Utilization of Agro-Industrial by Products Fruit or Vegetable for Extraction of Antioxidants*[43]

Usually valuable natural materials have been extracted with organic solvents. However, some of them are toxic, and the extraction conditions are often severe. For this reason, in solvent extraction a food grade solvent is recommended to be used.[53] The effects of different parameters (conditions of the preparation of peel samples, repeated extraction, organic solvents used and their concentration, temperature, etc.) on the extraction process were investigated by different authors.[54] To this point, significant research has been done on the polyphenols obtained from grape marc, soya bean seed coat, potato peels, sugar beet pulp, etc. Besides these, search for newer sources of natural antioxidants from economical materials, agricultural wastes is hot area of research in recent years.[20]

APPLICATIONS OF ANTIOXIDANTS

Role of Antioxidants in Food Industries

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes.[17] The superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutase's catalyzing the first step and then catalases and various peroxidases removing hydrogen peroxide.[19] People in today's world want to eat healthier food to stay fit and this is being achieved by incorporating unsaturated and polyunsaturated fats in the food products being marketed. As the human lifestyle and also its view towards food are changing thus there is an increased shift observed from convenient foods to ready to-eat product category. For this there is need of certain potential health protecting factors named as antioxidants.[55]

Antioxidants, both natural and synthetic, have a wide applications in food industries as they are used as food additives in fats and oils to help prolong the shelf life and appearance of many foodstuffs.[23] Thus, efforts are being made to reduce oxidation by increasing addition of antioxidants to food. Lipid oxidation is a major cause of quality deterioration in many types of natural and processed foods. It is usually undesirable in most foods because it leads to the development of rancidity and potentially toxic reaction products. One of the most effective means of retarding lipid oxidation in foods is to incorporate antioxidants as preservatives.[23,56] Synthetic phenolic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) effectively inhibit oxidation. For example, chelating agents such as EDTA, can bind metals reducing their contribution to the oxidation process.[23,57]

The search for effective methods to retard oxidative processes in meat and meat products has led researchers to investigate natural antioxidants. Addition of antioxidants to meat and meat products is known to be effective in metmyoglobin formation and lipid oxidation.[58] These preservatives include plant phenols as natural antioxidants such as vitamins (ascorbic acid [AA] and α-tocopherol (E306)), many herbs and spices (rosemary, thyme, oregano, sage, basil, pepper, clove, cinnamon, and nutmeg), and plant extracts (tea and grape seed) contain antioxidant components thus imparting antioxidant properties to the compound.[55,57]

While use of synthetic antioxidants (such as butylated hydroxytoluene and butylated hydroxyanisole) to maintain the quality of ready-to-eat food products has become common place, consumer concern regarding their safety has motivated the food industry to seek natural antioxidants.[57] The antioxidants obtained from plants are more functional towards improving the shelf life of food products and providing health promotion when compared to materials whose antioxidants have been removed during processing.[23] Thus, current researches are under investigation on the various extraction technologies and processing for plant extracts to be used as antioxidant additives for food industries.

Medical Application of Antioxidants

Antioxidants have important preventive roles not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases. They are effective in prevention of degenerative illnesses, such as different types of cancers, cardiovascular and neurological diseases, cataracts and oxidative stress dysfunctions.[6,59] Chronic diseases such as arteriosclerosis and cancer, which are the leading causes of death in the Western world, are likely to be mediated by free radical and lipid per oxidation mechanisms.[35] Antioxidants have been investigated and reported to play a specific role in the treatment of these diseases/disorders.[55]

Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials. Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity.[6]

Various research studies conducted so far have confirmed the role of antioxidants, viz., lanthanides, selenium, flavonoids, lycopene and glutathione as anti-cancerous compounds in bio-coordination chemistry. Recent developments in medicinal chemistry have become crucial for improving the design of the compound, reducing toxic side effects and understanding their mechanism of action.[55] Oxidants may play role in many diseases.[60] In the last decades, several epidemiological studies have shown that dietary intake of foods rich in natural antioxidants was correlated with reduced risk of coronary heart disease.[61] Dietary and natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic or health effects.[62,63]

Industrial Application

Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasoline to prevent the polymerization that leads to the formation of engine-fouling residues. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials.[57]

Nowadays, most food and pharmaceutical products contain synthetic antioxidants. These compounds are added to food in order to prolong product shelf life, mainly by preventing the oxidation of unsaturated double bonds of fatty acids. In pharmaceutical products antioxidants are added to enhance the stability of therapeutic agents that are susceptible to chemical degradation by oxidation.[64]

Adv Food Technol Nutr Sci Open J. 2019; 5(2): 38-49. doi: 10.17140/AFTNSOJ-5-155

Applications of plant extracts with antioxidant activity in food processing were investigated and more are under investigation in their uses for health paybacks, conservation processes improvement and shelf-life extension. The plant source and industrially recovered antioxidants can be incorporated into various food matrices including meats, oils, fruits, vegetables, spices and condiments, root crops, pulses and cereal products. Research and development on the application of plant origin extracts with respect to antioxidant activity in food processing requires outcomes for amble sources of antioxidants, innovative methods of extraction technologies, application techniques with their respective thresh hold level, successive effects and regulatory aspects to improve their oxidative stability.[63]

NATURAL ANTIOXIDANTS UTILIZATION IN THE AFRICAN CONTEXT

Being located within the tropical and sub-tropical climates has made Africa rich in enormous biodiversity resources. In Africa there is a vast amount of vegetables, fruits and mushrooms which are consumed for food and their medicinal purposes.[65,66] The use of traditional medicines prepared from plant sources is well known in different parts of the continent. This plant sources are widely used for preparing remedies in the form of alternative medicine. Antioxidants are particularly important among the numerous compounds found in plants because they might serve as precursors for the development of novel drugs.[11] Africa has many diverse varieties of plant resources like fruits and vegetables, spices and many other types of fruits and vegetables, which can be processed and utilized as a source of antioxidants.[65,67] Studied and evaluated some of African medicinal plants for their antioxidant activities. According to their study, considering the enormous biodiversity resource that Africa has, the attention given to the utilization of these resources is still rather very low.

The utilization of industrially processed natural antioxidants in the Africa context is very limited compared to utilization of imported synthetic antioxidants in the area of food processing industries application.[36] Consequently, there is a need for the stakeholders to engage and give a due attention towards the valuable plant resources present in the African continent. Top urgently, need to start in evaluating the potential utilization and processing of its enormous biodiversity resources as a potential source for natural antioxidants manufacturing in to the growing food industry.[67] This will help Africa to save a huge cost that has been invested for import of synthetic antioxidant in food industries and help the continent in a fight to disease and poverty. The use of natural antioxidants in food products of plant and animal origin is one of the new and intensive research areas.

Ethiopia is one of the major countries with a wide distribution and high potential for plant sources. Even though few studies have reported the nutritional value of various indigenous food ingredients consumed in Ethiopia, only limited information is available regarding their phenolic content and health beneficial properties.[68] Furthermore, there is shortage of organized information regarding antioxidants and related issues in Ethiopia.[69] Moringa tree (*Moringa Stenopetala*) is among the medicinal plant associated with naturally occurring antioxidants. *Moringa stenopetala* is widely used for treatment of high blood pressure, diabetes and also heart problems in the country. However, nowadays there are some emerging research works on antioxidant in Ethiopia.[69]

INFLUENCE OF NATURAL ANTIOXIDANTS IN HUMAN HEALTH

Normal molecules in the body have two (a paired group) electrons in their outer shell. A molecule with a single electron (unpaired) in its outer shell is called a free radical. Antioxidants are important in living organisms as well as in food because they may delay or stop formation of these free radicals by giving hydrogen atoms or scavenging them.[11] The destructive effects of free radicals can be prevented with the addition of antioxidants in the diet or by antioxidant supplements.[70]

When a specific antioxidant meets a free radical in the bloodstream at its appropriate activity site, it naturally combines with it and coverts the free radical to harmless water and oxygen. As a result, as antioxidant increases due to the supplementation of higher amounts of a greater variety of anti-oxidants, cellular damage lessens and performance and health improves.[9]

The antioxidants that have caused health problems, for some people, are primarily synthetic. The most problematic antioxidants appear to be BHA, BHT and TBHQ, with gallates in second place and have been used in food products, with some restrictions, since the late 1950s. Asthma, angioedema, dermatitis, excessive sweating, joint pains, stomach and eye problems are among the health problems in humans that have been linked with adverse reactions to BHA, BHT and/or TBHQ.[23,71] New data indicating that the synthetic antioxidants used in the industry could have carcinogenic effects on human cells resurface every year. Thus, the search for effective, non-toxic natural compounds with antioxidant activity has been intensified in recent years.[64,72,73] Since there are recent studies on the effect of antioxidants on human health reveal that some of currently available data's may not be true as they supposed or contradict with current findings. Thus, the positive effect of antioxidants in human health should be investigated intensively.[74]

FUTURE PERSPECTIVES ON NATURAL ANTIOXIDANTS TO REDUCE DISEASE BURDEN IN AFRICA

During the past decades a lot of research has been carried out around antioxidants and their effects on health. However, there is still a lack of a standard procedure to determine antioxidant activity across the majority of matrixes in order to produce consistent and undoubted results. The published results so far are conflicting and difficult to compare between each other. The antioxidant limitations and metabolism still pose a challenge to future research in this field, and researchers must try and overcome these drawbacks.[19] The level of intake (threshold level) of antioxidant nutrients desirable for optimal nutrition is still an open question, and there is little information on antioxidant bioavailability in vivo in humans.[40,75] Furthermore, to develop a more complete framework for the relevance of antioxidants for the ex-

pression of life history traits and trade-offs, it is suggesting that future studies should consider the full range of available antioxidants, their possible interactions, their environmental availability, and the potential for inter individual differences in anti-oxidant intake and uptake.[2,76] The current African traditional practices for food and medicines utilization of exotic plant resources as antioxidants shall be supported by evidence based research and development to support millions of Africans who has the resources in hand. Extraction and processing of antioxidants from African plant resources are expected to come *via* turning research into impact, and establishment of functional and nutraceutical industries for the same purpose to save millions from the current chaos.

CONCLUSION

Current research carried-out in the field of increases knowledge about naturally healthy compounds that are available in agricultural produces. Advance research has to performed on processing and utilization of agricultural and industrial by-products as a potential source for extraction of antioxidants. Investigations should be done on determination of threshold level of intake for antioxidants and related possible side effects.

The present review likewise provides a brief overview on oxidative stress mediated cellular damages and role of natural dietary antioxidants as functional foods in the management of human diseases. The imminent research focus can identify possible application of natural antioxidants, the most important; they can recognize them as an alternative to replace chemical synthesized antioxidants used in food industry improving the market of natural products.

The forthcoming research can focus on promoting the use of natural extracts and fulfilling consumer demands for healthier foods. Natural antioxidants use in food products will increase quality and added value. Hence, novel methodologies of extraction, purification, identification and quantification of natural antioxidants using environmentally friendly techniques need to be developed to improve the extraction yields and market value of natural products.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Brief Research Report

Evaluation of Different Varieties of Pea under Agro-Climatic Conditions of Gilgit-Baltistan

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ABSTRACT

Introduction
Pea (*Pisumsativum* L) is an important crop which is used both as food and fodder purpose. Peas are highly nutritious; pea is used both as vegetable and pulse.

Materials and Methods
Five pea varieties were evaluated for their performance at mountain agricultural research centre (MARC), Juglote, Gilgit during 2017. The varieties are climax, pasan, rondo, meteor and green feast.

Results
Differences among plant heights of pea varieties were significant with maximum plant height of 85.25 cm noted in plots of variety climax, while the minimum plant height of 45.75 cm was recorded in plots of variety rondo. Number of branches per plant of the different varieties ranged significantly from a minimum of 2.15 (green feast)to a maximum of 4.00 (meteor). Average number of pods per plant varied significantly between 10.84 and 13.71.Maximum pods per plant were found from variety climax (13.71) followed by green feast (13.58). Maximum pod length of 7.900 cm was recorded for variety Rondo, followed by variety green feast with pod length of 5.800 cm, while the minimum of 5.220 cm was recorded for the verity meteor. Maximum pod weight 26.29 kg per plot was obtained from variety climax, followed by 21.75 kg per plot from variety green feast, the minimum pods weight was recorded for variety meteor 16.20 kg per plot. Difference in pods yield of the five varieties were significant, with a maximum pods yield of 3585 kg per hectare recorded for variety climax, followed by 3545 kg per hectare for cultivar green feast and minimum of 2491 kg per hectare noted for variety rondo.

Conclusion
Therefore, variety climax being the highest yielder can be recommended to the pea growers of Gilgit-Baltistan for commercial cultivation.

Keywords
Evaluation, Pea, Varieties, Yield, pod, Gilgit, Baltistan.

INTRODUCTION

Pea is grown throughout the world for diverse uses as food and fodder. Well drained clay loam or silt loam soil with a pH range of 6-7.5 is better for pea, but it does not tolerate excessive acidity. Peas are highly nutritious and are a rich source of digestible protein (27.8%) along with carbohydrates (42.65%), minerals (calcium, phosphorus), vitamins, dietary fibers, antioxidant and sugars (5.67 g/100g) edible portion.[1] Pea is used both as vegetable and pulse. It can also be used in soups, canned, processed or dehydrated and can be consumed during offseason.

In Pakistan, the pea is an important crop, which plays a major role in the farmer's economy. It is the most common crop and enjoys a great commercial demand due to its nutritive value. It is cultivated during winter in plains and during summer in high-

lands.[2] It represents about 40% of the total trade in pulses. In 2011-2012, the crop was grown over an area of 15.8 thousand hectares with 105 thousand tonnes production of green pea and average yield was 166 mounds ha^{-1}.[3] In Pakistan, it is cultivated under an extensive range of agricultural regions, but the average yield per hectare is very low as compared to its potential and yield obtained in many other countries.

As compared to many other countries, the average yield of pea crop is very low in Pakistan which may be attributed due to the non-adoption of improved varieties. Santalla et al[4] have also reported that variability in old, unimproved varieties needs to be determined in order to create useful genetic variation for broadening the narrow genetic base of commercial cultivars and for making efficient use of available resources. The other factors like non-usage of recommended agronomic practices, application of improper fertilizer doses; diseases and harvesting losses also play an important role in yield reduction. According to Khan et al[5] the main hurdle in the way of increasing per hectare pea production is the weed competition. Sometimes season long crop-weed competitions reduce the green pod yield by up to 45-55%.[6] In addition to these, environmental factor such as rainfall also affects yield. McPhee and Muehlbauer have also reported that seed yield in pea is highly dependent on the environment and is particularly responsive to the amount and distribution of precipitation received during the growing season. Gupta et al[7] have also reported that seed yield in pea is highly dependent on environment and is particularly responsive to the amount and distribution of precipitation received during the growing season[8] have also reported about existence of considerable amount of genetic variability in pea. Keeping all these issues in view, present research work was designed to evaluate the available material for yield other agronomic traits under agro-climatic conditions of Gilgit-Baltistan. Based on our findings, high yielding variety will be recommended for the commercial cultivation of pea in Gilgit-Baltistan.

