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Physicochemical and Microbiological Properties of Probiotic Kiwi Whey-based Beverages

[1]Sahar H. S. Mohamed, [2]Eman M. Hegazy and [1]*Jihan M. Kassem

[1] Dairy Department, Food Industries and Nutrition Research Institute, National Research Centre (NRC),33th Tahreer Street, Dokki, Giza, Egypt.

[2] Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre (NRC).

Corresponding author:

Name: Jihan M. Kassem

Email

Address: jihankassem997@gmail.com

Phone Number: 01223567745

ORCID ID: 0000-0002-8462-6478

ABSTRACT

Background and Objectives: Preparing functional probiotic beverages using sweet whey is considered as one of the required alternatives instead of disposal and causing environmental pollution. The main target of this study was applying sweet whey, honeybee and kiwi fruit in addition to probiotic bacteria (*Leuconostoc mesenteroides* and *Lactococcus lactis diacetylactis)*to produce probiotic kiwi whey-based beverages. **Materials and Methods:** Nine different ratios of kiwi juice and honeybee were used as preliminary experiments to select the best product according to the judge's opinions. Two elected ratios; one has 10% kiwi fruit without any sweetener served as control, the other had 10% kiwi fruit and 15% honeybee served as treatment sample. These samples were examined for some chemical and microbiological analysis besides, color parameters and apparent viscosity. **Results:** Data revealed the treatment gained higher significant ($p \leq 0.05$) antioxidant activity (58.02%) compared to control one (43.51%). Also, there were differences in color parameters (L*, a* and b*) and apparent viscosity between the two samples. The addition of honey had a significant ($p \leq 0.05$) decrease in the viability of lactic acid bacteria. This viability was in demand border (10^6CFU mL^{-1}) until the twentieth day for the control, while they were at the limited numbers until the fifteenth day for treated samples. The treated sample appeared antifungal activity toward *A. flavus*, and they all were free from mycotoxins. **Conclusion:** A successful probiotic kiwi whey-based beverage had been produced as a new probiotic dairy product with kiwi fruit at 10% and honeybee at 15% accompanied by antioxidant properties.

Conflict of Interest: The author declares there is no conflict of interest among them.

Keywords: Functional beverages, probiotic bacteria, antioxidant activity, antifungal activity, mycotoxins.

1. INTRODUCTION

Sweet whey is the liquid that split up from the manufacturing of hard and semi-hard cheese. It includes important nutrients like proteins, lactose, vitamins and minerals. It is no longer insignificant as before [1].Getting rid of whey is considered a dangerous treatment that leads to environmental pollution as a result of the presence of high organic materials. Biological Oxygen Demand (BOD) of whey is almost 200 times more before treats it, which was uneconomical [2]. The whey production is about 187 million ton and increasing worldwide up to 2% per year until 2020**,** which means that its usage must be grown up [3]. Before about fifty years, whey beverages

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production had been started and continued till today with a broad scope of various whey beverages. Whey based fruits beverages whether fermented or not; are more suitable for health as compared to other drinks**.** They have very high nutrient value and good therapeutic properties[4].There are many literatures mentioned that fermented milk beverages with whey proteins improve cardiovascular health. Also these beverages help in lowing high blood pressure and triglyceride levels in the blood [2].

Kiwifruit belongs to the genus *Actinidia* (Actinidiaceae) and recently it has a better consumer acceptance worldwide, according to its health advantages. It has many benefits contain an antioxidants source, recovery the laxation of gastrointestinal, reducing blood lipid levels, and relief the skin disorders. Kiwifruit is rich in vitamin C, folate, potassium, dietary fibre and contain nutrients and biologically active phytochemicals which promote its antioxidant and anti-inflammatory actions that might help prevent cardiovascular diseases [5]**.**

On another side, the honeybee has a special formula and anti-microbial qualities composed of fructose & glucose and contain 4 to 5% fructo-oligosaccharides, which serve as prebiotic agents. Honey is a high-quality food rich in essential beneficial substances to guarantee balanced biological processes and reducing the formation of free radicals [6].

However, Lactic Acid Bacteria (LAB) plays an important role in food, especially in beverages. Functional beverages are considered as an attractive way to present LAB at the appropriate limits to the consumer. Among the LAB, the genera *Leuconostoc* and *Lactococcus* are utilizing in milk and dairy products. *Leuconostoc* strains are mesophilic, Gram-positive bacteria. They are obligatory hetero-fermentative *cocci*. They are associated with an enormous number of naturalist fermentations of food items, which drove the realization of their status as Generally Recognized as Safe (GRAS) [7]. Genera *Leuconostoc* is also characterized by their capability to grow in high sugar concentrations. It also improves the rheological properties of the product by its ability to produce exo-polysaccharides. The fermentation of citrate by some *Leuconostoc* is important, providing both diacetyl as an important flavor compound in dairy fermented products. Where, *Lactococcal* strains are the main acid-producing starters [7, 8]**.**

From another view, Aflatoxins are sorts of mycotoxin created by Aspergillus species of organisms such as *A. flavus* and *A. parasiticu*s. At least 14 types of aflatoxin are delivered in nature; aflatoxin B_1 is considered the foremost poisonous [9].**Moss, [10]** illustrated that Ochratoxin isa mycotoxin that comes in three secondary metabolite forms, A, B and C. All are produced by *Penicillium* and *Aspergillus* species. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice-making process. Also, same author reported that patulin is a toxin produced by *Penicillium expansum* which associated with a range of moldy fruits such as apples, pears, peaches and cherries. Although the considerable problems are financial with a critical loss of valuable food materials, there are a few examples that involving a role for mycotoxins in the safety of fresh fruits.

