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Effect of Drying by Vacuum Oven on Viability of *Lactobacillus Casei* and *Bifidobacterium Longum* Bacteria

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ABSTRACT

The reconstitution skimmed milk in concentrations (12, 20, 25) % were used for *Lactobacillus casei* (*Lb. casei*) and *Bifidobacterium longum* (*Bif.longum*) growth in 37°C for 24-48 hours after individually inoculation with 10% of starter. The highest growth rate was achieved by 20% (w/v). The logarithmic numbers of bacteria were 10.04 and 9.85 for *Lb. casei* and *Bif.longum* respectively, whereas titratable acidity percentages of fermented milk was 0.72 and 0.65 respectively. The pH values of the 20% reconstitution skimmed milk were to 4.6 and 4.9 respectively. Lactic acid bacteria cultures were dried by vacuum oven with or without protectants (dried skimmed milk). The drying affected were studied on viability of lactic acid bacteria. Drying in vacuum oven gave the best results when protectants material was added, logarithmic viable numbers were 9.14 and 8.93 for *Lb. casei* and *Bif.longum* respectively, followed by dried cultures without protectants which registered 9.06 and 8.81 respectively. The moisture contents were 4.14 and 4.0 % for *Lb. casei* and *Bif.longum* respectively in dried cultures by vacuum oven with protectants, while were recorded 4.35 and 4.23 % for *Lb. casei* and *Bif.longum* respectively for cultures were dried without protectants. Vacuum oven dried probiotics were used to produce bio yogurt as dried or rehydrated. Dried probiotic of *Lb. casei* and *Bif.longum* with yogurt starter achieved highest account of starters and improved sensual attributes compared with rehydrated probiotic and conventional yogurt.

Key words: *Bif. longum*; bio yogurt, *Lb. casei*;protectant, vacuum oven.

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INTRODUCTION

Due to personal health concerns, consumers expect food to be nutritious and disease preventative. This is reflected by the popularity of probiotic-based products. Lactic acid bacteria (LAB) are living microorganisms that improve microbial balance in gut flora and have a positive health impact. Food biotechnology relies heavily on lactic acid bacteria to produce a large variety of fermented foods, such as cheese, yogurt and sourdough bread .Probiotics play a key role in the food and dairy industries, and probiotics have been shown to exert positive effects on health status of the host. Powdered probiotics are convenient for subsequent processing and transportation and have longer shelf lives compared to those of liquid probiotics (Santivarangkna *et al*, 2008). The first probiotics introduced in research work were *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.(Jayaprakash *et al*, 2023). *Lactobacillus* is Gram-positive, non-spore forming, rod or coccobacilli ranging from (0.5-1.2) to (1-10) µm in size are observed in rich habitats containing carbohydrate such as food; fermented foods, habitats of heavy microbial host like the normal microbiota of mouth, female genital tract and gastrointestinal tract (GIT) of animals and humans (Lang *et al*, 2022). *Lactobacillus casei* is one of the most important types used in the manufacture of medicinal and therapeutic food products, as it is characterized by its high ability to resist

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extreme acid conditions in the stomach and bile salts all the way to the intestinal canal. A role in strengthening the immune system, preventing colon cancer, and lowering cholesterol levels by converting it to Coprostanol and then removing it, as well as helping in the digestion and absorption of nutrients, resisting diarrhea, and reducing acne (Dimitrellou *et al*,2019, Poddar *et al*, 2021). *Bifidobacterium longum* is present in the large intestine and vagina and has the ability to produce lactic and acetic acid, which work to prevent the growth of unwanted microorganisms in the intestinal flora by lowering the pH of the large intestine as well as its role in improving the immune system and has the ability to prevent the growth of ulcer-causing bacteria, as it is a type of intestinal flora in breastfed children, it can effectively settle in the intestines of infants and helps to reach the better weight in children born with very low weights (Nguyen *et al*,2016, Shokri *et al*, 2015). Probiotics work by keeping balance of useful bacteria in the gut, in order to help improve nutrient digestion, absorption, boost immunity, and reduce the disease threats .The loss of viability of probiotics during processing, storage, and after digestion has been widely documented, and it has been established that any food claiming to have a probiotic effect should contain at least 10^6 to 10^7 cfu ml^{-1} of viable probiotic bacteria, as health benefits are directly related to the number of viable cells present at the time of consumption. Considering the human health implications of this issue, as well as the economic ones, since the global probiotic market is projected to increase to around USD 110 billion by 2030 (Acosta-Piantini *et al*, 2024). Despite this importance, storage stability of probiotics was reported mostly from studies conducted with cells from standard industrial drying process, i.e. freeze drying. Alternatively, to freeze drying, the interest in alternative drying processes is increasing because of their lower costs compared to freeze drying. Nevertheless, only little information on storage stability of dried powder from the alternative drying processes is available. However, to the best of the authors' knowledge, there is no information available on the storage stability of probiotics dried by vacuum drying, which is a promising alternative drying process (Gul, 2017). Current technology has been developed to create dehydrated probiotics that are shelf-stable at room temperature, maintain a high concentration of living cells, and are acid-stable and bile resistant (Dimitrellou *et al*, 2016). For vacuum drying, the materials are maintained at a certain temperature and vacuum level to keep the material in a non-frozen state throughout the process, therefore, cells are metabolically active during the drying process, and the combination of different kinds of protectants may improve the survival of cells. Trehalose and sucrose are predominantly used saccharides protectants, sodium glutamate is frequently applied antioxidants, and polyvinyl pyrrolidone (PVP) and skim milk are colloidal protection compound and stabilization for cell membrane, respectively (Ying *et al*,2016). Yogurt was known for its high nutritional and hydration levels, ability to reduce lactose intolerance, increase beneficial bacteria such as *Bifidobacterium longum*, inhibit growth of intestinal pathogenic bacteria, and improve constipation. *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. casei*, and *Streptococcus salivarius* subsp. *thermophilus* are the major lactic acid bacteria used in yogurt production and given its improved nutrition, digestibility, unique flavor, and various physiological functions, the demand for yogurt is steadily increasing worldwide (Simões Bandiera *et al*,2013). In the present study, it was aimed to investigate the best percentage of fermented milk recovery for the development of *Lactobacillus casei* and *Bifidobacterium longum* and determined the viability of the rehydrated cells after vacuum drying by CFUs counts on plates with and without presence of skimmed milk as protective substance as well as the effeteness in bio yogurt production.

