Innovation of a new milk drink using natural immune-boosting ingredients

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Abstract

In light of what societies are currently suffering from poor economic conditions and widespread poverty and hunger, strengthening the body's immunity has become a major challenge. This study aimed to prepare a new milk drink using natural immune-boosting ingredients. Five samples of milk drink with different natural immune-boosting additives were prepared and subjected to approximate, quality, microbial, sensory evaluation and energy value calculations. The most important results of this study indicated that there were a significant enhance in the average percentages of carbohydrates, protein and ash in the new milk drink samples compare to fresh milk (10.68 ± 0.021, 7.12 ± 0.012, 0.83 ± 0.00, 14.87 ± 0.001, 3.24% ± 0.006, 0.23±0.007%), respectively. The microbial results indicated that total count of coliform bacteria did not exceed what is stipulated in Libyan Standard Specification No. (355/ 1992). A significant increase in the average total energy value of the new milk drink was indicated compared to fresh milk (101.57 ± 0.829, 62.54 ± 0.138 kcal/100 ml), and panelist prefer the new type of milk drink with a very good general acceptance rate. The study concluded to prepare 5 samples of the new milk drink with immune-boosting ingredients to suit consumers' tastes. This study recommended to introduce the new milk drink with in the healthy diet of the members of society as immune- boosting drink.

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Keywords: milk drink, immune-boosting, functional foods, propolis, energy value.

1. Introduction

A well-functioning immune system is essential to provide a good defense against disease-causing organisms. The immune system works by providing an exclusion barrier, by identifying and eliminating pathogens, and by identifying sources of non-threatening antigens and tolerating them (Philip& Calder, 2013), it also protects against viruses and diseases and produces antibodies to kill pathogens, differentiate between body cells and foreign antigens and builds a defense against this specific antigen **(**Chowdhury *et al*, 2020). The immune system works at all times, but its activity increases in the presence of pathogens, this results in a significant increase in the immune system's demand for nutrients to provide a ready source of energy which can be provided from external sources, the most important of which is a healthy diet. The lack of total energy or one or more of the essential nutrients weakens the function of the immune system and increases susceptibility to infection with infectious pathogens. (Noor *et al*., 2021). Numerous evidences suggest that various nutritional supplements from various herbs and fruits can reduce the risk or severity of viral infections by enhancing the immune response (Read *et al*., 2019). The active compounds in this plant help increase the immune response by stimulating the division of natural killer cells and macrophages and also prevent cell damage due to their antimicrobial properties, antioxidants and anti-inflammatory agents (Gasmi *et al*, 2023). Numerous studies have demonstrated the health benefits of some natural ingredients that can boost the functioning of the immune system, for example, taking 6-120 mg of cinnamon per day for 4-18 weeks led to a significant decrease in fasting glucose, total LDL cholesterol, improves blood sugar control and decrease fasting sugar by 8.4% (Allen *et al*, 2013). The basic mechanism of cinnamon in decreasing fasting glucose is

based on the hypothesis that it has an insulin-like effect which regulating insulin pathways by inhibiting glycogen synthase kinase and promoting glycogen synthesis in the liver (Cao *et al*, 2010, Cheng *et al,* 2012). Honey contains flavonoids, which have antitumor properties (Jaganathan and Mandal, 2009), and shown to be beneficial in reducing the activity of cancer cells in the breast and ovaries (Fauzi *et al.,* 2011). One recent study indicated that the mechanism of honey as anti-tumor refers to its interference in complex cell signaling pathways to encourage the formation of anti-mutagenic and anti-cell death pathways (Waheed *et al,* 2019). It's found that ginger powder helped reduce vomiting, improve the antioxidant capacity, enhance immune function, and reducing inflammatory response (An *et al*., 2019). Ginger extract is an effective dietary source that could enhance the immune function of piglets by improving the antioxidant capacity and the level of immunoglobulin in the sow colostrum (Lee *et al*., 2013). Curcumin helps maintain beta cells in the pancreas, which helps in treating diabetes (Akbar *et al.,* 2018), it's also reduce insulin resistance, blood sugar levels, and reduces inflammatory substances for diabetics (Hajavi, *et al*., 2017)