Accordingly; the broad objective of this study was producing probiotic kiwi whey- based beverages fortified with lactic acid bacteria (*Leuconostoc mesenteroides* and *Lactococcus lactis diacetylactis*) as probiotics and natural sweetener such as honeybee. Also, it considered as a trial for increasing the economic and commercial value of whey which is a valuable by-product. Antioxidant activity, pH values and microbiological properties of beverages had been evaluated. Color measurements and viscosity apparent were also determined.

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2. MATERIALS AND METHODS

The study was carried out at Dairy Department, Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre, Egypt.

2.1. Materials

2.1.1. Fresh sweet whey (7.2% total solids and 0.7% protein) was obtained from the Faculty of Agriculture, Cairo Univ. Giza, Egypt.

2.1.2. High-quality green Kiwi fruits (*Actinidiadeiciosa*) and sugar were purchased from the local market, Giza, Egypt.

2.1.3. The honeybee was also purchased from the El-Daghlia Governorate, Egypt.

2.1.4. Lactic acid bacteria (LAB)

Leuconostoc mesenteroides and *Lactococcus lactis diacetylactis* were obtained from the laboratory of dairy microbiology, Dairy Department, National Research Centre, Giza, Egypt.

2.1.5. Fungi produced toxin and Mycotoxins Standards:

Aspergillus flavus NRRL 3359 (aflatoxins produced) *A. ochraceus* NRRL 3174 (Ochratoxin A produced) and *Penicillium expansum* NRRL6069(patulin produced) were obtained from Standard Association of Australia 80- Astrum St., Orth Sydney, NSW.

Aflatoxins, ochratoxin A and patulin were purchased from Sigma, chemical company USA.

2.2. Experimental

2.2.1. Preparation of Kiwi juice

Fresh high-quality green kiwi fruits were washed, peeled, cut to pieces and pulped in a blender. The resultant homogenized mixture juice was filtered through a muslin cloth then heated in a water bath (70°C/2 min) and cooled immediately (2-4°C).The average compositions of green kiwi fruit are; protein (1.05%), total sugar (14.86%), total solids (26.06%), ash (0.58%) and total fiber were 2.39mg/100g.

2.2.2. Preliminary experiments of probiotic kiwi whey-based beverages

Nine beverages samples were prepared as mentioned below:

1- Three ratios (10, 15 and 20% v/v) of kiwi juice were individually added to whey samples without adding any sweetened materials and served as control samples. (C_1, C_2 and C_3) respectively.
2- Five percent of sugar were added to C_1, C_2 and C_3 to serve T_1,T_2 and T_3 respectively
3- Fifteen percent of the honeybee was added to C_1, C_2 and C_3 to serve T_4, T_5 and T_6respectively.

All blends were pasteurized at 65°C for 30 min, then cooled to 37°C and inoculated with 1% of (LAB) (1:1 of *Leuconostoc mesenteroides* and *Lactococcus lactis diacetylactis*) then filled in sterilized glass bottles, tightly closed and keep them in a refrigerator at5±2°C for 24hrs.Thereafter,sensorial evaluation was represented in Table (1) for these nine batches to select the best one for the judge's acceptability.

Table (1): Sensory evaluation of preliminary experiment of probiotic kiwi whey-based beverages

Parameters	C_1	C_2	C_3	T_1	T_2	T_3	T_4	T_5	T_6
Appearance(10)	6^{D}	6.1^{CD}	6.09^{CD}	7^{BCD}	7.08^{ABC}	7.16^{AB}	7.77^{AB}	8^{AB}	8.08^{A}
Odor (10)	5^{C}	5^{C}	5.08^{C}	6.23^{B}	6.42^{B}	6.72^{B}	8.08^{A}	7.65^{AB}	7.52^{A}
Taste (10)	4.5^{D}	5.08^{D}	4.93^{D}	6.54^{C}	6.35^{C}	6.58^{C}	8.28^{A}	7.58^{AB}	7.23^{BC}

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Total acceptability (30)	15.5^{C}	16.18^{C}	16.1^{C}	19.77^{B}	19.85^{B}	20.46^{B}	24.13^{A}	23.23^{AB}	22.83^{AB}

Footnote: Data expressed as a mean of 20 replicates. Means with the same letter are not significantly different (P≤ 0.05).
C_1: control sample with 10% v/v ratio of kiwi juice; **C_2:** control sample with 15% v/v ratio of kiwi juice; **C_3:** control sample with 20% v/v ratio of kiwi juice; **T_1:** treatment sample with 10% v/v ratio of kiwi juice and 5% sugar; **T_2:** treatment sample with 15% v/v ratio of kiwi juice and 5% sugar; **T_3:** treatment sample with 20% v/v ratio of kiwi juice and 5% sugar; **T_4:** treatment sample with 10% v/v ratio of kiwi juice and 15% honeybee; **T_5:** treatment sample with 15% v/v ratio of kiwi juice and 15% honeybee and **T_6:** treatment sample with 20% v/v ratio of kiwi juice and 15% honeybee.

