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# GROWTH AND VIABILITY OF *BIFIDOBACTERIUM LACTIS* BB-12 IN PROBIOTIC HIGH PROTEIN WHEY BEVERAGES

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## Abstract

The study concerned with the manufacture of probiotic high protein whey beverages with *Bifidobacterium lactis* BB-12 as starter culture by using sweet whey obtained directly from the soft cheese industry and supported with five protein sources by adding 2 g /100 mL each separately, three of which included the centers of whey proteins (WPC 80). (WPC 81) and whey protein isolate (WPI 90) and two types of powdered milk (skim and whole) in order to raise its total solids and enhancing its nutritional value, in which the mixture were Exposed to two kinds of heat treatments before incubation at 37 ° C for 24 hours. The live cell bacterial count was calculated with an estimate of the pH and the titratable acidity of the products. The probiotic bacteria showed good growth as the highest logarithm of the number of their live cells reached 8.73 CFU/mL in the whey beverage with protein WPC 80, and showed the shortest generation time 40 minutes for these bacteria, the results of the sensory evaluation showed the possibility of using these probiotic bacteria to develop high-protein fermented Beverages with improving their function and sensory properties. During which the whey supported with protein WPI 90 and sterilization treatment obtained the highest levels of probiotic high protein whey beverage evaluation.
***Keyword***: Bifidobacterium, lactis BB-12, Probiotic

## Introduction

Whey is the largest by-product in terms of quantity in the dairy industry. It is the remaining aqueous portion of rennet or sour milk after the removal of protein mass, which is about 85-90% of the volume of milk used in cheese-making processes, It retains about 55% of milk nutrients, Its consisting of lactose, water, whey proteins, minerals and fat residues (5, 93, 0.85, 0.53 and 0.36), respectively, is an ideal source of many bioactive peptides that have a positive impact on human health and have many roles (McSweeney and Fox, 2013; Mohanty *et al.*, 2015). For years, efforts have been made to convert large quantities of it into a suitable food product. Currently, whey is no longer a by-product but a co-product, including the introduction of whey proteins into the dairy industry (Alves *et al.*, 2014). A wide range of whey ingredients are available for use in the yogurt and fermented beverages industry, including sweet whey powder (SWP), whey protein concentrate (WPC) and whey protein isolate (WPI) (Shiby *et al.*, 2013). The need to develop whey-based beverages depends on the nutritional and functional properties of whey proteins, as well as on consumers' desire and tastes for innovative products with improved functions (Chavan *et al.*, 2015b). The global trend has recently increased to produce high-quality foods, including high-protein beverages from whey and fermented with probiotics because proteins are essential components of the body and are required in large quantities by all age groups, including athletes. These products are necessary for growth and metabolic regulation of humans (Singh *et al.*, 2018). There are numerous studies on the health benefits associated with the consumption of fermented dairy products. Therefore, the food industry develops new fermented milk products with health benefits each year. The effectiveness and metabolic activity of bacteria is an important feature of the inclusion of probiotics in beverages because bacteria need to survive in beverages during Shelf life, and the use of probiotics bacteria has increased in recent years for health purposes and benefits based on class and type of Bacteria (Mutukumira et al., 2015). probiotic are microorganisms (mostly bacteria) that have the ability to cross the upper digestive tract and reach the small intestine and stability in numbers as low as $10^6$ cfu \ g as a minimum to restore the natural balance of the host intestinal flora and events of health effect by preventing and treating many diseases, by including them in the production of therapeutic foods as in Fermented Dairy Products (Prakash *et al.*, 2016; De Prisco and Mauriello, 2016).

## Materials and Methods

### Probiotic bacteria:

*Bifidobacterium lactis* BB-12 from (Probiotic QUEST) was used as a capsule after activation as a manufacturing Probiotic starter culture.

### Culture media:

**(A) Liquid medium Man Rogosa Sharpe Broth(MRSb) :** The medium was used at pH 6.5 in the development and activation of bacteriostatic primer bacteria and prepared according to the instructions of the company equipped (Oxoid) and the addition of 0.05% of L-Cysteine to make it oxygen-free according to (Holt et al., (1994) .

