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Research Article
Enhancing the Nutritive Values of Ice Milk Based on Dry Leaves and Oil of *Moringa oleifera*

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Abstract

Objective: *Moringa oleifera*, described as an important nutritional supplement with a variety of pharmaceutical and functional properties. Therefore, the main purposed of this study was to improve the nutritional value and sensory quality of fortified ice milk by blending with *Moringa oleifera* dry leaves (MODL) and *Moringa oleifera* oil (MOO). **Methodology:** The MODL was combined into ice milk at three different concentrations (0.5, 1 and 1.5%) substituted with the milk solids not fat (MSNF) 12%, while ice milk base mix by MOO was prepared to contain 4, 6 and 8% oil as source of fat. **Results:** Addition of MODL increased the specific gravity, pH, total solid, overrun, fiber, minerals and vitamins (B1, B2, B6) content of ice milk. Also, adding MOO increased the total solid, oleic acid and vitamin E (α-tocopherol) content of ice milk. Furthermore, viscosity, melting resistance, cytotoxic activity and antioxidant activity increased in fortified ice milk with the addition of MOO and MODL. All these studied parameters improved with raising ratios of added MOO and MODL. Sensory evaluation revealed that the addition of MODL at ratio of 0.5% was higher than other treatments fortified with MODL. Moreover, the total score with comparable acceptability of ice milk with 4 and 8% MOO was lower than 6%. **Conclusion:** For the emergence of fortified foods by this nutraceuticales, it was recommended that MODL can be used at 0.5% addition and MOO can be used at 6% addition to formulate fortified ice milk to increased health benefits and suitable sensory aspects.

Key words: *Moringa oleifera*, ice milk, minerals, vitamins, antioxidant, sensory evaluation, cytotoxic activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa is extensively known and used for its health benefits. Among commoners, it has earned its name as 'the miracle tree' due to its amazing therapeutic abilities for various ailments and even some chronic diseases[1]. Through various studies, the *Moringa* was found to contain many necessary nutrients for example, vitamins, minerals, amino acids, beta carotene, antioxidants, anti-inflammatory, omega 3 and 6 fatty acids[2-4]. *Moringa oleifera* also consists of anti-microbial, anti-hypertensive, anti-inflammatory, anti-diabetic, anti-tumor and anti-cancer activities[5]. Almost every part of the tree is of value for food. *Moringa oleifera* is regarded as one of the most significant and useful because it is both a medicinal and functional food.

Moringa oil is useful externally for skin diseases[6]. Moreover, it has been used as general cooking and salad oil[7]. *Moringa oleifera* seed oil is agreeable taste, highly safe to eat[8] and resembles olive oil in its fatty acid composition[9]. Also, seed oil contains all the major fatty acids found in olive oil and for that reason can be used as a possible replace with the costly olive oil after some modifications. The *M. oleifera* oil has a elevated content of oleic acid (>70%) with saturated fatty acids comprising most of the residual fatty acid profile high oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying[10].

On the other hand, *Moringa oleifera* leaf powder is an specially nutritious vegetable tree with a range of possible uses; it is used as a micronutrient powder to treat diseases[11]. *Moringa* leaves can be eaten fresh, cooked or stored as dried powder for many months without refrigeration and reportedly without loss of nutritional value[12]. Also, *Moringa* leaves have a few specific benefits that contain extra vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and the protein value of *Moringa* leaves rivals that of milk and eggs. Also, the leaves and stem of *Moringa oleifera* are known to have large amounts of their calcium bound in calcium oxalate crystals[13,14]. Along with these specific nutrients, the leaves can be consumed to stimulate metabolism, thus aiding in weight loss because *Moringa* provides energy without sugar[15]. The leaves have high amounts of anti-inflammatory components and contain anti-toxin and anti-oxidant materials. In addition, the leaves of *Moringa oleifera* rich with total phenols, proteins, calcium, potassium, iron, magnesium, manganese and copper also good sources of phytonutrients such as tocopherols and ascorbic acid[16,17]. *Moringa* could be an extremely valuable food resource, it can be used in stimulating and healthy sauces, juices, spices, milk, bread and most importantly, instant noodles[18,19]. Fortification of dairy products is now achieved using numerous beneficial compounds derived from natural products, which development the quality and nutritive value of these products. Utilize *Moringa oleifera* oil enhanced the oxidative stability of vegetable oils and butter oil[20,21]. Also, *Moringa oleifera* leaves and oil improved the quality and extended the shelf life of sour cream without negative result on sensory, acceptability, flavor, body, texture and appearance[22].

