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### **HEALTH SCIENCES**

# *Moringa oleifera* potential as a functional food and a natural food additive: a biochemical approach

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**Abstract:** Several works have shown different aspects of the use of the plant *Moringa oleifera*. However, few review studies bring an approach to its use in food preparation, specifying its role as a functional food and its use as a natural additive, focusing on food biochemistry and including sensory acceptance and safety. Composed by multiple bioactive substances, *Moringa oleifera* has the potential to be used as a food additive, mainly as a preservative with the potential to prevent lipid oxidation and other unwanted chemical reactions that lead to product deterioration. Furthermore, it can improve the physicochemical characteristics of food, increasing its quality and shelf life. It also promotes nutritional improvement, elevating protein, mineral, and vitamin levels. Despite this, the sensorial characteristics of this plant result in a low consumer acceptance of the fortified products, which is a problem for the food industry. Apart from inconclusive works, some data involving Moringa's safety are contradictory, resulting in its commercialization prohibition in Brazil in 2019. This review focused on important data about Moringa use to contribute to the literature and to the food industry, describing information about this medicinal plant effects on food products.

**Key words:** bioactive compounds, food biochemistry, food fortification, medicinal plant, nutritional improvement.

### INTRODUCTION

With the growing interest in healthy lifestyle, functional foods have become popular and recurrent in the human diet. Consequently, this global market niche has been expanding constantly, and its innovation is being stimulated (Barauskaite et al. 2018, Farag et al. 2020). Functional food is defined as food products that incorporate or enhance ingredients that offer health benefits, in addition to nutritional values, modulating bodily functions, which can improve the immune response or decrease the risk of diseases and comorbidities (Farag et al. 2020, Contreras-Rodriguez et al. 2020). Each country, or trading blocs, defines and regulates the approval criteria for the use of a functional food independently (Stringheta et al. 2007).

In the food industry, food shelf life and their sensory characteristics maintenance during storage are important concerns. Hence, the use of food additives is essential in most industrialized products. The regulation for its use, although strict, is still controversial, as there are a large number of studies with divergent results. Moreover, there are different interpretations by the governments (Silva & Lidon 2016, Carocho et al. 2014). An alternative to synthetic food additives is the natural additives, obtained from plants, fungi, algae and animals, often used empirically by the population. Amongst the various beneficial effects that can be promoted by natural food additives, antimicrobial and antioxidant activity are the ones which arouse the biggest interest in the food industry (Bearth et al. 2014).

Moringa oleifera is one of the various medicinal plants that can be used both as a functional food and as a natural food additive. Belonging to the Moringacea family of perennial angiosperm plants, which includes 12 other species, this plant is widely cultivated in the Middle East, Africa, and Asia (Farid & Hegazy 2019, Hisam et al. 2018). Moringa has been used for centuries, with reference to its use in more than 80 countries, and researches about its nutritional values have been carried out since 1970. In 1998, the World Health Organization (WHO) promoted this plant as an alternative supplement to treat malnutrition. (Mahmood et al. 2010).

Moringa leaves contain important bioactive compounds, including vitamins, carotenoids, phenolics, flavonoids, glucosinolates, isothiocyanates, tannins, and saponins. Thereby it is commonly known for its medicinal power (Hisam et al. 2018). Countless benefits are assigned to this plant, such as antioxidant activity, hepatoprotective, anti-inflammatory and antihypertensive properties. It is also associated with hyperglycemia decreasing, showing an antidiabetic effect (Farid & Hegazy 2019). Hence, the plant is called "Miraculous Tree" and "Tree of Life" and is also recognized for the beneficial effects on water sanitation (Padayachee & Baijnath 2020).

Several attributions are given to *M.* oleifera. However, recently in Brazil ANVISA (National Health Surveillance Agency) banned the manufacturing, import, commercialization, advertising, and distribution of all foods containing Moringa owing to lack of evaluation and proof both of the safety of its use and the therapeutic claims attributed to it (BRAZIL 2019). Cytotoxicity, moderate organ toxicity, and histopathological changes were observed in studies carried out *in vivo* and *in vitro* (Asare et al. 2012, Kasolo et al. 2012).

Due to the contradictory data available in the literature, it is necessary to compile results about Moringa incorporation in functional foods and its use as a natural food additive. There is a gap in the literature regarding the potential of Moringa in food biochemistry, associated with its sensory acceptance and safety. Thus, this review aims to describe the *Moringa oleifera* utilization in food formulation, in addition to its potential to modify the biochemical properties of food products. Therefore, databases such as Google Scholar, Science Direct, and PubMed were used.

## CHARACTERIZATION OF Moringa oleifera

Moringa leaves contain high levels of fiber (11.23 g/100g), ash (4.56 g/100g), carbohydrates (56.33 g/100g), total proteins (9.38 g/100g), and lipids (7.76 g/100g). The plant is an excellent source of essential minerals (such as sodium, potassium, magnesium, phosphorus, iron, zinc, copper, calcium, and manganese). 17 amino acids were quantified, both essential and non-essential. The amino acids found in higher levels were leucine and lysine (94.36 and 69.13 mg/100g, respectively). Regarding vitamins, beta-carotene (vitamin A precursor), vitamins E, C, B1, B2, and B3 were identified (El Sohaimy et al. 2015).

El Sohaimy et al. 2015 and Coz-Bolaños et al. 2018 executed extractions with different solvents (water, ethanol 70% and methanol 70%) from the previously dried leaves, and the extracts exhibited a high content of total phenolic compounds and, consequently, of antioxidant activity.

