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The influence of adding whey protein powder in probiotic white soft cheese making

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Abstract

Probiotic white soft cheese is made by adding different levels of whey protein powder (WPP) (Nil, 0.5, 1.0 and 1.5%) to milk and inoculated with 0.75% Bif. bifidum. The resultant cheeses were stored at refrigerator temperature up to 28 days. The data concluded that, there were increases of TS, TA, SN, TN, SN coefficient, TVFFA and salt in water ratio with increasing storage periods up to 28 days and with increasing level of WPP in all treatments. The control samples (C- and C+) had lower and higher values of TS, TA, SN/TN, TVFFA & salt in water ratio and syneresis & firmness than that of other treatments, respectively. The cheese made from 1.5% WPP (T3) had higher values of TS, TA, SN, TN, SN coefficient, TVFFA, salt in water ratio than that of other treatments. There were increases of TC and Bif. bifidum counts with increasing storage periods at refrigerator temperature up to 28 days and with increasing level of WPP in all treatments. There were not detected psychotropic bacteria and coliform group counts in all treatments. Yeast and moulds were not detected in fresh cheese or after one week of storage, but they were detected and increase gradually with the progress of storage at 14 up till 28 days in most treatments. The data showed that, T2 stored for 7 days had the highest scores than that of the other treatments using of different levels of WPP. Whilst, T2 stored for 21days had the lowest scores than that of the other treatments.

Keywords: probiotic bacteria, whey protein, white soft cheese, sensory quality, acceptability.

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1. Introduction

Whey is the soluble fragment of milk, which separates from the curds during cheese manufacturing. Whey is an opaque liquid with a greenish-yellow color (Magenis, 2006). The components of whey include; α-lactalbumin, β-lactoglobulin, bovine serum albumin, lactoferrin, immunoglobulins, lacto-peroxidase enzymes, glycol-macropeptides, lactose, and minerals (Walzem *et al.,* 2002). There are three main types of whey protein which is obtained from various processing techniques that is used to separate whey protein. They are; whey powder (WP), whey concentrate (WPC) and whey isolate (WPI) (Shankar and Bansal, 2013). Whey proteins possess important features pertaining to human nutrition and health, which easily override their classical low added value. Additionally, antimicrobial and antiviral actions, immune system stimulation, and anti-carcinogenic activity have been claimed for such whey proteins as α-lactalbumin, β-lactoglobulin, lactoferrin, lacto-peroxidase, serum albumin and glyco-macropeptide (Madureira *et al.,* 2007). The availability of carbohydrate reservoir of lactose in whey and presence of other essential nutrients for the growth of microorganisms makes the whey one of the potential substrate for the production of different bio products through biotechnological means (Hofvendahl and Hahn-Hagerdal, 2000). Nowadays, utilization of WP and WPC to food and dairy products is considered to be one of the most important functional foods due to their effects either on the product characteristics and/or the health promotion (Shenana *et al.,* 2007). Probiotics are defined as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001), being Lactobacillus and Bifidobacterium the most frequently used genera (Sharma and Devi, 2014). Cheese may offer several advantages as a probiotic carrier due to its higher pH and fat content and harder consistency compared to fermented milks. These features provide more protection to probiotics not only during cheese production, ripening and storage, but also during the passage through the gastrointestinal tract, allowing bacteria to arrive in higher numbers at the target site after ingestion (Stanton *et al.,* 1998). So that, the aim of the present study was to examine the effect of different levels of WPP mix with 0.75% Bif. Bifidum on chemical, microbiological, rheological and organoleptic properties of resultant cheese.

2. Materials and methods

2.1 Materials

All chemicals used in this study were analytical grade supplied by Sigma BDH, and Difco chemical companies. Distilled water was used for the preparation of all solutions, Pyrex glassware were used throughout. Fresh buffalo's milk was obtained from the Herd of the Animal Production Department, Faculty of Agriculture, Al-Azhar University (Branch of Assiut), Egypt. Whey protein powder (WPP) was used as a powder obtained from locally market (Cairo, Egypt). Enzyme sources (Microbial rennet) was used as a powder obtained from DSM (France) with a commercial name (Fromase R 2200). Commercial sodium

chloride was obtained from El-Nasr Company, for salt (Alexandria, Egypt). Bifi. bifidum (EMCC 2203), were obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2.2 Methods