MATERIALS AND METHODS

The present investigation was carried out at experimental farm MARC Juglote Gilgit during 2017 to evaluate the suitable variety for the commercial cultivation of pea in Gilgit-Baltistan (Table 1). The experimental plot was laid out in randomized complete block design with four replications. The varieties used were climax, pasan, rondo, meteor and green feast. The seeds were sown on 1st week of March in a well-prepared bed size of 5 x 3 m^2. Row to row and plant to plant spacing was maintained at 45 x 20 cm. All the standard agronomic practices were followed throughout the growing season and recommended a dose of fertilizer was applied for the better nourishment of plants. The data were recorded during the mid of May and five plants were randomly selected for taking data. Observations were recorded on the basis of plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, pods length, pods weight, and pods yield kg ha^{-1}. The recorded data were subjected to the analysis of variance technique and the significant means were subsequently separated by the lysergic acid diethylamide (LSD) test Steel and Torrie (1984). The material required for trial was collected from vegetable program National Agricultural Research Center (NARC), Islamabad.

Table 1. *Physiochemical Properties of the Experimental Soil at MARC, Juglote, Gilgit*

Parameter	Value
pH	7.84
Electrical Conductivity (EC)	0.45
Organic Matter (OM)	0.12
Nitrogen	0.09
P_2O_5	1.23
K_2O	85.60
Lime Content	5.74
Texture Class	Silt Loam

RESULTS AND DISCUSSION

Plants Height

Statistical analysis of the data revealed that differences in plants height were significant (Table 2). Maximum plants height (85.25 cm) was attained by the plants of variety climax (85.25 cm) followed by green feast (82.54 cm), while the minimum was recorded in rondo (45.75 cm). This variation in plant height could be due to variation in the genetic make-up of different varieties. Environmental conditions caused variation in the hormonal balance and cell division rate that result in changes in the plant height of the different varieties. The results of this study are in agreement with Srivastava et al.[9] Million also reported that significant variability existed for the traits studied in field pea genotypes and plant height is among those traits having positive and greater influence. Similar differences in plant height among different pea cultivars were reported by Gentry.[10]

Table 2. *Evaluation of Different Pea Cultivars Under Agro-Climatic Conditions of Gilgit-Baltistan*

Treatment	Plant height (cm)	No of branches $plant^{-1}$	No of pods	Pod length (cm)	Pod weight (kg $plot^{-1}$)	Podyield (kg ha^{-1})
Climax	85.25^{a}	3.00^{ab}	13.71^{a}	5.230^{b}	26.29^{a}	3585^{a}
Pasan	62.00^{bc}	2.50^{ab}	10.84^{ab}	5.500^{b}	21.53^{ab}	2667^{ab}
Rondo	45.75^{c}	2.75^{ab}	11.41^{ab}	7.900^{a}	16.72^{bc}	2491^{b}
Meteor	77.50^{ab}	4.00^{a}	8.89^{b}	5.220^{b}	16.20^{c}	2673^{b}
Green feast	82.54^{a}	2.15^{b}	13.58^{a}	5.800^{b}	21.75^{ab}	3545^{a}
LSD 0.05	17.84	1.711	3.934	1.205	5.188	755.7

Number of Branches

Statistical analysis of the data showed that differences in a number of branches per plant of different varieties were statistically significant (Table 2). Maximum of 4.00 branches $plant^{-1}$ was recorded for variety Meteor, followed by 3.00 branches $plant^{-1}$ for variety climax was recorded. Minimum of 2.15 branches $plant^{-1}$ was recorded for variety green feast. More flowering in some varieties with more number of branches is an indication of more vegetative growth due to climatic conditions. It was observed

that some varieties had determined type growth and their plants bloomed and exhaust simultaneously, hence they have fewer branches.

Number of Pods

Statistical analysis of the data revealed that differences in a number of pods were significant (Table 2). A maximum number of pods 13.71 was recorded for variety Climax followed by variety green feast with 13.58 pods plant^{-1}, while the minimum number of pods of 8.89 were recorded for variety meteor. This variation in number of pods also be attributed to variation in genetic make-up and adaptability of these varieties to different environmental conditions. Significant differences for varieties with respect to the number of pods per plant were also reported by Kumar et al, Singh et al, and Chadha et al.[11-13]

Pod Length

Statistical analysis of the data revealed that differences in the length of pods were significant (Table 2). Maximum pod length of 7.900 cm was recorded for variety Rondo, followed by variety Green feast with 5.800 cm, while minimum pod length of 5.220 cm was recorded for variety Meteor. This variation in length of pods also is attributed to variation in genetic make-up and adoptability of these varieties to different environmental conditions. Similar findings were also observed by Ashraf et al.[14]

Pod Weight

Statistical analysis of the data showed that differences in pod weight of the different varieties were significant (Table 2). Variety Climax ranked first maximum pod weight of 26.29 kg plot^{-1}, variety Green feast with 21.75 kg plot^{-1} stood second. Variety Meteor produced the minimum pod weight (16.20 kg plot^{-1}). A higher number of pods plot^{-1} is attributed to the higher pods weight. These results are in conformation with those of Kokhar et al, Hatam & Amanullah, Hussain & Badshah.[15-17]

Fresh Pod Yield

Statistical analysis of the data revealed that differences in fresh pod yield of the different varieties were significant (Table 2). Maximum fresh pod yield of 3585 kg ha^{-1} was recorded for variety Climax followed by variety Green feast with 3545 kg ha^{-1}. Minimum fresh pod yield of 2491 kg ha^{-1} was recorded for the variety Rondo, followed by 2667 kg ha^{-1} for the variety Pasan. The result could be due to the fact that Climax gave more plant height (85.25 cm), a number of pods (13.71) and pods weight (26.29 kg plot^{-1}) as compared to other varieties of pea. Yield is determined by many factors such as soil, climate, and agronomic conditions. Crop with vigorous vegetative growth produces higher yield as it has a higher number of leaves which means more photosynthesis and ultimately results in more yield. Makasheva, Bhutia et al, Ihsan et al[11] Amjad, and Anjum.[18-21]

IRB APPROVAL

Approved by the institution Review board of MARC for conducting the research trial at MARC,
Juglote Gilgit-Baltistan.

CONCLUSION

It can be concluded that the variety climax was found to be superior in terms of plant height (85.25 cm), a number of pods per plant (13.71), pod weight per plot (26.29 kg) and pod yield (3585 kg ha^{-1}). The climax had the highest yield compared to the other varieties, hence it can be recommended to farmers for the commercial cultivation in both single and multiple cropping system of Gilgit-Baltistan.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

Influence of Heat Treatment and Microfiltration on the Milk Proteins Properties

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ABSTRACT

Heat treatments are the established food technology for commercial processing of milk. However, degradation of valuable nutrients in milk (as proteins) and its sensory characteristics occur during these processes due to substantial heat exposure. The most important reactions that occur during milk heat treatment are the whey proteins denaturation, its interactions with the casein micelles and aggregation/dissociation of the casein micelles. Microfiltration represents an emerging food processing technology allowing gentle milk preservation at lower temperatures for similar, or better, nutritive value, microbial removal, and shelf stability. Thus, the aim of this work is to review the existing studies on the effects of microfiltration on milk proteins by comparing with the effects of heating treatments.

Keywords
Microfiltration; Heat treatment; Milk proteins.

INTRODUCTION

The proteins are important constituents of the human diet since they are the principal source of nitrogen and essential amino acids. The properties and functionality of the protein depend on its amino acid composition and arrangement of the peptide bonds that stabilize the structure.[1] The functional properties of the proteins are related to various general characteristics such as molecular moisturizing, surface activity and type of protein-protein interactions, facilitated by the partial unfolding of structures.[2] Milk proteins play an important role as functional ingredients in foods, acting as emulsifying, foaming and gelling agents.[3]

Milk is a complex food from a molecular composition perspective which constitutes an important part of a human's diet, mainly because of its high nutritional value. Milk consists of various protein fractions, such as casein micelles and several whey proteins with different molecular weights.[4] Caseins representing approximately 80% of the total protein fraction in milk. These proteins have excellent surfactant properties in emulsions and foams, gelling properties, and thermal resistance to denaturation[5] because of their lack of complex secondary and tertiary structure. However, the casein micelles are composed of the proteins αS1-, αS2-, β-, and κ-caseins, and salts of Ca, P, Mg, and Zn.[1] In fresh milk, the caseins are present in the form of essentially spherical particles containing many protein molecules and amorphous calcium phosphate; these particles have in average 150 nm of diameter with sizes ranging from 15 to about 1000 nm in diameter. Apart from κ-casein, which is mostly found on/or near the external surface of the micelle, the caseins appear to be more or less evenly distributed within the micelle.[6] Casein micelles are in colloidal suspension in milk and are approximately 100 times larger than whey proteins (0.003 to 0.010 μm), which are soluble in milk.[7] However, the whey proteins are typical globular, highly structured proteins, most of which have been isolated, crystallized, and well characterized. The lactoglobulin fraction contains immunoglobulins G, A, and M, which are present at very high-levels in colostrum and play very important protective roles. The lactalbumin fraction contains two main proteins, α-lactalbumin and β-lactoglobulin, and several minor proteins, including blood serum albumin, lactoferrin, and vitamin-binding proteins; it contains several peptide hormones and about 70 enzymes.[1]

Heat treatment can damage the biological properties of milk components, impair protein availability, and promote intolerance and allergy.[5,7] It is now well established that high-temperature processing, especially ultra-high temperature (UHT), causes a series of effects on milk such as loss of available lysine,[8,9] and aggregation and denaturation of protein.[10-13] As a result, many chemical changes could also occur,[14-16] in addition to modifications on functional properties of milk proteins.[6] These changes inevitably affect the renneting, emulsifying, and foaming properties of the dairy products based on the processed milk.[17-20]

The rise of alternative technologies, such as microfiltration, can help prevent these problems while also assuring food safety. Membrane processes are increasingly used in the dairy industry for bacteria removal by microfiltration.[21] Microfiltration (MF) has gained significant attention in recent years as a processing method for the removal of microorganisms from milk.[22-29] With the advancement of membrane filtration technology, the use in the dairy industry has become more technically and economically feasible.[30-32] Furthermore, the MF process may preserve the bioavailability of the thermosensitive and active milk components, such as bioactive peptides, vitamins, and antioxidants. However, works that studied the effects of the microfiltration processes on milk proteins properties are scarce.

Therefore, this work aims to review the existing studies on the effects of milk microfiltration on milk proteins by comparing with the effects of heating treatments, like pasteurization and UHT technology. In other words, our work aims to review the application of milk microfiltration technology to obtain milk with better safety and quality which does not have its original features transformed by thermal processes.

Proteins Functional Properties

The functionality of food proteins refers to the physical and chemical properties that influence the performance of the proteins of food systems during processing, storage, preparation, and consumption. These characteristics influence the quality and sensory characteristics of food.[2] The functional properties of food proteins can be classified into three main groups: hydration properties, which are dependent on protein-water interactions (water absorption and retention, wettability, swelling, adhesion, dispersibility, solubility and viscosity); properties that are related to protein-protein interactions (precipitation and gelation); and surface properties (surface tension, emulsification and foaming characteristics).[33]

Defining and measuring protein functionality starts at the level of protein structure. A simple model for protein denaturation is:

$$N \leftrightarrow I \rightarrow D$$

In this model, a native (N) structure is reversibly converted to an intermediate (I) state where the tertiary structure is changed but much of the secondary structure remains, and further unfolding produces a denatured (D) state. There are various molecular properties associated with each state that have an impact on functional properties (Table 1). According to Foegeding and Davis,[34] molecular weight and primary structure will not be changed during the denaturation process and are considered constants. The isoelectric point can vary due to intermediate and denatured (unfolded) structures exposing charged amino acids to new local environments. The main changes are in a secondary and tertiary structure that can alter the surface exposure of amino acids. This cumulates in an increase in interaction potential, favoring aggregation, and the loss of structural epitopes for allergenicity. Therefore, determining the specific structural transitions during folding/unfolding for food proteins is essential to understand the molecular basis of functionality.[34]

Milk proteins are commonly used as food ingredients in food products not only for their nutritional properties but also for their functional and technological characteristics.[35] Milk proteins have a high nutritional value compared to other proteins, because

Table 1. *Molecular Properties Associated with Native (N), Intermediate (I) and Denatured (D) Structures of Proteins*[34]

Properties of the native state	Key factors	Change in N ⟶ I	Change in I ⟶ D
Molecular weight	Determines general polymer properties	No	No
Isoelectric point	Determines phase stability	Possible due to altered pK_as of functional groups	Possible due to altered pK_as of functional groups
Primary structure	Sequence of non-polar, polar and charged amino acids	No	No
	Sequential epitopes	No	No
	Bioactive peptides	No	No
Secondary structure	Amount of α-helix, β-sheets and other structures	Very little	Yes
Tertiary structure	Overall structure	Yes	Yes
	Structural epitopes	Yes	Yes
Surface topology	Groupings of non-polar, polar and charged amino acids in surface-assessable space	Yes	Yes

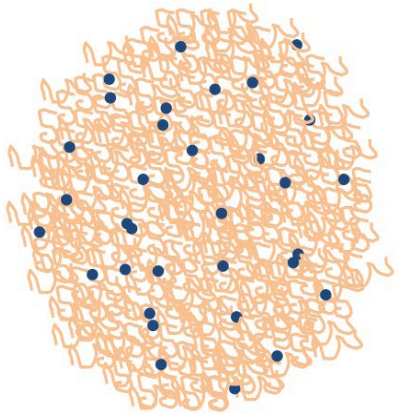
of their relatively high content of essential amino acids and better digestibility.[1] Milk consists of various protein fractions: casein micelles (d 50, 3=180 nm, isoelectric point: pH 4.6) and several whey proteins with different molecular weights (d=2-6 nm, isoelectric point: pH~5).[4]

Casein Functional Properties

Caseins are one of the most important and complex proteins in milk. These proteins have excellent surfactant properties in emulsions and foams, gelling properties, and thermal resistance to denaturation[5] because of their lack of complex secondary and tertiary structure. However, the casein micelles are composed of four individual gene product component, denoted αS1-, αS2-, β-, and κ-caseins, which differ in primary structure, type and degree of post-translational modification, and salts of Ca, P, Mg, and Zn.[35] The remainder of the micellar solids consists of inorganic material, collectively referred to as colloidal calcium phosphate (CCP) or micellar calcium phosphate (MCP).[36] Casein micelles (0.02 to 0.40 μm in diameter) are in colloidal suspension in milk, and are approximately 100 times larger than whey proteins (0.003 to 0.010 μm), which are soluble in milk.[7]

The internal structure of a casein micelle consists of a protein matrix in which calcium phosphate nanoclusters are dispersed (Figure 1). Attached to the surface of the nanoclusters are the centers of phosphorylation (nearby 3-5 phosphorylated amino acid residues) of the caseins. The tails of the caseins, much larger than the CCP clusters, then associate to form a protein matrix, which can be viewed as polymer mesh. The association of the tails is driven by a collection of hydrophobic interactions (weak interactions). The association is highly cooperative and originates in the weak interactions. It is the cooperative that leads to a stable casein micelle. Invariably, k-casein is thought to limit the process of self-association leading to stabilization of the native casein micelle.[36]

Figure 1. *Model Structure of the Casein Micelle Showing a More-or-less Homogeneous Protein Matrix Containing Calcium Phosphate Nanocluster-like Particles (•) Distributed with a Mean Spacing of 18 nm. There is No Distinct Hairy Layer, but Rheomorphic Polypeptide Chains Provide Steric Stabilization in the Outermost Limits of the Particle*[37]



Caseins exhibit specific interactions with calcium ions and salts. Because of their primary structures and specific tertiary, these proteins undergo post-translational phosphorylation.[35,37] This modification results in the formation of anionic clusters of the calcium-sensitive casein while a single residue is phosphorylated in k-casein, which is insensitive to calcium. The calcium-sensitive caseins, αs1-, αs2- and β-caseins are so called because of its extremely low solubility in the presence of Ca^{2+}.[38] It is very probable that these caseins have evolved from a common ancestral gene, while the k-casein has arisen from a different gene.[35]

