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ACKNOWLEDGEMENT

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The Diet Equation: Linking Food, Genetics and Wellness

OPEN ACCESS

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Recent research demonstrates that food affects our physiology, and genetic code, influencing health and life span. Our knowledge of diet and wellness has increased significantly, highlighting the connection between our genetic makeup and what we eat in our daily routine. This method changes our conviction about health and offers a modified path to a great lifestyle [1].

Historically, all types of dietary guidelines were based on the study of a large population and generally recommended to everyone. After the involvement of genetic research, the approaches have been different now, a study revealed that everybody processes food and absorbs nutrients differently according to their genetic makeup. For example, some people might metabolize caffeine slowly compared to others, whereas some people have a genetic tendency to lactose intolerance. As per the type of nutritional information, it is stated that nutrients can change the genetic information over the generations.

Nutritional genomics also known as nutrigenomics, explores the relationship between the human genome, diet, and health outcomes. Researchers reveal how our body responds to different types of food and nutrients, and also identify the interaction between specific food compounds and genes. Food contains micro and macronutrients and their breakdown activates the genetic switches to regulate the genome.

Furthermore, the development of individual genetic testing helps to identify the personal genetic makeup and design their food accordingly, helping to make decisions about the nutrients. The significant increase in cases of diabetes, cardiovascular disease, and obesity has been linked to the high sugar and fat content typical of Western lifestyles. Medical anthropologists and researchers have termed this phenomenon a 'disease of civilization'.

Despite these advancements in the field of nutrigenomics, the integration of genetics and diet is not without challenges. One of the major concerns is the interpretation and application of genetic information. Furthermore, there is a need for more rigorous clinical studies to validate the effect of a personalized based diet on genetic information.

Additionally, ethical considerations surrounding genetic data privacy and potential misuse of genetic information are paramount. Assurance that genetic data is handled with confidentiality and that personalized dietary recommendations are based on robust scientific evidence is crucial for maintaining public health.

With the advancement in genomics, data science, and biotechnology. We can anticipate a more nuanced understanding of how genetic variations influence nutritional needs and health outcomes.

In conclusion, the intersection of genetics, food, and wellness represents a promising frontier in personalized medicine. As research and technology progress, the diet equation will become increasingly sophisticated, paving the way for a future where our diets are as unique as our genetic blueprints.

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Review Article

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Nanotechnology in Food: Processing, Packaging, and Preservation

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ABSTRACT

Nanotechnology entails creating, characterizing, and employing structures with sizes ranging from 1 to 100, significantly influencing medicine, engineering, agriculture, and food. Nanomaterials hold potential for the development of high-quality, healthier, and safer foods improving shelf life and reducing contaminations. Food safety and security are gaining much attention globally to maintain a consistent supply of nutrient-rich and safe food. Many disciplines of food science have been changed by the rapid growth of nanotechnology, particularly those involving food storage, processing, functioning, packaging, transportation, and other safety considerations. This review focuses on current advancements in food nano-packaging, such as active, smart, and improved packing. Nano-encapsulation improves food processing by releasing bioactive chemicals, increasing bioavailability, and extending shelf life. Additionally, applications of nanotechnology in agriculture and food, including nano-sensors, nano-encapsulation, nanocomposites, food packaging, and nano-emulsions are discussed. Despite tremendous advancements in nanotechnology in food items, nanomaterials and nanoparticle toxicity are not fully understood. If the chemical mechanisms through which nanomaterials interact with food are not completely understood, we may face a nano-toxicity catastrophe, hence they must be further characterized and their usage must be carefully controlled.

INTRODUCTION

Nanotechnology is a multidisciplinary field that includes biotechnology, chemistry, engineering, and physics and implies the utilization of nano-materials with nanoscale structures ranging from 1-100 nm [1]. Nanotechnology provides interesting opportunities in the food industry, including quality control, food safety, as well as the development of new food flavors and additives [2]. Nanotechnology plays a vital role in the agriculture and food sector by manipulating nanomaterials for various purposes, including crop improvement, improving food safety and quality, and promoting human health using innovative methods [3]. Food spoilage can occur for numerous reasons, including spoilage due to pathogens and chemicals. In the food sector, food wastage results in significant losses. According to "Food and Agriculture Organization", above "1.3 billion metric tons" of edible food are wasted each year all over the supply chain, primarily due to inadequate post-harvest techniques, transportation, and storage facilities, as well as consumer and market food waste [4]. Nanotechnology is utilized in the food industry to create packages with improved mechanical or thermal properties and safety. Nano-sensors inserted in food packaging systems, are utilized to notify consumers when food has expired [5]. Various nanomaterials including Nano laminates, nano-clays, nanofibers, nano-emulsions, and nano-rods have been created to improve agricultural production and enhance food quality [6]. Nanomaterials are also employed to increase the protective features of food due to their unique capabilities. Furthermore, numerous nano-sensors and nano-packaging materials have been employed to boost sensitivity and specificity in detecting pesticides, microbial contamination, and

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hazardous substances. Food processing is further enhanced by nano-encapsulation, which allows for the release of bioactive chemicals, boosts food bioavailability, and extends food shelf life [7]. Nanotechnology has received attention from regulatory authorities such as FDA to improve food safety and quality. Regulatory authorities have proposed safety regulations and testing protocols for nanotechnology in food packaging and processing, although approaches differ by area [8]. This review discusses possible applications and utilization of nanotechnology using various nanomaterials in the food sector for better quality and shelf life through preservation, security, processing, and storage. The potential uses of nanotechnology in food pathogen detection, nutraceuticals, and possible negative impacts of nanotechnology on animal and human health are discussed.

Nanotechnology in Food Processing

Nano-emulsions

In the food sector, nano-emulsions are utilized to produce things like salad dressing, flavored oils, individualized drinks, sweeteners, and more [9]. Nano-emulsions are colloidal solutions that have oil-in-water emulsion properties, comprising small droplets (10–1000 nm) with lipophilic and amorphous surfaces [10]. The small size of nano-emulsions allows for the production or existence of a large surface area, which can be crucial for substantial interaction with a variety of bioactive chemicals absorbed in the digestive system. Moreover, nano-emulsions digest faster than traditional emulsions because they have more binding sites in the gastrointestinal tract for the enzymes amylase and lipase [11]. Because their properties, composition, and structure can be adjusted, the nano-emulsion-based approach effectively boosts the bioavailability of physiologically active compounds [12].

Nano-encapsulation

Nano-encapsulation involves packing substances into small structures through methods like nano-structuration, nanocomposites, or nano-emulsification to enable precise release of the core. Various forms of Nano-encapsulation, such as nanoparticles, Nano-spheres, and liposomes, are employed based on specific applications. These techniques find use in diverse areas, including the use of dietary supplements to mask unwanted flavors, facilitate the effective dispersion of insoluble supplements, and enhance the bioavailability, all without the need for surfactants or emulsifiers [13]. The application of nano-encapsulation has been employed to enhance the shelf life of tomatoes, and there is potential to extend this strategy for preserving other fruits and vegetables [14].

Nano-laminates

Nano-laminate films typically consist of two or more layers of manmade or natural polyelectrolytes mixed with nanoparticles (dendrimers, silica, or inorganic nanoparticles, etc.), connected through chemical or physical means. Layer-by-layer deposition is the most common synthesis technique, allowing the surface lamination of multiple nano-layers using various nanomaterials [15]. Various adsorbing compounds, such as charged lipids, bio-based or natural polyelectrolytes, and colloidal particles, can enhance the properties of different layers [20]. Additionally, active compounds like anti-browning agents, antioxidants, antimicrobials, enzymes, odors, and flavors can be incorporated into the films to prolong the shelf life and quality of packaged food products including sausages, vegetables, citrus fruits, and other meat products [16]. Nano-laminated coatings can also be produced from edible or bio-based ingredients, serving as edible nano-coated films [17].

Nanoparticles

At the Nanoscale, nanoparticles serve to enhance food's flow properties, color, and stability [18]. Nanoparticles containing plastic films as nanoparticles of silicate, titanium oxide, and zinc oxide serve to minimize the flow of oxygen inside the food containers helping to reduce moisture leakage by improving the shelf life of the food products [19]. Nanoparticles of Silicon dioxide are used as anticaking or drying agents in food packaging and help to absorb the molecules of water in food thus, showing hygroscopic applications [20]. Silver nanoparticles act as effective antibacterial as they can penetrate through biofilms, they also help in decomposing ethylene hence, improving the shelf life of various fruits as well as vegetables [21]. Other nanoparticles with antimicrobial activity are copper and its oxide, zinc oxide, magnesium oxide, selenium, cadmium, chitosan, telluride, and single-walled carbon nanotubes [22] (Table 1).

Table 1: Nanotechnology in Food Processing

Processing Techniques	Food Items	References
Nano-Emulsions	Fresh-Cut Pineapples, Chicken Breast Fillet, Lettuce, Milk	[23]
Nano-Encapsulation	Fruit Juices (e.g., Carrot, Grape, Pomegranate etc.)	[24]
Nano-Laminates	Meats, Fruits, Vegetables, Cheese, And Bakery Products	[25]
Nano-Particles	Apples, Meat	[12]

Nanotechnology in Food Packaging

Food packaging is crucial to ensure food safety as it is important to protect food from contamination and spoilage, enhance sensitivity by increasing the activity of enzymes, and minimize weight loss —26]. Using nanostructured and nano-modified materials for packaging is important to improve the shelf life of food products —27]. The applications of different nanoparticles in food packaging are briefly explained (Table 2).

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Table 2: Applications of Nanoparticles in Packaging of Food

Nano-particles	Matrix	Applications	Reference
Silver	Poultry Meat, Orange Juice, Asparagus, Fresh Cut Melon, Beef and Meat Exudates	Stops aerobic psychotropic, molds, and yeast growth, has antimicrobial properties against Escherichia coli and Staphylococcus aureus	[28]
Zinc Oxide	Liquid Egg Albumen, Orange Juice	Without affecting quality it decreases the growth rate of Lactobacillus plant arum, yeasts, molds,and salmonella	[29]
Titanium Oxide	Strawberry, Chinese Jujube	Decrease browning rate, ripening as well as senescence and decaying process	[30]
Silver Oxide	Apple Slice	Slows-down microbial spoilage	[27]

The use of nanotechnology in packaging has shown a great number of commercial applications in recent years [31]. The advantages of using nanoparticles in food packaging include enzyme mobilization, antimicrobial potential, O2 transport, and clues for factors associated with degradation[32].

Active Food Packaging

Active packaging uses nanoparticles of metal and metal oxides as antimicrobials such as Silver (Ag) and TiO2 [33]. Due to the semi-conducting properties of TiO2, it is frequently used as an adsorbent material, strain, or catalytic substrate with increased optical, photosensitive, and electrical outcomes [34]. Active packaging keeps the food secure from environmental components by acting as a barrier to outside conditions[35].

Smart Packaging System

Nanoparticles inserted in smart packaging systems, are used to detect any chemical changes inside as well as outside the food by helping in tracing fraud. It improves mechanical barrier, and antimicrobial properties as well as monitors food during its transport and storage [36]. Various types of sensors such as chemical and biosensors are used in smart packaging systems to monitor the quality and protection of packaged goods like pharmaceuticals, foods, and health or household products [37]. Smart packaging is used to improve the total quality of food including Quality indicator (QI) temperature indicator (TI), gas concentration indicator (GC), and time-temperature indicator (TTI) provides more ease and protection against tempering of packages and counterfeiting, theft[38].

Improved Packaging

The uniqueness of improved food packaging is that nanoparticles are added to this packaging to enhance its physical and as well as mechanical properties including biodegradability, strength, UV absorptivity, strength, and oxygen permeability. Metal oxides are incorporated into the polymers that can enhance properties like light permeability [39]. Nano clay is also added because it enhances the properties of the barrier against UV radiations and gases [40]. Using one type of nano-coating allows only one benefit like enhancing the shelf life of food but applying a coating of different materials makes the food in multiple ways e.g. taste, smell, security, and ripening time[41].

Nano-clays

Nano clays are Nanoparticles that are made by layered mineral silicates, stacked together [42]. They are well-known for being reasonably priced nano-fillers that strengthen polymer nanocomposites and enhance their mechanical, thermal, and barrier qualities for use in food packaging [43]. Different types of nano clays are added to enhance the properties of polymers. Two types of Nano clays montmorillonite (MMT, MMT-Na+) and organophilic MMT (organic modified MMT, OMMT) are more prominent and preferred because of their high surface area, large aspect ratio (500-1000), and compatibility with organic thermoplastics[44].

Nano-cellulose

Nano-cellulose is synthesized by the breakdown of the cellulose particles, has the ability of biodegradability, and are biopolymer that produces the minimum amount of carbon prints [45]. Nano-cellulose can be prepared through a process of microbial fermentation or can be isolated from plant sources. Nano-cellulose has particular characteristics that enable it to be used for food packaging its crystallinity, length, diameter of the fiber, and polymerization ability enable it to work as a mechanical barrier and its degradability, renewability, and some morphological properties enable it to reinforce bio-based materials [46]. Plant cellulose is already used in food packaging as cellophane, paper board, or also in the form of modified cellulose that is hydroxyl-propyl cellulose (HPC), carboxy-methyl cellulose (CMC), and cellulose acetate, methylcellulose(MC).

Nanofibers

Nanofibers are fabricated by the method of electrospinning, within the range of micro and nanoscale [47]. Numerous materials like ceramics and polymers can be treated into nanofibers [48]. Nanofibers have outstanding properties of high porosity and large surface-to-volume ratio. In comparison to polymeric films of equivalent thickness, nanofiber mats exhibit superior mechanical capabilities. The latest research shows that nanofibers with antimicrobial activities are launched in the market that show applications in drug delivery, food packaging, and tissue engineering[49](Table 3).

Table 3: Nanotechnology Food Packaging Techniques Used for Different Foods

Processing Techniques	Examples of Food	References
Active Food Packaging	Meat, Fish	[50]
Smart Packaging	Milk, Shrimp, Chicken Breast	[39]

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Improved Packaging	Beer	[31]
Nano-clays	Meat, Bread, Fruits,Vegetables, Dried Fruits,Cheeses, Coffee	[52]
Nano-cellulose	Fruits, Vegetables	[53]
Nanofibers	Spinach, Melons, Mangoes	[54]

Methods of Nano-encapsulation

Several methodologies are used to bring about nano-encapsulation to attain enhanced bioavailability and deliver desired substances safely and in a controlled manner. A vehicle with such characteristics and benefits usually consists of a core protected by a polymer membrane layer [55].

Emulsification

Emulsification is used to prepare nano-capsules in which two immiscible liquids are mixed via the use of a surfactant (Tween 20, Tween 40, etc.). It results in the formation of nano-emulsions of two types based on oil and aqueous media resulting in the suspension of a water molecule in an oily media or vice versa. The preparation of nano-emulsions via low and high-energy methods produces droplet sizes ranging from 20-200 nm. However, low-energy methods are preferred since they are cost-effective and depend mostly on the system's internal chemical energy [56]. Sol-gel methodology focuses on the formation of gel structure with an inorganic network. Firstly, a solution is prepared (sol) and subjected to solidifying and heating to stir up the inorganic and organic molecules in the mixture. It results in forming a 3D network with high versatility and potential for incorporating functionalities [57].