A balanced diet in general is also important for the immune system. Certain foods can help ensure get the right amount of vitamins, minerals, antioxidants, protein and healthy fats to reduce inflammation and support the immune system include: colorful fruits rich in antioxidants, vitamins and minerals such as those in avocados, fermented foods such as yogurt, and foods rich in fiber like whole grains, herbs such as turmeric and ginger, nuts especially walnuts, dark chocolate, and green tea (Shao *et al*., 2021). There are currently a number of functional foods that have been scientifically validated and contain properties that boost the immune system (Lopez *et al*., 2002) including functional yogurt that contains probiotics and micronutrient supplements that claim immune-boosting properties (Coleman *et al*., 2016). Milk, which contains antibodies that strengthen the immune system, and thus reduces the risk of many diseases (Pehrsson *et al*., 2000), as well as honey, which is a natural antioxidant and anti-inflammatory, and plays an important role in strengthening the immune system and reduce the chances of infection (Ahmed *et al*., 2018). In this context, these products provide a health benefit that exceeds the traditional nutrients that they contain, so they are used to prevent certain diseases (Vayalil, 2012). This study aims to prepare a new milk drink with some natural immune posting ingredients.

2. Methodology and methods

2.1 Purchase of raw materials and preparation of milk drink samples

Milk sample was purchased from a farm in the Bir El Helou/ Aljemail area/ Libya, and the rest of the ingredients like: nuts, avocado, mint, rosemary and honey were purchased from the local markets in the Aljemail area. Five samples of the new milk drink were prepared using different natural ingredient and percentage as showed in table (1). For preparation of samples, fresh full cream cow milk was used, honey, turmeric, cinnamon, cardamom, ginger, black pepper and propolis were the main component of the 4 first samples in addition to avocado fruit in sample (3 and 4) and nuts in sample (1 and 4). Sample (5) consist of milk, honey, turmeric, propolis, mint leaves and rose marry. The ingredients were mixed with the milk and the samples were subjected to pasteurization using HTST technique (High Temperature Short Time), in which, the temperature was raising to 90 ^{0}C for 3 mint and rabidly cool to 4 ^{0}C. The samples were filled while hot in sterile glass bottles and rabidly cooled to 4^{0}C.

2.2 Methods

2.2.1 Chemical analysis

The samples of the new milk drink were subjected to chemical analysis according to (AOAC, 1992). Fat. Protein, lactose density, solids non-fat SNF was determined using Milk analyzer (SAP-CB-05560)/BOECO/ Germany.

Total Soluble Solids (T.S.S) was determined using refractometer, which measures the specific density of liquids and thus the proportion of dissolved solids by measuring the rate of refraction of the substance**.**

Titerable acidity determined using titration (NaOH) 0.1 N and phenolphthalein as indicator, the acidity described as % of lactic acid**.**

pH value determined using multi- parameter handheld instrument (Lovibond/ SensoDirect 150), Tinometer/ Thailand**.**

Moisture content determined using dry oven method (150 ^{0}C/ 6 hrs.).
Total ash was determined using Muffle ferenece (550 ^{0}C/ overnight).
Sugars: the total sugars were determined using D- glucose and D- fructose enzymatic bio analysis method using alpha beta UV visible spectrophotometer (P/N 9423 UVA 1202E) at 340 nm /20 ^{0}C. The method based on spectrophotometric measurement of (NADPH) produced through the reaction, after addition of Hexokinase (HK), Phosphoglucose isomerase (PGI) and Glucose-6-phosphate dehydrogenase (G6PDH).
Minerals: a flame photometer (BWB Technologies)/ England was used with liquified petroleum gas and air as source of flame.Standard curve for every type of mineral was stablished. The concentration of every mineral was determined against the standard curve.

New milk drink samples: percentage of ingredients

		
Sample (1) Milk 88.0%, honey 8.80%, turmeric 0.17%, cinnamon 0.17%, ginger 0.17%, cardamom 0.08%, black paper 0.88%, propolis 0.017% and nuts1.76%.	**Sample (2)** Milk 90.0%, honey 9.00%, turmeric 0.22%, cinnamon 0.22%, ginger 0.18%, cardamom 0.22%, black paper 0.09% and propolis 0.09%.	**Sample (3)** Milk 88.0%, honey 8.80%, turmeric 0.22, cinnamon 0.22%, ginger 0.17%, cardamom 0.22%, black paper 0.08%, propolis 0.08% and avocado 2.20%
		
