

Yoghurt fortification with *Moringa oleifera*: nutritional and production aspects

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Feyisayo O. Adepoju^{1*}, Irina S. Selezneva¹,
Charles Odilichukwu R. Okpala^{2,3}

¹Ural Federal University, Institute of Chemical Technology, Technology for Organic Synthesis, Mira 19, 620002 Ekaterinburg, RF

²Wrocław University of Environmental and Life Sciences, Faculty of Biotechnology and Food Science, Department of Functional Food Products Development, 50-630 Wrocław, PL

³University of Georgia Athens, College of Agricultural and Environmental Sciences, UGA Cooperative Extension, GA 30602, USA

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*Corresponding author: besee010@gmail.com

Abstract

Yoghurt is a nutrient-dense dairy food product produced by lactic acid bacteria that enhances digestion and nutrient absorption, as well as sustains gut flora. However, it is a low-phenolic food. In addition to being a potential food delivery system, creating unique yoghurts with plant-derived ingredients has become more popular. Increasingly, *Moringa oleifera* remains a popular phytotherapeutic plant known for its antioxidant properties, given the presence of a wide range of phenolic compounds. Adding *M. oleifera* to yoghurt, therefore, would enhance both mineral and phenolic compositions, as well as promote the growth of probiotic bacteria. In this review, the nutritional and production aspects of *M. oleifera* to fortify yoghurt is discussed. The obtained findings on *M. oleifera*'s therapeutic properties consolidate it as a promising ingredient for yoghurt fortification, as demonstrated by its nutritional and functional impact on yoghurt. Given that yoghurt production requires adherence to specific standardized thresholds, the rich polyphenolic content of *M. oleifera*, which adds an astringent taste, requires future studies to overcome this challenge.

Keywords: *Moringa oleifera*; antioxidant; yoghurt; fortification; functional foods

Introduction

Consumers today are aware of their energy intake and its impact on health and are therefore concerned about the products they include into their diets. Besides, considering that the body sometimes produces excess oxidants that cause imbalance and oxidative damage to biomolecules and may eventually be responsible for the occurrence of several diseases, including cancer. Food products that provide psychological or physiological benefits in addition to the classical nutritional function are in demand. As a result, there is growing interest in foods containing functional ingredients and nutraceuticals that can benefit the body. In response to consumer demand, the food industry is attempting to fulfil these requirements by developing functional products with specific health benefits.

Yoghurt is one of the oldest fermented dairy products worldwide and is commonly produced from domesticated milk by lactic acid bacteria (especially *Lactobacillus bulgaricus* and/or *acidophilus*, as well as *Streptococcus thermophilus*) (Das et al., 2019; Ogunyemi et al., 2021). Furthermore, the active microorganisms in yoghurt, called probiotics, help to improve the balance between beneficial and undesirable bacteria in the intestinal tract. This stabilizes the gut microflora, lowers blood cholesterol, and improves immunomodulation, all of which are associated with a lower incidence of chronic diseases, such as gastrointestinal disorders and cancer (Ahmad et al., 2022). Yoghurt is considered a nutritious food that, in addition to its high digestibility and acceptability, improves lactose intolerance and continues to serve as an appropriate food vehicle for functional and nutritional ingredients for human wellness (Gahruie et al., 2015; da Silva et al., 2019). Fortifying food with natural additives is one of the best ways to boost the overall nutrient intake of food while minimizing adverse effects. The addition of additives, such as fruits, cereals, herbs, and plant extracts, to yoghurt can improve its rheological and antioxidant properties (Durmus et al., 2021; Wu et al., 2023; Ibhaze et al., 2022; Kiros et al., 2016; El-Sayed and Youssef, 2019).

Moringa oleifera Lam is one of the most cultivated cruciferous herbs in tropical and subtropical areas of Asia, Africa, and Central America. This crop is well-adapted to tough climatic conditions, allowing it to thrive across different geographical locations (Kou et al., 2018). Furthermore, the plant's vegetative parts have a long history of use as food, medicine, and water clarifier, making practically all plant parts versatile, particularly because of their high levels of dietary fiber, phenolic compounds, and macro- and micronutrients (Xu et al., 2019). *M. oleifera* extracts are used in traditional medicine in India, Malaysia, and Puerto Rico to treat anxiety, anaemia, diarrhoea, diabetes, and obesity (Ma et al., 2018; Luangpiom et al., 2013). Additionally, *M. oleifera* leaves are considered an excellent food source, rich in protein, vitamins, calcium, ascorbic acid, and antioxidant compounds such as flavonoids and phenols. Leaf extracts of *M. oleifera* have been shown to be useful in diabetes treatment, with the ability to lower blood sugar levels and improve antioxidant status (Olurishe et al., 2016; Pontual et al., 2012).

As a less expensive source of nutrients, *M. oleifera* can modulate the microbiota, just as prebiotics can improve cardiovascular health and enhance diets in developing nations (Fernandez and Marette, 2017). Therefore, *M. oleifera* may be more beneficial when paired with yoghurt to help people recover from both nutritional deficits and provide the body with the necessary nutrients (Ahmad et al., 2022). Taking all these factors into consideration, fortifying yoghurt with phenolic-rich additives, such as *M. oleifera*, seems to be an ideal way to maximize the benefits of consuming high phenolic compounds. However, yoghurt has unique properties that make it acceptable to consumers; therefore, it needs to be clarified whether the addition of *M. oleifera* will positively or negatively impact yoghurt. Given the dearth of understanding regarding the potential use of *M. oleifera* in yoghurt production, it is expected that specific aspects such as fermentation time, rheology, acidification, and physicochemical properties may well be altered (Dimitrellou et al., 2020). There is the need to learn more about the impact of *M. oleifera* in fortifying yoghurt quality towards improving human wellness. To supplement the existing information, this review compiles important information from relevant literature about *M. oleifera* fortifying yoghurt products, specifically the nutritional and production aspects. The therapeutic properties of the *M. oleifera* plant, yoghurt production, and its fortification (with *M. oleifera* powder and extracts) will be discussed, ending with limitations and directions for future studies.

