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# Impact of ultrafiltrated whey powder and lactulose on survival of *Bifidobacterium bifidium* and color characteristics of ultrafiltrated synbiotic cheese

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## ABSTRACT

During last decades, consumer awareness in importance of healthy intake has led their tendency towards the potential health benefits of particular foods and food ingredients. These conditions resulted in development of functional foods. In the current research, the effect of different concentrations of demineralized ultrafiltrated whey powder (DUWP) at levels of 0, 1 and 2% (W/V) and lactulose at levels of 0 and 1% (W/V) on symbiotic ultrafiltrated cheese were evaluated as a functional food. The color values, total acceptability and survival ability of *Bifidobacterium bifidium* during 60 days of storage at 4 °C were studied. Results showed that as the amount of lactulose and DUWP powders increased, the number of probiotics enhanced meaningfully, while the number of probiotic bacteria deceased with passing the storage time ($p<0.001$). However, the count of probiotic bacteria in samples containing lactulose and 1 or 2% DUWP at the end of 60 days of storage time were in the standard range of probiotic foods ($>10^7$ Log cfu/g). The colorimetric results showed that as the amount of DUWP and the storage time increased, the lightness (L*) decreased significantly ($p<0.001$); but addition of both powders had no significant effect on a* and b* values. Based on the obtained results, the synbiotic cheese containing 1% DUWP and 1% lactulose powder as compare to control sample had a higher count of probiotic bacteria. Meanwhile, no differences in color quality and total acceptability were found between these two samples. Therefore, this synbiotic ultrafiltrated cheese was determined as the best functional cheese sample.

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## 1. Introduction

Today, there are more than hundreds of types of cheese in the world, each of which has its own characteristics. One of the types of cheese is ultra-refined cheese. Iranian white cheese with ultra-refining method has the highest per capita consumption in Iran. In recent years, the use of ultra-refining process for cheese production has attracted much attention worldwide. This cheese is consumed immediately after production.[۱].

During the last few decades, the attention to nutrition science (the effect of diet on health and well-being) is increasing strongly. This has led to the design of new and healthier foods that reduce the risk of several chronic diseases. Foods designed in this way, functional foods[1] or they are called utilitarian. FromExamples of functional foods include foods containing biologically active substances or phytochemical compounds (vitamins, polyphenols, fatty acids, antioxidant compounds, etc.), dietary fibers, and foods containing probiotics. If prebiotic compounds are used together with probiotics, the term "synbiotic" is used, and this combination helps to improve the viability of probiotics in the product and expand the health benefits in the host. Synbiotic products are also in the category of functional foods.[۲].

One of the desirable features of synbiotic products is to improve the survival of probiotic bacteria in manufacturing and storage conditions as well as during passage through the digestive system. The viability of probiotics in foods is often affected by adverse environmental conditions in food matrices such aspH Acidity and the presence of such compounds $H_2O_2$is limited. On the other hand, the presence of prebiotic compounds has a positive effect on their survival, and therefore, a wide range of prebiotics have been introduced as probiotic protectors in milk-based foods and other foods.[۳].

In order to survive, microbial strains must have the ability to resist bile salts in the small intestine, stomach conditions, enzymes in the small intestine, and toxic metabolites produced during the digestion process. A necessary condition for the therapeutic effects of probiotic bacteria is their resistance and survival.[۴]. Among the probiotic bacterial strains with beneficial effects that can be used in fermented products is Bifidobacterium strain.[۵].

Bifidobacterium is an anaerobic lactic acid producing bacterium. Bifidobacterium bacteria are non-motile, non-sporing, gram-positive and anaerobic bacteria that make up a large part of the intestinal microflora of humans and other animals. These bacteria play a very important and effective role in limiting the formation of exogenous and pathogenic colonies. The presence of these bacteria in the human intestine has health-enhancing effects. Probiotic bacteria maintain the balance and improve the microflora population of rivers with their biological activity.[۶].

In addition to having prebiotic properties, lactulose is an effective drug in the treatment or prevention of many diseases.is used][۷]. In addition, lactulose works well in absorbing calcium and creates favorable mechanisms in regulating the immune system as well as beneficial effects on lipid metabolism and reducing the risk of cardiovascular diseases.Lactulose is produced by isomerization of lactose by chemical and enzymatic methods. A large number of catalysts such as alkaline agents, mixing reagents and enzymes are used for the isomerization of lactose to lactulose.][۸].