**(B) Solid medium Man Rogosa Sharpe Agar (MRSa):** used in counting live bacteria by pour plate method.

### Whey and Whole milk and Skim milk:

Was used soft cheese whey produced by the dairy factory College of Agricultural Engineering Sciences / University of Baghdad, and the use of whole milk powder from Millac company Ireland Origin, and the use of skim milk powder from the company Regilait French origin fat 0% and recovered by solids 12% (w/v) sterilizer device at a temperature of 121 C / 5 minutes It was used to activate the starter of bacteria and for industrialization.

**Whey proteins**

Three kinds of dried whey proteins were supplied from abroad through electronic shopping and shipped by DHL. Two of them are concentrates of whey proteins which contain protein ratio (80 and 81)% and the third protein is whey protein isolate which contains protein 90% and packed in special envelopes with weights ranging from (500-700) grams and support the liquid whey by 2 g / 100 ml.

**Manufacture of probiotic high protein whey beverage**

The ratio of solids to sweet whey was increased by addition of WPI and WPC, powdered skim milk and whole milk powder individually by 2 g/100 ml and then two heat treatments (slow pasteurization 63 C/30 min and sterilization 121 C/5 min) and then inoculated using the initiating therapeutic bacteria by 10% and incubation on The temperature is 37 C for 24 hours.

**Chemical approximate analysis and qualitative tests**

The percentage of fat, protein, lactose, total solids, and whey moisture was estimated using the German-origin Milkoscane FT2, and the lactose content of whey was estimated according to (Egan et al., 1981), Protein was determined by the KielDahl method according to( A.O.A.C, 2008), and ash was estimated by the method described in (A.O.A.C, 2010).The pH of the models was measured using a Beckman-equipped pH-meter at 25 ° C. The total acidity was estimated according to the method described by (Elmer, 1978).

**Microbial count**

Estimation of the number of therapeutic bacteria and the total number of whey models supported by the casting method in the pure plates (Speak, 1984).

**Sensory evaluation**

The sensory evaluation of the products was carried out according to a questionnaire form (Nelson and Trout, 1964) modified by (Al- Dhahir, 1999), The product was sensually evaluated by a group of specialists from professors and graduate students in the Department of Food Science \ University of Baghdad, as well as some workers in dairy factories Abu Ghraib.

**Statistical analysis**

Statistical Analysis System -SAS (2012) was used to analyze the data to study the effect of different coefficients on the studied characteristics according to a complete random design (CRD), Significant differences between the mean were compared with the test of the least significant difference (LSD).

## Results and Discussion

**Microscopic and phenotypic examinations of *Bifidobacterium lactis* BB-12:**

The phenotypic tests showed the characteristics of the colonies formed at the growth of the *Bifidobacterium lactis* BB-12 bacterial culture on MRS by the casting method in the pure plates and under anaerobic conditions for 48 hours as shown in Figure 1, Their colonies were circular and convex in shape, lustrous, soft to the touch and sticky, slightly yellowish-white and full-bodied edges, They are consistent with the characteristics of *Bifidobacterium* colonies, according to (Scardovi, 1986), It was noted that the aforementioned agricultural medium has allowed the growth of these bacteria well because it is a complex medium containing all the nutrients necessary for growth, especially when incubating in anaerobic conditions, which is an important requirement for these bacteria, While it did not get growth on the nutrient agar medium as confirmed by (Dave and Shah, 1996), the growth was lower on the skim milk medium compared to the hard agricultural medium MRS.

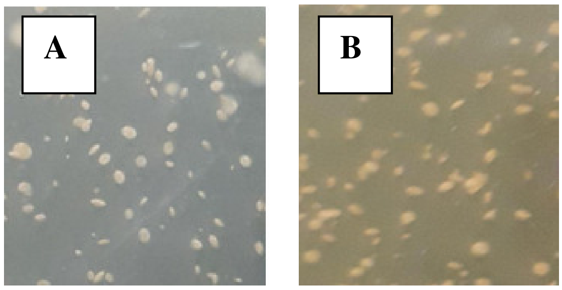


**Fig. 1 :** Bacteria colonies grown on the recovered skim milk medium (A) and on the hard agricultural medium MRS agar (B) by the pure plates at 37 ° C incubation for 48 h under anaerobic conditions.