MATERIALS AND METHODS

1. Starter media: The reconstituted skimmed milk (RSM) was prepared in proportions (12, 20, 25) % (w/v) and sterilized at 121 °C, 15 b/in^2 for 5 minutes, after which the treatments were inoculated with *Lactobacillus casei* and *Bifidobacterium longum* bacteria (supplied by

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the University of Baghdad / College of Agricultural engineering sciences/ Department of Food Sciences) by 10% and each separately, then incubated at 37°C until coagulation complete (Sang *et al*,2023).

2. Protectant agent preparation: Dried skimmed milk was used as a protective material when drying bacterial cultures. The method mentioned in (Robinson,2005) was followed. And modified as follows in the sterilization of dried sorting milk: 100 g of dried sorting milk was taken and spread in a sterilized stainless steel container in the form of a thin layer of about 1 mm, then an Ultra Violet ray source was placed at a height of 15 cm from the container was for three hours, after which it was transferred in a sterile manner in sterile bottles and kept until use, and the efficiency of sterilization was confirmed by detecting the total number of microorganisms, the coliform bacteria and yeasts and molds count (Houghtby *et al*,1992).

3. Sample preparation and vacuum drying: Vacuum drying was performed according to the method of (Cerrutti *et al*, 2000) with some modifications. The growth conditions, initial cell concentration, vacuum drying temperature, rehydration material and temperature were standardized in order to eliminate the effects of these factors on the viability of starter bacteria during vacuum drying. The bacterial cultures solution (100 ml) was mixed with equal volumes of the double concentrated protectants (dried skimmed milk) solutions. The aliquots of the mixed samples (2 ml) were added to a 4 × 25 mm tared dishes, which were placed in a vacuum oven (DZF-6051) to be dried at 40 °C under a vacuum of 0.07 mpa. The drying was finished approximately for a period of 24-28 hours.

4. Determination of bacteria viability within vacuum oven drying: For the determination of viable cells *Lactobacillus casei* and *Bifidobacterium longum*, diluted samples were pour plated on MRS agar plates (HiMedia, India) after a 10-fold serial dilution in peptone water. After solidification of the agar, individual bacterial cells were fixed, which allowed them to multiply during incubation and form colonies. The visible colonies developed after 24–48 h incubation; viable cell counts were determined after 48 h of incubation under aerobic conditions at 37°C. Colonies counted were then multiplied with the dilution factor to obtain total viable cell counts and recorded as logarithmic colony forming units per milliliter (log cfu ml^{-1}) of product (Poddar et al, 2021, Sang *et al*, 2021).

5. pH, titratable acidity and moisture: The pH of the starters cultures was evaluated using a pH meter (SD300-Garmany), and the titratable acidity (TA) percentage was determined using 0.5 N NaOH with 0.5 ml phenolphthalein as an indicator and is expressed as a percentage of lactic acid (Niamah, 2017). Moisture was determined according to the methods described by (AOAC , 2008).

6-Bio yogurt preparation: For starter preparation, *Lactobacillus casei* and *Bifidobacterium longum* were obtained from this study. Freeze-dried starter culture (*Streptococcus salivarius* subsp. *thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus*, were obtained from Sacco Company in Italy. For vacuum oven set (VOS) inoculation, we used powdered cultures directly inoculated into the milk. While for rehydrated cultures (RC) preparation, the vacuum oven dried probiotic *Lb. casei*, *Bif. longum* and freeze-dried *Lb. bulgaricus* cultures were activated separately in MRS broth (HiMedia, India) for 18– 24 h at 37°C under anaerobic conditions, whereas *St. thermophilus* cultures were activated in M17 broth (HiMedia, India) for 24 h at 37°C under aerobic conditions. Cultures were reactivated in a 10% skimmed milk suspension, incubated at 37-38°C for 18 hrs. The rehydrated starter cultures (RC) were ready to be inoculated into the milk. (4.28). About Bio-yogurt preparation, whole cow milk (2 liter) was mixed vigorously with 2% skimmed milk powder and 5% sucrose to ensure an efficient mixing.Then, the remaining 1L milk was added and mixed thoroughly. The mixture was heated at 94°C for 15 minutes, After subsequently; a cold incubation temperature (37°C) follows. The mix was aliquoted into three equal portions (1 L each). The first group was Conventional yogurt (CY) inoculated with

