Nutraceutical Advantages of Proteins from Millets on Status of Human Health- An Analysis

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ABSTRACT
In present times millets have been proved to become more significant than staple cereals in terms of productivity and climatic susceptibility. There are many different types of millets being produced in the world today. In this article we have compared various millet varieties available and worked out data about their proteins. Constituent proteins of millets contain leucine, isoleucine, valine, and phenylalanine which are just a few of the crucial amino acids, minerals, vitamins, phyto-chemicals, and anti-oxidant qualities that make these make superior than many cereals. Millets also contains calcium, iron, and zinc. Additionally, millets are useful in treatment of long-term ailments like cancer, CVDs, and diabetes. Millets have also been shown to be effective in reducing bacterial activity, celiac disease, and aging process. In spite of all the health advantages of millet's, anti-nutrients are thought to lower the bioavailability of minerals. Hence, to lower anti-nutrient content and raise mineral bioavailability, a variety of conventional and mechanical processing techniques have been devised, in combination with use of exogenous enzymes. More studies are needed to be carried out to develop novel processing methods, as well as, for analysis of mineral bioavailability of the millet-based foods through in vivo studies, in order to lead a healthy life and maintain a sustainable environment by the humankind. Through the paper we advise to increase millet intake in our daily food.
Key words: Health, millet, Cancer, Protein, Antimicrobial activity, Aging.

INTRODUCTION

The evolution of the Millennium Development Goal (MDG) resulted in the creation of the Sustainable Development Goal (SDG) Ritchie, H et al., (2018). Saleh, A.S. et al.,(2013) reported the second SDG objective, the United Nations committed in 2015 to achieve zero hunger and improving nutrition by 2030.Kumar, A. et al., (2018) studied the shifted food security metric of total calorie (prevalent during the MDG) to nutritional content in food for proper nourishment, as human beings' general physical and mental well-being is dependent on the consumption of nutritious food.Huang, J. et al., 2016, Devi, P.B. et al (2014) & Banerjee, S. et al., (2012) according to the 2016 Global Nutrition Report, almost 44% of the population in 129 countries suffer from severe malnutrition as a result of a nutrient-imbalanced diet.

Population expansion, declining production, and susceptibility to climate change are all predicted to turn the nutritional issues worse. Chandrasekara & Shahidi (2011) studied due to unpredictable variations in rain pattern, intensity, and distribution induced by climate changes, there has been a significant decline in water levels and increase in dry land mass. Jideani (2012) reported the potential for enhanced staple crop production is seriously damaged by the gradual rise in dry land and depth of ground water level.Gopalan, C. et al., (1971), Amadou, I. et al., (2013) & Nithiyanantham, S. et al., (2019) reviewed bigger grains are unable to provide a yield that can be sustained, millets become a preferable choice since they are able to grow luxuriantly on dry, infertile land. Millets provide substantial agricultural advantages over the production of main grains. For instance, poor rice production is a widely reported issue.

Anitha, S. et al., (2021) evaluated on the other hand, millets (pearl millet and finger millet) may thrive and provide better yields at soil salinities of 11–12 dS/m.Sharma, & Niranjan (2018) investigate the

Millets (proso millet and pearl millet) only need an average of 20 cm of rainfall each year, but rice requires 120–140 cm/year. Yadav, O.P. et al., (2012), Yadav & Rai (2013) & Nedumaran, S et al., (2014) reported millets are members of the C_4 cereal family, which has the advantages of efficient carbon dioxide absorption and better oxygen production, with high water holding qualities, and low input requirements. They therefore have dual advantage to the environment and are readily available. Millets have been proved to be very useful in overcoming issues like climate uncertainty and reducing atmospheric carbon dioxide all over the globe Reyes-García, V. et al., (2006). Together with the agricultural benefit, millets perform better as compared to common grains like rice and wheat in terms of nutritive content, as well as, being rich in amino acids, minerals, anti-oxidants, and health advantages. Vinoth & Ravindhran (2017), de Wet, J.M. et al., (1983) & Mishra, A et al., (2020) reported many studies have been done on millets' nutritional and biological relevance, processing techniques, intake of a variety of millet-based cuisines, and health benefits. Makwana, K (2023) studied the selection of blast resistant lines from diverse germplasm set of foxtail millet. Nesar Ahmad Nesar et al., (2023) reported effect of rice straw on weed populations, biomass and yield of wheat under zero and conventional tillage practices.