Moringa is a multifaceted plant. Most of its parts are edible, and it has therapeutics

values. Its seeds, flowers, and fruits (pods) have relevant nutrients and substances for feeding, as characterized by Fernandes et al. 2021 (Table I). Because of this, Moringa has been widely used as a supplement against malnutrition. Its leaves are the most used parts and best studied by diverse authors (Table II). They can be dried, ground, and stored for later use (Shiriki et al. 2015).

Many factors can affect the plants' composition, such as edaphoclimatic and biotic conditions. Techniques used in agronomy can also contribute to the differences in the plant's nutrients levels (Ziani et al. 2019). Thus, this can explain why there are variations in the composition of Moringa found in the literature.

# ANTIMICROBIAL POTENTIAL AND FOOD PRESERVATION

Aqueous, ethanolic and methanolic Moringa leaves extracts revealed broad antimicrobial potential. These extracts inhibited the growth of eight pathogenic bacteria and fungi species (*Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus epidermis*, *Staphylococcus aureus*, *Salmonella senftenberg*, *Escherichia coli*, *Bacillus subtilis*, *and Candida albicans*). This inhibition suggests that pharmaceutical and food industries can use Moringa leaves extracts as a natural antimicrobial (El Sohaimy et al. 2015).

Moringa leaves and seeds have been reported to contain antibacterial properties, acting against Gram-positive and Gram-negative bacteria (Saadabi & Zaid 2011, Rahman et al. 2009). The plant leaves contain pterigospermin, a compound that easily dissociates into two benzyl-isothiocyanate molecules, which has antibacterial and antifungal effects (Jayawardana et al. 2015). The presence of many phytochemicals in Moringa seeds can explain its antimicrobial activity, but, as well as in the leaves, benzyl-isothiocyanate is remarkable. This compound can act directly on microorganisms, interrupting the cell membrane synthesis or inhibiting essential enzyme synthesis, disrupting microorganisms' growth (Bukar et al. 2010).

Different Moringa leaves extracts showed activity against bacteria ranging from pathogenic to toxinogenic, such as *E. coli, S. aureus*, and *P. aeruginosa*, responsible for causing food deterioration and possibly causing foodborne diseases (Salem et al. 2015). However, the work conducted by Bukar et al. 2010 indicated that *Pseudomonas aeruginosa*, a resistant bacteria that defy researches in the food area, was sensitive only to the ethanolic extract of Moringa leaves at 200 mg/mL, demonstrating that Moringa extracts may have uses limitations in food products.

Moringa leaves and seeds extracts, obtained through percolation with the solvents ethanol and chloroform (1:10 w/v), showed sanitizers and preservatives properties. These extracts contain substances that act as antimicrobial agents against bacteria and fungi that are frequently associated with food spoilage and food contamination (Bukar et al. 2010). The extracts analyzed can be potential substitutes for artificial preservatives, satisfying the emerging consumers' interest, who have shown a change in nutritional habits with a preference for natural additives (Carocho et al. 2014).

One product that can be challenging for the food market is raw sugarcane juice, as it deteriorates shortly after extraction. To extend the validity of the product, manufacturers often use chemical preservatives. To offer other alternatives for this problem, Ramachandran et al. 2017 studied the effectiveness of combining different natural preservatives (Moringa leaf and seed extract, lemon, and ginger) at low

	Seeds	Flowers	Fruits
Moisture (%)	NA	81.40 ± 0.10	76.80 ± 0.90
Energy value (Kcal/100g)	522.20 ± 0.50	393.20 ± 0.10	389.70 ± 0.30
Carbohydrates (g/100g)	38.85 ± 0.03	67.20 ± 0.10	71.91 ± 0.04
Proteins (g/100g)	31.88 ± 0.08	19.83 ± 0.01	19.49 ± 0.06
Lipids (g/100g)	26.60 ± 0.10	5.02 ± 0.05	2.67 ± 0.06
Ashes (g/100g)	2.67 ± 0.01	7.95 ± 0.07	5.93 ± 0.05
Fructose (g/100g)	ND	1.51 ± 0.01	2.86 ± 0.04
Glucose (g/100g)	0.16 ± 0.04	3.30 ± 0.04	10.03 ± 0.08
Sucrose (g/100g)	1.70 ± 0.03	5.52 ± 0.07	4.92 ± 0.01
Trehalose (g/100g)	ND	0.75 ± 0.01	1.01 ± 0.05
Total sugars (g/100g)	1.86 ± 0.06	11.10 ± 0.10	18.80 ± 0.20
Oxalic acid (g/100g)	10.60 ± 0.20	1.82 ± 0.01	1.18 ± 0.01
Malic acid (g/100g)	ND	1.29 ± 0.02	1.30 ± 0.01
Ascorbic acid (g/100g)	ND	0.19 ± 0.01	0.65 ± 0.02
Citric acid (g/100g)	ND	3.19 ± 0.02	2.62 ± 0.01
Fumaric acid (g/100g)	tr	tr	tr
Total organic acids(g/100g)	10.60 ± 0.20	6.42 ± 0.01	5.75 ± 0.02
Palmitic acid - C16:0 (%)	7.00 ± 0.20	21.60 ± 0.20	10.40 ± 0.20
Stearic acid - C18:0 (%)	6.50 ± 0.20	4.23 ± 0.09	4.73 ± 0.07
Oleic acid - C18:1n9 (%)	69.40 ± 0.40	20,32 ± 0,01	48.80 ± 0.10
Linoleic acid - C18:2n6 (%)	0.69 ± 0.06	14.40 ± 0.50	8.50 ± 0.30
α-linolenic acid - C18:3n3 (%)	0.195 ± 0.005	22.30 ± 0.30	6.30 ± 0.40
Beenic acid - C22:0 (%)	6.68 ± 0.09	5.40 ± 0.50	9.10 ± 0.10
Lignoceric acid - C24:0 (%)	1.33 ± 0.01	5.00 ± 0.30	3.60 ± 0.20
Saturated fatty acids (%)	26.00 ± 0.40	40.84 ± 0.08	33.40 ± 0.50
Monounsaturated fatty acid (%)	73.10 ± 0.50	21.23 ± 0.04	49.00 ± 0.10
Polyunsaturated fatty acid (%)	0.89 ± 0.06	37.90 ± 0.10	17.50 ± 0.60
α-tocoferol (mg/100g)	3.36 ± 0.01	17.22 ± 0.09	4.67 ± 0.02
δ-tocoferol (mg/100g)	1.48 ± 0.03	2.68 ± 0.07	0.19 ± 0.01
Total tocoferol (mg/100g)	4.84 ± 0.01	19.90 ± 0.01	4.86 ± 0.03

