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Probiotic Yoghurt Made from Milk of Ewes Fed a Diet Supplemented with *Spirulina platensis* or Fish Oil

Ahmed B. Shazly[1], Mostafa S. A. Khattab[1*], Mohamed T. Fouad[1], Ahmed M. Abd El Tawab[1], Eltaher M. Saudi[2] and Mahmoud Abd El-Aziz[1]

Abstract

Purpose: Yoghurt is a widely consumed dairy product around the world. It has healing properties and characteristics that are important for human health. Our goal was to see how using ewes' milk fed *Spirulina platensis* (SP) or fish oil (FO)-supplemented diets affected the chemical, physical, and nutritional properties of yoghurt, as well as the activity and survival of starter and probiotic bacteria during storage.

Methods: The collected milk from each ewe group was preheated to 65 °C and homogenized in a laboratory homogenizer, then heated to 90 °C for 5 min, cooled to 42 °C, and divided into two equal portions. The first portion was inoculated with 2.0% mixed starter culture (*Lactobacillus bulgaricus and Streptococcus thermophilus*, 1:1), whereas the second was inoculated with 2% mixed starter culture and 1% *Bifidobacterium longum* as a probiotic bacteria.

Results: SP yoghurt had the highest levels of short chain-FA, medium chain-FA, mostly $C_{10:0}$, and long chain-FA, namely $C_{16:0}$, $C_{18:2}$ and the lowest levels of $C_{18:0}$ and $C_{18:1}$, followed by FO yoghurt. The addition of SP or FO to ewes' diets resulted in yoghurt with higher viable counts of *L. bulgaricus* and *S. thermophilus*, which were still $>10^7$ cfu/g at the end of storage, as well as a higher level of acetaldehyde content ($P<0.05$) as a flavor compound, than the control (C) yoghurt. The viscosity of SP yoghurt was higher than that of FO and C yoghurt; the difference was not significant. The addition of *B. longum,* a probiotic bacteria, to all yoghurt samples, improved antioxidant activities, particularly against ABTS• radicals, but reduced SP yoghurt viscosity. When *B. longum* was added, acetaldehyde content increased from 39.91, 90.47, and 129.31 µmol/100g in C, FA, and SP yoghurts to 46.67, 135.55, and 144.1 µmol/100g in probiotic C, FA, and SP yoghurts, respectively. There was no significant difference in sensory qualities among all the yoghurt samples during all storage periods.

Conclusions: Supplementing the ewes' diets with *Spirulina platensis* or fish oil can change the fatty acid composition of the resulting yoghurt. The starter culture's activity, flavor compounds, and some chemical, physical, and antioxidant properties of milk produced from these diets can all be improved, particularly in yoghurt treated with probiotic bacteria (*B. longum*).

Keywords: Ewes' milk, *Spirulina platensis*, Fish oil, yoghurt, Fatty acids, Antioxidant activities, Physical properties

Introduction

There has been a surge in interest and funding for research into innovative functional foods in recent years. As a result, global changes in consumer demand for natural and healthful foods, such as milk and dairy

*Correspondence: ms.khattab@nrc.sci.eg; msakhattab@gmail.com

[1] Dairy Department, Food Industries and Nutrition Research Institute, National Research Centre, Giza, Egypt
Full list of author information is available at the end of the article

products, are constantly expanding. Modification of animal diets with bioactive feed additions such as algae and fish oil (Madhusudan et al. 2011; Abo El-Nor and Khattab 2012; Khattab et al. 2022) or microalgae is one technique for creating such diets (Christaki et al. 2012; Hussein et al. 2020.(Those enriched with low saturated fatty acid (SFA) content and high quality polyunsaturated fatty acids (PUFA), which humans and animals cannot synthesize and can protect against diseases including cardiovascular disease, diabetes, atherosclerosis, skin diseases, and arthritis (Gouveia et al. 2008; Christaki et al. 2012).