Sample (4) Milk 86.1%, honey 8.61%, turmeric 0.21%, cinnamon 0.21%, ginger 0.17%, cardamom , black paper 0.08%, propolis 0.08%, nuts 2.15% and avocado 2.15%.	**Sample (5)** Milk 90.0%, honey 9.00%, turmeric 0.22%, mint leaves 0.36% and rose marry 0.36%	**All new milk drink samples and ingredients**

2.2.2 Microbial analysis

The samples were appropriate diluted to 10^3. Total viable count of the new milk drinks samples was determined using a total plate count agar by means of grown colonies after incubation at 30 – 35 °C for approx. 48 hours' colonies will be counted using an electronic colony counter. The bacterial concentration (colony forming units) in the new milk drink sample was then calculated using the following formula (Campbell *et al*. 2015).

$$\text{cfu/ml} = \frac{\text{Average count (number of colonies)}}{\text{(Dilution plated) x (Volume plated)}}$$

The coliform test was done according to Bacteriological Analytical Manual (Hitchins, *et al*, 2001)

2.2.3 Energy value

The energy value of the new milk drink determined using Bomb calorimeter (GDY/1A$^{+)}$/ Chongqing/ China., by measuring the heat produced by completely burnt of a given weight of sample under considerable pressure.

2.2.4 Panel test

A panel of 50 untrained (between 20 to 60 years old of both sexes) evaluated the sensory attributes of the new milk drink samples for color, flavor, taste, texture, and general acceptability. The test based on 5 points hedonic scale, the lowest score (1) for a bad option and (5) for an excellent option (Kilcast, 2010).

2.2.5 Statistical analyses

The obtained results were subjected to statistical analysis using the SPSS program (Version 21) and the LSD test was applied to calculate the value of significant differences at the level of significance $P \leq 0.05$ based on the Duncan 1955 method, and the results of the statistical analysis were monitored using the (mean ±SD) method.

3. Results and discussion

Table (1) Proximate analysis of the new milk drink

Samples	**Mean ± SD**				
	Moisture%	**Ash%**	**Protein%**	**Fat%**	**Carbohydrate%**
1	78.00±0.020	0.91±0.009	7.03±0.015	3.20±0.00	10.55±0.021
2	79.01±0.042	0.60±0.003	6.82±0.010	2.95±0.100	10.29±0.078
3	78.55±0.030	0.63±0.005	7.07±0.066	2.70±0.070	10.62±0.105
4	79.37±0.040	0.83±0.001	7.12±0.012	3.37±0.102	10.69±0.021
5	79.91±0.122	0.70±0.005	7.09±0.068	2.76±0.064	10.65±0.104
Pure milk	87.04±0.040	0.23±0.007	3.24±0.006	3.35±0.010	4.87±0.007
p- value	***0***.000	***0***.000	***0***.000	***0***.000	***0***.000
Comment	**S**	**S**	**S**	**S**	**S**

A one-way analysis of variance was used to compare the averages of the approximate analysis of the samples, S means that there is a significant difference between the samples at a significance level of 0.05

