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EFFECT OF CASEIN HYDROLYSATE ON THE QUALITY OF LOW FAT YOGURT

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ABSTRACT

The aim of the present study was to investigate the effect of 1.5% casein hydrolysate as well as combination with probiotic bacteria on the physical and chemical properties of low fat yogurt during 14 days storage at 4°C±1. Effects of casein hydrolysate by trypsin enzyme have been also investigated on acidification of the yogurt, water holding capacity (WHC), curd firmness and growth both commercial culture and probiotic bacteria during 14 days storage at 4°C±1. The obtained results showed the addition of 1.5% casein hydrolysates improved the water holding capacity (WHC) at the 1st day and during storage for 14 days at 4°C comparing to the control. Casein hydrolysates strongly decreased the fermentation and coagulation time of the yogurts. The rate of the decreases in pH was higher with casein hydrolysate in comparison with control. The sensory evaluation scores of low fat yogurts were improved by the addition of 1.5% casein hydrolysate. Also, casein hydrolysates improved flavour compounds from 1st day till 14th days of storage for all samples. The hydrolysates improved the viability of probiotic bacteria and starter culture during storage. Low fat yogurt without casein hydrolysates showed a decline of probiotic bacteria and lactic acid bacteria (LAB) counts after 7 days of storage at 4°C.

Key words: casein hydrolysate, low fat yogurt and probiotic bacteria.

INTRODUCTION

Yogurt is obtained through acid fermentation of milk by specific Lactic acid bacteria (Serafeimidou et al., 2012) and can be used as a vehicle for probiotic cultures (Lourens-Hattingh & Viljoen, 2001 and Costa et al., 2013). The consumption of sufficient amounts of these live microorganisms promotes health benefits (FAO WHO, 2001) and can positively influence the stabilization of the gut mucosal barrier (Kailasapthy & chin, 2000).

Consumption of full fat yogurt has declined due to the awareness of probable harmful effect of fat on consumer's health, thus dietary habits has tended to change in favor of low or non-fat yogurt (Brennan & Tudorica, 2006).

Textural and microstructural characteristics in yogurt are important parameters influencing consumer market acceptability (Park, 2007). The commercial success of food on the consumer market is related with sensory characteristics well accepted by the consumer, safety guarantees for consumption, and nutritional qualities (Cruz *et al.*, 2010). These parameters are governed by a three-dimensional milk proteins network formed with casein micelles aggregation (Tamime & Robinson, 2007 and Paseephol *et al*., 2008) in conjunction with denatured whey proteins through hydrophobic and electrostatic bonds (Paseephol *et al.*, 2008).

Thus, the protein content of milk is most important component influencing yogurt textural and chemical properties. In addition, the increase in protein content improves the amount of bound water, and consequently the gel firmness (Saxelin *et al.,* 2003). Milk protein hydrolysates are rich in small peptides and have a higher nutritive value than native milk protein (Choi *et al.,* 2012). Casein derived peptides play major role in enhancement of immune system (Korhonen & Pihlanto, 2001). These peptides also inhibit angiotensin converting enzymes (ACE) leading to regulate blood pressure (antihypertensive effect) (FitzGerald et al., 2004; Mizuno *et al.,* 2004 and Otte *et al.,* 2007). Guesdon *et al*., 2006 showed that αs1-casein hydrolysate prevented stress-induced sleep disturbance in rats. On the other hand, the hydrophilic amino acid residues including His, Lys, Glu and Ser, which derived from casein hydrolysate, were beneficial for bacterial growth (Zhang *et al.,* 2011).

The aim of this study is to evaluate the effect of (1.5%) casein hydrolysates supplementation as well as probiotic bacteria on the quality of low fat yogurt.

MATERIALS AND METHODS

Milk supply:

Fresh raw cow's milk was obtained from the faculty herds, Agriculture, Minia University. All samples were from the morning milking.