From Table (1) it could conclude that (T_4) was significantly (P≤0.05) the best compared to other treatments from the panels point of view. So, 10% kiwi fruit with 15% honeybee versus control sample (C_2) which contain (10% kiwi juice without sugar) followed applied. All samples were stored in a refrigerator at 5±2°C for 20 days. These two samples (C_2 and T_4) were analyzed in intervals every 5 days of storage for some chemical composition, antioxidant activity and microbiological examinations. Also, color parameters and viscosity apparent were evaluated.

2.3. Methods:

2.3.1. Sensory evaluation of probiotic kiwi whey-based beverage

Sensory attribution was followed up by 25 members in the Dairy department, National Research Centre. Nine samples were estimated at a fresh period to elect the best treatments. The evaluation degrees were included appearance, odor, taste and total acceptability.

2.3.2. Analytical procedure of probiotic kiwi whey-based beverage

Total Solids (TS %) content of beverage samples were determined according to AOAC [11], methods, 926.08. The pH values were measured using a digital pH meter (HANNA, Instrument, Portugal) with a glass electrode.

2.3.3. DPPH radical scavenging activity

Free radical scavenging activity of probiotic kiwi whey-based beverage samples were also performed according to the method of **Locattili *et al.*,** [12]using the DPPH (2,2-diphenyl^{-} ^{1}picrylhydrazil)**.**The radical scavenging activity (%) was calculated using the following equation:

Radical scavenging activity (%) = ($A_{control} - A_{sample}$) / $A_{control}$ x 100

Where: $A_{control}$ is the absorbance of a blank.
A_{sample} is the absorbance of a sample.

2.3.4. Color measurements

Color parameters were determined by using a Spectro-Colorimeter (Tristimulus Color Machine)[13]with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection model. The color was expressed in three terms of L, a and b. Where:
L: Value represents darkness from black (0) to white (100).
a: Value represents color ranging from red (+) to green (-).
b: Value represents yellow (+) to blue (-).

2.3.5. Apparent viscosity

Beverage samples were determined for apparent viscosity using a Brookfield Synchro- Lectric viscometer (Model LVT; Brookfield Engineering Inc. Stoughton,

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MA). Reading was taken at the speed of 4 to 50 s^{-1}using spindle-04 at 7°C for upward curve. Apparent viscosity wasexpressed as poise.

2.3.6. Microbiological analysis

2.3.6.1. Counting of total bacterial count and probiotic bacteria

The viability of LAB was detected using MSE medium[14] for *Leuconostoc mesenteroides*, incubated aerobically for 48h at 35°C, while M17 medium [15] was used for *Lactococcus lactis diacetylactis*, incubated aerobically for 48h at 30°C. Plate count agar medium [16] was used for enumeration of total bacterial count (TBC). Plates were incubated aerobically for 24h at 37°C. Potato Dextrose Agar was used for yeast and mold enumeration. Plates were incubated at 25°C for 5 days, according to **Marshall** [17]. Violet Red Bile Agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 h, according to **Marshall** [17]. Viable cells were calculated as follows [14]:

% Viability = (CFU at n week (s) of storage / initial CFU) x 100

2.3.6.2. Isolation and Identification of Fungi

The dilution plate count on Potato Dextrose Agar (PDA, Oxoid) was examined according to **Tafinta *et al*., (2013)** [18]. Fungi were isolated from all samples under investigation as mention above. Fungal isolates were identified according to **Barnett and Hunter** [19].

2.3.6.3. Detection of antifungal activity

0.5 ml from 48 h old cultures of *A. flavus* NRRL 3359, *A. ochraceus* NRRL 3174 and *P. expansu* NRRL6069 were transferred into separate sterile Petri dishes contained 20 ml of sterile media of potato dextrose agar (PDA, Oxoid) was poured into each dish. After solidification of PDA, agar wells were dug in plates with the aid of sterilized cork borer (6 mm diameter) 0.5 ml of the control and treated sampleswere put in the wells with properly labelling then the Petri dishes were incubated at 25°C for 7 days. The diameter of the clear zone of inhibition around the well (Zone of inhibition) was measured to the nearest millimetre using a transparent ruler after subtracting the well diameter [20, 21].

2.3.6.4. Detection of some Mycotoxins

Aflatoxins (B_1, B_2, G_1 and G_2), Ochratoxin A and patulin were detected in (kiwi, honeybee, sweet whey and probiotic kiwi whey-based beverages) according to **Hamed *et al*., (2017)** [22].

2.3.7. Statistical analysis

Statistical analysis of experimental data was performed by using **SAS**[23]**.**Probability equal ($p \leq 0.05$) was significant. Each assay was carried out in triplicate.

3. RESULTS AND DISCUSSION

3.1. pH values, Total Solids (TS) and antioxidant activity of probiotic kiwi whey-based beverages

The pH values were elucidated in Fig. (1).It was found that pH values for both samples gained acidic degrees either fresh or stored samples. As known, whey contained a remarkableratio of lactose so; it may be a reason to raise acidity for the conversion of lactose to lactic acid. Addition of honey; which has pH level between

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2-6 caused a tendency towards acidity compared to the control sample which didn't contain honey bee. During storage, the valuesof pH were significantly decreased ($p \leq 0.05$) in both control and treated samples. These results were agreed with **Hande and Chavan,** [24] who stated that the addition of kiwi fruit to *chhana* whey beverages decreased pH values till the end of the period (10 days). Same indication was observed by **Pandey *et al*.,** [25].