2.2.1 Manufacture of probiotic white soft cheese with whey protein powder

Probiotic white soft cheese was made by conventional method of making Domiati cheese according to the adopted method of Metwalli *et al.* (1982) with some modification as follow: Law fat buffalo milk (4% fat) was divided into five equal portions; WPP (Nil, 0.5, 1.0 & 1.5%), every part was heated to 72 ± 1◦C for 5 min, 4% salt (w/w) beside controls samples (with and without starter culture), rapidly cooled to 40-42◦C, and then starters and rennet were added as following:

- C^- : Nonstarter cheese. C^+: cheese with 0.75% Bifi. bifidum starter.
- T1: Adding 0.5% WPP + 0.75% Bifi. bifidum starter.
- T2: Adding 1.0% WPP+ 0.75% Bifi. bifidum starter.
- T3: Adding 1.5% WPP + 0.75% Bifi. bifidum starter.

The resultant cheese was packed in about 350 g of cheese in plastic cans of 1000 g capacity, filled with its own drained whey, then stored in refrigerator at 6±1°C. Samples were taken fresh and after 7, 14, 21 and 28 day for analysis.

2.2.2 Chemical analysis

- Total solids and ash contents: They were determined according to the AOAC (2000).
- Fat content: It was determined in milk and cheese by the conventional Gerber's method as described by Ling (1963).
- Salt content: It was determined according to the method described by Simov (1980).
- Total Nitrogen (TN): It was determined by the semi-micro kjeldahl as described by Ling (1963).
- Water-soluble nitrogen (SN) content: It was determined according to Kuchroo and Fox (1982).
- Total volatile free fatty acids (TVFFA): It was determined using the method described by kosikowski (1982).
- Titratable acidity: It was determined according to the method described by Ling (1963).
- PH measurement: pH values of the samples was determined using a pH meter (model 68 ESD 19713), USA.

2.2.3 Microbiological analyses

Total bacterial count, coliform, yeast and moulds of cheese samples were determined according to Marshall (1992). Psychotrophic bacterial count is estimated by plating the samples using the SPC procedure and incubating for 10 days at 7°C (Marshall, 1992). Bifidobacteria counts were enumerated according to

Dave and Shah (1996) using modified MRS agar medium (m-MRS), supplemented with 0.05% L-Cysteine HCl and 0.3% lithium chloride. The plates were incubated at 37°C for48 h under anaerobic condition.

2.2.4 Rheological testes

Curd firmness (CF): The penetration method described by Shalabi (1987). Curd syneresis: The volume of whey expelled within 60 min microbial rennet, was measured by the method described by Marshall (1982).

2.2.5 Organoleptic scoring

Organoleptic properties of cheese samples were evaluated according to the method of Pappas *et al*. (1996).

3. Results and Discussion

In this present investigation attempts have been made to judge by using of suitability WPP for making white soft cheese with Bifi. Bifidum. White soft cheese is made by combining different levels of WPP (0.5 ,1.0 and 1.5%) during heat treatment of milk to 72 ± 1°C for 5 min, then rapidly cooled to 40-42˚C and inoculated with Bifi. Bifidum (0.75%) (w/w) for each treatment. The resultant white soft cheeses were stored at refrigerator temperature (6 ± 2°C) up to 28 days. The obtained final products were evaluated for its chemical analysis, rheological properties, microbiological analysis and sensory evaluation.

3.1 The chemical composition of probiotic white soft cheese with different levels of WPP

Data presented in Table (1) illustrated the chemical composition of white soft cheese with Bifi. Bifidum made with different levels of WPP during storage periods at refrigerator temperature for 28 days.

3.2 Total solid contents (TS)

The data in Table (1) revealed that, the TS content of white soft cheese was affected by addition of different levels of WPP during storage periods at refrigerator temperature for 28 days. The TS content of white soft cheese found to increase with increasing the storage periods up to 28 days and with increasing the WPP level in all treatments. This increase could be attributed to the contraction of curd as a result of developed acidity during pickling period, which helps to expel the whey from the curd and the high level of total solids in the WPP. These results are in line with those reported by Dhuol and Hamid (2013). Moreover, the control samples (C+) had lowers TS than that of white soft cheese made with different levels of WPP; whilst, the control samples without culture (C-) had lower TS than that of control samples with Bifi. bifidum (C+). These results are in agreement with those obtained by Samy *et al.* (2013).

3.3 Titratable acidity and pH values

The data in the previous table revealed that, the TA and pH values of white soft

cheese was affected by combining storage periods at refrigerator up to 28 different levels of WPP and during days.

Table (1): Effect of different levels of WPP on chemical composition of white soft cheese with Bifi. bifidum held at 6±1°C up to 28 days.

