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DEVELOPMENT OF INNOVATIVE BEVERAGE BASED ON MILK PERMEATE FORTIFIED WITH DRIED LEAVES OF *Moringa oleifera* [1]

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ABSTRACT

This study was carried out to produce milk permeate beverage fortified with dried leaves of *Moringa oleifera* (DLMO) as innovative beverage. DLMO was added to permeate at concentrations of 0.5, 1 or 2%. Subsequently, the physiochemical, microbiological and organoleeptic properties of freshly innovative beverage and after 3, 7 and 10 days of storage at 5 ±25C were examined. Addition of DLMO had significantly increased the total solids, protein, carbohydrate and ash contents of beverage. Acidity values increased gradually in all treatments during storage period. Bifidobacteria counts were higher in innovative beverages fortified with DLMO compared to control during storage period (10 days). Mould&yeast and coliform bacteria were not detected in innovative beverage when fresh and till the end of storage. Innovative beverage fortified with DLMO can be considered a good source of minerals (K, Ca, Mg and Fe) for human nutrition. The results indicated that innovative beverage fortified with DLMO contained higher essential and non essential amino acids compared to control. Organoleptic properties of innovative beverage fortified with DLMO were highly acceptable during storage period.

INTRODUCTION

Food fortification is gaining momentum in recent years since it helps to maintain the quality of food keeping nutrient levels adequate to correct or prevent specific nutritional deficiencies especially in elderly, vegetarians, malnutrition and pregnant women. Micronutrient-fortified foods including beverages are becoming increasingly popular in many countries. Unfortunately, there is no single food that contains all of the micronutrients. Utilization of milk permeate and dry leaves of *Moringa oleifera* may consider as a best approach towards fighting micronutrients deficiencies in a cheap and sustainable manner.

Milk permeate (MP) can be used as value added fortifying ingredients in other food including nutritional beverage and infant formula. Although still viewed as a waste products of process, milk permeate may hold the key to future product innovation. The composition of permeate approximates electrolyte beverage being sold as sports drinks (**Geilman *et al* 1992 and Williom, 2001).** This beverage is fall into functional nutraceutical beverage category (**Williom, 2001).** Milk permeate beverages are known for their genuine thirst quenching refreshing nature with some health benefits. These beverages can also offer good potential profit margins **(Suresha and Jayaprakasha, 2003 and Beuncler *et al* 2005)**. Functional beverage can be successfully made using MP fortified with fruits as natural sources of antioxidants as well as improving the nutritive value. These beverages are necessary for health protection, so they are introduced for patient in hospital or gents in clinically touristy venue **(EL-Sayed *et al* 2007).** Herb beverage blend with milk permeate are one of the promising trends of wide and diversified potentialities **(Abdel–Salam *et al* 2004).**

Moringa oleifera (family: Moringaceae) known as a natural gift has an impressive range of medicinal uses with high nutritional value. *Moringa oleifera* is the most nutrient rich plant yet discovered. It provides a rich and rare combination of nutrients, amino acids, antioxidants, anti aging and anti-inflammatory properties used for nutrition and healing. Moringa is sometimes called mother's best friend and miracle tree. Since 1998, the world health organization has promoted Moringa as an alternative to imported food supplied to treat malnutrition (**Johnson and Pharm, 2005; Sreelatha and Padma, 2009 and Mahmoud *et al* 2010).**

Moringa oleifera is a miracle tree with a great indigenous source of highly digestible proteins, calcium, Iron and vitamin C. It contains all the essential nutritional elements that are essential for livestock and human beings **Ashfaq *et al* (2012)**. Morringa fresh leaves contain seven times the vit-

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amin C of oranges, four times the vitamin A of carrots, four times the calcium of milk, three times the potassium of banana and two times the protein of yoghurt. In dried leaves the micro nutrient content is even more ten times the vitamin A of carrots, 17 times the calcium of milk, 15 times the potassium of bananas, 25 times the iron spinach and 9 times the protein of yoghurt. However vitamin C drops to half that of arranges **(Mahmoud *et al* 2010; Manzoor *et al* 2007 and Monica, 2005).** Leaves can be eaten fresh, cooked, or stored as dried powder for months without refrigeration and reportedly without loss of nutritional value. Leaves can be used for food fortification (**Fuglie, 1999; Lockelt & Calvert, 2000 and Mahmoud *et al* 2010)**. The product, *Moringa oleifera,* will be marketed in the U.S under the name Zija. Many commercial products like Zija soft drink, tea and nutraceuticals are available all over the globe (**Johnson and Pharm, 2005).**

The present study was designed to fortify the milk permeate with different concentration of dry leaves of *Moringa oleifera* to produce new func

tional milk permeate beverage as innovative beverage.