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2% yogurt starter was made with *Lb. bulgaricus* (Lb) and *St. thermophilus* (St) and served as a control yogurt. The second was probiotic yogurt with vacuum oven set (VOS) cultures inoculated with 2% (Lb: St: *Lb. casei*: *Bif. longum* is 1:1:2:2). The third portion was probiotic yogurt with rehydrated cultures (RC) inoculated with same the second portion but after reactivation with skimmed milk medium at 37°C for 18 hours. The fermentation of bio-yogurt samples was stopped after 37°C incubation when they reached a pH of 4.6–4.8, and they were stored at 4°C for 21 days. The bio-yogurt samples were examined every 0, 7, 14, and 21 days to perform bacteriological and sensual tests. (Azizkhani and Tooryan. 2016., Lestari et al, 2022). To determination of culture viability, Bio yogurt samples were added to ¼ Ringer's solution and the appropriate serial dilutions were prepared. *S. thermophilus* was enumerated using M17 agar supplemented with lactose at 45°C, *Lb. bulgaricus* was enumerated using MRS agar, adjusted to pH 5.2 at 45 °C and *Lb. casei* was enumerated using lithium propionate MRS (LP-MRS) agar (lithium chloride 2 g l^{-1} and sodium propionate 3g l^{-1} at 37°C (Dimitrellou et al, 2016). The *Bif. longum* viable cell counts was carried out by using the MRS agar with addition of 0.5% dicloxacillin stock solution, 1% lithium chloride stock solution, as well as 0.5% cysteine hydrochloride stock solution per 1liter of medium to inhibit yogurt lactic acid bacteria according to the pour plate method and was incubated anaerobically at 37˚C for 48 h (Azizkhani and Tooryan. 2016). For Sensory evaluation**,** the yogurt samples were tested for their sensory characteristics as described by Dimitrellou et al. (2016). More specifically, the evaluation was conducted by 20 untrained panelists familiar with the consumption of yogurts. All samples were coded with random three-digit numbers. The yogurts were evaluated for color, sweet odor, sourness, smoothness, sweetness, viscosity, and overall acceptability using a 9-point hedonic scale ranging from 1 (“dislike extremely”) to 9 (“like extremely”). The hedonic test was done at the 3th weeks of refrigeration storage.

RESULTS AND DISCUSSION

Figures (1 and 2) show the logarithm count of *Lb. casei* and *Bif. longum* in reconstituted skimmed milk (RSM) at (12, 20, 25) % after 24 and 48 hours of incubation respectively. The logarithm counts *Lb. casei* and *Bif. longum* was registered 9.80 and 9.60 respectively in RSM at 12% after 24 hours of incubation and reached 10.04 and 9.85 log cfu ml^{-1} respectively at 20%, then recorded 9.95 and 9.79 at 25% of same medium after 24 hours of incubation. The results noted decreasing of bacterial counts after 48 hours compared with 24 hours for trails which registered (9.89, 9.66) log cfu ml^{-1} at 20 %, and (9.56, 9.34) log cfu ml^{-1} at 25% of RSM respectively. Results were agreed with (1) that increasing the incubation period for bacterial cultures leads to a decrease in their live numbers as a result of the continued production of organic acids. It was observed that RSM at 20% gave the best vitality for *Lb. casei* and *Bif. longum* bacteria when grown at 37 °C for 24 hours. The results showed that the viability of *Lb. casei* and *Bif. longum* in RSM sorting rate of (12,20,25) % falls within the highest counts of starters which range between 10^{9}-10^{10} cfu ml^{-1}, the ability of lactic acid bacteria to maintain their viability in RSM (Chávez and Ledeboer. 2007).

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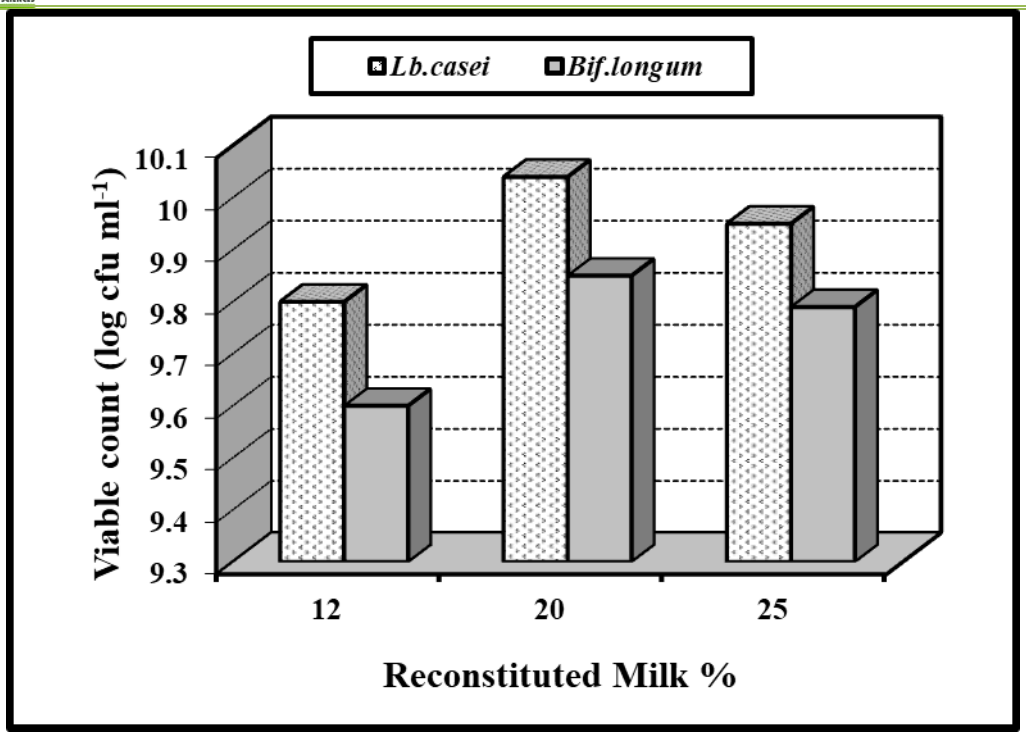