On the other side, as shown in Fig. (2), there were no observable significant differences ($p > 0.05$) between total solids in the control and treated sample either fresh or during the storage period. Total solids were recorded at 6.33% for the fresh control sample and 6.28% after 20 days of storage period. While, treated sample was recorded 20.43 and 20.46 % for fresh and at the end of the storage period, respectively. The same trend was noticeable with **Chauhan*et al*.,** [26]established that no significant difference was observed during storage for TS contents of herb mixed beverage.

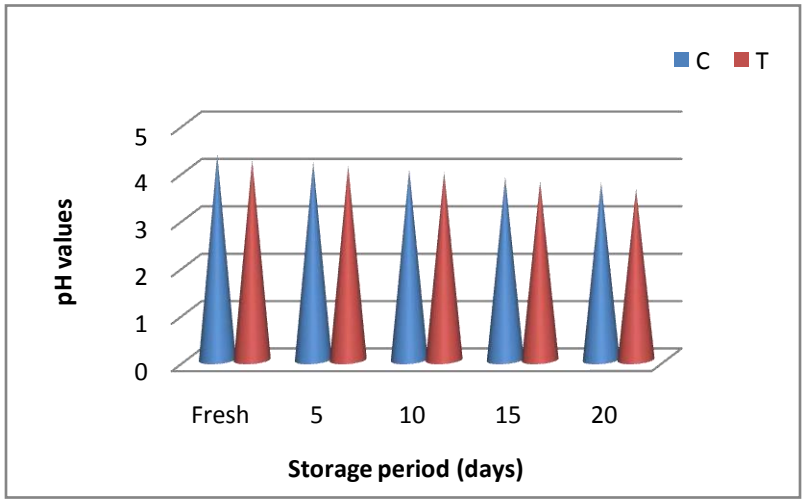

Fig. (1): pH Values of probiotic kiwi whey-based beverages during the storage period.
C: control sample with 15% v/v ratio of kiwi juice
T: treatment sample with 10% v/v ratio of kiwi juice and 15% honeybee

As well, the antioxidant activity of probiotic kiwi whey-based beverage during storage had been illustrated in Fig. (3).It was found out that the treated sample had higher antioxidant activity (%) than the control sample either fresh or stored. The treated sample contained three main sources ofantioxidant agents which were whey, kiwi juice and honeybee. Whey has potent antioxidant activity, like cysteine-rich proteins that contain a thiol (sulfhydryl) group serves as an active reducing agent in preventing oxidation and tissue damage. The high content of sulfur-containing amino acids in whey protein is relevant to its potential to increase the antioxidant capacity of the body. The amino acids methionine and cysteine are precursors of glutathione and taurine which called 'body's own' antioxidants [2]. While, kiwi fruit is a highly nutritional fruit due to its high level of vitamin C and its strong antioxidant including carotenoids, lutein, phenolics, flavonoids and chlorophyll[27]. At the same trend, honey contains a significantly high level of antioxidants, both enzymatic and non-enzymatic, including catalase, phenolic acids, flavonoids, carotenoids, organic acids, ascorbic acids, amino acids and Maillard reaction products. Phenolic compounds commonly

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found in honey include phenolic acids, flavonoids and polyphenols [24].

As it was expected, there was a significant reduction ($p \leq 0.05$) in antioxidant activity in all samples during the storage period. Antioxidant activity for the control sample was 43.51% at zero time and reached 39.02% at the end of the storage period. As well treated sample gained 58.02% antioxidant activity at a fresh time; then obtained 51.32% at the end of the trial. The same trend was found by **Bhat and Singh,** [28] who represented that vitamin C and antioxidant activity of whey-guava and RTS (ready-to-serve) beverages had decreased during 60 and 90 daysof storage due to the oxidation of vitamin C.

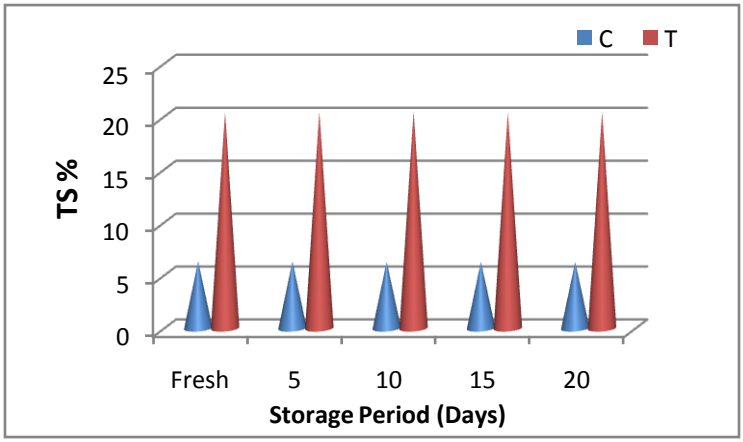

Fig. (2): TS (%) of probiotic kiwi whey-based beverages during the storage period.