Therapeutic effects of *M. oleifera*

M. oleifera possesses various phytochemicals, some with therapeutic properties, including antioxidant, antidiabetic, anti-inflammatory, and antitumor aspects. The aerial parts of *M. oleifera* plants and their bioactive components (Figure 1) reveal how physiological and environmental factors influence the existence of various compounds, including phenolic acids, flavonoids, tannins, glucosinolates, etc. Therapeutic effects of *M. oleifera* are presented in subsequent sub-sections, specifically antioxidant, antimicrobial, and anticancer aspects.

Antioxidant activity

Biochemical reactions in the human body produce unstable free radicals like reactive oxygen species (ROS) that, in excess, can damage essential macromolecules such as proteins, lipids, carbohydrates, and nucleic acids. Thus, antioxidants would stabilize the free radicals and prevent such damage to macromolecules (Swati et al., 2018). *M. oleifera* contains antioxidant micronutrients such as zinc and selenium, vitamins A, C, and E, and other antioxidant pigments (α and β -carotene, xanthine, chlorophyll, lutein, and others) (Hodas et al., 2021). *M. oleifera* leaves, pods, and seeds contain such antioxidant compounds as rutin, quercetin, caffeoylquinic acid, and kaempferol. For example, *M. oleifera* leaves were reported to have 89.8 mg/100 g quercetin, 36.3 mg/100 g



Figure 1. Aerial parts of *M. oleifera* plants and their bioactive components

kaempferol, 2.9 mg/100 g isorhamnetin, and 129 mg/100 g total flavonoids, excluding apigenin and luteolin (Yang et al., 2008). Phenolic compounds could also be found in *M. oleifera* leaves, roots, flower, seed, and bark. Myricetin and quercetin ($1530 \pm 10 \mu\text{g/g}$, $985 \pm 4 \mu\text{g/g}$) in the leaves, as well as gentisic acid and biochanin A ($85 \pm 2 \mu\text{g/g}$, $45 \pm 1 \mu\text{g/g}$) in the roots, had significantly higher concentrations than others (Prabakaran et al., 2018). Elsewhere, the antioxidant capacity of methanolic extracts from the leaves, root, and stem bark of *M. oleifera* revealed IC_{50} values of 30, 16, and 38 μL , respectively. More so, *in-vitro* evaluation of the methanolic extracts of the leaves, root, and stem bark in the 2-deoxyguanosine assay model showed IC_{50} values of 40, 72, and 58 μL , respectively (Atawodi et al., 2010). To further contextualize this sub-section, Table 1 summarizes some recent reports of the antioxidant potential of *M. oleifera* (Wang et al., 2019; Soliman et al., 2020; Sailaja et al., 2021; Gupta et al., 2012; Amara et al., 2021; Vongsak et al., 2015; Cheraghi et al., 2017; Karthivashan et al., 2015; Edeogu et al., 2019; Khalil et al., 2020; Jaiswal et al., 2013; Abou-Zeid et al., 2021; Alqahtani and Albasher, 2020).

Antimicrobial activity

Practically all *M. oleifera* plant parts, from bark, roots, seeds, flowers, to leaves, would exhibit antimicrobial

activities (Arora and Arora, 2021). Isolated from various parts of *M. oleifera*, both pterygospermin and isothiocyanates would exert both antibiotic and antifungal properties (Islam et al., 2021). Ethanol extract of *M. oleifera* leaves/seeds would show antimycotic activities *in vitro* against *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* (Chuang et al., 2007). Antimicrobial activities of *M. oleifera* seed extracts were associative with the presence of moringin (4-(α -L-rhamnosyloxy) benzyl isothiocyanate) (Padla et al., 2012; Wen et al., 2022). Whilst crude chloroform extract of *M. oleifera* bark displayed promising antibacterial/antifungal activity (Nikkon et al., 2003), those of leaf (ethanol, methanol, and chloroform) would be anti-bactericidal, specifically against such gram-negatives as *Pseudomonas aeruginosa* and *Shigella shinga*, and gram-positives as *Escherichia coli*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus B haemolytica* (Masurekar et al., 2015).

Anti-cancer activity

M. oleifera leaves/bark are considered as potential anti-cancer agents, as shown in Table 2. Extracts from *M. oleifera* leaf would inhibit the viability of hepatoma and acute

Table 1. Some reports about the antioxidant potential of *M. oleifera*

Active components	Duration	Study model	Outcomes	References
Niazirin (5 and 25 µM, 10 and 40 mg/kg/day)	14 days after induction of diabetes	High glucose induced VSMCs and STZ-induced diabetic mice	Niazirin inhibits cell proliferation by inhibiting the expression of Nox4, which results in the deactivation of PKCζ thus, decreasing the production of ROS	Wang et al., 2019
Hydro-alcoholic leaf extract (300 mg/kg)	12 days	Methotrexate-induced oxidative stress in mice	Extract regulates renal oxidative stress by upregulating Nrf2 and HMOX1 genes and downregulating NFκB	Soliman et al., 2020
Moringa isothiocyanate-1 (MIC-1, 80 mg/kg)	3 days	LPS-induced acute inflammation in mice	MIC-1 increases nuclear translocation of Nrf2, leading to increased transcriptional activity of AREs, antioxidant genes and reduced nuclear accumulation of NFκB	Sailaja et al., 2021
Methanolic extract of pods (150 and 300 mg/kg/day)	21 days treatment post-induction of diabetes	STZ-induced diabetes in Wistar rats	<i>M. oleifera</i> extract exerted cellular antioxidant defences by protecting β-cells from ROS-mediated damage and reduced lipid peroxidation with concomitant increase in SOD, CAT, and GSH	Gupta et al., 2012
Sprout extract (100 µg/mL)	24 h	DEHP-induced Human neuroblastoma	<i>M. oleifera</i> extract decrease levels of ROS by modulating the expression of antioxidant genes (Nrf2 and HO-1)	Amara et al., 2021
Leaf extract (100 µg/mL); Astragalin (100 µg/mL); crypto-chlorogenic acid (50 µg/mL); Isoquercetin (5 µg/mL)	24 h	H ₂ O ₂ -induced ROS in HEK-293 cells	<i>M. oleifera</i> leaf extract, isoquercetin, and crypto-chlorogenic acid suppressed the production of ROS by increasing the mRNA expression levels of antioxidant enzymes, SOD, CAT, and HO-1.	Vongsak et al., 2015
N, α-L-rhamnopyranosyl vincosamide (200-2000 µg/mL)	14 days	Doxorubicin -induced cardiac toxicity in rats	N, α-L-rhamnopyranosyl vincosamide mediated cardioprotective effect by blocking lipid peroxidation in cardiac tissue through its antioxidant potential	Cheraghi et al., 2017
Methanolic leaf extract (250 mg/kg)	14 days	Lead-induced neurotoxicity in Wistar rats	Increased activities of SOD, GPx, GR, GSH, and CAT and decreased MDA and NO levels	Alqahtani and Albasher, 2020
Ethanol leaf extract (400 and 800 mg/kg)	7 days	Tilmicisin-induced renal damage in Sprague Dawley rats	Decreased renal levels of hydrogen peroxide and MDA, and increased SOD and GPx	Abou-Zeid et al., 2021
Aqueous leaf extract (200 mg/kg)	21 days	STZ-induced diabetic in Wistar rats	Increased SOD, GST, and CAT levels and decreased MDA levels in tissues	Jaiswal et al., 2013
Ethanol leaf extract (400 and 800 mg/kg)	7 days	Tilmicisin-induced cardiac damage in Sprague Dawley rats	<i>M. oleifera</i> extract modulate total ROS level by decreasing the levels of MDA, protein carbonyl, and 8-OHdG	Khalil et al., 2020
Seed oil (5 mL/kg)	16 days	Gentamicin-induced oxidative nephrotoxicity in Wistar rats	Increased renal levels of SOD, CAT, GSH, and GPx and decreased levels of MDA, IL-6, IL-18, TNF-α, NO, iNOS, and NFκB	Edeogu et al., 2019
Ethanol leaf extract (100 and 200 mg/kg)	24 h	Acetaminophen-induced hepatotoxicity in male Balb/c mice	Decreased hepatic levels of MDA and increased levels of SOD, CAT, and GPx, by upregulating the expression of NQO1, HO-1, Gsta2, GCLM, and Nrf-2	Karthivashan et al., 2015