**Photos of the bacteria *Bifidobacterium lactis* BB-12**

Microscopic images of *Bif. lactis* BB-12 culture slides in MRS and 12% reconstituted skim milk medium and in WB1, WB2, WB3 and WB4 whey beverages when incubated at 37 °C for 24 hours were shown in Figure (2) shows. Microscopic examination of the cells of these bacteria that they were convergent qualities in the mentioned media where they were short and heterozygous bacillus with a curved form swollen from the center and was single or bilateral and sometimes organized in the form of short chains, which is positive for dye gram and non-formation of spores and these attributes are consistent with what indicated (Willey *et al.*, 2008).

| *Bifidobacterium lactis* **BB-12** | | | | | |
|---|---|---|---|---|---|
| **Whey beverages** | | | | **Culture media** | |
| WB1 | WB2 | WB3 | WB4 | MRS broth | Skim milk |

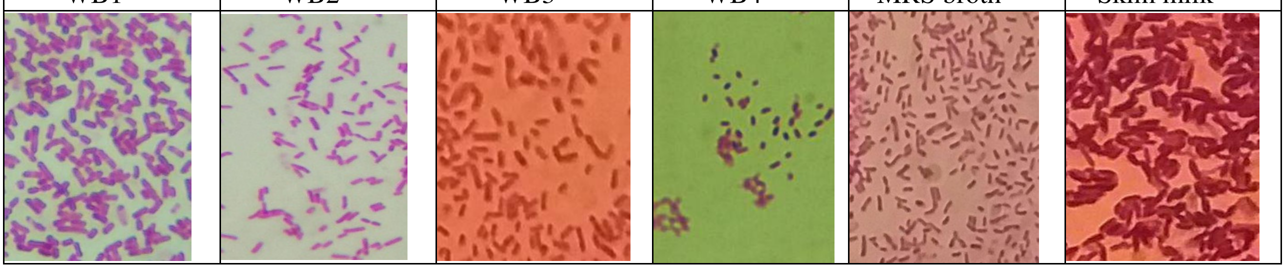

**Fig. 2 :** Microscopic images of *Bif. lactis* BB-12, WB1 represents the whey beverage fortified with WPI 90, WB2 represents the whey beverage fortified with WPC 81, WB3 represents the whey beverage fortified with WPC 80, WB4 represents the whey beverage fortified with Skim milk powder.

**Chemical composition of whey**

The results of chemical analysis of sweet whey under study obtained from dairy laboratory College of Agricultural Engineering Sciences / University of Baghdad were shown in Table (1), where it was found that the results were normal, except fat percentage was high in the whey under study, which is 0.2% compared with the percentages mentioned in Previous studies are 0.05% (Barukčić et al., 2019), This may be due to thrombus slicing in smaller cubes than recommended with violent clotting or because of the length of time between thrombus slicing and filling, or because the thrombus temperature increases during slicing, resulting in an increase in clarity of fat and then loss with whey (Yaseen et al., 2012), The decrease of total solids content in the whey under study is 5.6 compared with 6.0 in the previous studies it may be due to the low percentage of total solids in the milk source used in the cheese industry and the result of the whey being studied.

**Table 1 :** The chemical composition of sweet whey

| Ash | Lactose | protein | Fat | Total solids | Water | Components % |
|---|---|---|---|---|---|---|
| 0.5 | 4.3 | 0.6 | 0.2 | 5.6 | 94.4 | Sweet whey |

**Manufacture of probiotic high-protein whey beverages**

Table (2) shows the effect of whey protein isolates, whey protein concentrates and milk powder separation using two heat pasteurization and sterilization treatments on the physical properties of bio-whey beverages using *Bif. lactis* BB-12, the highest Primary pH in sterile WB3 was 6.20 and the lowest in sterile WB4 was 6.00, With no significant differences between all treatments, The highest pH after fermentation was found to be sterile WB4 of 4.90 and the lowest in pasteurized WB1 was 4.64, with no significant differences between all treatments, this is consistent with (Božanić *et al*., 2004) and (Marafon *et al*., 2011) found that no significant differences in pH were found between treatment containing whey protein and control. The highest pH decrease was in sterile WB2 which was 1.52 and the lowest in sterile WB4 was 1.10, With no significant differences on the probability level of 0.05 between all treatments.

The Primary acidity of all sterilized heat treatment models increased compared to the slow pasteurization treatment and the highest was 0.20% in the sterilized WB1 model, The lowest in WB3 and WB4 is 0.15% pasteurization, with no significant differences between all models, It was found that the highest titrate acidity value was in the sterile WB1 treatment of 0.79% and the lowest in the pasteurized WB4 treatment of 0.58%, With no significant differences on the probability level of 0.05 between all treatments. this is consistent with (Vargas Lopez and Alfonso, 2013) that the addition of WPI leads to increased acidity of the final product compared with the control model, and agreed with ( Dave and Shah, 1998b) that high thermal treatment during manufacturing processes increases the availability of amino acids and peptides needed for the growth of lactic acid bacteria, this is reflected in the increased production of lactic acid, and what (Akalin *et al*., 2007) explained of the same effect when adding whey proteins In addition to whey blends, high temperatures during the heat treatment process have increased the availability of amino acids and peptides.