Properties	Storage (days)	Control samples		Treatments		
		C-	C+	T1	T2	T3
TS %	Fresh	32.26	33.93	36.52	36.90	37.46
	7	32.43	35.31	37.88	38.01	38.78
	14	33.53	36.92	38.17	38.67	39.11
	21	34.92	37.24	38.87	39.22	39.78
	28	35.78	38.16	39.84	40.35	41.17
Acidity %	Fresh	0.15	0.175	0.175	0.175	0.175
	7	0.175	0.200	0.200	0.200	0.250
	14	0.2	0.325	0.400	0.400	0.450
	21	0.325	0.425	0.55	0.625	0.625
	28	0.51	0.625	0.725	0.775	0.875
pH	Fresh	6.9	6.6	6.6	6.7	6.7
	7	6.6	6.13	6.15	6.18	6.08
	14	6.11	5.84	5.72	5.72	5.65
	21	5.31	5.19	5.19	5.08	5.03
	28	5.08	5.00	4.85	4.77	4.72
TN %	Fresh	1.47	1.54	1.75	1.78	1.79
	7	1.67	1.85	1.92	2.06	2.18
	14	1.82	1.85	2.02	2.18	2.22
	21	1.89	2.04	2.16	2.22	2.32
	28	2.03	2.18	2.34	2.51	2.77
SN %	Fresh	0.108	0.155	0.166	0.171	0.173
	7	0.160	0.182	0.201	0.237	0.256
	14	0.191	0.251	0.277	0.317	0.358
	21	0.217	0.253	0.355	0.471	0.552
	28	0.346	0.481	0.556	0.608	0.678
(SN/TN) × 100	Fresh	7.33	10.06	9.49	9.63	9.65
	7	9.58	9.84	10.47	11.50	11.74
	14	10.49	13.54	13.71	14.53	16.16
	21	11.48	12.40	14.58	16.68	19.52
	28	17.04	22.04	23.72	24.20	24.46
TVFFA (ml 0.1 N NaOH/100 g cheese)	Fresh	10.15	10.16	10.21	10.25	10.61
	7	10.94	11.25	11.54	11.81	12.36
	14	11.46	11.93	12.11	12.75	13.11
	21	12.15	12.83	13.49	13.75	14.31
	28	16.11	18.1	20.45	21.33	22.12
Salt in water ratio	Fresh	4.61	4.68	4.72	5.19	5.52
	7	4.57	4.99	5.33	5.52	5.77
	14	4.84	5.40	5.73	6.01	6.25
	21	5.04	5.53	5.91	6.24	6.39
	28	5.10	5.90	6.18	6.40	6.73
F/DM × 100	Fresh	27.89	29.47	26.01	24.93	24.29
	7	29.29	30.30	27.72	27.62	26.82
	14	29.99	30.34	28.82	28.45	27.61
	21	30.07	30.88	29.59	28.56	27.65
	28	30.74	31.45	30.12	29.26	27.93

TS = Total solids, TN = Total nitrogen, SN = soluble nitrogen, TVFFA = Total volatile free fatty acids F/DM = Fat / Dry Matter.

The acidity of white soft cheese found to increase with increasing of WPP percentages and during storage periods at refrigerator up to 28 days. The control samples had lower acidity than that of white soft cheese made with different levels of WPP. The control samples (C-) had lower acidity than that of control samples (C+). The differences among cheese treatment in acidity and pH values might be attributed to the availability of carbohydrate reservoir of lactose in WPP and presence of other essential nutrients for the growth of Bifi. Bifidum and ability to ferment lactose during pickling periods. These results are in agreement with those obtained by El-Abd *et al*. (2003) and Amin *et al*. (2013), who found that the use of different starter cultures in Domiati cheese manufacture increased the acidity. Whilst, the pH values of white soft cheese found to decrease with increasing of WPP concentration and during storage period at refrigerator up to 28 days. Moreover, the control samples had higher pH than that of white soft cheese made with Bifi. Bifidum made with different levels of WPP. These results are in agreement with those obtained by Perotti *et al*. (2014). The control samples (C-) had higher pH than that of control samples (C+). In the same order, these results are in agreement with those obtained by Edgaryan *et al*. (2007) and Samy et al. (2013).14.3 and 14.43 in 1st and 2nd seasons, respectively, indicating of the organic fertilizer.