Drying or Solvent Removal

This method mainly involves the removal of organic solvents like ethanol, methanol dichloromethane, etc. which can dissolve the polymer, which causes adverse effects in certain environmental conditions, to produce a powdered form of Nano capsule through Spray drying or Freeze drying. In the former, the atomizer disperses the liquid into a medium containing hot dry gas, resulting in solvent loss in a drying chamber. Freezing, however, relies on the sublimation of solvent from frozen to the gaseous state through surrounding temperature. The main problem in drying is losing original physio-chemical properties to some extent along with reduced product recovery [58]. The better technique, however, is spray drying since it operates with simple controls and provides cost-effective results [59].

Electro Hydrodynamic Processes

Electrospinning and electrospray are applicable in the production of Nano-microcapsules with high feasibility and potential. This process involves the ejection of liquid polymer solution that contains polyvinyl alcohol (PVA) or poly-capro-lactone (PCL) dissolved in organic solvents, through an atomic nozzle into an area that is controlled with varying electric fields allowing them to stretch immensely and solidify via cooling or evaporation, the resulting particles are collected in form of sheets [58]. The variation in electrospray from electrospinning stems from the varicose instability of finely charged molecules when the concentration of polymers is low. However, a rapid formation can be obtained through this strategy [60].

Nanotechnology in Food Safety

Food safety is a persistent health issue comprising foodborne diseases (FBD) due to insufficient food handling procedures, contaminated food supply, and inadequate cleanliness. Consumer sickness and FBDs are frequently connected, showing significant medical expenses, and decreased revenue and efficiency. To ensure food safety, advanced technologies such as the development of nano-sensors are utilized for the preservation and processing of food [61]. Nano-sensors can detect and provide signals for the assessment of the physical or chemical qualities of any particle that has contaminated food.

Radio Frequency Identification Sensors (RFID)

People nowadays want safer and healthier foods. To ensure food safety a complex and well-structured system RFID is developed [62]. RFID can detect any tangible object, it consists of a reader and a transponder. The reader is a device that emits radio waves in the form of an electromagnetic field [63]. This field provides energy for the radio tags to operate. The tag is a small device that is made up of an antenna and a microchip. The microchip and an antenna allow the tag to receive and transmit data [64]. RFID tags come in passive, semi-passive, and active forms [65]. RFID is mainly used to detect changes in food such as chemical changes, pH, humidity, temperature, and gas changes. The recorded changes are sent to the control system [66].

Gas Sensor

Foods that contain high levels of oxygen cause browning by food pigment oxidation and fat oxidation [67]. Most spoilage caused by bacteria and fungi needs oxygen to grow and these sensors measure oxygen content in the food. These sensors are made to detect gasses that come from the metabolism of microorganisms and are released when food spoils [68]. Gas sensors can be broadly categorized into two primary groups according to the type of transducer they use: electrochemical (potentiometric, amperometric, and conductor) and optical (colorimetric, gas-induced fluorescence change) [69]. Limit detection (LOD), power consumption, response and recovery durations, sensitivity, selectivity, and other parameters all play a part in how well these sensors perform in commercial applications [70]. The gas sensors are usually used to detect volatile organic compounds compromises of organic acids, esters, aliphatic alcohols, polyphenols, aldehydes, ketones, and amino acids [71].

Sensor for Food Pathogens and Contamination

Nanotechnology-based sensors are utilized to detect pathogens and contamination in food [72]. Nano-sensors have potential for quick pathogen detection because of

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their sensitivity, and specificity which are derived from antibody-antigen interaction [73]. Furthermore, because of their small size, nanomaterials can attach to bacterial cells, amplifying signals and extending detection limits. Commercially available sensors such as Toxin Guard and Food Sentinel Systems, are nano-sensors based on antigen-antibody interaction [74]. This detector uses thin polymer films with immobilized antibodies to detect pathogens that cause food-borne diseases. The change in configuration or color suggests the presence of pathogens [75].

Toxin Detection

Quantum dot sensors are used to detect the toxins and pesticides present in the food. Using water-soluble bi-conjugated QDs, toxins and enterotoxin (produced by S. aureus and E. coli) can be identified. The benefit of these artificially created aqueous QDs comprises extended photo stabilities, wide absorption, stability, and a highly compatible, highly specialized emission spectrum. Because of their unique optical and magnetic characteristics, they can be combined with various biomolecules to form hybrid, integrated biosensors with targeted, sensitive detection capabilities [76]. QDs can be assembled in an assembly and coated with a coating of chitosan, thio-glycolic acid, and organophosphorus hydrolase to detect the harmful chemical (paraoxon) produced by the organophosphorus insecticide parathion [77](Table 4).

Table 4: Nano-sensors Used for Safety Detection in Various Food Items

Nano-sensors	Food Items	References
RFID Sensors	Meat, Fruit, and Dairy Products	[53]
Gas Sensors	Chicken, Apples, Pears, and Kiwis	[71]
Food Sentinel Systems (Sensor for Food Pathogens and Contamination)	Fish, Poultry, Meat,	[74]
Quantum Dot Sensors	Milk, Egg, Chicken, Vegetables, Water	[78]

Nanotechnology for Detection of Mycotoxins in Food and Feed

About a million species in the fungal kingdom are used for different industrial activities, including manufacturing chemicals and antibiotics. By fermentation, fungi are also essential to the synthesis of food and drink [79]. On the other hand, more than 400 fungal species are harmful to people and can result in endemic or infectious diseases. When mycotoxin-producing fungi are found in poisoned food and feed, they may be extremely harmful to both human and animal health [80]. Aspergillus, Fusarium, and Penicillium are prominent fungal genera that produce mycotoxin; among these, aflatoxins, ochratoxin A, zearalenone, fumonisins, and trichothecenes are of particular concern because of their potential health and economic effects [81].

Mycotoxin Toxicity and Regulations

Mycotoxin exposure can have severe, life-threatening consequences in addition to harmful effects including damage to the DNA, oxidative stress, and cell death. The degree of toxicity can vary greatly. The International Agency for Research determines mycotoxin carcinogenicity in Cancer [82]. Mycotoxin maximum values in food and feed have been set by regulatory authorities such as the EU Scientific Committee for Food (SCF) and the World Health Organization (WHO) to protect public health. Effective detection techniques are required to ensure respect for these standards and minimize financial damages [83].

Challenges in Mycotoxin Detection and Conventional Methods

Mycotoxins have low concentrations (parts per billion) in food and feed, making detecting them in trace amounts difficult. Conventional techniques like enzyme-linked immunosorbent tests (ELISA) and the use of high-performance liquid chromatography (HPLC), although sensitive and specific, are time-consuming, costly, and take a lot of time [84]. In contrast, rapid screening tests are not as effective or reliable as they should be. Because of this, there is an increasing need for quick, affordable, and trustworthy methods for mycotoxin detection in quality food management [79].

Biosensors for Mycotoxin Identification

Biosensors are emerging as a valuable tool for the early detection of food spoilage, poisonous fungi, and mycotoxins. These devices combine a biological sensing element with a transducer to offer sensitivity, simplicity, and fast analysis [85]. There are many types of biosensors, including piezoelectric, optical, and electrochemical biosensors, which introduce nanomaterials to boost signal strength and sensitivity. For instance, gold nano-rods embedded in optical biosensors are used to detect aflatoxins, quantum dots are used for mycotoxin detection in food beverages, and silver nanoparticles for ochratoxin detection [86](Table 5).

Table 5: Nano-Biosensors for the Detection of Mycotoxins in Food

Nano-biosensors	Mycotoxin Detected	Food Item	References
Immuno-Chromato-Graphic Nano-Biosensors	Zearalenone (ZEN)	Corn	[86]
Fluoro-Immunoassay Nano-Biosensor	Aflatoxin B1	Peanuts	[86]
Electrochemical Biosensor (Aunps/COF/Apt)	Zearalenone (ZEN)	Corn Flour	[87]
Electrochemical Biosensor (Nafion/G/Aunps/Phno2/Ab)	Deoxyniva-lenol (DON)	Cereals	[87]
Electrochemical Immuno-Sensor	PAT	Apple Juice	[88]

Safety Concerns of Nanotechnology in Food Industry

Because of nanotechnology developments, nanoparticles' use in the food business is expanding, causing serious safety risks and regulatory difficulties. Risks related to nanoparticle usage in food items are receiving immediate

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attention due to limited knowledge of their toxicity and the release of allergens and heavy metals [89]. Nanoparticles have the potential to cause unexpected health hazards by interfering with biological processes, damaging DNA, and affecting different parts of the cell. Complete toxicity studies are necessary due to the possibility of organ accumulation and the wide range of effects that nanoparticles might have on various tissues [90]. Although organic nanoparticles are usually considered non-toxic, the lack of international rules and differences in regulatory strategies, such as those used by the FDA and EFSA, emphasize the necessity of uniform safety evaluations and precise recommendations. Achieving a balance between using nanotechnology's advantages in food processing and protecting human health requires thorough investigation, clear laws, and public participation in decision-making [91].

Safety Concerns

To ensure food safety, it is essential to address the potential for nanoparticles to migrate from packaging materials into food products. A thorough understanding of the functional properties and toxicity of nanomaterials at the nanoscale will greatly improve the practical applications and safety standards of nanotechnology. It is important to recognize and address the potential health risks, toxicity issues (organ and tissue toxicity), and environmental concerns associated with nanoparticles [92]. There has been a noticeable advancement in the use of innovative nanotechnology in the food sector, even though the possible toxicity of nanomaterials is still not fully known and the FDA has given general approval for the use of nanotechnology in the food industry. The FDA does not categorically ascertain products containing nanomaterials or utilizing nanotechnology as inherently safe or harmful. Instead, the FDA will regulate nanotechnology products within its existing statutory authorities, in line with the specific legal standards applicable to each type of product under its jurisdiction. The FDA supports innovation and the safe use of nanotechnology in FDA-regulated products through enhanced scientific expertise and tools necessary to assess the safety and effectiveness of products under balanced regulatory oversight [93].

CONCLUSIONS

It was concluded that nanotechnology provides multiple methods for improving food safety throughout the supply chain. These improvements, which range from nano-sensors to RFID devices, allow for the accurate detection and control of pollutants. Regulatory and safety concerns remain significant needing extensive toxicity assessments. Nonetheless, nanotechnology's ability to revolutionize food safety measures is apparent. FDA has given general approval for the use of nanotechnology techniques and nanoparticles in food packaging, processing, and preservation with proper safety assessments but there are still many health risks associated with the use of nanotechnology in the food industry. Continued research and collaboration are required to obtain the full benefits and protect consumer health in modern food systems.

Authors Contribution

Conceptualization: AJ
Methodology: AJ, SI, AA[1], MB, AA[2], SU
Formal analysis: MBS, MW
Writing review and editing: AJ, MW, MAH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Nutritional Analysis of Wheat Flour at Hyderabad for Detection of Essential and Toxic Metals

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ABSTRACT

Wheat flour is basic diet in Asian countries. Quality of wheat flour and milling process has been changing day by day which have profound impact on nutrition value of wheat flour. **Objective:** To carry out Nutritional assessment of whole and refined wheat flour grinded locally at 13 mills of Hyderabad to determine presence of essential and toxic metals. **Methods:** Whole and refined wheat flour were randomly collected from 13 flour mills of Hyderabad for determination of moisture, ash, fat, fiber, carbohydrates, proteins, essential (Fe, Zn and Mn) and Toxic (Cd, Cr, Pb and Cu) metals with standard scientific methods. **Results:** High moisture has been recorded in F.M.13 mill in whole and refined flour as 12.5% and 11.8% respectively where as low moisture 7.1% has been found in whole flour in F.M.10 mill and 7.4% in refined flour in F.M.01 mill. F.M.04 contains high Iron in whole and refined wheat flour as 0.91±1.1 and 0.74±0.5mg/kg respectively. Zinc content has been high in F.M.10 and F.M.11 as 9.95±5.6mg/kg and 8.66±5.1mg/kg respectively. Cadmium has been high in F.M.09 as 0.06±0.01mg/kg in refined flour whereas Lead has been high in F.M.09 as 0.28±0.13mg/kg in whole wheat flour. **Conclusions:** Carbohydrates have been high whereas fiber and protein has been low in refined flour. Fe, Zn and Mn has been significantly low whereas Cd, Pb, Cr and Cu has been significantly high in refined wheat flour. It is concluded that consumption of whole wheat flour is better than refined wheat flour.

INTRODUCTION

Human beings have been using cereal grains as food since birth. Corn, Oat, Barley seeds are grown worldwide, however; wheat grains are popular worldwide due to flavor/taste, multiple uses (bakery items etc) and availability in the market. In Asia, wheat grains (Triticum aestivum) are grinded to make flour for meals or bakery items and refined flour etcetera [1]. In ancient times wheat was grinded in small grinders at home after proper washing and cleaning. With advancement; large flour mills were installed to meet bulk supply and demand of the market [1-3] and elaborate procedures of washing, drying, cleaning/segregation of rotten wheat were cut short while compromising the quality of flour. From nutritional point of view; our diet should ideally contain 50% carbohydrates (glucose and fiber) in wheat flour [4]. Short shelf life of whole wheat flour introduced refined form of flour in the market by removing extra fiber from whole grain flour thereby increasing its shelf life and making it easy to digest by all ages. It gained rampant popularity in market due to easy making of bakery items; however; less fiber and more percentage of glucose proved it harmful for diabetic and obese people. Discreet use of bleach was also noticed with few mills to improve colour and rihiology of dough and enhance its shelf life [5]. Heavy metal accumulation in soil and wheat crops can lead to insoluble complexes that hinder fertilizer uptake. The growing need for food safety has raised concerns about pesticides, heavy metals (Pb, Cd, Cu, Zn, and Co), and toxins [6, 7]. However, refined flour

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becomes more vulnerable to contamination during grinding process due to chemical treatment and might cause disorders [8, 9]. In this research work wheat flour samples have been collected for analysis of nutritional assessment and presence of essential and toxic metals to determine quality of wheat flour.