lymphoblasts (Khalafalla et al., 2010). Such active ingredients as niazimicin, glucomoringin, glucosinolates, and β-sitosterol-3-O-β-D-glucopyranoside makes *M. oleifera* a promising anticancer candidate (Berkovich et al., 2013). Indeed, benzyl isothiocyanate promotes apoptosis by producing intracellular reactive oxygen species that lead to cancer cell death (Wu et al., 2021). Cold-water extract of *M. oleifera* leaf could be an anti-proliferative agent that inhibits cancer cell growth (Jung

et al., 2015). Anti-proliferative effect may induce reactive oxygen species in cancer cells, which lead to apoptosis shown by upregulation of apoptotic pathway members, caspase 3 and caspase 9 (Hermawan et al., 2012). Elsewhere and through the activation of the intrinsic/extrinsic pathway, *M. oleifera* extracts would induce cell death in different tumour cells (Do et al., 2020; Madi et al., 2016; Akinlolu et al., 2021; Asaduzzaman et al., 2017; Das et al., 2021).

Table 2. Some reports about the anticancer potential of *M. oleifera*

Active components	Duration	Study model	Outcomes	References
Water soluble leaf extract (50-200 µg/mL)	48 h	HepG2 cells and HFA in immunodeficient nude mice	Induces apoptosis by overexpressing apoptotic genes such as cleaved caspase-3 and cleaved PARP, and downregulating Bcl-xL genes.	Jung et al., 2015
Moringin (4-(α -L-rhamnosyloxy) benzyl isothiocyanate) (1.64-16.4 µM)	24 - 72 h	SH-SY5Y and WI-38 cells	Moringin induced p53-mediated cell cycle arrest by upregulating p21 and Bax, as the increased transcription of Bax leads to permeabilization of the mitochondrial outer membrane and the activation of caspases 9 and 3, which trigger the intrinsic apoptotic cascade and induce apoptosis by preventing the nuclear translocation of NFκB	Cirmi et al., 2019
Aqueous leaf extract (0.1-2.0 mg/mL)	72 h	Panc-1, p34, and COL0357	Inhibits the NFκB signalling cascade by blocking the translocation of p65 and IκB α , leading to apoptosis and cell death.	Berkovich et al., 2013
Soluble leaf extract (0-400 µg/mL)	24-48 h	A549 cells	<i>M. oleifera</i> reduces tumour proliferation and intracellular ROS and induces apoptosis by activating cleaved caspase 3 and JNK and inhibiting NFκB	Jung, 2014
n-hexane fraction of seed (0.25-4.0 mg/mL)	24 h	MCF7 and MDA-MB-231 and normal cell line: MCF 10A	Seed extract upregulates nucleophosmin and hnRNPF levels, thereby inducing apoptosis and cell cycle arrest in cancer cells	Adebayo et al., 2019
Moringin (8-24 µM)	24 h	CCF-STTG1 cells (human grade IV astrocytoma)	Moringin induces oxidative stress-mediated apoptosis by activating p53 and Bax, inhibiting Bcl-2, and modulating the expression of Nrf2 and CK2 α	Rajan et al., 2016
Methanolic leaf extract (40-640 µg/mL)	24 h	Normal and carcinogenic prostate cell	Induces cell cycle arrest by increasing Bax/Bcl-2 ratio and decreasing the GLI-1 and SMO mRNA expression in treated cells, hence deregulating the hedgehog signalling pathway.	Khan et al., 2020
Ethanollic flower extract (0.07-100 µg/mL)	24-48 h	PC-3 cells	Induces apoptosis via caspase dependent pathway by upregulating the expression of several apoptosis-related proteins and downregulating p-Akt and Bcl-2	Ju et al., 2018
Seed lectin (1.5-16 µM)	24-48 h	B16-F10 cells and GN cells	Increased ROS levels induce apoptosis by the intrinsic pathway of activating caspase-9 as well as cell death by the extrinsic axis via the activation of caspase 8	De Andrade Luz et al., 2017
Methanolic leaf extract (40-640 µg/mL)	24 h	DU145	Leaf extract downregulates Bcl-2 and upregulates Bax expression, inhibits tumour growth and induces apoptosis by downregulating Notch-1 and Hes-1	Khan et al., 2020
Aqueous leaf extract (0.1-10 mg/mL)	24 h	SNO cells	<i>M. oleifera</i> proapoptotic effect - increased caspases 9 and -3/7 activities and decreased ATP - results in the induction of cell death in SNO cells via the intrinsic pathway	Tiloke et al., 2016
Ethanollic fruit extract (50-100 µg/mL)	24-72 h	A2058 and HaCaT cells	Fruit extract induces apoptosis via the mitochondrial signaling pathway by increasing the production of ROS and Bax and decreasing the expression of Bcl-2, which results in the disruption of the mitochondrial membrane followed by activation of cleaved caspases -3 and -9	Goun et al., 2017
Ethyl acetate leaf extract (25-75 µg/mL)	24-48 h	A375 cells	Cell death mechanism involves the activation of caspases -3 and -9 (caspase-dependent pathway) and activation and translocation of apoptosis-inducing factor (AIF) into the nucleus (caspase-independent pathway).	Do et al., 2021
Aqueous leaf extract (1-500 µg/mL)	24 h	HepG ₂ and PBMC and noncancerous human kidney cells: Hek293	Increased levels of ROS resulted in significant oxidative DNA damage in treated cells, as indicated by increased DNA fragmentation and γH2AX levels. Increased Smac/DIABLO and caspase-9 activity activates caspase-3, which leads to increased cleaved PARP1, which induces apoptosis through the intrinsic pathway	Tiloke et al., 2019