Incorporating marine supplements or plant oils rich in 18:2n-6 into a ruminant's diet is an effective nutritional strategy for altering milk and increasing polyunsaturated fatty acids (PUFA) such as cis-9,trans-11 conjugated linoleic acid (CLA) and 22:6n-3 in bovine (Toral et al. 2012). Spirulina, *Arthrospira platensis,* is an edible blue-green microalgae that is high in proteins (up to 70%) and contains a variety of minerals and vitamins, including vitamins B_{12}, B_1, B_2, B, and vitamin E, as well as carbohydrate contents (15-20%) composed of glucose and glycogen, lipids (up to 7%), and essential fatty acids such as linoleic acid and γ-linolenic acid (Wells et al. 2017). Spirulina is a valuable resource for natural antioxidants, such as phycocyanin pigments, carotenoids, and phenolic compounds (Soni Arora and Rana 2017; Wells et al. 2017). The findings suggest that Spirulina could be used as a feed source for various animal species. It has been associated with improved animal development and nutritional product quality (Bichi et al. 2013). Cows given dietary Spirulina exhibited a 21% increase in milk production, according to Kulpys et al. (2009). Furthermore, Simkus et al. (2008) found that cows receiving Spirulina had higher milk fat (between 17.6 to 25.0%), milk protein (up by 9.7%), and lactose (up by 11.7%) than cows receiving no Spirulina.

Because fish oil is one of the greatest sources of long-chain PUFA, like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the human diet, its insufficient consumption might have a significant impact on health (Freitas and Campos 2019). Omega-3 PUFAs are known to protect against cardiovascular disease, reduce the incidence of some types of cancer and autoimmune illnesses, and are necessary for the healthy development of brain and retina functions (Sokoła-Wysoczańska et al. 2018). Ruminant products such as milk, dairy products, and beef have been criticized for their high levels of SFA and low levels of ω-3 PUFA (Kliem and Shingfield 2016; Rodriguez-Herrera et al. 2017). Fish oil and microalgae have been used in most research that has attempted to improve the health characteristics of milk and dairy products as the main dietary source of ω-3 PUFA (Givens and Gibbs 2006).

Probiotics are live microorganisms that benefit the host when given in sufficient amounts. The health benefits of functional foods can be further boosted by supplementing them with specific lactic acid bacteria, which are the most commonly utilized probiotic cultures in dairy products and beverages (El-Kholy et al. 2016; El-Shenawy et al. 2019). Probiotic bacteria provide a variety of health benefits, the most important of which is viability, or the ability to survive in the gastrointestinal tract in a certain number, improve the microbial balance of the digestive system, and survive in a variety of environments. Yoghurt fortified with probiotics has been shown to have various health benefits, including anti-diabetic effects in diabetic rats (Abbas et al. 2017; Terpou et al. 2019). Furthermore, adding probiotic-fermented dairy products will improve the functional qualities of nutrients considered "functional food" (El-Shenawy et al. 2016; Arowolo and He 2018). Hence, combining probiotics with fermented milk modified, which can be used as dietary supplements in dairy products to achieve high efficiency in increasing the growth of probiotics. The nutritional importance of the mixed form of milk modified and probiotics can be considered highly nutritious and cost-effective due to a senior count of probiotic bacteria (Markowiak and Śliżewska 2017).

As a result, it's reasonable to predict that a diet supplemented with fish oil and Spirulina alga, both of which are high in unsaturated fatty acids and secondary fatty components, will improve the fatty acid profile and also the physical and nutritional qualities of milk. Also, incorporating these supplements into yoghurt milk promotes the ability of probiotic bacteria and improves nutritional and physicochemical qualities, making it a functional dairy food. Therefore, this work was aimed to see how using milk from ewes fed *Spirulina platensis* or fish oil-supplemented diets affected the chemical, physical, and health aspects of yoghurt, as well as the activity and survival of starter cultures (*L. bulgaricus* and *S. thermophiles*) and probiotic bacteria (*B. longum*) during storage.

Materials and methods

Materials

A Spirulina alga, *Spirulina platensis,* was obtained from the marine toxins Laboratory, National Research Centre, Egypt. Fish oil was purchased from the local market, Cairo, Egypt. Starter culture *(Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus)* and probiotic bacteria *(Bifidobacterium longum* ssp. *longum* 35624 ATCC) were obtained from stock cultures of Dairy Microbiology Lab., National Research Centre, Cairo, Egypt. Sigma-Aldrich, USA, provided 1-diphenyl-2-picrylhydrazyl (DPPH•) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•). All chemicals and reagents were analytical grade and came from various sources.