METHODS

A cross sectional analytical study was conducted at Institute of Biochemistry, University of Sindh, Jamshoro from June 2022- June 2023. Initially survey of shops and consumers was carried out and it was found that 13 mills have been supplying two types of wheat flour at shops of Hyderabad and adjoining areas. A sample each of whole and refined flour was collected during three different stages i.e. grinding, packing and storage, of all 13 mills (3x13=39 samples each of whole and refined flour). Samples were quantitatively tested for moisture, ash, total carbohydrates, total protein, iron (Fe), zinc (Zn), manganese (Mn), cadmium (Cd), cromium (Cr), lead (Pb) and copper (Cu). Flour mills were coded as Gul Star flour mill (FM.01), Hameed flour mill (FM.02), Geo flour mill (FM.03), Sukkur flour mill (FM.04), Jubilee flour mill (FM.05), Al. Mustafa flour mill (FM.06), Hyderabad flour mill (FM.07), Sun Shine flour mill (FM.08), Syed flour mill (FM.09), Super Shine Roller flour mill (FM.10), Ghori flour mill (FM.11), Aghaz flour mill (FM.12) and Al.Noor Roller flour mill (FM.13). **Wheat Flour Samples:** 500g samples of whole and refined flour were collected in polyethylene bags from each mill during grinding, packing and storage (3 x 13=39+39=78). Labeled all samples and brought them to laboratory for nutritional assessment and metal analysis. **Moisture Content:** Took 5g of each sample (whole and refined flour) in Petri dish and carried out weight on digital machine. Covered the sample in aluminum foil and put it in oven at 130°c for 120mins. Sample was repeatedly heated after every 10mins until final weight became constant [10]. Moisture (%)= Obtained weight/total weight of sample x 100. **Ash Content:** 5g of each sample was put in crucible and placed in furnace at 580°c for two hours. Carried out its weight on normalization and repeated the procedure until complete carbon was burnt out of it [10]. Ash (%)=Weight of Ash/total weight of sample x 100. **Total Fat:** Soxhlet apparatus has been used for determination of total fat [10]. Fat (%)= Obtained weight/total weight of sample x 100. **Total Fiber:** McCleary 2023 has been used for the determination of fiber [11]. Fiber (%)= Obtained weight/total weight of sample x 100. **Analysis of Carbohydrates:** Took 5g of each sample in conical flask and added 5ml of 2.5 N HCl in it. Took the mixture and placed it on hot water bath for 3hrs. Cooled it and added 0.1ml normal sodium bicarbonate until sparkle ended. Diluted it with 100ml distilled water and filtered it. **Procedure:** Took 1ml of sample and 4ml of Anthrone solution and had put it on boiling water bath for 10mins. Checked; absorption of sample on double beam spectrophotometer at 630nm. Determined standard of different concentrations and found concentration of total carbohydrates against standard curve [12]. **Analysis of Proteins:** 5g of each sample was taken in conical flask. Added 10ml phosphate buffer (pH from 7.4-7.6) in it and put it in shaking water bath overnight. Centrifuged the solution, filtered it and filtrate was collected, diluted with 10ml distilled water and kept it for boiling water bath for 10mins. Added 0.1ml of Folin coicaltau and kept the solution at room temp for 30-60mins. Checked; absorption at 750nm with double beam spectrophotometer [13]. **Analysis of Metal Sample Preparation:** 1g of grinded whole and refined flour was put in a beaker and added 2M H2SO4 and HNO3. Placed beaker on hot plate at 100°c for 2hrs and covered it with glass plate to prevent any kind of evaporation. Added 50ml distilled water and filtered it. Filtrate was used for metal analysis. Atomic absorption spectrometry was used for metal analysis, wavelength of metals; Cadmium (228.8nm), Lead (217nm), Chromium (357.9nm), Copper (324.7nm), Iron (248.3nm), Zinc (213.9nm) and Maganese (279.8nm) have been used [14]. Mean ± SD of carbohydrates, protein, Fe, Zn and Mn, Cd, Cr, Pb and Cu have been determined by descriptive analysis. p-values have been calculated by Independent samples t-test. (Note: a sample each of whole and refined flour collected during three different stages i.e. grinding, packing and storage, of all 13 mills (3x13=39 samples each of whole and refined flour) and analyzed. Obtained results have been used to find mean and standard deviation and p-values and <0.05 p-value has been considered statistically significant. Data entry and analysis has been done with SPSS version 22.0 [15].

RESULTS

Table 1 shows percentage of moisture, ash, fat and fiber and Mean ± SD of carbohydrates and protein of 13 mills. F.M.12 has lowest ash content of 0.69% and F.M.01 has 0.98%. Carbohyderates have been high in whole flour in F.M.04 and F.M.08 as 73.26±5.4g/100g and 73.43±3.1g/100g whereas in refined flour F.M.04 and F.M.09 have 80.95±4.6g/100g and 80.24±6.5g/100g respectively. Protein content has been found high in whole flour in F.M.11 and F.M.13 as 10.19±1.9g/100g and 10.11±1.9g/100g respectively. High protein was also found in refined flour in F.M.11 and F.M.13 as 8.78±1.5g/100g and 8.84±1.3g/100g respectively.

Table 1: Nutritional Evaluation of Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Carbohydrate (g/100g) Mean ± SD	Protein (g/100g) Mean ± SD
F.M.01	8.7[a]	1.32[a]	0.7[a]	0.82[a]	68.46 ± 4.4[a]	9.21 ± 0.9[a]
	7.4[b]	0.98[b]	0.8[b]	0.79[b]	73.672.0[b]	8.08 ± 1.7[b]
F.M.02	10.0[a]	0.84a	0.6[a]	1.16[a]	66.06 ± 7.9[a]	8.24 ± 0.8[a]

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	10.8[b]	0.74b	0.8[b]	0.93[b]	69.28 ± 3.1[b]	7.75 ± 0.6[b]
F.M.03	9.5[a]	0.95a	0.9[a]	0.7[a]	70.52 ± 5.7[a]	9.42 ± 1.6[a]
	9.2[b]	0.8b	1.2[b]	0.69[b]	75.86 ± 6.4[b]	8.79 ± 1.1[b]
F.M.04	8.5[a]	0.9a	1.18[a]	0.86[a]	73.26 ± 5.4[a]	9.93 ± 1.34[a]
	8.1[b]	0.76b	1.6[b]	0.81[b]	80.95 ± 4.6[b]	8.12 ± 1.2[b]
F.M.05	11.4[a]	0.84a	0.7[a]	0.69[a]	72.03 ± 7.2[a]	8.25 ± 3.3[a]
	11.0[b]	0.78b	0.9[b]	0.65[b]	76.57 ± 4.6[b]	8.51 ± 0.7[b]
F.M.06	9.6[a]	1.16a	0.9[a]	0.73[a]	68.72 ± 8.7[a]	8.66 ± 0.7[a]
	9.0[b]	0.79b	1.21[b]	0.59[b]	73.0 ± 5.7[b]	7.96 ± 1.0[b]
F.M.07	8.5[a]	0.88a	0.6[a]	0.87[a]	69.63 ± 6.3[a]	9.66 ± 2.8[a]
	8.4[b]	0.75b	0.81[b]	0.83[b]	67.9 ± 3.1[b]	8.41 ± 1.8[b]
F.M.08	7.5[a]	1.16a	0.75[a]	0.92[a]	73.43 ± 3.1[a]	7.89 ± 1.5[a]
	7.6[b]	0.79b	0.8[b]	1.02[b]	77.95 ± 74.86[b]	8.64 ± 0.5[b]
F.M.09	9.0[a]	0.98a	1.31[a]	0.76[a]	71.79 ± 4.9[a]	8.65 ± 2.3[a]
	9.6[b]	0.86b	1.05[b]	0.75[b]	80.24 ± 6.5[b]	7.11 ± 2.0[b]
F.M.10	7.1[a]	1.41a	0.75[a]	1.26[a]	72.49 ± 7.9[a]	8.72 ± 0.9a
	8.6[b]	0.96b	1.1[b]	0.85[b]	75.62 ± 4.8[b]	7.99 ± 1.5[b]
F.M.11	10.2[a]	0.92a	0.71[a]	0.82[a]	70.69 ± 5.1[a]	10.19 ± 1.9[a]
	10.4[b]	0.78b	0.75[b]	0.75[b]	73.64 ± 2.8[b]	8.78 ± 1.5[b]
F.M.12	12.2[a]	0.9a	0.72[a]	1.35[a]	65.41 ± 6.6[a]	8.25 ± 1.0[a]
	11.2[b]	0.69b	0.8[b]	0.91[b]	69.67 ± 4.3[b]	8.32 ± 1.2[b]
F.M.13	12.5[a]	0.86a	1.15[a]	1.0[a]	72.78 ± 3.7[a]	10.11 ± 1.9[a]
	11.8[b]	0.71b	1.47[b]	0.89[b]	76.1 ± 5.9[b]	8.84 ± 1.3[b]

[a]Whole Wheat Flour [b]Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour.

Table 2 shows mean, standard deviation, minimum, maximum and p value of carbohydrates and protein of 13 mills.

Table 2: Statistical Assessment of Carbohydrates and Protein Concentration in Whole and Refined Wheat Flour at Hyderabad, Sindh(n=39)

Nutritional Assessment	Min-Max (g/100g) Mean ± SD	Min-Max (g/100g)	p-Value
Carbohydrates	70.41 ± 2.6[a]	65.41-73.43[a]	0.01
	74.65 ± 4.02[b]	67.9-80.95 [b]	
Protein	9.01 ± 0.79[a]	7.89-10.19[a]	0.02
	8.25 ± 0.49[b]	7.10-8.84 [b]	

[a]Whole Wheat Flour(n=13) [b]Refined Wheat Flour(n=13)

*Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour. Independent sample t-test has been used for p value and <0.05 has been considered statistically significant.

Table 3 shows Fe, Zn and Mn in whole and refined flour in 13 Mills at Hyderabad.

Table 3: Determination of Essential Metals in Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Iron (mg/Kg) Mean ± SD		Zinc (mg/Kg) Mean ± SD		Manganese (mg/Kg) Mean ± SD	
F.M.01	0.62 ± 0.5[a]	0.39 ± 0.4[b]	6.27 ± 3.1[a]	5.52 ± 2.5[b]	9.34 ± 5.6[a]	7.98 ± 4.3[b]
F.M.02	0.52 ± 0.4[a]	0.41 ± 0.3[b]	5.83 ± 2.7[a]	4.35 ± 2.1[b]	6.89 ± 3.6[a]	6.35 ± 2.8[b]
F.M.03	0.71 ± 0.4[a]	0.71 ± 0.4[b]	6.35 ± 3[a]	6.54 ± 3.1[b]	7.61 ± 3.2[a]	7.19 ± 3.5[b]
F.M.04	0.91 ± 1.1[a]	0.9 ± 1.1[b]	7.11 ± 3.5[a]	6.71 ± 3.5[b]	5.59 ± 1.3[a]	6.11 ± 1.9[b]
F.M.05	0.73 ± 0.6[a]	0.72 ± 0.4[b]	8.01 ± 4.2[a]	7.45 ± 3.1[b]	6.66 ± 2.4[a]	6.95 ± 2.5[b]
F.M.06	0.49 ± 0.3[a]	0.35 ± 0.3[b]	6.94 ± 3.5[a]	7.81 ± 4.2[b]	8.48 ± 4.9[a]	7.1 ± 3.6[b]
F.M.07	0.61 ± 0.4[a]	0.43 ± 0.6[b]	8.16 ± 4.7[a]	7.68 ± 3.9[b]	10.18 ± 6.1[a]	9.34 ± 5.4[b]
F.M.08	0.51 ± 0.4[a]	0.66 ± 0.4[b]	9.45 ± 5.8[a]	8.35 ± 4.5[b]	8.04 ± 4.3[a]	7.18 ± 3.6[b]
F.M.09	0.6 ± 0.5[a]	0.51 ± 0.42[b]	7.46 ± 4.3[a]	6.53 ± 3.9[b]	7.69 ± 3.8[a]	8.04 ± 4.3[b]
F.M.10	0.62 ± 0.5[a]	0.5 ± 0.6[b]	9.95 ± 5.6[a]	8.52 ± 4.6[b]	9.93 ± 5.6[a]	9.39 ± 5.5[b]
F.M.11	0.46 ± 0.4[a]	0.37 ± 0.4[b]	8.89 ± 5.01[a]	8.66 ± 5.1[b]	9.62 ± 5.9[a]	8.88 ± 5.1[b]
F.M.12	0.52 ± 0.4[a]	0.46 ± 0.5[b]	6.88 ± 3.4[a]	6.34 ± 3.3b	8.81 ± 4.5[a]	8.42 ± 3.7[b]
F.M.13	0.55 ± 0.5[a]	0.74 ± 0.5[b]	8.56 ± 4.1[a]	8.13 ± 4.4[b]	7.56 ± 4.2[a]	8.1 ± 3.8[b]

[a]Whole Wheat Flour [b]Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour.

Table 4 shows toxic metals(Cd, Pb, Cr and Cu)in 13 Mills.

Table 4: Cadmium, Lead, Chromium and Copper in Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Cadmium (mg/Kg) Mean ± SD	Lead (mg/Kg) Mean ± SD	Chromium (mg/Kg) Mean ± SD	Copper (mg/Kg) Mean ± SD
F.M.01	0.02 ± 0.02[a]	0.19 ± 0.20[a]	0.32 ± 0.05[a]	0.28 ± 0.2[a]
	0.03 ± 0.01[b]	0.23 ± 0.13[b]	0.18 ± 0.05[b]	0.32 ± 0.3[b]
F.M.02	0.04 ± 0.03[a]	0.24 ± 0.12[a]	0.25 ± 0.14[a]	0.26 ± 0.1[a]
	0.04 ± 0.02[b]	0.26 ± 0.12[b]	0.27 ± 0.22[b]	0.24 ± 0.2[b]
F.M.03	0.03 ± 0.01[a]	0.18 ± 0.14[a]	0.20 ± 0.07[a]	0.27 ± 0.2[a]
	0.02 ± 0.01[b]	0.24 ± 0.16[b]	0.19 ± 0.14[b]	0.28 ± 0.2[b]
F.M.04	0.03 ± 0.01[a]	0.20 ± 0.22[a]	0.26 ± 0.21[a]	0.23 ± 0.1[a]
	0.03 ± 0.01[b]	0.28 ± 0.12[b]	0.31 ± 0.13[b]	0.22 ± 0.3[b]
F.M.05	0.02 ± 0.03[a]	0.24 ± 0.13[a]	0.23 ± 0.22[a]	0.28 ± 0.2[a]
	0.03 ± 0.02[b]	0.22 ± 0.17[b]	0.21 ± 0.05[b]	0.30 ± 0.3[b]
F.M.06	0.03 ± 0.01[a]	0.27 ± 0.11[a]	0.25 ± 0.14[a]	0.24 ± 0.2[a]
	0.05 ± 0.02[b]	0.33 ± 0.11[b]	0.31 ± 0.20[b]	0.26 ± 0.3[b]
F.M.07	0.03 ± 0.02[a]	0.23 ± 0.12[a]	0.24 ± 0.11[a]	0.23 ± 0.1[a]
	0.03 ± 0.01[b]	0.26 ± 0.14[b]	0.21 ± 0.11[b]	0.27 ± 0.2[b]
F.M.08	0.01 ± 0.01[a]	0.27 ± 0.14[a]	0.16 ± 0.21[a]	0.27 ± 0.2[a]
	0.05 ± 0.02[b]	0.28 ± 0.15[b]	0.17 ± 0.12[b]	0.29 ± 0.2[b]
F.M.09	0.02 ± 0.01[a]	0.28 ± 0.13[a]	0.28 ± 0.13[a]	0.31 ± 0.2[a]
	0.06 ± 0.01[b]	0.24 ± 0.14[b]	0.26 ± 0.11[b]	0.32 ± 0.2[b]
F.M.10	0.03 ± 0.02[a]	0.23 ± 0.21[a]	0.24 ± 0.12[a]	0.32 ± 0.2[a]
	0.05 ± 0.01[b]	0.27 ± 0.11[b]	0.25 ± 0.23[b]	0.31 ± 0.2[b]
F.M.11	0.04 ± 0.01[a]	0.25 ± 0.22[a]	0.14 ± 0.05[a]	0.23 ± 0.1[a]
	0.03 ± 0.01[b]	0.28 ± 0.12[b]	0.21 ± 0.05[b]	0.32 ± 0.3[b]
F.M.12	0.02 ± 0.01[a]	0.26 ± 0.31[a]	0.22 ± 0.13[a]	0.32 ± 0.3[a]
	0.04 ± 0.01[b]	0.28 ± 0.1[b]	0.27 ± 0.22[b]	0.28 ± 0.2[b]
F.M.13	0.04 ± 0.02[a]	0.28 ± 0.21[a]	0.20 ± 0.11[a]	0.26 ± 0.2[a]
	0.03 ± 0.01[b]	0.32 ± 0.05[b]	0.18 ± 0.05[b]	0.29 ± 0.2[b]

[a]Whole Wheat Flour [b]Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour.