The highest advanced acidity value in the sterile WB1 was 0.59% and the lowest was the pasteurized WB4 of 0.43%, With no significant differences on the probability level of 0.05 between all treatments. The WB1 sterile treatment was significantly different with all treatments except WB2 which was 0.54% and the pasteurized and sterile WB3 were 0.50% each.

**Table 2 :** Manufacturing of whey beverages (WB1, WB2, WB3, WB4) Bio-enhanced by *Bif. lactis* BB-12

| Acidity % | | | pH | | | Heat Treatment | Whey Beverages |
|---|---|---|---|---|---|---|---|
| **Developed** | **Titratabled** | **Primary** | **Decline** | **After fermentation** | **Primary** | | |
| 0.48 | 0.64 | 0.16 | 1.45 | 4.64 | 6.09 | P | **WB1** |
| 0.59 | 0.79 | 0.20 | 1.45 | 4.70 | 6.15 | S | |
| 0.47 | 0.64 | 0.17 | 1.36 | 4.77 | 6.13 | P | **WB2** |
| 0.54 | 0.72 | 0.18 | 1.52 | 4.65 | 6.17 | S | |
| 0.50 | 0.65 | 0.15 | 1.35 | 4.80 | 6.15 | P | **WB3** |
| 0.50 | 0.68 | 0.18 | 1.50 | 4.70 | 6.20 | S | |
| 0.43 | 0.58 | 0.15 | 1.31 | 4.87 | 6.18 | P | **WB4** |
| 0.45 | 0.61 | 0.16 | 1.10 | 4.90 | 6.00 | S | |
| 0.193 NS | 0.259 NS | 0.066 NS | 0.503 NS | 0.622 NS | 0.702 NS | **LSD Value** | |

*****(P<0.05) WB1: whey beverage fortified with WPI 90, WB2 : whey beverage fortified with WPC 81, WB3 : whey beverage fortified with WPC 80, WB4 : whey beverage fortified with Skim milk powder. P : Pasteurization, S : Sterilization.

**Logarithmic numbers of living bacterial cell and growth curve**

Figure (3) shows the growth curve and Table (3) the logarithm of living cells of *Bifidobacterium lactis* BB-12 at the time (24, 21, 18, 15, 12, 9, 6, 3, 0) during their growth in (WB1, WB2, WB3, WB4, WB5) and per treatment. The effect of whey protein isolate, whey protein concentrates, dried skim milk, and whole milk powder was found in the live numbers of *Bif. lactis* BB-12 The treatment WB3 surpassed the rest of the treatments with the highest logarithm of 8.73 followed by the treatment WB1 where the logarithm of live number 7.95, The lowest logarithm of WB4 was 6.77, and these results were comparable to those found by (Herrero and Requena, 2006), (Slačanac *et al*., 2010) and (Slačanac *et al*., 2013) when studying the viability of *Bifidobacterium lactis* BB-12 in bovine milk after a certain period of fermentation.

