



Studies on the use of *Lactobacillus rhamnosus* in white soft cheese manufacture

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Abstract

White soft cheese is made by using different levels of *Lactobacillus rhamnosus* (0.0, 0.2, 0.4, 0.6 and 0.8%) (Control, T1, T2, T3 and T4) before incubation of milk. The resultant cheeses were stored at refrigerator temperature 6c up to 28 days. The data concluded that, TS, TA, SN, TN, SN/TN, TVFFA were increased and salt in water increased by increasing the *L. rhamnosus* level in all treatments. The control samples without culture (C) had lower TS, TA, SN, TN, SN/TN, TVFFA and salt in water ratio than that of with different levels of *L. rhamnosus*. The cheese made with 0.8% *L. rhamnosus* starter (T4) had higher values of TS, TA, SN, TN, SN/TN, TVFFA and salt in water ratio than that of other treatments. There were increased on syneresis and firmness with increasing levels of *L. rhamnosus*. Microbiologically, there were increases of TC, and lactobacilli counts with increasing storage periods up to the end of storage periods in all treatments. There were not detected psychotrophic bacteria and coliform group counts in all treatments. Yeast and moulds were not detected in fresh cheese and 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. Moreover, the data showed that T3 stored for 28 days had the highest scores than that of the other treatments using different levels of *L. rhamnosus*. Whilst, T3 stored for 7days had the lowest scores than that of the other treatments.

Keywords: *Lactobacillus rhamnosus*, white soft cheese, sensory quality, starter.

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1. Introduction

Functional foods are defined as “foods that through specific beneficial physiological action, contribute to the health of the consumer” (Corbo *et al.* 2001). They can include probiotics, prebiotics and symbiotic (Champagne and Gardner, 2005). Probiotics are “live microorganisms which when administered in adequate numbers confer a health benefit on the host” (FAO/WHO, 2001). Most common probiotic strains used in dairy foods belong to *Lactobacillus* (*Lactobacillus acidophilus*, *L. johnsonii*, *L. gasseri*, *L. crispatus*, *L. casei/paracasei*, *L. rhamnosus*, *L. reuteri*, *L. plantarum*) and *Bifidobacterium* (*Bifidobacterium lactis*, *B. bifidum*, *B. infantis*, *B. breve*, *B. animalis*, *B. adolescentis*) (Saxelin, 2008). To have a beneficial effect on the gut microbiota, probiotics have to be in such an excess (approximately 10^8 - 10^9 CFU ml⁻¹) so as their amounts were higher than 10^6 CFU ml⁻¹ at the end of the shelf-life. The exact mechanism of action of probiotics has not been explained, but a number of aspects of the way it affects the human body was found, including beneficial effect on the intestinal microflora; prevention of intestinal infections; increased tolerance to lactose; improving the immune system; anti-allergic effects; prevention of cardiovascular disease and prevention of cancer (Czerwionka-Szaflarska and Romańczuk, 2008). Because of their health benefits, probiotic bacteria are increasingly incorporated into dairy products, such as fermented milks; milk beverages; cheese and baby foods (Rybka and Kailasapathy, 1995). *Lactobacillus rhamnosus* GG represents a probiotic strain which was clinically

studied and was found to enhance human natural resistance and healthy digestive system (Hickey, 2005). The strain was able to inhibit adhesion of *Clostridium histolyticum*, *Cl. difficile* and *Salmonella enterica*. The combination of *L. rhamnosus* GG with *L. rhamnosus* LC705 inhibited growth of *Staphylococcus aureus*, *E. coli* and *S. enteric* (Rodgers, 2001). Since many probiotic microorganisms are sensitive to the concentration of oxygen, carbon dioxide and salt, high and freezing temperatures and acidic environments (Cruz *et al.*, 2009 and Fortin *et al.*, 2011). Since many dairy products are fermented, it is common to find levels of acidity that may affect the probiotics viability (Roy, 2005). Cheese is a dairy product which has a good potential for delivery of probiotic microorganisms into the human intestine due to its specific chemical and physical characteristics compared to fermented milks (higher pH value and lower titratable acidity, higher buffering capacity, greater fat content, higher nutrient availability, lower oxygen content and denser matrix of the texture). White soft cheese is the main white cheese normally processed from buffalo or cow milk, sometimes admixture of both. The unique step of manufacture is the salt addition to cheese milk directly before renneting (El-Baradei *et al.*, 2007). It is consumed fresh or after 3-6 months of ripening period in pickling solution. Probiotic bacteria in cheese especially lactobacilli possess several peptidases, which can hydrolyze peptides to oligopeptides and amino acid and induce change in flavor, body & texture and consequently in sensory properties of the cheese (Soufa and Saad, 2009). Adding *L. rhamnosus* up to 0.75% improved the organoleptic properties of the resultant cheese. Fat, TN, salt, ash,