Fortification of yoghurt with *M. oleifera*

Why is yoghurt fortification important?

Food fortification involves the addition of essential nutrients using staple foods as delivery vehicles to a target population especially where nutrients are minute or insufficient (Liyana and Hettiarachchi, 2011). Notwithstanding the fortification purpose, both vehicle and fortifier must be compatible with the chemical/food matrix and avail nutritionally to achieve healthy food (Oyeyinka and Oyeyinka, 2018). Moreover, fortificants should not influence the taste, texture, colour, or flavour of the overall food product. Indeed, several factors can influence sensory, nutritional, and physicochemical properties of yoghurt, for example, the amount of fat in milk, the type of milk used (buffalo, goat, sheep, etc.), the production technique, additives and functional ingredients, and the starter culture used (Buttriss, 1997). Given the relatively short shelf life compared to other dairy products, such as cheese, the high water activity and rich nutritional composition make yoghurt vulnerable to spoilage microorganisms (Santos et al., 2018). Yoghurt contains free amino acids and bioactive peptides, which arise from the proteolytic activity of lactic acid bacteria during fermentation. Some lactobacilli can create bacteriocins and hydrogen peroxide, which is made proactive by certain bacteria cultures believed to synthesize and boost the presence of vitamin B in yoghurt (LeBlanc et al., 2015). Yoghurt stands out for its nutritional components, alongside prophylactics and therapeutic benefits, despite its slightly low pH that reduces pathogenic infection and gastric juice secretion (O'Connell and Fox, 2001). However, yoghurt remains a low source of phenolic compounds despite its therapeutic effects on casein, whey proteins, and traces of various antioxidant compounds (Niero et al., 2017; Dubrovskii et al., 2019).

Recently, there has emerged an increasing interest to develop unique-flavoured yoghurts, which might be seen as an ideal carrier of nutrients/functional ingredients in the human diet. Besides, the choice of adding different mineral salts (including organic and inorganic salts) to yoghurt, which adds to the complexities of food matrices, remains challenging. For instance, such salt addition tends to change yoghurt's mouthfeel and pH. However, the popularity of functional yoghurts prepared with plant-derived components or additives such as fruits, vegetables, seeds, or even plant extract have risen (Roy et al., 2015; Alenisan et al., 2017; Hamed et al., 2020; Ahmad et al., 2022). Functional foods would possess various phytochemicals that facilitate their bioactivity, which helps to mitigate certain disease conditions/risks. Indeed, the addition of either fruits, herbs or plant extracts to yoghurt remains crucial, either as adaptogens (natural substances that increase the body's resistance to stress) or as functional ingredients with additional functions other than supplying nutrients (Dubrovskii et al., 2019).

Functional impact of *M. oleifera* in yoghurt

M. oleifera contains several classes of phytochemicals besides minerals and carotenoids that can support the growth of lactic acid bacteria and exert antimicrobial and antioxidant scavenging activities. Antioxidants play an essential role in safeguarding against oxidative stress and maintaining a balance between the generation of active oxygen species and the quantity of endogenous antioxidants. Male albino rats exposed to lead acetate-induced oxidative stress received oral doses of green tea and *M. oleifera* yoghurt (88.2 mg/kg/day) for five weeks. The results showed a significant reduction in liver weight and levels of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as a superior lowering effect on plasma total cholesterol, triglycerides, and low-density lipoprotein upon consumption of *M. oleifera* yoghurt (El-Ziney et al., 2017). Other groups of authors further elucidated that *M. oleifera*-fortified yoghurt had increased radical-scavenging activity of up to 40 % in a dose-dependent manner during three weeks of cold storage, as well as an increase in the expression of antioxidant proteins in human colon cells (Zhang et al., 2019). Such results might be attributed to the formation and degradation of mineral and phenolic compounds based on the interactions between lactic acid bacteria and compounds present in *M. oleifera*.

Furthermore, a study on the influence of probiotic yoghurt supplemented with *M. oleifera* leaf powder (4.3 g) as a source of micronutrients in the gut, oral, breast milk, and vaginal microbiotas of pregnant women (n = 56) in Tanzania revealed that *M. oleifera*-probiotic yoghurt provided a safe and inexpensive food for pregnant women without adversely changing the gut and oral microbiota, as well as improving the gut microbiota of new-borns (Bisanz et al., 2015). A previous study on the potential use of *M. oleifera* probiotic yoghurt for heavy metal exposure in vulnerable populations, including pregnant women (n = 60) and children (6–10, n = 44), found that probiotic yoghurt consumption had a protective effect against increases in mercury (3.2 nmol/litre; P = 0.035) and arsenic (2.3 nmol/litre; P = 0.01) levels in pregnant women's blood and no significant changes in children's blood (Bisanz et al., 2014).