# Table I. Nutritional value and composition of *Moringa oleifera* seeds, flowers and fruits<sup>a</sup>.

<sup>a</sup> Data reported by Fernandes et al. 2021. ND, not detected. tr, traces. temperature (2.0 °C). The authors found that the combination of Moringa seed extract and lemon at 10% v/v promoted a satisfactory antimicrobial effect, with a minimum inhibitory concentration (MIC) of  $25 \,\mu$ L/mL against 15 bacterial strains. This combination promoted the highest acceptance among all samples, and was more effective against bacterial growth than sodium benzoate (0.1%) and sodium metabisulfite (0.5%), chemical preservatives used as standard at its maximum allowed concentration.

To gather information on the Moringa application as a natural food additive in livestock products, Singh et al. 2015 described works carried out with the plant in a review article. The authors indicated that the dietary supplementation of goats, pigs, and broiler birds with Moringa leaves improves attributes such as meat color, odor, and lipid profile. It also improves the chemical composition and oxidative stability, without causing adverse effects on carcass characteristics (Singh et al. 2015).

The same work describes that Moringa leaf powder applied in meat products improved safety during the storage, reducing microbial counts. It also improved its nutritional value, ameliorated its physicochemical, sensory quality, and microbiological characteristics, and reduced the production cost (Singh et al. 2015).

	Ziani et al. 2019	Leone et al. 2015	Yaméogo et al. 2011
Energy value (Kcal/100g)	376.0 ± 1.0	NP	362.4
Carbohidrates (g/100g)	56.6 ± 0.5	NP	NP
Proteins (g/100g)	22.8 ± 0.3	31.47 ± 0.12	27.6 ± 0.9
Lipids (g/100g)	6.5 ± 0.2	6.65 ± 0.28	21.6 ± 0.3
Ashes (g/100g)	14.1 ± 0.5	10.79 ± 0.01	9.1 ± 0.1
Fructose (g/100g)	0.36 ± 0.02	0.47 ± 0.07	NP
Glucose (g/100g)	0.42 ± 0.004	2.41 ± 0.20	NP
Sucrose (g/100g)	3.04 ± 0.07	2.50 ± 0.08	NP
Total sugar (g/100g)	3.82 ± 0.09	NP	36.6 ± 0.0
Sodium - Na (mg/100g)	319.00 ± 6.0	307.65 ± 1.49	ND
Magnesium - Mg (mg/100g)	382.00 ± 1.0	562.49 ± 9.07	313.0 ± 0.0
Potassium - K (mg/100g)	1626.00 ± 1.0	NP	2250.0 ± 2.6
Calcium - Ca (mg/100g)	2785.00 ± 1.0	1839.10 ± 12.32	2100.0 ± 2.0
Iron - Fe (mg/100g)	39.00 ± 3.0	17.03 ± 0.79	19.8 ± 0.0
Zinc - Zn (mg/100g)	3.37 ± 0.09	2.48 ± 0.01	2.2 ± 0.0
Copper - Cu (mg/100g)	0.81 ± 0.01	ND	NP
Manganese - Mn (mg/100g)	5.21 ± 0.03	NP	NP

NP, not performed.

ND, not detected.

The crude aqueous extract from Moringa leaves raised softness and juiciness in buffalo ground meat, suggesting that it can execute a proteolytic activity, as indicated by Shi et al. (2019). Aqueous extract can also prevent TBARS increase during storage, being more effective than the synthetic additive BHT (butylated hydroxytoluene, E321).

To improve the quality and prolong the shelf life of sour cream, Salem et al. 2015 evaluated the addition of aqueous leaf extract and Moringa oil to the product. The results showed that both the addition of the oil and the Moringa leaves extract did not modify the samples' composition. Furthermore, the oil and extract did not lead to a standard of identity problems of the sour cream. All the products containing extract or Moringa oil resulted in good sensory evaluation, with an acceptable taste, texture, and appearance throughout the storage period. Changes in pH were observed in all treated samples during storage. Lipolytic and proteolytic bacteria, yeasts, and molds were present in the control samples after 15 days of storage, but not in the products fortified. Therefore the authors concluded that the aqueous Moringa leaves extract and Moringa oil can be added to the sour cream, lengthening its stability and, thus, extending its shelf life (Salem et al. 2015).