Fig 1. Viable count of *Lb. casei* and *Bif. longum* (log cfu ml^{-1}) in RSM after 24 hours

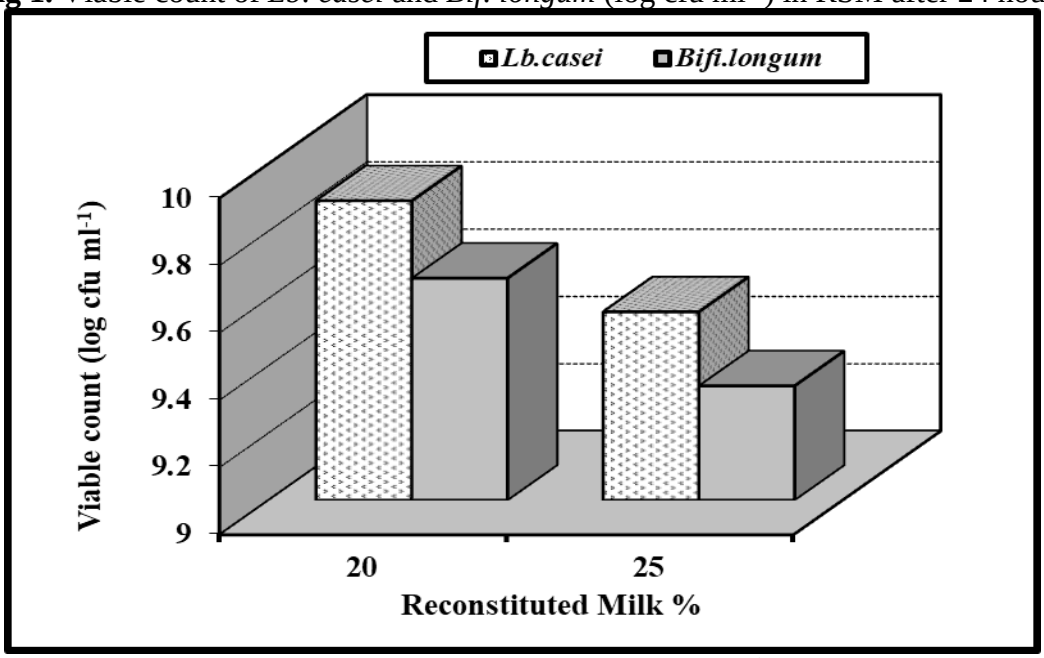

Fig 2. Viable count of *Lb. casei* and *Bif. longum* (log cfu ml^{-1}) in RSM after 48 hours

RSM was used as the inexpensive carbon source for bacterial growth and key protective substance, which prevents bacterial damage by stabilizing cell membrane components in spray drying of bacteria. It owns all beneficial features of RSM and contains vitamins like thiamine, riboflavin and niacin, which are required for the growth of *B. bifidum* (Shokri *et al*,2015). However, the sub-lethal treatment caused by environmental factors (high and low temperature, pH, osmotic, peroxide, bile salt, redox potential, starvation, etc.) allows the bacteria to activate their defense system against intracellular damage and enhances the viability of probiotics after drying and storage (Nguyen *et al*,2016). Figure (3) shows *Lb. casei* and *Bif.longum* bacteria cultures acidity at 12% in RSM were 0.67 and 0.61, respectively, while registered 0.72 and 0.65, respectively at 20% of media and recorded 0.69 and 0.62 respectively at 25%, in the same medium after 24 hours.

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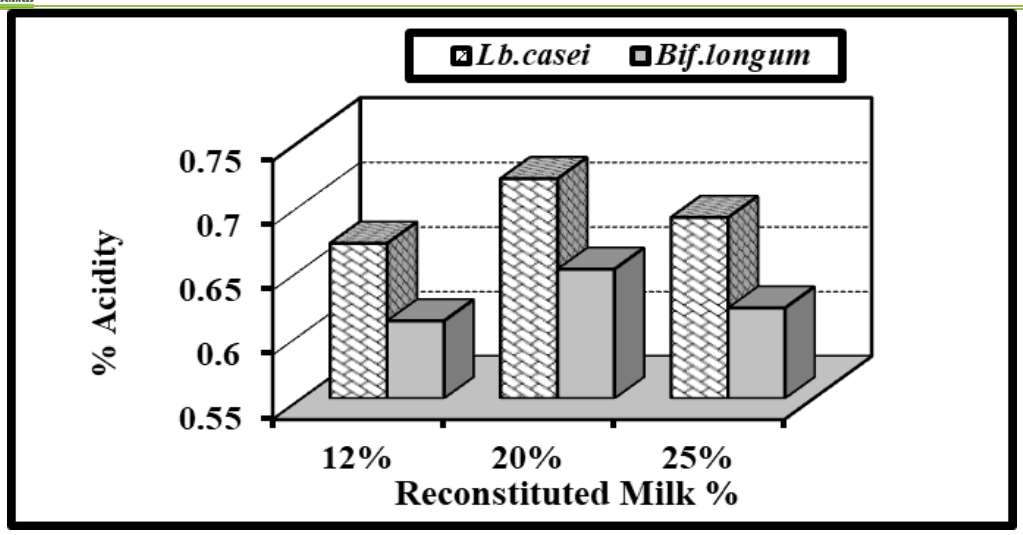

Fig 3. Total acidity of RSM inoculated with *Lb. casei* and *Bif. longum* after 24 hours