MATERIALS AND METHOD

a) Materials Required:

(i) **Equipments:**

•Spectrophotometer

•Glass or polystyrene cuvettes

(ii) **Chemicals/reagents:**

• Bradford reagent

• Bovine serum albumin (BSA)

(iii) **Glassware and others:**

• Test-tubes

• Pipettes

• Micro centrifuge tubes

b) Reagent Preparation:

• Protein extraction buffer:

Tris HCl pH 8.1 ------10 mM

EDTA pH 8.0----------10 mM

β-Mercaptoethanol---- 5 mM

PMSF ----------0.1 mg/ml

• **BSA stock solution**: 2.0 mg/ml in extraction buffer

c) Procedures

Protein Extraction

1. Weighed 1-1.5g leaves/seeds/roots/flowers harvested from test plants. Added 1ml of protein extraction buffer per gram of leaf in a cold mortar and pestle.
2. Ground the tissue in the presence of fine sand or liquid nitrogen until a thick paste was produced. Collected the paste and placeed them in a 1.5 ml micro-centrifuge tube and centrifuge for 20 minutes at 12000 rpm at 4^{o}C.
3. Transferred the supernatant to another 1.5 ml micro-centrifuge tube.
4. Placeed about 100µl of the supernatant in a tube for quantification of the extracted protein by Bradford method.

Protein Estimation by Bradford method

1. Used a 100 µl l bottle of protein extract (10–100 µg). Since we are unaware of the protein content of the extract, a preliminary test was performed. To create a volume of 100 µl, combined two different extract strengths—20 µl and 55 µl with extraction buffer. After adding 5 ml of the colourant reagent, and thoroughly mixed these. While preparing the standards, separate tubes containing 5, 10, 20, 30, 40, 50, and 100 µl of bovine serum albumin (BSA 2.0 mg/ml stock in extraction buffer) were prepared. To achieve the appropriate volume, each tube required 100 µl of extraction buffer. Also, added 5ml of the dye reagent to these tubes and thoroughly mixed by vortexing. Compared the absorbance at 595 nm (OD595) to a blank for the reagent. After five minutes but before an hour has passed, compared the absorbance at 595 nm (OD595) to a reagent blank (100 µl of extraction buffer with 1 ml of dye reagent).

2. By comparing the extract's protein concentration to the BSA standard curve, determined its protein content. Prepared a more suitable dilution if the OD 595 for the diluted extract was too high or too low.

3. Because various proteins have very diverse capacities for binding dye, they respond differently in the experiment. For example, bovine serum albumin has a high optical density 595 values, which makes it

only partially representative of proteins. For convenience, we used it with whole leaf extract. As to determine the concentration of a certain protein was required, we utilized a purified form of the same protein as a standard.