The addition of MOO and MODL into the ice milk was planned to raise the nutritive value of the product, specifically increasing the fiber, antioxidant, oleic acid content to the ice milk. Moreover ice milk is a delicious nutritious frozen dairy product, which is usually consumed for all sections of the people and thus, offering more health benefits to the customers.

The objective of this study was to characterize and evaluate functional properties of MOO and MODL, using ice milk as a product, which serves as a vehicle for transport this nutrient and large health benefits. Also, estimate the sensory acceptability and quality of the ice milk to determine the acceptable level of addition of MOO and MODL in ice milk. In addition, evaluate anticancer effect of produced ice milk using cell line.

MATERIALS AND METHODS

Materials: Fresh buffalo's milk was obtained from the Animal Production Research Institute, Ministry of Agriculture. Skim milk powder (medium heated, fat 1.25%, moisture 4%) Kasyiat, Sp. 700, poland was obtained from local market. Commercial grade granulated cane sugar produced by Sugar and Integrated Industries Co., at Hawamdia was obtained from the local market. Carboxy methylcellulose (CMC) BDH chemicals Ltd., Poole, England. Vanillin (Boehringer Manheim GMB, Germany) was obtained from the local market. The MOO and MODL were obtained from National Research Center herb plant unit.

Methods

Preparation of ice milk with oil and dry leaves of *Moringa oleifera*: Ice milk base mixes was prepared by MOO and MODL as follow: By MODL ice milk base mix was prepared to contain 8% milk fat, 12% milk solids not fat (MSNF), 16% sugar and 0.25% CMC as stabilizer. The MSNF 12% was substituted with MODL at levels 0.5, 1.0 and 1.5%. While ice milk base mix by MOO was prepared to contain 4, 6 and 8% MOO, 12% milk solids not fat (MSNF), 16% sugar and 0.25% CMC as stabilizer. The manufacturing procedure was carried out as described by Marshall and Arbuckle[23] and Schmidt[24]. All mixes were heat treated at 80±1°C for 30 sec, then cooled to 4°C and suitable amount of vanilla was added, aged in the fridge at 4°C for 20 h, frozen and whipped in the ice milk maker (Model: BL1380) for 30 min. The ice milk was collected at an exit temperature of -5.5°C. The resultant product was filled into polystyrene cups (cap. 100 mL) covered and hardened in a deep freezer at -20°C for 24 h before analysis. Three replicates were done for every treatment.

Chemical analysis: The pH values of ice milk samples were measured using laboratory pH meter model (HANNA, Instrument, Portogal). Ash and total solid content were determined according to AOAC[25].

Physical analysis

Specific gravity: The specific gravity of the mix and the final ice milk was determined according to Winton[26].

Overrun and melting properties of oil and dry leaves of *Moringa oleifera* ice milk: Three batches of each type of MOO and MODL of ice milk were evaluated for overrun and melting assessment[24,27]. The overrun was calculated using the equation:

$$\text{Overrun (\%)} = \frac{\text{Vol. of ice milk-Vol. of mix used}}{\text{Vol. of mix used}} \times 100$$

Meltdown of frozen ice milk was determined according to Arndt and Wehling[28], by carefully cutting the foamed plastic cups from the ice milk samples (~50 g), placing the samples onto wire mesh over a glass funnel fitted on conical flask and measure the amount of ice milk drained into the conical flask at 30°C every 15 min until the entire sample had melted.

Apparent viscosity of oil and dry leaves of *Moringa oleifera* ice milk (cP sec): Ice milk mix samples were gently stirred 5 times in clockwise direction with a plastic spoon prior to viscosity measurements. Apparent viscosity was measured at 25°C using a Brookfield digital viscometer (Middleboro, MA 02346, USA). The sample was subjected to shear rates ranging from 3-100 S-0 for upward curve. Viscosity measurements were expressed as centipoises (cP sec) and were performed in triplicate.