Table III contains a summary of the use of Moringa in food products made by different authors and the benefits provided by the plant.

# FOOD PRODUCTS INCORPORATED WITH *Moringa oleifera* AND ITS SENSORY ATTRIBUTES

Cardines et al. 2018 evaluated the Moringa seed extracts thickening action in yogurt formulations. The authors obtained the extracts by saline extractions, followed by ultrafiltration, and added it at 1.5% (v/v) in yogurt fermented

by probiotic lactic culture. The seed extracts promoted thickening and improved the product's characteristics by increasing the protein content, reducing syneresis values, and forming a more cohesive casein net.

Nadeem and Imran 2016 reported favorable characteristics of the oil extracted from Moringa seeds. The authors signalized that the plant seeds have 35 to 40% oil content, with a light yellow color and pleasant nutty flavor. For these sensory characteristics, besides low peroxides levels, different areas can use Moringa oil without needing to pre-process it (refining, bleaching, and deodorization), being suitable for both food and non-food applications, such as the production of biodiesel and cosmetics formulations.

Moringa seed oil has high oxidative stability, is resistant to auto-oxidation, and can be used to stabilize other edible oils, acting as an antioxidant and preventing their deterioration after a storage time. The addition of Moringa oil in sunflower, canola, and soybean oil promoted a lower concentration of primary and secondary oxidation products throughout the storage period (Nadeem & Imran 2016). Oleic acid is the principal fatty acid in Moringa oil (75 - 77% of fatty acids). This high content may raise HDL cholesterol and reduce serum cholesterol and triglyceride levels. Therefore, Moringa seed oil can be considered a functional food (Nadeem & Imran 2016).

Nadeem et al. 2013 studied the effects of ethanolic leaves extract of Moringa on the stabilization of butter. The extract addition in all concentrations evaluated did not cause negative changes in the final composition of the product. The incorporating at 600 and 800 ppm efficiently inhibited the oxidative process and also promoted a decrease in the free fatty acids formation after 90 days of storage under refrigeration. However, the highest concentration

Authors	Food product	Additives	Benefits promoted	Negative results
Ramachandran et al. 2017	Sugarcane juice	Moringa seed extract and lemon	Antimicrobial effect against 15 bacterial strains (MIC: 25µL/mL)	-
Singh et al. Restructured 2015 <sup>*</sup> chicken slices <sup>a</sup>		Moringa leaves	Lower microbial counts throughout storage	-
	Frankfurter- type sausage <sup>b</sup>	Moringa leaves	Increased crude protein content and reduced fat levels	_
	Hamburguers <sup>c</sup>	Moringa leaves	Nutritional value, physicochemical, microbiological and sensory quality improvement; production cost decrease	-
	Buffalo ground meat <sup>d</sup>	Aqueous Moringa leaves extract	Organoleptic properties improvement	_
	Goat meat hamburgers <sup>e</sup>	Aqueous Moringa leaves extract	Prevented TBARS increase during storage	-
	Marinade for smoked fish <sup>f</sup>	Moringa leaves	Antibacterial and antifungal effects	-
Jayawardana et Chicken al. 2015 sausages		Moringa leaves	Reduction of TBARS values during storage	Leaf concentration above 0.50% modifies sensory characteristics negatively
Salem et al. Sour cream Aque 2015 leave		Aqueous Moringa leaves extract/oil	Lower peroxide values during storage; growth inhibition of lipolytic and proteolytic bacteria, yeasts and molds	-

### Table III. Summary of the benefits promoted by the incorporation of Moringa oleifera in different food products.

<sup>\*</sup> Data based on the review article of Singh et al. 2015.

<sup>a</sup> Original data by Najeeb et al. 2014.

<sup>b</sup> Original data by Teye et al. 2013.

<sup>°</sup>Original data by Sharaf et al. 2009.

<sup>d</sup> Original data by Hazra et al. 2012.

<sup>e</sup> Original data by Das et al. 2012.

<sup>f</sup> Original data by Adeyemi et al. 2013.

(800 ppm) caused a negative sensory acceptance of the product, with a significant change in the product's organoleptic properties. Residual flavor and aroma of phenolic compounds, known to have an astringent taste, were observed (Nadeem et al. 2013, Angelo & Jorge 2007).

To verify the addition of interesterified Moringa oil effects on the physicochemical characteristics and oxidative stability in ice cream, Nadeem et al. 2016 substituted the milk fat with Moringa oil at three levels (10, 20, and 30%). The authors concluded that no significant changes in pH, fat content, protein, mineral content, and total solids occurred, comparing the control sample (without the oil addition). There were no differences in the ice cream viscosity or in melting time. This result diverges from other studies that replaced milk fat with vegetable oils, which caused a decrease in the melting time. This effect is due to the interesterification process of Moringa oil that promotes an increase in its temperature melting point, going from 18.0 to 35.6°C (Nadeem et al. 2016).

Interesterified Moringa oil, at all levels used, increased the oleic acid level and the storage stability. The supplemented ice cream samples became less susceptible to the formation of oxidation products. There was a reduction in the loss rate of oleic and linoleic acids. The supplementation also decreased peroxide and p-anisidine values. However, it was not effective in interrupting the formation of free fatty acids during three months of storage (Nadeem et al. 2016).

