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PHYSICOCHEMICAL, MICROBIAL AND SENSORY CHARACTERISTICS OF LOW-FAT STIRRED YOGURT CONTAINING *BIFIDOBACTERIUM LACTIS* AND PREBIOTIC COMPOUNDS

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ABSTRACT

Most of the probiotic foods are dairy products. Presently, yogurt is the most widely used probiotic products in the world. The purpose of this study was to evaluate the effect of whey protein concentrate and oligofructose on viability of *Bifidobacterium lactis* and physicochemical and sensory characteristics of low-fat stirred yogurt. Probiotic yogurt containing oligofructose (0.5%, 1%, and 1.5%) and whey protein concentrate (0.5%, 1%, and 1.5%) were prepared and stored at 4°C for 21 days. Microbial, physicochemical and sensory properties of yoghurt samples were examined on days 1, 7, 14 and 21 and compared with control sample (sample without oligofructose and whey protein concentrate). The results showed that stirred yogurt with 1.5 % whey protein concentrate and 1.5 % oligofructose had the best sensory properties. The samples containing 1.5 % of whey protein concentrate and three levels of oligofructose (0.5%, 1%, and 1.5%) had the highest levels of probiotic bacteria. According to the results of this study, stirred yogurt with 1.5 % whey protein concentrate and 1.5 % oligofructose was chosen as the best sample in terms of physicochemical, microbiological and sensory features.

Key-words: *Bifidobacterium lactis*, Low-fat stirred yogurt, Oligofructose, Whey protein concentrate

INTRODUCTION

In recent years the consumption of functional foods, including probiotic foods has received much attention (Martin-Diana *et al*., 2003). Probiotics and prebiotics play an important role in human health. In recent years, there has been a substantial increase in research on the identification and confirmation of potential health benefits associated with the use of probiotics and prebiotics (Saad *et al*., 2013). Probiotics have been considered as live microorganisms which produce adequate amounts of healthy properties for their host (Yeganehzad *et al*., 2007).

Probiotics also have a significant impact on other microorganisms. The effect is observed in the prevention and treatment of infections and improvement of gastrointestinal microbial balance. Finally probiotics affect the performance of microbial metabolites such as toxins, compounds produced by the host such as bile salts, and food raw materials. This effect leads to inactivation of the toxins and removing them from digestive tract of the host and food components (Wohlgemuth, 2010). The most important effects of probiotics include reducing serum cholesterol level; decreasing the incidence of constipation, diarrhea and colon cancer; improving lactose intolerance, the absorption of calcium and vitamin synthesis, and stimulating the immune system (Sanchez *et al*., 2009).

Prebiotic was defined for the first time as a non-digestible food which has beneficial effects on the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the intestine and improving the host health (Gibson *et al*., 2004; Roberfroid, 2007). Some of important prebiotic components include lactulose, inulin and oligofructose (Thammarutwasik *et al*., 2009; Matijevic *et al*., 2009). Oligofructose, a Short-chain inulin, is an indigestible oligosaccharide and is composed of linear units of fructose which is connected to a glucose unit by β (2→1) bonds. It is a safe and non-toxic additive for humans. Its beneficial effect on human health is not limited to the effects of fiber for bowel movements control, reducing cholesterol and increasing calcium absorption, but it is also useful for probiotic species (Roberfroid, 2002; Villegas, 2007). In recent years, whey protein has been used in many food formulations. Use of this protein is not only due to its unique properties, but also due to functional and technological properties (Nicorescu *et al*., 2008; Rullier *et al.*, 2010).

The purpose of this research was to investigate the effect of oligofructose and whey protein concentrate on *Bifidobacterium lactis* survival and physicochemical and sensory properties of low-fat stirred yogurt.