Methods

Experimental design–animals and feeding

In a complete randomized design, thirty lactating Barki ewes weighing 40±2.3 kg were randomly assigned to three experimental groups 7 days post-parturition and followed for 60 days. According to NRC, ewes were separately housed in pens (1.5 m^2/ewe) with free access to water and an experimental diet (ad libitum) to meet their nutritional needs. The diets consisted of a 60:10:30 concentrate feed mixture, clover hay, and bean straw, respectively. Table 1 shows the chemical composition of the ingredients and diet. The experimental diets included a control diet with no additions (C), a control diet supplemented with 10 mL fish oil/kg DM, and a control diet supplemented with 5 g *Spirulina platensis* /kg DM. All diets were given twice daily at 07:00 and 17:00 hr, and milk samples were taken from each animal in the morning and evening. Each animal group's sample was mixed samples of a fixed percentage of the evening and morning yield.

Yoghurt making

The collected milk from each ewes group was preheated to 65 °C and homogenized in a laboratory homogenizer (EURO TURRAXT 20b, IKA Lobo Technik 27000 min G1). The homogenized milk was heated to 90 °C for 5 min, cooling to 42 °C, and divided into two equal portions. The first portion was inoculated with 2.0% mixed starter culture (*L. bulgaricus* and *S. thermophiles*, 1:1), whereas the second was inoculated with 2% mixed starter culture and 1% *B. longum* as a probiotic bacteria (2:1). All treatments were poured into 150 mL plastic cups and incubated at 42 °C until homogenous coagulation was achieved (Hassan et al. 2015). The yoghurt samples were stored at 5±2 °C for 15 days.

Chemical analysis

Total solids, fat, total nitrogen, and ash content of yoghurt were determined using AOAC (2007). The protein content was obtained by multiplying the percentage of TN by 6.38. A laboratory pH meter with a glass electrode was used to measure the changes in pH in the yoghurt samples during storage (HANNA, Instrument, Portugal. A water soluble nitrogen/total nitrogen ratio (WSN/TN ratio) was used to determine the level of proteolysis in the yoghurt samples during storage, according to Innocente (1997). The concentration of acetaldehyde in the yoghurt samples was measured using a spectrophotometer (Shimadzu, 240-UV–Vis, Japan) as described by Less and Jago (1970).

Table 1 The chemical composition of the ingredients and experimental diet (g/kg DM)

Item	CFM	Clover	Bean straw	Diet
Dry matter	883.4	900.1	376.5	883.0
Organic matter	948.3	911.5	925.1	937.7
Crude protein	138.3	85.9	66.3	111.5
Ether extract	139.3	105.6	94.7	122.6
Neutral detergent fiber	319.0	516.1	583.9	418.2
Acid detergent fiber	134.4	325.3	434.9	243.6
Hemicelluloses	184.6	190.8	149.0	174.5
Non-structural carbohydrate	351.7	203.9	180.2	285.5
Ash	51.7	88.5	74.9	62.3

CFM Concentrate Feed Mixture

Fatty acids profile

The fatty acid methyl ester of yoghurt samples was prepared according to the method of AOAC (2007). Fatty acid methyl esters were injected into (HP 6890 series GC) apparatus provided with a DB-23 column (60 m x 0.32 mm x 25 μm). Carrier gas was N 2 with flow rate 2.2 mL/min, splitting ratio of 1:50. The injector temperature was 250 °C and that of Flame Ionization Detector (FID) was 300 °C. The temperature setting was as follows: 50 °C to 210 °C/min and then held at 210 ° C for 25 min. peaks were identified by comparing the retention times obtained with standard methyl esters.

Antioxidant activities of yoghurt

Antiradical activities of yoghurt samples were estimated in yoghurt supernatant using stable DPPH radicals (DPPH•) and stable ABTS radicals (ABTS•) assays according to Brand-Williams et al. (1995) and Re et al. (1999), respectively. Briefly, 20 g of yoghurt were centrifuged at 4000 g for 5 min before filtered through Whatman filter paper No 1. 100 mL of yoghurt supernatant was added to 3.9 mL of DPPH working solution (25 mg DPPH/L methanol) or ABTS working solution (7 mM ABTS solution with 2.45 mM K2S2O8). After incubation for 30 min in the dark at room temperature (25±2 °C), the degree of decolorization was measured in a spectrophotometer (Shimadzu spectrophotometer, UV–Vis. 1201, Japan) at 517 nm for the DPPH• and 700 nm for the ABTS• radical-scavenging activity assays. Control solutions, DPPH and ABTS solutions without yoghurt supernatant, were prepared in the same manner as the assay mixture. The following formula was used to determine both ABTS• and DPPH• scavenging activities:

$$\text{Yoghurt antiradical activity } (\%) = [(A_0 - A_1)/A_0] \text{ x } 100$$

A_0 is the absorbance of the control (DPPH or ABTS solution), and A_1 is the absorbance of the sample.