MATERIALS AND METHODS

Materials

Milk permeate was obtained from Animal Production Research Institute Agric. Res. Center. Dokki, Giza. Dry leaves of *Moringa oleiferia* powder was obtained from Breadbasket of Egypt Association. Sugar was obtained from local market. Citric acid was obtained from El- Gomhoria Company. Probiotic bacteria *Bifido. bifidum Bb12* were obtained from Microbiology Lab. at Dairy Department, National Research Center, Dokki, Egypt. The compositions of milk permeate and dry leaves of *Moringa oleiferia* are shown in **Table (1).**

Table 1. Chemical composition (%) of *Moringa oleiferia* dry leaves and milk permeate.

Materials	TS	Fat	Protein	Ash	Carbohydrate (by difference)	pH
Dry leaves of Moringa powder	96.75	2.30	44.20	10.81	38.20	6.08
Milk permeate	5.09	0.00	0.38	0.42	4.29	7.17

Preparation of innovative beverage

1. Preliminary experiments

Preliminary experiments were carried out to select the suitable percentages of sugar (3,5,7 and 10%),citric acid (0.05,0.1,0.15 and 0.2%)and concentration of dry leaves of *Moringa oleiferia* (0.5,1,2,3,4,5%) to be added to milk permeate. The organoleptic properties of the prepared beverages were examined immediately after processing. The results showed that beverages that ranked highest organoleptic were obtained by addition of (0.5, 1and 2%) dry leaves of *Moringa oleiferia*. The optimum percentages of sugar and citric acid were: 7% and 0.1% respectively in all treatments.

2- Experimental procedure

Fresh milk permeate was used to prepare the innovative beverage by adding 7% sugar and 0.1% citric acid, then heated at 705C for 15 sec. and cooled to 405C. The stock was divided into four portions, the first portion was served as control and the other three portions were fortified with o.5, 1, and 2% of dry leaves of *Moringa oleiferia* (DLMO) for T1, T2 and T3 respectively. Each portion was inoculated with 2% *Bifido. bifidum Bb12* and incubated for 4 hr at 40 5C. The prepared beverages were filled into sterilized bottles and stored in refrigerator. The resultant beverages were analyzed when fresh, after 3, 7and 10 days of storage at refrigerator (5±2ºC).

Method of analysis

Moisture, fat, ash and total protein contents were determined according to **AOAC, (2007)**.Total carbohydrates in innovative beverage were calculated by differences as described by **Ceirwyn, (1995).** Titratable acidity of beverages was determined according to **Richardson, (1986)**. The pH values were measured using a digital laboratory pH meter (HI 93 1400, Hanna instruments) with glass electrode. Specific gravity of resultant beverages was determined as described by **Winton (1958)** at 20ºC. Colour index of beverage samples was determined according to the method of **Hendel *et al* (1950).** Mineral contents were analyzed using Atomic absorption 3300 Perkin Elmer USA according to **AOAC, (2007).** Amino acid contents of beverages were determined using a Waters

2690 HPLC system separation module as described by **Cohen *et al* (1989).** Protein efficiency ratio (PER) was estimated according to the following equation proposed by **Alsmeyer *et al* (1974)** equation. **PER=-0.684+0.456 (Leucine) -0.047 (Proline).**

Biological value (BV) was estimated using the equation reported by **Mitchell and Block, (1946). BV=49.9+10.53 PER.**

Microbiological estimation

Bifidobacteria was determined according to the method described by **Dave and Shah, (1996),** whereas, moulds & yeast and coliforms were determined according to **Marshall, (1992).**

Sensory evaluation

Samples of resultant beverage were judged by a panel of 10 judges selected on the basis of their consistency in scoring. The samples were scored for flavour (out of 50 point), appearance (out of 25 point) and colour (out of 25 point) as suggested by **Abel-Salam *et al* (2004).** All data were analyzed by the General Linear Models procedure of **SAS (1990)**. Least significant difference test was performed to determine differences in means at P≤0.05.