Table 5 showed mean, standard deviation, minimum, maximum and p value of iron, zinc, manganese, cadmium, lead, chromium and copper of 13 mills.

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Table 5: Statistical Assessment of Fe, Zn, Mn, Cd, Pb, Cr and Cu in Whole and Refined Wheat Flour at Hyderabad, Sindh (n=13*)

Metals	(mg/Kg) Mean ± SD	(mg/Kg) Min-Max	p-Value
Iron *15mg/Day	0.6 ± 0.11[a]	0.461-0.89[a]	0.03
	0.50 ± 0.13 [b]	0.36-0.74 [b]	
Zinc *10-15mg/Day	7.68 ± 1.28[a]	5.83-9.95[a]	0.04
	7.12 ± 1.27[b]	4.35-8.66[b]	
Manganese *12.2mg/Day	8.18 ± 1.41[a]	5.59-10.19[a]	0.03
	7.77 ± 1.1[b]	6.11-9.42[b]	
Cadmium *1.50µg/Day	0.02 ± 0.004[a]	0.016-0.027[a]	0.04
	0.03 ± 0.003[b]	0.021-0.031[b]	
Lead *0.30mg/Day	0.241 ± 0.034[a]	0.173-0.281[a]	0.02
	0.263 ± 0.038[b]	0.204-0.332[b]	
Chromium **1.50µg/Day	0.22 ± 0.045[a]	0.145-0.285[a]	0.02
	0.24 ± 0.048[b]	0.177-0.31[b]	
Copper ***2-3mg/Day	0.276 ± 0.027[a]	0.235-0.321[a]	0.04
	0.287 ± 0.031[b]	0.224-0.327[b]	

*Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour

aWhole Wheat Flour (n=13) bRefined Wheat Flour (n=13) **FAO/WHO Tolerable limit [14]. ***copper [15]. Independent sample t-test has been used for p value and <0.05 has been considered statistically significant.

DISCUSSION

Moisture in flour is very important for bakers and millers. In this research; moisture content has been 7-10% which is good for long storage of flour. All 13 mills have shown <1% of moisture which indicates good quality of flour. Whole and refined flour contains less ash percentage in mill flour which indicate low quantity of essential minerals. Czaja T *et al.*, in 2020 and Liu Y *et al.*, in 2023 also reported that moisture and ash were key parameters of nutritional assessment [16, 17]. In current analysis both types of flour contain low percentage of fat which is good as it delays rancidity [1-4]. Almost all mills have been found with low fiber in both types of flour. Nowadays mill owners are selling fiber separately at high cost. Meng Y *et al.*, in 2023 reported that wheat fiber have been good in diet for human health as it lowers glucose level [5]. Carbohydrates are important compound of wheat flour; however; their excessive use especially glucose can cause Diabetes and Obesity etcetera. Mean of all 13 mills shows less proteins and high carbohydrates in refined flour. In developed countries; sell of flour without adding essential metals is not allowed; however; this aspect is neglected in our country. Iron, zinc and manganese are essential nutrients of human metabolism and their deficiency can cause severe disorders. Sample from F.M.04 contained high Iron in whole flour and refined flour as 0.91±1.1 and 0.74±0.5mg/kg respectively. Zinc content has been high in F.M.10 and F.M.11 9.95±5.6mg/kg and 8.66±5.1mg/kg respectively. F.M.10 contained high Mn in both types of flour as 9.93±5.6mg/kg and 9.39±5.5mg/kg. Cadmium has been high in refined flour in F.M.09 as 0.06±0.01mg/kg. Lead has been high in whole flour in F.M.09 as 0.28 ± 0.13mg/kg. Copper has been high in whole flour as 0.32±0.3mg/kg in F.M.12 and F.M.01 contained high copper i.e. 0.32 ± 0.3mg/kg in refined wheat flour. All mills contained statistical significantly low Fe (p-0.03), Zn (p-0.04) and Mn (p-0.03) in refined flour than whole flour. Jiang Z *et al.*, in 2023 observed that biofortified of wheat with Fe and Zn was useful for human diet [2]. Wheat flour samples produced in Corum had higher Pb concentrations (2.009, 1.617, 1.574, 2.201, and 1.915 mg/kg) as compared to guideline values of 0.2 mg/kg. whereas no statistical difference has been found in concentrations of zinc and cadmium in wheat of similar and different cultivation lands [18]. In 16 different districts of Shanghai city, various mycotoxins and heavy metals have been studied in rice, maize, soybean and wheat flour [19]. These metals accumulate in our body cells after exposure; get attached to the cells membrane, carry out mimic reaction and produce toxicity [20].

CONCLUSIONS

Refined flour has slightly higher amount of carbohydrates and lower amount of proteins than whole flour. However, overall difference in values has been not more than few grams. Four toxic metals i.e. Cd, Cr, Pb, and Cu have been found in more concentration in refined flour than whole flour; whereas essential metals i.e. Fe, Zn, and Mn have been found high in whole flour than in refined flour. However, obtained results were well within maximum food limits laid down by FAO/WHO. It is concluded that whole wheat flour is better than refined wheat flour.

Authors Contribution

Conceptualization: SM
Methodology: SM
Formal analysis: SM, AMS
Writing, review and editing: MM, SH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Development and Quality, Chemical and Sensory Evaluation of Nutritive Herbal Blend

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ABSTRACT

Herbal blends have obtained popularity due to their health benefits, good fragrance and antioxidant capacity. Herbal tea is a famous drink due to its low cost, attractive taste and aroma. **Objectives:** To assess the nutritional properties of herbal blends and to develop an herbal blend using locally available herbs. **Methods:** The developed herbal blend was 40% rose, 15% lemongrass, 15% Tulsi leaves, 10% cinnamon, 10% ginger and 10% fennel. Rose, Tulsi and lemongrass leave was dried in a hot air oven. All ingredients were ground. Then this prepared ground herbal mixture was subjected to proximate analysis for moisture, ash content, crude protein, crude fat and crude fiber. Afterwards, physiochemical and sensory tests of prepared tea were done to check the pH, colour and sensory evaluation of the tea. Then the developed tea was subjected to phytochemical and antioxidant activity assays to check total phenolic contents and 1,1-Diphenyl-2-picrylhydrazyl. Proximate analysis and physiochemical analysis of the product were done at regular intervals. **Results:** Sensory characteristics and consumer acceptability of mixed herbal blends as tea alternative was evaluated through the hedonic survey. Sensory scores were higher for the prepared herbal blend as compared to the control. **Conclusions:** It was concluded that the developed herbal blend possessed significant nutritional value and antioxidant activity, making it an attractive alternative to tea. Its pleasant taste and ability to stay stable over time indicate that herbs can be used to make healthy, inexpensive drinks. Further studies are required to enhance its functional applications.

INTRODUCTION

The herbal blend is the form of a mixture of leaves, seeds, and roots of various plants. Herbal tea is an infusion of a mixture of herbal blend or powder and hot water, commonly ingested for its remedial and invigorating properties, including inducing relaxation. Having the capacity to help with stomach-related issues; herbal teas often have purging properties and strengthen the immune system. Pakistan has a rich history of folk use of plants, with people, especially those living in remote villages, using indigenous plants as medicines, and this knowledge has been passed orally from generation to generation. Herbal tea is consumed all over the world. Fruits, roots, and seeds were also used by local and traditional practitioners for curing many diseases. The traditional medicinal usage of plants for curing human ailments is vital to indigenous communities in the northern parts of Pakistan, which is considered a valuable local sociocultural heritage [1]. Herbal blend provides a lot of information and contains material on several elements of ethno-pharmacological study. As a response to illnesses with several etiologies or

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by a potential multifunctional element of the mixture as opposed to the action of individual ingredients, one may study the significance of this blend in folk medicine. For decades, many herbal blends have been employed to improve health [2]. Although the study of this blend is extremely challenging, its results are encouraging. Awareness of the ingredients in this traditional blend can assist in making some new blends that can improve individuals' health [3]. The conventional medicinal use of plants for the treatment of human ailments is important to Indigenous families in the northern parts of Pakistan which is considered useful local sociocultural heritage [4]. In recent years, due to awareness of the health attributes of herbal tea in the modern world, its consumption has increased significantly. As people are taking a keen interest towards natural plant remedies, the consumption of herbal tea will also increase significantly. It will also increase in size of the market for herbal tea. Knowledge of the composition of these traditional blends can help develop new blends that can improve the health of citizens. The healthcare systems of many developing countries were based on traditional herbal remedies. About 80% of the human population depends on traditional ethno-remedies of plant origin, and three-fourths of the world population cannot afford modern medicines. The major player in the herbal tea industry in the whole world is South Africa. Their main products are their local rooibos tea and honey-bush teas. These two products have a very significant marketplace [5]. Studies on other herbal tea having ingredients other than rooibos and honey-bush are increasing at a high rate. So, there is a high potential for other herbal teas in the market.

This study aims to assess the physicochemical and sensory properties of herbal blends and to develop an herbal blend using locally available herbs.

METHODS

Some raw materials used in this research as cinnamon, dried ginger and fennel were purchased from the local market of Multan in a dried form but rose petals, lemon grass and tulsi were collected from the field of Muhammad Nawaz University of Agriculture Multan. Rose petals, lemongrass and tulsi leaves were washed thoroughly and dried in a hot air oven at the B block laboratory of the Muhammad Nawaz University of Agriculture Multan. Rose, lemongrass and tulsi leaves were washed first to remove the dirt and all types of other contaminants. Ginger, cinnamon and fennel which were collected from the local market also washed properly. This process is known as sedimentation. Then oven dry at 50°C until constant weight. After drying these dried herbs were ground by using a grinder. Then it was sieved into 40 mesh sieve and stored at 4°C until use. All the ingredients which were dried in a hot air oven, were mixed according to the research plan. Herbal tea blend (T1) has the following ingredients rose petal 40%, lemongrass 15%, fennel 15%, dried ginger 10%, cinnamon 10% and tulsi leaves 10%. A mixture of this natural herbal blend was ground in a powder form. This mixed herbal blend is packed in a tea pack then in a zipper bag and also stored in a jar. During the extraction of herbal tea factors which were must be considered were infusion temperature, length of infusion, type of water which is infused, the ratio of tea to water and the type of tea used. Herbal tea aqueous extract was made according to the developed research plan. It was prepared by steeping the already made treatment 2-gram sample in 200 ml distilled water at 95°C for 3 minutes in an Erlenmeyer flask covered with aluminium foil to prevent removal of all necessary phytochemicals on a water bath [6]. After exactly 3 minutes, an herbal infusion was made. Then this herbal infusion was filtered through Whattmann filter paper No 41 cooled to 20°C and stored at room temperature in a cool and dark place [7]. Market-available herbal tea was taken as control (To) and Treatment (T1) was the developed herbal tea blend (explained above). Storage study was done at 15-day intervals to check the quality of the prepared herbal tea blend. Proximate analysis (moisture content, ash content, crude fiber, crude protein and crude fat) of the product was done following the method of [8]. Total carbohydrates were determined by the subtraction of percentages of moisture content, crude protein content, crude fat content and total ash present in this herbal blend. pH is defined as the logarithm to the tenth of the base of H+ activity reciprocal. An electrolytic cell with two electrodes attached was standardized in a buffer solution of pH 4.0. Afterwards, the dipping of electrodes into test samples occurred. A voltage relative to the pH of the solution was developed and we directly read the pH of the test sample solution as shown by the instrument [8]. Total phenolic content is based on the reaction of the F.C. reagent with the test sample. It results in the formation of blue chromophores because of the reduction of phosphor-tungstic and phosphor-molybdic acid in an alkaline medium in the presence of phenolic compounds [9]. DPPH is a stable radical having a dark red colour and chemically is (1,1-Diphenyl-2-picrylhydrazyl). When any antioxidant scavenges its free radicals, its colour changes from red to yellow at 515 nm wavelength. DPPH reagent was prepared by dissolving 4 mg of DPPH in methanol. Then 50 µl of sample was added to 2ml of DPPH solution. Then mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 minutes. The absorption was measured at 515 nm wavelength in a UV-visible spectrophotometer [10]. For the determination of mineral content, 1 g powder of the sample was poured into a 100 ml beaker. Purposely, 7 ml HNO3 was poured into the beaker followed by leaving the sample overnight. Then the sample was taken and 3 ml HCIO4 was also poured and subjected the sample for digestion on a hot plate at a temperature of

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about 180ºC. The hot plate was turned off when about 2-3 ml sample left. After cooling, the digested sample was diluted with 100 ml distilled water. Dilution was done by adding the sample and distilled water followed by tilting the flask for the uniform mixing of supernatant. Dilution was done in a 100 ml plastic bottle. Then prepared sample was used for the mineral profile as potassium sodium and calcium on a flame photometer while heavy metals like iron and zinc were accessed by atomic absorption spectrophotometer. A triplicate sample was run for the determination of the mean by the following method [8]. A hedonic survey of consumer acceptability of the prepared herbal tea was conducted in the Department of Food Science and Technology, MNS University of Agriculture Multan. A total of 30 respondents, including students, lecturers, some industrial members and other staff members of the University, as well as family members, participated in the survey. The respondents were then asked about their degree of liking of the teas in terms of taste, aroma, colour, mouthfeel and overall acceptability, based on a 9-point hedonic scale Triplicate analyses were performed to ensure data reliability. Significant differences were assessed using ANOVA procedures [11]. The Completely Randomized Design (CRD) was computed using STATISTIX 8.1 software.

RESULTS

Mean values of moisture content of herbal blend at 0 days, 15 days and 30 days was approximately 7.27%, 7.38% and 7.47%. In this herbal blend rose and tulsi has more moisture content while other ingredients have less amount of moisture (Table 1).

Table 1: Mean Value of Moisture % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	10.05 ± 0.28	7.29 ± 0.19	8.78 ± 0.22^a
15 Days	10.1 ± 0.2	7.38 ± 0.17	8.74 ± 0.18^a
30 Days	10.09 ± 0.23	7.47 ± 0.11	8.76 ± 0.17^a
Means	10.08 ± 0.23^a	7.3 ± 0.15^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of ash contents of the herbal blend were 4.13%, 4.15% and 4.18% at 0 days, 15 days and 30 days. As ash content in cinnamon is very low 2.4% while lemongrass has more ash content which is 17.1% (Table 2).