Casein has a long history as essential ingredients for food and due to its open and flexible structure, caseins have different functionality. It can be widely used in several kinds of products.[1] The casein has excellent solubility and thermal stability at pH above 6.[35] Moreover, due to their amphiphilic structure, these proteins are useful for emulsifying, water binding, thickening, creaming/foam, and gel formation.[2,38] The products based on commercial casein available as food ingredients are acidic casein, casein obtained by enzymatic coagulation, caseinates and coprecipitates.[35] The functionality of these commercial products depends on the type of food in which they are added.[35]

One of the simplest ways to alter the functionality of the proteins is by mild heat treatments that alter protein structure and cause aggregation but do not result in large scale protein precipitation.[34] Denaturation controlled or partial during various isolation steps is generally desirable, as it helps in maintaining an acceptable protein solubility, which is often a prerequisite for the functionality of these proteins in food products.[2]

Proteins are often isolated using isoelectric precipitation. The casein micelles are destabilized irreversibly by isoelectric precipitation.[38] These collapses of the micellar structure of casein are due to several factors, including the solubility of colloidal calcium phosphate and the change in the balance of hydrophobic and electrostatic interactions between different types of casein.[39] The composition of the precipitated proteins usually changed, compared with materials in their native form. These changes at the molecular level may have an impact on protein functionality.[39]

Studies have shown the relationship between the structure of the casein modified by the processing and its functionality.[33,40-42] According to Raikos[39] the degree of protein denaturation induced by heat treatment, under the given chemical environment, was concluded to be the key factor which determines the interfacial functionality of milk proteins with subsequent effects on the emulsion properties. In these and other modifications, i.e., Maillard type reactions to form protein/sugar adjuncts, deamidation, enzymatic crosslinking or enzymatic hydrolysis, usually alter the functionality of the protein.[34]

Whey Proteins Functional Properties

Whey proteins constitute 20% of the proteins in milk and represent an excellent source of functional proteins of high nutritional value.[35] The most important are the globular proteins β-lactoglobulin (β-LG; approximately 3.2 g/L) and α-lactalbumin

(α-LA; approximately 1.2 g/L), representing 70 to 80% of the whey proteins. Besides these, the whey also contains serum albumin (BSA; approximately 0.4 g/L) and immunoglobulins (Ig; approximately 0.7 g/L), which are derived from blood.[39] Whey proteins are very desirable as nutritional ingredients due to its high concentration of sulfur amino acids.[35]

β-Lactoglobulin is a globular protein with a monomer molecular weight of 18.4 kDa and accounts for about 50% of the protein in bovine whey isolate. The secondary structure is composed of 16% α-helix, 58% β-sheet and 25% random coil.35 Native β-LG has nine strands that are folded into two β-sheets: sheet 1 contains strands B, C and D, and part of strand A (A1); sheet 2 contains strands E, F, G and H, part of strand A (A2) and strand I. One side of sheet 1 is hydrophobic and the other side is hydrophilic. Sheet 2 is also hydrophobic on one side and faces the hydrophobic side of sheet 1, thus creating a very hydrophobic cavity, which is nevertheless filled with water. There is also another hydrophobic region on the side of sheet 2, where the three-turn helix lies above it and along strands F, G and H. β-LG has two disulfide bonds and one free Cys(CysH).[43]

The α-LA accounts for about 20% the protein in bovine whey, has a molar mass of 14.2 kDa, and is stabilized by four disulfide bonds and does not contain a free thiol group. However, one of the disulfide bonds (Cys6-Cys120) is more sensitive to cleavage than the other three because of its lower inherent stability.[44] The protein also exists in a number of environment-dependent conformations, including the holo (native, calcium-bound) form, which is the major form in milk.[43]

The BSA is a single polypeptide of 582 amino acid residues with a molecular weight of 66,433 Da and exists in a multidomain structure with complex ligand-binding specificities.[43] It is characterized by an overall oblate shape and consists of three domains (I, II and III), each stabilized by an internal network of disulfide bonds. The primary structure has 17 disulfide bridges that hold the molecule in a structure consisting of nine loops. It contains one free thiol group, Cys34. The secondary structure is composed of 76% helix, 10% turn, and 23% extended chain, and no β-sheet.[2]

Whey protein denaturation is one of the main effects of milk heating which causes modification on the chemical and nutritional properties.[45] The concentration or isolation of whey protein, which represent excellent functionality and nutritional properties, is economically feasible for use as the basis of many dairy products, cheese, and other protein ingredients.[35] Whey protein ingredients are used for a variety of functional applications in the food industry.[3,46-48] The milk proteins and particularly whey proteins are commonly used as emulsifying and foaming agents in diverse food products thanks to their unique interfacial properties.[49]

Unlike the caseins, whey proteins are unstable to heat and this influences the physical and chemical properties of milk products.[43] On the other hand, the manufacture of most dairy products involves heat treatment.[35] Studies have been conducted on the mechanism of denaturation and aggregation of whey proteins during heating and on ways of preventing such changes which are critical to improving their stability.[45]

Effects of Heat Treatments on Milk Proteins Structure

Heat treatment of milk and milk products is an essential operation in commercial dairy processes in order to provide acceptable safety and shelf life.[50] The most important reactions that occur during milk heat treatment are the whey proteins denaturation, its interactions with the casein micelles and aggregation/dissociation of the casein micelles.[51] Denaturation alters several important properties of proteins from the viewpoint of food technology. The denatured protein is generally less soluble or even insoluble, promotes an increase in feed viscosity and the reactivity of their side groups are intensified.[2]

The denaturation also affects the three-dimensional conformation and functional characteristics of the milk proteins besides the degree of heat denaturation varies depending on the intensity of the thermal treatment applied.[35] Changes in protein conformation can affect the thermodynamics of binding with water because they may change the availability of polar sites or hydration sites. The transition from the compact globular conformation of the protein molecule for random conformation can result in an increase of the available surface area and exposure peptides and amino acid side chains.[52]

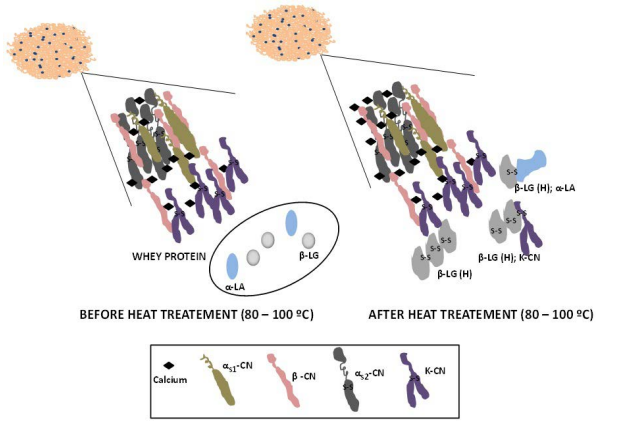
Among the milk proteins, whey proteins are the most thermolabile. As been described in the literature, susceptibility of whey proteins to heat denaturation results from their high level of secondary and tertiary structure.[35,43,53-55] Because denaturation of whey proteins occurs rapidly at temperatures above 70 °C, commercial heat treatments denature at least a portion of these proteins.[35] The functionality of whey proteins is very sensitive to the extent of denaturation. Major whey proteins exhibit thermostability to structural unfolding in the order α-lactalbumin<albumin<immunoglobulin<β-lactoglobulin. However, the thermal unfolding of α-lactalbumin is reversible so that denaturation, as measured by irreversible changes, indicates an order of increasing the thermostability of IgG<serum albumin<β-lactoglobulin<α-lactalbumin.[56,57] The thermal behavior of the whey proteins is ruled primarily by the properties of β-lactoglobulin, which are affected by the pH, lactose, sodium chloride, calcium, and other ions.[58] In particular, β-lactoglobulin and serum albumin command protein aggregation *via* thiol-disulfide exchange and oxidation-reduction reactions during the heat treatment.[55] Swaisgood[35] report that β-lactoglobulin, are considerably less soluble and more sensitive to precipitation by calcium ions than are their native counterparts when denatured.

The degree of heat denaturation of the whey proteins varies depending on the intensity of the thermal treatment of the milk in during the processing of different products.[59] The kinetics of thermal denaturation of whey proteins is complicated, with a significant effect on the ranges between 80-100 °C.[60] The dena-

turation reaction shows a non-linear Arrhenius relationship; there is a noticeable change dependent on the temperature around 80-90 °C for both α-LA and β-LG. The apparent activation energy is in the range of 260-280 kJ.mol^{-1} for β-LG and 270-280 kJ.mol^{-1} for the α-LA at temperatures below 90 °C.[56,57] At higher temperatures the activation energy is lower, ranging from 54 to 60 kJ.mol^{-1} for β-LG and 55 to 70 kJ.mol^{-1} for the α-LA, indicating chemical interactions (aggregation).[60]

Pasteurization or UHT processing causes partial, irreversible unfolding of β-lactoglobulin, thereby exposing hydrophobic surface and the sulfhydryl group. The subsequent interaction with κ-casein, stabilized by a sulfhydryl-disulfide interchange, alters the surface properties of casein micelles (Figure 2).[55] According to Fox1 the κ-casein, in particular, has the ability to react with sulfhydryl group of denatured whey proteins, possibly in these form: [κ-CN]-SH+HS-[β-lactoglobulin]+O_2→[κ-CN]-S-S-[β-lactoglobulin]+H_2O[κ-CN]-SH+SS=[α-La]→[κ-CN]-S-S-[α-La]-SH. These complexes are co-precipitated with caseins when the whey is separated from caseinic phase, resulting in an increase of the N content and in the size of casein micelles.[43] Therefore, the heat-induced association of whey protein (especially β-lactoglobulin) with casein micelles alters micelle properties and also increases heat stability.[35]

Figure 2. *MEffect of Heating Milk at 90 °C. The Native β-lactoglobulin (β-LG) Dimer Dissociates and the Monomer Undergoes Internal Disulfide-bond Interchange to Give Reactive Monomers that React with k-casein (k-CN) at the Surface (Outer Region) of the Casein Micelle. Native β-LG Monomers can also form an Adduct with α-lactalbumin (α-LA), which then Gives Rise to α-LA Dimers and α-LA:β-LG Dimers. In the Severely Heat-treated Samples, αs2-CN also forms Disulfide Bonds with Other Proteins*[55]



On the other hand, the α-lactalbumin does not present SH groups but present four S-S/mol groups. In order to connect α-lactalbumin with the κ-casein, these S-S groups must first be broken by oxygen. Besides being more heat resistant, the functional group of α-lactalbumin is more difficult to connect to the κ-casein, so it is strongly relevant to the thermal stability of milk.[55]

Oldfield, Singh, and Taylor[61] suggested that there are at least three possible kinds of denaturation of β-LG that can associate with the micelles: (i) β-LG monomer unfolded, (ii) self-aggregating β-LG and (iii) aggregate LG β-/α-LA. The relative association rate of these species with the casein micelles depends on the heating gradient, which in turn affect the relative rates of breakdown and formation of different aggregated species. At higher temperatures and faster heating rates, all whey proteins begin to unfold in a very short period of time, thus presenting an opportunity for more monomers of unfolded β-LG to auto aggregate, which in turn could make an association with the caseins micelles less efficiently. These β-LG aggregates could protrude from the surface of the casein micelles promoting a steric effect for new β-LG associations. Furthermore, these aggregates may have their reactive sulfhydryl groups within the array, and thus unavailable for sulfhydryl-disulfide transfer reactions with κ-casein micelle. The formation of β-LG unfolded can be promoted by long periods of heating at low temperature or by heating at a slow rate until the desired temperature. These monomeric molecules of β-LG enter the capillary layer of κ-casein and have easier accessibility to the sulfhydryl group.

Besides time and heating temperature, several other factors influence the extent of association of denatured whey proteins with the casein micelles, which include the pH of the milk prior to heating, the soluble calcium and phosphate concentrations and the concentration of solids in milk.[55] According to Swaisgood[35] pasteurization (71.7 °C for 15 s) or UHT processing (142-150 °C for 3-6 s) irreversibly increases the amount of colloidal calcium phosphate at the expense of both soluble and ionized calcium and soluble phosphate. Consequently, the pH also decreases, due to the release of protons from primary and secondary phosphates. The calcium transformed in tertiary calcium phosphate does not come entirely from the serum because heating also causes dissociation of calcium bound to protein. Thus, pasteurization, and especially sterilization, affects the size distribution of micelles, leading to an increase in the abundance of both large and small micelles. Moreover, heat treatments of increasing severity are accompanied by increased production of dehydroalanine residues, due to β-elimination of disulfide bonds and phosphoseryl residues, increased deamidation of asparaginyl and glutaminyl residues, and increased Maillard browning. Cross-linking of protein during heating can result from the reaction of dehydroalanine residues with ε-amino groups of lysyl residues to form lysinoalanine or reaction with sulfhydryl groups of cysteinyl residues to form lanthionine.

Due to the growing consumer demands for better retention of the nutritional value, sensory attributes, and longer shelf stability of milk, the emerging technologies processes have become more interesting for the dairy industry.[62] One of these emerging technologies that no present the changes in proteins functionality that the heat treatment presents, is the microfiltration process (MF).[63] Incorporation of MF in the milk manufacturing process can improve the microbial and sensory quality of milk.[31,64] Moreover, generally enable high flow rates, improve the yield, reduce the processing costs and shown higher quality compared to conventional heat treatments.[62]

Predicting the Effect of Microfiltration on the Properties of Milk Proteins

The commercial processing of milk are carried out almost entirely by heating temperature processes.[62] Pasteurized milk currently has a short shelf life (about 14 days) due to the presence of remaining bacteria,[65] their enzymes[66] and somatic cells[67,68] that are not eliminated by this process. On the other hand, UHT treated milk has a longer shelf life, but there is major damage to the molecular structure of milk proteins. It has been reported that heat temperature processes can lead to several changes in milk, including denaturation of whey proteins, the interaction of whey proteins with caseins, inactivation of indigenous enzymes, as well as the destruction of certain nonstarter lactic acid bacteria.[22] Therefore, as reported by Elwell and Barbano[24] there is a desire in the fluid milk processing industry for an HTST pasteurization process that will produce fluid milk with a refrigerated shelf life of 60 to 90 days. Nevertheless, there are other technologies currently being researched that may eventually replace the pasteurization or the UHT processes.[69] These include the membrane filtration process, i.e. microfiltration technologies.[70]

The microfiltration represent an emerging food processing technology in several coutries allowing gentle milk preservation at lower temperatures and shorter treatment times for similar, or better, microbial inactivation and shelf-life stability when applied in a hurdle approach compared to heat temperature processes.[62] This process is based on the selective permeability of a membrane for one or more of the constituents of the liquid. Selectivity depends on both the type of membrane (different cut-off) and process conditions.[70-72] The liquid submitted to this process is divided into two fractions: the fluid retained by the membrane (i.e., the retentate), which results in a higher concentration of the components having a bigger size than the average pore diameter of the membrane, and the liquid going through the membrane (i.e., the permeate or the microfiltrate) (Figure 3).[73]