Determination of crude fiber of dry leaves *Moringa oleifera* ice milk: Crude fiber determinations were performed using the method of AOAC[29]. Ice milk sample (3 g) with MODL was mixed with sulfuric acid [(200 mL, 1.25%, (w/v)]. The mixture was boiled under reflux condenser for 30 min and filtered through a gooch crucible provided with asbestos mat, then thoroughly washed with hot distilled water. The residue was boiled with aqueous sodium hydroxide solution [200 mL, 1.25%, (w/v)] under reflux condenser for 30 min and then filtered through a gooch crucible as described above. The residue was washed with distilled water and then dried at 100°C to constant weight. The ash was determined and subtracted from the dry weight of treated material to give the fiber content.

Sensory evaluation: Sensory evaluation of different treatment of ice milk was carried out according to Arbuckle[27] for flavor (50 points), body and texture (40 points), melting property (5.0 points) and appearance (5.0 points). The scoring panel consisted of the 12 staff member of Dairy Department, National Research Centre.

Determination of vitamins

Preparation of vitamin standards: A stock standard solution (100 μg mL^{-1}) of thiamin, riboflavin and pyridoxine were prepared with MQ water and a standard solution (100 μg mL^{-1}) of vitamin (E) α-tocopherol was prepared with acetonitrile. All standards were stored at 4°C before use.

Determination of fat-soluble vitamin concentration (vitamin E) by HPLC: Vitamin E (α-tocopherol) was determined by HPLC analysis after an extraction from different ice milksamples as described by Escriva *et al*.[30]. The HPLC analysis was performed with an Agilent 1260 HPLC system (Agilent Technologies, USA), equipped with a quaternary pump, auto sampler injector with 20 μL fixed loop injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was ODS H optimal (150×4.6 mm, 5 μm film thicknesses). The column was kept at room temperature. Aliquot (20 μmL) of the standard or a sample was injected and then eluted with the mobile phase of acetonitrile/methylene chloride/methanol (70:20:10, v/v/v) at an isocratic flow rate of 1 mL min^{-1} with a total runtime of 15 min. Detection wavelength for detection of α-tocopherol was set at 250 nm. The retention time of α-tocopherol was about 9.644 min. The limit of detection was found to be 0.02 mg kg^{-1}. Vitamin E (α-tocopherol) content was further expressed in mg/100 g of fresh weight.

Determination of water-soluble vitamin

Vitamin thiamin (B1), riboflavin (B2) and pyridoxine (B6): Vitamins B1, B2 and B6 were determined by HPLC analysis after extraction from different ice milk samples according to Albala-Hurtado *et al.*[31]. The HPLC analysis was performed with an agilent 1260 HPLC system (Agilent Technologies, USA), equipped with a quaternary pump, auto sampler injector with 20 μL fixed loop injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was C18 Zorbax XDB (250×4.6 mm, 5 μm film thicknesses). The column was kept at room temperature at a flow rate of 0.8 mL min^{-1} with a total runtime of 12 min. Separation of vitamins was carried out by gradient elution with methanol (A) and 1% TFA containing water (B). The elute composition was initially 8% A+92% B, held for 2 min and changed linearly to 92% A+8% B in the next 4 min and held for 6 min. Detection wavelength for detection of thiamin, riboflavin and pyridoxine was set at 254 nm.

Determination of minerals: Mineral contents were determined by atomic absorption 1100B, Rerken Elemer as described by Sa'adatu and Mshelia[32].

Gas-liquid chromatographic analysis of fatty acids methyl esters: The fatty acid composition of the MOO sample used in this study was identified and measured using gas liquid chromatography on a Hewlett Packard Model 6890 with a flame ionization detector using capillary column 30.0 m×530×1.0 μm. The carrier gas used was nitrogen set at a flow rate of 15 mL min^{-1} and split-ratio of 8:1. Esterification of fatty acid for methyl ester preparation was carried out accordingly to Luddy *et al.*[33].

Determination of peroxide value and acidity for *Moringa oleifera* oil: Peroxide value and acidity for MOO were determined according to AOAC[34] and IUPAC[35].

Determination of antioxidants

Total phenolic compounds: The extract solution prepared by diluting 2 g from ice milk with methanol overnight filtered and then completed to 50 mL with methanol. Aliquot of the extract (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) containing sodium carbonate (75 g L^{-1}, 4 mL). The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (9.4×10^{-3} to 1.5×10^{-1} mg mL^{-1}) and the results were expressed as mg of Gallic Acid Equivalents (GAE) g^{-1} of extract[36].