The highest acidity values of *Lb.casei* and *Bif.longum* cultures were noted at 20% of RSM when grown at 37 °C for 24 hours, which may be contains high lactic acid bacteria counts that work on consuming lactose sugar and converting it into lactic acid. As well as the suitability of water activity and viscosity of the medium to the growing organisms (Tripathi and Giri, 2014). The results on Figure (4) showed the pH values of RSM in different proportions that inoculated with *Lb.casei* and *Bif.longum* bacteria cultures. Results recorded 4.8 and 5.2 respectively at 12%, and registered 4.6 and 4.9, respectively at 20%, while the pH values were 4.7 and 5.1 respectively at 25% The most decreasing in the pH value were noted at 20% of RSM treated with *Lb.casei* and *Bif.longum* bacteria compared to other trails. This is due to an increase in lactose consumption and the production of lactic acid which leads to an increase in acidity and decrease in the pH value, because this medium contains high numbers of the aforementioned bacteria (Strasser *et al*, 2009). The microbial contamination tests, and by detecting coliform bacteria, yeasts and molds, showed that the trails under studied were completely free of these contaminants, which means that the conditions for the development of *Lb.casei* and *Bif.longum* bacteria are safe.

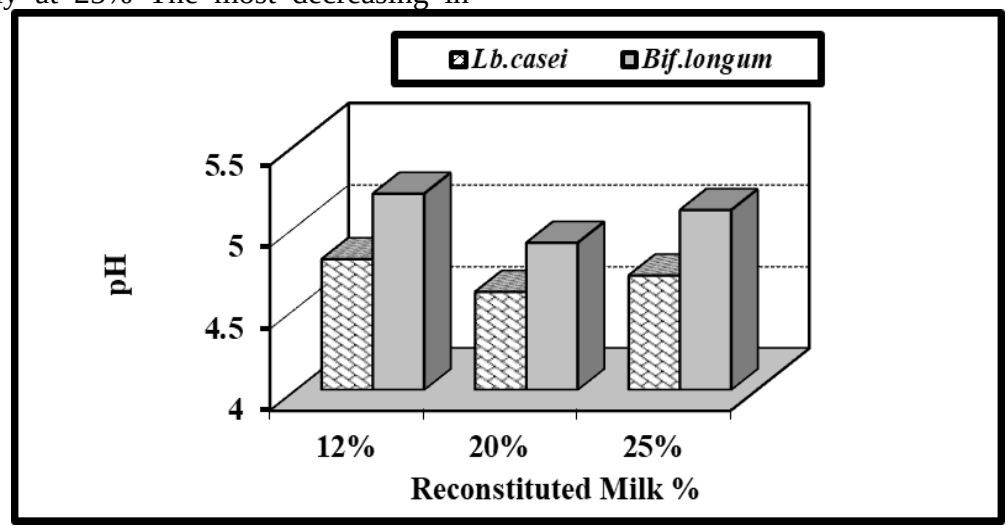

Fig 4. pH values of RSM inoculated with *Lb. casei* and *Bif. longum* after 24 hours.

Khaleel & Thaer (25) confirmed the ability of *Lb. acidophilus* and *Bifidobacterium* spp. has a distinct inhibitory capacity against types of pathogenic bacteria, yeasts and molds. The antibacterial activity of probiotic Lactobacilli act against different pathogenic bacteria through multifunctional properties by secreting antimicrobial (organic acids, bacteriocins, H_2O_2, lactic acid), counteracting the spread within the colonized body or competing for

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nutrients and binding sites .It was observed from Figure (5) that the average moisture content medium in the control treatment was 83.7% and in cultures of *Lb.casei* and *Bif.longum* bacteria was 82.6% and 82.5% respectively in 20% of RSM after 24 hours of incubation. A certain amount of water must remain in the dehydrated state and the optimum moisture content needs to be determined for a satisfactory survival rate of the strain of investigation, e.g. for freeze dried *Lactobacillus salivarius* the optimum moisture content was reported to range from about 2.8% to 5.6% (Strasser *et al*,2009).Vacuum drying is one of the promising drying processes, with high moisture evaporation rate, and allows the avoidance of the severe thermal inactivation (Zhang *et al*, 2020).

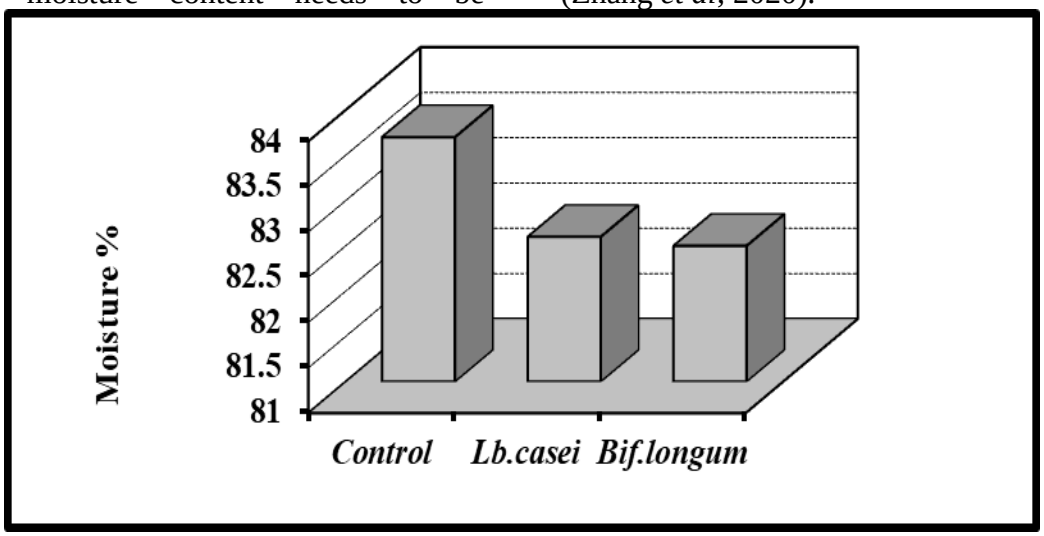

Fig 5. Moisture content of RSM inoculated by *Lb.casei* and *Bif.longum* after 24h