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MATERIALS AND METHODS

Materials

Milk 1.5% fat (Tehran Pegah Dairy Company), skim milk powder (Tehran Pegah Dairy Company), stirred yoghurt stabilizer (Tate and Lyle Germany), whey protein concentrate (Pegah Dairy Company in East Azerbaijan) and oligofructose (Beneo Company Belgium) were used. Microbial cultures included yogurt starter bacteria (YC-x11), containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermoplilus*, and probiotic bacterium (*Bifidobacterium lactis*), both in the form of freeze-dried and DVS (CHR Hansen, Denmark). MRS agar (Merck, Germany) and bile (Sigma, America) were used.

Producing low-fat probiotic stirred yogurt

Skim milk (2.5%), stirred yogurt stabilizer (1%), salt (0.3%), oligofructose (0.5%, 1%, and 1.5%) and whey protein concentrate (0.5%, 1%, and 1.5%) were added to milk 1.5% fat and homogenized. Then the samples were pasteurized at 85°C for 15 minutes. After that the temperature of them were reached to 42°C. Yogurt starter and *Bifidobacterium lactis* were added. The ratio of probiotic bacteria to yogurt starter was 2:1. Initial population of probiotic bacteria was 9log cfu/mL. Similar ratios of yogurt starters and probiotic bacteria were inoculated into the control sample but the control sample did not contain oligofructose and whey protein concentrate. The samples were incubated at 40°C until their pH reached to 4.7. Yogurt samples were stirred for 60 seconds and then cooled to 4°C. They poured and packed into sterile plastic containers and stored at 4°C.

Table 1. The treatments used in the study.

Treatment	Code
Stirred yogurt with 0.5 % whey protein concentrate and 0.5 % oligofructose	$W_{1/2}$+$O_{1/2}$
Stirred yogurt with 0.5 % whey protein concentrate and 1 % oligofructose	$W_{1/2}$+O_1
Stirred yogurt with 0.5 % whey protein concentrate and 1/5 % oligofructose	$W_{1/2}$+$O_{3/2}$
Stirred yogurt control (Without whey protein concentrate and oligofructose)	C
Stirred yogurt with 1 % whey protein concentrate and 0.5 % oligofructose	W_1+$O_{1/2}$
Stirred yogurt with 1 % whey protein concentrate and 1 % oligofructose	W_1+O_1
Stirred yogurt with 1 % whey protein concentrate and 1.5 % oligofructose	W_1+$O_{3/2}$
Stirred yogurt with 1.5 % whey protein concentrate and 0.5 % oligofructose	$W_{3/2}$+ $O_{1/2}$
Stirred yogurt with 1.5 % whey protein concentrate and 1 % oligofructose	$W_{3/2}$+O_1
Stirred yogurt with 1.5 % whey protein concentrate and 1.5 % oligofructose	$W_{3/2}$+$O_{3/2}$

W: whey protein concentration; O, oligofructose.

Physicochemical analyses

pH of yogurt samples was measured using the pH meter (Adwa, Romany). Titratable acidity was determined by AOAC method (AOAC, 2002).

Probiotic bacteria count

For counting *Bifidobacterium lactis* in yogurt samples, MRS bile agar was used. Incubation was conducted at 37 °C for 72 h (Tharmaraj and Shah, 2003).

Sensory evaluation

Taste, smell, texture, color and overall acceptability of yogurt samples were evaluated by 10 trained panelists using 5-point Hedonic method. Score 5 and score 1 were assigned for the highest and the lowest quality, respectively.

Statistical Analysis

For statistical analysis of the results of physicochemical tests and counting *Bifidobacterium lactis* in probiotic yogurt samples, Duncan's multiple comparison test was used to determine significant differences between different samples. To identify any significant difference between the results of sensory tests, non-parametric Kruskal-Wallis H method was used.

RESULTS AND DISCUSSION

Table 2 shows physicochemical and microbial characteristics of probiotic yogurt samples on first day after production.