Health Benefits of Millets

In India, urbanisation has caused a substantial impact on consumption pattern of food grains, as decreasing use of some grains like millets while boosting consumption of animal-based foods, oil, refined sugar, fat, and alcohol. This consumption pattern has contributed to an increase in the burden of non-communicable illnesses, which account for around 71% of all deaths worldwide Mishra, A et al., (2022), Ludwig, D.S. et al., (2018) & Wu, G (2016). Chethan & Malleshi (2007), ICMR, N. (2010), Aggarwal, V et al., (2013) observed Furthermore, the current consumption pattern has an essential influence in the production of oxidative stress. The imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues causes oxidative stress. Aggarwal, V et al., (2013) determined it is also that ROS contribute to cellular ageing, diabetes, mutagenesis, DNA damage, and carcinogenesis. When DNA, the genetic material in the human body, is broken, it causes a variety of disorders, including cancer Hambidge, M. (2000). According to some studies, an increase in oxidative stress can significantly contribute to inflammatory diseases (arthritis, vasculitis, adult respiratory disease syndrome), cardiovascular diseases, gastric ulcer, neurological disorder diseases (Alzheimer's, Parkinson's, muscular dystrophy), acquired immune deficiency syndrome, and many others Dykes & Rooney (2007) & Sokół-Łętowska, A et al., (2007).Pradeep & Sreerama (2017) studied to combat oxidative stress, the human body has many systems for creating antioxidants (naturally produced or supplied externally through food), which operate as free radical scavengers in preventing and repairing ROS-induced damage. As a result, it aids in the enhancement of the immune defense system, minimizing the risk of degenerative diseases. Millets are a good source of antioxidants Asharani, V.T et al., (2010) & Pathak, R. K. et al., (2018). Adom & Liu (2002) reviewed Hence, millets' free radical scavenging ability not only lowers ROS, but also provide an effective means of prevention and therapy of radical-mediated diseases. Thus, millet eating reduces the risk of the aforementioned degenerative diseases by inhibiting oxidative stress. Fr. T. A. A. Raj S.J. et al., (2023) observed preparation of Hand-Made Chocolates and the Nutritional Composition of its Ingredients.

Millets against Diabetes & Cardiovascular Disease

Diabetes is a chronic metabolic condition characterized by hyperglycemia and altered protein, carbohydrate, and lipid metabolism Pandey, B et al. (2020).Misra, A. et al. (2011) observed the dietary glycemic load is closely connected with an increased risk of diabetes, using a natural inhibitory diet is preferable in the management of hyperglycemia. Alae-Carew, C., et al. (2019), Balwan & Kour (2021) & Sharifi-Rad, M et al. (2020) assessed Along with fiber which is important for glycemic regulation. Millet is a good diet for diabetics due to its high dietary fiber and mineral content, as well as, it contains slow digesting starch with leucine. However, some in vivo investigations have revealed that millet-based foods have a hypoglycemic impact following intervention Pizzino, G. et al.(2017).

Sastre, J. et al. (2000) studied diabetes clearly increases the risk of cardiovascular disease (CVD) by three to eightfold. In terms of CVD risk, low density lipoprotein (LDL) and high density lipoprotein (HDL) have opposing effects Takabe, W. et al.(2001) i.e., every 1 mg/dL rise in LDL raises the risk of CVD by 2%, while every 1 mg/dL increase in HDL lowers the risk of CVD by 2-3%. Triglyceride is another risk factor for CVDs; a link between high triglyceride levels and CVD risk has existed for decades Kawanishi, S. et al.(2001). Among the several strategies for fighting CVDs, decreasing LDL has had the most successful results. As a good measure to reduce the risk of CVDs, a diet that lowers LDL cholesterol should be recommended. Heim Jr. (2002) reported Niacin corrects lipoprotein abnormalities by reducing LDL and triglyceride levels. Lobo, V. et al. (2010), Pham-Huy, L. A. et al. (2008) & Subba Rao & Muralikrishna (2002) observed niacin-rich foods need to be recommended for reduction in the incidence of CVDs. Kam, J. et al.(2016) observed the millets are a rich source of Niacin in the cereal group, so undoubtedly, millet has the ability to reduce LDL and thereby treat CVDs.

In keeping with the preceding research, in vivo investigations prove that millet-based meals cut LDL and increase HDL while decreasing triglycerides (without enhancing LDL level). D. C. et al. (2013) reported the another in vivo investigation, on the other hand, discovered a significant decrease in blood glucose and cholesterol levels without affecting HDL. Definitely to summarize, millets can be recommended to reduce the risk of CVDs Greenwood.