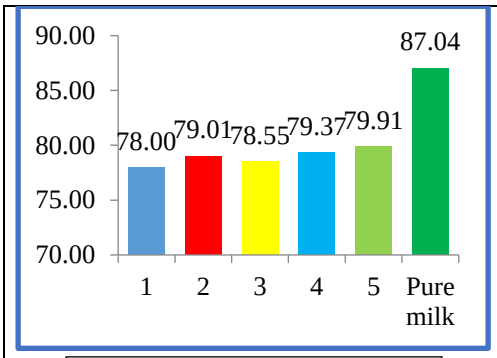

Figure (1) Moisture content%

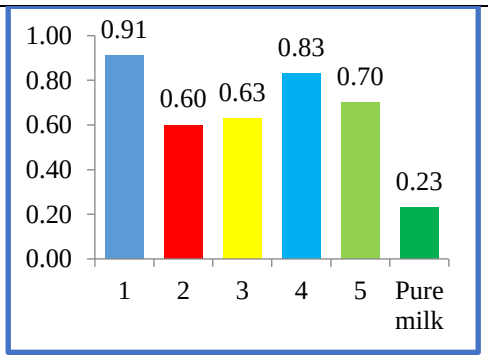

Figure (2) Ash content%

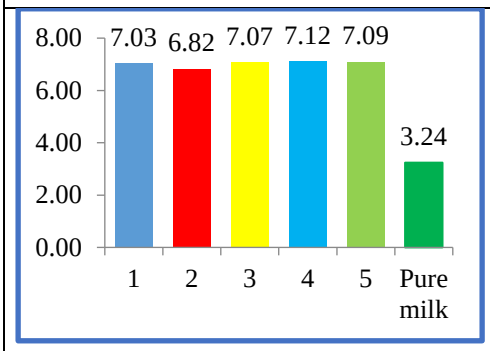

Figure (3) Protein content %

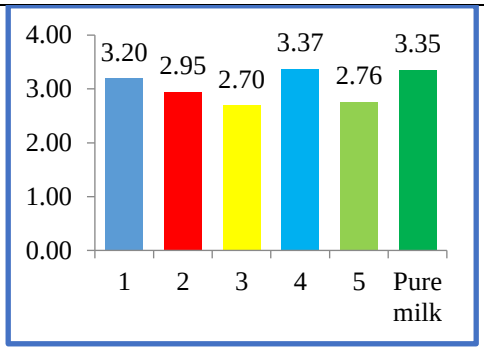

Figure (4) Fat content %

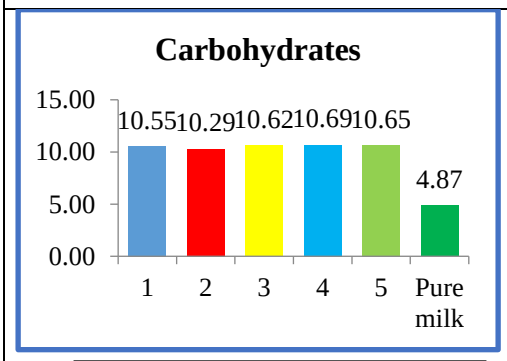

Figure (5) carbohydrate %

The role of nutrients in the immune function many and varied. It is clear that adequate and balanced availability of these elements is very necessary to provide a good immune response. Good nutrition creates an immune system capable of responding well to threats. Table (1) and figures (1,2,3,4,5) illustrated the proximate analysis of the new milk drink. It is noted that there is a significant increase in the percentage of carbohydrates, protein and ash (10.69±0.021, 7.12±0.012, 0.83±0.001%) compared to raw milk (4.87±0.071,3.24±0.006, 0.23±0.007) respectively. In addition to energy, the new milk drink provides a high percentage of protein and ash (mineral). Many researchers suggested that the immune system needs a number of nutrients, such as proteins, which play an

important role in the maturation, differentiation, and response of immune cells (Calder, 2013). On the other hand, other additives in the composition of the new milk drink have a significant impact on strengthening the immune system. It was found that ginger and turmeric acts as an anti-inflammatory by inhibiting the Cox 2 enzyme, which is part of the chemical pathway that produces chemicals increase inflammation (London, 2010). From the results obtained in this study, it's obvious that the addition of immune-boosting ingredients has a significant role in increasing the level of vital ingredients like carbohydrate, protein and ash (minerals).

Table (2) Quality characteristics and energy value of the new milk drink

Samples	Mean ± SD			
	pH	TSS	Acidity	Total energy value (kcal)
1	6.66±0.021	19.90±0.141	0.22±0.071	99.12±0.144
2	6.33±0.014	19.50±0.707	0.20±0.014	94.96±1.181
3	6.59±0.000	18.20±0.283	0.21±0.021	95.06±0.190
4	6.46±0.014	17.90±0.141	0.24±0.021	101.57±0.829
5	6.56±0.007	17.00±0.000	0.20±0.028	94.86±0.927
Pure milk	6.65±0.007	13.10±0.141	0.22±0.007	62.54±0.138
p- value	0.000	0.002	0.843	0.000
Comment	S	S	NS	**S**

A one-way analysis of variance was used to compare the averages of the material analyzes for the samples. S means that there is a significant difference between the samples at a significant level of 0.05.