Mouminah 2015 formulated cookies using blends of wheat flour and Moringa dried leaves (95:5, 90:10, and 85:15 w/w). The addition of Moringa in the formulation increased total ash, protein, fibers, iron, calcium, and magnesium levels. Although the fat content of the formulations with the plant showed a slight increase, the addition of nutrients and beneficial health compounds in a dose-dependent manner indicates that the use of Moringa as an ingredient provides a better nutritional profile to the food. Samples containing 5.0% of leaves did not show a sensory difference compared to the control sample, whereas samples containing 10 and 15% had a acceptability decreasing (Mouminah 2015).

A summary of positive and negative biochemical changes in foods supplemented with Moringa is available in Table IV.

### NUTRITION AND FUNCTIONAL BENEFITS

One of the most studied and published aspects involving the applicability of Moringa in food is its nutritional benefits, especially in populations with a diet low in essential nutrients. Thus, this plant has been used for formulations of supplements against malnutrition and as an additive or fortifier of the most varied foods (Leone et al. 2018).

The use of dried and powdered Moringa leaves is relatively common to fortify dairy drinks, increasing the drink's nutritional benefits. Singh et al. 2015 reported in a review article that these milk products incorporated with Moringa presented good sensory acceptance, except for the color. The use of dry leaves in the curd improved nutritional, microbiological, and organoleptic properties. In buttermilk, the leaves demonstrated the potential to increase health benefits and sensory attributes. Moringa seed extract was incorporated into cottage cheese, increasing yield, protein, and mineral content (Singh et al. 2015).

Madane et al. 2019 added Moringa flower extract in chicken nuggets formulation (1.0 and 2.0%) to evaluate its potential as an antioxidant dietary fiber or as a functional ingredient, based on nutritional content, quality, storage stability, and product acceptability. The authors concluded that the extract enhanced dietary fibers level and extended the product shelf life by increasing its lipid stability. Furthermore, it acted as an antioxidant and did not affect the product acceptability (Madane et al. 2019).

Hekmat et al. 2015 elaborated probiotic yogurts with Moringa leaves in different proportions. The work indicated that, although studies show that the seeds of the plant have an antibacterial effect, the use of Moringa as a functional ingredient did not inhibit the growth of bacteria present in yogurt, except for the

Authors	Food product	Additives	Evaluation	Benefits promoted	Negative results
Cardines et al. 2018	Yogurt	Moringa seed extract	Thickening action	Yogurt thickening; protein levels increase; syneresis values decrease; more cohesive casein net	_
Nadeem & Imran 2016	Sunflower, canola, and soybean oil	Moringa seed oil	Stability	Oxidation product levels decreased	-
Nadeem et al. 2013	Butter	Ethanolic Moringa leaves extract	Stability	Inhibition of oxidative process; decrease in the free fatty acids formation	The addition of extract at 800 ppm promotes a decrease in product acceptance; significantly altered organoleptic properties
Nadeem et al. 2016	lce cream	Interesterified Moringa seed oil	Physicochemical characteristics and oxidative stability	Increase in oleic acid content; higher storage stability; formation of oxidation products decreased	It was not effective in preventing the formation of free fatty acids
Mouminah 2015	Cookies	Moringa leaves	Sensory evaluation and nutritional properties	Provided better nutritional profile (increased the content of total ash, protein, fibers, iron, calcium and magnesium)	Significant reduction in product acceptability with supplementation above 10%

Table IV. Biochemica	aspects in foods	fortified with	Moringa oleifera.

formulation with higher doses of the plant and without sugar. That suggests that the Moringa addition in yogurt formulation can stimulate bacteria growth, and may have prebiotic properties. Moringa leaves at 0.5% enhanced the product's nutritional value, but did not increase the vitamin A levels in the yogurt (Hekmat et al. 2015).

Shiriki et al. 2015 blended maize, soybean, and peanuts in a ratio of 60:30:10, based on the protein content to provide 16 g of protein for each 100 g of food, content recommended for infant diets. The authors divided the mixture into four samples, one part used as control (without leaves addition) and three parts fortified with 5, 10, and 15% Moringa leaves powder. Protein, fiber, and ash levels increased considerably with the product's fortification (from 16.04% to 17.59%, 2.25% to 4.42%, and 1.40% to 2.50%, respectively). Contrarily, fat and carbohydrate decreased, with a consequent decrease in energy value. Protein efficiency ratio (PER) and net protein ratio (NPR) improved significantly with fortification up to 10%, indicating an increase in protein quality, even though all samples had lower PER values than recommended by the Protein Advisory Group. However, 15% fortification decreased PER and NPR values. At this level, a bitter taste is noticeable and, therefore, the animals' consumption was lower. The feed conversion efficiency (FCE) and apparent digestibility (AD) had a similar tendency to PER and NPR, as the proportion of Moringa leaf powder increased. The augment of PER, NPR, FCE, and AD values of the fortified products may be a consequence of the content of proteins and micronutrients increased that the Moringa powder provided (Shiriki et al. 2015).