SN content, TVFFA, shilovich number and scores of organoleptic properties increased as pickling period proceeded, while moisture content and TN content decreased (Kebary, 2015). Egyptian consumers are cheese consumer more than fermented milk products (Motawee, 2013). So, it will be great benefit to produce soft cheese from heat treated milk with suitable starters to produce healthy traditional soft cheeses. Therefore, the objectives of this study were to study the possibility of making a good quality probiotic white soft cheese using *L. rhamnosus* obtained from Dairy Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt and monitor the changes of cheese quality during pickling period.

2. Materials and methods

2.1 Materials

2.1.1 Study area

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 (Assiut branch), Assiut, Egypt. Enzyme sources (Microbial rennet) were 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). *Lactobacillus rhamnosus* obtained from Dairy

Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

2.2 Methods

2.2.1 Manufacture of probiotic white soft cheese

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:

Lowfat buffalo milk (4% fat) was divided into five equal portions; every part was heated to $72\pm 1^\circ\text{C}$ for 5 min, 4% salt (w/w) was added, rapidly cooled to $40\text{--}42^\circ\text{C}$, and then starters and rennet were added as following:

- C: Nonstarter cheese.
- T1: Adding 0.2% (w/w) *L. rhamnosus*.
- T2: Adding 0.4% (w/w) *L. rhamnosus*.
- T3: Adding 0.6% (w/w) *L. rhamnosus*.
- T4: Adding 0.8% (w/w) *L. rhamnosus*.

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

2.2.2 Chemical analysis

Total solids contents were determined according to the AOAC (2000). Fat content was determined in milk and cheese by the conventional Gerber's method as described by Ling (1963). Salt content was determined according to the

method described by Simov (1980). Total Nitrogen (TN) was determined by the semi-micro kjeldahl as described by Ling (1963). Water-soluble nitrogen (WSN) content was determined according to Kuchroo and Fox (1982). Total volatile free fatty acids (TVFFA) was determined using the method described by Kosikowski (1982). Titratable acidity was determined according to the method described by Ling (1963). 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 and yeast and moulds of cheese samples were determined according to Marshall (1992). Lactobacillus counts on MRS agar after incubation for 48 h at 32°C. Psychotrophic bacterial count is estimated by plating the samples using the SPC procedure and incubating for 10 days at 7°C (Marshall, 1992).

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 *L. rhamnosus* for making white soft cheese. White soft cheese is made by combining different levels of *L. rhamnosus* (0.2, 0.4, 0.6 and 0.8) before incubation of milk. The resultant white soft cheeses were stored at refrigerator temperature ($6\pm 2^{\circ}\text{C}$) 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 white soft cheese with different levels of *L. rhamnosus*

Data presented in Table (1) illustrated the chemical composition of white soft cheese made from different levels of *L. rhamnosus* during and storage 28 days at refrigerator temperature.

3.2 Total solid contents (TS)

The results in Table (1) indicated that, the TS content of white soft cheese was affected by different levels of *L. rhamnosus* during as well as storage periods. The TS content of white soft cheese increased as the pickling period advanced and by increasing the *L. rhamnosus* level due to the decrease in the moisture content in cheese and increasing the bacterial counts. These results are in an agreement with these reported by Badawi and Kebary (1996) and Kebary *et al.* (2015). Moreover, the control samples (C) had lowers TS than that of white soft

cheese made with different levels of *L. rhamnosus*. These results are in agreement with those obtained by Samy *et al.* (2013).

3.3 Titratable acidity (TA %)

The data Table (1) revealed that, the TA% of white soft cheese was affected by different levels of *L. rhamnosus* as well as during storage periods. The acidity of white soft cheese *increased with increasing of L. rhamnosus levels and during storage period at refrigerator up to 28 days*. Moreover, the control samples had lowers acidity than that of white soft cheese made from different levels of *L. rhamnosus*. The differences among cheese treatment in acidity might be attributed to the growth rate of *L. rhamnosus* and ability to ferment lactose during pickling period. These results are in agreement with those obtained by El-Abd *et al.* (2003) and Samy *et al.* (2013), who found that the use of different starter cultures in Domiati cheese manufacture increased the acidity.