Starters culture:

Yogurt starter culture consisted of (*Streptococcus salivarius subsp thermophilus*, *Lactobacillus delbureckii subsp. Bulgaricus* and *Bifidobacterium coagulans*) were obtained from

Cairo Microbiological Resource center (MIRCEN), Faculty of Agriculture Ain Shams University. The organisms were inoculated at (1:1:1).

Manufacture of yoghurt

The whole or low fat milk (1.5% fat) was heated in boiling water bath for 30 min, at 85°C and cooled to 42°C under running tap water, than inoculated with yoghurt starter culture (*Streptococcus salivarius subsp thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). Inoculated milk samples were incubated at 42 °C until pH reached 4.6. Fermentation was stopped by cooling the fermented milk to 4°C in refrigerator.

The manufacture of plain yoghurt without additives (Control 1), low fat yoghurt 1.5% fats (Control 2), low fat yogurt with probiotic bacteria (Treatment 1), low fat yogurt with casein hydrolysate (Treatment 2) and low fat yoghurt with probiotic bacteria and casein hydrolysate (Treatment 3).

Acid casein preparation

Acid casein was prepared from cow skim milk. Milk was acidified to pH 4.6 with 1 M HCl at 40°C. Casein precipitate was washed three times with acidified water (pH4.6) then, the precipitate was suspended in water and the pH was adjusted to 7.0 with 1 M NaOH, reprecipitated at pH 4.6 then the casein was dried.

Casein Hydrolysate preparation

Trypsin enzyme was dissolved in 50 mM phosphate buffer at pH 7.0, The acid caseins were reconstituted in jennes & Koops buffer (1962) to have a final casein concentration of 2.5g $100mL^{-1}$. The pH of casein solutions were adjusted to pH 7.0 by adding 1 M NaOH and completely suspended before adding the enzyme. The enzyme solution was added into casein solution with an enzyme to substrate ratio [E/S] of 1/100 and the hydrolysis was carried out at 37 °C for 1h. The reactions were terminated by heating the enzyme casein mixture at 100°C in boiling water bath for 10 min. The resulting mixture was rapidly cooled to ambient temperature in the ice-water bath and then added to cow milk just before yoghurt preparation.

Preparation of casein hydrolysate yoghurt

The low fat milk (1.5%) was heated in boiling water bath for 30 min, at 85°C and cooled to 45°C under running tap water. The heated milk was supplemented with cow casein hydrolysate each at 0.5; 1.0 and 1.5% (v/v). The milk samples were inoculated then fermented with common yoghurt culture (*Streptococcus salivarius subsp thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) at 42 °C until pH reached 4.6. Fermentation was stopped by cooling the fermented milk to 4 °C in refrigerator. The products were sensory evaluated by panelists. The concentration 1.5% was chosen.

Chemical analysis

Titratable acidity & pH

Yogurt samples were analyzed for titratable acidity according to Ling (1963). pH was measured

using an E 512 type pH meter (Switzer land).

Acidification

The pH of the fermented milk was monitored of 40c by using (E 512 type pH meter (Switzer land)) after calibration it with fresh pH=4.0 and 7.0 standard buffers. The time taken for the pH to reach 4.6 was calculated as the fermentation time. This assay was performed in three replicates of each sample.

Acetaldehyde Content Determination

Acetaldehyde contents of samples were determined by Lees and Jago method **(**Lees & Jago, 1969).

Determination of Diacetyl and Acetoin:

Acetoin and diacetyl in yogurt samples were determined according to Westerfeleld, (1945).

Determination of curd firmness

Firmness of the formed gel (curd) was determined by the penetration method as described by Ibrahim, (1983).

Water holding capacity of yoghurt

Water holding capacity (WHC) was determined using the method of Keogh and O'Kennedy (1998). Samples (40g) were centrifuged at 3000 g for 20 min at 4° C (using Hraeus Christ GMBH centrifuge, H. Jurgens & Co. Bremen). The clear supernatant (*W*) was poured off, weighed and the water-holding capacity (WHC, g100g) was calculated as:

$WHC = (Y - W)/Y \times 100.$

Microbiological analyses

Total microbial count:

The total bacteria count (TBC) was estimated using Nutrient agar as described by Chalmers, (1962).