To determine the consequences of Moringa leaves addition in snacks, Zungu et al. 2020 substituted a flour blend (50% wheat flour and 50% cornflour) for 0% (control), 1, 3, and 5% (w/w)of powdered Moringa leaves and evaluated the products' color, texture, nutritional components, and acceptability. They verified that the snack color was affected as the Moringa leaves increased, changing from light brown (control) to dark green with brown traces, which can be due to the chlorophyll present in the plant leaf. The leave addition at higher levels caused a reduction in the snacks' breaking force, making them more crumbly (Zungu et al. 2020). Formulation at 1% of Moringa leaves was the only well accepted by the public. At this level, the Moringa leaves promoted an increase in mineral content and higher calcium, magnesium, potassium, and iron levels were observed. The fortified snacks can be a possibility to improve the food and nutrition of children from communities with low economic levels and malnutrition (Zungu et al. 2020).

In the same intention of formulating snacks to counter malnutrition, Lopez & Bhaktikul 2018 defined a formulation containing maize flour, cassava root, and Moringa leaves at 1%, which resulted in good acceptability. The authors observed positive results in the protein, moisture, and ash content (Lopez & Bhaktikul 2018).

Rocchetti et al. 2020 replaced durum wheat with powdered Moringa leaves at 5, 10, and 15 g/100g (w/w) for fresh pasta formulation. The authors investigated the bioaccessibility of phenolic compounds and starch digestibility and concluded that the fortified pasta had 152 phenolic compounds, with the highest content in the formulation containing 15% of leaves. Low molecular weight phenolic compounds were the most abundant, identified as equivalents of tyrosol, flavonoids, and phenolic acids (Rocchetti et al. 2020). The replacement of durum wheat by Moringa promoted a decrease in the rapidly digestible starch, which causes a fast elevation of blood glucose levels. Also, it increased the slowly digestible starch levels, which provides prolonged glucose release (Rocchetti et al. 2020). In contrast, the resistant starch levels, considered as a prebiotic food, decreased as there was an increase in Moringa levels in the formulation (Rocchetti et al. 2020, Pereira 2007).

When added to food products, Moringa promotes many beneficial effects. In the literature, several studies report these improvements. Despite the advantages associated with the plant, there are limitations in its applicability, generally associated with the negative sensory characteristics promoted to the fortified products. Boateng et al. 2018 described in a review article some studies that observed certain disadvantages concerning the organoleptic properties of foods fortified with Moringa leaves powder. Unwanted descriptions of sensory attributes have been reported, emphasizing the taste and color. Herbaceous and bitter flavor increases as the Moringa content is high. A study demonstrated that the minimum percentage of powdered Moringa leaves for significant improvements in nutritional value is 10%, the same level in which acceptability becomes lower. Another relevant aspect is that the plant leaf powder is not satisfactory to replace wheat flour in the formulation of bread once it may cause undesirable changes in its characteristics (Boateng et al. 2018).

Since Moringa can cause unwanted sensory changes to the fortified products, more studies are necessary to determine the appropriate level of the plant addition. This level needs to promote beneficial effects, maintaining product acceptability. Besides, new studies on the toxicity and safety of using Moringa need to be carried out (Boateng et al. 2018).

A possible alternative to improve biochemical, functional, and nutritional aspects without causing a negative sensory change is the microencapsulation of Moringa extracts. Microencapsulation is a technique typically used to promote protection and resistance to bioactive compounds (Zorzenon et al. 2020). Thammarat & Airouyuwa 2020 microencapsulated the Moringa leaf extract by the spray drying method, using pea protein isolate and isolated soy protein as encapsulating agents, with a ratio of encapsulated material to wall material in 1:4 w/w.

A summary of Moringa supplementation effects on food products is available in Table V.

# TOXICITY AND FOOD SAFETY

There is a lack of researches about the safety of Moringa and its possible adverse reactions. Since it is a plant, there may be an idea that the higher the dose consumed, the greater the benefits.

Thus, Asare et al. 2012 carried out a study to identify possible acute toxicity with the suprasupplementation of aqueous Moringa leaves extract. The authors used human peripheral blood mononuclear cells and ministered gradual doses of extract to induce cytotoxicity. Acute toxicity was verified using two groups of mice, one receiving low doses (1000 mg/ Kg body weight) while the other received high doses (3000 mg/Kg body weight). The authors determined that extract levels at 20 mg/mL presented cytotoxicity. They discarded the occurrence of toxicological syndromes since the animals showed no differences in behavior, and there was no mortality. The animals that received a supra-supplementation at the highest level (3000 mg/Kg) did not have hepatorenal toxicity. Furthermore, the hematological results were within normality (Asare et al. 2012).

The administration of aqueous Moringa extract. at both doses. reduced the levels of serum chloride. There was a reduction in sodium at the highest dose, suggesting that Moringa can reduce the aldosterone effects in the reabsorption of sodium from the renal tubules. Therefore, the plant has hypotensive and diuretic properties. The increase in blood urea observed was not considered pathological since no change in the serum creatinine level (renal dysfunction marker) occurred, suggesting a physiological response to the high levels of nitrogen compounds, especially proteins, provided by the plant. Serum albumin levels decreased, indicating the presence of toxic substances in the animals' bodies, such as isothiocvanate and glycoside cvanides. The dose of 3000 mg/Kg was genotoxic, but the exact level for its beginning has not been determined yet (Asare et al. 2012). Figure 1 contains a schematic representation of the results discussed.

To verify liver, renal and cardiac toxicity, along with histopathological changes in liver, kidney, and heart, Kasolo et al. 2012 evaluated the sub-acute toxicity of aqueous and ethanolic Moringa leaves extracts. The authors provided different single daily doses of extracts to albino Swiss rats for 30 days. Four groups received the aqueous extract, and four other groups received the ethanolic one. The authors calculated the doses based on the median lethal dose (LD50).