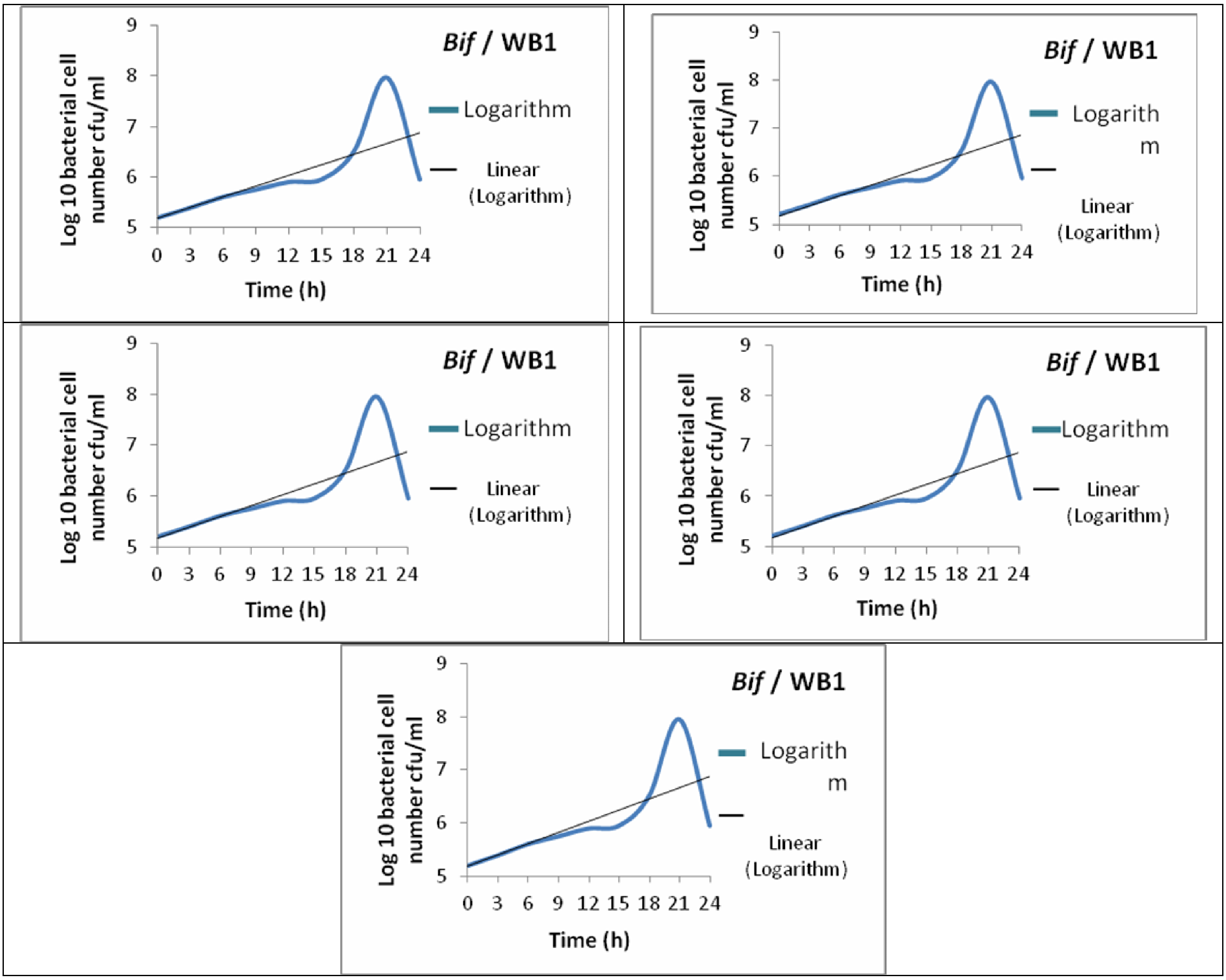


**Fig. 3 :** Growth curve of *Bif. lactis* BB-12 as it grows in milk )WB1, WB2, WB3, WB4, WB5) WB1: whey beverage fortified with WPI 90, WB2 : whey beverage fortified with WPC 81, WB3 : whey beverage fortified with WPC 80, WB4 : whey beverage fortified with Skim milk powder.

**Table 3 :** Logarithm of cell numbers of *Bif. lactis* BB-12 as it grows in milk )WB1, WB2, WB3, WB4, WB5)

| Logarithmic number / treatment | | | | | Times |
|---|---|---|---|---|---|
| **WB5** | **WB4** | **WB3** | **WB2** | **WB1** | |
| 6.11 | 5.24 | 5.15 | 5.60 | 5.39 | 0 |
| 5.46 | 5.53 | 5.36 | 5.47 | 5.20 | 3 |
| 5.61 | 5.76 | 5.63 | 5.69 | 5.61 | 6 |
| 5.82 | 5.77 | 5.82 | 5.90 | 5.75 | 9 |
| 5.90 | 5.84 | 6.00 | 6.00 | 5.90 | 12 |
| 6.54 | 5.90 | 6.00 | 5.90 | 5.95 | 15 |
| 6.60 | 6.71 | 7.69 | 7.20 | 6.51 | 18 |
| 7.69 | 6.77 | 8.73 | 5.90 | 7.95 | 21 |
| 6.56 | 6.69 | 6.04 | 5.60 | 5.95 | 24 |

WB1: whey beverage fortified with WPI 90, WB2 : whey beverage fortified with WPC 81, WB3 : whey beverage fortified with WPC 80, WB4 : whey beverage fortified with Skim milk powder.