Count of Lactic Acid Bacteria (LAB) group:

Counting the numbers of LAB group was used by the MRS agar (Biolife) as recommended by the Standard Methods for Examination of Dairy Products (1985). The MRS agar plates were incubated at 37 C for 48 h for lactobacillus counts.

Count of *Bifidobacteria*

Total viable *Bifidobacteria* counts were enumerated on modified Lactobacilli MRS (Oxoid Basing Stoke UK), according to methods described by Vinting and Mistry (1993).

Count of yeast and moulds

The enumeration of yeasts and moulds was made as recommended by the Standard Methods for Examination of Dairy Products (1985).

Sensory evaluation:

Sensory evaluation was performed by the staff members of the Dairy science department and was measured according to Bodyfelt *et al*., (1988) as follows flavour (40 points), body and texture (30 points) and appearance and colour (30 points).

Panelists evaluated all yogurt samples after storage for 1, 3, 7 and 14 days at 4 °C ±1.

RESULTS & DISCUSSION

pH & acidity

Results in **Table1** represent the changes in pH of yogurts made from low fat cow milk (1.5% fat). Addition of casein hydrolysates and probiotic bacteria to milk yogurts

has a strong effect on pH changes during fermentation time. The rate of pH decrease was higher with treated samples in comparison with that of control. It means that the casein hydrolysates promote the growth of yogurt culture and probiotic bacteria.

Casein hydrolysates probably contained small peptides and free amino acids which promote the growth of probiotic bacteria and yogurt culture. The pH of control decrease from 4.6 at zero time to 4.44 after 14 days of storage at 4°c. On the other hand the pH of treatment samples with probiotic bacteria and casein hydrolysates exhibited a more decrease than control product. However, the acidity was characterized with increasing trend throughout the incubation period with all samples. These results are in a good agreement with those obtained by Lucas *et al.*, (2004) Sodini *et al.*, (2005); Zhao, *et al.*, (2006); and Saleh *et al.*, (2014).

Table (1): Changes in pH and titratable acidity of yogurt samples during storage at 4°C

Treatments	storage period (Days)	TA%*	pH	Δ pH
Full fat yogurt Control 1	Zero	0.84	4.60	___
	1	0.87	4.54	0.06
	3	0.88	4.51	0.09
	7	0.90	4.49	0.11
	14	0.92	4.44	0.16
Low fat yoghurt (LFY) control 2	Zero	0.82	4.62	-----
	1	0.85	4.56	0.06
	3	0.87	4.52	0.10
	7	0.89	4.48	0.14
	14	0.91	4.40	0.18
LFY+ probiotic T1	Zero	0.83	4.61	------
	1	0.90	4.57	0.04
	3	0.94	4.53	0.08
	7	0.94	4.47	0.17
	14	0.97	4.40	0.21
LFY+ casein hydrolysate (CH) T2	Zero	0.84	4.6	------
	1	0.88	4.54	0.06
	3	0.92	4.50	0.1
	7	0.93	4.46	0.14
	14	0.97	4.33	0.27
LFY+CH+ probiotic T3	Zero	0.85	4.6	------
	1	0.89	4.54	0.06
	3	0.92	4.52	0.08
	7	0.94	4.47	0.13
	14	0.98	4.31	0.27

*TA= Titratable acidity

Acidification

Fig (1) presents changes in fermentation time of yogurt samples. Addition of casein hydrolysates to milk yogurt has a strong effect on fermentation time. The fermentation time of yogurt samples with added casein hydrolysates were shorter than both control and samples with probiotic bacteria. Meanwhile**,** Zhao *et al.*, (2006) reported that the casein hydrolysates strongly decreased the fermentation and coagulation time of the yogurts. Supplements of casein hydrolysates decrease the coagulation time from 3.40h in control to 3.0h.