Milk is the only suitable food for the evolution of mammals. Milk is a good

[1]- Functional food

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source for providing energy needed by humans because it is a source of carbohydrates (lactose), a source of nitrogen (proteins) and a rich source of calcium suitable for bones. Also, milk has other benefits, for example, many of the biological activities of milk are related to the components present in milk, which biologically active components are mainly present in milk serum or whey.[۹. Blue cheese is a by-product of cheese production. This yellow liquid contains a lot of lactose and mineral compounds such as calcium and phosphorus. Considering the high biological value of whey proteins compared to casein and egg proteins, its use as a nutrient has become important. [۱۰.Nowadays, cheese protein products are used as a valuable component in the production of food products because of their excellent nutrients with unique functional characteristics and high nutritional value.]11 and 12.From cheese, products such as protein products, derivatives of serum proteins (lactoparasidase, lactoferrin), peptide concentrates and solutions such as calcium salts are obtained. It should be noted that whey is one of the sources of organic pollution, and the use or disposal of whey has been the main concern of dairy industry experts around the world, because whey is an important environmental pollutant. On the other hand, this material contains valuable components that should not be wasted, and its organic compounds such as protein, lactose and fat can be recycled by various biotechnological processes and reused as a food component. Today, a significant volume of afterwater or rennet resulting from the ultra-refining process (permeate) is created during the preparation of cheese, which contains completely different compounds compared to the usual rennet. Compared to the powder made from sweet cheese, the product made from Permit cheese has a relatively lower quality; The powder prepared from Permit contains more lactose and salts and less amount of cottage cheese proteins. Therefore, in order to increase the usability of Permit powder in the formulation of food products, the amount of its salts has been reduced, and in this way, a product called ultra-refined water cheese powder with reduced salts.[2DUWP) is produced and marketed][۱۳. Therefore, the present study was conducted in order to investigate the effect of adding lactulose powders and powdersDUWP As prebiotic compounds, the viability of probiotic bacteria was investigated, as well as the color characteristics and general acceptability of synbiotic ultra-refined cheese.

## 2- Materials and methods

### ۲-۱- Materials used

In order to produce ultra-refined cheese samples, high quality cow's milk available in Pegah Khuzestan factory was used. Lactulose from Sigma (USA) and Permit powder or ultra-refined milk effluent with reduced salts (DUWP) used in this research was purchased and used from Baltic Food Industry Company (Russia). Powder ingredientsDUWP It included 3.3% moisture, 3.8% protein, 88.5% lactose, 3.5% ash, and 0.9% fat. Standard rent with brand nameChey-Max from Christian Hansen Denmark and mesophilic starter powders

[2]- Demineralized whey powder

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CHOOZIT 230 (Contains a mixture of bacteria*Lactococcus lactis subspecies Cremoris*And*Lactococcus lactis subspecies lactis*) and thermophilic starterYo-Mix 532 (contains*Streptococcus thermophilus* And*Lactobacillus delbruecki subspecies bulgaricus*) was purchased from Danisco company in Germany. Probiotic starter powder containing bacteria*Bifidobacterium Bifidium subspecies used in this research*From Christian Hansen Company[3] Denmark was purchased. The mentioned powders are of the typeYOU and were kept at -18 degrees Celsius until use. Other chemicals used in this research were of high quality and were mostly purchased and used from Merck, Germany.

### ۲-۲- Cheese production method

Farapaloude cheese was produced in Pegah milk factory in Shush Khuzestan from fresh whole milk (3.3% fat) and with suitable physicochemical and microbial quality according to [14] and [15] methods. The received raw milk was subjected to bactofugation in two stages and then subjected to the process of pasteurization and ultra-refining. After passing through the ultra-refining membrane, the milk is permeated into two parts[4] Or the passage part (cheese juice) and retentate[5] or Natraveh (containing 31% solid matter and about 16% fat) was divided. Homogenization operation after adding powderDUWP (zero levels, 1 and 2 percent,W/V) and lactulose (zero and 1% levels,W/V) in addition, at a pressure of 70 bar by the homogenizer modelJHG-Q60-P60 (Ronghe machinery, China) and then it was pasteurized by a plate pasteurizer at a temperature of 75 degrees Celsius for 15 seconds. It should be noted that the levels of powdersDUWP and lactulose in this research was determined after preliminary tests. Next, Natravah went to a plate pasteurizer and was pasteurized up to 78 degrees Celsius for 16 seconds. After that, mesophilic and thermophilic starter powders were added in the amount of 0.025% each, and probiotic starter powder in the amount of 0.05% along with rennet in the amount of 0.03%. After placing Natrave in 400 grams of cheese containers, the packages enter the coagulation tunnel at a temperature of 31-30 degrees Celsius for 25 minutes to coagulate the clot, and after exiting the tunnel and coagulation of the cheese, parchment paper was placed on the surface of the cheese. At the end, before sealing the packages with aluminum foil, 2% salt (weight/weight) was added to the containers containing cheese. Finally, the cheese packages were kept in a greenhouse at a temperature of 25-27 degrees Celsius and after 18-24 hours and ripeningpH The samples were transferred to the cold room at 5 degrees Celsius. In this research, the samples of Iranian white cheese are extrapolated with probiotics (without lactulose powder andDUWP) as a control sample with synbiotic cheese samples (containing different amounts of lactulose powder andDUWP) were evaluated on days 1, 30 and 60 in terms of viability of probiotics as well as color characteristics and general acceptability.