Table 2: Mean of Ash Contents % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	12.47 ± 1.29	4.13 ± 0.15	8.3 ± 0.72^a
15 Days	12.44 ± 0.60	4.15 ± 0.13	8.3 ± 0.36^a
30 Days	12.43 ± 0.66	4.18 ± 0.15	8.29 ± 0.4^a
Means	12.4 ± 0.85^a	4.1 ± 0.14^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of crude protein contents in herbal tea at 0 days, 15 days and 30 days' storage were 6.36%, 6.19% and 6.06%. As protein contents in rose are low 2.5% while tulsi has more amount of protein which was 16.1% (Table 3).

Table 3: Mean of Crude Protein % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	7.45 ± 0.05	6.36 ± 0.24	6.9 ± 0.14^a
15 Days	7.34 ± 0.09	6.19 ± 0.32	6.7 ± 0.2^a
30 Days	7.35 ± 0.07	6.06 ± 0.48	6.6 ± 0.27^a
Means	7.3 ± 0.1^a	6.2 ± 0.34^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

The mean values of crude fat contents of the herbal blend at 0 days, 15 days and 30 days' storage were 3.99%, 3.99% and 3.84% respectively. Rose has less amount of crude fat which was 0.6% while fennel has more crude fat which is 9.1% (Table 4).

Table 4: Mean of Crude Fat % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	4.246667 ± 0.13	3.99 ± 0.28	4.1 ± 0.2^a
15 Days	4.203333 ± 0.10	3.99 ± 0.28	4.09 ± 0.19^a
30 Days	4.216667 ± 0.08	3.84 ± 0.22	4.0 ± 0.15^a
Means	4.2 ± 0.1^a	3.9 ± 026^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

The mean values of crude fiber contents of the herbal blend at 0 days, 15 days and 30 days' storage were 19.75%, 19.46% and 19.42%. As rose has a low amount of crude fiber 3% while crude fiber in tulsi leaves was 30% (Table 5).

Table 5: Mean Value of Crude Fiber % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	24.26 ± 0.38	19.75 ± 0.88	19.54 ± 0.63^a
15 Days	24.23 ± 0.55	19.46 ± 0.60	21.8 ± 0.57^a
30 Days	24.11 ± 0.19	19.42 ± 0.46	21.7 ± 0.32^a
Means	24.2 ± 0.37^a	19.5 ± 0.64^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

The mean values of carbohydrate contents of the herbal tea sample are 73.31%, 73.27% and 73.18% at 0 days, 15 days and 30 days' storage respectively (Table 6).

Table 6: Mean of Carbohydrate % of Herbal Tea Sample

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	68.40 ± 0.10	73.31 ± 0.87	72.3 ± 0.48^a
15 Days	68.45 ± 0.08	73.27 ± 1.01	72.3 ± 0.04^a
30 Days	68.23 ± 0.20	73.18 ± 1.14	72.2 ± 0.67^a
Means	76.2 ± 0.19^a	68.3 ± 0.9^b	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values pH values of herbal tea at 0 days, 15 days and 30 days' storage were 6.21%, 6.23% and 6.28% respectively. According to the table, the highest pH value was found at 30 days of storage and the lowest pH value was observed at 15 days of storage (Table 7).

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Table 7: Mean pH Value of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	4.246667 ± 0.13	3.99 ± 0.28	4.1 ± 0.2[a]
15 Days	4.203333 ± 0.10	3.99 ± 0.28	4.09 ± 0.19[a]
30 Days	4.216667 ± 0.08	3.84 ± 0.22	4.0 ± 0.15[a]
Means	4.2 ± 0.1[a]	3.9 ± 026[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of the TPC value of herbal tea at 0 days, 15 days and 30 days' storage were 667.6, 667.47 and 667.44 mg GAE/g, respectively. According to the table, the highest TPC value was found at 1 day and the lowest TPC value was observed at 30 days of storage (Table 8).

Table 8: Mean of TPC Mg GAE/G of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	580.80 ± 0.72	667.6 ± 0.53	623.2 ± 0.62[a]
15 Days	579.43 ± 1.25	667.47 ± 0.38	623 ± 0.8[a]
30 Days	579.33 ± 1.15	667.44 ± 0.33	623 ± 0.74[a]
Means	579 ± 1.04[a]	667 ± 0.41[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of DPPH value of herbal tea at 0 days, 15 days and 30 days' storage were 80.13%, 79.96% and 79.93% respectively. According to the table, the highest DPPH value was found at 0-day storage and the lowest DPPH value was observed at 30 days' storage (Table 9).

Table 9: Mean of DPPH % of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	90.13 ± 2.30	80.13 ± 0.77	85.11± 1.5[a]
15 Days	89.2 ± 1.31	79.96 ± 0.86	84.4 ± 1.08[a]
30 Days	88.3 ± 1.52	79.93 ± 0.92	83.6 ± 1.21[a]
Means	88 ± 1.7[a]	79.8 ± 0.85[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mineral analysis was done to assess quantities of minerals in the prepared herbal tea blends. It was found that treatment To had a higher value of calcium with a mean of 376 ± 4 mg/L and To also had a higher percentage of potassium with a mean of 419 ± 5 mg/L. T1 had slightly higher magnesium content with a mean value of 64 ± 0.5 mg/L as compared to To with a mean value of 63 ± 0.4 mg/L (Figure 1).

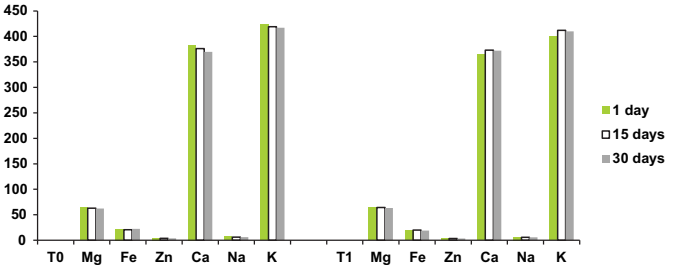

Figure 1: Mean for Minerals mg/L of Herbal Tea Sample

Herbal tea is a good source of iron (Fe), sodium (Na), zinc (Zn), potassium (K), magnesium (Mg), and calcium (Ca). The herbs are good dairy-free sources of calcium. One cup (166 grams) of tea contains about 2/3 of the calcium found in 1 cup of whole milk. The prepared products T0 and T1 were analyzed for mineral profiling. The mean values of the taste of tea were checked using a 9-hedonic scale at 1 day, 15 days and 30 days of storage. These showed the results at 1 day, 15 days and 30 days were 6.8533, 7.3600 and 6.9433, respectively. The highest taste score was found at 30 days' storage and the lowest taste score was recorded at day 1 (Table 10).

Table 10: Mean Value of Taste of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	5.55 ± 0.18	6.85 ± 0.60	6.2 ± 0.39[a]
15 Days	5.52 ± 0.19	7.36 ± 0.97	6.6 ± 0.52[a]
30 Days	5.43 ± 0.05	6.94 ± 0.34	6.2 ± 0.19[a]
Means	5.51 ± 0.14[a]	6.9 ± 0.6[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of the colour of tea at 1 day, 15 days and 30 days' storage were 7.1267, 7.6133 and 6.9900, respectively. The highest colour % was found in 15 days of storage and the lowest colour value was observed in 30 days of storage (Table 11).

Table 11: Mean Value of the Color of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	6.10 ± 0.35	7.12 ± 0.10	6.5 ± 0.2[a]
15 Days	5.97 ± 0.33	7.61 ± 0.16	6.79 ± 0.24[a]
30 Days	5.93 ± 0.11	6.99 ± 0.21	6.4 ± 0.16[a]
Means	5.95 ± 0.26[a]	7.24 ± 0.15[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of aroma of tea at 1 day, 15 days and 30 days' storage were 7.8900, 8.93 and 7.8767, respectively. The highest aroma was found in 15 days of storage. The lowest aroma was observed at 30 days' storage. (Table 12)

Table 12: Mean Value of Aroma of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	5.82 ± 0.15	7.88 ± 0.02	6.8 ± 0.08[a]
15 Days	5.73 ± 0.23	8.93 ± 0.42	7.1 ± 0.32[a]
30 Days	5.7 ± 0.3	7.8 ± 0.85	6.4 ± 0.15[a]
Means	5.75 ± 0.22[a]	8.2 ± 0.43[b]	

T_0=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of mouth feel of tea at 1 day, 15 days and 30 days' storage were 6.0333, 6.7767 and 5.9533, respectively. The highest mouth feels were found at 15 days of storage. The lowest mouth feel was observed in 30 days of storage (Table 13).

Table 13: Mean Value of Mouth Feel of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	5.6 ± 0.36	6.03 ± 0.05	5.73 ± 0.20[a]

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15 Days	5.68 ± 0.45	6.76 ± 0.05	6.02 ± 0.60[a]
30 Days	5.44 ± 0.48	5.95 ± 0.50	5.6 ± 0.49[a]
Means	5.52 ± 0.43[a]	6.2 ± 0.43[b]	

T_o=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

Mean values of overall acceptability of tea at storage of 1 day, 15 days and 30 days were 7.3067, 7.5067 and 6.9667, respectively. According to the table, the highest overall acceptability was found at 15 days of storage and the lowest overall acceptability was observed during 30 days of storage. This acceptability of herbal tea was measured using a 9-hedonic scale (Table 14).

Table 14: Mean value of Overall Acceptability (%) of Herbal Tea

Storage Effects	T_0	T_1	Mean ± S.D
1 Day	5.89 ± 0.31	7.3 ± 0.726	4 ± 0.51[a]
15 Days	6.2 ± 0.34	7.5 ± 0.506	6 ± 0.42[a]
30 Days	5.82 ± 0.84	6.9 ± 0.566	4 ± 0.7[a]
Means	5.9 ± 0.48[a]	7.2 ± 0.59[b]	

T_o=Control, T_1=Herbal tea blend. Different letters 'a' and 'b' indicate significant difference

DISCUSSION

Analysis of variance of the proximate analysis indicated significant results among treatments. Proximate composition was measured with the highest amount of moisture content and ash content was measured at 30 days of storage. Moisture content was measured and the highest amount of moisture % was measured at 1 day of storage shown in table 1. Contrasting results were also measured in which they compared the proximate analysis of cinnamon and lemongrass for their nutritive value [12]. They noted that the moisture of cinnamon was 5.6% and lemongrass was 10.12%. The value of cinnamon was very close to the current study. The ash content was measured and the highest amount of ash % was observed at 1 day of storage shown in Table 2. Contrasting results were also analyzed by [12], they compared the proximate composition of dried and fresh tulsi leaves. The ash content of both fresh and dry samples was 3.57% and 22%, respectively. It was shown that drying increased the ash contents of dried dry leaves. The crude protein was measured and the highest value of protein % was observed at 1 day of storage shown in Table 3. Contrasting results were also found in which researchers determined the protein content of ten local herbs and spices [13]. Crude fat was measured and the highest value of fat % was measured at 1 day of storage shown in Table 4. Contrasting results were also measured in which they calculated the nutritional composition of cinnamon [14]. The fiber content was measured and the highest value of fibre % was observed at 15 days of storage shown in Table 5. Contrasting results were also measured in which they determined the proximate and mineral composition of tulsi varieties [15]. The carbohydrates were measured and the highest value of carbohydrates % was observed at 1 day of storage shown in Table 6. Contrasting results were also measured, which determined the proximate composition of lemongrass varieties [16]. They calculated that lemongrass contained 63% carbohydrates, lower than the current reported value. This may be due to seasonal and regional differences. Crude protein, crude fat and crude fiber were measured in the highest amount at 1-day storage. Carbohydrates also show high results at day 1 and low values at 30 days of storage [17]. Analysis of Variance of the phytochemical analysis indicated non-significant results of tea at different times [18]. The pH value was measured and the highest value of pH was observed at 30 days of storage shown in Table 7. Contrasting results were reported by [16], in which they carried out a comparative study of the pH value of some essential spices. Antioxidant activity was measured and the highest amount of TPC was measured at 30 days of storage. Contrasting results were also measured by [19], in which they studied the antioxidant activity of green cardamom E. cardamom. They used ethanoic extract from cardamom seeds to measure total phenolic contents and DPPH. Results revealed that the total phenolic contents of cinnamon were 2.47 mg GAE/g at a concentration of 25 microgram/ml. From TPC analysis, they concluded that cinnamon was a rich source of polyphenols. While DPPH was measured in the highest amount at 1 day indicating the synergetic effect of all ingredients in increasing antioxidant activity than alone ingredient. Contrasting results were also measured by [20], in which they compared the antioxidant activity of fennel and sage essential oils. They noticed that at a concentration of 200 µg/ml, fennel essential oil showed 100% radical scavenging activity than sage (50.34%). They concluded from their study that fennel essential oil had the highest value of DPPH, PV, TBA and BCB. They also concluded from their results that fennel and sage essential were powerful antioxidants in stabilizing the essential oil to a greater period than synthetic antioxidants such as BHT, PG and BHA. Minerals such as Ca, K, Mn and Fe show the highest values at 15 days of storage. Results of [21] for the mineral profile of herbal tea and close to the results of this study, a little difference is due to differences in ingredients. Sensory analysis showed highly significant results among treatments. Sensory evaluation was done with a 9-hedonic scale and five qualities attributed to taste, colour, aroma, mouth feel and overall acceptability. Taste evaluation's highest score was obtained at 30-day storage and the highest score of aroma, mouth feel and overall acceptability were given at 15-day storage while the highest colour was obtained at day 1 analysis [22]. The study was conducted by [23] to assess the sensory characteristics of flower tea. In terms of taste, herbal tea stored for 30 days achieved a higher score compared to flower tea. Aroma results showed that herbal tea had the highest aroma score observed at 15 days, then flower tea. In terms of colour, herbal tea scored higher, reaching the highest peak at 15 days compared to flower tea. However, overall acceptability was superior in herbal tea, particularly

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at 15 days while jasmine flower tea had the lowest acceptability score.

CONCLUSIONS

It was concluded that herbal tea is a very popular beverage and is now gaining popularity worldwide, due to its variety of teas including rose tea, cinnamon tea, lemongrass tea, tulsi tea ginger tea etc. It was a mixture of plant parts. That is made from dried herbs, flowers and spices. It is not considered a true tea plant Camellia sinensis. This tea was made up of 40% rose petals, 15% lemongrass, 15% tulsi leaves, 10% cinnamon, 10% fennel and 10% ginger. Analysis and sensory were done at 1 day, 15 days and 30 days of storage and the best result was found at 15 days' storage. The results of the current study indicated that results of 15 days of storage were best of in antioxidant activity. This could be due to the synergetic effect of all ingredients rather than individual ingredients to show the highest amount of antioxidant activity. The developed tea was liked by the majority of personnel due to being caffeine-free, having a good aroma and a lot of health benefits from all-natural ingredients.