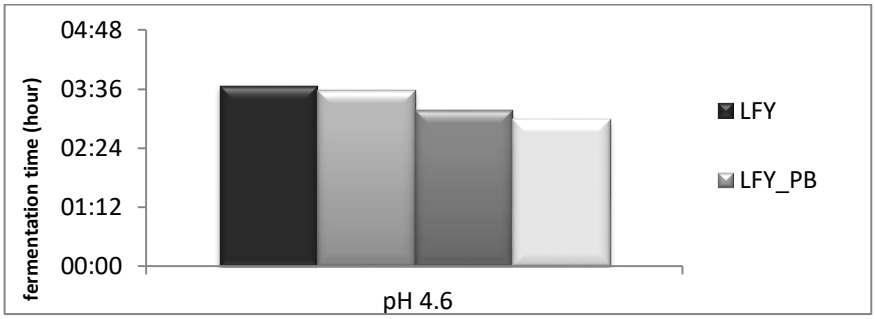

Fig (1) Fermentation times of yogurt with added probiotic and casein hydrolysate to reach pH 4.6

*LFY=low fat yogurt, LFY+PB= low fat yogurt = probiotic bacteria, LFY+CH= low fat yogurt + casein hydrolysates and low fat yogurt + probiotic bacteria + casein hydrolysates.

The coagulation time of samples were 03.40, 03.35, 03.10 and 3.00 hours (control low fat yogurt, probiotic bacteria, casein hydrolysates and probiotic + casein hydrolysates) respectively Fig (1). The greatest decrease in coagulation time occurs in the treated samples with casein hydrolysates. The present data agree with those obtained by Oliveira *et al*., (2001); Sodini *et al.*, (2002); Lucas *et al.*, (2004); Zhao *et al*., (2006) and Saleh *et al.*, (2014).

Water hold capacity (WHC):

WHC of food is generally synonymous with its ability to bind or hold entrapped or bulk phase water. Consequently WHC is dependent on the extent of protein-protein and protein – water interactions in the gel matrix (Parnell-clunies *et al.*, 1986 and Tamime and Robinson, 1999).

Changes in (WHC) of yogurt samples are presented in Fig (2). The lowest (WHC) had control yogurt sample Low fat yogurt (51%). Data showed that there is no difference between low fat yogurt prepared with commercial starter and with probiotic bacteria. While yogurt with 1.5% casein hydrolysate presented higher WHC (54%). During storage the WHC% of yogurt increased for all samples. The results showed that the values of WHC increased by the addition of casein hydrolysate so, the WHC values were potentially affected by

the increase of protein content as milk protein exhibit amphilphilic trait (Le *et al.*, 2011).

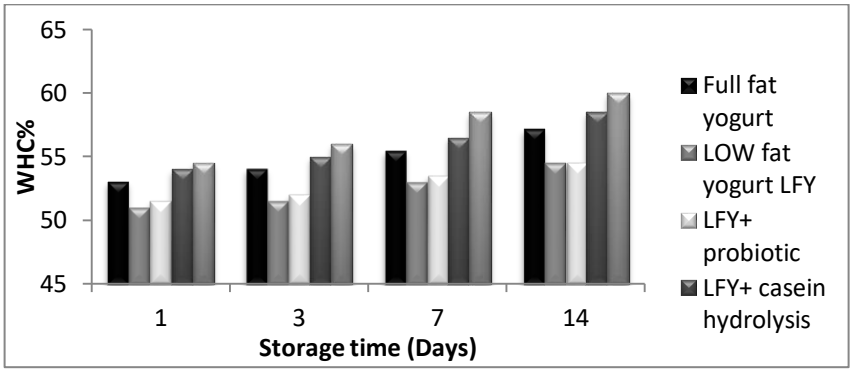

Fig. (2): Water holding capacity (WHC) of yogurt samples during storage at 4°C

Curd firmness:

Firmness characteristics are important criteria for evaluating the quality of yogurt. As can be observed from **Fig (3)** there was difference in firmness between the treatments. The addition of 1.5% casein hydrolysate increased firmness of low fat yogurt.