Figure (6) shows the moisture content of RSM at 20%, which is inoculated with *Lb.casei* and *Bif.longum* bacteria and dried in the vacuum oven. Results were registered (4.35, 4.23) % respectively in the models not supported by the protector, while the moisture content were 4.14% and 4.0 % respectively in the dried forms by protectant presence. Protectants relieve the damage caused by permeable swell during rehydration (Chanyuan *et al*, 2012).

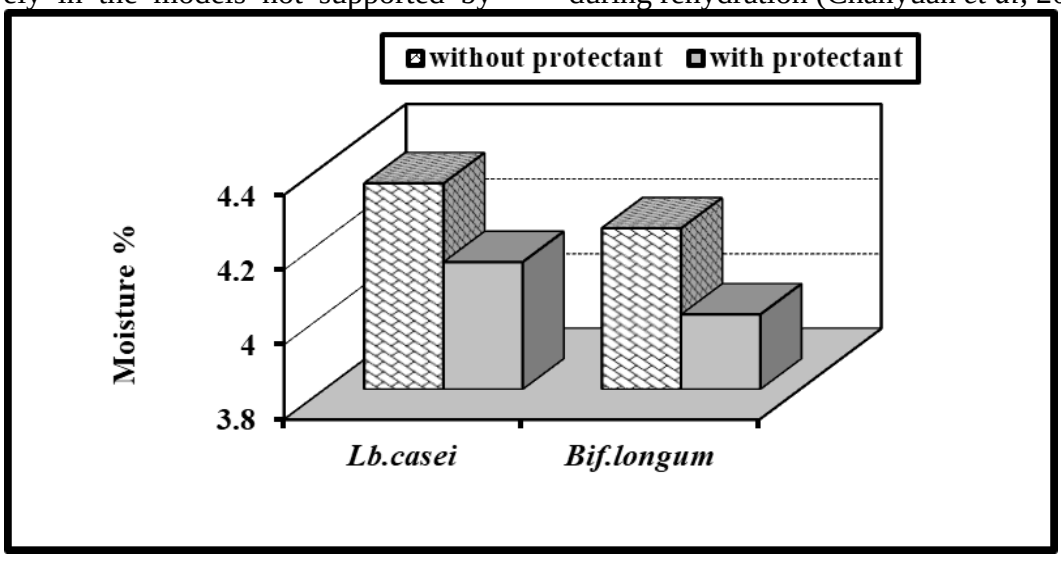

Fig 6. Moisture contents of dried bacterial cultures by vacuum oven

The logarithm numbers of *Lb.casei* and *Bif.longum* bacteria after vacuum oven dried were 9.06 and 8.81 respectively in unsupported protectant, while it was reached 9.14 and 8.93 respectively in presence of the protectant (Amakiri and Thantsha,2016). Moreover, previous studies have shown that a water activity ($_{aw}$) of around 0.1 or a water content around 4% is preferable because at this water activity deteriorative reactions, such as lipid oxidation, are limited (Broeckx *et al*,2010).

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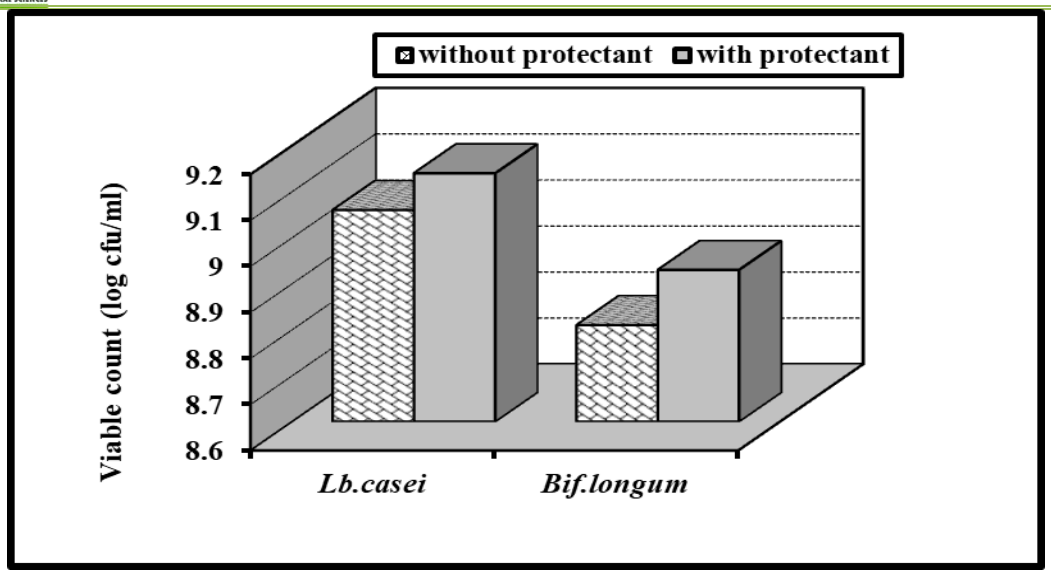