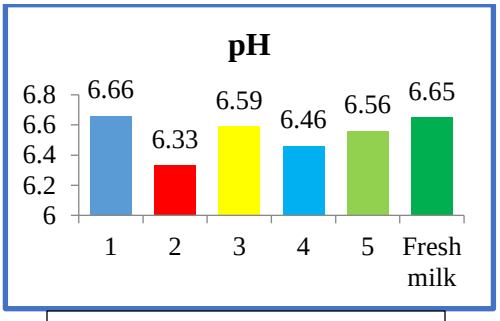

Figure (6) pH

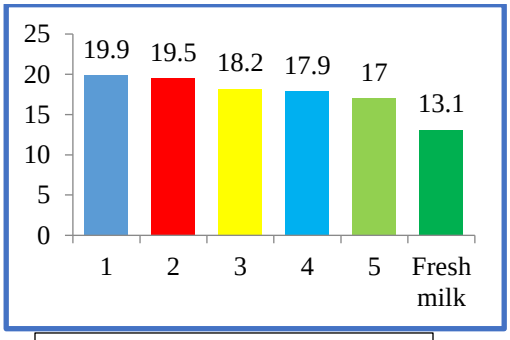

Figure (7) Total Soluble Solids (TSS)

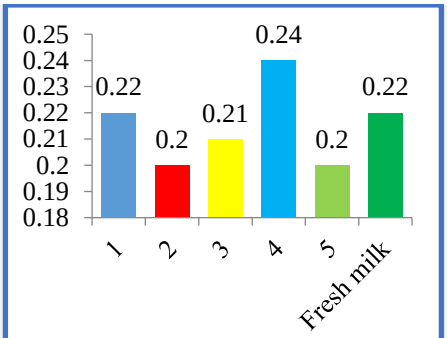

Figure (8) Titratable acidity)

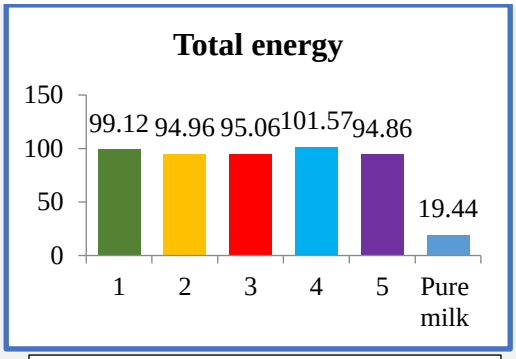

Figure (9) Total energy Kcal/100ml

Table (2) and figure (6,7,8) represented quality characteristics of the new milk drink samples in compare with pure milk. It is noted that there is a significant difference in the mean of pH value and total soluble solids, and there is no difference between the samples in the average of acidity, as the probability value is greater than 0.05 (0.843). Sample (2) represent lowest levels of pH and acidity, while the sample (5) the lowest levels of total solids, high levels of pH and total soluble solids, and sample (3) has the highest level of acidity (0.24). From the results obtained its noticed that the addition of immune-boosting ingredients help to increase the total soluble solids (TSS) of the new milk drink without affecting the average acidity.

The total energy value of the new milk drink samples compares to pure milk. It is noted that there is a significant difference in the average energy value of the drink milk samples (all levels of significance are less than 0.05). Sample (2) was the lowest total energy value, and sample (4) was the highest energy value. Referring to the components of the two samples, it is noted that sample (4) contains nuts and avocados, which helped in raising its fat and protein content, while sample (2) was contained only the main components of the new milk drink formula. From nutritional point of view, the new milk drink shows a significant increase in total energy value compare to pure milk which will enhance the nutritional value of the new milk drink.

Table (3) Sugars, density and solids non- fat (SNF)

Samples	**Mean±SD**					
	Sucrose	**Glucose**	**Fructose**	**lactose**	**Density**	**SNF**
1	0.00±0.000	6.77±0.115	0.16±0.021	4.82±0.149	1.07±0.000	19.19±0.036
2	0.00±0.000	6.60±0.300	0.00±0.000	4.43±0.087	1.07±0.001	18.71±0.139
3	0.00±0.000	6.67±0.252	0.20±0.006	4.76±0.110	1.07±0.006	19.30±0.186
4	0.00±0.000	6.63±0.153	0.00±0.000	4.83±0.025	1.07±0.000	19.44±0.038
5	0.00±0.000	6.67±0.252	0.00±0.000	4.81±0.090	1.07±0.001	19.36±0.193
Pure milk	0.00±0.000	0.00±0.000	0.00±0.000	4.86±0.010	1.03±0.000	8.86±0.015
p- value	0.000	0.000	0.001	0.000	0.000	0.000
Comment	**S**	**S**	**S**	**S**	**S**	**S**

A one-way analysis of variance was used to compare the averages of the sensory evaluation. S means that there is a significant difference between the samples at a significant level of 0.05