Millets against Cancer

Millet grains contain phenolic components such as phenolic acids, flavonoids, and tannins, making them antinutrients that lower the incidence of colon and breast cancer in animals. Chandalia, M et al., (2000) studied an invivo study revealed that a novel 35 KD protein called Fibroin-modulator-binding protein (FMBP) extracted from foxtail millet suppresses the growth of colon cancer cells by inducing G1 phase arrest and the loss of mitochondrial trans-membrane potential, which results in apoptosis (programmed cell death) in colon cancer cells via caspase activation. It is observed that another in vivo study presented that foxtail millet food supplementation aids in the treatment of colitis-associated colorectal cancer via activating gut receptors Liu, P. et al.,(2003). The study also discovered that a millet-based diet helped to suppress the Signal Transducer and Activator of Transcription (STAT)-3 signaling pathway 2. Ejeta, G et al., (1987) assessed STAT is a transcription factor family that plays an important role in cancer cell uncontrolled cell proliferation, angiogenesis, and apoptosis evasion.

Millets and Antimicrobial Activity

Further, millets' secondary metabolites have been found to contain antibacterial and antifungal properties. Studies have been shown that millets prevent growth of bacterial infections such as E.coli, B. cereus, L. monocytogens, P. mirabilis, S. typhi, P. aeruginosa, and Y. enteroclitica and millet have been shown to have antifungal properties, also Abdelgadir, M et al., (2005).

Millets against Celiac Disease and Aging

Mao, X. et al., (2020) reported Celiac disease is the most common disorder, affecting people all over the world. It is an autoimmune condition caused by a strange adaptive response to gluten-containing cereals. Jali, M. V. et al.,(2012) observed that a gluten-free diet may be able to solve this condition, because millets are gluten-free, millet-based beverages are the best alternative for people with celiac disease.

The presence of antioxidants and β-carotene aids in the maintenance of health and ageing, and millet grains are high in antioxidants. Shobana, S et al., (2009) studied thein vitro investigation discovered the inhibitory impact of methanolic extracts of finger millet and kodo millet on collagen glycation and cross-linking. As a result, a millet-based diet become beneficial to individuals as anti-aging agent. In nutshell, it can be suggested that millet consumption can aid in the prevention and treatment of different diseases, and operates as a nutraceutical.

Table 1. Health benefits of Millets

Disease	Millets responsible for	References
Diabetes	Proso millet, finger millet, little millet	Saleh, A. S et al., (2013), Yadav & Rai (2013) & Shukla & Srivastava (2014).
Cardio vascular diseases	Proso millet, foxtail millet	Mishra, A et al., (2022), Devi, P. B. et al., (2014), ICMR, N. (2010) & Sokół-Łętowska et al., (2007).
Cancer	Proso millet, foxtail millet	Jideani (2012), HM, K. (2015) & Gordon, & Rifkind (1989).
Anti-microbial activity	Proso millet, finger millet, foxtail millet	Miller, M. et al., (2011), Prasad, M.P. et al., (2016) & Devi, P. B. et al.,(2014).
Aging	Finger millet and kodo millet	Tharifkhan, S. A. et al., (2021), Abdelgadir, M. et al.,((2005) & Chait & Eckel (2016).

Nutritional Bioavailability

Bioavailability is the amount of nutrients taken which are absorbed and used through regular metabolic pathways McKenney (2004). So as to determine the dietary adequacy, the bioavailability of nutrients finds greater weight than the nutrient content of the meal as millets are high in minerals. However, due to presence of anti-nutrients such as phytates, polyphenols, and tannins, only a tiny amount is available for human absorption Balk, E. et al., (2017). Both dietary and physiological factors influence nutrient bioavailability in millets Allen, J. P et al., (1997). Hence it is advisable that reducing anti-nutrients while increasing nutrient content should be the desired Moharana, A. et al., (2020). Undoubtedly, millet processing has a major impact on nutritional bioavailability in humans Finkelstein, J. L. et al.,(2015) & Prasad, M.P. et al., (2016).