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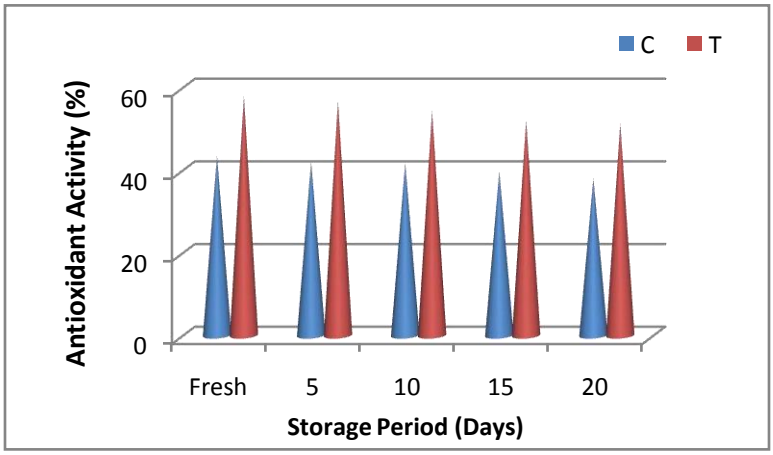

Fig. (3): Antioxidant activity (%) of probiotic kiwi whey-based beverages during the storage period.

See footer of Fig. (1)

3.2. Color Parameters of probiotic kiwi whey-based beverages

Color parameters for both control and treated samples (C & T) had been presented in Table (2).It was found that there was a significant difference ($p \leq 0.05$) between them. The L* values wereraised in the control sample either fresh or stored samples compared to treatment samples thendecreased significantly ($p \leq 0.05$) gradually after 10 days of the storage period in all samples.During the storage period treatment, samples slop to darkness; it could be due to the degradationof the phenolic compounds and vitamin C. The a* values tend to negative values which indicatedgreen color related to both kiwi fruit and whey color.

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Similar to L* values; a* values weredecreased during the storage period in both samples. Also, after 10 days of the trial, the b* valueswere significantly ($p \leq 0.05$) decreased in control and treatment samples. As **Chou *et al.,*** [29] displayed that kiwi fruit color tends to green color in all color parameters (L*, a* and b*).

Table (2): Color parameters of probiotic kiwi whey-based beverages***** during the storage period.

Samples	L*	a*	b*
Fresh			
C	56.76^{Ad}	-2.40^{Aa}	25.97^{Ab}
T	52.50^{Bc}	-2.10^{Aa}	25.81^{Aa}
5 Days			
C	60.76^{Aa}	-2.04^{Ab}	27.86^{Aa}
T	55.43^{Ba}	-1.50^{Bb}	25.33^{Bb}
10 Days			
C	59.23^{Ab}	-1.83^{Abc}	26.80^{Ab}
T	54.11^{Bb}	-1.48^{Bb}	23.76^{Bc}
15 Days			
C	57.74^{Ac}	-1.72^{Abc}	25.89^{Ab}
T	54.27^{Bb}	-1.44^{Bb}	21.95^{Bd}
20 Days			
C	55.43^{Ae}	-1.54**Ac**	23.45^{Ac}
T	51.65^{Bc}	-1.38^{Ab}	20.12^{Be}

Different capital letters between samples (C& T) in the same period had a significant difference ($p \leq 0.05$).
Different small letters between different periods for each sample had a significant difference ($p \leq 0.05$).
***** See footer of Table (1).

3.3. Apparent viscosity of probiotic kiwi whey-based beverages

The viscosity of control and treatment samples was exhibited in **Fig. (4 a-e)** at fresh and during the storage period. The conducted Figure declared that the treatment samples had gained higher viscosity versus the control samples either fresh or during the storage period. The addition of honeybee seemed to increase the viscosity of treatment samples till the 10 days of storage period then decreased till the end of the period. On the other hand, the control sample's viscosity had been increased after 5 days of storage period till the end. The viscosity reduction after 10 days of storage for the treated sample could be due to the addition of honeybee that caused decreased in the viability of *Leuconostoc mesenteroides* as exhibited in microbiological results. These data were in harmony with **Izadi *et al.,*** [30] who elucidated that the apparent viscosity of yoghurt either control or treatment samples were decreased after 7-14 days of storage period.