` From the above table (3), it is noted that there is a significant difference in the average chemical properties of the milk drink (all levels of significance are less than 0.05). The pure milk sample contains the lowest rates of sucrose, glucose, fructose, density and solid non-fat. Sample (2) contains the lowest rates of lactose compared to the other samples. The samples of the new milk drink were distinguished by containing no sucrose and little or negligible percentage of fructose. The results of the analysis showed that most of the available sugars in the milk drink samples is glucose, which may mean that a quick and effective source of energy. It is also noted from the above table that the density and non-fat solid materials increased in the new milk drink compared to pure milk, this may be due to the increase in the total soluble solids (TSS) ratio resulting from the addition of natural immune-boosting ingredients.

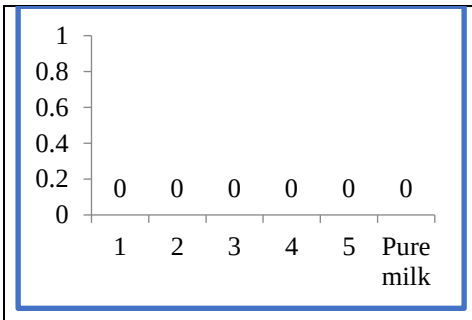

Figure (10) Sucrose%

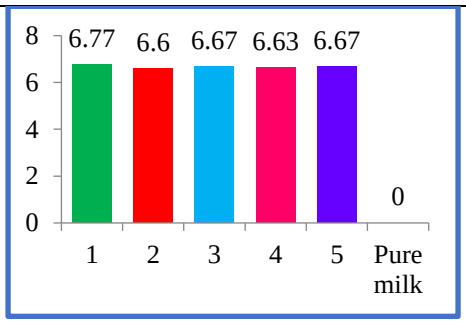

Figure (11) Glucose%

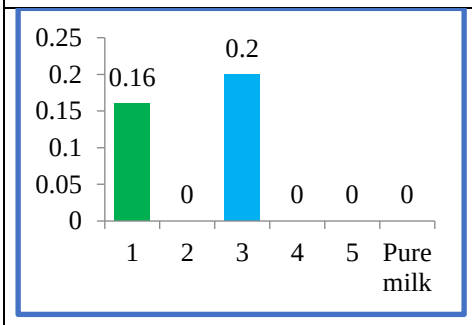

Figure (12) Fructose%

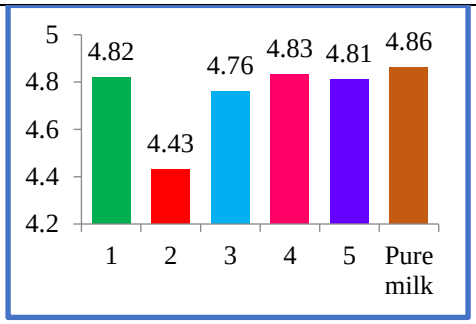

Figure (13) Lactose%

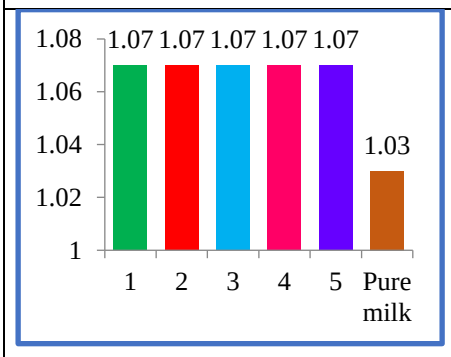

Figure (14) Density

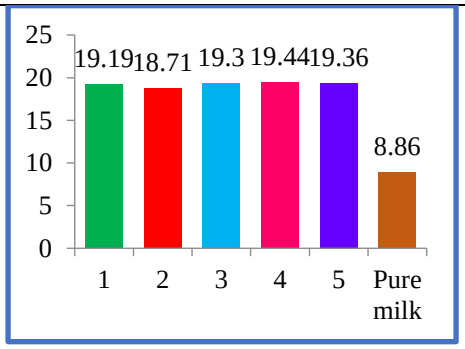

Figure (15) Solids Non-Fat

A one-way analysis of variance was used to compare the averages of the sensory evaluation. S means that there is a significant difference between the samples at a significant level of 0.05

Table (4) Analysis of minerals (mg/ml)