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3.4 Titratable acidity (TA %)

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during pickling period. These results are in agreement with those obtained by El-Abd *et al.* (2003) and Samy *et al.* (2013), who found that the use of different starter cultures in Domiati cheese manufacture increased the acidity.

Table (1): Effect of different levels of *L. rhamnosus* on chemical composition of white soft cheese held at 6±1°C up to 28 days.

Properties	Storage (days)	Treatments				
		Control (C)	T1	T2	T3	T4
TS %	Fresh	30.12	31.23	31.49	31.54	31.66
	7	32.05	32.09	32.49	32.57	33.33
	14	33.50	33.59	33.96	34.58	35.27
	21	34.20	34.67	35.13	35.70	36.38
	28	34.90	35.30	36.11	36.61	38.06
Acidity %	Fresh	0.175	0.195	0.215	0.235	0.255
	7	0.195	0.265	0.275	0.275	0.290
	14	0.230	0.265	0.275	0.280	0.320
	21	0.230	0.285	0.285	0.300	0.350
	28	0.250	0.295	0.300	0.350	0.400
pH	Fresh	6.78	6.77	6.76	6.67	6.64
	7	6.76	6.68	6.60	6.48	6.38
	14	6.73	6.64	6.64	6.44	6.32
	21	6.51	6.48	6.44	6.40	6.28
	28	6.40	6.34	6.30	6.28	5.90
TN %	Fresh	1.500	1.520	1.544	1.560	1.600
	7	1.612	1.633	1.675	1.711	1.745
	14	1.716	1.825	1.881	1.912	2.010
	21	1.908	2.010	2.010	2.050	2.275
	28	2.070	2.213	2.356	2.356	2.475
SN %	Fresh	0.103	0.145	0.156	0.164	0.172
	7	0.156	0.164	0.173	0.179	0.191
	14	0.173	0.195	0.215	0.223	0.334
	21	0.211	0.243	0.324	0.356	0.445
	28	0.355	0.445	0.509	0.536	0.632
(SN/TN) × 100	Fresh	6.87	9.54	10.10	10.51	10.75
	7	9.68	10.04	10.33	10.46	10.95
	14	10.08	10.68	11.43	11.66	16.62
	21	11.06	12.09	16.12	17.37	19.56
	28	17.15	20.11	21.60	22.75	25.54
TVFFA (ml 0.1 N NaOH/100 g cheese)	Fresh	9.89	9.97	9.97	10.11	10.22
	7	10.28	10.54	10.86	11.33	12.50
	14	11.15	11.47	12.34	13.18	14.45
	21	12.63	12.85	13.29	14.16	16.44
	28	15.24	16.65	17.39	19.48	21.23
Salt in water ratio	Fresh	4.18	4.44	4.52	4.81	4.87
	7	4.30	4.49	4.80	4.88	4.99
	14	4.45	4.67	5.19	5.24	5.44
	21	4.50	5.10	5.29	5.33	5.53
	28	4.62	5.15	5.51	5.54	5.83
F/DM × 100	Fresh	29.38	28.82	28.90	29.17	30.01
	7	28.08	28.36	28.93	29.47	28.80
	14	28.36	29.71	29.45	29.50	29.77
	21	29.82	29.74	29.35	30.81	29.55
	28	30.09	30.31	29.08	31.41	31.53

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

3.5 pH values

The data in Table (1) revealed that, the pH values of white soft cheese was affected by different levels of *L. rhamnosus* as well as during storage period at refrigerator temperatures up to 28 days. The pH values of white soft cheese found to decrease with increasing levels of *L. rhamnosus*. Moreover, the control samples had higher pH than that of white soft cheese made with different levels of *L. rhamnosus*. These results are in agreement with those obtained by Perotti *et al.* (2014). The pH values of white soft cheese found to decrease with increasing of the storage periods at refrigerator temperatures up to 28 days. In the same order, these results are in agreement with those obtained by Edgaryan *et al.* (2007) and Samy *et al.* (2013).