Bacteriological analysis

Yoghurt samples were diluted and subsequently plated in duplicate onto selective media. The MRS agar medium was used to enumerate *L. bulgaricus* (Mohamed et al. 2017), while *S. thermophiles* was enumerated on M17

agar (Hussein et al. 2017). *B. longum* was determined according to Blanchette et al. (1996) using modified MRS agar supplemented with 0.05% L-cysteine-HCl. All plates were incubated at 37 °C anaerobic for 4 days.

Physical properties

Water holding capacity (WHC)

The WHC of yoghurt samples was determined by the centrifuge method according to Akalın et al. (2012) with some modifications. Twenty grams of yoghurt (10 °C) were centrifuged for ten min at 5,000 xg. The pellet was weighed after the separated whey was removed. The following equation was used to determine the WHC:

$$\text{WHC}\ (\%) = \left[1 - \left(\text{Pellet weight}/\text{Initial sample weight}\right)\right] \text{x } 100$$

Structure viscosity

Before viscosity measurement, the yoghurt sample was gently swirled 5 times in a clockwise direction with a plastic spoon. A Brookfield digital viscometer (Model DV-II, Canada) connected with spindle-4 was used to determine structure viscosity at 7 °C. The yoghurt sample was treated to selected rpm ranging from 3.0 to 30 for an upward curve. Structure viscosity was expressed as a Pascal (Pa s).

Sensory evaluation

According to Mohebbi and Ghoddusi (2008), experienced judges selected from staff members of the Dairy Department, National Research Center, Egypt, evaluated the yoghurt samples for sensory attributes (appearance, body & texture, flavor) on a 9-point hedonic scale (9 excellent, 1 unacceptable). Yoghurt samples were presented in three-digit coded white plastic containers and tasted 15 min after leaving the refrigerator.

Statistical analysis

Statistical analysis was performed using the GLM procedure with SAS (2004) software. Analysis of variance (ANOVA) and Duncan's multiple comparison procedure were used to compare the means. A probability of $P<0.05$ was used to establish statistical significance.

Results and discussion

Composition of yoghurt

The chemical composition of probiotic and non-probiotic yoghurts made from milk produced by ewes fed a diet supplemented with *Spirulina platensis* (SP) or fish oil (FO) was not significantly different (Table 2). Total solids, proteins, fat, lactose, and ash content ranged from 14.22 to 14.68, 4.28 to 4.42, 3.93 to 4.15, 4.86 to 5.20, and 0.95 to 0.98%, respectively. This means that feeding on SP or FO had no significant effect ($P>0.05$) on the percentage of milk ingredients. Similar observations were found in milk produced from cows fed a diet supplemented with flaxseed and soybean oil (Hassan et al. 2020) or *Spirulina platensis* microalgae (Lamminen et al. 2019).

Fatty acids composition of yoghurt

Table 3 shows the fatty acid content of probiotic and non-probiotic yoghurt made from milk of ewes' on a diet supplemented with *Spirulina platensis* or fish oil. In general, *Spirulina platensis* (SP) and fish oil (FO) supplements had a significant effect on the fatty acid composition of milk. Short-chain FA (SCFA), medium-chain FA (MCFA), and saturated FA (SFA) content were the highest in SP yoghurt, while unsaturated FA (USFA) and long-chain FA content (LCFA) were the lowest. The SCFA, MCFA, and SFA increased by 77.67, 31.03, and 14.63%, while USFA and LCFA decreased by 19.66 and 9.73% compared to control (C) yoghurt, respectively. In particular, $C_{4:0}$ (46.67%), $C_{10:0}$ (49.49%), $C_{12:0}$ (43.53%),

Table 2 Chemical composition of probiotic and non-probiotic yoghurts made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil

Treatments	Yoghurt composition				
	Total solids	Fat	Protein	lactose	Ash
	(%)				
C	14.23±0.08	4.01±0.07	4.34±0.05	4.88±0.17	0.95±0.07
SP	14.68±0.21	4.10±0.05	4.42±0.12	5.16 ±0.20	0.97±0.09
FO	14.53±0.11	3.93±0.10	4.28±0.09	5.01±0.15	0.98±0.02
PC	14.22±0.05	4.00±0.05	4.37±0.13	4.86±0.31	0.97±0.13
PSP	14.64±0.15	4.15±0.13	4.41±0.21	5.08±0.18	0.96±0.01
PFO	14.56±0.09	4.05±0.09	4.29±0.22	5.20±0.25	0.98±0.06
p-value	0.605	0.344	0.05	0.048	0.001

C, yoghurt made from ewes' milk fed a control diet; SP, yoghurt made from ewes' milk fed *Spirulina platensis*; FO, yoghurt made from ewes' milk fed fish oil; PC, probiotic yoghurt made from ewes' milk fed a control diet; PSP, probiotic yoghurt made from ewes' milk fed *Spirulina platensis*; PFO, probiotic yoghurt made from ewes' milk fed fish oil

Table 3 Fatty acids profile of probiotic and non-probiotic yoghurts made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil

Fatty Acids	Yoghurt treatments					
	C	SP	FO	PC	PSP	PFO
$C_{4:0}$	1.79	2.62	1.97	1.52	2.12	1.12
$C_{6:0}$	1.24	2.48	2.02	0.96	2.28	1.61
$C_{8:0}$	1.27	2.54	1.81	1.05	2.83	1.48
$C_{10:0}$	4.97	7.43	5.16	4.02	8.29	5.38
$C_{10:1}$	0.11	0.23	0.19	0.05	0.30	0.20
$C_{12:0}$	2.55	3.66	2.62	1.70	4.43	3.06
$C_{14:0}$	8.53	10.25	9.36	8.74	10.35	10.63
$C_{14:1}$	0.13	0.09	0.13	0.11	0.10	0.09
$C_{14:2}$	0.50	0.34	0.40	0.49	0.31	0.34
$C_{15:0}$	0.92	0.89	0.89	0.92	0.86	0.95
$C_{15:1}$	0.30	-	0.22	0.30	0.17	-
$C_{16:0}$	24.14	30.60	27.60	25.59	29.91	30.01
$C_{16:1}$	1.50	1.58	1.87	1.59	1.75	2.10
$C_{17:0}$	1.12	0.62	0.55	1.29	0.58	0.57
$C_{17:1}$	0.34	-	0.14	0.46	0.16	-
$C_{18:0}$	12.08	6.76	7.22	12.84	6.01	7.22
$C_{18:1}$	31.73	22.98	26.16	31.38	22.84	27.75
$C_{18:2}$	3.74	5.17	4.98	3.85	4.87	4.52
$C_{18:3}$	0.51	0.50	0.51	0.57	0.53	0.52
CLA	0.60	0.81	1.21	0.69	0.73	1.12
$C_{20:0}$	0.28	-	0.29	0.42	-	-
$C_{22:0}$	0.71	0.47	3.84	0.62	0.45	0.46
SCFA	4.30	7.64	5.80	3.53	7.23	4.21
MCFA	16.79	22.00	17.86	15.11	23.78	19.7
LCFA	77.97	70.38	75.48	82.52	68.86	75.22
SFA	59.6	68.32	63.33	59.67	68.11	62.49
USFA	39.46	31.7	35.81	41.49	31.76	36.64

C, yoghurt made from ewes' milk fed a control diet; SP, yoghurt made from ewes' milk fed *Spirulina platensis*; FO, yoghurt made from ewes' milk fed fish oil; PC, probiotic yoghurt made from ewes' milk fed a control diet; PSP, probiotic yoghurt made from ewes' milk fed *Spirulina platensis*; PFO, probiotic yoghurt made from ewes' milk fed fish oil