Fig 7. Viable count of *Lb. casei* and *Bif. longum* after vacuum oven drying

A decrease is observed within the limits of one logarithmic cycle after vacuum oven drying compared without drying. Santivarangkna et al. (2008) found that the therapeutic bacteria accounts decreased after drying process as a result of the bacterial stresses were exposed to during the drying. It is noted that the dried bacterial cultures with protectant presence was superior compared to the dried bacterial cultures in the absence of the protector, due to their retention of less moisture as well as the short time for drying (Morgan *et al*, 2006, Yang *et al*, 2023). The survival rates of spray dried lactic acid bacteria varies with species, even strain of microorganism from 33.05% to 92.54% after 60-day storage at 5°C, spray-dried yogurt starter cultures with moisture content of 4.09-4.24% were packaged in polyethylene vacuum packed and store at 4°C. Cells viability of spray-dried cultures were maintained at 10^9 cfu g^{-1} at 4°C for up to 4 weeks (Utami *et al*, 2016). Foerst et al. (2012) demonstrated that vacuum drying, especially when dried with appropriate protectant, could be a promising method to produce dried probiotic cells with high stability for storage at non-refrigerated temperatures. It should be kept in mind that an appropriate protectant against drying inactivation may not effectively stabilize cells during storage and both aspects must be considered together. It was observed that the decrease in bacterial account of dried bacteria in the vacuum oven in the presence of the protective material was less compared with the same conditions in the absence of the protective material. A protective substance provides protection for the bacterial cells towards the stress by drying process. As well as increasing its ability to maintain its vitality for preventing or reducing its loss (Tripathi and Giri, 2014, Zhang *et al*, 2020). Gul (2017) was concluded, the addition skimmed milk (SM) as protectant agent in vacuum drying significantly increased the survival of entrapped *Lb. casei* Shirota cells. In addition, oxidation of lipids and protein denaturation has been effective on viability during the storage. Thus, protective mechanism of SM during storage might be forming a semipermeable wall around living cells and reducing the rate of water from the intermediate environment. Heat stress is known to produce heat-shock proteins (HSPs) in many bacterial strains which are induced by microwave vacuum drying can potentially work a role on survival of probiotic during storage (Li *et al*, 2018). Protective pretreatments could be applied to LAB to enhance resistance. Stress adaptation mainly happens with changes in membrane fatty acid composition, production of stress proteins and accumulation of compatible solutes that improve the survival in a variety of stresses. Moreover, cross-protection and stress adaptation improve cell survival during the freeze-drying process and the area is developing rapidly (Gao et al, 2022). Table (1) shows bacteriological characteristics of yogurt treatments, VOS probiotic yogurt appeared highest numbers of vacuum dried

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probiotics compared with RC probiotic yogurt. *Lb. casei* registered 8.67 log cfu g^{-1} in VOS yogurt immediately after production whereas, recorded 8.62 log cfu g^{-1} in RC yogurt at the same time. *Bifidobacterium longum* count reached 8.23 log cfu g^{-1} in VOS yogurt and 8.13 log cfu g^{-1} in RC yogurt within production. Starters accounts were decreased through storage periods at cooling condition. Lang et al. (2022) noted the *Lb. plantarum* A3 co-cultured with traditional fermentation bacteria has a good lactic acid producing ability, which could short the curding time also that during mixed culture condition, Lb. *bulgaricus* can promote the growth and acid reproduction of S. *thermophilus* during milk fermentation process of yogurt production. The decrease in probiotic count though production may be attributed to the depletion of sugars in the fermented milks. As well as the low pH and the accumulation of organic acids resulting from growth and fermentation. The decrease in Lactobacilli count at the end of the storage was explained as a result of increase in Titratable acidity (Fadawy *et al*,2023). Amakiri and Thantsha (2016) were concluded when *Bifidobacteria* are sensitive to low pH, continuous exposure of the RC cells to the acidic environment of yoghurt would cause a reduction in their numbers. At last week's storage of yogurt, there was a reduction of microbial populations probably caused by adverse conditions, such as lack of nutrients. Although some variations and reductions were observed during the storage period, *Lb. casei* populations remained always above the minimum recommended for a probiotic food which 10^6 cfu g^{-1} (Simões Bandiera *et al*,2013).

Table 1. Microbiological characteristics (log cfu g^{-1}) of bio yogurt produced with *Lb. casei* and *Bif. longum* during storage at 4 °C

Type of bio yogurt				
Starters	**Day**	**CY**	**VOS**	**RC**
S. thermophilus	0	8.53	8.58	8.55
	7	8.40	8.47	8.43
	14	8.28	8.33	8.31
	21	8.10	8.24	8.16
Lb. bulgaricus	0	8.40	8.50	8.43
	7	8.30	8.38	8.32
	14	8.11	8.21	8.18
	21	7.88	8.06	7.94
Lb. casei	0	-	8.67	8.62
	7	-	8.58	8.53
	14	-	8.31	8.27
	21	-	8.07	8.02
Bif. longum	0	-	8.23	8.16
	7	-	8.14	8.05
	14	-	8.06	7.91
	21	-	7.89	7.78

CY: conventional yogurt, VOS: probiotic yogurt with vacuum oven set, RC: Probiotic yogurt with rehydrated cultures

Hossain et al. (2024) were mentioned an increase in storage time leads to a higher production of lactic acid. Which lead to eventually results in a nutrient deficiency for lactic acid bacteria to entire a phase of decline in their population known as the death phase. Decreasing *Lb. bulgaricus and S. thermophilus* count may be due to dropping in pH. High acidity was due to the consumption of lactose and the production of lactic acid. In addition, the secondary metabolites in the environment will increase during incubation period more than that storage period and this affected the viability starter bacteria cells .The organoleptic evaluation of yogurt with probiotics was shown in table (2). It could be seen that the overall acceptability and various indicators of

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the yogurt containing probiotics were significantly higher than those of the control yogurt (CY). It showed that the addition of probiotics improved the organoleptic properties of yogurt, especially in terms of sweet odor, sweetness and viscosity. Moreover, probiotic starters *Lactobacillus casei* and *Bifidobacterium longum* are better used as VOS cultures than rehydrated cultures.