### ۲-۳- Colorimetry test

The color of cheese samples using a colorimeter. Minolta CR300 series, Minolta Camera Co It was done in thata* (a value) ،b* (b value) AndL* (L value) They indicate redness, yellowness and whiteness respectively].[۱٦

### ۲-٤- Microbial tests

[3]- Chr. Hansen

[4]- permeate

[5]- retention

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In order to evaluate the survival of lactic acid bacteria[6] (LAB) and probiotic bacteria, 25 grams of cheese from each sample with 200 ml of peptone water(Acumedia Manufacturers, USA) was mixed and well homogenized and then made up to 250 cc with peptone water. In this way, the dilution is 0.1 Was prepared. Then the necessary dilutions were prepared in test tubes containing peptone water. To cultivate bacteriaLAB FromMRS agar (Merck, Germany) and probiotic bacteria (Bifidobacterium bifidum) FromMRS agar contain ۰/۱۵ Bile salts (Merck, Germany) were used. For cultivation using the porplate method Plates were used for bacteriaLAB and probiotics were incubated at 37 degrees Celsius for 48 and 72 hours under anaerobic conditions][۱۷.

### ۲-۵- Overall acceptance evaluation

Ability to accept samples of ultra-purified synbiotic cheese by20 Some of the students and professors of food industry of Khuzestan University of Agricultural Sciences and Natural Resources were evaluated. This evaluation was done based on a 9-point preference test. Before evaluation, the samples were taken out of the refrigerator for 30 minutes and at ambient temperature (℃ ۲±22) were kept so that the temperature of all the samples during the evaluation is the same and does not affect the overall acceptance results. Cheese samples after ripening (48 hours after production) in the first, 30 and۶۰ were evaluated][۱۸.

### ۲-۶- Statistical Analysis

In this research, according to two variables lactulose (at two levels) and powderDUWP (at 3 levels), 6 cheese treatments were produced and cheese samples were compared with each other in terms of microbial characteristics, color and general acceptability during two months of storage (at intervals of 1, 30 and 60 days after production). According to the production of cheese samples in 3 repetitions, a total of 54 samples were produced. Results by completely random design in factorial format with the help of statistical programSPSS (SPSS Inc.، Chicago, ed۲۰) analysis and average results were compared with the help of Duncan's test at the 5% level.

## ۳- Results and discussion

### ۳-۱Counting probiotic bacteria andLAB

The viability and survival of probiotics are important factors in the evaluation of probiotic products. The survival of probiotics in food products until consumption is the most important determining factor in probiotic products. The effective inoculation of probiotic bacteria into cheese requires that these probiotics survive, as well as maintain their survival during processing without adversely affecting the sensory properties of the probiotic product.[۱۹ . Milk-based products are a favorable environment for the growth of probiotics. It is mentioned in the standard of probiotic products that the acceptable level of probiotics in dairy products should be minimumcfu/gr $۱۰^{۷}$ May they have healing properties][۲۰.

The results of the effect of adding sweet water cheese powder with reduced solutes and lactulose on the microbial characteristics of different samples of ultra-contaminated synbiotic cheese during 60 days of storage at 4 degrees Celsius are presented in Tables 1 and 2.Results

[6]- Lactic acid bacteria

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indicating the significant effect of all three variables Permit powder, lactulose powder and storage time on the number of bacteriaLAB was investigated (0.001*P*<). As can be seen in Table 2, with the increase of powder levelsDUWP and lactulose, noBacteriaLAB There was a significant increase.Number of bacteriaLAB In samples containing۰٪ ۱ And۲ Powder percentageDUWP Respectively43/8, 59/8 andLog cfu/g 8/78 was determined.The amount of bacteriaLAB Examples due to addition۰ And۱ The percentage of lactulose powder is also respectively 47/8 AndLog cfu/g 74/8 Was determined. alsoCounting resultsLAB Show activityLAB It increased significantly in the first 30 days and then decreased significantly until the end of storage time (Table 2).Studies have shown that due to the continued activity of bacteriaLAB and produce higher levels of acidity at the end of storage, significantly reducing activity levelsLAB It can be seen ]۲۱ [. On the other handIslam

and colleagues [22]Decrease during storage periodLAB Cheese was attributed to low moisture content, high salt content and low storage temperature of the product. increase in numberBacteriaLAB As a result of adding lactulose in synbiotic stirred yogurt ]23 [ and the addition of desalted cheese juice and inulin in ultra-refined cheese]15 [ It has already been reported. However, contrary to these results, Ozer et al]24 [reported that the addition of lactulose and inulin to bifidus yogurt had an effect on the noBacteriaLAB does not have. The findings showed that the interaction between lactulose-storage time and Permit powder-storage time was also significant.