$C_{16:0}$ (26.76%), $C_{18:2}$ (38.23%), and CLA (35.00%) showed the greatest increase, whereas $C_{18:0}$ (-44.04%) and $C_{18:1}$ (-27.57%) showed the greatest decline. These findings disagree with Christaki et al. (2012) and Kouřimská et al. (2014) in cow's milk and goat's milk, respectively. Excessive algal supplementation may negatively impact feed intake, ruminal metabolism, milk production, and lipids composition (Altomonte et al. 2018). When ewes were fed fish oil, a comparable but less pronounced change in the fatty acids of FO yoghurt was found. $C_{18:0}$ and $C_{18:1}$ content decreased, whereas $C_{16:0}$, $C_{18:2}$, and CLA content increased, with the latter being more pronounced than in SP yoghurts. These findings are consistent with those of Shingfield et al. (2003) in milk fat of cows fed fish oil, but they differ from those in milk fat of Holstein cows fed fish oil or soybean oil (Fatahnia et al. 2008). Except for a modest drop in the level of SCFA (-5.36%), $C_{18:0}$ (-11.10%) of probiotic SP yoghurt and $C_{18:2}$ (-9.23%) of probiotic FO yoghurt when probiotic bacteria (*B. longum*) were added, no apparent changes in fatty acids content were identified when compared to non-probiotics.

Viable counts of starters and probiotic

The main cultures in yogurt are *L. bulgaricus* and *S. thermophilus. Bifidobacteria* and other probiotic bacteria cultures can be added to yoghurt. *B. longum* is a multifunctional, clinically effective probiotic with a long history of safe use in the treatment of gastrointestinal, immunological, and infectious diseases in humans (Wong et al. 2019). Fig 1 shows the viable counts (log_{10} cfu/g) of yoghurt starters (*S. thermophilus* and *L. bulgaricus*) and probiotic bacteria (*B. longum*) after 15 days of storage at 5±2 °C. The counts of *L. bulgaricus* and *S. thermophilus* in both SP and FO yoghurts were greater than in C yoghurt;

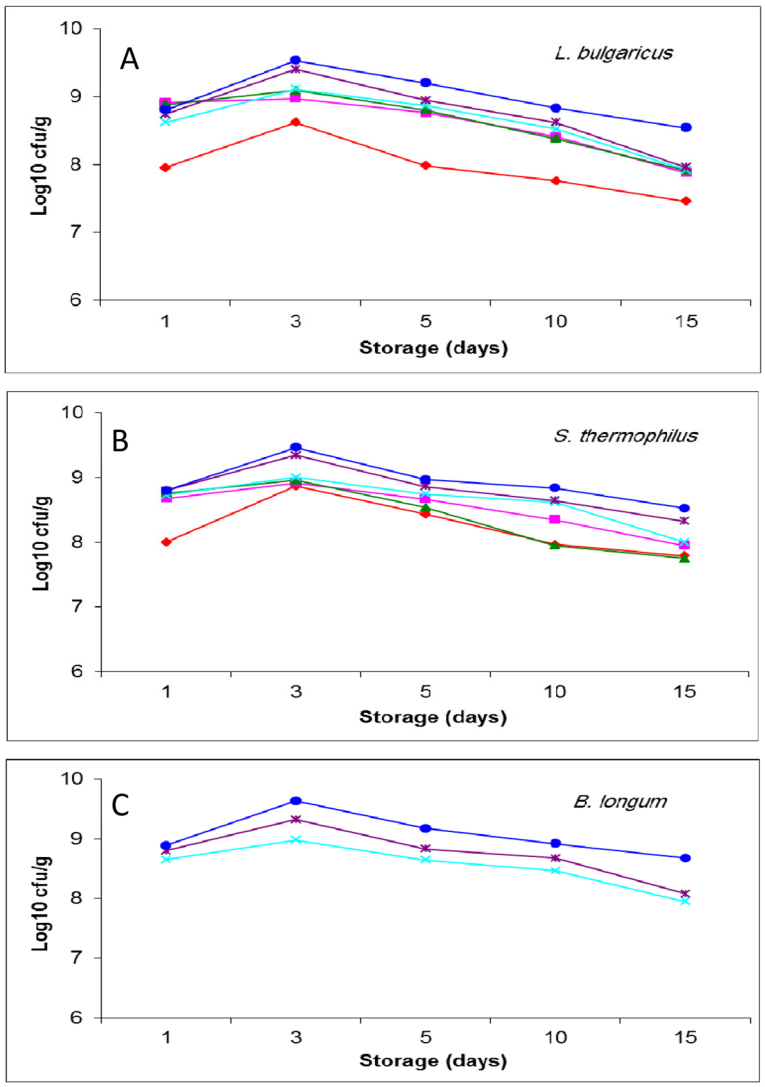

Fig. 1 Changes in viable counts of starter cultures and probiotic bacteria of yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2 °C for 15 days. —◆—, C yoghurt; —■—, SP yoghurt; —▲—, FO yoghurt; —×—, probiotic C yoghurt; —✳—, probiotic SP yoghurt; —●—, probiotic FO yoghurt