RESULTS AND DISCUSSION

Chemical composition of different treatments of innovative beverages fortified with DLMO is presented in **Table (2).** The results indicated that addition of DLMO at all levels have significant effect on TS, protein, carbohydrate and ash contents. The highest value was recorded with innovative beverage fortified with 2% DLMO than that of control. The protein content increased with increasing the level of DLMO in the permeate from 0.49% (control) to 0.98% (T3). Protein content was increased by approximately 100% at 3% addition level. The increase in protein content was due to the presence of which have dry leaves of *Moringa oleifera* higher concentration of protein.

Table 2. Physiochemical analysis of fresh innovative beverages fortified with different levels of dry leaves of *Moringa oleiferia*.

	Treatments*			
Character assessed	**Control**	**T1**	**T2**	**T3**
T S (%)	11.78C	12.39B	12.91AB	13.66A
Protein (%)	0.49C	0.65B	0.91A	0.98A
Fat (%)	0.00C	0.00C	0.10B	0.15A
Ash (%)	0.46C	0.54B	0.63AB	0.70A
Carbohydrate (%)	10.83B	11.20AB	11.27A	11.85A
		Fresh		
Color Index	2.15	3.54	5.35	6.00
		10 days		
	2.17	4.43	5.80	6.75

* Control : beverage without dry leaves of *Moringa oleiferia*
T1, T2, T3 : beverage with 0.5,1,2 % dry leaves of *Moringa oleiferia* respectively**.**
A,B,C : Means with the same letter among treatments are not significantly different

The quality of protein DLMO is better than all vegetable proteins and similar to egg and milk proteins as it contains all the essential amino acids in appreciable amounts **(Juliani *et al* 2008 and Ashfaq *et al* 2012).** Protein incorporation in the beverage is a convenient means of offering consumers nutritional value and other specific benefits and also to food marketers. Many functional beverages for sale today are marketed based on their health and wellness**.** Thus, proteins are believed to be more satiating than carbohydrates or fats, suggesting their use in beverages targeted for weight control and another target for people not consuming animal protein **(Meenatchi, 2012).** Ash, TS and carbohydrate contents were increased by 36.96, 15.66 and 9.42 respectively at 2% level (T3). This increase could be attributed to the high ash, TS, and carbohydrate contents in DLMO.

Colour is one of the most important quality attributes of food. The first impression of the quality and acceptability of a particular food is judged upon its appearance. It is well known that colour index readings are related to the colour intensity. From **Table (2)** it could be noticed that the control

beverage (without Moringa) had lighter colour than that fortified with DLMO either fresh or at the end of storage period (10 days). The results also indicated that, addition of DLMO to milk permeate led to increase the values of colour index (darker colour) of beverages. Moreover the darker colour was increased with increasing the added ratio of DLMO to the beverage. However, the darker colour of the beverage fortified with DLMO could be attributed to the presence of pigments in DLMO. *Moringa oleifera* contains specific plant pigments and unique phytochemicals with demonstrated potent of antioxidant such as carotenoids, isothiocyanates, chlorophyll, phenolic and flavonoid **(Ashfeq *et al* 2012 and Rajanandh & Kavith, 2010).** From the same table it could be also seen that, the values of colour index of all beverages fortified with DLMO were gradually increased during storage period. This increase could be due to the oxidation process of colour during storage which may led to easy release of pigments. These results are in accordance with **Kgatla *et al* (2011)** who found that the colour is affected by natural enzymes, oxidation of ascorbic acid and the Millard reaction, which depends on the content of reducing sugars, proteins, and temperature.

Data in **Fig. (1)** show the changes in total acidity and pH values of different beverage treatments during cold storage. It could be seen that acidity values of beverage of different treatments had gradually increased during storage period. This may be due mainly to the fermentation of carbohydrates present. Generally, the acidity content ranged from 0.4–0.43% in fresh beverage and increased during storage to be 0.45- 0.52%. The addition of DLMO increased acidity compared to control in fresh and during storage period **(Salem *et al* 2013)**. This may due to stimulatory effect of DLMO on starter culture. Moreover, **Mahdian and Tehrani, (2007)** reported that high total solids content improved the growth and activity of starter cultures and increased acidity. Changes in pH values of all treatments were in opposite trend to that of acidity. The pH of the whey proteins beverage increased as more permeate was added to each beverage **(Beucler *et al* 2005)**.