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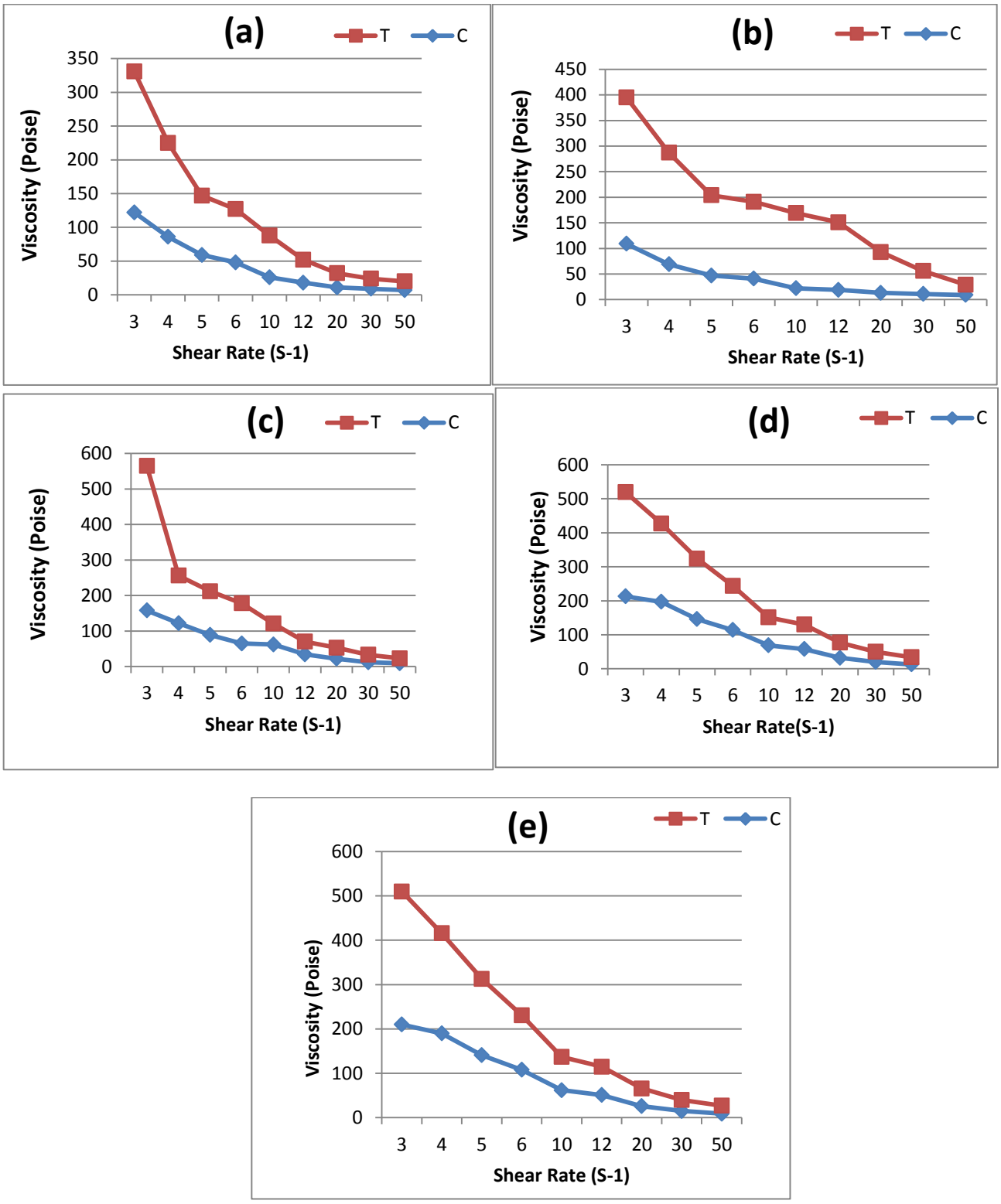

Fig. (4): Apparent viscosity of probiotic kiwi whey-based beverages during the storage period (a): Fresh, (b): 5 days, (c): 10 days, (d): 15 days and **(e)**: 20 days

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3.4. Microbiological analysis of probiotic kiwi whey-based beverages

3.4.1. Counting of total bacterial count and probiotic bacteria

In the present study, the viability of *Leuconostoc mesenteroides* significantly ($p<0.05$) increased during the storage period until 10 days, then significantly ($p<0.05$) decreased with the end of the storage period in the control sample **(Fig. 5 a)**. The same observation was shown in the viability of total bacterial count (8.2 log CFU mL^{-1}). Also, the numbers of *Lactococcus lactis diacetylactis* were increased until 5 days (9.4 log CFU mL^{-1}) of the storage period, then decreased (6.7 log CFU mL^{-1}) with increasing the period **(Fig. 5 b and c)**. The addition of honeybee had a significant decrease on the viability of *Leuconostoc mesenteroides*(6.5 log CFU mL^{-1}), *Lactococcus lactis diacetylactis* (6.5 log CFU mL^{-1}) and total bacterial count (6.7log CFU mL^{-1}) during 10 days of storage period if compared with control (8.3 log CFU mL^{-1}). *L. mesenteroides* decreased as the fermentation products increased, due to their sensitivity to acid conditions [31]. It was observed that the highest loss in the viability of *Leuconostoc mesenteroides* was on the 20^{th} day of storage for both of the control and treatment samples, respectively. In the case of *Lactococcus lactis diacetylactis* the loss was 29.5 and 44.2% for the control and treatment samples, respectively. Although, the decrease in the numbers of lactic acid bacteria, from the previous results, it was cleared that the viability of lactic acid bacteria was in the required border (10^6 CFU mL^{-1}) until the 20^{th} day during cold storage in the control samples, while they were within the required limits until the 15^{th} day for treatment samples under the same storage conditions.

It considered that honey has a prebiotic activity due to the variety of oligosaccharides it contains. Also, the prebiotic characteristic of honey can vary with different composition as a result of various types of honeybee. It may be because of the different types of plants that visited by the bees [15]. Oligosaccharides such as inulin and oligo fructose, which are found in most of thefood such as vegetables, fruits, milk, and honey, considered as the main prebiotics used in the food industry [32]. However, the presence of honey and green kiwi fruit resulted reducing in pH values of probiotic kiwi whey-based beverages as shown previously in **Fig.(1),** which cause a deficiency in the number of *Leuconostoc mesenteroides, Lactococcus lactis diacetylactis* and total bacterial count in the treatment samples, compared to the control one **Fig(5a, b and c)**. *Leuconostoc* is one of the most lactic acid bacteria that can grow on high concentrations of sugars and osmotic pressure. It can also grow on fruit mashes and some classical beverages in Africa [33].