The difference in the firmness of yogurt could be attributed to the protein matrix structure of the gel (Tamime *et al.*, 1991). Fig(3) showed that the lowest firmness of the control (2) low fat yogurt (23.5 gm) due to the reduction of fat content while the firmness increased to (26.2 gm) with the samples containing casein hydrolysate and probiotic bacteria.

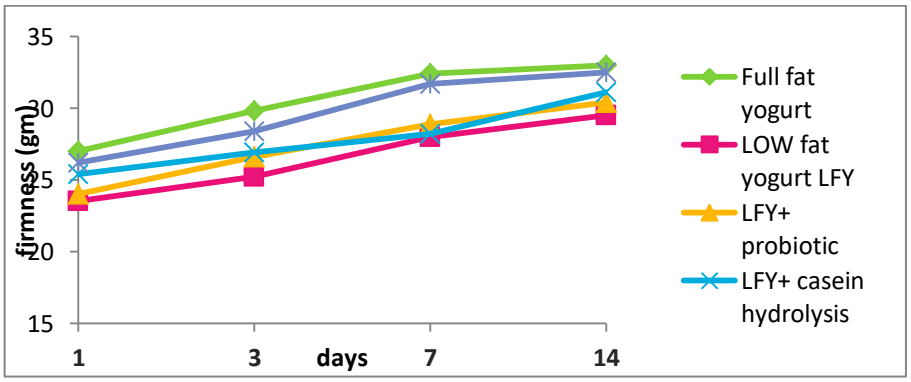

Fig. (3): Changes in curd firmness of yogurt samples during storage at 4°C.

Firmness for all samples increased during storage time till 7 days and start decreased after that. The addition of casein hydrolysate improved the texture characteristic and stability of low fat yogurts during storage. Our results are in agreement with Zhao *et al.*, (2006).

The addition of the casein hydrolysates significantly ($P<0.05$) increased the hardness and adhesiveness of the yogurts Saleh *et al.,* (2014).

Flavour compounds

Basically; aroma is affected by the compulsory presence of minor constituents without fermentative origin. Flavours are determined by presence of carbonylic acids (Acetaldehyde, acetoin, acetone and diacetyl (Denoni *et al.,* 1998 and Cheng, 2010). The acetaldehyde, acetoin and diacetyl contents obtained from 1st and 14th of storage are presented in Table (2).

Results in Table (2) showed the concentration of diacetyl, as O.D at 540nm, starter culture with full fat yogurt or low fat yogurt produced higher quantities of diacetyl than yogurt with probiotic and yogurt with casein hydrolysate. During storage at refrigerator all yogurt preparations showed appreciable increase in diacetyl content except those made with *Bifidobacterium* starter culture which showed a gradual decrease up to the storage period. This results in agreement with Hassanein (1998).

Results in Table (2) also show the concentration of acetoin, expressed as O.D at 540 nm in yogurt samples. It is interesting to note that the standard starter culture produced more acetoin than probiotic starter. The amounts of acetoin produced were relatively higher than diacetyl in all yogurt samples. Data showed that the maximum increase was observed at 3ed day especially with control (1, 2) 0.583, 0.547 respectively. During storage all the yogurt samples showed a steady increase in acetoin content till the end of storage period Contrary to our results **(**Ozer *et al.,* 2007 and Güler *et al.,* 2009) didn't determine diacetyl and acetoin in yogurts.

Data showed that the rate amount of acetaldehyde depends on the strain and growth condition. Table (2) showed that the acetaldehyde content was higher in yogurt with probiotic bacteria starter and yogurt with casein hydrolysate at first day and during storage period.

The production of acetaldehyde by standard starter culture for both full and low fat yogurt less than probiotic bacteria starter and casein hydrolysate.

Tamime & Deeth, (1980) and Chaves *et al.,* (2002) reported that acetaldehyde can be formed from acetic acid by aldehyde dehydrogenase. It was noticed that increase in acetaldehyde content was occurred in all yogurts samples with probiotic bacteria starter and casein hydrolysate combined with common yogurt starter culture.