RESULT & DISCUSSION

In present paper, we analyzed different types of millets using the Bradford method for estimation of protein levels, which yielded a variety of results. We recorded maximum amount of protein in foxtail millet and minimum amount of protein in finger millet (as indicated by mean values). In one study analysis of the physic-chemical, functional and nutritional components of millet, barnyard grass and rice, was done to compare the sensory quality and nutritional value of millet and barnyard grass foods with those of rice. Their analysis of physic-chemical and functional properties showed that the thousand-grain weights of millet, barnyard grass and rice were 2.5, 3.0 and 18.3 g, and the thousand-grain volumes were 1.6, 13, 2.0 and 7.1 ml, respectively. The water absorption of millet, barnyard grass and rice were 1.90, 1.96 and 1.98 ml/g, respectively, and the water solubility indexes were 2.8, 1.2 and 1.0%, respectively Verma, S et al., (2015).

Table 2. Estimation of Standard Protein curve

Sr. NO.	**Protein**		**Extraction Buffer (µl)**	**Bradford Reagent (ml)**	**Incubated for 30 min. at room temp. recorded before 1 hr**	**O.D. at 595 before 1 hr**
	(µl)	**(µg)**				
01	-	-	100	5ml		00.00
02	5	10	95			
03	10	20	90			0.20
04	20	40	80			
05	30	60	70			0.40
06	40	80	60			
07	50	100	50			0.80
08	100	200	-			

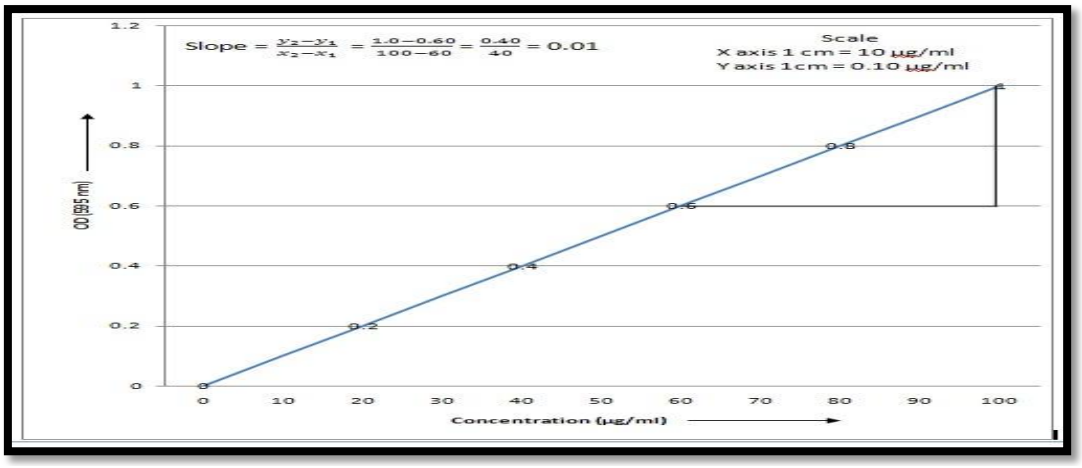

Fig 1. Standard curve for estimation of Protein

Table 2. Protein content of the Pearl Millet seeds.

Sr. NO.	**Protein**		**Extraction Buffer (µl)**	**Bradford Reagent (ml)**	**Incubated for 30 min. at room temp. recorded before 1 hr**	**O.D. at 595 nm**
	(µl)	**(µg)**				
01	-	-	100	5ml		00.00
02	5	10	95			11.20
03	10	20	90			11.28
04	20	40	80			11.38
05	30	60	70			11.50
06	40	80	60			11.65
07	50	100	50			11.79

Table 3. Protein content of the Foxtail Millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 nm
	(µl)	(µg)				
01	-	-	100	5ml		00.00
02	5	10	95			11.21
03	10	20	90			11.79
04	20	40	80			13.25
05	30	60	70			13.98
06	40	80	60			14.46
07	50	100	50			14.91