The rats that received aqueous extract showed less weight gain, suggesting a reduction in the animals' consumption of food or less body fat deposition. Moringa leaves may cause hypocholesterolemia (*in vitro* study). Therefore,

Table V. Summary of nutritional and functional aspects promoted by the addition of Moringa oleifera in food	
products.	

Authors	Food product	Additives	Evaluation	Benefits promoted	Negative results
Singh et al. 2015 <sup>*</sup>	Curd <sup>a</sup>	Moringa leaves	Chemical, microbiological and organoleptic properties during storage	Improvement in nutritional, microbiological and organoleptic properties	-
	Buttermilk <sup>♭</sup>	Moringa leaves	Nutritional value	Protein, ash, iron, calcium and vitamins levels increase	-
	Cottage cheese <sup>c</sup>	Moringa seed extract	Product yield and quality	Increase in yield, protein and mineral content	-
Madane et al. 2019	Chicken nuggets	Moringa flower extract	Potential as a functional ingredient	Dietary fibers levels increase; shelf life extended (lipid stability)	-
Hekmat et al. 2015	Probiotic yogurt	Moringa leaves	Nutritional value and possible health benefits	Leaves levels at 0.5% enhances the nutrients content of the product	1.0% Moringa leaves caused a strong and undesirable flavor to the yogurt
Shiriki et al. 2015	Maize, soy and peanuts blend		Nutritional value	Protein, fiber, ash and micronutrients levels increased; fortification up to 10% resulted in higher protein quality	The fortification at 15% promoted a decrease in protein quality (lower intake due to unpleasant taste)
Zungu et al. 2020	Snack	Moringa leaves	Nutritional composition and acceptability	Mineral content improved	Color alteration (from light brown to dark green); fortification makes snack more crumble
Lopez & Bhaktikul 2018	Snack	Moringa leaves	Chemical composition and sensory analysis	Good results in terms of protein, moisture and ash content	-
Rocchetti et al. 2020	Fresh pasta	Moringa leaves	Phenolic bioaccessibility and digestibility	Rapidly digestible starch fractions decrease; increased levels of slowly digestible starch	Resistant starch contents decrease
Thammarat & Airouyuwa 2020	Cookies	Moringa leaves extract microencapsulated	Sensory acceptance	Better stability of total polyphenols; there was no decrease in acceptability	-

<sup>\*</sup> Data based on the review article of Singh et al. 2015. <sup>a</sup> Original data by Salem et al. 2013. <sup>b</sup> Original data by Nadeem et al. 2012. <sup>c</sup> Original data by Mahami et al. 2012.

its aqueous extract can be used in herbal medicines for weight loss (Kasolo et al. 2012).

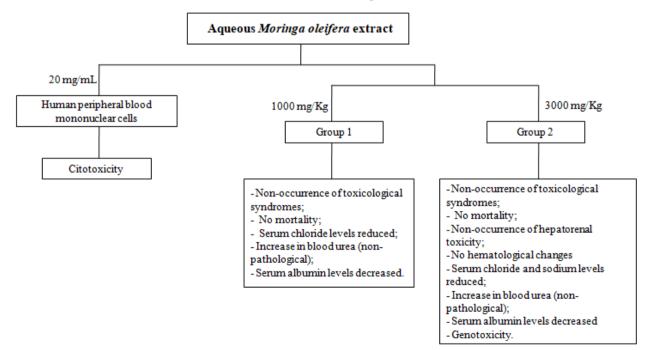
All groups that received aqueous extract had a significant increase in the mean value of total white blood cells, Cl<sup>-</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>. This result is contrary to the work described by Asare et al. 2012, where there was a decrease in serum chloride and sodium levels in rats treated with aqueous Moringa extract. Kasolo et al. 2012 identified higher levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bilirubin in the rats that received the aqueous extract. These increases indicate damage to the liver and the biliary system. The serum alkaline phosphate increase, in particular, is associated with the incidence of cholestasis, which is also a confirmation by the total bilirubin increase in rats. Despite the bilirubin increase, the rats did not show visible jaundice, suggesting that most bilirubin was not conjugated. Kasolo et al. 2012 found no differences in serum urea and

creatinine in all groups, another disagreement with Asare et al. 2012.

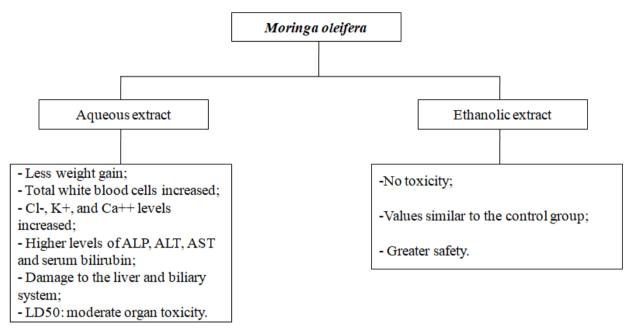
Most histopathological changes occurred in the animals that received doses equivalent to LD50. Aqueous extract single daily doses, according to LD50, are associated with moderate hepatic necrosis, glomerulonephritis, tubulointerstitial nephritis, and chronic myocarditis. Hence, aqueous Moringa extract is related to moderate organ toxicity. The ethanolic Moringa extract did not show any toxicity characteristics.

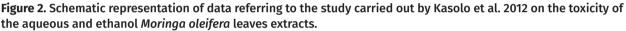
A schematic representation of the results is available in Figure 2.