Table (2): Concentration of flavour compounds in yogurts samples.

Treatments	storage period (Days)	Diacetyl	Acetoin	Acetaldehyde (ppm)
Full fat yogurt Control(1)	1	0.281	0.441	50.6
	3	0.452	0.583	52.6
	7	0.584	0.663	57.2
	14	0.630	0.682	54.2
Low fat yogurt Control(2)	1	0.278	0.384	48.4
	3	0.441	0.547	50.6
	7	0.547	0.641	55.0
	14	0.589	0.668	52.6
Low fat yogurt + probiotic (T1)	1	0.289	0.371	57.2
	3	0.333	0.415	61.6
	7	0.442	0.488	63.8
	14	0.389	0.498	64.2
Low fat yogurt + Casein hydrolysate (T2)	1	0.304	0.338	55.0
	3	0.381	0.421	57.2
	7	0.455	0.505	61.6
	14	0.478	0.520	62.7
Low fat yogurt + Casein hydrolysate + Probiotic(T3)	1	0.308	0.402	59.4
	3	0.392	0.450	61.6
	7	0.471	0.523	66.0
	14	0.423	0.560	68.7

Viability of bacteria at the end of fermentation and storage time:

The changes in the counts of probiotic and yogurt microorganism at the end of fermentation and during the cold storage are presented in Table (3). As shown, there are differences among the viable counts of the mentioned bacteria. As demonstrated in Table (3) *S. thermophilus* and *L. bulgaricus* showed similar final proportions of cells (approximately 10^6 cfu/g) and maintained the same cell number throughout the storage period in control (1, 2). However, casein hydrolysate addition increased the LAB counts at initial stage and during storage period compared to the control. The growth of probiotic organisms during fermentation time and storage period is presented in **Table (3)**. The casein hydrolysates increase the probiotic counts at initial stage compared to the control (1, 2). It indicated that the hydrolysates enhanced the growth of probiotic organisms. During storage period the yogurt with the hydrolysates have more probiotic bacteria than the yogurt without hydrolysates.

Table (3): Viability of bacteria of yogurt samples during storage at 4°C for 14 days.

Treatments	(Days) storage period	T.C NA	Lactobacilli (MRS)	probiotic (MRSL)	Lactococcus M17	Molds & yastes
Full fat yogurt control (1)	Zero	9×10^6	5.0×10^6	_____	4.0×10^6	ND*
	1	9.4×10^6	5.2×10^6		4.4×10^6	ND
	3	13.8×10^6	8.0×10^6		6.0×10^6	ND
	7	14×10^6	8.3×10^6		6.5×10^6	ND
	14	13×10^6	8.1×10^6		6.3×10^6	ND
Low fat yoghurt control (2)	Zero	9.1×10^6	5.4×10^6	_____	4.6×10^6	ND
	1	10×10^6	5.7×10^6		5.0×10^6	ND
	3	14×10^6	8.1×10^6		6.4×10^6	ND
	7	14.6×10^6	8.7×10^6		6.8×10^6	ND
	14	13.2×10^6	8.5×10^6		6.4×10^6	ND
Yogurt+ probiotic bacteria T1	Zero	5.4×10^7	2×10^7	4.0×10^7	1.1×10^7	ND
	1	5.6×10^7	2.2×10^7	4.2×10^7	1.2×10^7	ND
	3	7.4×10^7	4×10^7	6.0×10^7	2×10^7	ND
	7	7.2×10^7	6×10^7	4.9×10^7	2.6×10^7	ND
	14	6.8×10^7	5×10^7	4.1×10^7	2.510^7	ND
Yogurt + casein T2	Zero	10×10^6	6×10^6	_____	4.8×10^6	ND
	1	11.2×10^6	6.4×10^6		5.2×10^6	ND
	3	15.2×10^6	8.8×10^6		6.8×10^6	ND
	7	15.6×10^6	9.2×10		7.1×10^6	ND
	14	15.5×10^6	9×10^6		7×10^6	ND
LF Y+ probiotic + CH T3	Zero	5.8×10^7	2.8×10^7	4.2×10^7	1.1×10^7	ND
	1	6.1×10^7	3×10^7	4.6×10^7	1.3×10^7	ND
	3	8.0×10^7	5×10^7	6.4×10^7	2.4×10^7	ND
	7	7.7×10^7	6.2×10^7	5.3×10^7	2.8×10^7	ND
	14	7.5×10^7	6×10^7	5.2×10^7	2.7×10^7	ND