Table 4. Protein content of the Finger Millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 nm
	(µl)	(µg)				
01	-	-	100	5ml		00.00
02	5	10	95			06.90
03	10	20	90			07.20
04	20	40	80			07.29
05	30	60	70			07.34
06	40	80	60			07.89
07	50	100	50			08.10

Table 5. Protein content of the Proso Millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 nm
	(µl)	(µg)				
01	-	-	100	5ml		00.00
02	5	10	95			09.85
03	10	20	90			09.99
04	20	40	80			10.11
05	30	60	70			11.80
06	40	80	60			12.35
07	50	100	50			13.20

Table 6. Protein content of the Burnyard Millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 nm
	(µl)	(µg)				
01	-	-	100	5 ml		00.00
02	5	10	95			06.12
03	10	20	90			06.25
04	20	40	80			07.40
05	30	60	70			08.56
06	40	80	60			10.89
07	50	100	50			12.30

Table 7. Protein content of the Kodo Millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 nm
	(µl)	(µg)				
01	-	-	100	5 ml		00.00
02	5	10	95			08.20
03	10	20	90			08.85
04	20	40	80			09.45
05	30	60	70			09.98
06	40	80	60			10.12
07	50	100	50			10.50

Table 8. Protein content of the Little millet seeds.

Sr. NO.	Protein		Extraction Buffer (µl)	Bradford Reagent (ml)	Incubated for 30 min. at room temp. recorded before 1 hr	O.D. at 595 n.m
	(µl)	(µg)				
01	-	-	100	5ml		00.00
02	5	10	95			06.50
03	10	20	90			08.91
04	20	40	80			10.12
05	30	60	70			12.56
06	40	80	60			13.47
07	50	100	50			14.75

Table 9. Comparative study of different types of Millet Protein.

Sr. no.	Types of Millet	Average Estimated Protein
1.	Pearl Millet	09.82
2.	Foxtail Millet	11.37
3.	Finger millet	06.39
4.	Proso millet	09.61
5.	Burnyard millet	07.36
6.	Kodo millet	08.16
7.	Little millet	09.47