Studies on evaluations of possible toxicities of Moringa are still scarce, and some data found in the literature are controversial. New tests that evaluate its toxicokinetics, acute, subchronic, and chronic toxicity are essential. Moreover, studies about Moringa neurotoxicity, immunotoxicity, and allergenicity are required. This evaluation will help to define clearly the dosage considered safe.



**Figure 1.** Schematic representation of data referring to the study carried out by Asare et al. 2012 on the toxicity of the aqueous *Moringa oleifera* leaves extract.





# OTHER BENEFITS ASSOCIATED WITH Moringa oleifera CONSUMPTION

Moringa contains several substances associated with functional and medicinal benefits. These compounds can regulate osmotic control, enzymatic and hormonal activities. They can also act in several metabolic pathways. Moringa arouses interest for its application as a functional food or as a nutraceutical, especially its leaves and seeds. Researchers report the plant's ability to intervene in human diseases, which can bring positive results in the treatment of diabetes, obesity, inflammation, cancer, hypertension, and infections caused by microorganisms (Udechukwu et al. 2018).

Despite the interest, Moringa products development focused on health applications has a key impediment: the lack of data and clear evidence of its efficiency and safety, principally concerning chronic diseases. Yet, there is preclinical evidence that can stimulate more rigorous studies. Currently, the therapeutic use of Moringa is carried out in South Asia medicine. Its use is performed widely in the Ayurvedic and Unani medical systems, traditional in India. The plant is a bet for the intervention of diseases based on diet, however, further studies are essential to develop this potential (Udechukwu et al. 2018, Kumar et al. 2010).

To evaluate the chemical composition of powdered Moringa leaves, their sensory acceptability, their ability to inhibit the enzyme  $\alpha$ -amylase *in vitro*, and their effect on the postprandial glycemic response, Leone et al. 2018 supplemented meals for volunteers from the refugee camp in southwest Algeria. 20 g of powdered Moringa leaves (corresponding to 8.0% of the meal weight, stipulated based on the literature and informal tests by the authors on sensory acceptability) were added to the meals of 17 diabetic and 10 non-diabetic volunteers. The diabetic individuals were patients with type 2 diabetes for at least one year, without being submitted to insulin therapy, treated only with oral hypoglycemic agents.

The results showed that Moringa leaves reduce the  $\alpha$ -amylase activity, a key enzyme in the digestion of carbohydrates in the diet, suggesting a decrease in the postprandial glucose level. It also suggests a reduction in the starch hydrolysis rate mediated by enzyme and glucose absorption by the intestine. Indeed, supplementation with Moringa leaves determined a lower increase in postprandial glycemia in diabetic individuals, who in general had a mean change in the baseline concentration of postprandial glycemia when they consumed the supplemented meal, compared to the meal control, without supplementation. In the case of non-diabetic individuals. there was no effect. Thus, the constant consumption of Moringa leaves can improve glycemic control in diabetics and can be a hypoglycemic herbal medicine (Leone et al. 2018).

Regardless of the positive results found by Leone et al. 2018, there was a low acceptance, especially on the flavor of supplemented meals with Moringa leaves, mainly due to the bitter taste. It is necessary to evaluate the effect of lower doses of supplementation. In addition, studies that assess long-term effects on blood glucose need to be carried out (Leone et al. 2018). To better evaluate the hypoglycemic effect and other benefits attributed to the plant, the bioavailability and bioaccessibility of Moringa phytochemicals should be better elucidated (Udechukwu et al. 2018). The polyphenolic compounds found in the plant, for example, usually contain sugar molecules, which can prevent their intestinal absorption. In particular, glycosides linked to rhamnose must undergo hydrolysis by the intestinal microbiota and therefore are absorbed with difficulty. Biologically active compounds present in Moringa extracts may be involved in complex interactions, which may hinder their accessibility and availability. An explanation of the bioavailability of plant

compounds may assist in determining the right ingestion levels so that the desired physiological effects occur (Udechukwu et al. 2018).

### CONCLUSIONS

This review showed relevant aspects, not yet described with this approach, of Moringa oleifera use in food products, gathering data on its characterization, antimicrobial, and food preservation potential. This work collected information about food biochemistry and chemistry attributes, compiling data on Moringa safety and toxicity. Therefore, this study contributes to the literature and encourages new researches with Moringa. This plant has several properties that can be explored in many potentialities, mainly acting as a natural food additive and food preservative due to its bioactive substances and compounds with antimicrobial and antioxidant activity. The plant has a high potential to be used in functional food formulation, promoting nutritional and health benefits. However, dose-effect and sensory attributes still need a better evaluation. Despite being promising, Moringa use must be analyzed to define a safe dosage since studies are either scarce or inconclusive, and some data are contradictory. Thus, studies to elucidate its toxicology are necessary. Another barrier that deserves attention is its sensory acceptance, which is low, especially when Moringa is incorporated in higher concentrations. Studies point out that attributes such as its color and its particular herbal flavor can be a problem, especially when larger doses of the plant is added to the foods formulations. Even with limitations, the use of Moringa has grown worldwide, and new technological properties of this plant have been discovered, especially in the area of food.

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Fabiane Hodas identified the literature sources, carried out and drafted the manuscript. Maria Rosa Trentin Zorzenon participated in the chosen topics' decisions and made the proper corrections. Paula Gimenez Milani was the work supervisor, who guided and revised the manuscript. All authors gave their final approval for submission.