Samples	Mean±SD			
	P	**K**	**Ca**	**Mg**
1	306.00±2.000	286.00±3.000	179.00±2.000	68.00±2.000
2	517.33±0.577	348.33±11.719	235.00±2.000	259.00±3.511
3	571.33±0.577	386.67±23.965	143.67±1.155	85.67±1.155
4	306.67±59.475	833.00±3.000	272.00±2.000	136.00±1.000
5	375.00±1.000	962.00±2.000	327.33±2.548	118.67±3.512
P- value	*0*.000	*0*.000	*0*.000	*0*.000
Comment	**S**	**S**	**S**	**S**

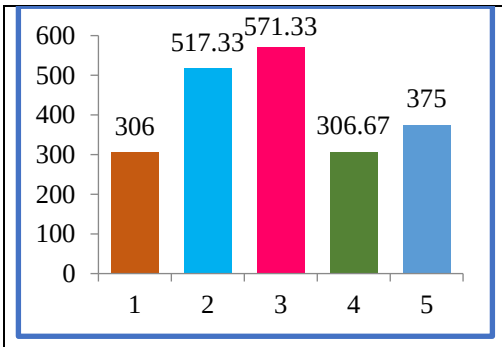

Figure (16) P (PPM)

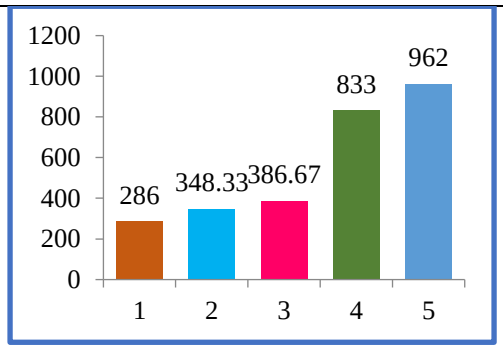

Figure (17) K (PPM)

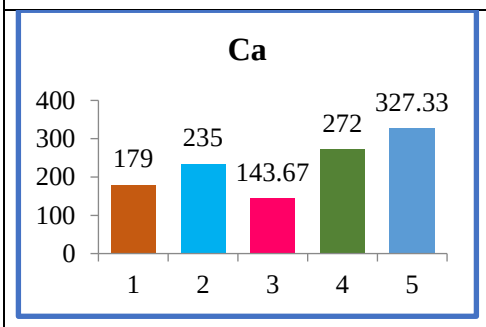

Figure (18) Ca (PPM)

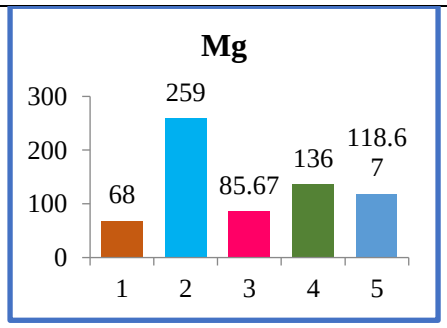

Figure (19) Mg (PPM)

Table (4) and figure (16,17,18, 19) show the determination of mineral in the new milk drink samples. It is noted that there is a significant difference in mineral content of the new milk drink (all levels of significance are less than 0.05). Sample (3) contains the highest levels of phosphorus (571.33±0.577), and sample (5) contains the highest levels of potassium and calcium (962.00±2.00, 327.33±2.548) respectively, and sample (2) contains the highest levels of magnesium (259.00±3.511). Previous study shows that the percentage of P, K, Ca and Mg of pure milk is (103.55, 166.88, 136.45 and 140,63) respectively. (Visentin *et al*., 2018). In comparison, it obvious the new milk drink samples rich in minerals and this certainly due to the high nutrients of the ingredients.

Table (5) Total count and coliform bacteria (cfu/ml)

	1	**2**	**3**	**4**	**5**
Total count	3	4	2	4	3
Coliform	Absent	4	5	2	2

The results of the microbial analysis of the new milk drink samples shown in Table (5), the samples were pasteurized at 90 degrees for 3 minutes, packaged while hot, and then cooled to 4 minutes, indicated that sample (1) was free of coliform bacteria, where the sudden heating and cooling of samples (pasteurization) is sufficient to reduce the number of bacteria. The total count of microbes did not exceed 10 bacterial growths per ml, as the obtained results agreed with what was stipulated in local and international standards (East African Standard, 2006, Libyan standard 1992), provided that the milk remains fit for consumption unless the growth of coliform bacteria not exceeds 10 growths per ml. Pasteurized milk usually contains strains of bacteria that are resistant to heat treatment (Rolyea *et al*., 1998).