**Table 1.** The results of analysis of variance (ANOVA) of the effect of demineralized ultrafiltrated whey powder (DUWP) and lactulose powder on Microbial characteristics of probiotic ultrafiltrated cheeses during 60 days storage at 4 °C

| Treatments | df | Mean Square | |
|---|---|---|---|
| | | LAB | Probiotic bacteria |
| **DUWP** | 2 | $0.548^{**}$ * | $0.675^{***}$ |
| **Lactulose** | 1 | $0.984^{**}$ * | $1.242^{***}$ |
| **Storage Time** | 2 | $0.711^{**}$ * | $7.613^{***}$ |
| **DUWP× Lactulose** | 2 | $0.007^{NS}$ | $0.039^{NS}$ |
| **DUWP×Storage Time** | 4 | $0.069^{**}$ * | $0.035^{NS}$ |
| **Lactulose ×Storage Time** | 2 | $0.169^{**}$ * | $0.144^{*}$ |
| **DUWP**× *Lactulose* × *Storage Time* | 4 | $0.003^{NS}$ | $0.014^{NS}$ |
| **error** | 36 | 0.208 | 1.272 |

NS, *, ** and *** respectively indicate: non-significance, and significance at p<0.05 and p<0.01, p<0.001 levels.

**Table 2.** Microbial characteristics of synbiotic ultrafiltrated cheeses containing demineralized ultrafiltrated whey powder (DUWP) and lactulose during 60 days storage at 4 °C

| Bacterial Count (Log cfu/g) | Storage Time (Day) | *$P_0L_0$ (Control) | $P_1L_0$ | $P_2L_0$ | $P_0L_1$ | $P_1L_1$ | $P_2L_1$ |
|---|---|---|---|---|---|---|---|
| **LAB** | 1 | 8.32±0.09$^{N}$ ot | 8.38±0.09$^{Not}$ | 8.43±0.07$^{No}$ t | 8.37±0.08$^{Not}$ | 8.43±0.07$^{No}$ t | 8.47±0.07$^{That}$ |
| | 30 | 8.78±0.15$^{B}$ ut | 8.97±0.10$^{Ad}$ 8.03±0.09$^{Cd}$ | 9.24±0.04$^{Ab}$ c | 9.12±0.07$^{And}$ 8.17±0.09$^{Cc}$ | 9.32±0.01$^{Ab}$ 8.40±0.04$^{Bb}$ | 9.67±0.02$^{And}$ 8.68±0.06$^{Not}$ |
| | 60 | 7.85±0.09$^{T}$ his | | 8.20±0.02$^{Cc}$ | | | |
| **Probiotic bacteria** | 1 | 8.07±0.23$^{A}$ b | 8.17±0.04$^{Oop}$ s | 8.30±0.14$^{Oo}$ ps | 8.19±0.07$^{Oops}$ 8.02±0.21$^{Ab}$ | 8.28±0.13$^{Oo}$ ps | 8.40±0.31$^{Aa}$ 8.58±0.29$^{Aa}$ |
| | 30 | 7.81±0.23$^{A}$ b | 7.95±0.21$^{Ab}$ 6.79±0.21$^{Bcd}$ | 8.08±0.11$^{Ab}$ 7.02±0.13$^{Bb}$ c | 6.95±0.16$^{Bcd}$ | 8.23±0.26$^{Oo}$ ps | 7.63±0.16$^{Not}$ |
| | 60 | 6.65±0.24$^{B}$ d | | | | 7.28±0.09$^{Bb}$ | |

P0, P1 and P2 are 0, 1 and 2% levels of whey permeate and L0 and L1 are 0 and 1% lactulose, respectively. Different small and capital letters indicate significant differences ($p<0.05$) in each row (treatments) and column (days) for each cheese characteristics, respectively.

As can be seen in Tables 1 and 2, the concentration of Permit powder and lactulose increasedNumberBacteriaLAB cause a significant increase in the survival of bacteria*Bifidobacterium Bifidium* in production samples (٠٠١/٠*P*<). This was despite the fact that the number of probiotic bacteria in the samples decreased significantly during the storage period.The findings also showed that, apart from the interaction between lactulose and storage time, there was no significant interaction between other test variables. (٠٥/٠*P*<).

The number of probiotic bacteria in the samples containing٠٠ ١ And٢ Powder percentageDUWP Respectively7/61, 7/78 andLog cfu/g 8/20 was determined.The amount of probiotic bacteria in the samples due to addition٠ And١ The percentage of lactulose powder is also respectively٦ 5/٧ And95/٧ Was determined. As in the table٢ It can be seen, although at the beginning of the storage time, the number of bacteria*Bifidobacterium bifidium* In all treatments more thanLog cfu/g $10^8$ was determined, but at the end of the storage time, only two treatments 5 (containing 1% of powdersDUWP and lactulose) and 6 (containing 2%DUWP and 1% lactulose) containing

The number of probiotic bacteria is higher than the standard ($10^7$) They were. At the end of the storage time bacteria *Bifidobacterium Bifidium* In the examplesProduced synbiotic cheese containing 1% powderDUWPand 1% lactulose powder Log cfu/g (Treatment 5) 7/28 and sample containing 2% powderDUWPand 1% lactulose powder (treatment 6)Log cfu/g 7/63 was determined.