Chemical composition of *M. oleifera*-fortified yoghurt versus non-fortified yoghurt at different concentrations or extracts is shown in Table 3. Hassan et al. (2016) claimed that after adding 0.5 %, 1 %, 1.5 %, and 2 % *M. oleifera* leaf powder to yoghurt made from buffalo milk, the optimal supplementation ratio was 0.5 %. Compared to plain yoghurt, the authors observed that adding *M. oleifera* to yoghurt increased total solids, fat, total protein, and amino acids, especially alanine, leucine, tyrosine, and glutamic acid. The increase in the amino acid content of fortified yoghurt may be related to the numerous nutrients in the *M. oleifera* plant, as well as the fact that during fermentation and storage, due to the action of lactobacilli, proteolysis increases, leading to the formation of bioactive peptides that enhance the activity of streptococci. On the contrary, Akajiaku et al. (2018) reported that adding different proportions of *M. oleifera* leaf powder

to yoghurt had no significant changes on total solids and ash, while significantly increasing the percentage of total protein (Table 3).

Bikheet and co-authors also observed that the addition of ethanol and water extracts of *M. oleifera* (1, 3, and 5 %) to yoghurt enhanced its vitamin C and mineral contents (Fe, Ca, K, and P), total solids, total flavonoids, total phenols, total proteins, and antioxidant capacity after production and during storage (Bikheet et al., 2021). This result is further corroborated by a different study that found that adding *M. oleifera* to yoghurt increased the antioxidant scavenging activity, total protein, dietary fibre, volatile fatty acids, and acetaldehyde composition of the fortified yoghurt either fresh or during storage (Al-Ahwal et al., 2017). In addition, yoghurt supplementation by *M. oleifera* has been reported to improve its textural and chemical properties (El-Gammal et al., 2017). Elsewhere, a study on low-fat yoghurt reported that the addition of *M. oleifera* powder resulted in an increase in the water holding capacity (WHC) with a significant decrease ($p < 0.05$) in the syneresis when compared to the control sample during the first week of storage. The increase in water holding capacity was postulated to be due to the interaction between the particles of the *M. oleifera* powder and the casein matrix in the yoghurt. However, the study showed a significant increase ($p < 0.05$) in syneresis during the second week of storage (Adepoju and Selezneva, 2020). Cardines et al. (2018) prepared yoghurt supplemented with different *M. oleifera* seed extracts at concentrations of 0.5 and 1.5 % (v/v). Seed extract enhanced the acidification of yoghurt and led to higher consistency indices than the control yoghurt. The fortified yoghurts were characterized by higher protein content and significantly lower syneresis, which depicted the compacted networks of the three-dimensional network of aggregated casein micelles (Figure 2). Elsewhere, the addition of permeated (5, 10, and 15 mL/L) and concentrated (1, 2, and 3 mL/L) seed extracts of *M. oleifera* to yoghurt revealed that concentrated additives presented viscoelastic behaviour, improved protein and fat compared to plain yoghurt, and significant pH decrease with storage. By adding 2 mL/L, the produced yoghurt had increased firmness, as confirmed by compact/homogenous microstructure and reduced susceptibility to syneresis (Quintanilha et al., 2021).

The antimicrobial effects of 1 and 2 % ethanolic extracts of pomegranate peels and *M. oleifera* leaves on the viability of *E. coli* in yoghurt were investigated. It was found that 2 % ethanolic extracts of pomegranate peels and *M. oleifera* produced peak inhibition against *E. coli* during the storage period (AM et al., 2019). Fortification of yoghurt with 0.05, 1.0, and 2 % Moringa extract improved the growth of *Streptococcus thermophilus*, *Bifidobacterium longum*, and *Lactobacillus acidophilus* during fermentation, decreased syneresis by up to 21 %, increased WHC by 17 %, and increased viscosity (Zhang et al., 2019). Similarly, *M. oleifera* would support the growth of probiotic cultures of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus acidophilus* (Hekmat et al., 2015). Besides, the polyphenols present in *M. oleifera* leaf prevented spoilage by decreasing the pH of yoghurt and inhibiting the microbial (fermentative) spoilage caused by yeasts and moulds (Georgakouli et al., 2016). To further support the above discourse, a summary of the biochemical and functional changes in yoghurt is presented in Table 4.

Advantages of *M. oleifera* in comparison to other natural antioxidants

Considering the capacity of *M. oleifera* to fortify yoghurt, understanding the advantages associated with it is important. For example, a clinical study on malnourished individuals found that consuming 10 g of powdered *M. oleifera* leaves per day for six months resulted in a quicker recovery rate (36 ± 16.54 days) and an increase in average weight gain of 8.9 ± 4.3 g kg⁻¹ per day with no significant increase ($p > 0.05$) in haemoglobin level. Thus, it is recommended as an effective alternative for addressing malnutrition in children (Zongo et al., 2013). Another study on the effect of using *M. oleifera* leaves powder (25 g/ day) to improve anaemia in children with iron deficiency anaemia aged 6-24 months for six months revealed a significant decrease in anaemia prevalence in the intervention group by 53.6 % and an increase in mean haemoglobin (10.9 g/dL) versus the control group (13.6 %, Hb 9.4 g/dL) (Shija et al., 2019).

Moreover, several authors have used *M. oleifera* as a fortifier in yoghurt production, with the primary purpose of improving

Table 3. Chemical composition of *M. oleifera*-fortified yoghurt versus non-fortified yoghurt at different concentrations or extracts

Chemical components	(Hassan et al., 2016)		(Akajiaku et al., 2018)		(Al-Ahwal et al., 2017)	
	Plain yoghurt	<i>M. oleifera</i> yoghurt (0.5 %)	Plain yoghurt	<i>M. oleifera</i> yoghurt (2 %)	Plain yoghurt	<i>M. oleifera</i> extract yoghurt (10 %)
Total protein (%)	6.91	7.10	30.68±2.44	56.80±0.71	3.92±0.11	3.98±0.11
Ash (%)	N/R	N/R	0.40±0.00	0.50±0.42	0.83±0.02	0.90±0.03
Fat content (%)	6.00	6.10	1.50±0.71	0.60±0.71	6.6±0.06	6.6±0.06
Total solids (%)	18.46	18.73	21.0±1.41	20.0±2.26	15.60±0.15	15.86±0.10
Dietary fibre (%)	N/R	N/R	N/R	N/R	0.42±0.04	0.62±0.04
TVFA	9.76	11.90	N/R	N/R	0.37±0.06	0.42±0.07
Acetaldehyde	6.60	9.65	N/R	N/R	162.4±13.2	166.33±12.31

N/R means not reported; TVFA means total volatile fatty acids (0.1N NaOH 10 g⁻¹ of Yoghurt); Acetaldehyde (mole 100 g⁻¹ of yoghurt).