Antioxidant activity: The free radical scavenging activity (FRS) of the different ice milk samples was measured using DPPH (2,2-Diphenyl-1-picrylhydrazy). Estimation of FRS activity: 2, 2-Diphenyl-l-picrylhydrazy (DPPH) radical scavenging activity of each ice milk methanolic extract was estimated according to Elmastas *et al.*[37]. The 2, 2-Diphenyl-l-picrylhydrazy (DPPH) 0.1 mM was prepared in absolute methanol. One milliliters of 0.1 mM solution of DPPH was added to 3 mL of each ice milk methanolic extract at concentration 300 μL mL^{-1}. The mixture was shaken vigorously and allowed to stand at room temperature in darkness for 30 min. The absorbance (A) was measured spectrophotometrically at 517 nm. Butylated Hydroxyl Toluene (BHT) was used as positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical analysis: All measurements were done in triplicates and analyzed according to Statistical Analysis System (SAS)[38].

RESULTS AND DISCUSSION

Table 1 refers to properties of ice milk containing different ratios of MODL and MOO. Specific gravity for ice milk and mixes presented in Table 1, specific gravity significantly decreased with increased amount of MODL and MOO added to different treatments. Overrun significantly increased from 67.96-72.53 with increase MODL from 0.5-1.5%, while overrun significantly decreased from 60.64, 56.57 and 54.34 with increase MOO from 4, 6 and 8%, respectively. The highest overrun recorded at ice milk treated with 1.5% MODL and 4% MOO. Also, pH values of ice milk mixes indicated in Table 1. The pH in mixes prepared with MODL showed that significant increase in pH values with the increase MODL amount (0.5, 1.0 and 1.5%). The pH values slightly significantly decreased in ice milk mixes made with MOO to record the significant decreased at treatment with added 8% MOO. Moisture and total solids also presented in Table 1. Moisture significantly decreased by increase of MODL and oil. The highest moisture value was at ice milk mixture prepared with 4% MOO but the lowest moisture was at ice milk mixture prepared with 4% MOO. The high moisture content reflects the lowest total solids content. The lowest total solids were at treatment 4% MOO while the highest total solids noticed at ice milk mixture

Table 1: Physical and chemical properties of ice milk containing different ratios of dry leaves and oil of *Moringa oleifera*

	Treatments					
	MODL (%)			MOO (%)		
Properties	0.5	1.0	1.5	4	6	8
Specific gravity for mixes	1.03^{D}	1.01^{E}	0.99^{F}	1.13^{A}	1.11^{B}	1.09^{C}
Specific gravity for ice cream	0.94^{D}	0.88^{E}	0.88^{F}	1.04^{A}	0.99^{B}	0.96^{C}
Overrun	67.96^{C}	70.76^{B}	72.53^{A}	60.64^{D}	56.57^{E}	54.34^{F}
pH	6.54^{C}	6.57^{B}	6.61^{A}	6.48^{D}	6.44^{E}	6.40^{F}
Moisture	60.11^{C}	60.33^{B}	58.53^{D}	66.99^{A}	55.11^{E}	52.82^{F}
Total solid	39.89^{D}	39.67^{E}	41.48^{C}	33.01^{F}	44.89^{B}	47.18^{A}
Ash	0.87^{F}	0.90^{E}	0.99^{D}	1.26^{A}	1.23^{B}	1.18^{C}
Fiber	0.28^{C}	0.55^{B}	0.83^{A}	-	-	-

Data expressed as mean of 3 replicates, means in the same letters are not significantly different ($p \leq 0.05$)

prepared with 8% MOO. Arbuckle[27] reported that the manufacture of ice milk with variation and quality of milk solids not fat source have a significant effect on the physical and chemical properties of ice milk mixes and the resultant products. Fiber and ash contents also presented at Table 1, increase of add MODL from 0.5-1.5% significantly increased the fiber and ash contents to recorded highest present 0.83 and 0.99%, respectively at 1.5% MODL. High ash in ice milk content MODL is generally linked to high mineral content of samples[39].