**Generation time measurement**

Generation time was measurement for *Bif. lactis* BB-12 when grown in whey beverages WB1, WB2, WB3, WB4 and WB5, to identify the speed of growth of these types of bacteria in the product, reaching (54,90, 40, 124.1 and 93.9) minutes, respectively, and agree with both (Gupta *et al.*, 2006; Oliveria, 2011) which varied for species of lactic acid bacteria between 1.5-1 hours.

It turns out that the shortest generation time for *Bif. lactis* BB-12 is 40.0 minutes in the WB3 whey beverage, and this result was comparable to what (Cicvarek *et al.*, 2011), which found the shortest generation time for *Bif. lactis* BB-12, was 38 minutes when studying the effect of adding CMP on the growth of these bacteria.

**Identify the effect of reinforcement with different protein sources on the sensory qualities of therapeutic whey beverages**

Table (4) shows the effect of reinforcement with different protein sources on sensory qualities of whey therapeutic beverages (WB1, WB2, WB3, WB4, WB5) and one day old in the refrigerator at a temperature of (5 ± 1 ºC), where the highest flavor value was for the treatment WB1 was (29.4) and lowest in WB5 (17.8), With significant differences in probability level (P <0.05) between them, and the treatments WB1, WB2 and WB3 differed significantly with WB4 and WB5, and agreed with what (Skryplonek et al., 2019) found in fermented acid whey with probiotic *Bif. Lactis* BB-12, He explained that to the bacteria *Bif. Lactis* BB-12 has the ability to manufacture acetaldehyde aromatic compound , an essential ingredient in the smell of fermented milk beverage that is manufactured from Lactose or amino acids are all present in the whey, As reported (Magalhães *et al*., 2011), the production of acetaldehyde from whey may be as dense as in milk.

The results showed the highest textures in WB1 (28.6) and lowest in WB4 (25.4) With significant differences in probability level (P <0.05) between them, and it did not differ significantly with other treatments As for the live numbers of the initiating bacteria in the treatments, there is a superiority of treatment WB3 and obtained 12 degrees and the lowest in the treatment WB4 and is 6 degrees, while the highest titrate acidity value in the treatment WB1 and reached 7.9 and the lowest in the treatment WB5 and reached 6.0 with a significant differences on the level of probability (P <0.05) between them, and the highest appearance value was in WB1 which was 8.5 and the lowest in WB5 was 6.1, with a significant differences on the level of probability (P <0.05) between them, and the treatments WB1, WB2 and WB3 differed significantly with WB4 and WB5.

**Table 4 :** Sensory evaluation form for Probiotic high protein whey beverages )WB1, WB2, WB3, WB4, WB5)

| **Total (100)** | **Appearance (10)** | **Acidity** (10) | **Bacteria count** (15) | **Textures (30)** | **Flavored (35)** | **Treatment** |
|---|---|---|---|---|---|---|
| 83.4 | 8.5 | 7.9 | 9 | 28.6 | 29.4 | **WB1** |
| 81.6 | 8.4 | 7.2 | 9 | 28.2 | 28.8 | **WB2** |
| 81.2 | 8.0 | 6.8 | 12 | 27.1 | 27.3 | **WB3** |
| 61.8 | 6.3 | 6.1 | 6 | 25.4 | 18.0 | **WB4** |
| 65.5 | 6.1 | 6.0 | 9 | 26.6 | 17.8 | **WB5** |
| 5.943 * | 1.318 * | 0.941 * | 2.66 * | 3.07 * | 4.39 * | **LSD Value** |

***(P<0.05)** WB1: whey beverage fortified with WPI 90, WB2 : whey beverage fortified with WPC 81, WB3 : whey beverage fortified with WPC 80, WB4 : whey beverage fortified with Skim milk powder.

As for the total sensory characteristics 100% in the treatments, WB1 surpassed the rest of the samples and obtained a score of 83.4%, followed by treatment WB2 and WB3 (81.6 and 81.2)% respectively, while the treatment WB4 obtained the lowest evaluation 61.8%, and also showed some significant differences between the treatments, we conclude that the treatments supported by whey proteins WB1, WB2 and WB3 obtained a higher degree of evaluation than the samples supported by skim milk and whole milk are WB4 and WB5 respectively.

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