According to many references, fermented milk must present counts of total lactic bacteria of at least 10^6cfu/ g[34].Whey acts as a protective source to microorganisms, also fruit such as kiwi had nutrient sources that containing minerals, vitamins, dietary fibers and antioxidants is a promising carrier for probiotic bacteria [35]

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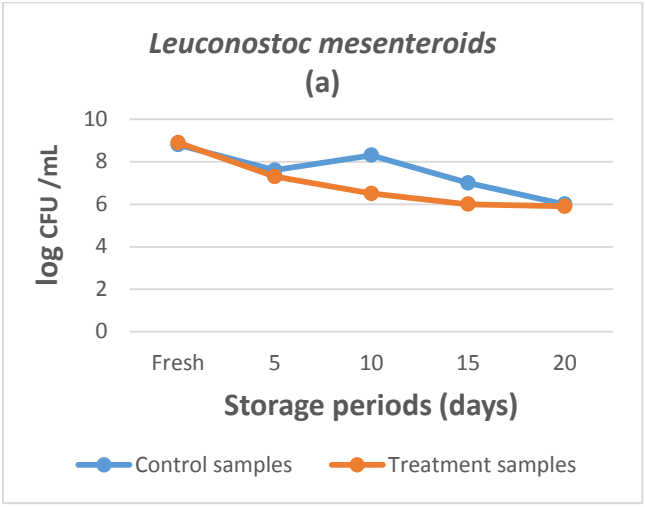

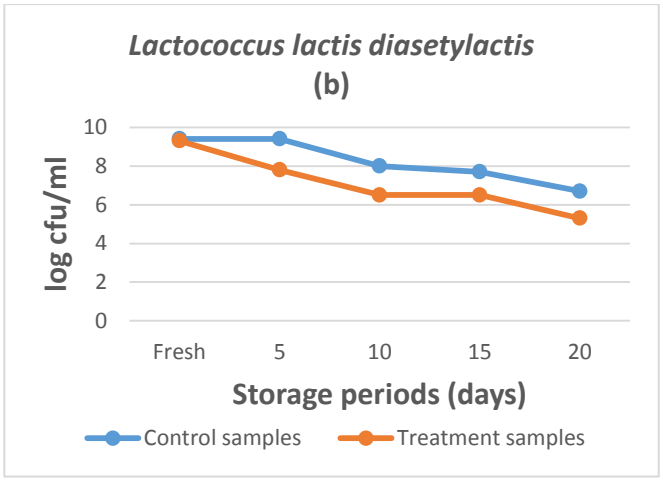

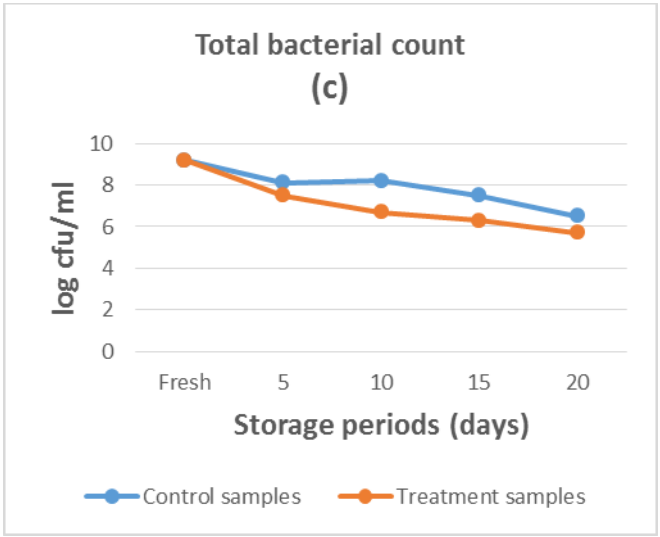

Fig. (5): The viability of LAB (a) *Leuconostoc mesenteroids* (b) *Lactococcus lactis diacetylactis* and (c)TBC in probiotic kiwi whey-based beverage samples during the storage period.

See footer of Fig. (1)

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4.3.2. Isolation and Identification of fungi

Six fungi were isolated and identified as *Aspergillus flavus* (sweet whey and green kiwi fruit), *Aspergillus niger* (green kiwi fruit), *Aspergillus versicolor* (honeybee), *Penicillium spp* (sweet whey and green kiwi fruit), *Fusarium spp* (green kiwi fruit) and *Alternaria spp* (sweet whey) as presented in Table (3). The results showed that kiwi fruit contained the highest number of fungi isolated when compared to other samples. Despite the high water activity of most fruits and vegetables, the low pH, high levels of sugars and nutrients element especially of fruits gives fungi a competitive advantage over the majority of bacteria and mold spoilage is uncommon[10].These results were agreed with **Pescheck**, ***et al***.,[36] who isolated *Penicillium solitum* from kiwi fruit. Both *A. flavus* which has been directly correlated with adversehealth effects, such as liver cancer and *A. niger* is considered parasitical fungi on man and animals. They cause many diseases called Aspergillos.