Increasing the number of probiotic bacteria as a result of increasing water cheese powder or lactulose previously in synbiotic cheese.15[٠ Synbiotic yogurt]23[and fermented milks]25[ It has been reported. Also, in confirmation of the findings of this research, many researchers such as Kardarleliaet al. [26], Ozturkaglu-Bodak et al. [27], Ozer et al. [28]To increase




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the activity of probiotic bacteria in the presence of different concentrations of prebiotic compoundshave mentioned

Kardarlelia et al.]٢6 [In accordance with the findings of this research, the effect of the mixture of inulin, oligofructose and honey on the growth of probiotics such as*Lactobacillus acidophilus* And*Bifidobacterium lactis* They investigated in Swiss cheese. In the studies of these researchers, the reason for increasing the growth of probiotics indicated the conversion of lactate to propionate, acetate and butyrate by probiotics. Anyway, when indigestible carbohydrates such as oligosaccharides (fructo-oligosaccharide) are available to probiotics, it causes the accumulation of lactates, followed by the production of butyrate and the growth of probiotics, and this depends on the type of probiotics. Boylston et al.]١٩ [Also, the difference in the survival rate of probiotic bacteria according to the type of treatment used, the rate of metabolizing the compounds and the rate of reductionpH attributed to the consumption of prebiotic compounds. Zamora-Vega et al.]٢9 [Also in a research, the number of probiotic bacteria decreased during 30 days of storage at temperature°CThey reported 4.These researchers reported that the number of probiotic bacteria in the samples containing inulin as a prebiotic compound was higher than the control sample; At the same time, with micro coverage of probiotic bacteria*Saccharomyces boulardi*[7] Along with the composition of cactus mucilage, sodium alginate and inulin, viabilityProbiotics increased.

**٣-٢- Evaluation of cheese color during storage**

Molecular uniformity and type of microstructure can affect the brightness of food. In materials such as cheese, light passes through the surface layers and scatters. The porosity of the casein network tissue and milk fat globules affects the amount of light scattering. In milk and dairy products, the amount of light scattering is largely dependent on the accumulation of casein micelles and colloidal particles of milk, especially colloidal calcium phosphate and to some extent fat globules. Findings of statistical analysis of different concentrations of powderDUWP and lactulose on the color indicators of Iranian white cheese in the table٣ Is visible.

The results showed that the powderDUWP cause significant changes in the indexL* The samples were (٠٠١/٠*p*<), but this variable has an effect on the indicesa* Andb* did not have(٠5/٠<*p*). Also, adding lactulose on any of the indicatorsCheese color had no significant effect (٠5/٠<*p*). However, as in the tables3 and 4 It can be seen, all the color indicators were affected by the storage time. On average, the brightness index of the samples containing٠٠ ١ And٢ Powder percentageDUWP Respectively٥٣/88, 02/88 and 17/87 were determined.The brightness of the samples due to addition٠ And١ The percentage of lactulose powder is also respectively٠٠ / ٨٨And٧٨/٨٧ was determined Although a significant difference was observed in terms of brightness at the beginning and in the middle of the storage period between the produced cheese samples, but at the end of 60 days of storage, no difference was observed in this regard.٠5/٠*p*>) and the reason for that was the significant effect of storage time on reducing the brightness of cheese samples, especially the control sample.

Although with increasing powder percentageDUWP Indicator valuesa* Andb* was decreasing and increasing, but these changes were not significant. However, as

[7]- *Saccharomyces boulardi*

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can be seen in Table 4, there is a significant difference in opinionIndicatora* It was observed at the end of 60 days of storage. Indicatora* Samples containing۰٫ ۱ And۲ Powder percentageDUWP Respectively۵۰/۲- ، ۵۵/۲- And۶۳/۲- and the amount of the indexa* Examples due to addition۰ And۱ The percentage of lactulose powder respectively۵۵/۲- And۵۶/۲- It was determined. Average indexb* due to addition ۰٫ ۱ And۲ Powder percentageDUWP To the examples in order۵۳/۰۹ ۶۶/۹ And9/92 was determined andAverage indexb* as a result of adding 0 And۱ The percentage of lactulose powder respectively۶۶/۹ And۷۴/۹ was determined

Rostamabadi et al.]1[In confirmation of the results of this research, they stated that the increase in the hydration of proteins and the decrease in free water droplets affect the light reflection and the whiteness of the samples during the processing period, and as a result, they reduce the brightness of the samples. Danesh et al.]30[Contrary to the present results, they stated that the use of whey proteins in the formulation of traditional Iranian white cheese caused the texture of the cheese to become more porous, and due to the creation of more pores, the microstructure of the cheese resembles the structure of a beehive; A condition that certainly leads to an increase in light refraction and an increase in the brightness of the samples. Anyway, the reason for the lack of effectpowderDUWPOn the lightness of the cheese in this research, probably the low amount of whey proteins in the powderDUWPis.In addition, Danesh et al.]31[In another study, the effect of combining transglutaminase enzyme and blue cheese proteins on Iranian Farapaloudhi cheese was investigated. These researchers also stated that the reason for the higher brightness of the samples in ultra-refined cheese is factors such as the increase in the number of serum holes and the creation of porous texture in the cheese samples.