Adding DLMO to innovative beverage was accompanied by high level of Ca, K, Fe and Mg due to the high contents of these elements in DLMO **(Table, 3). Owusn *et al* (2011)** reported that dried leaves powder of *Moringa oleifera* can serve as an excellent source of minerals. The calcium content in innovative beverage fortified with DLMO (2%) was 116.40 and 30.00 mg/100g for innovative beverage and control respectively. Studies indicated that intake of calcium rich food during childhood and adolescence is important determinant of peak bone mass and future risk of osteoporosis **(Kohlmeier, 2003).** Consuming 100g of this innovative beverage provide about 14.60 % of the Recommended Dietary Allowance (RDA). The addition of permeate to diet improves the bioavailability of calcium **(Kansal, 2002 and El sayed *et al* 2007).** The highest value of K was recorded with innovative beverage (180.00mg/100g). Potassium plays a major role in body electricity (heart impulse transmission and muscle contraction) and enzyme activation. Inadequate intake leads to low plasma potassium concentration with an increased risk of heart arrhythmia, stroke and muscle weakness **(Kohlmeier, 2003).** Hence, 100g of innovative beverage provide about 11.60 % of the RDA. The addition of DLMO increase the Fe content of the innovative beverage as permeate is considered poor sources of Fe **(Abd El-Salam, 2000)**.100g of innovative beverage provide a bout 0.09 % of the RDA. Also, the addition of DLMO improved the Mg content of the beverage. 100g of innovative beverage provide a bout 18.40 % of the RDA.

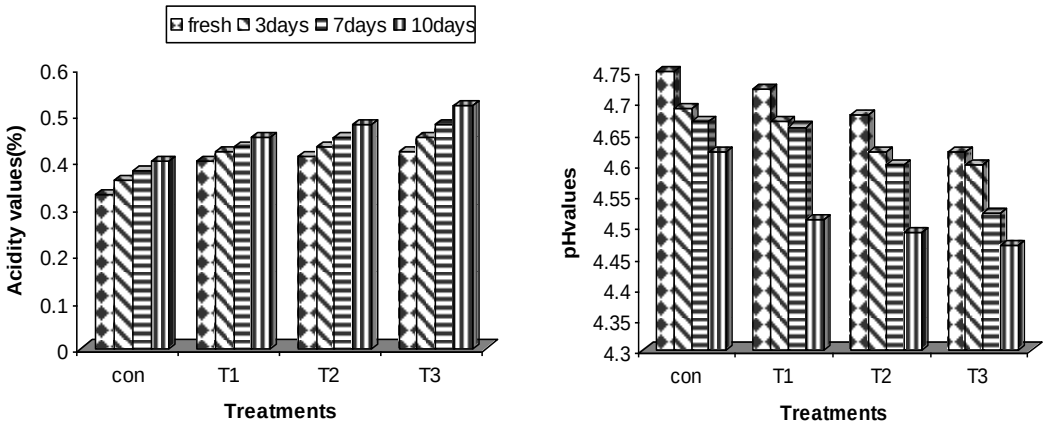

Fig. 1. Acidity (%) and pH values of innovative beverage as affected by using different levels of dry leaves of *Moringa oleiferia* (DLMO) when fresh and during storage at refrigerator (5±2ºC).

Table 3. Mineral contents (mg/100g) of innovative beverage fortified with 2% dry leaves of *Moringa oleifera* (T3) compared to the Recommended Daily Allowances (RDA) %.

Minerals (Mg/100g)	Means RDA*/(mg)	Permeate	% from RDA	Beverage(T3)	% from RDA
Ca	800.0	30.00	3.79	116.40	14.60
K	1550.0	140.00	9.03	180.00	11.60
Fe	10.00	0.005	0.05	0.85	0.09
Mg	200.0	11.00	5.50	36.80	18.40

* RDA recommended by **WHO/FAO 2000**

Amino acids analysis

The amino acids composition of protein plays an important role in determining the biological and nutritive value of protein. As the nutritional value of beverages are reflected by the composition of essential amino acids when compared to the recommendations of United Nations. Amino acids of innovative beverage (T3) expressed as mg/ 100ml protein compared to control are shown in **Table (4 & 5).** The results show that lysine, valin, isolucine, leucine and phenyl alanine are higher in beverages fortified with DLMO compared with control. This may be due to dominating protein content in DLMO is nutritionally high. Data in **Table (4 & 5)** revealed that beverage fortified with DLMO contain higher sulfer amino acids and aromatic amino acid compared with control. This is due to the containing of Moringa leaves all essential amino acid, which are the building units of proteins.