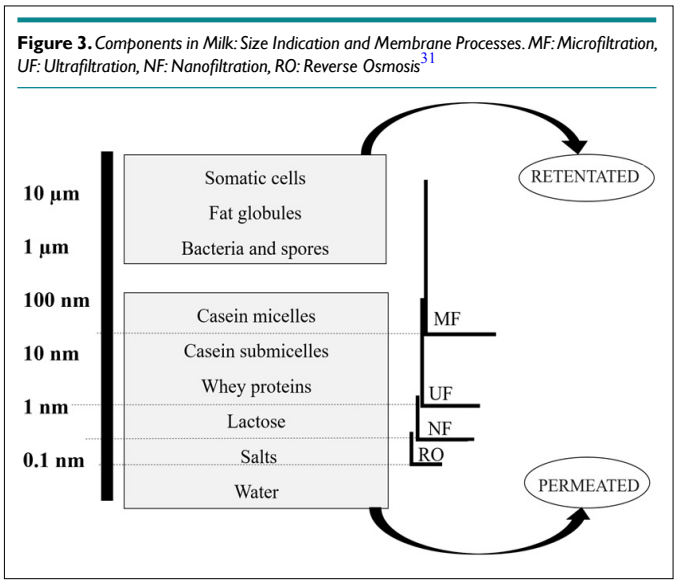


Figure 3. *Components in Milk: Size Indication and Membrane Processes. MF: Microfiltration, UF: Ultrafiltration, NF: Nanofiltration, RO: Reverse Osmosis*[31]

However, as related by Brans et al,[31] whole milk is a complex and challenging feed for microfiltration membranes because of the broad particle size distribution (1 nm-20 µm) coming from the fat globules and natural and/or seasonal variations in composition. Bacteria spores and somatic cells present in raw milk are not affected by the HTST heat treatment used in the processing of most dairy products, whereas they can be physically removed by microfiltration. If not removed or killed, spores can compromise the quality and shelf life of milk and other dairy products, such as milk powder and cheese.[68] At the same time, high somatic cells count can lead to increased proteolytic and lipolytic activity in milk, thus compromising the flavor, texture, and shelf life of dairy products.[74-77] Alternatives to already existing "cold sterilization" processes based on cross-flow microfiltration with ceramic membranes[28] are being the focus of milk microfiltration. In the case of "cold sterilization", today's commercially available technology based on fine ceramic filters, still has to be combined with final heat treatment in order to guarantee the safety of the final product. Furthermore, due to the fact that the bacteria and spores are smaller than fat globules, the milk needs to be skimmed before the membrane process in order that the microfiltration can be effective against the milk pathogens and spores.[78] On the other hand, the smaller the ceramic pores, the higher the microbiological quality in the final product but on the process side, unfortunately, more fouling occurs due to milk proteins retention in the membrane and, thus, decreases the productivity of the whole operation.[79]

Membrane processes are used to improve the microbial quality of milk and dairy fluids, as well as are also applied for preserving the functional properties of milk proteins.[71] Avalli et al[73] reported that the mechanic stress of the transmembrane pressure affects minimally the milk proteins in comparison with heat temperature processes. But when the skim milk passes through the microfiltration membrane the proteins and minerals tend to form aggregates in the pores and this problem may decrease the flux and modify its properties and the quantity of the protein in the permeate.[80] It has been reported that proteins with low internal stability as β-CN, α-LA, BSA, and immunoglobulin (IgG) tend to adsorb on all surfaces, even onto electrostatically repelling surfaces.[81,82] Thus, proteins with diameters much smaller than the membrane pore typically cause pore constriction, while those with a diameter comparable to the membrane pore may cause pore blocking, and proteins larger than the pores can be retained on the membrane surface and cause cake formation.[4,83] Gesan-Guiziou et al[84] found that as membrane resistance (fouling) increased, the transmission of whey proteins decreased during the MF of skim milk. On the other hand, another membrane property that needs to be considered is it's hydrophilic–hydrophobic character because this is known to affect the strength of the interaction between a protein and a surface.[78] Specifically, proteins tend to adsorb more extensively and less reversibly at hydrophobic surfaces than at hydrophilic surfaces.[85] According to Gao et al,[86] ceramic membranes are hydrophilic and this property does not preclude the deposition of proteins onto its surface. As related by Wang et al[85] this behavior may occur because whereas proteins tend to unfold and spread their hydrophobic core over hydrophobic surfaces, their charged and polar functional groups

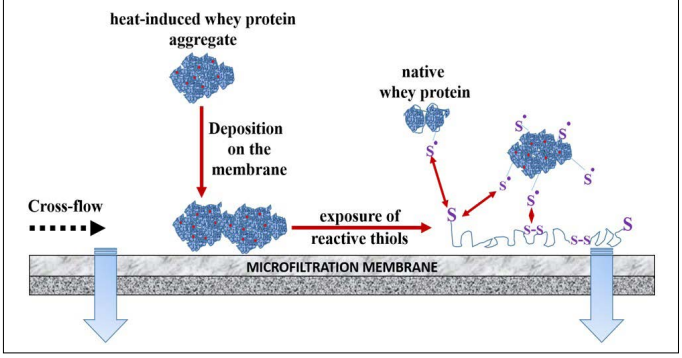
tend to interact with hydrophilic surfaces. In the case of CN micelles, it is known that the hydrophilic ends of κ-CN molecules are preferentially located at the surface of micelles, which would explain the ceramic membrane–CN interactions.[78]

Investigations on the minor whey protein bovine serum albumin (BSA) conducted by Chandavarkar[87] and Kim et al[88] have shown that protein aggregates are formed in shear flow during cross-flow filtration, which is deposited on the membrane surface afterward. In contrast to BSA, the major membrane foulant during whey filtration, β-Lg, is not sensitive to shear forces.[89] Kelly, Opong, and Zydney,[90] as well as Kelly and Zydney[91] and Kelly and Zydney,[92] found that BSA-aggregates catalyze fouling when deposited on the membrane. The molecular mechanism involved in the fouling reaction was found to be based on the exposure of reactive thiol-groups in the deposit. This reactive initial deposit then serves as a nucleation site for further thiol oxidation and thiol-interchange reactions. Despite the fact that the proteins varied significantly in size, molecular structure and originated from various animal protein sources (Table 2), Kelly and Zyndey[93] observed that intensity of membrane fouling was correlated to the number of free thiols of the respective protein (Figure 4).

Table 2. *Molecular Characteristics of whey Proteins*[11,98]

Protein	Size (kDa)	pI	-SH	-S-S-	Concentration in whey (g/L)	Mass portion (%)
β-Lactoglobulin	18.3	5.13	1	2	3	60
α-Lactalbumin	14.2	4.2-4.5	0	4	1.2	20
Bovine serum albumin	66.4	4.7	1	17	0.4	3
Immunoglobulin G	161	-	-	-	0.6	10
Lactoferrin	76.1	9	0	17	0.02	<0.1

Figure 4. *Impact of whey Protein Aggregates on Membrane Fouling*[110]



Like as BSA, β-Lg contains one free sulfhydryl group (Table 2). Hence, membrane fouling based on thiol-interchange reactions as well as thiol oxidation probably also applies for β-Lg or other whey proteins. This reaction pathway *via* thiol/disulfide reactions is known for β-Lg aggregation during thermal processing.[94] Additionally, for the major whey protein β-Lg it was found that gel network formation is facilitated in the presence of calcium.[95,96] Based on this, Marshall, Munro, and Trägårdh[97] assumed that calcium-induced cross-linking was involved in β-Lg deposit formation.[98]

Besides these properties, Kühnl et al[99] related that the microfiltration flux during cross-flow filtration depends on the pH of milk and showed that the casein micelles where size constant in the pH 5.9-6.8 range. Acidification of milk from pH 6.8 to 5.9 leads to a strong reduction of hydrophilic repulsion between casein micelles while the micelle size remains the same.[99] In addition to acidification, this behavior can also be described by a model which incorporates van der Waals and electrostatic interactions as well as hydrophilic and hydrophobic Lewis Acid-Base interactions. In accordance, Steinhauer et al[4] reported that a less repulsion between casein micelles, in turn, results in a flux drop, i.e. that change in colloidal interaction is a basic problem in cross-flow filtration.

In addition, minerals are found in association with casein, whey protein, and fat globule membranes.[35] Furthermore, the minerals in the whey phase of milk are present as free ions, salts, or in association with whey proteins. According to Svanborg et al,[100] these minerals may pass through the MF membrane, and their distribution after MF fractionation will influence the functional properties of the fractions. For example, the treatment of milk with MF will increase the calcium (Ca) content and the buffering capacity of the retentate, therefore delaying the pH reduction during traditional cheese making.[101] As reported by Kaombe et al[102] for pasteurized milk, the temperature of the MF process also may influence the Ca content on dialysates and permeates. On the other hand, even relatively mild heat treatments, such as HTST, could most likely affect the composition and protein yield of the MF permeate. Increasing the temperature is expected to decrease the viscosity of the permeate,[103] and if no change in membrane resistance occurs the transmembrane pressure required to maintain a constant flux is expected to decrease as the temperature is increased.[104] Moreover, heat denaturation of whey proteins may also change the performance of the membranes during MF.[100]

Hurt et al[104] clarified that increasing the temperature of MF from 50 to 65 °C could also cause denaturation of whey proteins and possible association with casein (CN) micelles. Long et al[105] reported that only 3.4% of β-Lactoglobulin was associated with κ-CN after 20 min at 65 °C, and thus when they was covalently associated, the yield of whey protein in the permeate would be reduced. In addition, aggregates of β-LG and α-LA have also been found when heated at 75 °C,[106] being unable to pass through the MF membrane. It was shown that whey protein hydrophobicity increases as a consequence of protein unfolding, e.g. under heat-treatment.[107] Another reason for protein unfolding and an increase in hydrophobicity is surface denaturation, as it is reported for the adsorption of several proteins including β-LG at hydrophobic surfaces. Unfolding was found to increase when repulsive electrostatic interaction forces were reduced.[108] It can be assumed that hydrophobic whey protein aggregates can potentially serve as adsorption sites and induce protein unfolding during deposit layer formation on membranes. Steinhauer et al[109] confirm that β-LG and its heat-induced aggregates are the major

species made responsible for membrane fouling, forming a highly reactive deposit and accelerate membrane fouling (Figure 4). Therefore, deposited aggregates serve as nucleation sites for thiol oxidation and disulfide reactions between deposited particles and native protein. Some works confirm this theory since aggregates formed during whey heat-treatment enhance membrane fouling.[97,110-112]

Briefly, the microfiltration is an emerging technology that can improve the quality of milk and dairy products once that changes in protein properties do not occur as far as in milk produced by heat treatments. The most important reactions that occur during milk heat treatment are the protein denaturation of whey proteins, its interactions with the casein micelles and aggregation/dissociation of the casein micelles. The microfiltration also has the advantages of retaining spores of microorganisms and somatic cells that no are affected by heat treatments, that can damage the milk and dairy products quality and safety. However, the microfiltration, considerate a mechanical process, makes that the milk continues to be considered raw and in Brazil (as in other countries) is not possible to sell raw milk. On the other hand, heat treatments are the established food technology for commercial processing of milk in order to provide acceptable safety and shelf life. The shelf life of pasteurized milk in around 8 days while for the microfiltrated milk is close to 30 days.[113] Finally, how is not possible yet to produce only microfiltered milk in Brazil, several works recommend the use of the two technologies (microfiltration and pasteurization) for preserving the better as is possible the quality of the milk proteins and the others constituents.[62,114]

Final Considerations

The microfiltration, a mechanical process, affects minimally the properties of milk proteins as compared to heat treatments. However, only the use of membrane filtration process is not completely understood since the microbiological quality cannot always be guaranteed, and these may bring some disadvantages to the consumer. Therefore, more studies regarding the microbiological safety of microfiltered milk must be performed in several countries. On the other hand, the heat treatment in the fluid milk technologies is already established in the industry worldwide. Despite the negative influence on the milk proteins, it is a safer methodology to produce this kind of products nowadays. The use of heat treatment prior to microfiltration may induce a higher membrane fouling because of the formation of protein aggregates. Meanwhile, microfiltration in combination with pasteurization can be an alternative to extend the shelf life of pasteurized milk by removal microorganisms, spores, and somatic cells of raw milk.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Demonstration of Technologies and Training of Growers for Handling and Value Addition of Fruits and Vegetables in Gilgit-Baltistan

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ABSTRACT

Introduction

The present activity was conducted to execute fruit handling, processing, preservation, dehydration and value addition trainings in Gilgit-Baltistan, to control wastages/losses of fruits and vegetable which is above 60% of total production.

Objectives

To prepare fruit pulp for fruit preservation using potassium metabisulphite ($K_2O_5S_2$). To develop household level methods for development of value added products like fruit jam, tomato paste, mix vegetable pickle and dehydrated apricot.

Methods

The research work for method development was carried out at Pakistan Council of Scientific & Industrial Research (PCSIR) Skardu, Gilgit-Baltistan, Pakistan. Methods were developed with recommended dosages of chemical preservatives. A total of two days training courses were conducted focusing fruit handling, processing, preservation, dehydration and value addition of fruits and vegetables at 4 different locations in 4 districts of Gilgit-Baltistan (Skardu, Diamer, Hunza and Shigar).

Results

In each district, 4 training courses were conducted at 4 different locations and in each training there were 30 participants. The participants included fruit growers/farmers. A total of 480 fruit growers were trained in all the 16 training courses in 4 districts. The farmers also trained in use of fruit processing machinery as pulpier, washer, cutter, etc.

Conclusion

The basic objectives of training were to control wastages/losses of fruits, income generation of fruit growers through sale of fruit, value added products and to contribute to ensure food security issue in Gilgit-Baltistan.

Keywords

Trainings; Value addition; Fruits; Vegetables; Wastage; Gilgit-Baltistan.

INTRODUCTION

Gilgit-Baltistan (GB) is one of the most important parts of Pakistan (geographically it is connected with China and India) which extends over an area of 27,188 sq miles. Administratively, Gilgit-Baltistan is distributed into 10 Districts (Gilgit, Skardu, Diamer, Astore, Ghanche, Ghizer, Hunza, Nagar, Shigar and Kharmang) having a population of 2 million people. The main issue in Gilgit-Baltistan is food security, as cultivated lands are less than one kanal per capita.[1,2] The people of GB are totally dependent on wheat supplied through Government on subsidized rates from Punjab.[3,4] The climatic conditions of GB are suitable for abundant, delicious, high-quality fruits and vegetables. Fruits and vegetables are the main source of income generation for the area. According to agriculture statistics 2014,[5] the pre- and post-harvest losses of fruits and vegetables are 50% and according the survey of center for public health initiatives (CPHL) project PCSIR[6] these losses exceed 60% of produce. The total fruit production in GB

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is 1,49,769 metric tons (Apricot 1,08,588 tons, Apple 19,054 tons, Grapes 6,413 tons, Pear 2,579 tons, Peach 3,308 tons, Pomegranate 4,287 tons, Cherry 2,256 tons, Mulberry 9,092 tons, Walnut 5,992 tons, Almond 1,700 tons and Sea buckthorn 3,600 tons). Due to lack of processing, preservation, testing, transportation, communication and research a large amount of fruits, vegetables are wasted and do not reach the markets as the fruits are highly perishable. To cope with growing demand of fruits and vegetables without bringing more land under cultivation, prevention of post-harvest losses can play a vital role. Training farming community in processing, preservation, dehydration and value addition of fruits and vegetables can control post-harvest losses.[7,8]

Demand of chemically preserved fruit pulp and semi-processed fruits is on the rise among the food industry of Pakistan. Demand has also risen for dehydrated fruit, pulp and other value-added products in the western market. A number of importers from Europe have shown their interest in the import of dry apricot from Gilgit-Baltistan.[9] According to Baltistan Culture Development Foundation (BCDF) Skardu, Gilgit-Baltistan, Pakistan there was an order of 3,500 tons dried apricot from a single party from the United Kingdom during the year 2014, but they failed to collect 10 tons of dehydrated dry apricot of international standard. This project aims to address this issue through training of selected progressive farmers of 4 districts of GB which will be extended to all 10 districts. The training sessions will enable farmers to process their products according to Hazard analysis and critical control point (HACCP), World Health Organization (WHO) and Food and Agriculture Organization (FAO) recommended standards, with recommended chemical preservatives/doses and to produce quality value-added products in accordance with international requirements. This would not only help to uplift the socio-economic conditions of the farmers but help to cater local and international demands.