the organoleptic, nutritional, and functional aspects. Most beneficial features of such fortification include increased antioxidant properties, amino acid content, increased textural qualities of the fortified yoghurt, increased total viability count of both lactic acid bacteria and probiotic strain *Lactobacillus rhamnosus*, and increased nutritional quality of the fortified yoghurt. Similar to *M. oleifera*, several fruit pulps, cereals, vegetables, and extracts have been introduced to yoghurt

to improve its functional and nutritional qualities, serving as a gelling, water-binding, and thickening agent (Vénica et al., 2020; Nandakumar et al., 2021; Illupapalayam et al., 2014; Barakat and Hassan, 2017). A study on the sensory and chemical properties of yoghurt fortified with pumpkin fiber (0.5, 1.0, and 1.5 %) revealed increased viscosity and lowered syneresis, but protein decreased with an increase in fibre concentration (Bakirci et al., 2017). Another study

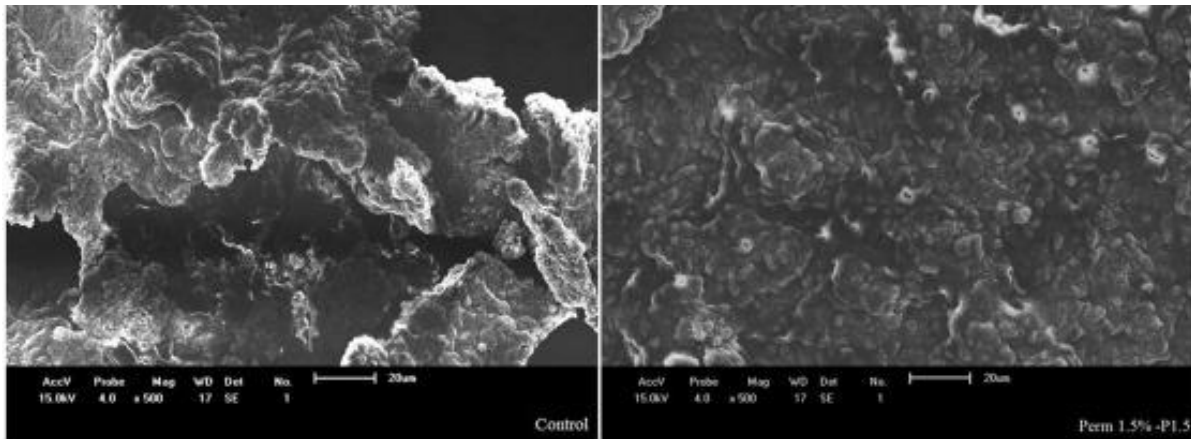


Figure 2. SEM of plain yoghurt (left) with permeated seed extract fortified yoghurt (right) (Source: Cardines et al., 2018)

Table 4. Preparation of functional yoghurt with *M. oleifera* and its properties

Functional Ingredient	Dairy source	Concentration	Outcomes	References
Leaf powder	Liquid whole milk	0, 0.5, 1, 1.5, and 2 % with mango flavour	Leaf powder increased total solids, protein, and acidity and decreased syneresis and sensory scores during storage. 1% fortified yoghurt scored highest for appearance, aroma, taste, body, and overall acceptability	Saeed et al., 2021
Leaf extract	Yoghurt mix	0 and 0.9 %	The leaf extract increased total phenolic content (18.31 mg/GAE) and DPPH radical scavenging capacity (78 % inhibition), increased syneresis, reduced viscosity and firmness on storage, and lowered the acceptance rate for flavor, smell, and overall acceptability	Shokery et al., 2017
Leaf flour	UHT bovine milk	0, 3, 5, and 7 %	Increased vitamin C content in fortified yoghurt (21.99 and 30.04 mg)	Aznury et al., 2020
Leaf extract	Cow milk (3.2 % milk fat)	0, 1, 3, and 4 %	<i>M. oleifera</i> reduced fermentation time, increased mineral elements, total phenolic content, and antioxidant capacity, decreased syneresis and increased water holding capacity on storage, and improved sensory scores compared to control	Lisak Jakopović et al., 2022
Leaf extract	Raw bovine milk	0, 2, 4, and 6 %	Leaf extract improves cohesiveness, decreases firmness and consistency, increases viable counts of <i>S. thermophilus</i> , and inhibits <i>L. bulgaricus</i> , and improves utilization of iron and manganese by starter cultures during yoghurt fermentation.	Nduti et al., 2018
Root powder	Soy milk, whole cow milk	0 and 0.1 %	Root powder increased copper, manganese, and iron, decreased zinc, calcium, magnesium, potassium, and sodium, lowered pH and titratable acidity, increased ash, crude protein, fiber, and fats, and decreased carbohydrates.	Ponka et al., 2022
Leaf extract	Fresh buffaloes' milk	0, 0.2, 0.4, and 0.8 %	decreased pH and increased protein, total solids, and fat during storage, inhibited the growth of yeast and mold and decreased the viable count of lactobacilli and streptococci during storage.	Saad and Elkhtab, 2019
Seed powder	Full cream powdered milk	0, 0.5, 1, 1.5, 2 and 2.5 %	The addition of seed powder increased shelf stability by inhibiting yeast and mold growth both at refrigerator and ambient temperatures, and the total aerobic count decreased with the advanced storage period for all fortified samples as compared to plain yoghurt.	Obasi et al., 2019
Leaf extract	Fresh cow milk	0, 0.5, 1, 1.5, 2, and 2.5 %	<i>M. oleifera</i> extract increases consistency and firmness and reduces pH and fermentation time. The yoghurt with the highest extract concentration had the maximum probiotic viability.	Rupa and Vijay, 2022

on the fortification of yoghurt with fenugreek and *M. oleifera* seed flour (0.1 and 0.2 %, respectively) reported the latter with higher total phenolic content, antioxidant capacity, antibacterial activity, and mineral content (Ca, P, K, and Fe). In contrast, fenugreek seed flour yoghurt obtained significantly higher microbial viability count than plain yoghurt and *M. oleifera* yoghurt (Dhawi et al., 2020). Shokery et al. (2017) prepared yoghurt supplemented with green tea extract and *M. oleifera* leaves extract at 1 and 0.9 %. The workers found increased syneresis with *M. oleifera*, while green tea decreased it. However, both bio yoghurts were characterized by higher contents of phenolic acids and improved antioxidant activities than plain yoghurt, which may offer range of yoghurt products with additional health benefits to consumers. Thus, compared to other widely used plant antioxidants, *M. oleifera* can improve the properties of yoghurt and yield extra nutritious products.