3.4 Soluble and total nitrogen

The obtained data in Table (1) revealed that; the SN and TN of white soft cheese made with different levels of WPP found to increase with increasing of WPP percentages and during storage periods at refrigerator up to 28 days in all treatments. This increase in TN and SN could be due to the increasing in TS content & high level of TN in WPP and the activity of proteinases & peptidases released from Bifi. bifidum which resulted in higher proteolysis in cheese, respectively. Moreover, the control samples had lower SN and TN than that of white soft cheese made with different levels of WPP. Whilst, the control samples (C^-) had lower SN and TN than that of control samples (C^+). These results are in agreement with those obtained by Samy *et al*. (2013) and Jelena *et al*. (2014).

3.5 Soluble nitrogen coefficient (SN/TN)

The obtained data in Table (1) revealed that, the soluble nitrogen coefficient found to increase with increasing of WPP percentages and during storage periods at refrigerator up to 28 days in most treatments. In addition, the control samples had lower values of soluble nitrogen coefficient than that of white soft cheese made with different levels of whey protein powder in most treatments. Moreover, the control samples (C-) had lowers SN coefficient than that of control samples (C+) in all treatments.

3.6 Total volatile free fatty acids content (TVFFA)

The obtained data in Table (1) revealed that, the TVFFA content found to increase

with increasing of WPP percentages and during storage periods at refrigerator up to 28 days in all treatments. In addition, the control samples had lower values of TVFFA than that of white soft cheese made with different levels of WPP. This increase in TVFFA could be due to the lipolytic activity of Bifi. bifidum. Moreover, the control samples (C^-) had lowers TVFFA than that of control samples (C^+). These results are in agreement with those obtained by Kebary *et al*. (2015).

3.7 Salt in water ratio

The obtained data in Table (1) revealed that, the salt in water ratio found to increase with increasing of WPP percentages and during storage periods at refrigerator up to 28 days in all treatments. This increase in salt in water ratio could be due to the decrease in the moisture content in cheese made with different levels of whey protein powder. These results are in agreement with those obtained by Kebary *et al*. (2015). In addition, the control samples had lower values of salt in water ratio than that of white soft cheese made with different levels of WPP. Moreover, the control samples (C^-) had lowers salt in water ratio than that of control samples (C^+).

3.8 Fat / Dry Matter (F/DM)

The obtained data in Table (1) revealed that, the F/DM was increase gradually as the storage periods progressed up to 28 days in all treatments and decrease with increasing WPP levels in most treatments. This increase in fat content could be due to the decrease in the moisture content in cheeses. These results are in agreement with those obtained by Kebary *et al*. (2015). On the other hand the decrease in the fat content with increased levels of WPP may be due to the low level of fat content in the WPP. In addition, the control samples had higher values of fat/DM than that of white soft cheese made with different levels of WPP. Moreover, the control samples (C^-) had lowers fat/DM than that of control samples (C^+).

Table (2): Effect of different levels of WPP on rheological properties of fresh white soft cheese with Bifi. Bifidum .

Properties	Treatment				
	C-	C+	T1	T2	T3
Syneresis (ml/100 g)	37.5	38.0	34.0	32.0	28
Firmness (g)	21.08	23.05	21.07	20.95	19.09

3.9 Rheological properties of probiotic white soft cheese made with different levels of WPP

Data presented in Tables (2) illustrated the rheological properties of white soft cheese with *Bifi. Bifidum* made with different levels of WPP during storage periods at refrigerator temperature for 28 days. The obtained data observed that, the

control samples had the higher values of syneresis as well as firmness values than that of other treatments using different levels of WPP. In addition, the data observed that the control samples (C-) had lower values than that of control samples (C+). The lower values of syneresis and firmness might be due to the whey proteins and its effects on water binding.

3.10 Microbiological analyses of white soft cheese with Bifi. bifidum with different levels of WPP

Data presented in Tables (3) illustrated the microbiological analyses of white soft cheese made with different levels of WPP during storage periods at refrigerator temperature for 28 days.

Table (3): Effect of different levels of WPP on microbiological properties of probiotic white soft cheese held at 6±1°C up to 28 day (Logcfu/g-1).