Authors Contribution

Conceptualization: MS, HN
Methodology: SM, MS, MSEA, MHUGH
Formal analysis: SM, MS, MSEA, MHUH
Writing review and editing: SM, MAK, HN, UM, AM, TBQ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Anthropometric and Socio-Economic Determinants of Dietary Diversity Scores in University Students

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ABSTRACT

Eating habits, particularly, diversity of diets of university students, are not reported. **Objectives:** To investigate the dietary habits with special consideration of the dietary diversity score of university students at Bacha Khan University Charsadda (BKUC). **Methods:** A cross-sectional study design was conducted on a sample (n=200) of students from various teaching departments. Data on anthropometry and dietary intake were collected. Dietary diversity score (DDS) was calculated by employing a normal food category score scheme. Data on anthropometrics (height, weight, BMI, waist circumference (WC), and waist-to-hip ratio (WHR), and socioeconomic were recorded in a questionnaire. Group differences were evaluated using one-way ANOVA and t-tests for evaluating differences in DDS based on SES and the anthropometric measures. **Results:** As expected, significantly higher scores of DDS were found in the subjects who had higher economic status ($p<0.001$). A high negative relationship existed between DDS and anthropometrics (BMI, WC, and WHR), especially in the female students ($p<0.05$). The intake of grain, fruit, vegetable, dairy, and protein, and an improved intake in DDS quartiles ($p<0.01$), once again validated DDS as a solid measure of dietary diversity and intake. **Conclusions:** It was concluded that higher DDS are associated with better intake of special food categories and better anthropometric indicators. These findings reinforce the importance of promoting dietary diversity to promote health and prevent chronic disease.

INTRODUCTION

In the context of human nutrition and health, and disease prevention, dietary diversity (DD) is a developing field [1]. DD has been considered one of the qualities of a healthy diet on a global scale [2]. To attain dietary adequacy and optimal growth and development, a diverse diet that includes all the food groups, meat, vegetables, fruits, grains, and dairy products is necessary [3]. "The number of different foods or food groups consumed over a given reference period" [4] has been defined as dietary diversity. An omnivorous diet that includes the right macro- and micronutrients may increase hunger, especially in kids, and lower the risk of developing chronic illnesses like cancer, diabetes, metabolic syndrome, and cardiovascular diseases (CVDs) [5]. One indicator that has been considered is the Dietary Diversity Score (DDS). DDS, which has been linked to socioeconomic status (SES) and anthropometric measurements, is becoming more widely acknowledged as a crucial indicator of the overall quality of diets [6, 7]. Although previous research highlights the connection between DDS and nutritional outcomes, little is known about how underlying factors like income and nutrition-related knowledge influence dietary patterns and food choices, especially in lower- and middle-income environments [8]. Understanding the factors that influence dietary diversity is particularly important in Pakistan, where nutritional shifts are taking place, and underweight and overweight youth malnutrition still exists. Additionally, university students are in a special time of life

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where they have more freedom to choose their foods and are more susceptible to bad eating habits. In Pakistan, they are still a community with little knowledge of eating habits. As a result, this study is supported not only by the importance of dietary diversity as a public health indicator on a global scale but also by the local need to comprehend the relationships between dietary diversity and variables like SES, waist circumference (WC), body mass index (BMI), and waist-to-hip ratio (WHR) in a university setting. By concentrating on Bacha Khan University, Charsadda students, this study seeks to produce context-specific insights that could guide focused interventions to encourage young people to adopt healthy eating habits.

This study aims to investigate dietary habits with special consideration of the dietary diversity score of university students.

METHODS

This cross-sectional descriptive study design was conducted at the Bacha Khan University, Charsadda. The sample was calculated to identify significant DDS associations with corresponding sociodemographic or behavioral variables (e.g., income, education, frequency of meals). A power analysis was performed using G*Power version 3.1 based on the following assumptions: Effect size (Cohen's f^2) = 0.15, reflecting a medium effect size that is typical for cross-sectional DDS studies, using statistical power ($1 - \beta$) = 0.90, to have a 90% chance of finding a true association, significance level (α) = 0.05, for two-tailed tests and number of predictors = 6–8 (e.g., age, gender, household size, income level, education, meal frequency, food access, physical activity). Based on these criteria, the minimum sample size needed was 150 participants for multiple linear regression. In order to enhance generalizability and allow for potential non-response or missing data (~20%), the sample target was elevated to 180–200 participants, providing adequate statistical power for subgroup comparisons. In addition, as no exact local prevalence of low DDS in university students in KP was known, reference prevalence rates were assumed from regional reports that indicated 30–50% of young adults possess low dietary diversity, particularly amongst students in semi-urban areas. Students from all academic departments (n=16)(Faculty of Sciences and Faculty of Arts and Humanities) of the Bacha Khan University Charsadda, were chosen at random to serve as sample subjects. Convenience sampling was used for the selection of participants based on the population at each of the 16 academic departments. Participants with diabetes, cardiovascular diseases, any kind of malignancy, or any other infection history in the last 6 months from the time of data collection were not allowed to participate in the study. Prior written informed consent was taken from all the participants. Data on demographics (sex, age, marital status, education, socioeconomic status (SES) were collected by a structured self-report survey. A questionnaire was administered, which gathered the information of socio-economic status (gender, age, marital status, current education degree/program, and economic status). Participants were asked to report their average monthly household income to gauge their economic condition. Each of the three categories, namely "good", "medium", and "weak", was made as on their responses to their economic status. Anthropometrics (weight, height, WC, and HC) were measured using standard protocols as previously reported [9]. BMI was calculated from height and weight data. Subjects were categorized according to BMI following WHO criteria; BMI < 18.5kg/underweight; the normal range was 18.5-24.9kg/m2, overweight- 25.0-29.9kg/m2, and obese-30 kg/m2.Waist-to-hip ratio (WHR) was calculated by dividing WC by hip circumference. High values were defined as ≥ 1 for men and ≥ 0.8 for women [10]. Dietary data were collected in a semi-quantitative food frequency questionnaire (FFQ) that included food items and a standard portion size [11]. Data were analyzed by SPSS (Version: 20.0; IBM Corp., Chicago, IL, USA). Individual variables in both genders were examined for normalcy using the Kolmogorov-Smirnov test. The $\chi 2$ test was used to look at the impact of gender on DDS in each of the DDS quartiles. To report on the characteristics of the subjects under study, descriptive statistics were used. The qualitative factors were presented as frequency graphs and tables, whereas the quantitative data were presented as mean ± standard deviation. Kruskal-Wallis and Mann-Whitney U tests were used for analysis to find the difference between SES and DDS. ANOVA was used to determine the difference between DDS and anthropometric indices. Differences were considered significant at $p<0.05$. The study was approved by the ASRB, BKUC. Ethical clearance was obtained from the Ethics Review Board of BKUC. We separated diets into five main categories—grains, fruits, vegetables, meats, and dairy products in order to calculate DDS [12]. Subgroups were created by further segmenting the main groups. A person must eat at least half of a food group every day to be classified as a consumer of that group. As previously reported, each food group was awarded two points. Four quartiles were created from the DDS: less than 3.0, 3.0–5.5, 5.6–8.5, and greater than 8.5. Ten was the highest DDS. The Food Guide Pyramid was used to classify DDS [12].

RESULTS

The Dietary Diversity Score (DDS) variations among the different socioeconomic attributes of the students are shown. Mean DDS was slightly non-significantly higher in male (4.32) than in female (4.11) ($p>0.05$). DDS was significantly impacted by marital status, with married

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students having a significantly higher mean DDS (5.11) than those single (3.31) ($p<0.05$). Additionally, there was a notable difference in DDS between various self-reported economic levels. As expected, the highest DDS (4.24) was reported by participants in strong economic status, followed by those in medium (3.19) and weak (2.11) economic conditions ($p<0.05$). However, there was no significant correlation between educational attainment and DDS ($p>0.05$), suggesting that respondents' better dietary diversity was not always correlated with higher education (Table 1).

Table 1: Comparison of DD Across Sex and Other Socioeconomic Characteristics

Parameters		n (%)	Mean of SD	p-Value*
Sex	Male	120 (60.0%)	4.32 ± 1.23	0.208
	Female	80 (40.0%)	4.11 ± 1.12	
Marital Status	Married	45 (22.5%)	5.11 ± 1.34	0.000
	Single	155 (77.5%)	3.31 ± 1.45	
Self-Reported of Economic Status	Good	40 (20.0%)	4.24± 1.56	0.000
	Medium	105 (52.5%)	3.19 ± 1.57	
	Weak	55 (27.5%)	2.11 ± 1.67	
Educational Level	BS	130 (65.0%)	4.11 ± 1.56	0.831
	MS/MPhil	66 (33.0%)	4.24± 1.76	
	PhD	4 (2.0%)	4.32 ± 1.32	

DDS=dietary diversity score; socioeconomic status; *p-value=calculated using Kruskal-Wallis (for comparison involving two groups) and Mann-Whitney U (for comparison involving more than two groups) tests.

The distribution of several anthropometric parameters among male and female students across quartiles of the DDS is analyzed. Overall, there was a pattern where better DDS was linked to better anthropometric profiles, especially in females. A statistically significant rise in height was observed in both genders across DDS quartiles (p for all trends<0.05), indicating a potential relationship between dietary variety and physical stature, maybe as a result of long-term nutrition. Although the difference was only statistically significant in females ($p=0.015$), weight fell across DDS quartiles in both sexes, suggesting that greater dietary diversity may be linked to healthier body weights in women. Similar to this tendency, BMI decreased considerably across DDS levels in both genders (p for all trends<0.05), indicating that people with more varied diets are better at managing their weight. WC and WHR showed a comparable inverse relationship. In males (WC: $p=0.020$; WHR $p=0.019$) and in females (WC: $p=0.014$; WHR $p=0.003$), both measures dramatically dropped as DDS increased. This suggests that people with more diversified diets have better fat distribution and may be at lower risk for cardiometabolic disease. Along with decreasing WC and WHR, hip circumference (HC) increased significantly in females ($p=0.020$) but did not alter much in males ($p=0.300$). This could indicate that women with higher DDS have healthier body compositions. All things considered, these results highlight a strong correlation between greater dietary diversity and better anthropometric results, particularly for women. This suggests that encouraging a diverse diet may improve physical health and possibly reduce the risk of obesity-related disorders (Table 2).

Table 2: Anthropometric Parameters of Male And Female Students Across Quartiles of the DDS

Anthropometric Parameters	Sex	Quartiles of DDS				p*	p**
		Q_1 (< 3.0)	Q_2 (3.0 to 5.5)	Q_3 (5.6 to 8.5)	Q_4 (> 8.5)		
Height (cm)	M (85)	166.0 ± 12.3	164.7± 8.23	167.8 ±8.90	171.3 ± 47.9	0.030	0.004
	F (105)	154.0 ± 11.2	158.1 ± 5.73	159.7 ± 8.6	161.1 ± 6.8	0.010	
Weight (kg)	M (85)	65.1 ± 18.9	64.5± 11.5	62.7 ± 10.9	60.5 ± 7.9	0.106	0.000
	F (105)	56.7 ± 13.7	55.9.26 ± 7.8	54.6 ± 11.3	52.7 ± 6.6	0.015	
BMI (kg/m^2)	M (85)	23.6 ± 4.6	23.7± 3.6	22.2 ± 3.8	20.6 ± 3.5	0.016	0.243
	F (105)	23.9± 5.3	22.4 ± 3.7	21.4 ± 3.6	20.3 ± 3.1	0.043	
WC (cm)	M(85)	91.3 ± 3.86	90.2 ± 6.8	90.1 ± 9.2	88.3 ± 10.0	0.020	0.900
	F (105)	86.2 ± 10.86	83.6 ± 7.8	83.8 ± 10.3	82.70 ± 8.7	0.014	
HC (cm)	M (85)	102.1 ± 6.7	102.6 ± 4.9	102.6 ± 6.5	103.1± 9.8	0.300	0.010
	F (105)	100.5 ± 7.9	101.6 ± 8.1	102.7± 7.5	104.2 ± 8.7	0.020	
WHR	M (85)	0.89 ± 0.07	0.88 ± 0.05	0.88 ± 0.07	0.85 ± 0.10	0.019	0.000
	F (105)	0.85 ± 0.08	0.82 ± 0.07	0.82 ± 0.09	0.79 ± 0.07	0.0.3	

BMI=body mass index; DDS=dietary diversity score; WC=waist circumference; HC=hip circumference; WHR=waist-to-hip ratio; p*-value was calculated using analysis of variance. p**=comparison between means of male vs female. Values are presented as mean ± SD

A distinct and statistically significant pattern emerges from the study of food category intake across Dietary Diversity Score (DDS) quartiles: people with higher DDS reported eating more portions of all food groups, vegetables, grains, fruits, dairy products and meat, and cereals. Compared to those in the lowest quartile (Q1), who reported only 1.4 ± 3.7 and 1.2 ± 2.33 servings/day, respectively, participants in the highest DDS quartile (Q4) consumed significantly more nutrient-dense foods, especially fruits (4.7 ± 3.5 servings/day) and vegetables (3.7

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±3.62 servings/day). Likewise, consumption of meat, dairy, and grains rose steadily with DDS, showing that those with more varied diets not only consume more of each food group, but also consume more of each one (Table 3).

Table 3: Comparison of Differences of Food Servings among Quartiles of DDS

Food Groups	Quartiles of DDS				p*
	Q_1 (<3.0)	Q_2 (3.0–5.5)	Q_3 (5.6–8.5)	Q_4 (>8.5)	
No of Students	6141	34	15	10	-
Grains	0.11 ± 0.22	0.7 ± 0.84	1.5 ± 0.7	1.9 ± 1.0	0
Meat and Cereals	0.1 ± 0.7	0.8 ± 1.2	1.2 ± 0.8	2.7 ± 1.7	0.001
Vegetables	1.2 ± 2.33	1.62 ± 2.7	2.0 ± 2.5	3.7 ± 3.62	0.04
Fruits	1.4 ± 3.7	1.9 ± 1.65	2.1 ± 2.8	4.7 ± 3.5	0.001
Dairy Product	0.02 ± 0.10	1.2 ± 0.5	1.5 ± 0.8	2.5 ± 1.1	0.001

DDS=dietary diversity score.*p-value=calculated by ANOVA/Kruskal-Wallis test. Values are as mean ± SD

DISCUSSION

A positive association between DDS and daily intake of all major food groups existed in this study. Participants in higher DDS quartiles consistently consumed more servings of grains, vegetables, fruits, meat, and dairy, reflecting better diet quality and nutrient adequacy. These results reinforce DDS as a practical indicator of healthy eating patterns and highlight the value of dietary diversity in promoting balanced nutrition. This study observation is in line with the results of other studies that indicate that the greater the intake of important food groups, the higher the Dietary Diversity Score (DDS). In Malaysia, Tiew *et al.*, achieved a similar finding that a high DDS level indicated greater consumption of fruits, vegetables, and protein-rich food [13]. On the same note, a study by Esfahani *et al.*, and Azadbakht *et al.*, revealed that those with high DDS ate more meats of all major food groups and obtained their nutrient adequacy ratios much better [14, 15]. In this research, the correlation is high compared to the patterns found otherwise observed between DDS and some of the significant anthropometric characteristics, particularly among the female participants. The body weight, BMI, WC, and WHR also showed a downward trend with an increase in DDS, especially in female. This suggests that those with more diversified diets typically have healthier body compositions [16]. This confirms earlier findings that better weight management and decreased central adiposity are associated with a more varied diet [17], which frequently reflects a larger intake of fruits, vegetables, and foods high in fiber [18]. According to research, a varied diet may help avoid undernutrition and overweight by promoting a more balanced calorie intake and better metabolic health [19]. This is supported by the inverse link between DDS and BMI. In the present study, although both sexes' height increased statistically significantly with DDS, this could be due to the cumulative impact of improved nutrition in early childhood for those who continue to eat a variety of foods. It's interesting to note that hip circumference (HC) rose considerably with DDS in females but not in males. This, along with decreases in WC and WHR, may suggest that women with greater DDS have healthier fat distribution patterns. The findings also highlight how crucial it is to encourage diversified, well-balanced meals that are full of all the necessary food groups in order to improve public health outcomes, particularly in countries where monotonous diets may predominate. Diet diversity can help in the management of malnutrition widely prevalent in Pakistan [20-22].