For export of value-added fruit products, each product must contain its nutritional profile amounts of preservatives, expiration date and testing/analysis certificate from any ISO17025 certified accredited laboratory. The project will address this issue with purchase of the required laboratory testing equipment through this project. Sometimes, even the hygienic and best quality fruits/value-added products are rejected during export due to inappropriate packaging material, large in volume and weight, unattractive and without required printed information in packaging material. Packaging machines were used to train the farmers, the training included packaging that is recommended, attractive, low in volume/size and with required information supplied in the packing materials.

In order to minimize these post-harvest losses of fruits and vegetables, PCSIR established a Demonstration-Cum Training Center in Skardu, Gilgit-Baltistan, Pakistan which is equipped with processing and testing/analysis, qualified and skilled scientist/food technologists. Now PCSIR Demonstration-Cum Training Center Skardu has been upgraded to PCSIR Laboratories Skardu. It has been providing processing, testing and research facilities in Gilgit-Baltistan. Fruits and vegetables processing training provided to farmers during the past few years have yielded excellent results.

Pakistan Council of Scientific & Industrial Research (PCSIR)Skardu has also been extending the expertise of their scientists, processing and Lab. facilities to the other allied departments and Non-Government Organizations (NGOs)such as, Agha Khan Rural Support Program (AKRSP),Mountain Areas Farmer Support Organization(MAFSO), Ghizer Women Development Social Welfare Organization (GWDSWO), Agribusiness Support Fund (ASF), Baltistan Cultural Development Foundation (BCDF) and Agriculture Department to conduct fruit and vegetable dehydration and preservation training at Demonstration Cum Training Center Skardu, Gilgit-Baltistan, Pakistan.

To realize the need of processing of fruits and vegetables, Karakoram International University (KIU) started BSc (Hons) and MSc (Hons) degree programs at Department of Agriculture and Food Technology, Gilgit where students are educated/trained about processing of fruits. The processing training for the majority of farmers needs to be given in the future to control the existing losses/wastages of fruits though skill development in processing. The PCSIR, Skardu needs financial support to conduct processing, preservation, dehydration and value addition of fruits and vegetables training throughout the Gilgit-Baltistan (all 10 Districts) and to procure some essential Lab. equipment. Therefore, a proposal for these endeavors has been submitted to Agriculture Linkage Program (ALP) Pakistan Agriculture Research Council (PARC), Islamabad.

MATERIALS AND METHODS

Fruits and vegetables (i.e. apple, tomato, apricot and cabbage leaves) were purchased and different trials took place to develop household-level value-added products. The most liked formulas were determined through an organoleptical evaluation panel of PCSIR experts and selected for the compilation of a Fruit Processing Booklet (mentioned by Larmond E). During these initial trials, the pulp was preserved at the recommended dosage of potassium metabisulphite ($K_2O_5S_2$), i.e. 1 gram for 1 litter pulp.[10] Tomato paste was prepared from over-ripened tomatoes, apricot dehydration was done without chemical preservatives and with chemical preservatives, and for vegetable pickle household level methods were developed.[11] These methods were compiled in the form of a fruit processing booklet entitled "*Processing, Preservation, Dehydration and Value Addition.*"

The physicochemical analysis of developed jam, pulp and tomato paste was carried out such as pH, total soluble solids, acidity (%), sugar acid ratio, Brix, reducing sugar, and non-reducing sugar using the recommended methods of Association of Analytical Communities (AOAC).[12] The same samples were organoleptically evaluated for color, taste and overall acceptability by a panel of 10 experienced judges using Nine Point Hedonic Scale (as described by Larmond). After printing of the booklet, other required items for fruit processing training were purchased from the local market i.e. fruit processing chemicals (potassium metabisulphite, citric acid and pectin), head covers,

facemasks, disposable gloves, pen, writing pads, etc. The training were conducted in collaboration with Agriculture Department of concerned district, while in some locations the training was conducted with Local Support Organizations (LSO) and Welfare Organizations (WOs). Before the start of training, Memorandum of Understandings (MoUs) were signed with the collaborating partners.

Fruit Processing Training

Two days fruit handling, processing, preservation and value addition training started from District Diamer, Gilgit-Baltistan. The principal investigator and his team including the female training facilitator, along with mobile training unit and its operator proceeded to district Diamer from PCSIR Laboratories, Skardu. Training material i.e. apples, tomatoes, vegetables, spices for pickle were purchased and the training started from the first location "Chilas" of district Diamer. First day of training started at 9:00 am. Registration of trainees started, a folder consisting of two days training schedule, writing pad, pen, fruit processing booklet, facemask, head cover, disposable gloves and a list of contacts of fruit processors in GB were given to each trainee. Then Deputy Director, Agriculture Department District Diamer delivered a welcome address and brief about the agricultural scenario of the District, after that the Principal Investigator briefed about the objectives of fruit processing training. Then practical training on development of apple pulp at household level started.

Development of Apple Pulp and its Preservation

Initially, 10 kg apples were taken, washed and cut it into pieces, poured in 5 liters of water and started cooking. After proper cooking, removed the steel pan from stove and allowed to cool for 10 minutes. Then filtered the cooked apples with muslin cloth pressed by hand. The received pulp was weighed and according to weight, added 1 gram potassium metabisulphite per liter pulp i.e. 1000 ppm (Awan and Rehman).[13] After proper mixing, the pulp was packed in 1 litter sterilized bottles.

Preparation of Fruit Jam

The second practical for the first day of training was preparation of fruit jam at household level. A 5 liter preserved apple pulp was put into a stainless steel pan and started cooking; then added sugar (550 g/liter pulp), citric acid (4 grams/liter pulp), pectin (5 grams/liter pulp) and mixed thoroughly. The mixture cooked until the required brix (68 ºBrix). The jam was allowed to cool for 5 to 10 minutes to lower the temperature almost below 85 ºC, that protects glass jars from burst with high temperature and packed in sterilized glass jars, capped and labeled (Awan and Rehman).[13]

Preparation of Tomato Paste

Tomatoes were washed, cut into pieces, and pulp extracted using the pulpier machine fitted in the mobile training unit (MTU). The pulp was weighed and put it into a cooking pan and started cooking until 50 ºBrix was reached (checked with digital refractrometer). After that, added salt (5 g/liter), vinegar (5 g/liter) and 1 g potassium metabisulphite.[10] Removed the fire, cooled the paste and packed into bottles.

Preparation of Vegetable Pickle

The second day started with practical preparation of vegetable pickle. Cabbage leaves were washed, cut into pieces and put into a cooking pan to start cooking. Added salt 25 g/kg after slight cooking; drained water and put leaves in perforated trays to dry. Then add dry red chili powder (10 g/kg), mustard seed (10 g/kg), coriander seed (8 g/kg) and mixed mustered oil up to lid of jar. Finally, mixed it properly and filled in glass jars and capped.[13]

Dehydration of Apricot

The second practical of day 2 was dehydration of apricot. Fruit growers of the area were trained in dehydration of apricot. Apricot Variety "Halman" which is best for dehydration was used. Two methods i.e., organic dehydration and inorganic dehydration were utilized.[14]

Organic Dehydration

Apricots harvested from tree were washed and pitted (kernels of apricot removed). The de-pitted apricots were kept in wooden trays and kept in open sun for dehydration. On clear, sunny days dehydration is completed within 6 days. The dehydrated apricots were cooled before packing in polyethylene bags and sealed.

Inorganic Dehydration

In inorganic dehydration, the solution of potassium metabisulphite (2 g/liter of water) was prepared. The apricots were de-pitted and dipped in this solution for 10 minutes, then the apricots were kept in wooden trays and kept in the open sun for dehydration.

Physicochemical Analysis

The physicochemical analysis of the jam, tomato paste, vegetable pickle, and dehydrated apricot was carried out, such as pH, total soluble solids, acidity (%), sugar acid ratio; reducing sugar and non-reducing sugar were carried out using the recommended methods of AOAC.[12] The samples organoleptical evaluation included for color, taste, and overall acceptability by trainees by using the Nine Point Hedonic Scale described by Larmond.[15]

Demonstration of Use of Fruit Processing Machinery

The Mobile Training Unit (MTU) was equipped with fruit pulpier, washer, cutter, mincer, potato peeler etc. Demonstration of fitted machines occurred during fruit processing training. The fruit growers practically performed use of these machines. The training process was performed on the same pattern for all 4 districts of GB.

RESULTS AND DISCUSSION

The methods developed for fruit jam, tomato paste, vegetable pickle and dehydration of apricot (organically and inorganically). The organoleptically recommended best methods were compiled and 1400 copies of fruit processing booklets were printed. The accepted formulae are shown in Table 1, the two days fruit handling, processing, preservation, dehydration and value addition training were given according to these methods/recipes.

Training on Fruit Processing

Conducted 16 training courses, each lasting 2 days, consisting of 491 fruit growers/farmers trained at fruit harvesting, handling processing, preservation, dehydration and value addition during the reporting year in 4 districts of Gilgit-Baltistan (Skardu, Diamer, Hunza and Shigar). The training were given to 16 groups at 16 different location, 4 groups/location in each district and one group consist of 30-36 participants (Table 2). All the required preservatives, processing chemicals, bottles, head covers, facemasks, gloves, pen, writing pads, training schedule, processing booklets and fruit survey questionnaires in Urdu were provided to the trainees in a folder. The fruit growers/farmers practically performed all the training activities to get hands-on training. The officials and trainees appreciated the material provided in the fruit processing booklet. All trainees participated with keen interest. After fruit processing and preservation training, it is expected that the fruit growers/farmers will preserve their fruits, and the wastage of fruits will be minimal and they are likely to get maximum benefits from their produce.

Fruit Value-added Product Development Training

The training were conducted at household level i.e. how to prepare these products using kitchen utensils' no additional utensil/equipment were purchased except fruit processing chemicals. The recommended methods/recipes/formulas were used for product development. The fruit growers/farmers practically performed this value-added product development. After practical training the chemicals were provided to each trainee from project (i.e. Potassium Metabisulphite 40 g, Citric Acid 30 g and Pectin 30 g) and given task to prepare Apple/Apricot jam at their homes at the evening of the first day of training. More than 58% training participants prepared Apple/Apricot Jam at their homes after receiving training using the provided chemicals. The list of trainees is shown in Table 3 developed jam at home after practical. The quality of developed jam was good but some training participants have not properly judged the time of removal of jam from stove. The 2nd day the jams prepared by trainees were examined by project team and the trainees were explained about the quality of their jam, their deficiency were identified and were advised how to eradicate them. The value-added products receive premium prices in local and international markets. Prolonged fruit shelf life will help in developing the cottage industry (small scale fruit processing industry) at the village level in Gilgit-Baltistan, which will be a new avenue of income generation and source of income generation for the mountain communities.

Dehydration of Apricot

Apricot dehydration training both organically and inorganically with recommended standards were given to 16 groups consist of 491 selected fruit growers in 4 districts. Dehydration of apricot was common and most of the farmers dehydrate their fruits but unfortunately they do not follow recommended methods, and dehydration occurs in a non-hygienic manner with banned methods and overdose of chemical preservatives.

During training it was noted that some farmers dehydrate their fruits with chemical preservatives (Potassium Metabisulphite) without any measurements and they have no idea about the recommended dosage. During training it was strictly told to the farmers about use of preservatives within recommended dosages (i.e. 1 g/kg of fruit). Some farmers/growers also preserve their fruits through smoke of raw Sulphur (a traditional practice of the area); it was stringently advised to avoid this practice. It was also noted during training that some fruit growers dehydrate their fruits on the roadside and other places and that they do not take care for the quality of product and hygiene of product or self-hygiene. In all training, lectures on product quality and hygiene was given and its importance was practically demonstrated i.e., to cover their head with head covers, use of facemask and gloves while working and the trainers advised that it protects you and the product.

Table 1. *Recommended Methods/Recipes for Development of Apple Jam, Tomato Paste and Vegetable Pickle at House Hold Level for Farmers/Fruit Growers*

Apple Jam		Tomato Paste		Vegetable Pickle	
Name of Ingredient	**Quantity**	**Name of Ingredient**	**Quantity**	**Name of Ingredient**	**Quantity**
Apple pulp	1 liter	Tomato pulp	1 liter	Cabbage leaves	1 kg
Sugar	550 g	Salt	5 g	Salt	30 g
Citric Acid	4 g	Vinegar	10ml	Red chili powder	10 g
Potassium metabisulphite	1 g	Potassium metabisulphite	1 g	Coriander seed	10 g
Pectin	5 g	-	-	Mustard seed	10 g
Food color	As desired	-	-	Nigella seed	10 g
-	-	-	-	Mustard oil	Up to cover

Table 2. *Two Days Fruit Processing, Preservation and Value Addition Trainings During 1st Year of Project*

Group	District	Location	Collaborating Partner	Date	Participants
1	Skardu	PCSIR Laboratory	Agriculture Department Skardu	13-14, Feb- 2018	30
2	Diamer	Agri. Nursery Chilas	Agriculture Department Diamer	19-20, Feb-2018	30
3	Diamer	Agri. Nursery Goner Farm	Agriculture Department Diamer	21-22, Feb-2018	30
4	Diamer	Agri. Nursery Tangir Juglot	Agriculture Department Diamer	23-24, Feb-2018	30
5	Diamer	Agri. Nursery Darel Gumari	Agriculture Department Diamer	25-26, Feb-2018	30
6	Hunza	Khana Abad Jamatkhana	Agriculture Department Hunza	10-11, May-2018	30
7	Hunza	Gulkin Community Center	Agriculture Department Hunza	12-13, May-2018	30
8	Hunza	Passu community Center	Agriculture Department Hunza	14-15, May-2018	30
9	Hunza	ShishkatJamatKhana	Agriculture Department Hunza	16-17, May-2018	30
10	Skardu	Tormik Community Center	LSO Tormik	18-19, July-2018	36
11	Skardu	Astana Grace Academy	Agriculture Department Skardu	20-21, July-2018	35
12	Skardu	Baghardu High School	Rang Yul Welfare Organization	23-24, July-2018	30
13	Shigar	Agri. Nursery Hasoopi	Agriculture Department Shigar	29-30, Aug-2018	30
14	Shigar	Agri. Nursery Choka	Agriculture Department Shigar	31-1, Sep-2018	30
15	Shigar	Makunja	Kisaan Cooperative Society Shigar	2-3, Sep-2018	30
16	Shigar	GulabPur	Agriculture Department Shigar	4-5, Sep-2018	30
Total Fruit Growers/Farmers Trained					491

Table 3. *Number of Training Participants Developed Fruit Jam at their Homes after Receiving*

G.#	District	Training Location	No of trainees Developed Apple jam at home	No of trainees Developed Apricot Jam at home	Date	No of Trainees	%age
1	Skardu	PCSIR Laboratory	12	-	13-Feb-2018	30	40%
2	Diamer	Agri. Nursery Chilas	8	-	19-Feb-2018	30	26.6
3	Diamer	Agri. Nursery Goner farm	-	14	21-Feb-2018	30	46.6
4	Diamer	Agri. Nursery Tangir Juglot	10	-	23-Feb-2018	30	33.3
5	Diamer	Agri. Nursery Darel Guma	14	-	25-Feb-2018	30	46.6
6	Hunza	KhanaAbaridJamatkhana	17	-	10-May-2018	30	56.6
7	Hunza	Gulkin Community Center	20	-	12-May-2018	30	66.6
8	Hunza	Passu community Center	15	-	14-May-2018	30	50
9	Hunza	ShishkatJamatKhana	8	-	16-May-2018	30	26.6
10	Skardu	Tormik Community Center	22	-	18-July-2018	36	61.1
11	Skardu	Astana Grace Academy	-	25	20-July-2018	35	71.4
12	Skardu	Baghardu High School	-	23	23-July-2018	30	76.6
13	Shigar	Agri. Nursery Hasoopi	-	27	29-Aug-2018	30	90
14	Shigar	Agri. Nursery Choka	24	-	1-Sep-2018	30	80
15	Shigar	Makunja	26	-	2-Sep-2018	30	86.6
16	Shigar	GulabPur	20	-	4-Sep-2018	30	66.6
		Total	196	89		491	58.04

Demonstration Use of Fruit Processing Machinery

The participants were trained on use of fruit processing machinery for fruit pulp extraction, fruit/vegetable peelings, fruit crushing, chips cutting machinery, fitted on Mobile Training Unit (MTU). Skill development on the use of fruit processing machinery will be helpful in processing and preservation of fruits at commercial scale and in developing processing industry at Gilgit-Baltistan level. The farmers appreciated and took keen interest in the use of machinery. In some training locations, there was no road access for MTU; hence demonstration of commercial-scale machinery fitted in MTU could be performed.