Properties	Storage periods (days)	Treatment				
		C-	C+	T1	T2	T3
Total count	Fresh	7.79	7.91	7.92	7.98	8.02
	7	7.85	7.93	7.97	8.79	8.82
	14	8.59	8.65	8.71	9.06	9.15
	21	8.66	8.73	8.91	8.93	10.29
	28	8.71	9.8	9.98	10.06	11.06
Bif. bifidum	Fresh	ND*	7.67	7.77	7.78	8.07
	7	ND	7.83	7.89	8.46	8.47
	14	ND	8.47	8.51	8.53	8.59
	21	ND	8.51	8.54	8.56	8.71
	28	ND	8.98	9.66	9.74	9.79
Psychotrophic bacteria	Fresh	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND
	21	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND
Coliform group	Fresh	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND
	21	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND
Yeast & Moulds	Fresh	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND
	14	3.58	3.54	3.53	3.5	ND
	21	3.65	3.57	3.54	3.53	3.50
	28	3.74	3.69	3.67	3.6	3.56

3.11 Psychotrophic bacteria and coliform group counts

The obtained data in Table (3) showed that, there were not detected psychotrophic bacteria and coliform group counts in all treatments in fresh cheese and after storage period in refrigerator temperatures up to 28 days. This might be due to high hygienic condition during making cheese and pickling period and the development in

the acidity in cheese when fresh and during the pickling period. These results are in agreement with those obtained by Kebary *et al*. (2015).

3.12 Yeast and moulds counts

The obtained data in Table (3) illustrated that, yeast and moulds were not detected in fresh cheese or after one week of storage, but they were detected and increase gradually with the progress of storage at 14 days up till 28 in all treatments. In addition, the control samples (C^- and C^+) had highest counts of yeasts and moulds than the other treatments after storage periods on 14 days.

3.13 Organoleptic properties of probiotic white soft cheese with made with different levels of WPP

Data presented in Tables (4) illustrated the organoleptic properties of white soft cheese with Bifi. Bifidum made with different levels of WPP during storage periods at refrigerator temperature for 28 days. The obtained data observed that, the organoleptic properties such as; flavor, body & texture and appearance & color of white soft cheese with Bifi. Bifidum made from low fat buffalo's milk were affected by addition of levels of WPP and during storage periods at refrigerator temperature up to 28 days.

Table (4): Effect of different levels of wpp on organoleptic properties of white soft cheese with Bifi. Bifidum held at 6±1°C up to 28 days.

Properties	Storage periods (days)	Treatment				
		C-	C+	T1	T2	T3
Flavor (50)	Fresh	43.25	42.00	42.00	42.25	42.75
	7	42.25	47.00	37.75	49.50	42.75
	14	41.00	38.80	39.50	39.50	37.50
	21	38.35	37.50	36.50	36.00	38.00
	28	40.00	39.40	36.20	41.40	37.00
Body and texture (40)	Fresh	34.75	34.00	34.50	33.50	33.75
	7	35.00	37.50	36.25	37.00	33.75
	14	34.17	33.67	32.50	33.17	33.67
	21	31.25	29.25	28.75	28.00	31.00
	28	37.80	36.00	37.20	35.80	31.60
Appearance and color (10)	Fresh	8.00	7.50	8.00	7.50	7.50
	7	8.25	8.25	8.75	8.00	7.50
	14	7.70	7.80	7.70	8.00	7.20
	21	7.75	7.75	7.75	8.00	7.80
	28	9.20	8.80	9.00	8.80	8.20
Total scores (100)	Fresh	86.00	83.50	84.50	83.30	84.00
	7	85.50	92.80	82.80	94.50	84.00
	14	82.80	80.30	79.70	80.70	78.30
	21	77.80	74.50	73.00	72.00	76.80
	28	87.00	84.20	82.40	86.00	76.80

3.14 Flavor

The obtained data in Table (4) observed that, the sample of T2 stored for 7 days had the highest values of flavor than that of the other treatments using different levels of WPP. In addition, the sample of T2 stored for 28 days had the lowest flavor than that of the other treatments.

3.15 Body and texture

The obtained data in Table (4) observed that, the C^- sample stored for 28 days had the highest values of body and texture than that of the other treatments using of different levels of WPP. In addition, the samples of T2 sored for 21 days had the lowest body and texture than that of the other treatment.

3.16 Appearance and color

The obtained data in Table (4) observed that, the control sample C^- stored for 28 days had the highest values of appearance and color than that of other treatments using of different levels of WPP. In addition, the samples of T3 stored for 14 days had the lowest appearance and color than that of the other treatments.

3.17 Total scores

The obtained data in Table (4) showed that, samples of T2 stored for 7 days had the highest scores than that of the other treatments using of different levels of WPP. Whilst, the samples of T2 stored for 21days had the lowest scores than that of the other treatments. In addition, the control samples (C^-) had higher scores than that of the control samples (C^+) in most treatments.

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