Table (6) Sensory evaluation of the new milk drink

Samples	**Mean ± SD**				
	Color	**Flavor**	**Taste**	**Texture**	**General acceptability**
1	3.66±1.118	3.48±1.216	3.42±1.326	3.46±1.199	3.80±1.143
2	3.42±1.18*0*	3.02±1.253	2.94±1.376	3.00±1.195	3.16±1.405
3	4.22±0.648	3.92±0.900	4.16±0.889	4.12±0.961	4.32±0.819
4	4.20±1.010	4.18±1.004	4.12±1.062	3.96±1.124	4.34±0.982
5	4.06±1.150	3.56±1.128	3.54±1.182	3.62±1.260	3.82±1.224
p- value	*0*.000	*0*.000	*0*.000	*0*.000	*0*.000
Comment	**S**	**S**	**S**	**S**	**S**

A one-way analysis of variance was used to compare the averages of the sensory evaluation. S means that there is a significant difference between the samples at a significant level of 0.05, from 1 to 1.80 bad, from 1.81 to 2.60 acceptable, from 2.61 to 3.40 good, from 3.41 to 4.20 very good, from 4.21 To 5 excellent.

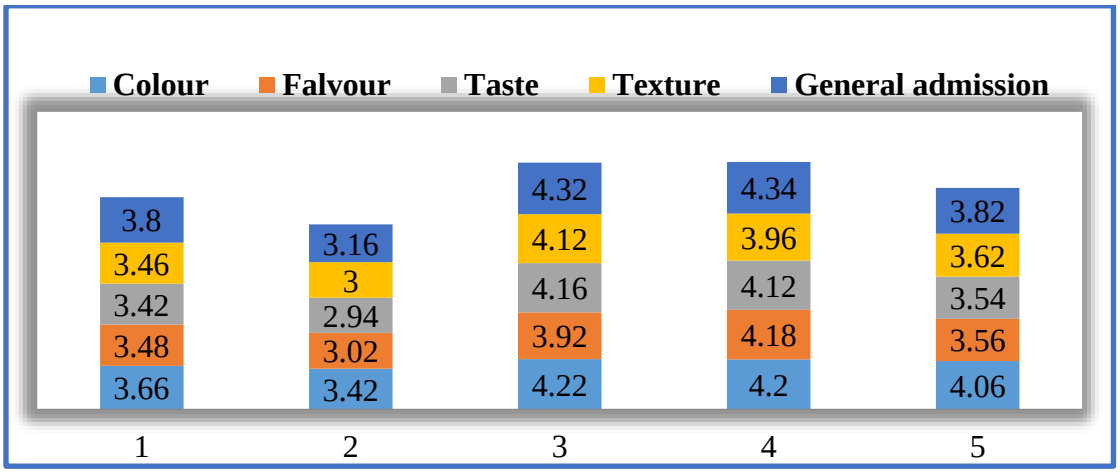

Figure (20) sensory evaluation

The use of aromatic healthy spices in the production of the new milk drink samples increased its biologically active compounds, health benefits and sensory properties. From the above table (6) and figure (20), the panelist - generally- prefer the new milk drink samples specially in flavor and test, its obvious panelist prefer sample 3 and 4

more than the other samples, this may be due to the nuts flavor in both samples. General acceptance of the second sample was (good), and first and fifth samples were (very good).

4. Conclusion

In this study, 5 samples of the new milk drink were prepared using natural immune-boosting ingredients. Chemical analyses of the new milk drink, showed a significant increase in the percentage of carbohydrates, protein and total solids, compared to fresh milk. This corporation enhanced the Total Soluble Solids (TSS), Sold Non Fat (SNF), density and total energy value. Samples show acceptable microbiological quality and average very good organoleptic characteristics. These results lead to conclude that incorporation of natural immune- boosting ingredients in the new milk drink, might be efficient to formulate new product with novel characteristics, and could by qualified as a functional food with very good consumer's acceptability. To the best of our knowledge, this is the first scientific research to innovate a new milk drink with natural immune boosting ingredients in Libya. This study also provides a basis for developing novel immune boosting new milk drinks.

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