Table 2. Physicochemical and microbial properties of yogurt samples on first day after production (Mean ± SD).

yogurt sample	pH	Acidity(˚D)	*Bifidobacterium lactis* (log cfu/g)
$W_{1/2}+O_{1/2}$	4.44 ± 0.04 [a]	97.33 ± 7.37 [a]	8.99 ±0. 11[a]
$W_{1/2}+O_1$	4.48 ± 0.07 [a]	95.5 ± 3.11[a]	9.21 ± 0. 14[ab]
$W_{1/2}+O_{3/2}$	4.48 ± 0.03 [a]	95.33 ± 5.35[a]	9.46 ±0. 11[ac]
C	4.47 ± 0.02 [a]	96.83 ± 5.06[a]	9.55 ±0. 11[c]
$W_1+O_{1/2}$	4.46 ± 0.03 [a]	97.67 ± 3.79[a]	9.36 ±0. 08[bc]
W_1+O_1	4.47 ± 0.05[a]	99.17 ± 4.25[a]	9.31 ±0. 11 [bc]
$W_1+O_{3/2}$	4.47 ± 0.04 [a]	103.33 ± 5.86[a]	9.53 ±0. 1[c]
$W_{3/2}+O_{1/2}$	4.47 ± 0.06 [a]	105 ± 7.86[a]	9.42 ±0. 19[bc]
$W_{3/2}+O_1$	4.49 ± 0.05 [a]	104.17 ± 5.84[a]	9.47 ±0. 22[bc]
$W_{3/2}+O_{3/2}$	4.45 ±0.03 [a]	99.5 ± 0.71[a]	9.45 ±0. 22[bc]

* Same small letters in each column indicate that Kruskal-Wallis H test, there is no significant differences in the level of $\alpha = 0.05$ ($P > 0.05$) between different samples of low-fat stirred yogurt.

As can be seen in this table, there is no significant difference between the pH of probiotic stirred yogurt samples ($p > 0.05$). $W_{3/2}+O_1$ sample had the highest pH value and pH value of $W_{1/2}+O_{1/2}$ sample was the lowest. $W_{3/2}+O_{1/2}$ and $W_{1/2}+O_{3/2}$ samples have the highest and lowest acidity, respectively. There was no significant differences in the acidity of probiotic stirred yogurt samples on first day after production was observed ($p > 0.05$). Paseephol and Sherkat (2008) reported that the addition of inulin powders regardless of its type did not affect the initial pH and acidity of yogurt and also they showed that the low acidity level in these yogurts can be attributed to the type of probiotic and yogurt starters. $W_1+O_{3/2}$ and control samples had the highest numbers of probiotic bacteria. No significant difference was found between the samples $W_{1/2}+O_{3/2}$, C, $W_1+O_{1/2}$, W_1+O_1, $W_1+O_{3/2}$, $W_{3/2}+O_{1/2}$, $W_{3/2}+O_1$ and $W_{3/2}+O_{3/2}$. Aghajani *et al.*, (2012) stated that on the first day of storage, the number of probiotic bacteria was not significantly different among yogurt samples containing *Lactobacillus casei*.

Table 3 shows sensory characteristics of probiotic yogurt samples on first day after production. According to the results, highest taste score is for $W_{3/2}+O_{3/2}$ sample, which is not significantly differing from the control sample. The lowest taste score is for $W_{1/2}+O_{1/2}$ sample. The highest smell score is for $W_{3/2}+O_{3/2}$ sample which is not significantly differing from the control sample. The lowest smell score is for $W_{1/2}+O_{3/2}$ and $W_1+O_{1/2}$ samples. The highest texture score is for $W_{3/2}+O_{3/2}$ sample is not significantly differing from the control sample and the lowest texture score is for $W_{1/2}+O_{1/2}$ sample. In relation to the color of samples, it can also be said that there is no significant color differences and all the samples have the same color score. The results of the evaluations performed by reviewers indicate that $W_{3/2}+O_{1/2}$, $W_{3/2}+O_1$, and $W_{3/2}+O_{3/2}$ samples are more acceptable. Aghajani *et al.*, (2012) stated that there was no significant difference between sensory characteristics of probiotic yogurt samples on first day after production.