the difference being significant only in counts of *L. bulgaricus* on days 1, 3, and 5. A slight increase in the viable counts of starter cultures was found when *B. longum* was added ($P>0.05$(. This is comparable to Sharma et al. (2014), who found that using probiotic bacterial cultures promotes the growth of favored microorganisms while crowding out potentially harmful bacteria. When compared to probiotic C yoghurt, both probiotic SP and probiotic FO yoghurts had a similar, small rise in *B. longum* counts ($P>0.05$). During storage, all yoghurt samples exhibited a little increase in viable counts ($P>0.05$) until day 3, then decline thereafter, the decrease being significant only at day 15 ($P<0.05$). However, the viable counts of *S. thermophiles*, *L. bulgaricus* and *B. longum* were still $>10^7$ cfu/g at the end of storage. A similar trend was observed by Mani-López et al. (2013). Sarvari et al. (2014, b) mentioned that the decline of viability for *bifidobacteria* was gradual and steady during storage. *B. longum* loses viability during storage due to a large amount of acid, hydrogen peroxide, and maybe bacteriocins produced by *L. bulgaricus* (Sarvari et al., 2014, b). However, *B. longum* in FO yoghurt was more stable during storage than SP or C yoghurts. This could be due to the presence of bioactive components with high antioxidant activity (Table 3) in FO yoghurt, which absorb molecular oxygen and thereby prevent B. longum (anaerobic bacteria) from dying.

Biochemical changes

The biochemical changes of probiotic and non-probiotic yoghurt made from milk produced by ewes fed SP or FO during storage at 5±2 °C for 15 days are presented in Table 4. The highest acetaldehyde content was found in SP yoghurt ($P<0.05$), followed by FO yoghurt ($P<0.05$) and C yoghurt. The high acetaldehyde content in SP yoghurt may be due to the high levels of SCFA and MCFA (Table 3), which improve acetaldehyde formation .The acetaldehyde content of yoghurt increased when *B. longum* was added as a probiotic; the increase was significant only in PFO yoghurt compared to FO yoghurt ($P<0.05$). Tamime and Robinson (2007) reported that the protein composition of yoghurt and the bacteria culture and ratio in mixed strains influence the formation of aroma compounds such as acetaldehyde. Proteolytic activities of yoghurt starter cultures, for example, increased acetaldehyde formation by producing threonine in goat's milk.

The FO yoghurt also had the highest WSN/TN ratio and the lowest pH; however, the difference was insignificant ($P>0.05$). An increased starter activity in FO yoghurt; increased viable counts of starters could explain the high WSN/TN ratio and low pH (Fig 1). Acetaldehyde content dropped ($P<0.05$) while the WSN/TN ratio increased throughout the storage period of 15 days; the difference was significant only in acetaldehyde content ($P<0.05$). Acetaldehyde content appears to be positively associated with pH value; acetaldehyde content decreases as pH value decrease (El-Shenawy et al. 2019). Similar findings were made in yoghurt containing plant polysaccharides (Hussein et al., 2011) and yoghurt containing plant mucilage (Hassan et al. 2015). However, all yoghurt samples showed a significant decrease ($P<0.05$) in pH at day 5, after which the decrease was not significant ($P>0.05$). The changes in pH during storage were also similar in symbiotic low-fat yoghurts (Ramchandran & Shah 2010). According to Mani-López et al. (2013), the pH of yoghurts and fermented milks decreased during storage due to residual microbial activity (post-acidification).