Fatty acids content of MOO showed at Fig. 1. The main fatty acid in *Moringa Oliefera* was oleic acid (81.75%) as noticed in Fig. 1. Oleic acid the highest fatty acids content showed in MOO. Abdulkarim *et al.*[40] informed that the oleic acid content inclined to be higher (up to 78.5%) compared to plants grown in the tropics. This support previous studies which recommended that MOO is collected of highly unsaturated fatty acids covering 80.4% polyunsaturates mainly oleic acids (67.9%)[41]. Palmitic acid, stearic acid, arachiddic acid and palmito oleic acid found in MOO at concentration of 7.07, 6.62, 2.76 and 1.81%, respectively.

Analysis of *Moringa oliefera* oil: The DPPH scavenging activity (mM trolox equivalent kg^{-1}) of MOO was 101.2 mM kg^{-1}. Acidity (%) of MOO was found 0.91% and peroxid value also was 19.5 meq kg^{-1}. This high value due to cold pressed of MOO fat soluble vitamins in MOO δ-tocopherol, γ-tocopherol and α-tocopherol was 129, 461 and 595 µg/100 g, respectively.

Apparent viscosity of ice milk (cP sec) ice milk mixture samples made with different ratios of dry leaves and oil of *Moringa oleifera*: Apparent viscosity described as the amount of force requisite to shift one layer of fluid in relation to another in the ice milk mix. Viscosity values (cP) are plotted as a function of time (sec) in Fig. 2, to show the flow behavior of ice milk samples with different amount from MODL and MOO. The viscosity behavior is influenced by the complex hydrodynamic properties (i.e., size, shape and hydration potential). The value of apparent viscosity in the treatment with MOO (4, 6 and 8%) ice milk was found to be significantly higher than the treatment with MODL. Also, the viscosity of ice milk significantly increased with the increased of oil% added to the ice milk mix treatments; these data are in agreement with that reported by El-Sayed *et al.*[42] who substituted milk fat with sesame oil in the manufacture of functional milk beverage fortified with kiwi pulp (20.0% w/w) and sesame oil (0.5, 1.5 and 3%). The increase in viscosity of the ice milk made with MOO may be attributed to changes to protein conformation and changes in acidity[43]. The addition of *Moringa oliefera* in the ice milk can create some changes in the ice milk matrix and interaction between protein and fatty acids resulting in more open texture and by reducing the interfacial tension which reduces the viscosity[44]. The increase of viscosity in the samples made with MODL; this may be due to the fiber contents in MODL that increased with amount added from 0.5-1.5%, which is responsible for gel forming viscous as well as particle size and high water holding capacity of fibers[45], also this agreement with Salama *et al.*[46]. The results revealed that, adding of different amount from MODL and MOO significantly increases the viscosity with increasing the addition. Use of MOO and MODL in this study significantly affects in apparent viscosity of different ice milk mixes.

Melting properties of ice milk samples made with different ratios of *Moringa oleifera* dry leaves and oil: The data presented in Table 2 refers to melting resistance (Loss percentage after) of ice milk samples made with different ratios of MODL and MOO. Melting resistance of ice milk was expressed as the loss in weight percent of the initial weight of the tested samples. The melting resistance ice milk samples made with different ratios of MODL and MOO significantly increased with increasing the time of the test. The melting resistance of ice milk samples made with different ratios of