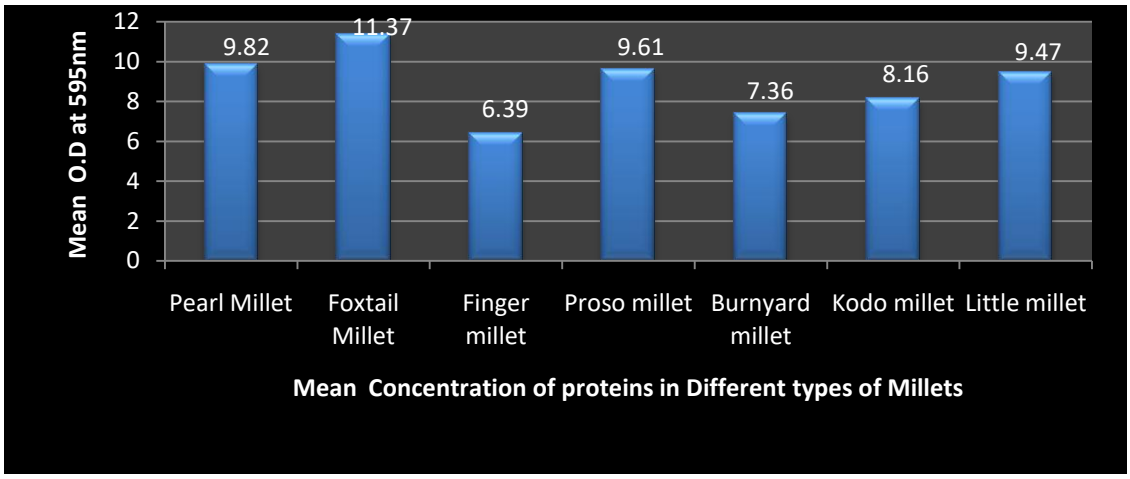

Fig. 2 Histogram showing Mean OD vs. Mean concentration

With an increasing population globally, there is a dire need that nutritional and health problems caused by the unbalanced use of high-calorie fast food must be addressed properly. Problems such as obesity, diabetes, cardiac arrest, osteoporosis, and depression are all so-called modern life style diseases. There are so many grains that are economically viable and tasty, but may not prove healthy. Currently, people have become extra health conscious. Millets are one of the best solutions for finding high nutritional and health benefits in times of pandemic. Researchers have also proved that millets are better alternatives to other grains. These contain high energy, protein, vitamins, minerals and phytochemicals. Millet value-added products have the potential to address negative impacts on agriculture and food security. Therefore, we focused on the nutritional value, health benefits and processing techniques of millet and its value-added products to promote healthy consumption Mishra, P. et al., (2021). Millets play a pivotal role in traditional diets in many parts of the globe. Millets have many beneficial properties such as drought resistance, high yield in water-scarce areas, combined with high nutritional value. Millets are rich in phytochemicals, but the types and amounts vary widely between and within species. The processing techniques used for grains, such as husking and dehusking, malting, fermentation and heat treatment, etc., affect the phenolic content and mainly contribute to the reduction of phenolics. Therefore, processed millet foods and beverages turn to contain significantly lower levels of phytochemicals than other grains. Ambati and Sucharitha (2019) reported evidence suggests that due to the action of these phytochemicals, foods and beverages made from millet have functional and health-promoting effects, especially anti-diabetic, anti-obesity and cardiovascular diseases, and play a positive role in the human immune system. Billions of people around the world suffer from food insecurity and malnutrition. The United Nations has set a global goal to end hunger by 2030, but we are still a long way from achieving it. Climate change, population growth and economic slowdown have affected food security for a decade or more. Many countries now face simultaneous challenges of under-nutrition and over-nutrition. It is imperative that, food systems need to be transformed to achieve food and nutrition security. One way to get closer to our goal is to provide affordable, healthy and nutritious food for all. As a nutritious grain, millets have the potential to play a key role in the fight against food insecurity and malnutrition together. Nutritious grains are rich sources of essential macro- and micronutrients, carbohydrates, protein, fiber, lipids and phytochemicals. It is observed that nutrient content and digestibility of millet are significantly affected by processing techniques Nanje G et al., (2022). Yadav, & Mandhan (2023) studied the low-carbohydrate diets were recently used to treat various illnesses. The low- carbohydrate diets have emerged as a substitute for medicines. Weight reduction and its maintenance are the key targets to dietary modulations. Millets have the potential to contribute to food security and nutrition, but they remain an underutilized crop. Gopinath & Kumar (2023) assessed the small millets are potentially high nutrient rich crops with great value to human consumption and animal feed. Small millets include small seeded crops of Poaceae family like foxtail millet, finger millet, kodo millet, proso millet, barnyard millet and little millet

CONCLUSION

Millets are more important in terms of productivity and climatic susceptibility than staple cereals. These are superior to other cereals because of the presence of important amino acids (such as leucine, isoleucine, valine, and phenylalanine), minerals (calcium, iron, and zinc), vitamins, phytochemicals, and anti-oxidant characteristics. In addition to above millets have potential to treat chronic diseases such as diabetes, CVDs, and cancer. Millets have also been shown to be effective against antibacterial activity, celiac disease, and ageing. Despite all of above benefits of millet, the presence of anti-nutrients definitely reduces mineral bioavailability. So, various traditional and mechanical processing methods, as well as, with intervention of exogenous enzymes, are available to reduce anti-nutrient content while increasing mineral bioavailability. However, more research is needed not only to design a novel processing technology, but also to investigate the mineral bioavailability of millet-based foods through in vivo investigations. As concluding remarks we advice that increased millet intake will make life healthier and create a sustainable environment for better human survival.

The authors declare that they have no conflict of interest.

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