It is very rare for vegetable to contain all of these amino acids. These leaves could be a great boon to people who do not get protein from meat **(Ashfaqu *et al* 2012 and Manzoor *et al* 2007).** Generally, addition of DLMO to the beverages had considerable effect on their contents of essential and non essential amino acids. The highest value was recorded with beverage fortified with DLMO. Beverage fortified with DLMO has highest level of argenine and histidine amino acids that important for infant who are unable to make enough protein for their growth requirements. Thus, Moringa can be utilized in fortifying foods such as sauces, juices, bread and instant noodles. **Johnson and Pharm, (2005)** reported that *Moringa olifera* contain 19 of the 20 prominent protein amino acids. It also contains all of the eight amino acids considered essential.

It is also clear from **Table (4)** that Protein Efficiency Ratio (PER) was higher in innovative beverage fortified with DLMO compared to control, being 20.16 and 4.89 for innovative beverage fortified with DLMO (T3) and control respectively. The highest value of PER of innovative beverage fortified with DLMO may be due to its well balanced amino acid.

The highest Biological Value (BV) for innovative beverage fortified with DLMO may be due to high PER value of innovative beverage. The values were 262.18 and 101.39 for innovative beverage (T3) and control respectively.

Table 4. Essential amino acids of innovative beverage fortified with 2% of dry leaves of Moringa *oleiferia*.

Amino Acid(mg/100ml)	Control	Innovative Beverage(T3)
	Essential amino acids	
Methionine	1.00	7.28
Cystine	2.13	9.92
Total sulfur amino acids	3.13	17.20
Phenylalanin	4.45	30.11
Tyrosine	0.29	8.87
Total aromatic amino acids	4.74	38.98
Lysine	9.45	33.67
Theronine	4.80	20.89
Valine	5.90	30.35
Leucine	10.15	45.75
Isoleucine	5.48	25.40
Tryptophane	---	----
Histidine	2.80	13.97
Total essential amino acids	46.45	226.21
PER	4.89	20.16
BV	101.39	262.18

Table 5. Non essential amino acids of innovative beverage fortified with 2% of dry leaves of Moringa *oleiferia*.

Amino Acid (mg/100ml)	Control	Innovative Beverage(T3)
	Non essential amino acids	
Argnine	2.69	28.89
Aspartic acid	11.59	62.95
Alanine	4.23	30.53
Proline	8.60	29.39
Glutamic acid	29.22	74.18
Glycine	8.82	27.57
Serine	5.48	18.14
Total non essential amino acids	70.63	271.65

Microbiological examination

The changes of viable count of bifidobacteria during storage period are presented in **Table (6).** Data indicated clearly that the viability of bifidobacteria remained higher for 7 days of cold storage and then started to decline. The results also, indicated that the bifidobacteria was higher in innovative beverage fortified with DLMO compared to control. This would suggest that bacterial population were stimulated by adding DLMO which could be due to the high nutritional value of DLMO such as carbohydrate, proteins, minerals and vitamins. Moreover, increases in the level adding DLMO led to increase bifidobacteria count. Concerning the therapeutic benefits the product should contain, at least 106 viable cell of bifidobacteria per gram to realize its therapeutic properties **(Salem and EL-Shibiny, 2003).** On the other hand, **Chandan, (1999)** found that under proper distribution and handling practices, the active cultures probiotic yoghurt at the time of consumanption will be a minimum of 107 cfu/gm. As can be seen **(Table 6)** innovative beverages fortified with dry leaves of *Moringa oleifera* showed this count during storage period (7days). As a result of high hygienic conditions during manufacturing and storage, moulds& yeasts and coliform bacteria were not detected in all treatments when fresh and throughout storage period. These results are in line with those reported by **Salem *et al* (2006) and Hareedy *et al* (2008).**

Sensory evaluation

It is evident from the results of sensory evaluation that addition of different concentrations of DLMO had significant effect on flavour, colour and appearance. As the concentration of DLMO was increased up to 1% (T2), the score of flavour, color, appearance as well as total score for innovative beverage was significantly increased then decreased at T3. The highest scores for flavour were in the innovative beverage T1 and T2 during storage period.