CONCLUSIONS

It was concluded that the dietary diversity score is positively related to health parameters assessed by anthropometry and also socio-economic status in a group of students of Bacha Khan University, Charsadda.

Authors Contribution

Conceptualization: ST
Methodology: ST, AZ, IA
Formal analysis: ST, AZ, IA
Writing review and editing: ST, AZ, IA

All authors have read and agreed to the published version of the manuscript.

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Exploring Relationships between Common Healthy Behaviors in Adolescents Using Innovative Social Network Analysis

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ABSTRACT

Data on the interrelationship between common healthy behaviors is not available for adolescents. **Objective:** To explore the relationships among common healthy behaviors in a population sample using network analysis. **Methods:** A random sample of 250 adolescents (34% female) was selected from a population of adolescents, who visited the Nutrition, Education, Awareness and Training (NEAT) Clinics during January-March, 2024. Data was collected on a validated questionnaire. This study applied network centrality analysis to investigate six health related behaviors non-smoking, fruit intake, vegetable intake, proper sleep, eating breakfast, and walking. Centrality measures (betweenness, closeness, strength, and expected influence) were used to assess the role and influence of each behavior within health behavior networks. **Results:** The overall network density was high (0.80; 12 edges out of total 15 possible edges), suggesting that the healthy behaviors in the sample population were tightly interconnected. Such a dense network indicates that changes in one behavior may potentially influence others, supporting the need for multi-behavior interventions. With mean scores grouped between 2.3 and 2.6, moderate variability, symmetric distributions, and full-scale utilization, descriptive analysis showed that teenagers modestly engaged in six fundamental health behaviors. This suggests that while these behaviors are present, they are not widely adopted in the population. **Conclusion:** Walking and eating breakfast were identified as key lifestyle habits in adolescent health networks, suggesting that they may be targeted strategically for treatments aimed at preventing obesity.

INTRODUCTION

Obesity is an ongoing, worldwide public health issue [1, 2], with substantial contributions to the load of non-communicable chronic diseases like diabetes, cardiovascular disease and some cancers [3-5]. It is well accepted that obesity is determined not solely by individual influences, but by intricate interactions between several lifestyle factors [6]. Notwithstanding rigorous health promotion campaigns, obesity prevalence continues to escalate, particularly in middle- and low-income nations, necessitating a more subtle insight into behavioral determinants. Healthy behaviors like routine physical activity, adequate sleep, breakfast consumption [7-9], the intake of fruits and vegetables, and not smoking have been shown to protect against obesity [11]. These behaviors, however, are not isolated; they tend to co-occur and interact in ways that will either promote health or add to risk. Recognizing these patterns is necessary for creating comprehensive and effective interventions. Network analysis provides a new and compelling structure for analyzing inter-behavioral relationships [12]. In contrast to classical statistical approaches that emphasize single effects, network analysis views behaviors as interlinked nodes in a system. This enables researchers to find central behaviors with potential to dominate others, identify clusters of co-occurring habits, and reveal structural properties of behavioral networks that might be used to guide interventions. The current study will apply network analysis to examine the associations between prevalent

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healthy behaviors in a population sample. In particular, it was aimed to characterize key behaviors as central nodes in the behavior network, determine the modular organization of behavior clusters, and gain insights that will inform more focused, system-level obesity prevention efforts. Recognizing how health behavior is connected can provide useful insight for constructing effective public health interventions. Instead of considering behaviors such as diet, physical activity, or sleep separately, network analysis enables us to look at how these habits group and affect each other within practical behavioral systems. This research explores the centrality and impact of six typical health behavior categories non-smoking, fruit eating, vegetable eating, good sleeping, breakfast eating, and walking. Through the application of network centrality metrics like betweenness, closeness, strength, and expected influence, we determine what behaviors are most embedded within an individual's lifestyle networks and are more on the periphery. This method tells us not only what habits are most effective in advancing healthy living, but also how behavioral patterns vary along demographic and geographic lines.

Such information is vital to where health promotion efforts are most likely to build upon larger positive lifestyle change.

METHODS

The cross-sectional analytical study sought to examine the interrelations of prevalent healthy lifestyle behaviors that are likely to play a role in preventing obesity, employing a network analysis strategy. The aim was to map behavioral co-occurrence patterns and detect important behaviors that could be points of leverage for intervention. Male and female adolescents aged 16-20 years were recruited from the population of those who had visited the NEAT (Nutrition, Education, Awareness, and Training) Clinics for nutrition and health counselling in January-March. 2024. NEAT registered with cooperative society Pakistan, has been actively assisting studies related to health and nutrition [13-16]. The sample size for this cross-sectional study was estimated using the standard formula for calculating a single population proportion: $n=Z2 \cdot p \cdot (1-p)/d2$; Where: n = required sample size; Z = Z-score for 90% confidence level (1.645); p = assumed prevalence of the outcome (in this case, 70% prevalence of healthy BMI among adolescents based on previous NEAT Clinic records); d = margin of error (5% or 0.05). Putting the values into the formula. Thus, the required minimum sample size was rounded to 227 participants [17]. This ensures adequate power to estimate the proportion of adolescents with healthy BMI within a 5% margin of error at a 90% confidence level. As a precaution against non-response, the target sample size was a 10% increase, and the final sample size was about 250 adolescents. Participants were selected using a consecutive sampling technique. All eligible adolescents who visited the NEAT Clinic during the study period (January to March 2024) and met the inclusion criteria were invited to participate. This approach ensured that every individual meeting the criteria during the specified timeframe had an equal opportunity to be included, thereby reducing selection bias while maintaining feasibility in a clinical setting. Questionnaire data were gathered from a sample of 250 respondents aged 16years and older. The questionnaire items were modified and modeled against tested instruments utilized in public health and behavioral nutrition studies (Table 1). Every item represents essential concepts of lifestyle behavior and conforms to WHO and national health guidelines for daily habits that impact non-communicable disease (NCD) risk. Content Validity was maintained by incorporating central lifestyle behaviors (physical activity, diet, sleep, smoking) proven to influence health outcomes, aligned with the WHO STEPwise Approach to NCD Risk Factor Surveillance exported elsewhere [18]. Construct Validity is informed by the Health-Promoting Lifestyle Profile-II (HPLP-II) and the Global School-based Student Health Survey (GSHS), both of which have been shown to possess acceptable psychometric properties across different populations [19]. Public health and nutrition experts' (n = 3) expert review was employed to verify the appropriateness and understandability of each question. The scoring scheme (0–4 scale) was developed from behavioral nutrition literature's practices (e.g., frequency and dietary diversity scoring), providing interval-level data for statistical analysis [20]. The respondents self-reported their practice of the following health-related behaviors: 1) Regular walking; 2) Consumption of fruit; 3) Intake of vegetables; 4) Adequate sleep; 5) Consumption of breakfast; 6) Non-smoking status. Responses were coded between 0-4; where 0=no consumption; 4=high consumption (for variables fruits and vegetables consumption) and for other variables, for instance for appropriate sleep (0=poor sleep; 4=highly appropriate sleep); for breakfast consumption (0=rarely consuming; 4=daily consuming); for walking (0=no walking; 4=daily walking). For network building, a binary co-occurrence matrix was built from the co-occurrence of behavior across participants [21]. Each behavior as a node was represented, and edges (links) between nodes represented the frequency of co-occurrence between two behaviors. The edges were weighted with pairwise Phi coefficients, representing the strength of association between the behaviors. The weighted, undirected network obtained was plotted using the igraph and qgraph packages in R. We calculated centrality measures for every node: 1) Strength

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centrality to find most connected behaviors; 2) Closeness centrality to identify nodes with shortest average paths to other nodes; 3) Between centrality to find behaviors that play bridge roles. Edges were visually scaled by weight, and node sizes were scaled by strength centrality. Colors and positions were optimized using the Fruchterman-Reingold algorithm for readability. In order to determine the clusters of behavior, community detection was carried out with the walk trap algorithm. Networks were created for the total sample, and also for male and female as well as rural and urban dwellers separately. All network analyses were conducted in R using relevant packages including igraph, qgraph, and bootnet. Responses were coded between 0-4; where (for walking) 0 = Never 1 = Once a week; 2 = 2–3 times/week; 3 = 4–6 times/week; 4=Daily. Similarly, (for variables fruits and vegetables consumption) 0 = None; 1 = Less than once/day; 2=Once/day; 3 = Twice/ day; 4=More than twice/day; for appropriate sleep (0 = Very poor; 1=Poor; 2=Average; 3=Good; 4=Excellent); for breakfast consumption (0 = Never/rarely; 1 =1–2 days/week; 2=3–4 days/ week; 3=5–6 days/week; 4=Daily); for non-smoking status (0=Regular smoker; 1=Occasionally; 2=Recently quit; 3=Non- smoker, but exposed; 4=Never smoked or exposed). The table 1 outlined the six key health behaviors assessed in adolescents along with their respective response categories (Table 1).

Table 1: Description of Health Behavior Items and Response Options Used in the Adolescent Health Network Analysis

S. No.	Behavior	Description	Response Options
1	Regular Walking	How often do you engage in walking for at least 30 minutes a day?	0 = Never1 = Once a week2 = 2–3 times/week3 = 4–6 times/ week4 = Daily
2	Fruit Consumption	How often do you consume fresh fruits in a day?	0 = None1 = Less than once/day2 = Once/day3 = Twice/ day4 = More than twice/day
3	Vegetable Consumption	How often do you eat vegetables (cooked or raw)?	0 = None1 = Less than once/day2 = Once/day3 = Twice/ day4 = More than twice/day
4	Adequate Sleep	How would you rate your sleep in terms of adequacy (7–9 hrs)?	0 = Very poor1 = Poor2 = Average3 = Good4 = Excellent
5	Breakfast Consumption	How often do you eat breakfast in a typical week?	0 = Never/rarely1 = 1–2 days/week2 = 3–4 days/ week3 = 5–6 days/week4 = Daily
6	Non-Smoking Status	Are you currently smoking?	0 = Regular smoker1 = Occasionally2 = Recently quit3 = Non-smoker, but exposed4 = Never smoked or exposed

RESULTS

Utilizing measures of central tendency, dispersion, and shape of distribution, distribution of six key healthy behaviors among adolescents was examined (Table 2). On a 0 to 4 scale, the average scores for all the behaviors walking (M = 2.61), breakfast eating (M = 2.63), sleep (M = 2.47), fruit consumption (M = 2.36), nonsmoking (M = 2.43), and vegetable consumption (M = 2.41) were bunched at 2.3 to 2.6, indicating that the sample had accepted these behaviors on a moderate level. Standard deviations indicated moderate behavior adoption diversity ranging from 1.67 to 1.73. All the values of items' skewness ranging from -0.117 to 0.087 were close to zero, indicating that the distributions were relatively symmetric. Light-tailed relative to normal was suggested by repeatedly negative kurtosis values ranging from -1.21 to -1.31. The full range of the scale was used, indicated by the minimum observed score of 0 across all behaviors and the maximum score of 4. Descriptive data overall indicate that the healthy behaviors are indeed in the population but are not yet highly practiced and differ moderately from person to person.

Table 2: Mean Scores of Healthy Behaviors

Variables	Frequency (%)	Skewness	Standard Error of Skewness	Kurtosis	Standard Error of Kurtosis	Minimum	Maximum
Non-smoking	2.43 (1.71)	0.037	0.086	-1.31	0.172	0	4
Veg-Intake	2.41 (1.69)	0.079	0.086	-1.207	0.172	0	4
Proper-Sleep	2.47 (1.73)	0.068	0.086	-1.311	0.172	0	4
Fruit-Intake	2.3 (1.69)	0.087	0.086	-1.226	0.172	0	4
Eating-Breakfast	2.62 (1.67)	-0.117	0.086	-1.21	0.172	0	4
Walking	2.61 (1.71)	-0.1	0.086	-1.261	0.172	0	4

Network Structure

The behavioral network comprised seven nodes, each representing a healthy lifestyle behavior: Walking, Proper Sleep, Eating Breakfast, Vegetable Intake, Fruit Intake, and Non-Smoking. All nodes were interconnected with varying edge weights, indicating positive associations between behaviors. The edge thickness reflected the strength of co-occurrence or correlation; for example, strong ties were observed between Eating Breakfast and Non-Smoking, and between Fruit Intake and Vegetable Intake, suggesting these behaviors often co-exist in individuals.

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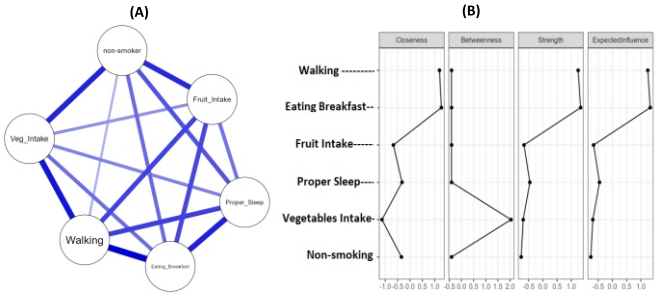

Figure 1: Network plot. The overall network consists of seven nodes connected through edges (A). the centrality plot defects centrality measures: closeness, betweenness, strength, and expected influence (B).

Centrality Measures

To determine the most central behaviors in the network, centrality measures were calculated: Degree Centrality: Fruit Consumption, Adequate Sleep, and Walking had the highest degree centrality, meaning that they were most often linked to other behaviors. Betweenness Centrality: Breakfast Consumption showed high betweenness, functioning as a bridge among clusters of physical activity/sleep and eating habits. Closeness Centrality: Walking and Non-Smoking had high closeness scores, indicating that they were able to effectively reach or influence other nodes in the network.

Modularity and Community Detection

With modularity optimization, the network divided into two major clusters: Cluster 1 (Lifestyle + Physical Activity): Walking, Proper Sleep, and Non-Smoking. Cluster 2 (Dietary Behaviors): Eating Breakfast, Fruit Intake, and Vegetable Intake. This suggests a modular distinction between lifestyle and dietary behaviors, albeit with bridging nodes such as Eating Breakfast that link the two clusters.