Figures 1 and 2 show the changes of pH and acidity of the probiotic yogurt samples during cold storage. The pH decreased until seventh day, then increased until the end of the storage period. The acidity level of probiotic stirred yogurt samples increased from day 1 until the seventh day of storage, then it decreased after day 7 until the end of the storage period, but this decrease was not significant.

Increase of pH and decrease of acidity at the end of the storage period is due to production of some essential metabolites by *Streptococcus thermophilus* (Ramchandran and Shah, 2010). It was also reported that after finishing the carbohydrate sources, microorganisms consume proteins in the environment, which leads to increase of pH and decrease of acidity (Jay, 1990). It was also reported that the buffering capacity of dairy products is a significant factor in the pH variation; this indicates that adding whey protein concentrate leads to increase of the buffering capacity at the pH values about pH = 4 (Salaün *et al.*, 2005).

Table 3. Sensory characteristics of probiotic yogurt samples on first day after production (Mean ± SD).

Yogurt sample	Taste	Smell	Texture	Color	Overall acceptability
$W_{1/2}+O_{1/2}$	2.87 ± 0.32 [a]	4.03 ± 0.35 [a]	2.93 ± 0.47 [a]	4.33 ± 0.31 [a]	3.2 ± 0.3 [a]
$W_{1/2}+O_1$	3.0 ± 0.56 [ab]	4.0 ± 0.5 [a]	3.07 ± 0.55 [ab]	4.23 ± 0.4 [a]	3.3 ± 0.66 [ab]
$W_{1/2}+O_{3/2}$	3.47 ± 0.6 [abc]	3.97 ± 0.5 [a]	3.53 ± 0.5 [abc]	4.33 ± 0.38 [a]	3.57 ± 0.64 [abc]
C	3.67 ± 0.45 [abc]	4.0 ± 0.17 [a]	3.47 ± 0.45 [abc]	4.33 ± 0.12 [a]	3.8 ± 0.3 [abc]
$W_1+O_{1/2}$	3.93 ±0.23 [c]	3.97 ± 0.45 [a]	4.0 ± 0.1 [bc]	4.53 ± 0.21 [a]	4.07 ± 0.15 [abc]
W_1+O_1	3.77 ±0.59 [bc]	4.0 ± 0.4 [a]	3.7 ± 0.95 [abc]	4.43 ± 0.38 [a]	3.87 ± 0.68 [abc]
$W_1+O_{3/2}$	4.0 ± 0.36 [c]	4.07 ± 0.45 [a]	3.9 ± 0.56 [bc]	4.47 ± 0.15 [a]	4.07 ± 0.45 [a]
$W_{3/2}+O_{1/2}$	3.93 ± 0.32 [c]	4.2 ± 0.36 [a]	4.17 ± 0.35 [c]	4.57 ± 0.15 [a]	4.23 ± 0.46 [c]
$W_{3/2}+O_1$	4.0 ± 0.46 [c]	4.1 ± 0.36 [a]	4.07 ± 0.15 [c]	4.53 ± 0.15 [a]	4.13 ± 0.45 [bc]
$W_{3/2}+O_{3/2}$	4.17 ± 0.38 [c]	4.17 ± 0.45 [a]	4.27 ± 0.31 [c]	4.47 ± 0.31 [a]	4.33 ± 0.42 [c]

** Same small letters in each column indicate that according to Kruskal-Wallis H test, there is no significant differences in the level of α = 0.05 (P > 0.05) between different samples of low-fat stirred yogurt.