Table 2. Sensory evaluation of probiotic yogurts treated with VOS culture, RC culture and compared with conventional yogurt CY at 3th week of refrigeration storage

	color	Sweet odor	sourness	smoothness	sweetness	viscosity	Overall acceptance
CY	7.7	6.3	7.2	7.8	5.4	8.2	7.7
VOS	8.5	7.5	6.3	7.1	6.5	8.4	8.8
RC	8.3	6.8	7.3	7.7	5.2	7.8	8.3

The fermentation capability of rehydrated cultures might be decreased because they are used more than one time (Lestari *et al*, 2022). On the contrary, vacuum oven dried cultures are usually stable in terms of viability and activity as well as being convenient in handling, storage, marketing and application. Similarly, significant improvement of sensory characteristics was also previously recorded in dairy products containing *Lb. casei* as an adjunct or starter culture (Dimitrellou *et al*,2019). Several studies pointed out that *Lactobacillus casei* is highly capable of utilizing the proteins in the whey and produces abundant peptides, which was associated with the higher acceptance of probiotic yogurt group (Yang *et al*, 2023). The presence of encapsulated *Bif. longum* in yogurt, change was considered minor or not noticeable to the human eye (Amakiri and Thantsha, 2016). Son et al. (2023) found the viscosity of the yogurt tended to increase with fermentation time, and the viscosity increased as the *Bif. longum* inoculation concentration increased. This may be due to interaction between exopolysaccharide (EPS) produced by *Bif. longum* and the milk protein, which affect the property of the gel structure in fermented milk and acidity.

CONCLUSION

Reconstituted skimmed milk was a good media for growth and viability of *Lb. casei* and *Bif. longum*. Vacuum oven drying is an excellent method for preserving probiotics. Skimmed milk was an effective protectant agent for maintaining viability and resistance to vacuum oven condition when added with probiotic bacteria. The accounts of probiotics were negatively affected through drying, but it remained always above the minimum recommended for a probiotic food which 10^6 cfu g^{-1}. *Lb. casei* and *Bif. longum* were Contributed to improving bacteriological and sensual properties of bio yogurt. VOS probiotic form was the excellent way to produce bio yogurt compared with RC probiotic form. However, rehydrated vacuum oven dried probiotic can enhance characteristic of bio yogurt.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

AUTHOR'S CONTRIBUTION STATEMENT

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تأثير التجفيف بالفرن المخلخل على حيوية بكتريا *Lactobacillus casei* و*Bifidobacterium longum*

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المستخلص

أُستعمل الحليب المجفف منزوع الدسم المسترجع بتراكيز (12، 20، 25)٪ لتنمية بكتريا *Lactobacillus casei* (*Lb. casei*) و *Bifidobacterium longum* (*Bif.longum*) في 37 درجة مئوية لمدة 24-48 ساعة بعد التلقيح بالبادئ بنسبة 10٪ بشكل مفرد لكل منهما. حقق تركيز 20٪ (وزن / حجم) اعلى معدل نمو. إذ بلغت الأعداد اللوغاريتمية 10.04 و 9.85 لبكتريا *Lb. casei* و *Bif.longum* على التوالي، وبلغت النسبة المئوية للحموضة التسحيحية في الحليب المخمر 0.72 و 0.65 على التوالي. أما قيم الرقم الهيدروجيني للحليب المسترجع منزوع الدسم بنسبة 20٪ فقد بلغت 4.6 و 4.9 على التوالي. جُففت مزارع بكتيريا حامض اللاكتيك بواسطة الفرن المخلخل مع أو بدون المادة الحامية (حليب مجفف منزوع الدسم). دُرس تأثير التجفيف على عيوشية بكتريا حامض اللاكتيك. أعطى التجفيف في الفرن المخلخل أفضل النتائج عند إضافة مواد الحماية وبلغت الاعداد اللوغاريتمية 9.14 و8.93 لبكتريا *Lb. casei* و*Bif.longum* على التوالي، تليها المزارع المجففة بدون مواد حماية والتي سجلت 9.06 و8.81 على التوالي. وكانت نسبة الرطوبة 4.14 و4.0٪ في المزارع المجففة في الفرن المخلخل لبكتريا *Lb. casei* و*Bif.longum* على التوالي مع وجود المواد الواقية، وسجلت 4.35 و 4.23 % على التوالي للمزارع البكتيرية المجففة بدون وجود المادة الحامية. أُستعملت مزارع المعززات الحيوية المجففة في الفرن المخلخل أو المسترجعة في انتاج اليوغرت الحيوي. حققت مزارع المعززات الحيوية المجففة (*Lb. casei* و*Bif.longum*) مع بادئ اليوغرت التقليدي أعلى قيم في الأعداد اللوغارتمية للبوادئ المستعملة والصفات الحسية للمنتج مقارنة مع مزارع المعززات الحيوية المسترجعة بعد التجفيف في الفرن المخلخل.

الكلمات المفتاحية: *Bif. Longum*، اليوغرت الحيوي*Lb. casei*، المادة الحامية ، الفرن المخلخل.