Yoghurt production process using *M. oleifera*

A block diagram of yoghurt production with *M. oleifera* is shown in Figure 3. It can be argued that streptococci possess higher aerotolerance compared to lactobacilli, which, given the well-established growth ratio of 4:1, would suggest a recognized synergistic interaction, especially when fermentation kicks off. Further, *Lactobacillus* avails the needed peptides that enhance the growth of streptococci because of its greater and more significant proteolytic activity. More so, the initial fermentation taken over by the cocci is believed to depress the redox potential, producing formate (methanoate), CO₂, and pyruvic acid as by-products. Methanoate would, therefore, encourage the growth of lactobacillus given by decreased oxygen, which in tandem accelerates the whole fermentation process. Post-fermentation, the yoghurt's temperature is rapidly dropped to about 4-5 °C via chiller/heat exchanger to stop the cultures' fermentative activity, inhibit enzyme activity, and initiate the cold gelatinization of the curd.

M. oleifera can also be added after fermentation, followed by cooling, before serving (Kuikman and O'Connor, 2015). When probiotic yoghurt was refrigerated at 4 °C, the addition of *M. oleifera* had growth-promoting effects on *Lactobacillus rhamnosus* GR-1, a probiotic bacterium (Van Tienen et al., 2011). Moreover, *Lactobacillus rhamnosus* GR-1's growth may be boosted by *M. oleifera*, but potentially only at a greater concentration (*M. oleifera* at 1 %) and when sugar is present (Hekmat et al., 2015). Compared to the control, the supplementation of yoghurt with *M. oleifera* extract would reduce the fermentation period in a dose-dependent manner (Zhang et al., 2019). Besides, increased fermentative activity of bacterial starter cultures may corroborate the flavonoid, phenolic, and organic acid contents of *M. oleifera*-enriched yoghurt (Rodríguez-Pérez et al., 2016). To achieve health benefits, bacterial colony formation must exceed 10⁶ CFU mL⁻¹, which makes the

potential growth-promoting effects of *M. oleifera* crucial to extending the probiotic yoghurt's shelf-life. Combined with other adjuncts, *M. oleifera* leaf powder would improve yoghurt's nutritional/sensory properties (Kechagia et al., 2013). The effects of adding different adjuncts (banana, avocado, and sweet potatoes) showed improved taste and overall acceptability of *M. oleifera*-enriched yoghurt (Kuikman and O'Connor, 2015).

Despite specific standardized thresholds, such as milk fat content, the overall quality of yoghurt varies globally. Among the very common yoghurt categories, especially those based on fat level, are skimmed, low-fat, and high-fat yoghurt. Yoghurt styling and nutritional qualities are greatly influenced by additives such as sweetening and flavouring agents, preservatives, and functional ingredients. *M. oleifera* and its extracts have served as functional ingredients that enrich yoghurts (Bikheet et al., 2021) and have an added value during yoghurt production. Raw *M. oleifera* leaves can be washed, dried in an oven, and ground to make powder or extract. The extract can then be boiled or macerated in water. The milk base is heated in a homogenizer at 65–70 °C and 15–20 MPa pressure to reduce the fat globule diameter and improve the mixing of the extract with milk casein (Adepoju and Selezneva, 2020). Moreover, the milk base is pasteurized to destroy pathogens, denature whey protein, inactivate milk enzymes, reduce redox potential, and eliminate inhibitory substances at 85 °C for 20–30 min or 90–95 °C for 5 min, chilled to 40–45 °C, which is ideal for lactic acid bacteria growth. 2–4 % of the bulk starter or the amount specified on the commercial starter culture for direct vat inoculation or direct vat set at 40–43 °C are used to inoculate the milk base (Nagaoka, 2019). Yoghurt fermentation starts with the action of bacterial cultures, which could either be a mixed culture comprising of *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) or other bacteria mixtures, on pasteurized milk. By acting on complex macromolecules in milk, such as proteins, fats, and carbohydrates, *S. thermophilus* and *L. bulgaricus* carry out three key metabolic processes, namely glycolysis, proteolysis, and lipolysis, converting them into simpler and readily absorbable nutrients (Buttriss, 1997). In addition, the fermentation process is carried out in an aseptic vat or containers at the optimum temperature for lactic acid bacteria (40–43 °C) until the pH drops to 4.5–4.7, followed by rapid cooling to stop any further decrease in pH.

Toxicology and safety

There is a need for additional synthesis of relevant information on the safety and toxicity of *M. oleifera* in human studies, especially in relation to effective dose intake. Nevertheless, there are an eclectic number of studies on the therapeutic effects of *M. oleifera* in different preclinical models, and animal studies, when properly conducted, provide useful indicators of safety in humans. Oral administration of *M. oleifera* leaf extract at supra-supplementation levels of