3.6 Total nitrogen and soluble nitrogen contents (TN& SN)

The obtained data in Table (1) revealed that, the TN as well as SN contents of white soft cheese was affected by different levels of *L. rhamnosus* during storage periods at refrigerator temperature up to 28 days. The TN and SN contents of white soft cheese found to increase as the pickling period advanced and by increasing the *L. rhamnosus* level in all treatments. This increase in TN and SN could be due to the increasing in TS content. Moreover, the control samples had lower TN and SN than that of white

soft cheese made with different levels of *L. rhamnosus*. These results are in agreement with those obtained by Samy *et al.* (2013), Jelena *et al.* (2014) and Kebary *et al.* (2015).

3.6 Total nitrogen and soluble nitrogen contents (TN& SN)

The obtained data in Table (1) revealed that, the TN as well as SN contents of white soft cheese was affected by different levels of *L. rhamnosus* during storage periods at refrigerator temperature up to 28 days. The TN and SN contents of white soft cheese found to increase as the pickling period advanced and by increasing the *L. rhamnosus* level in all treatments. This increase in TN and SN could be due to the increasing in TS content. Moreover, the control samples had lower TN and SN than that of white soft cheese made with different levels of *L. rhamnosus*. These results are in agreement with those obtained by Samy *et al.* (2013), Jelena *et al.* (2014) and Kebary *et al.* (2015).

3.7 Soluble nitrogen coefficient (SN/TN)

The obtained data in Table (1) revealed that, the soluble nitrogen coefficient was increase gradually as the storage periods progressed up to 28 days of all treatments. In addition, the control samples had lower values of soluble nitrogen coefficient than that of white soft cheese made with different levels of *L. rhamnosus*.

3.8 Total volatile free fatty acids content (TVFFA)

The obtained data in Table (1) revealed that, the TVFFA content was increase gradually as the storage periods at refrigerator temperature progressed up to 28 days of all treatments. In addition, the control samples had lower values of TVFFA than that of white soft cheese made with different levels of *L. rhamnosus*. This increase in TVFFA could be due to the lipolytic activity of *L. rhamnosus*. These results are in agreement with those obtained by Kebary *et al.* (2015), who found that the TVFFA of UF cheese with *L. rhamnosus* increased in all cheese treatments during the pickling periods.

3.9 Salt in water ratio

The obtained data in Table(1) revealed that, the salt in water ratio was increase gradually as the storage periods progressed up to 28 days of 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 *L. rhamnosus*. These

results are in agreement with those obtained by Kebary *et al.* (2015), who found that the salt in water ratio of UF cheese with *L. rhamnosus* increased with the pickling periods advanced and with the high levels of *L. rhamnosus* due to the decrease in the moisture content in cheese. In addition, the control samples had lower values of salt in water ratio than that of white soft cheese made with different levels of *L. rhamnosus*.

3.10 Rheological properties of white soft cheese with different levels of *L. rhamnosus*

Data presented in Table (2) illustrated the rheological properties of white soft cheese made with different levels of *L. rhamnosus* during storage periods at refrigerator temperature for 28 days. The obtained data observed that, the control samples had the lower values of syneresis and firmness than that of other treatments using different levels of *L. rhamnosus*. In addition, the data showed that there were increases on syneresis and firmness with increasing levels of *L. rhamnosus*. The higher values of syneresis and firmness might be due to acid development.

Table (2): Effect of different levels of *L. rhamnosus* on rheological properties of fresh white soft cheese.

Properties	Treatment				
	C	T1	T2	T3	T4
Syneresis (ml/100 g)	23	26	28	32	35
Firmness (g)	20.62	22.86	23.91	24.62	27.69

3.11 Microbiological analyses of white soft cheese with different levels of *L. rhamnosus*

Data presented in Table (3) illustrated the microbiological analyses of white soft cheese made with different levels of *L. rhamnosus* as well as during storage periods at refrigerator temperature for 28 days.

3.12 Total bacterial counts

The obtained data in Table (3) illustrated that, the control sample (C) had lower total bacterial counts than that of other treatments. These results are in agreement with those obtained by Kebary *et al.* (2015). Furthermore, the total bacterial counts found to increase as the pickling periods advanced and by increasing the *L. rhamnosus* level in all treatments. In addition, the T4 samples had higher total counts than that of other treatments.

Table (3): Effect of different levels of *L. rhamnosus* on microbiological properties of white soft cheese held at 6±1°C up to 28 days (Log cfu/g-1).