CONCLUSION

The findings of these training activities showed that fruit processing training are likely to help control wastages/losses of fruits and vegetables. Value addition of fruits is expected to become a source of income generation so that the growers can get maximum benefits of their fruits/produce. Ultimately, this will

become a step towards self-sufficiency of the people of Gilgit-Baltistan. Skill development in use of fruit processing machinery will lead towards the establishment of fruit processing industry in GB.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Contamination Status of Water, Fish and Vegetable Samples Collected from a Heavy Industrial Area and Possible Health Risk Assessment

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ABSTRACT

Aim

The present study was conducted to extract information about heavy metal pollution in water of Karnaphuli river and to assess the risk to public health occurred from consumption of heavy metal contaminated foodstuff like fish and vegetables collected from the adjacent area of Karnaphuli river which receives a huge amount of industrial and domestic wastes from kalurghat heavy industrial area, Chittagong, Bangladesh.

Methods

Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer (Model: Epsilon 5) was used as major analytical technique for determining elemental concentration. For assessing toxicity level of analyzed foodstuffs and associate health risk problem some indices like metal pollution index (MPI), health risk index (HRI) and hazard index (HI) were also estimated.

Results

The mean value of physicochemical properties like pouvoir hydrogène (pH), electrical conductivity (EC), total dissolved solid (TDS), salinity of river water were found 6.8, 745.5 µS/cm, 458.2 mg/L, 747.4 µS respectively showing that those values are much lower than the Department of Environment (DoE) of Bangladesh suggestive value, indicating safe for irrigation but EC and salinity are higher than the DoE suggestive value for drinking water. In water, the mean concentration of heavy metals in Karnaphuli river was found in the sequence of Fe>K>Cr>Mn>Zn>Cu>As=Ni=Hg>Pb. Chromium, Manganese, Iron, Zinc, and Mercury concentrations are higher than World Health Organization (WHO) standard 2011, United States Environmental Protection Agency (USEPA) 2009 and Bangladesh Standard for drinking water but other elements are within the safe limit. All metal concentrations in water are below the Bangladesh Standard for Irrigation except Iron (Fe). The decreasing trend of heavy metals (mean) in all the vegetable was Fe>Zn>Cu>Cr>Ni>Pb>Co and for all fish was Fe>Mn>Zn>Cr>Ca>Se>Co>Cu>K. Metal pollution index (MPI) for fish and vegetable is high enough to cause any detrimental effect on human. Estimated daily intake (EDI) value for fish followed a decreasing sequence Fe>Mn>Zn>Cr>Cu>Ni=As>Pb and for vegetable samples Fe>Zn>Cu>Mn>Cr>Ni>Pb>As respectively. Health risk assessment (HRI), and hazard index (HI) value are less than one for fish but HI value is greater than one for most of the vegetable samples analysed.

Conclusion

From the overall study it can be concluded that the mean value of physicochemical parameters (pH, EC, TDS, salinity) in river water were much lower than the DoE of Bangladesh suggestive value, indicating safe for irrigation but not safe for drinking. Fishes are safer for human consumption than vegetables collected from that particular area and hence, suggested to consume those vegetables at lower amount in the diet to reduce any detrimental effect.

Keywords

Health risk index (HRI); Hazard index (HI); Metal pollution index (MPI); Toxic effect; Vegetables; Fishes.

INTRODUCTION

Heavy metal contamination in aquatic environment is a critical concern, due to toxicity of metal and their accumulation in aquatic habitats. Heavy metals, in contrast to most pollutants, are not biodegradable and they undergo a global ecological cycle in which natural waters are the main pathways.[1] A large part of the heavy metal input ultimately accumulates in the estuarine zone and continental shelf, since these areas are important sinks for suspended marine and associated land-derived contaminants.[2]

Food safety is a major public concern worldwide especially in a country like Bangladesh where population is a great problem. The increasing demands for food and food safety have drawn the special attention of researchers to the risks associated with consumption of contaminated foodstuffs i.e. pesticides and heavy metals.[3,4] Heavy metal contamination is a major problem of the environment as they are one of the major contaminating agents of the food supply.[4] This problem is receiving more and more attention all over the world, in general and in developing countries in particular. Among the heavy metals some are toxic such as Cd, Pb, Cr, Hg, As, etc. and some are essential such as Fe, Zn, Mn, Cu, Ni, Co, Si, etc. The biological half-lives of these heavy metals are non -biodegradable and thermo-degradable and thus their accumulation readily reaches to the toxic levels.[5] They have the potential to accumulate in different body organs and thus produce unwanted side effects.[6-8]

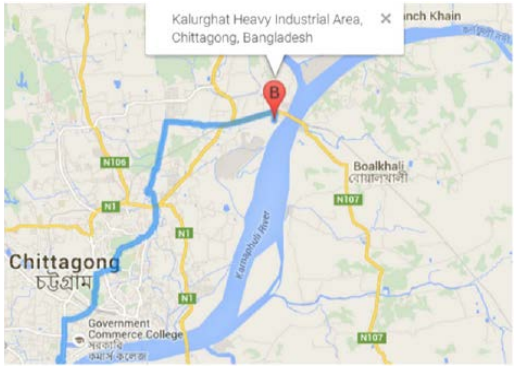
Kalurghat area in the port city, Chittagong, Bangladesh has grown up as a rapidly expanding industrial area in the country. Different industries were set up there according to the data of Chittagong Chamber of Commerce and Industry. These industrial units directly discharge untreated toxic effluent into Karnaphuli river[9] and hence metals remain in the ecosystem eventually move from one compartment to the other within the food chain. These toxic metals not only pollute the river waters but also pose a threat to the aquatic biota. The increase in residue levels of heavy metal content in water and biota will result in decreased productivity and increased health risk in case of human beings. For better understanding of heavy metal sources, their accumulation in the water seems to be particularly important issues of present-day research on risk assessments. The water and food reserves and resources of Bangladesh have not been considered seriously for long time and hence extensive and comprehensive research on the pollution of fish as well as vegetable samples grown in contaminated water is urgently needed. Present study was therefore sketched to quantify the level of heavy metal accumulation by water, fish and vegetable samples collected from the kalurghat heavy industrial area at Karnaphuli river under Chittagong district, Bangladesh and to assess the possible health risk that may associate due to dietary intake of those foodstuffs.

MATERIALS AND METHODS

Study Area

The study has been carried out in Karnaphuli river near Kalurghat heavy industrial area (Figure 1) located in 5 no. Mohra ward sub-locality, Chittagong District, Bangladesh. 22°23'48.66" latitude and 91°52'59.22" longitude can be mapped to closest address of kalurghat heavy industrial area, Chittagong, Bangladesh (Figure 1). Kalurghat area is loaded with huge number of industries, most of which discharge their effluents in the Karnaphuli river without any prior treatment, unthinkable in these days. Even more, Karnaphuli is being polluted by agricultural runoff resulting in reduced amount of oxygen available and thus harming aquatic life in the river. Vegetable samples, usually irrigated by the river water, were collected from embankment of the river in addition to the different variety of small fishes and surface water collected directly from the river.

Figure 1. *Map of the Study Area*



Water Sample Collection

Ten water samples (500 ml) were collected in March 2015 along the downstream of river (Rw-1, Rw-2, Rw-3, Rw-4, Rw-5, Rw-6, Rw-7, Rw-8, Rw-9, Rw-10) which started from CNB drain (a drain next to Chandgaon Kalurghat heavy industrial area) that carries most of the effluents of Kalurghat industrial area. Starting point of water sample collection was the junction of the river and drain. Others were collected 10, 20, 30, 40, 50, 60, 70, 80, and 90 m away from the starting point. During sampling, cleaned plastic bottles rinsed three times with river water were used to obtain representative samples. Water samples were collected from about 10 cm depth of surface water to avoid air penetration. pouvoir hydrogène (pH), electrical conductivity (EC), total dissolved solid (TDS), temperature and salinity of water samples were measured *in-situ* and rest were preserved with 10% nitric acid in refrigerator for further analysis.

Collection of Vegetable and Fish Sample

A total of six vegetable samples viz; brinjal (*Solanum melongena*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), string bean (*Vigna sesquipedalis*), lady's finger (*Hibiscus esculentus*), Hyacinth bean (*Lablab niger*) and five species of fish samples localy named kachki fish (*Coricasoborna*), poa fish (*Otolithoides pama*), chingri fish (*Macrobracium lamarre*), chiring fish (*Apocryptes bato*), shamuk (*Neritaspengleriana*) were randomly collected in triplicate,

from different locations of the study area during the period of March to April 2015. The samples were tagged with proper identification number and carried to the laboratory for analysis.

Water Sample Preparation

For X-ray fluorescence (XRF) measurement of elements, a volume of 500 ml of each collected water sample filtered with Whatman 41 filter paper was taken in a clean weighed porcelain dish followed by addition of 2 gm of cellulose powder (analar grade) and evaporated on water bath. The sample after evaporation to dry mass was further dried under IR lamp at about 70 ºC for two hours to remove the trace of moisture and weighed. For homogeneous mixing, the dry mass was then transferred to a carbide mortar and ground to fine powder using a pestle. Each powdered sample was pressed into a pellet of 2.5 cm diameter with a hydraulic press pellet maker (Specac) using 10 tons of pressure.

Preparation of Vegetable and Fish Sample

The vegetable samples were cut into suitable pieces with a stainless steel knife, washed first with tap water several times and rinsed with deionized water three times. The inedible parts of all fish samples were removed with a stainless steel knife. The remaining edible part of the samples were washed with tap water repeatedly and then rinsed with deionized water three times. All vegetables and fish samples were then taken into porcelain dishes separately. Each dish with particular sample was marked with an identification number and placed in an oven at around 70 ºC for overnight drying which was continued until a constant weight was obtained. The dried mass of each sample was then transferred to a carbide mortar and ground to fine powder using a pestle. Each powdered sample was pressed into a pellet of 2.5 cm diameter with a hydraulic press pellet maker (Specac) using 10 tons of pressure.

Measurement of Physicochemical Properties

pH, EC, TDS, temperature and salinity were determined by using Multimeter HACH-USA-Sension 378 which is equipped with glass electrode. The electrode is rinsed and calibrated with distilled water. Meter was kept in gentle position through the water column while a reading was being taken.

Sample Analysis

Sample irradiation with X-ray beam: Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer (Model: Epsilon 5) was used as major analytical technique for determining elemental concentration. The irradiation of all real samples was performed by assigning a time-based programmed, controlled by a software package provided with the system. The standard materials were also irradiated under similar experimental conditions for construction of the calibration curves for quantitative elemental determination in the respective samples. The generated X-ray spectra of the materials were stored into the computer.

Construction of calibration curve and method validation: A direct comparison method based on EDXRF technique was used for elemental concentration measurement.[10] In comparison method, standards are set to construct the calibration curves. Again key to this comparison method is that both the standard and the samples have to be of similar matrix, so that they can produce identical sensitivity and thus matrix effects are nullified. Hence to comply with the fact, three lab-synthesized cellulose-based multi element standards (cellu-1, cellu-2, and cellu-3) were used to construct the calibration curves[11] for carrying out elemental analysis in water samples. The calibration curve constructed for each element was based on its K X-ray and L X-ray line sensitivity as a function of its atomic number. To justify the accuracy of the curves, a groundwater sample were analyzed under the constructed calibration curve and in another method named total reflection X-ray fluorescence (TXRF) respectively. All the results were found within the acceptable limit.[12] For vegetables and fish samples, precision and accuracy of the method was checked through analysis of spinach/NIST 1570a and tuna fish homogenate/IAEA-350 respectively. Obtained values were found in good agreement with the certified values and the percentage of relative error and coefficient of variation in almost all the elements were less than 10%.[13,14]

Data Analysis

Metal pollution index: Metal Pollution Index (MPI) was computed to determine overall heavy metal concentrations in different food stuff analyzed. This index was obtained by calculating the geometrical mean of concentrations of all the metals in different food stuff[15] following the equation bellow:

$$MPI\ (mg\ kg^{-1}) = (Cf_1 \times Cf_2 \times \ldots\ldots Cf_n)^{1/n}$$

Where Cf_n=Concentration of n number of metal in the sample.

Health risk index (HRI): The health risk index was calculated as the ratio of estimated exposure of test vegetables and fishes and oral reference doses.[16] The oral reference doses (R_fD) represents an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. Oral reference dose for Cr, Ni, Cu, Pb, Mn and Zn were 1.5, 0.02, 0.04, 0.004, 0.033 and 0.30 (mg/kg bw/day) respectively;[17] 10-60 (mg/kg bw/day) for Fe;[18] 0.002 (mg/kg bw/day) for Hg.[19]

The R_fD for inorganic arsenic is 0.0003 (mg/kg bw/day) based on hyper pigmentation, keratosis and possible vascular complications in human.[20]

The estimated daily intake (EDI) of each metal in this exposure pathway is calculated by the equation:

$$\text{Estimated Daily Intake (EDI)} = \frac{C_{metal} \times D_{food\ intake}}{B_{average\ weight}}$$

where, C_{metal}, $D_{food\ intake}$, and $B_{average\ weight}$ represent the heavy metal concentrations in foodstuff (mg kg^{-1}), daily intake of foodstuff and average body weight, respectively. According to the food consumption, survey,[21] Bangladeshi people per person per day consumes vegetable: 0.089 kg/day[22] and fish: 0.03 kg/day.[23] The average body weight (baverage weight) was taken 70 kg for adults according to World Health Organization (WHO).[24]

Health risk of consumers due to intake of metal contaminated foodstuffs was assessed by using HRI. A HRI greater than 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a HRI bellow 1 means that there is a chance of non-carcinogenic effects, with an increasing probability as the value increases. The HRI was calculated by using the equation suggested by Wang et al.[25]

$$HRI=DIM/RfD$$

It has been reported that exposure to two or more pollutants may result in additive and/or interactive effects. The hazard index (HI) of heavy metals for individual foodstuff was also calculated which is the arithmetical sum of the individual metal HRI[26]:

$$\text{Hazard Index (HI)}=\text{HRI (toxicant 1)}+\text{HRI (toxicant 2)}+\ldots \text{HRI (toxicant n)}$$

Statistical Analysis

To assess the contamination level of heavy metal, mean, minimum, maximum and standard deviation of water, fish, and vegetable samples were performed using Microsoft Excel (version 2007).