The frequency of occurrences of fungi species was exhibited in Table (4).Presented results revealed that *A. flavus* and *Penicillium spp* had the highest occurrence in substrates under this study with a frequency of 25% followed by *A. niger*, *A. versicolor*, *Fusarium spp* and *Alternaria spp* in which they had the lowest occurrence with a frequency of 12.5%. The results were compared with the investigation of **Tournas *et al*.,** [37] who found that thesixty-five pasteurized fruit juice samples were tested for fungal contamination. Twenty–two percent showed fungal contamination However, the results indicated that honeybee has the least occurrence of fungi when compared to other samples, where only *A. versicolor* was isolated (Table, 4). The isolated fungi are of economic and public health importance. These findings suggested that fruits and processed fruit products should be handled carefully with consideration given to fungal contaminants, including nonpathogenic fungi to control the quality them.

Table (3): Isolation and identification of fungi.

Substrates	Fungi isolate
Sweet whey	*Aspergillus flavus, Penicillium* spp, *Altrnaria* spp.
Honeybee	*Aspergillus versicolor*
Green Kiwi fruit	*Aspergillus flavus, Aspergillus niger, Penicillium spp, Fusarium spp*

Table (4): Frequency of occurrence of fungal species.

Fungi isolate	Substrates	Number isolated	Frequency of occurrences %
A. flavus	Sweet whey, Green Kiwi fruit	2	25
A. niger	Green Kiwi fruit	1	12.5
A.versicolor	Honey bee	1	12.5
Penicillium spp.	Sweet whey, Green Kiwi fruit	2	25
Fusarium spp.	Green Kiwi fruit	1	12.5
Alternaria spp.	Sweet whey	1`	12.5

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4.3.3. Detection of antifungal activity:

In the current study, control and treated samples were used as antifungal activity against tested fungal *A. flavus* NRRL 3359, *A. ochraceus*NRRL3174 and *Penicillium expansum* NRRL6069.The results in Table (5) displayed different inhibition against tested fungal. The data appeared that the treated sample manifested antifungal activity toward *A. flavus* NRRL 3359 growth which recorded 35 mm after 7 days of incubation periods. While, the inhibition zone recorded 12 and 10 mm for both *A. ochraceus*NRRL3174 and *Penicillium expansum* NRRL6069,respectively. On the other hand, the antifungal activity of the control sample against the three tested fungi *A. flavus* NRRL 3359, *A. ochraceus*NRRL3174 and *P. expansum* NRRL6069 were 25, 10 and 6 mm, respectively. Our results were compared with data of **DeMera and Angert** [38] who reported that honey from different phyto-geographic regions varied in their ability to inhibit the growth of bacteria and yeasts, suggesting that botanical origin plays an important role in influencing the antimicrobial activity. In addition, there are a great variety of components, including phenolic acids, flavonoids and other biomolecules, in a different biological activity of honey is mainly attributed to the phenolic component.

Also, the presence of Lactic Acid Bacteria (LAB): *Leuconostoc mesenteroides* and *Lactococcus lactis diacetylactis* in beverages under investigation enhanced the antagonisticactivity. The mode of action of LAB as an antifungal agent can be summarized, for their production of several compounds such as hydrogen peroxide, acetic acid, reuterin, diacetyl and bacteriocins which contribute to its preserving capabilities [39].

Table (5): Detection of antifungal activity of control and treated samples.

Samples	*A. flavus* NRRL 3359	*A.ochraceus* NRRL 3174	*P. expansum* NRRL6069
Control	25	10	6
Treatment	35	12	10

The activity was determined by a well diffusion assay, and diameters of the inhibition zones were measured and expressed as millimetres (mm).

4.3.4. Diagnosis of some mycotoxins:

Aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A and patulin were detected of all samples under investigation. The data represented that all samples were free from all these mycotoxins. These results were conformable with, **Hegazy**[40] who stated that about 30 samples of fresh tomato collected from Giza Governorate were free from aflatoxins and ochratoxin A**.** Also**, Moss** [10]reported that the most significant mycotoxins are not normally a problem in fresh fruits and vegetables.

5. CONCLUSION

The goal of this research was to add a new product to the Egyptian market. This product is characterized by a high health aspect, as well as to benefit from a by-product that was dumped into the sewage, causing environmental pollution. Acquired results suggested that adding 10% kiwi juice and 15% honeybee gave the final product an acceptable taste as increasing antioxidant and probiotic activity. This product is valuable according to its high antioxidant activity and probiotics effect. Also, the

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product showed promising antifungal activity, especially on *A. flavus*.Besides, this study was trying to make a practical use of the sweet whey in producing a new healthy and milky product instead of throwing the whey into the sewers and increasing the environmental pollution.

6. SIGNIFICANCE STATEMENT

This study was an attempt to take advantage of the sweet whey resulting from the manufacture of semi and hard cheese, which was dumped into the sewage and causing environmental pollution. It is known that whey contains many important whey protein fractions such as β- lactoglobulin, α- lactalbumin, immunoglobulins and lactoperoxidase that are healthy, fighting many diseases. It also contains lactoferrin fraction which advanced researches have proven its impact in resisting the newly defined coronavirus (COVID˙19). Therefore, several studies should be using whey protein fractions on feeding experimental rats. Also, probiotic bacteria have a magnificent effect on whey protein fractions and give many healthy bioactive and opioid peptides, which have a great biological effect on the human being. Additionally, honey also raises the immunity of the human body because it contains important nutrients, minerals and vitamins.

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