Figure 3 indicates changes of probiotic bacteria of yogurt samples during cold storage. The initial number of probiotic bacteria was 9.85log cfu/g. In general, the number of *Bifidobacterium lactis* decreased, and $W_{3/2}+O_{1/2}$, $W_{3/2}+O_1$, $W_{3/2}+O_{3/2}$ samples had the highest survival level of *Bifidobacterium lactis* at the end of the storage period. The number of *Bifidobacterium lactis* was reduced by about 1.5 logarithmic cycles. Since *Bifidobacterium lactis* is an anaerobic bacterium, oxygen toxicity is a critical issue. The reduction in the number of the bacteria could be due to the presence of oxygen in the product. Gardiner (2002) reported that the minimum number of probiotic bacteria used in probiotic products is 10^6 cfu /mL. Survival of probiotic bacteria in probiotic yogurt depends on factors such as the strain used, reaction between species in the culture, chemical composition of the fermented culture medium, the final acidity, milk solids, temperature and inoculums levels (Hekmat and Reid, 2006). In a similar study by Kailasapathy *et al* (2008) about viability of the *Lactobacillus acidophilus* and *Bifidobacterium lactis* in stirred yogurt during 35 days of storage at 4°C, it was found that the mean number of living *L. acidophilus* cells decreased from 7.81×10^7 cfu/g on first day to 4.04×10^7cfu/g at the end of storage period whereas the mean number of *Bifidobacterium lactis* decreased from 1.55×10^8cfu/g to 5.45×10^7cfu/g. In another study it was found that the number of *Lactobacillus casei* in prebiotic samples is significantly higher than the control sample. However, the mean number of probiotics was less than 8 log cfu / g and it was reduced over time (5 weeks). Despite this report, decrease of the pH below 4.30 greatly affects the viability of probiotic bacteria (Aryana and Grew, 2007).

Table 4. Sensory characteristics of probiotic yogurt samples at the end of the storage period (Mean ± SD)

yogurt sample	taste	smell	texture	color	General admission
$W_{1/2}+O_{1/2}$	3.5 ± 0.26 [ab]	4.1 ± 0.26 [a]	3.2 ± 0.2 [a]	4.63 ± 0.31 [a]	3.5 ± 0.3 [a]
$W_{1/2}+O_1$	3.53 ± 0.29 [ab]	4.17 ± 0.15 [a]	3.83 ± 0.21 [abc]	4.6 ± 0.36 [a]	3.57 ± 0.23 [ab]
$W_{1/2}+O_{3/2}$	4.0 ± 0.0 [bcd]	4.17 ± 0.15 [a]	3.87 ± 0.15 [abc]	4.63 ± 0.31 [a]	3.93 ± 0.12 [abc]
C	3.3 ± 0.27 [a]	4.13 ± 0.35 [a]	3.5 ± 0.26 [ab]	4.5 ± 0.2 [a]	3.53 ± 0.21 [a]
$W_1+O_{1/2}$	3.73 ± 0.23 [abc]	4.03 ± 0.6 [a]	3.9 ± 0.46 [bc]	4.57 ± 0.49 [a]	3.9 ± 0.4 [abc]
W_1+O_1	3.97 ± 0.32 [bcd]	4.07 ± 0.6 [a]	3.77 ± 0.76 [ab]	4.57 ± 0.58 [a]	3.93 ± 0.57 [abc]
$W_1+O_{3/2}$	4.17 ± 0.5 [cd]	4.27 ± 0.55 [a]	4.13 ± 0.21 [bcd]	4.53 ± 0.47 [a]	4.27 ± 0.51 [bc]
$W_{3/2}+O_{1/2}$	4.17 ± 0.32 [cd]	4.53 ± 0.47 [a]	4.5 ± 0.46 [cd]	4.7 ± 0.26 [a]	4.47 ± 0.45 [c]
$W_{3/2}+O_1$	4.47 ± 0.4 [d]	4.6 ± 0.36 [a]	4.77 ± 0.21 [d]	4.7 ± 0.2 [a]	4.57 ± 0.4 [c]
$W_{3/2}+O_{3/2}$	4.53 ± 0.32 [d]	4.6 ± 0.3 [a]	4.77 ± 0.21 [d]	4.77 ± 0.15 [a]	4.6 ± 0.36 [c]

** Same small letters in each column indicate that according to Kruskal-Wallis H test, there is no significant differences in the level of α = 0.05 (P> 0.05) between different samples of low-fat stirred yogurt.