RESULTS AND DISCUSSION

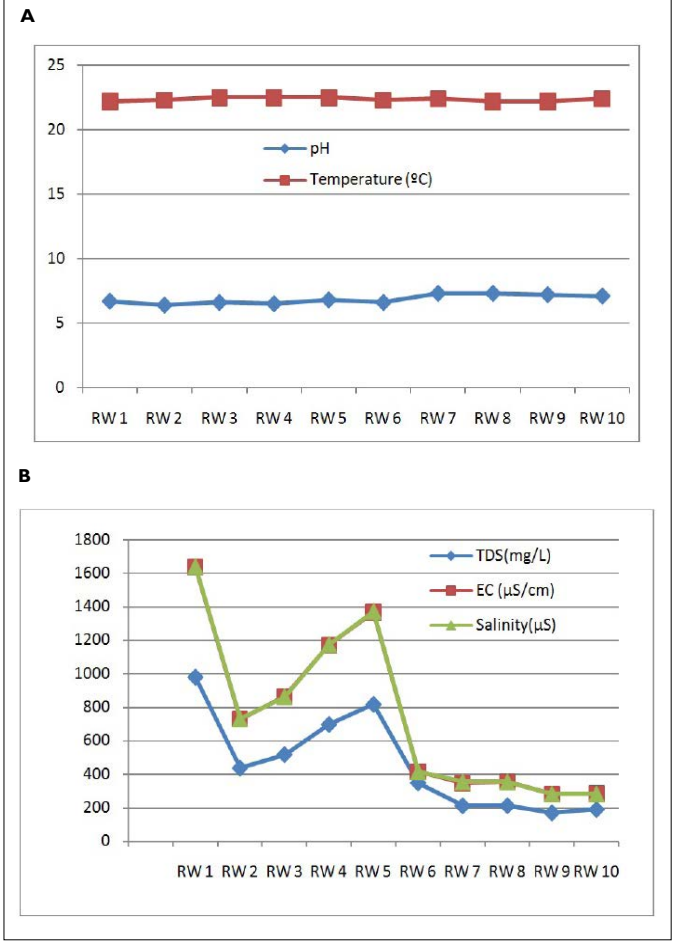
Physicochemical Properties in River Water

The physicochemical properties for river water are shown in Table 1 and Figure 2. The pH value of the study area ranges from 6.4 to 7.3 and the highest pH was observed for point RW-8 (70 m away from RW-1). Most fish can tolerate pH value of about 5.0 to 9.0 and hence pH value of water is in comfortable range for aquatic life. The level of total dissolved solid fluctuates between 190 to 979 mg/L. The highest value was observed for RW-1 (at the junction of CNB drain and Karnaphuli river) while the least value was detected at point RW-10 (90 m away from RW-1). Excessive TDS value can reduce water clarity, hinder photosynthesis, and lead to increased water temperature. However, the TDS value recorded in the entire points were within the WHO guideline of 1000 mg/L for the protection of fisheries and aquatic life and for domestic water supply. EC and salinity ranges from 282-1368 µS/cm and 283-1372 µS respectively. Department of environment (DoE) of Bangladesh, suggested value for EC in drinking water (also called BD Standard) is 320 µS/cm which is high enough in the present study which may be due to the fact that Bay of Bangle is only 40 km away from the sampling site. It is found that physicochemical properties in river water except EC and salinity are within the DoE suggestive value for drinking water. Temperature of water in the river was found uniform.

Table 1. *The Physicochemical Properties of Karnaphuli River Water*

Sample ID	pH	TDS (mg/L)	Electrical Conductivity (µS/cm)	Salinity (µS)	Temperature
RW 1	6.7±0.2	979±19	1637±23	1640±27	22.2±0.1
RW 2	6.4±0.2	437±23	730±29	731±21	22.3±0.1
RW 3	6.6±0.2	518±17	865±21	864±24	22.5±0.1
RW 4	6.5±0.2	697±18	1172±19	1175±19	22.5±0.1
RW 5	6.8±0.2	817±14	1368±23	1372±12	22.5±0.1
RW 6	6.6±0.2	349±20	416±18	416±25	22.3±0.1
RW 7	7.3±0.1	212±23	346±11	355±13	22.4±0.1
RW 8	7.3±0.1	213±22	355±12	355±11	22.2±0.1
RW 9	7.2±0.1	170±22	282±18	283±19	22.2±0.1
RW 10	7.1±0.1	190±17	284±19	283±17	22.4±0.1

Figure 2. *Physico Chemical Parameters in Water Samples of Karnaphuli River*



Concentration of Heavy Metal in Water

The concentration (Table 2) of Cr in river water ranged from

Table 2. *Concentration of Different Elements in River Water*

Elements	pHW									
	RW1	**RW2**	**RW3**	**RW4**	**RW5**	**RW6**	**RW7**	**RW8**	**RW9**	**RW10**
Cr	0.178±0.002	0.163±0.006	0.161±0.003	0.159±0.005	0.173±0.002	0.166±0.007	0.170±0.001	0.167±0.004	0.167±0.002	0.151±0.001
Mn	0.100±0.001	0.074±0.007	0.060±0.001	0.053±0.002	0.055±0.003	0.074±01.001	0.118±0.002	0.059±0.002	0.056±0.001	0.237±0.007
Fe	4.520±0.390	2.214±0.107	1.575±0.113	1.534±0.210	1.925±0.139	2.936±0.114	4.537±0.143	3.645±0.110	2.117±0.099	2.964±0.158
Ni	0.005±0.00	0.006±0.00	0.005±0.001	0.004±0.001	0.005±0.001	0.002±0.00	0.002±0.00	0.005±0.001	0.001±0.00	0.005±0.001
Cu	0.022±0.002	0.014±0.001	0.018±0.001	0.010±0.001	0.018±0.002	0.023±0.001	0.018±0.001	0.012±0.004	0.011±0.001	0.015±0.003
Zn	0.025±0.004	0.028±0.001	0.013±0.00	0.022±0.003	0.033±0.001	0.038±0.002	0.034±0.001	0.034±0.002	0.003±0.00	0.045±0.006
As	0.002±0.00	0.005±0.00	0.005±0.00	0.005±0.00	0.003±0.00	0.005±0.00	0.005±0.001	0.001±0.00	0.006±0.001	0.005±0.001
Hg	0.003±0.00	0.005±0.00	0.004±0.00	0.004±0.00	0.001±0.00	0.002±0.00	0.006±0.00	0.002±0.00	0.005±0.001	0.005±0.00
Pb	0.004±0.00	0.003±0.00	0.002±0.00	0.005±0.001	0.002±0.00	0.003±0.00	0.003±0.00	0.002±0.00	0.001±0.00	0.003±0.00

0.151 to 0.178 mg/L, which is much higher than the drinking water standard (0.050 mg/L) set by WPCB WHO[24] and technical remote viewing (TRV) (0.117 mg/L) assigned by United States Environmental Protection Agency (USEPA). Concentration of Cr in surface water at Kalurghat of Karnaphuli river was observed 0.809 mg/L by Das.[27] The value of manganese ranged from 0.053 to 0.237 mg/L in water sample, which exceeds both drinking water standard (0.1 mg/L) assigned by TRV (0.12 mg/L) and WHO,[22] (0.5 mg/L). The values of Fe in water ranges from 1.534 to 4.520 mg/L, thus at all points it exceeded both drinking water standard (0.3 mg/L) assigned by ECA[28] and TRV (1 mg/L) by USEPA. Das et al[27] reported the concentration of Fe is 35.325 mg/L at Kalurghat, which is much higher than the present value. The value of Nickel ranges from 0.001 to 0.006 mg/L which is within drinking water standard (10 mg/L) assigned by WPCB, TRV (0.052 mg/L) by USEPA and permissible limit of 0.02 mg/L given by WHO.[24] Das et al[27] observed the concentration of Ni 0.685 mg/L at Kalurghat. So it is apparent that the concentration of Ni found in the Kalurghat area is not harmful. The value of Copper ranged from 0.010 to 0.023 mg/L which is within the permissible limit (1 mg/L) set by the ECA[28] but higher than TRV value (0.009 mg/L) at all points, on the other hand all points are within the WHO, 2003 recommended value (2 mg/L), whereas Das et al[27] reported 0.711 mg/L of Cu in water at Kalurghat. The concentration of Zn ranges from 0.003 to 0.045 mg/L which is within drinking water standard (5 mg/L) and Zn in the investigated water course showed lower than the recommended value of 5 mg/L according to WHO.[24] In a previous study, it was observed[27] that the concentration of Zn was 0.731 mg/L at Kalurghat. The concentration of As in river water at different points ranges from 0.001 to 0.006 mg/L, which was within the safe limit for drinking (0.05 mg/L) assigned by ECA 1995[28] and TRV (0.15 mg/L) by USEPA. The concentration of Hg, ranges from 0.001 to 0.006 mg/L, exceeds both drinking water standard (0.001 mg/L) assigned by ECA 1995 and TRV (0.000012 mg/L) by USEPA and highest concentration of Hg was found in RW-7 (60 m away from RW-1) and lowest in RW-5 (40 m away from RW-1), showed a random distribution in water. The concentration of lead in the water sample ranges from 0.001 to 0.005 mg/L. Highest concentration was found at RW-4 (30 m away from RW-1) and lowest at RW-9 (80 m away from RW-1) showing a gradual decrease to the downstream points but Lead concentrations at all the points exceed the TRV assigned value by USEPA (0.0025 mg/L). In a study Das et al[27] reported, concentration of Pb in surface water at Kalurghat area of Karnaphuli river was 0.772 mg/L which was higher than the present study.

Heavy Metal in Aquatic Animal

Some aquatic fish collected from Karnaphuli river locally known as kachki (*Coricasoborna*), poa (*Otolithoides pama*), chingri (*Macrobracium lamarre*), chiring (*Apocryptes bato*) and aquatic animal locally known as samuk (*Neritaspengleriana*) are analyzed for heavy metals. The results obtained are shown in Table 3. Value of Chromium (Cr) ranges from 14.0 (*Neritaspengleriana*) to 16.3 (*Coricasoborna*) mg/kg respectively and all values of Chromium was found higher than the WHO standard[22] of 0.05 mg/kg. Islam et al[29] found highest concentration of Chromium in chiring fish (*Apocryptes bato*). Cr is an essential trace element in human and some animal but in excess, it could have undesirable lethal effect on fish and wildlife. Estimated Manganese (Mn) ranged from 14.1 to 216.1 mg/kg. Highest concentration was found in Samuk (*Neritaspengleriana*) and lowest in Kachki fish (Coricasoborna) but all values of Mn were found higher then WHO Standard[22] of 0.01 mg/kg. Among the heavy metals analysed, Fe was found as the most abundant. Its lowest and highest amount was found in poa (*Otolithoides pama*) (51.9 mg/kg) and samuk (*Neritaspengleriana*) (319.9 mg/kg) respectively. Akoto et al[30] reported a lower value of Iron in fishes from Fosu Lagoon, Ghana. In the present study, iron was found higher than WHO Standard[22] of 50 mg/kg for all the investigated aquatic animal and fishes. Amount of Cu in the fish muscles were found in the range of 3.4 to 10.6 mg/kg. Highest was found in chingri fish (*Macrobracium lamarre*) and lowest in samuk (*Neritaspengleriana*). FAO/WHO in 2001[31] established limits for Cu in fish as 30.0 mg/kg for human health risk concerns. Concentrations of Cu in these samples were far below the threshold value, therefore regular consumption of fish with such low amount of Cu could not lead to any serious health risk. Zinc in all the fish species were extremely high as compared to the amount of other micronutrients that were considered in this study. The maximum amount of Zn recorded in the kachki fish (*Coricasoborna*) was 70.6 mg/kg and the minimum was 21.5 mg/kg in samuk (Neritaspengleriana). FAO recommended concentration of Zn is 30 mg/kg for safe human consumption.[32] The amount

Table 3. *Heavy Metal in Fish Samples*

Element (mg/kg)	Kacki Fish	Poa Fish	Chingri Fish	Chiring Fish	Samuk	Safe Value (mg/kg)
Cr	16.3±1.1	15.0±0.0	14.4±0.7	15.3±0.5	14.0±0.6	0.05[a]
Mn	14.1±0.6	15.7±2.0	16.3±1.4	16.2±1.1	216.1±0.3	0.01[a]
Fe	82.4±3.8	51.9±0.7	84.9±1.2	196.5±5.1	319.9±10.6	50[a]
Ni	<0.24	<0.24	<0.24	<0.24	<0.24	80[c]
Cu	5.4±0.5	5.2±1.3	10.6±0.8	4.5±0.2	3.4±1.9	30[a]
Zn	70.6±2.1	35.0±0.5	40.1±0.3	30.6±13.1	21.5±1.0	50[a]
As	<0.38	<0.38	<0.38	<0.38	<0.38	0.001[b]
Pb	<0.001	<0.001	<0.001	<0.001	<0.001	-

a-WHO Standard 2004; b-FAO/WHO 1989; c-US FDA 1

of nickel (Ni), arsenic (As) and lead (Pb) were too low to detect by the instrument. In a study, Shakir et al[33] also stated the high-level of chromium and manganese which exceed the safe limit and Cu, Zn, Ni, As were within the safe limit, showing no threat to the human consumption in a wild carp fish from selected sites of a river loaded with municipal and industrial wastes in Pakistan. In the present study, the decreasing trend of heavy metal (mean) in fishes is Fe>Mn>Zn>Cr>Ca>Se>Co>Cu>K. Khan et al[13] also reported a similar trend (Fe>Zn>Mn>Co>Cu) for fish of Buriganga river.

Heavy Metals in Vegetables

Concentration of heavy metal in vegetable samples analysed are shown in Table 4. Chromium (Cr) in the investigated vegetables ranged from 0.6 to 7.4 mg/kg. Highest was found in string bean (*Vigna Sesquipedalis*) and lowest in tomato (*Lycopersicon esculentum*). Concentration of chromium in all the vegetable samples were higher than WHO/Food and Agriculture Organization (FAO) standard[34] of 2.3 mg/kg. Jolly et al[14] reported a lower level of Cr in radish, amaranthus, tomato and cauliflower collected from Rooppur area of Bangladesh. Concentration of nickel (Ni) in the investigated vegetables ranged from 0.65 to 5.43 mg/kg. Highest was found in string bean (*Vigna Sesquipedalis*) and lowest in tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*). Ni concentration was found lower than EU Standard[35] of 67 mg/kg and thus vegetables are not contaminated by nickel. Copper (Cu) is an essential part of several enzymes and it is necessary for the synthesis of haemoglobin but can cause harm at high concentrations.[33] Concentration of Cu in vegetables was in the range of 4.6 to 10.3 mg/kg. Highest was found in tomato (*Lycopersicon esculentum*) and lowest in lady's finger (*Hibiscus esculentus*). According to WHO/FAO,[34] safe limit for Cu in vegetables is 73.0 mg/kg for human and hence the value of Cu was far below the suggestive value therefore regular consumption of vegetables with such low amounts of Cu could not lead to any serious health risk so far as Cu is concerned. The maximum value of Zn recorded in the lady's finger (*Hibiscus esculentus*) was 57.1 mg/kg and the minimum was 39.4 mg/kg in brinjal (*Solanum meiongena*), which was within the guideline value of 100 mg/kg for safe human consumption[34] and hence vegetables are not contaminated by zinc. Lead (Pb) in the investigated vegetables was found from 0.57 to 0.91 mg/kg. Highest value was found in lady's finger (*Hibiscus esculentus*) and lowest in hyacinth bean (*Lablab niger*). Concentration of Pb in all the vegetable samples was within the WHO/FAO[34] suggestive value of 5 mg/kg. Among the heavy metals Fe was the most abundant metal and its highest and lowest value was found in string bean (*vigna Sesquipedalis*) 162.2 mg/kg and lowest in tomato (*lycopersicon esculentum*) 98.3 mg/kg, which was lower than WHO/FAO standard,[31] of 425 mg/kg. Manganese and mercury was present in very low amount to detect by the system . The trend of heavy metals (mean) in vegetables is Fe>Zn>Cu>Cr>Ni>Pb>Co. Khan et al[13] also reported, the trend of metals in vegetables as Fe>Mn>Zn>Cu>Co for vegetables collected from Buriganga river embankments.