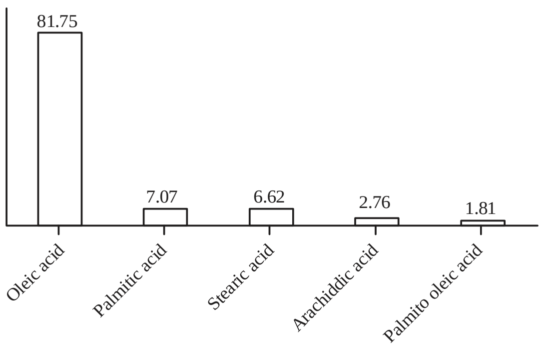

Fig. 1: Fatty acid content of *Moringa oleifera* oil

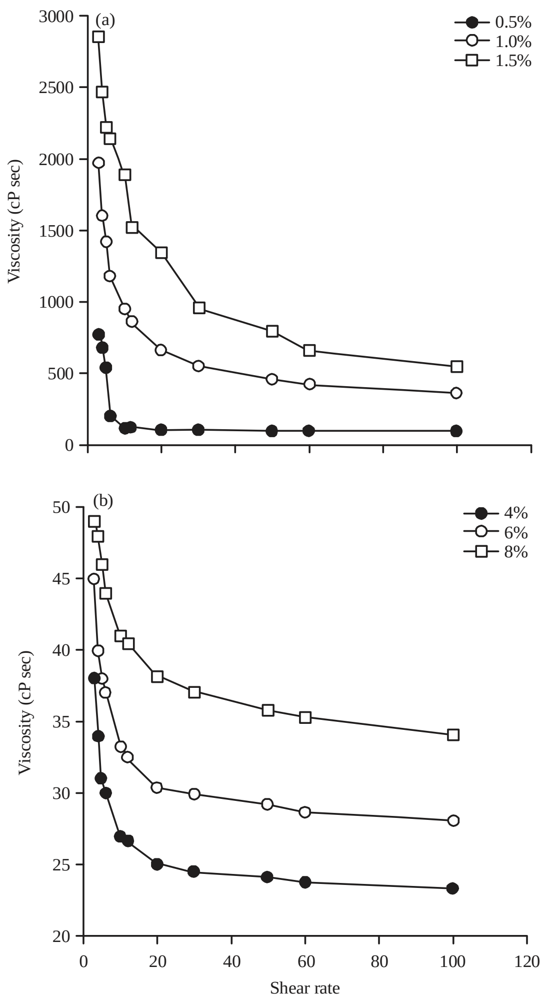

Fig. 2(a-b): Viscosity of ice milk mixture samples made with different ratios of dry leaves and oil of *Moringa oleifera*

MOO ice milk significantly different compared with MODL ice milk treatment, Akalin *et al.*[47] exposed that there is a opposite relative between fat content and hardness as observed in this study. Furthermore, unsaturated fats are unable to create a product with less body and less creamy[48]. Addition of MODL and MOO significantly affect in melting resistance of ice milk as showed in Table 2.

Table 2: Melting resistance (Loss percentage after) of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*

	Treatments					
	MODL (%)			MOO (%)		
Time (min)	0.5	1	1.5	4	6	8
15	9^{Da}	5^{Db}	5^{Db}	0.75^{Dc}	0.6^{Dc}	0.3^{Dc}
30	26^{Ca}	21^{Cb}	17^{Cc}	14^{Cd}	12^{Ce}	8^{Cf}
45	45^{Ba}	42^{Bb}	40^{Bc}	32^{Bd}	31^{Bde}	30^{Be}
60	60^{Aa}	52^{Ac}	50^{Ad}	56^{Ab}	55^{Ab}	52^{Ac}

Data expressed as mean of 3 replicates, means in the same column showing the same capital letters are not significantly different ($p \leq 0.05$), means in the same raw showing the same small letters are not significantly different ($p \leq 0.05$)

Mineral and vitamins content in ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*: From Table 3, observed that adding MODL to ice milk was accompanied by high levels of P, K, Ca, Mg, Fe, Zn and Na owing to the high content of these elements in MODL. Therefore, ice milk fortified with 1.5% MODL can be considered a good source of minerals. According to previous studies, *Moringa oleifera* have a great source of highly edible proteins, Ca, Fe and carotenoids[2].

Absence of Fe can result in anaemia and in defining the nutritional potential of *M. oleifera*, Oduro *et al.*[49] found Fe level of 282.9 mg kg^{-1} in the leaf. Iron content of ice milk1.5% MODL increased from 0.18 to 0.46 mg/100 g while P, K, Ca, Mg, Na and Zn increased from 69.3-82.2, 97.7 to 120.9, 86.33-104.4, 14.0-21.9, 36.9-48.0 and 0.30-0.39 mg/100 g, respectively compared with ice milk 0.5% MODL. Owusu-Ansah *et al.*[50] mentioned that dried leaf powder of *Moringa oleifera* contain 368 mg of Mg, 1324 mg of K, 204 mg of P and 28.2 mg of Fe. In other side, observed that minerals content of ice milk fortified with MOO declined by increasing the addition level of MOO. Iron content of ice milk 4% MODL decreased from 0.11-0.03 mg/100 g while P, K, Ca, Mg, Na and Zn decreased from 38.9-33.9, 91.1-80.4, 82.5-66.8, 8.5-8.1, 37.8-28 and 0.31-0.17 mg/100 g, respectively compared with ice milk 8% MOO. Vitamins content in fortified ice milk improved by increasing the addition level of MODL Table 3.