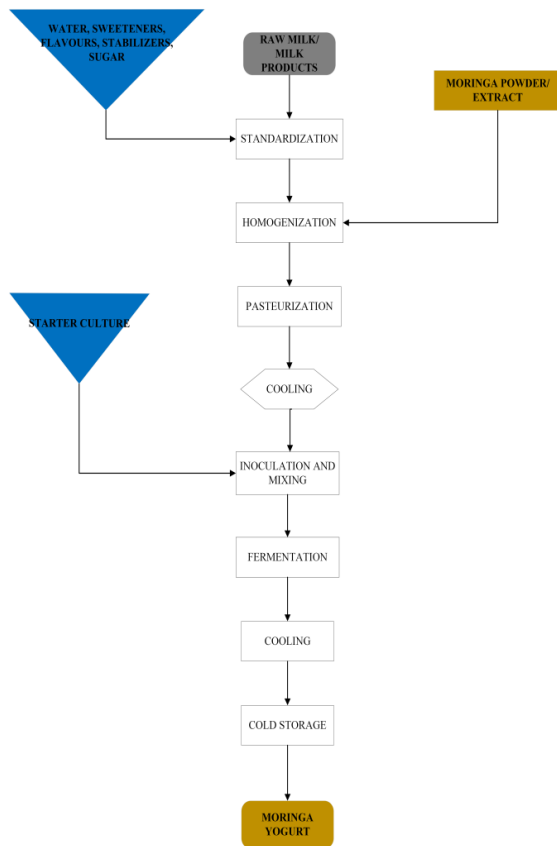


Figure 3. Block diagram of yoghurt production with *M. oleifera*

3000 mg kg⁻¹ to rats did not result in any hepatorenal toxicity or haematological alterations after acute exposure. However, the authors claimed that *M. oleifera* is genotoxic at a dose of 3000 mg kg⁻¹ (Asare et al., 2012). Another study on the acute (5000 mg kg⁻¹) and subacute toxicity (40-1000 mg kg⁻¹) of *M. oleifera* extract in rats revealed an increase in liver enzymes ALT and ALP ($p < 0.001$) with no adverse histopathological changes, but the consumption of the leaves should not exceed a maximum of 70 g per day to avoid accumulative toxicity of essential elements over extended periods (Asiedu-Gyekye et al., 2014).

A double-blinded, randomized, placebo-controlled trial evaluating the efficacy of feeding *M. oleifera* leaf capsules to early postpartum patients to increase breast milk volume revealed there was no significant difference in breast milk volume on the third day of postpartum between the intervention and the control group (73.5 vs. 50 mL, $p = 0.19$). However, the amount of breast milk in the intervention group was 47 % higher than that in the control group (Fungtammasan and Phupong, 2022). Another randomized crossover study examining the effects of *M. oleifera* leaf extract (500 mg dry extract) on plasma glucose concentrations and antioxidant status in healthy volunteers showed that

M. oleifera significantly improved antioxidant capacity in humans, without causing hypoglycemia (Ngamukote et al., 2016). Studies on the potential toxicity of *M. oleifera* are currently lacking, and some information in the literature is debatable. Hence, it is necessary to conduct studies on the toxicokinetic, acute, sub-chronic, and chronic toxicity, as well as studies on the allergenicity, immunotoxicity, and neurotoxicity of *M. oleifera*.

Limitations of incorporating *M. oleifera* to yoghurt and future prospects

Although several studies have successfully created one form of *M. oleifera*-enriched yoghurt or another (refer to Table 4), striking a balance on how the bioactive ingredients of *M. oleifera* would be incorporated per serving of yoghurt remains debatable. The main challenge is related to the observed sensory properties when *M. oleifera* extract/leaves are incorporated into yoghurt especially at high concentrations (Trigo et al., 2022). As achieving the recommended dietary allowance of this bioactive ingredient in functional product such as yoghurt without producing an off-taste, not only proves challenging, the rich polyphenolic content of *M. oleifera* may equally be associated with the astringent taste that occurs in milk products (O'Connell and Fox, 2001). Fortification of yoghurt with *M. oleifera* extract, even at a high concentration of 2.5 % w/v yoghurt, showed no adverse effect on lactic acid bacteria; however, the sensory properties of the final product were poorly accepted (Rupa and Vijay, 2022). Potential solutions might include the initial blanching of the leaves before drying, encapsulation of the extracts, as well as introduction of fruit flavours and fruits into yoghurt.

Higher concentrations of *M. oleifera*, which could increase syneresis and reduce firmness/viscosity of fortified yoghurt, could be a minor challenge during storage (Shokery et al., 2017). Achieving a balance between fortification levels using this bioactive ingredient is required, especially within the dietary recommended limits to ensure the sensory property of the final *M. oleifera*-fortified yoghurt becomes acceptable. Thus, the optimum fortification level of *M. oleifera* in yoghurt needs further investigation, with increased focus on sensory, physico-chemical, and antioxidant properties. Furthermore, more chronic toxicity studies using *M. oleifera* on hepatic, renal, hematopoietic, cardiac, and reproductive changes are warranted. This is because there are currently limited human studies on the toxicity levels of *M. oleifera*, especially considering that yoghurt can be appealing to different consumers of different age groups and for different benefits.

Importantly, the *M. oleifera* plant continues to be a rich source of micronutrients, macronutrients, and bioactive compounds that facilitates the growth of lactic acid bacteria with promising effects on antioxidant and rheological properties of yoghurt. Moreover, the low phenolic content of yoghurt not only makes it a potential food vehicle, but also, a target for food fortification with plant ingredients. Owing to

its nutritional, antioxidant, and antimicrobial properties, *M. oleifera* can be positioned as a functional ingredient in dairy technology to help alleviate the situations of malnutrition in low-income countries. Technological challenges such as syneresis and sensory properties involving a *M. oleifera* fortified yoghurt, however, still needs to be addressed.

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Obogaćivanje jogurta biljkom *Moringa oleifera*: prehrambeni i proizvodni aspekti

Sažetak

Jogurt je hranjivi mliječni prehrambeni proizvod proizveden pomoću bakterija mliječne kiseline koji pospješuje probavu i apsorpciju hranjivih tvari, te održava crijevnu floru. Međutim, riječ je o namirnici niskog sadržaja fenola. Budući da se jogurt smatra visokovrijednim prehrambenim proizvodom koji ljudski organizam učinkovito opskrbljuje hranjivim tvarima, razvoj jedinstvenih jogurta obogaćenih sastojcima biljnog podrijetla postaje sve popularniji. *Moringa oleifera* važna je fitoterapeutska biljka poznata po svojim antioksidativnim svojstvima, s obzirom na prisutnost širokog spektra fenolnih spojeva. Obogaćivanje jogurta dodatkom *M. oleifera* stoga bi poboljšalo mineralni i fenolni profil te pospješilo rast probiotičkih bakterija. Ovaj rad daje pregled prehrambenih i proizvodnih mogućnosti biljke *M. oleifera* za obogaćivanje jogurta. Terapeutska svojstva biljke *M. oleifera* ukazuju na visoki potencijal njezine primjene u obogaćivanju jogurta u svrhu poboljšanja nutritivnih i funkcionalnih svojstava. S obzirom da proizvodnja jogurta zahtijeva pridržavanje specifičnih standardiziranih pragova u pogledu okusa, bogat sadržaj polifenola *M. oleifera* koji daju oporost, zahtijeva provedbu istraživanja kojima bi se pronašle mogućnosti za prevladavanje ovog problema.

Ključne riječi: *Moringa oleifera*; antioksidansi; jogurt; obogaćivanje; funkcionalna hrana

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