*ND= Not detected

The hydrolysates improve the viability of LAB and probiotic bacteria. This is in accordance with Dave & Shah, (1997); Lucas *et al.*, (2004). Data in Table (3) showed that the growth of starter culture organisms decrease after 7 days storage. On the other hand, the decline of the probiotic bacteria counts could be retarded by the hydrolysate, these results agreed with Oliveira *et al.*, (2001); Zhao *et al.*, (2006).

Table (3) showed that the molds and yeasts were not detected in all samples when fresh and till the fourteen day storage. This may be due to the hygienic conditions where the manufacturing procedures took place. Similar results have been reported by Salem *et al.*, (2007), Taha *et al.*, (2007).

Sensory Evaluation

The effect of casein hydrolysates supplement on the organolepyic properties are

presented in Table (4). Yogurt samples with 1.5% casein hydrolysate and probiotic bacteria (T3) scored (80) points compared with the control (1) full fat yogurt scored (84).

The Organoleptic properties of yogurt were markedly decreased with decreasing the level of fat. Therefore the lowest score observed with low fat yogurt control (2) and yogurt with probiotic bacteria (T1).

No differences were found between control (1) samples and yogurt samples supplemented with 1.5% casein hydrolysate. The sensory scores for acceptability of yogurt increased with addition of 1.5% casein hydrolysates. Results revealed that using of casein hydrolysate with probiotic culture enhanced the body and texture for T3 comparing with low fat yogurt control (2) using probiotic culture with normal starter enhanced body, texture and flavour more than low fat yogurt control (2) but less than casein hydrolysate.

It can be concluded that using casein hydrolysate with probiotic culture in associate with common yogurt culture was most preferable in low fat yogurt in all features of the sensory properties at zero time and during storage at 4°C ±1 for 14 days.

Table (4): Effect of casein hydrolysates supplement on the organoleptic properties.

	Flavour & Aroma	Appearance & colour	Body & Texture	Total
Score	40	30	30	100
Full fat yogurt (C1)	32	26	26	84
Low fat yogurt (C2)	28	22	20	70
Yogurt+ probiotic bacteria (T1)	30	22	21	73
Yogurt + casein hydrolysate (T2)	29	24	24	77
LFY+ probiotic +CH (T3)	30	25	25	80

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تأثير الكازين المتحلل على جوده الزبادي المنخفض فى نسبه الدهن

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أجريت هذه الدراسة بهدف دراسة تأثير إضافة 1.5% من الكازين متحلل مع البروبيوتك بكتيريا علي جودة الزبادي منخفض في نسبة الدهن. تم دراسة إضاقة الكازين المتحلل بواسطة إنزيم التربسين علي كلا من وقت التجبن وقوة الخثرة ومعدل نمو بكتيريا البادي لمدة 14 يوم علي 4°م±1 وجد أن إضافة الكازين المتحلل أدي إلي انخفاض وقت التجبن بنسبة عالية وكذلك أدى إلى انخفاض رقم الـ pH وحسن من معدل نمو كلا من بكتيريا البادئ والبروبيوتك وأدي إلي زيادة مركبات النهكة. وجد أن الزبادي المضاف إليه كلا من الكازين المتحلل والبروبويتوك كان علي درجة عالية من الجودة من حيث الطعم والقوام والتركيب مقارنة بالكونترول