Cohesion and Network Density

The network density was high overall (0.80; 12 edges out of total 15 possible edges), which implies that the healthy behaviors in the sample population were densely connected. Such a dense network implies that changes in one behavior can potentially have an influence on others, which supports the necessity of multi-behavior interventions.

DISCUSSION

Applying network centrality measures such as degree, closeness, and betweenness, the current research analyzed the centrality of six health behaviors: not smoking, having breakfast, walking, sleeping enough, and eating fruits and vegetables. These measures can be applied to enable researchers to identify critical leverage points for intervention programs by determining how central a behavior is in a network of co-occurring lifestyle habits [22, 23]. The findings indicate that walking and eating breakfast across all subgroups consistently demonstrate the highest values of centrality, indicating they are most likely to influence and drive other positive behaviors. Walking is a low-intensity physical activity that improves cardiovascular health and mental well-being and eating breakfast, in specific, has been consistently associated with enhanced cognitive function, metabolic control, and reduced BMI in teenagers [24-26]. Non-smoking was also identified as a bridging behavior for men, which connects otherwise weakly related behaviors. This indicates that its effects transcend its direct health benefits and can also assist individuals in adopting other positive habits [27]. Eating fruits, vegetables, and obtaining sufficient rest all have low centrality scores and are seemingly peripheral in every network. This means that adolescents' schemas of health behavior do not consider such activities as interdependent or that they are practiced less often with other behaviors. Peripheral activities might have a lesser effect on overall lifestyle patterns and require more specific, stand-alone interventions, as stated by [28, 29]. In creating integrated interventions that increasingly link nutrition quality and sleep hygiene to leading lifestyle pathways, these results underscore the importance of strengthening core, high-centrality behaviors such as eating breakfast and walking. This approach is consistent with systems-based models of public health that emphasize equal attention to behavioral clustering and scalability of interventions.

CONCLUSIONS

Walking and eating breakfast were identified as key lifestyle habits in adolescent health networks, suggesting that they may be targeted strategically for treatments aimed at preventing obesity.

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Authors Contribution

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Formal analysis: IA
Writing, review and editing: IA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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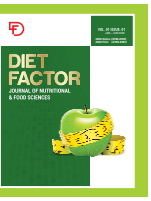

Original Article

OPEN ACCESS

Factors Affecting Obesity Among Adults Attending the University of Lahore Teaching Hospital, Pakistan

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ABSTRACT

Obesity is one of the major problems these days, and it is the root cause of many fatal health conditions, including hypertension, diabetes, and cardiovascular diseases. **Objective:** To find out the factors affecting obesity among Adults aged between 20-60 years attending the University of Lahore Teaching Hospital, Pakistan. **Methods:** A cross-sectional study based on a sample size of 100 participants was conducted. Non-probability convenience sampling was used. Adults of both genders aged 20-60 years attending the University of Lahore Teaching Hospital, Pakistan, were selected. SPSS version 21.0 was used to analyze the data. **Results:** In a sample of 100, 78% of them were found to be obese, and 22% of them were overweight. Female were more inclined to obesity (53%) in comparison to male (47%); married people (57%) were more predisposed to obesity in comparison to those who were single (43%). Obesity was statistically linked with psychological determinants (Stress, emotional eating), fast food consumption, consumption of sugary foods, high levels of cholesterol, and physical inactivity ($p<0.05$), and junk food preference, cholesterol levels, and daily exercise, as the p-value was <0.05. **Conclusions:** The study observed obesity as a major issue among the adult population and identified major correlations of obesity with marital status, BMI, physical inactivity, stress, and diet.

INTRODUCTION

An irregular and extreme fat buildup that harms the body and health is defined as 'Obesity' and Overweight. Body mass index (BMI) is a weight-for-height ratio calculated to get an idea of whether an individual is overweight/or obese. To calculate BMI (in kg/m2), a person's weight (kilograms) is divided by the square of his/her height (meters)1. For adults, the World Health Organization (WHO) describes overweight and obesity as: 'BMI more than or equal to 25 signifies overweight, whereas BMI more than or equal to 30 signifies a condition known as obesity [1]. Researchers declared obesity and overweight as contributing factors in increasing mortality rates, posing a risk to life. For example, being overweight can result in gestational diabetes during pregnancy, which can lead to high birth weight. Excessive birth weight is also an indicator of overweight and obesity in adulthood [2, 3]. It has psychological, economic, social, and medical health concerns, such as respiratory issues, cerebrovascular and cardiovascular diseases, digestive disorders, cancer, as well as type 2 diabetes [4]. Dyslipidemia development, type 2 diabetes, and hypertension in adults are primary risk factors of obesity [5]. Obesity has been increasing swiftly all over the world throughout recent decades [6]. In 2014, over 1.9 billion people, aged 18 years or above, were noted as obese worldwide [7]. Even in economically developing nations like Pakistan, the incidence rate of overweight and obesity has

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been gradually increasing. According to the Pakistan Demographic and Health Survey (PDHS), the prevalence of overweight and obesity among adults has shown a steady upward trend in recent years [8]. In urban areas such as Lahore, this rise is particularly concerning due to lifestyle factors and dietary transitions [9]. The World Health Organization (WHO) also reports that obesity-related health burdens are increasing in low- and middle-income countries, including Pakistan [10]. Lahore, one of Pakistan's most populous cities, has experienced rapid urbanization and socioeconomic change. Increased access to energy-dense fast food, reduced physical activity, and more sedentary occupations have significantly altered lifestyle patterns [2]. Cultural norms, especially those affecting women's mobility and outdoor engagement, limit opportunities for physical activity, increasing their risk of obesity [11]. As economic conditions have improved in urban areas, lifestyle-related health challenges have emerged alongside improved access to healthcare and infrastructure. Households with better economic means often outsource physical chores and consume high-calorie processed foods, contributing to metabolic conditions such as obesity, type 2 diabetes, and hypertension. These patterns mirror global urban trends, but they also highlight the unique intersection of culture, economy, and health behaviour in Pakistani cities like Lahore. Furthermore, obesity is considered to be a major factor behind the onset of different diseases, and it is increasing significantly among the population of Pakistan [12]. The data on obesity among adults is scarce in Lahore, although the urban population is increasing and the number of obese people is on the rise. There is little research on the level of local adult populations since the majority of research features either children or national trends. The present study fills that gap by finding out some of the major factors that are associated with obesity among adults attending a ubiversity in Lahore.

This study aims to find the factors affecting obesity among Adults aged between 20 and 60 years attending the University of Lahore Teaching Hospital, Pakistan.

METHODS

A cross-sectional study was conducted at the University of Lahore Teaching Hospital, Pakistan. The study duration was 4 months from May 2019 to August 2019, and the sample size included 100 overweight adults. A non-probability convenience sampling technique was used. The sample selection was based on inclusion criteria, which included adult male and female attending or admitted to the University of Lahore Teaching Hospital, who reside in Lahore, Pakistan. BMI was calculated using directly measured height and weight recorded at the time of data collection. After obtaining written informed consent, data were collected using a pre-tested data collection tool. (questionnaire/proforma). The questionnaire was reviewed by field experts for face validity and pre-tested for clarity and relevance on a small pilot sample (n=100). The data were then tabulated and analyzed using SPSS version 21.0.

RESULTS

Among 100 subjects, 47 were male and 53 were female. 22 subjects were overweight, 78 subjects were obese. 43 were single while 57 were married. Furthermore, 77 participants were stressed/depressed, 75 were feeling frustrated, 77 were feeling lonely, and 81 were eating food due to emotional factors. 49 were consuming sugary foods over healthy foods, and 82 were preferring fast/junk foods over natural foods. 65 participants used to eat out rather than eat food at home. Moreover, 58 subjects were eating snacks while using a computer or watching television. 49 subjects used to sleep for less than 6 hours. 71 were doing regular exercise, and 29 subjects were not doing any kind of exercise. Additionally, 13 subjects were non-diabetic and 87 subjects were diabetic. Moreover, 64 participants had high cholesterol levels out of 100 participants (Table 1).

Table 1: Frequency Distribution of Different Attributes (n=100)

Attributes	Subgroups		Frequency
BMI (Weight Status)	Overweight	–	22
	Obese	–	78
Gender	Male	–	47
	Female	–	53
Marital Status	Single	–	41
	Married	–	57
Psychological Factor	Stress/Anxiety /Depression	Yes	77
		No	23
	Frustration	Yes	75
		No	25
	Loneliness	Yes	77
		No	23
	Emotional Eating	Yes	81
		No	19
Types of Food Intake	Sugary food	Yes	49
		No	51
	Junk food/Fast food	Yes	82
		No	18
Lifestyle Factors	Eating Practices	Eat Out	65
		Eat at Home	35
	Snack Eating While Watching Television /Computer	Yes	58
		No	42
	Sleeping Time	Sleep Less Than 6 Hours	49
		Sleep More Than 6 Hours	51
	Exercise	Yes	71
		No	29

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The BMI of the participants was significantly associated with the psychological factors like stress/ anxiety /depression, frustration, loneliness and emotional eating, as the p-values were 0.000, 0.001, 0.001 and 0.000, respectively(Table 2).

Table 2: Association Between Psychological Factors

BMI	Yes	No	Total	p-value
Stress/Anxiety/Depression				
Over weight	9	13	22	0.000
Obese	68	10	78	
Total	77	23	100	
Frustration				
Over weight	10	12	22	0.001
Obese	65	13	78	
Total	75	25	100	
Loneliness				
Over weight	11	11	22	0.001
Obese	66	12	78	
Total	77	23	100	
Emotional Eating				
Over weight	8	14	22	0.000
Obese	73	5	78	
Total	81	19	100	

BMI of the participants was significantly associated with the intake of sugary foods and preference for fast/junk foods over healthy foods, as the p-values were 0.001 and 0.004, respectively(Table 3).

Table 3: Association Between BMI And Types of Food Intake

BMI	Yes	No	Total	p-value
Sugary Food Intake				
Over weight	4	18	22	0.001
Obese	45	33	78	
Total	49	51	100	
Fast/Junk Food Preference				
Over weight	13	9	22	0.004
Obese	69	9	78	
Total	82	18	100	

DISCUSSION

The study was directed to assess the factors causing obesity among participants of Lahore aged 20-60. Non-probability convenience sampling technique was used. The results revealed that out of 100 subjects, 22 were overweight, while 78 were obese. Moreover, 87 of them were suffering from diabetes mellitus, and 64 were suffering from high cholesterol levels due to higher BMI. The results indicated that female were at increased risk of being obese compared to males. The results are similar to a research study that was performed to observe the frequency of obesity by gender. Then a study established that women are at increased risk of being obese because women involve themselves less in physical activities as compared to male. Males mostly do physical activities; therefore have less chance of being obese, while female prefer fewer physical activities, and physical activity is directly proportional to obesity. People who do more physical activities, such as playing physical games, e.g., cricket, or do physical activity, have less frequency of being obese. On the other hand, females reported prolonged television viewing and prefer to do such less physically demanding activities; as a result, they are at higher risk of being obese [15]. In this study, the results concluded that marital status also plays a significant role in obesity. According to the results, 57 obese people were married, while only 43 people were not married. The results were almost similar to a study that was conducted to find the frequency of obesity among married single people. The study showed that men and women who live in a relationship tend to gain more weight compared to singles. The results concluded that 52 people spend more than 2 hours working on a laptop or computer in a day, while 34 people spend 2 hours on the computer. It was concluded that people who spend more time on the laptop/computer are at a higher risk of being obese [16]. A higher stress rate was observed in obese participants. A similar work was conducted in order to measure the association between continued financial tension and ensuing obesity. The survey was based on questionnaire completion and person-to-person interviews regarding their fitness, well-being, earnings, and services. The study was conducted over three years. The results of this Australian study showed a positive correlation between prolonged financial stress and resulting obesity [10]. The results showed that 54 people eat sugary food, while only 46 eat sugar-containing food. The present study supports the finding of cross-sectional analysis of 396 Chilean adults, among whom increased sugar consumption was significantly related to BMI and total body weight, despite confounding factors [17]. 51 people revealed that they eat fast food in their daily routine, while 49 do not. A similar finding was made when a study was conducted that examined study if perspective, viewpoint, and nutritional awareness were associated with obesity amongst low-earning women of Hispanic and African-American backgrounds. The results concluded that African American people have more nutritional knowledge; therefore at a lower risk of obesity, while other people who do not have nutritional knowledge are at a higher risk of obesity. Fast food contains a high rate of fats and calories; therefore, people who mostly eat fast food are at increased risk of obesity compared to people who eat home-made food [18]. Out of all the participants, 65 preferred to eat out, while 35 participants liked to eat at home. A similar result was observed by a survey-based

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study, which observed whether there was any correlation concerning exposure to take-away food options, body mass, and take-away food consumption. This study was conducted in the United Kingdom (UK) and looked at working adults' home and workplace environments. The results indicated a positive association between consumption of takeaway food and BMI in different fields covered, including home, workplace, and travelling environments, with a greater probability of obesity [19]. The study differs from current work as the researchers narrow down eating out to take-away food options. According to the results, 58 people have snacks while watching TV, and only 42 people indicated that they do not eat snacks while watching TV. Similarly, a study was conducted on American adults from 1988 to 2010. The study found that people who consume high-calorie foods and spend most of their time on computers or watching TV are at a higher risk of obesity as compared to other people. The results showed that 68 participants reported that they spend four to five hours a day watching TV or playing games on the computer [20]. The present study associated less sleep time as a determinant of obesity. Results showed that 60 subjects were sleeping less than 6 hours, and 40 subjects were sleeping more than 6 hours. Another work also presented similar results. A study was conducted to determine whether sleep duration is linked to obesity among adults. It was established that shorter sleep time was considerably linked with the possibility of obesity in the future. However, of all the reviewed literature, around four articles signified distinct outcomes with respect to both genders (men and women). One of the studies had only involved female participants, while one study was conducted on male gender merely [21]. Our results depicted that a lifestyle without exercise and physical activity is related to higher BMI (obesity). Similar results were observed in a study conducted on American adults, which tended to look into the association involving obesity, physical activity and caloric consumption [22]. Results showed that adults with no reported physical exercise and activity in their free time were declared to have 1.7% more fluctuations (among men) in their ideal BMI and 8.3% more fluctuations in women, compared to their counterparts who have a scheduled ideal level of physical activity in their free time [22]. The results of this study also manifested that obese people are at a higher risk of getting ailments. The results were the same with the study that was conducted by the NHS that people who are obese are at a higher risk of getting diseases. Obesity was the basic cause of many diseases, especially diabetes, hypertension, and heart problems. Therefore, people who are diagnosed with hypertension or diabetes are advised to exercise to reduce weight by a doctor [23].

CONCLUSIONS

It was concluded that the overweight condition in adults has been linked with modifiable factors of the lifestyle like poor food habits, physical inactivity, disrupted sleeping patterns, and stress. Awareness creation programs and behaviour change interventions would be useful in curbing the prevalence of obesity, as they address these issues. There is a need to conduct more research with even bigger and more representative samples to establish these findings. Future studies should include a formal power analysis and larger samples to enhance precision and generalizability.

Authors Contribution

Conceptualization: RS, AI
Methodology: SF, ZM
Formal analysis: SJ, SK
Writing review and editing: SK, SJ, ZM
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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