Properties	Storage periods (days)	Treatment				
		C	T1	T2	T3	T4
Total count	Fresh	5.477	9.057	9.328	9.756	10.324
	7	5.544	9.332	9.633	10.127	10.477
	14	7.029	9.544	10.477	10.826	11.382
	21	7.491	9.643	10.732	11.091	12.279
	28	7.857	10.898	11.544	12.269	12.340
<i>L. rhamnosus</i>	Fresh	ND	8.049	8.468	9.061	9.354
	7	ND	8.369	9.068	9.591	9.826
	14	ND	8.477	9.283	10.423	10.892
	21	ND	9.491	9.643	10.799	11.544
	28	ND	9.755	10.057	11.068	12.346
Psychotropic 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	3.740	3.672	3.643	3.544	ND
	21	3.763	3.707	3.643	3.568	3.544
	28	3.806	3.755	3.681	3.623	3.477

3.13 *L. rhamnosus* counts

The obtained data in Table (3) illustrated that, the T1 samples had the lower values of *L. rhamnosus* counts than that of other treatments, while T4 samples had higher counts than that of other treatments. In addition, the data showed that, the *L. rhamnosus* counts are increase with increasing levels of *L. rhamnosus* and with increasing storage periods at refrigerator temperatures up to 28 days. These results are in agreement with those obtained by Kebary *et al.* (2015).

3.14 *L. Psychotropic bacteria and coliform group counts*

The obtained data in Table (3) illustrated that, there were not detected psychotropic bacteria and coliform group counts in all treatments in fresh cheese and after storage period at 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).

3.15 *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) had higher counts of yeasts and moulds than that of other treatments.

3.16 *Organoleptic properties of white soft cheese with different levels of L. rhamnosus*

Data presented in Table (4) illustrates that the organoleptic properties of white soft cheese made with different levels of *L. rhamnosus* 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 made from buffalo's milk were affected by addition of different levels of *L. rhamnosus*, as well as during storage periods at refrigerator temperature up to 28 days.

3.17 *Flavor*

The obtained data in Table (4) observed that, the T3 and control samples stored for 28 day had the highest values of flavor than that of the other treatments using different levels of *L. rhamnosus*. Whilst, the samples of T3 and T2 stored for 7 and 28 days had the lowest, respectively.

3.18 *Body and texture*

The obtained data in Table (4) observed that, the T3 and control samples stored

for 28 day had the highest values of body and texture than that of other treatments with different levels of *L. rhamnosus*.

Whilst, the samples of T3 samples stored for 7 and 21 days had the lowest body and texture than that of other treatments.

Table (4): Effect of addition of different levels of *L. rhamnosus* on organoleptic properties of white soft cheese held at $6\pm 1^\circ\text{C}$ up to 28 days.

Properties	Storage periods (days)	Treatment				
		C	T1	T2	T3	T4
Flavor (50)	Fresh	39.6	41.6	43.5	43.2	42.8
	7	41.5	42.8	42.0	37.0	44.5
	14	41.8	41.8	43.8	40.8	41.3
	21	43.3	42.3	38.3	44.3	38.3
	28	46.3	44.3	38.0	46.7	44.3
Body and texture (40)	Fresh	33.4	36.0	35.8	34.4	37.0
	7	38.0	35.5	35.3	32.5	37.8
	14	33.3	33.0	36.8	35.8	33.5
	21	38.0	35.5	38.0	32.5	37.8
	28	38.5	38.0	38.0	39.0	38.0
Appearance and color (10)	Fresh	7.4	8.6	8.8	8.2	8.0
	7	9.3	8.5	9.3	8.0	8.8
	14	7.8	8.0	8.5	8.3	7.8
	21	8.7	7.7	8.3	9.0	8.3
	28	9.3	8.7	8.7	8.7	8.7
Total scores (100)	Fresh	80.4	86.2	88.1	85.8	87.8
	7	88.8	86.8	86.6	77.5	91.1
	14	82.8	82.8	89.1	84.9	82.6
	21	90.0	85.5	84.6	85.8	84.4
	28	94.1	91.0	84.7	94.4	91.0

3.19 Appearance and color

The obtained data in Table (4) observed that, the control samples stored for 7 and 28 days and T2 stored for 7 days had the highest values of appearance and color than that of other treatments using different levels of *L. rhamnosus*. Whilst, the control samples fresh and T1 stored for 21 days had the lowest appearance and color than that of the other treatments.

3.20 Total scores

The obtained data in Table (4) showed that, T3 and control samples stored for 28

days had the highest total scores than that of other treatments using different levels of *L. rhamnosus*. Whilst, T3 stored for 7 days and the control sample fresh had the lowest scores than that of the other treatments.

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