Table 6. Bifidobacteria, Coliform, Moulds& Yeast counts (log cfu/ml) of innovative beverages fortified with different levels of dry leaves of *Moringa oleiferia*(DLMO).

Type of Strain	Storage period (day)	Treatments*			
		Con.	T1	T2	T3
Bifidobacteria	Fresh	7.30	8.60	8.78	8.90
	3	6.95	8.30	8.69	8.78
	7	6.85	7.78	7.90	7.95
	10	5.60	6.30	6.95	6.85
Coliform	Fresh	N.D**	N.D	N.D	N.D
	3	N.D	N.D	N.D	N.D
	7	N.D	N.D	N.D	N.D
	10	N.D	N.D	N.D	N.D
Mould&Yeast	Fresh	N.D	N.D	N.D	N.D
	3	N.D	N.D	N.D	N.D
	7	N.D	N.D	N.D	N.D
	10	N.D	N.D	N.D	N.D

*see Table (2).
**Not detected.

Table 7. Sensory evaluation of innovative beverage fortified with different levels of dry leaves of *Moringa oleiferia* (DLMO).

Sensory attributes		Treatments*			
		Control	**T1**	**T2**	**T3**
			Fresh		
Flavor	**(50)**	46^{ABa}	46^{ABa}	47^{Aa}	44^{Bb}
Color	**(25)**	23^{ABa}	23^{ABa}	24^{Aa}	22^{Bab}
appearance	**(25)**	22^{ABab}	24^{Aa}	24^{Aa}	21^{Bab}
Total score	**(100)**	**91^{BCab}**	**93^{ABa}**	**95^{Aa}**	**87^{Cb}**
			3 days		
Flavor	**(50)**	46^{ABa}	47^{Aa}	47^{Aa}	44^{Bb}
Color	**(25)**	23^{Aa}	23^{Aa}	23^{Aa}	22^{Aab}
appearance	**(25)**	22^{ABab}	23^{Aa}	23^{Aa}	21^{Baba}
Total score	**(100)**	**91^{ABab}**	**93^{Aa}**	**93^{Aa}**	**87^{Bb}**
			7 days		
Flavor	**(50)**	45^{ABab}	46^{Aa}	46^{Aa}	43^{Bbc}
Color	**(25)**	22^{ABab}	22^{ABab}	23^{Aa}	21^{Bab}
appearance	**(25)**	22^{ABab}	23^{Aa}	23^{Aa}	20^{Bb}
Total score	**(100)**	**89^{Ab}**	**91^{Aab}**	**92^{Aab}**	**84^{Bbc}**
			10 days		
Flavor	**(50)**	44^{ABb}	45^{Aab}	45^{Aab}	41^{Bc}
Color	**(25)**	22^{Aab}	21^{ABab}	22^{Aab}	20^{Bb}
appearance	**(25)**	21^{ABab}	22^{Aab}	22^{Aab}	20^{Bb}
Total score	**(100)**	**87^{Ab}**	**88^{Ab}**	**89^{Ab}**	**81^{Bc}**

* see **Table (2).**
A,B,C : Means with the same letter among treatments are not significantly different (P≤0.05)
A,b,c : Means with the same letter during storage period are not significantly different (P≤0.05)

Results in **Table (7)** showed significant differences in score for colour between some treatments in fresh and during storage period. It could be concluded that the addition of high concentration of DLMO tended to make the colour greener. But the low level of DLMO gave acceptable green color **(Salem *et al* 2013).** However, the level of criticism for T3 (high concentration of DLMO) was not adverse as the people already add green apple and Kantalub in the beverage. Results in **Table (7)** also show significant differences in appearance scores among treatments. The highest score for appearance was noted for T2. Score for appearance was significantly decreased as the concentration of DLMO was increased in innovative beverage. Moreover, at higher concentration level of DLMO resulted more fiber in the innovative beverage which settles down at the bottom and the samples were shaken well before sensory evaluation.

In conclusion, a new functional beverage (innovative beverage) can be successfully made using milk permeate fortified with DLMO as source of mineral, protein and essential amino acids which improve nutritive and health value of resultant beverage. These beverages are necessary for health and could be introduced for patient in hospital or quest in clinical touristy venue.

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