Vitamins B1, B2, B6 and E increased from 0.026-0.041, 0.513-0.772, 0.032-0.039 and 0.126-0.129 mg/100 g, respectively. The increase in vitamins content was owing to the presence of these vitamins at the MODL in high ratios. This agreement with Johnson and Pharm[51] described that

Table 3: Mineral and vitamins content in ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*

	Minerals and vitamins (mg/100 g)										
Treatments (%)	P	K	Ca	Mg	Na	Fe	Zn	E	B1	B2	B6
MODL 0.5	69.3^{C}	97.7^{C}	86.3^{C}	14.0^{C}	36.9^{C}	0.18^{C}	0.30^{BC}	0.126^{C}	0.026^{C}	0.513^{C}	0.032^{C}
MODL 1.0	73.2^{B}	114.3^{B}	95.4^{B}	18.7^{B}	41.3^{B}	0.29^{B}	0.34^{AB}	0.128^{C}	0.031^{BC}	0.674^{B}	0.037^{B}
MODL 1.5	82.2^{A}	120.9^{A}	104.4^{A}	21.9^{A}	48.0^{A}	0.46^{A}	0.39^{A}	0.129^{C}	0.041^{B}	0.772^{A}	0.039^{A}
MOO 4	38.9^{D}	91.1^{D}	82.5^{D}	8.5^{D}	37.8^{C}	0.11^{D}	0.31^{BC}	0.258^{B}	0.24^{A}	0.367^{D}	0.027^{D}
MOO 6	35.9^{E}	85.6^{E}	76.3^{E}	8.3^{E}	31^{D}	0.09^{E}	0.25^{C}	0.266^{B}	0.24^{A}	0.320^{F}	0.023^{E}
MOO 8	33.9^{F}	80.4^{F}	66.8^{F}	8.1^{F}	28^{E}	0.03^{F}	0.17^{D}	0.381^{A}	0.23^{A}	0.333^{E}	0.019F

Data expressed as mean of 3 replicates, means in the same letters are not significantly different ($p \leq 0.05$)

Table 4: Antioxidant activity (%) and total phenol content (mg g^{-1}) of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*

Treatments (%)	Total phenol (mg g^{-1})	Antioxidant activity (%)
MODL 0.5	139.07	39.16
MODL 1.0	199.35	63.44
MODL 1.5	251.65	81.77
MOO 4	11.54	9.76
MOO 6	12.12	11.55
MOO 8	12.28	12.66

Moringa oleifera contains fat-soluble vitamins 16.3 mg of vitamin A and 113 mg of vitamin E. On other hand, there is a slight decrease on vitamins B1, B2, B6 by increasing the addition level of MOO in ice milk but there is a significant increase on vitamin E in the same treatments.

Antioxidant activity (%) and total phenol content (mg g^{-1}) of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*: Antioxidant activity determined by DPPH (%) and total phenol (mg g^{-1}) of ice milk made by different ratios of MODL and MOO demonstrated in Table 4. Total phenols (mg g^{-1}) increased with increaseing the ratios added from MODL and MOO to ice milk samples. Total phenol was highest in ice milk samples made with MODL; the highest phenol value noticed with 1.5% MODL to record 251 mg g^{-1}, while 12.28 mg g^{-1} with 8% MOO. Antioxidant activity also increased with increase the amount added from MODL and MOO and was higher in ice milk samples with MODL more than MOO.

These results agreements with Salem *et al.*[52] whom reported that antioxidant activity increased by increasing MODL level of addition, so this variation could be attributed to the high antioxidant activity in MODL. The high antioxidant activity of MODL may be to the presence major amount of phytoconstituents and potential therapeutic[53,54]. Furthermore, MOO rich in natural antioxidants such as oleic acid[55] and contains antiradical molecules like tocopherols and phenolics.