**Table 3.** The results of analysis of variance (ANOVA) of the effect of demineralized ultrafiltrated whey powder (DUWP) and lactulose powder on color characteristics of probiotic ultrafiltrated cheeses during 60 days storage at 4 ˚C

| Treatments | df | Mean Square | | |
|---|---|---|---|---|
| | | L* | a* | b* |
| **DUWP** | 2 | $8.553^{***}$ | $0.0089^{NS}$ | $0.722^{NS}$ |
| **Lactulose** | 1 | $0.900^{NS}$ | $0.003^{NS}$ | $0.081^{NS}$ |
| **Storage Time** | 2 | $23.793^{***}$ | $0.728^{***}$ | $1.656^{*}$ |
| **DUWP× Lactulose** | 2 | $0.362^{NS}$ | $0.022^{NS}$ | $0.001^{NS}$ |
| **DUWP×Storage Time** | 4 | $0.185^{NS}$ | $0.117^{NS}$ | $0.004^{NS}$ |
| **Lactulose ×Storage Time** | 2 | $0.111^{NS}$ | $0.076^{NS}$ | $0.32^{NS}$ |
| **DUWP×** $Lactulose \times Storage\ Time$ | 4 | $0.073^{NS}$ | $0.013^{NS}$ | $0.002^{NS}$ |
| **error** | 36 | 0.850 | 0.053 | 0.437 |

NS, *, ** and *** respectively indicate: non-significance, and significance at p<0.05 and p<0.01, p<0.001 levels.

**Table 4.** Color characteristics of synbiotic ultrafiltrated cheeses containing demineralized ultrafiltrated whey powder (DUWP) and lactulose during 60 days storage at 4 ˚C

| Color | Storage | *$P_0L_0$ | $P_1L_0$ | $P_2L_0$ | $P_0L_1$ | $P_1L_1$ | $P_2L_1$ |
|---|---|---|---|---|---|---|---|

| Values | Time (Day) | (Control) | | | | | |
|---|---|---|---|---|---|---|---|
| L* | 1 | 89.71±0.37$^{O}$ ops | 89.40±0.54$^{Oo}$ ps | 88.87±0.46$^{Ab}$ c | 89.88 ±0.34$^{Aa}$ | 88.96±0.65$^{A}$ bc | 88.30±0.28$^{A}$ nd |
| | 30 | 88.35 ±0.89$^{ABa}$ | 87.89±0.85$^{AB}$ ab | 87.03±0.84$^{AB}$ ab | 88.39 ±0.76$^{Not}$ | 87.34±1.13$^{O}$ ops | 86.42±0.94$^{B}$ b |
| | 60 | 87.45 ±0.98$^{Not}$ | 87.20±1.29$^{Not}$ | 86.35±1.52$^{Not}$ | 87.41 ±0.82$^{Not}$ | 87.26±1.39$^{A}$ a | 86.06±1.25$^{N}$ ot |
| a* | 1 | -2.67 ±0.13$^{Aa}$ | -2.72 ±0.17$^{Aa}$ | -2.73 ±0.39$^{Aa}$ | -3.03 ±0.10$^{Aa}$ | -2.82 ±0.16$^{Aa}$ | -2.76±0.25$^{Aa}$ |
| | 30 | -2.39 ±0.11$^{Bb}$ | -2.50 ±0.41$^{Aa}$ -2.44 ±0.10 Abc | -2.53 ±0.36$^{Aa}$ -2.69 ±0.08$^{Ad}$ | -2.41 ±0.25$^{Not}$ | -2.48±0.40$^{Aa}$ | -2.46±0.22$^{Aa}$ |
| | 60 | -2.27 ±0.07$^{Chapter}$ | | | -2.18 ±0.13$^{Not}$ | - 2.33±0.16$^{Oop}$ s | - 2.62±0.09$^{Acd}$ |
| b* | 1 | 9.15 ±0.43$^{Aa}$ | 9.31 ±0.48$^{Aa}$ | 9.61±0.37$^{Aa}$ | 9.20 ±0.14$^{Aa}$ | 9.35 ±0.59$^{Aa}$ | 9.60±0.38$^{Aa}$ |
| | 30 | 9.53 ±0.67$^{Aa}$ | 9.68 ±0.83$^{Aa}$ | 9.91±1.01$^{Aa}$ | 9.68 ±0.56$^{Aa}$ | 9.87 ±0.62$^{Aa}$ | 10.10±0.77$^{A}$ a |
| | 60 | 9.78 ±0.43$^{Aa}$ | 9.89 ±0.48$^{Aa}$ | 10.16±0.37$^{Aa}$ | 9.85 ±0.14$^{Aa}$ | 9.90 ±0.59$^{Aa}$ | 9.60±0.38$^{Aa}$ |

P0, P1 and P2 are 0, 1 and 2% levels of whey permeate and L0 and L1 are 0 and 1% lactulose, respectively. Different small and capital letters indicate significant differences ($p<0.05$) in each row (treatments) and column (days) for each cheese characteristics, respectively.