Table 4. *Heavy Metal in Different Vegetable Sample*

Element (mg/kg)	Brinjal	Cucumber	Tomato	String bean	Lady's finger	Hyacinth bean
Cr	1.0±0.1	3.2±0.8	0.6±0.1	7.4±0.7	5.1±0.2	6.0±1.2
Mn	<0.63	<0.63	<0.63	15.0±2.2	<0.63	13.7±1.7
Fe	113.5±1.1	105.9±0.8	98.3±0.9	162.2±1.4	124.3±1.1	123.9±1.7
Ni	0.7±0.0	<0.65	<0.65	5.4±0.8	2.1±0.4	3.9±1.1
Cu	10.0±0.5	10.0±0.3	10.3±0.6	9.8±0.5	4.6±0.9	9.2±0.7
Zn	39.4±1.5	52.1±0.8	40.9±0.6	54.8±0.7	57.1±1.0	44.7±0.7
As	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Pb	0.90±0.03	0.64±0.11	0.70±0.56	0.64±0.11	0.91±0.04	0.57±0.11

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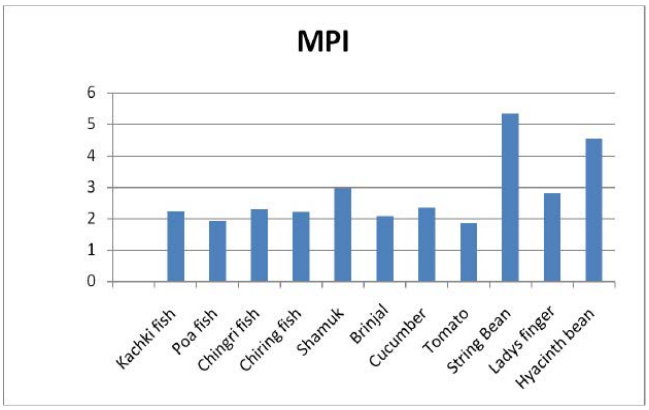
Metal Pollution Index for Vegetable and Fish/Aquatic Animal

Metal pollution index (MPI) is suggested to be a reliable and precise method for metal pollution monitoring. In this study MPI is calculated by considering concentration of Mn, As and Ni as 0.63, 0.02 and 0.65 mg/kg respectively for all vegetables where the concentrations were not detected by the analytical system. Among different vegetables, MPI (Figure 3) followed a decreasing sequence of string bean>hyacinth bean>lady's finger>cucumber>brinjal>tomato. On the other hand, among different fishes and aquatic animals (Figure 3), metal pollution index (MPI) followed a decreasing sequence of shamuk>chingri fish>kachki fish>chiring fish>poa fish. Shamuk is supposed to be more polluted because they live on sediment, which contain excess amount of heavy metal due to metal deposition on sediment surface. Islam et al[34] reported heavy metal accumulation was high in chirring fish and low in poa fish of Karnaphuli river. Vegetables are found to contain higher MPI value than fishes which may be due to the uptake of higher amount of heavy metal available from polluted soil. Khan et al[13] also reported that, vegetables contain higher MPI than fish and thus suggested that this foodstuff might cause human health risk. According to Pan et al,[35] the soil acidification trend and the decrease in soil organic matter were bound to increase the accumulation of heavy metals in agroproducts. Present findings agreed with the findings of Singh et al[36] of waste water irrigated site in north east Varanasi and the sequences was as lady's finger>tomato>brinjal.

Figure 3. *Metal Pollution Index in the Various Food Stuffs*



Estimated Daily Intake (EDI) of Metals through Different FoodStuffs

Daily intake of metals by consuming different food stuffs are shown in the Table 5. Usually different food items (fish and vegetables in this case) are consumed variably by different segment of populations throughout the year in different time; therefore it is realistic to consider the mean value of the particular elements in different foodstuffs respectively and hence the intake values have been calculated by considering the mean value of element in different fish and vegetable samples respectively and also taking the minimum detectable limit (MDL) values as the concentrations of those elements whose concentrations were too low to detect by the system. However it is observed from the table that intake of all the elements are far below the reference value (R_fD) suggested by different agencies. Shirin et al[37] reported to find estimated daily intake of metal (EDI) of Fe, Cr, Mn, Zn, As Cr, Ni and Pb were within the permissible value with an exception of Mn, As and Pb in the vegetable samples collected from the fertilizer factory polluted area. Jolly et al[38] also reported to calculate EDI value of Fe, Cu,, Mn, Zn, Co, Cr, V, Ni, Pb and Cd in vegetables collected from Rooppur area of Bangladesh and found all the values were within the permissible limit only Cd showed an alarming value. On the other hand in a study Rahman et al[39] estimated daily intake of metal (Cr, Mn, Zn, As, Pb) in marine fish samples followed a sequence Zn>Mn>As>Pb>Cr and all the values were within the safe value which is identical with the present study.

Health Risk Index (HRI) for Different Foodstuffs Analysed

Fish is one of the most popular food and sources of nutrition for human thus, intake of trace elements form consumption of fish, especially toxic elements is one of great concern for human health. To evaluate the health risk to human through the consumption of fish collected from Kalurghat heavy industrial area, HRI was estimated. The predominant pathways for heavy metal uptake, target organs, and organisms' sensitivity are highly variable and are dependent on factors such as metal concentrations, feeding behaviour and growth rates of fish.[40] The increasing demand of food safety has accelerated the research regarding the risk associated with consumption contaminated by heavy metal.[41] Result of health risk assessments (HRI) of the various heavy metals considered in this study is presented in Table 6. The calculated

Table 5. *Estimated Daily Intake of Metal (DIM) Through Different Foodstuffs*

Metal	Mean conc. of Fish sample (n=5)analysed (mg/kg)	Intake by human from fish(mg/kg bw/day)	Mean conc. of Veg. sample(n=6) analysed (mg/kg)	Intake by human from veg. (mg/kg bw/day)	RfD mg/kg bw/day	Refference
Cr	15	0.0064	3.8	0.0048	1.5	US EPA-IRIS, 2006
Mn	55.8	0.0239	5.2	0.0066	0.033	US EPA-IRIS, 2006
Fe	147.12	0.0631	121.35	0.1543	10-60	US EPA, 1989
Ni	0.24	0.0001	2.23	0.0028	0.02	US EPA-IRIS, 2006
Cu	5.82	0.0025	8.98	0.0114	0.04	US EPA-IRIS, 2006
Zn	39.56	0.0170	48.17	0.0612	0.30	US EPA-IRIS, 2006
As	0.3	0.0001	0.02	0.0000	0.0003	US EPA, ,2002
Pb	0.001	0.0000	0.73	0.0009	0.004	US EPA-IRIS, 2006

Table 6. *Health Risk Index (HRI) for Heavy Metals in Different Foodstuffs*

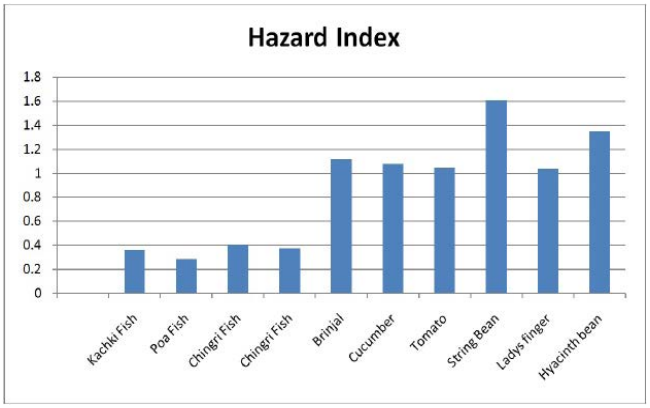
Heavy Metal	Health Risk Assessment (HRI)									
	Fish/ aquatic animal				Vegetables					
	Kachki fish	Poa fish	Chingri fish	Chiring fish	Brinjal	Cucumber	Tomato	String Bean	Lady's finger	Hyacinth
Cr	5.1×10^{-3}	4.7×10^{-3}	4.5×10^{-3}	4.8×10^{-3}	8.8×10^{-4}	2.7×10^{-3}	4.9×10^{-4}	6.3×10^{-3}	4.3×10^{-3}	5.1×10^{-3}
Mn	4.8×10^{-2}	5.3×10^{-2}	5.5×10^{-2}	5.5×10^{-2}	5.7×10^{-3}	5.7×10^{-3}	5.7×10^{-3}	1.4×10^{-1}	5.7×10^{-3}	1.2×10^{-1}
Fe	5.6×10^{-2}	3.5×10^{-2}	5.7×10^{-2}	1.3×10^{-1}	2.1×10^{-1}	1.9×10^{-1}	1.8×10^{-1}	3.0×10^{-1}	2.3×10^{-1}	2.3×10^{-1}
Co	7.6×10^{-2}	7.8×10^{-2}	1.1×10^{-1}	8.6×10^{-2}	1.1×10^{-2}	2.3×10^{-2}	1.2×10^{-2}	1.2×10^{-2}	5.0×10^{-3}	1.1×10^{-2}
Cu	6.3×10^{-3}	6.2×10^{-3}	1.2×10^{-3}	5.3×10^{-3}	3.2×10^{-1}	3.2×10^{-1}	3.3×10^{-1}	3.1×10^{-1}	1.5×10^{-1}	2.9×10^{-1}
Zn	1.1×10^{-1}	5.5×10^{-2}	6.3×10^{-2}	4.8×10^{-2}	1.7×10^{-1}	2.2×10^{-1}	1.7×10^{-1}	2.3×10^{-1}	2.4×10^{-1}	1.9×10^{-1}
Ni	-	-	-	-	4.5×10^{-2}	4.1×10^{-2}	4.1×10^{-2}	3.5×10^{-1}	1.3×10^{-1}	2.5×10^{-1}
Pb	-	-	-	-	2.9×10^{-1}	2.0×10^{-1}	2.2×10^{-1}	2.0×10^{-1}	2.0×10^{-1}	1.1×10^{-1}

HRI through the consumption of fish was less than 1, indicating that there is no potentially significant health risk associated with the consumption of fish collected from Karnaphuli river, even though the concentration of toxic elements found in fish muscles were above the limit set by national and international standard. But there is the need for a continuous monitoring of contamination level of these metals especially Pb since they can accumulate to toxic levels. Among the heavy metals examined in this study, Zn in kachki fish (*Coricasoborna*), Co in chingri fish (*Macrobracium lamarre*), and Fe in chiring fish (*Apocryptes bato*) show higher potential health risk. The health risk associated with heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb) in locally grown vegetables near kalurghat heavy industrial area were calculated as well and results are shown in Table 6. HRI for individual toxic element was found lower than one (1) for most of the vegetables. String bean has maximum HRI value of 1.61 which indicates maximum risk to public health and shows higher HRI value for Cr, Mn, Fe, and Ni. As HRI value of Pb in brinjal, Co in cucumber, Cu and Cd in tomato, Zn in lady's finger are more close to one, these metals in foodstuff may show health risk in near future. In a study Singh et al[41] reported, HRI value for Pb was higher in all the leafy vegetables. Khan et al[42] reported, Cd, Pb, and Mn have HRI>1 in food crops from wastewater irrigated land in Pakistan. Jolly et al[14] also reported the calculated HRI for Mn, Cd, and Zn was higher than 1 in vegetable samples collected from Rooppur area of Bangladesh.

Hazard Index (HI)

The HI is the cumulative non-carcinogenic effects of multiple elements exposed to consumption of one or more foodstuffs. The calculated HI (Figure 4) for fish and vegetables followed the decreasing order of chiring (*Apocryptes bato*)>chingri (*Macrobracium lamarre*)>kachki (*Coricasoborna*)>poa (*Otolithoides pama*) and string bean (*Vigna Sesquipedalis*)>Hyacinth bean (*Lablab niger*)>brinjal (*Solanum meiongena*)>cucumber (*Cucumis sativus*)>tomato (*Lycopersicon esculentum*)>lady's finger (*Hibiscus esculentus*) respectively. When the HI exceeds 1.0, there is concern for health hazard and hence HI values found more than 1.0 for all the analysed foodstuffs are suggested to not consume.

Figure 4. *Value of Hazard Index in Different Food Stuffs*



CONCLUSION

Present study was conducted to elucidate the status of environmental implication of metals in the water, commonly consumed vegetables and fishes and the possible health risk from consuming those food stuffs collected from Kalurghat heavy industrial area near Karnaphuli river. The average value of physicochemical properties like pH, EC, TDS, salinity in river water showing those properties are much lower than the DoE of Bangladesh suggestive value, indicating safe for irrigation but EC and Salinity are higher than the DoE of Bangladesh suggestive value for drinking water. The mean concentration of heavy metal in Karnaphuli river water followed a sequence of Fe>K>Cr>Mn>Zn>Cu>As=Ni=Hg>Pb. Comparing the present value with the previous study it was found that the pollution level has been decreased which may be due to implement of proper effluent treatment procedures and consciousness about harmful health effects of such pollutants. On the other hand, comparing with national and international standard it can be said that, the Karnaphuli river body near Kalurghat heavy industrial area is still contaminated by Chromium, Iron, Manganese, Zinc and Mercury.

For fish samples, heavy metal concentrations are within the safe value as defined by the recognized authorities with an

exception of Chromium, Manganese, Iron. On the other hand, the MPI, for all the fish samples are quite high. Consumption of foodstuff with elevated levels of heavy metals may lead to high level of accumulation in the body causing different diseases like thalassemia, dermatitis, brain and kidney damage and cancer. Among different aquatic animals, metal pollution index (MPI) followed a decreasing sequence of *Neritaspengleriana*>*Macrobracium lamarre*>*Coricasoborna*>*Otolithoides pama*>*Apocryptesba*. Result of HRI, HI for fishes and aquatic animals was found less than 1, as fish experience less contamination because pollutants became diluted in presence of river current and hence revealed no association of health risk or hazard for the consumers. In all vegetable samples, metal concentrations were found within the safe value recommended by WHO/FAO 2007,[42] FAO/WHO-Codex alimentarious commission 2001[34] except Cr. Metal pollution index (MPI) followed a decreasing sequence of lady's finger/okra (*Hibiscus esculentus*)>cucumber (*Cucumis sativus*)>string bean (*Vigna Sesquipedalis*)>hyacinth bean (*Lablab niger*)>brinjal (*Solanummeiongena*)>tomato (*Lycopersicon esculentum*). Vegetables are found to contain higher MPI value than fishes. HRI was found below one (1) but HI is greater than one (1) in all varieties of vegetables and thus regular monitoring is essential.

Bangladesh is facing a crucial choice between industrialization and environmental protection and therefore government policy should ensure balanced development and thus approaches should be more preventive than corrective. Therefore, water, vegetables and fishes should be monitored on regular basis in order to minimize the toxicity build-up inside the river.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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