Sensory properties of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*: Table 5 showed that sensory evaluation of ice milk samples made with different ratios of MODL and MOO by expert members. Data presented that the addition of MODL and MOO significantly decrease the overall acceptability with the increase of addition from MODL and MOO, while the significantly highest acceptability noticed at ice milk treated with 6% MOO (91.44). In the samples made with MODL, the best flavor found with ice milk with 0.5% MODL (42.75) while the best body and texture and melting properties was noticed at treatment with 1.5% MODL may be this for the high fiber content in the sample with 1.5% MODL. In the samples made with MOO. The best flavor and body and texture were with sample made by 6% MOO, while the good melting properties found at sample with 8% MOO as shown in Table 2. The high appearance was at samples made with lowest addition from dry leaves and oil (0.5 and 4%). This results agreement with Apilado *et al.*[56] found that use of malunggay powder lower than 0.5% should be recommended in the production of cream cheese.

Cytotoxic activity of ice milk samples containing different ratios of MODL and MOO: Cytotoxic activities of ice milk samples containing different ratios of MODL and MOO in three human cancer cells colon, breast and hepatocellular carcinoma cell line are shown in Table 6. The results revealed that treatments contain MOO displayed a slim effect on colon and hepatocellular carcinoma human cancer cells this effect increase by raising ratios of MOO with values 3.5, 7.9 and 11.8% to colon cell line in addition, 9.2, 17.1 and 22.6% to hepatocellular carcinoma cell line, respectively. Singh *et al.*[57] reported that nature of saturated and unsaturated fatty acids contained in MOO attributed to cytotoxic effect toward cancer cells. This anticancer effect may due to the action of fatty acids which growth the process of free radical generation and lipid peroxidation in cancer cells. Also, treatments contain MODL showed a good effect on colon cancer cells this influence increase by increasing percentages of MODL with values 16.9, 26.2 and 36.4%, respectively. On the other hand, the treatment containing 1.5% MODL have a minor effect on breast and

Table 5: Sensory evaluation of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*

	Treatments of different concentration of dry leaves and oil of *Moringa oleifera*					
	MODL (%)			MOO (%)		
Properties	0.5	1.0	1.5	4	6	8
Flavour (50)	42.75^{C}	40.33^{D}	36.33^{E}	45^{B}	47^{A}	43^{C}
Body and texture (40)	35.33^{D}	37.67^{B}	39.89^{A}	33.35^{F}	36.45^{C}	35.12^{E}
Melting properties (5)	4.13^{C}	4.22^{B}	4.65^{A}	3.55^{F}	3.79^{E}	4.00^{D}
Appearance (5)	3.80^{C}	3.20^{D}	3.00^{E}	4.50^{A}	4.20^{B}	3.90^{C}
Total score (100)	86.33^{B}	85.42^{D}	83.87^{E}	82.85^{F}	91.44^{A}	86.02^{C}

Data expressed as mean of 3 replicates, means in the same letters are not significantly different ($p \leq 0.05$)

Table 6: Cytotoxic activity (%) test of ice milk samples made with different ratios of dry leaves and oil of *Moringa oleifera*

	Treatments of different concentration dry leaves and oil of *Moringa oleifera*					
	MODL (%)			MOO (%)		
Cell line type	0.5	1.0	1.5	4	6	8
Colon	16.9	26.2	36.4	3.5	7.9	11.8
Breast	0.00	0.00	7.50	0.00	0.00	2
Hepatocellular carcinoma	0.00	0.00	15.4	9.2	17.1	22.6

hepatocellular carcinoma cell line and there is no effect in low concentration. Loo[58] illustrated that phenolic phytochemicals can prepare of high oxidative stress only in specific cancer cells also; flavonoids block the enzymes which produce estrogen decrease of estrogen-induced cancers. This means that good influence improved with high presented concentrations of MODL and MOO in this product. The effect on cancer cells tested in this study consider low compared with the high effect of MOO and MODL on cancer cell while the increase amount of MODL and MOO more than the ratios used in this study will be more effective.

CONCLUSION

The addition of MODL and MOO into the ice milk was envisioned to increase the nutritive value of the product also, develops new ice milk and stimulating flavors of acceptable ice milk and suitable taste to wide variety of consumers. In addition, increase the antioxidant, vitamins, minerals and fiber content of the ice milk. Thus, offering more health benefits to the consumers and may protect from different type of cancer.

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