Joan et al.]32[Decreasing the amount of fat caused by reducing the lightness of the cheese samples was proposed. These results are presented by Rodan et al.٣3 [ also confirmed. While Torabi et al.]٣4 [In the review of ultrapurified synbiotic cheese containing powderDUWP and inulin showed that the brightness of the optimal sample at the end of the storage period is higher than other produced samples, these researchers considered the reason for the increase in brightness to be tissue porosity.

### ٣-٣- Overall acceptance evaluation

Sensory characteristics of the samples play a significant role in the acceptability of cheese by consumers. The two processes of lipolysis and proteolysis play an essential role in sensory characteristics. It should be noted that the secondary changes that occur simultaneously with these processes are responsible for partial and general changes in the sensory characteristics of cheese. The main substrate of this process is caseins, lipids and compounds soluble in milk. Also, with the passage of storage time, due to microbial activity and enzymatic interactions, the characteristics of the scab change due to biochemical reactions, and because of this, new characteristics are created in the aroma, taste, and texture of the samples.35[.

The results of statistical analysis of the effect of three powder variablesDUWP, lactulose powder and storage time on the overall acceptance of cheese samples are shown in Figure 1. Variance analysis of the results of the overall acceptance study indicates the significance of the independent effect of the powderDUWP And it was lactulose powder. The obtained data showed that by increasing the amount of powderDUWP and lactulose powder, the overall acceptance rate decreases. These results are due to the negative effect that lactulose powder and powderDUWP It can be justified based on the texture and flavor of the samples. On average, overall acceptance of samples containing 0, 1 and 2 percent




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powderDUWP 8.43, 8.34 and 8.08 respectively (0٠1/٠*P*<). However, there is a significant difference between the control sample and the sample containing 1% powderDUWP did not see Also, the overall acceptability of the samples due to the addition of 0 and 1% of lactulose powder was determined as 8.34 and 8.23, respectively.٠٥/٠*P*<). As can be seen in Figure 1, although by increasing the amount of powdersDUWP and lactulose, the overall acceptance score of ultra-refined synbiotic cheese samples decreases, but there is a significant difference between the control sample (score 8.47) and the sample containing 1% of each of the powdersDUWP and lactulose (score 8.28) was not present. Jirsarai et al.]٣6[In results similar to the results of this research (the general acceptance of the product containing 1% powdersDUWP and lactulose) showed that there was no significant difference between the control sample of probiotic ultra-refined feta cheese and the sample containing 2% inulin and lactulose mixture until the middle of the storage period, although the control sample had a lower overall acceptance score at the end of the 60-day storage period, contrary to the results of the present study. Ben Musa and colleagues]٣7[ also reported the improvement of the overall acceptability of the product when lactulose was used in the study of the effect of lactulose on yogurt samples. These researchers attributed the reason for this to the greater acceptance of sweet yogurt in Western countries. However, these researchers also showed that increasing the concentration of lactulose from a certain level onwards causes a significant decrease in the overall acceptability of the samples.٣7[.

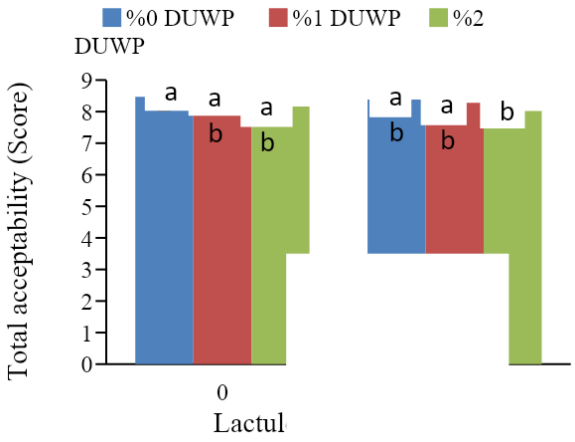


**FIG 1.** Effect of addition of demineralized ultrafiltrated whey powder (DUWP) and lactulose powder on total acceptability of synbiotic ultrafiltrated cheeses

Also, the results of this research showed that with the passage of storage time, the scoreOverall acceptance of cheese samples significantly(0٠1/٠*P*<) Decreased. Meanwhile, there was no significant difference between the overall cheese acceptance score on the 1st and 15th day. On average, the total acceptance of ultra-refined cheese samples was determined as 8.56, 8.48, and 7.82 in the periods of 1, 30, and 60 days of storage, respectively. Along with the results of this research, Jirsarai et al.٣6[In investigating the effect of inulin and lactulose on the sensory properties of probiotic ultra-refined feta cheese samples during 60 days of storage, they reported that the quality of the cheese samples decreased significantly over time.