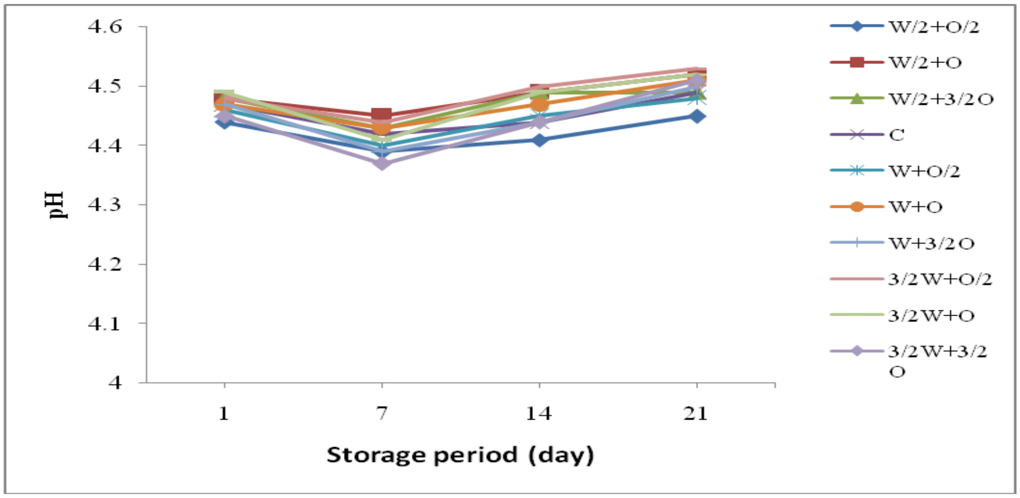

Fig. 1. Changes of pH of yogurt samples during storage period

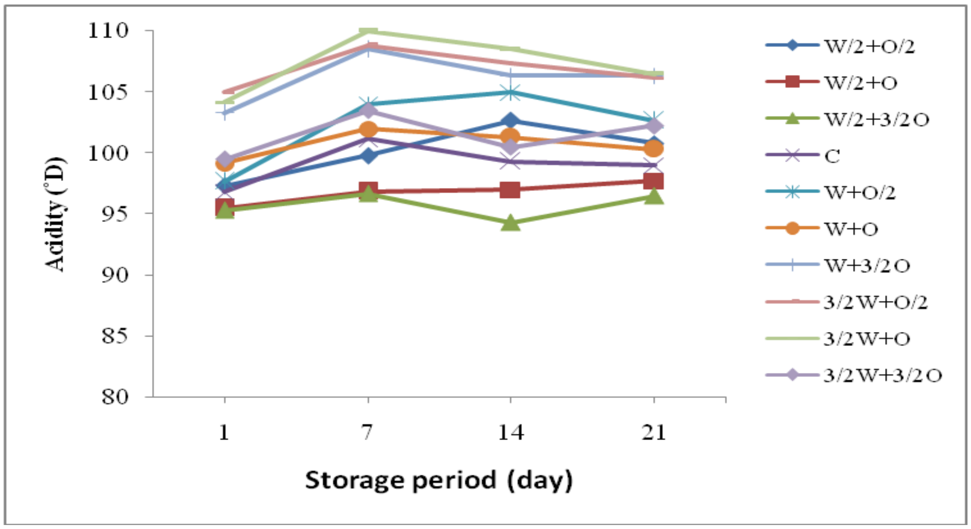

Fig. 2. Changes of acidity of yogurt samples during storage period

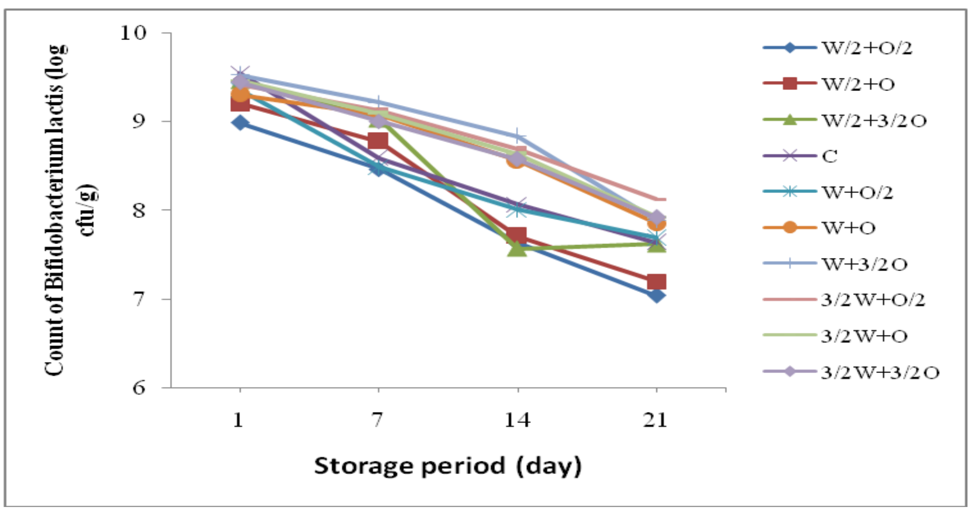

Fig. 3. Changes of probiotic bacteria count of yogurt samples during storage period

Table 4 shows sensory characteristics of yogurt samples at the end of the storage period. The highest taste score was for $W_{3/2}$+$O_{3/2}$ sample and the control sample had the lowest taste score. $W_{3/2}$ + O_1 and $W_{3/2}$ +O $_{3/2}$ samples had the highest smell score and W_1+ O $_{1/2}$ sample had the lowest smell score among probiotic stirred yoghurt samples. The texture score for all types of probiotic stirred yogurt was ascending with a gentle slope during the storage period, and $W_{3/2}$+ $O_{1/2}$, $W_{3/2}$ + O_1, $W_{3/2}$ + $O_{3/2}$ samples had highest texture score. At the end of the storage period, $W_{3/2}$+$O_{3/2}$ and control samples had the highest and lowest color score, respectively. The score of overall acceptability was ascending for all types of yogurt during storage. At the end of storage period, $W_{3/2}$ + $O_{1/2}$, $W_{3/2}$ + O_1 and $W_{3/2}$ + $O_{3/2}$ samples had higher overall acceptance score, but this trend was descending for the control sample. A similar study has reported that the adding prebiotics to dairy products leads to increasing consistency and improving mouth feel (Golob *et al*., 2004). Similarly, several studies performed by a number of researchers also suggest improvement of overall acceptability of probiotic cheese when equivalent amount of inulin and oligofructose are added. It was also reported that the use of oligofructose in dairy products will improve the structure and texture of the final product. The researchers also found that the acceptability of yogurt containing prebiotic was higher than control sample (Matigevic *et al*., 2009). Some studies have shown that during storage period, sensory characteristics of probiotic yogurt are reduced to lower than typical yogurt, unless stabilizers or prebiotic compounds are used (Hekmat and Reid, 2006). Ranadheera *et al*., (2012) reported that fruity stirred yogurts had higher aroma and taste rather than control sample. Also recorded scores for texture, taste and overall acceptance indicated that adding fruit juice will leads to positive effects on the sensory characteristics of the product. Our findings are similar to results of Ranadheera *et al*., (2012).

CONCLUSION

Based on the results, increasing the amount of whey protein concentrate and oligofructose in probiotic yogurt enhances the quality of products compared to the control sample. The results also show that using prebiotics in the formulation of probiotic yogurt bring a positive effect on yogurt due to improved growth and viability of probiotic bacteria during storage period.

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