## ٤- Conclusion

The results of the current research indicate that the use of appropriate concentrations of

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powderDUWP And lactulose in ultra-refined cheese formulation can produce products with desirable characteristics. It is worth noting that although factors such as The type of treatment, the rate of metabolization of compounds and the rate of reductionpH Due to the consumption of prebiotic compounds by probiotics, the number of probiotic bacteria decreases, but the results of this research showed that the number of bacteria in samples of synbiotic ultra-processed cheese containing high levels of lactulose andDUWP After two months of storage in the refrigerator more than the standard (Log cfu/g $10^7$) Was. At the end of 60 days of storage, the amount bacteria *Bifidobacterium Bifidium* In the examplesSynbiotic cheese containing 1% powderDUWPand 1% lactulose powder Log cfu/g 7/28 and the sample contains 2% powderDUWPand 1% lactulose powderLog cfu/g It was 7/63. Regarding the color index, the results showed that the addition of powderDUWP cause significant changes in the indexL* samples, but this variable has an effect on the indicatorsa* Andb* did not have Also, adding lactulose to any of the indicatorsCheese color had no significant effect. However, with increasing amountsDUWP،The brightness index value and overall acceptance score of synbiotic cheese samples decreased, but there was a significant difference in this respect between the control sample and the sample.Contains 1% powderDUWPand 1% lactulose powderdid not have. Therefore, a sample of ultra-processed synbiotic cheese contains ۱ Powder percentage DUWPAnd 1% lactulose powder was identified as the best sample.Therefore, by using the mentioned formulation, it is possible to produce products with health-enhancing properties that can maintain their probiotic properties until the end of 60 days of storage..

## ۵- Appreciation

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**مقاله علمی_پژوهشی**

# تأثیر پودر آب‌پنیر فراپالایش و لاکتولوز بر قابلیت زنده‌مانی باکتری *بیفیدوباکتریوم بیفیدیوم* و ویژگی‌های رنگ پنیر سین-بیوتیک فراپالوده

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**چکیده**

در طول چند دهه اخیر، آگاهی مصرف‌کنندگان در مورد اهمیت دریافت مود غذایی سلامت‌بخش منجر به تمایل آن‌ها به سمت مصرف مواد غذایی خاص و افزوده‌های غذایی شده است که مزایای سلامت-بخش قابل توجهی به‌همراه خود دارند. این شرایط منجر به توسعه غذاهای فراسودمند شده است. در پژوهش حاضر، تاثیر غلظت‌های مختلف پودر آب‌پنیر فراپالوده املاح‌گیری شده (DUWP) در سطوح صفر، ۱ و ۲ درصد (W/V) و لاکتولوز در سطوح ۰ و ۱ درصد، (W/V) بر پنیر سین‌بیوتیک فراپالوده به‌عنوان یک غذای فراسودمند مورد ارزیابی قرار گرفت. قابلیت زنده‌مانی باکتری‌های *بیفیدوباکتریوم بیفیدیوم*، شاخص‌های رنگ و پذیرش کلی محصول طی ۶۰ روز نگهداری در دمای ۴°C بررسی شد. نتایج نشان داد که با افزایش پودرهای لاکتولوز و DUWP، تعداد پروبیوتیک‌ها در نمونه‌های پنیر افزایش و با گذشت زمان نگهداری تعداد آن‌ها کاهش معنی‌داری یافت ($p$<۰/۰۰۱)؛ هرچند در پایان زمان ۶۰ روز نگهداری، تعداد باکتری‌های پروبیوتیک در نمونه‌های حاوی پودرهای لاکتولوز و سطوح ۱ و ۲٪ DUWP همچنان بالاتر از محدوده‌ی استاندارد غذاهای پروبیوتیک (Log cfu/g ۱۰$^{۷}$<) قرار داشت. نتایج رنگ‌سنجی نشان داد میزان روشنایی (L*) تحت تأثیر پودرDUWP و گذشت زمان نگهداری به-طور معنی‌داری کاهش یافت ($p$<۰/۰۰۱) اما افزودن پودرلاکتولوز و DUWP تأثیری بر شاخص‌های a* و b* نگذاشت. براساس نتایج به‌دست آمده، نمونه پنیر سین‌بیوتیک حاوی ۱٪ پودر DUWP و ۱٪ پودر لاکتولوز از شمارش باکتری‌های پروبیوتیک بالاتری نسبت به نمونه شاهد برخوردار بود؛ درحالی که اختلافی میان دو نمونه مذکور از نظر کیفیت رنگ و پذیرش‌کلی مشاهده نگردید. بنابراین این نمونه پنیر سین‌بیوتیک فراپالوده به‌عنوان بهترین نمونه فراسودمند مشخص گردید.