Antioxidant activity

As shown in Table 5, probiotic yoghurt had stronger antioxidant activity against DPPH• and ABTS• radicals than non-probiotic yoghurt; the difference being significant only for ABTS• radicals ($P<0.05$). Sah et al. (2014) focused

Table 4 Biochemical changes of probiotic and non-probiotic yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2 °C for 15 days

Treatments	Storage periods (days)			
	1	5	10	15
pH values				
C	4.58^{Ae} ±0.05	4.35^{Af}±0.04	4.27^{Af}±0.11	4.30^{Af}±0.08
SP	4.56^{ABe}±0.06	4.34^{Af}±0.10	4.31^{Af}±0.10	4.28^{Af}±0.09
FO	4.44^{Be} ±0.04	4.29^{Af}±0.02	4.26^{Af}±0.08	4.24^{Af}±0.02
PC	4.52^{Ae} ±0.07	4.28^{Af}±0.03	4.25^{Af}±0.05	4.29^{Af}±0.07
PSP	4.47^{ABe}±0.11	4.26^{Af}±0.03	4.23^{Af}±0.02	4.32^{Af}±0.06
PFO	4.39^{Ae} ±0.09	4.24^{Af}±0.02	4.22^{Af}±0.03	4.30^{Af}±0.04
WSN/TN ratio (%)				
C	8.74^{B}±0.31	8.77^{B} ±0.43	9.05^{B} ±0.29	9.69^{B}±0.43
SP	9.63^{AB}±0.09	9.68^{AB}±0.08	10.03^{AB}±0.50	10.71^{B}±0.28
FO	10.06^{AB}±0.31	10.1^{AB}±0.33	10.45^{AB}±0.75	11.48^{AB}±0.24
PC	9.85^{AB}±0.29	9.87^{AB}±0.03	10.19^{AB}±0.86	10.94^{AB}±0.57
PSP	10.65^{AB}±0.36	10.66^{AB}±0.51	10.99^{AB}±0.28	11.81^{A}±0.68
PFO	11.19^{A}±0.36	11.24^{A} ±0.61	11.56^{Aa} ±0.86	12.52^{A}±0.14
Acetaldehyde (µmol/100 g)				
C	39.91^{Ce}±2.05	37.92^{Ce}±2.21	33.92^{Ce}±1.98	16.96^{Cf}±3.35
SP	129.31^{Ae}±5.65	122.34^{Ae}±4.17	111.44^{Af}±3.22	66.87^{Ag}±6.43
FO	90.47^{Be}±5.60	85.94^{Be}±3.94	70.56^{Bf}±2.28	42.34^{Bg}±3.67
PC	46.67^{Ce}±4.33	44.33^{Ce}±2.16	39.67^{Ce}±3.83	19.83^{Cf}±2.91
PSP	144.15^{Ae}±4.57	136.94^{Ae}±3.47	115.32^{Af}±7.66	69.19^{Ag}±7.51
PFO	135.55^{Ae}±7.67	128.77^{Ae}±4.38	105.73^{Af}±3.65	63.44^{Ag}±5.74

^{A,B,C} letters within the same column differ significantly at $P<0.05$; ^{e,f,g} letters within the same row differ significantly at $P<0.05$; C, yoghurt made from ewes' milk fed a control diet; SP, yoghurt made from ewes' milk fed *Spirulina platensis*; FO, yoghurt made from ewes' milk fed fish oil; PC, probiotic yoghurt made from ewes' milk fed a control diet; PSP, probiotic yoghurt made from ewes' milk fed *Spirulina platensis;* PFO, probiotic yoghurt made from ewes' milk fed fish oil

Table 5 Radical scavenging activities of probiotic and non-probiotic yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2 °C for 15 days

Treatments	Storage periods (days)			
	1	5	10	15
DPPH• radicals scavenging activity (%)				
C	13.07^{B}±1.00	13.30^{B}1.07	14.10^{B}±0.92	15.51^{B}±1.77
SP	13.96^{B}±1.81	13.23^{B}±2.22	14.03^{B}±0.59	15.46^{B}±0.47
FO	14.71^{AB}±0.67	15.01^{AB}±0.31	15.92^{AB}±0.98	17.53^{AB}±1.04
PC	16.23^{AB}±1.08	16.56^{AB}±0.31	17.55^{AB}±1.06	19.33^{AB}±0.49
PSP	14.81^{AB}±0.96	14.94^{AB}±0.35	15.84^{AB}±0.67	17.44^{AB}±0.56
PFO	18.49^{A}±0.51	18.71^{A}±0.40	19.77^{A}±0.65	21.68^{A}±1.45
ABTS• radicals scavenging activity (%)				
C	23.67^{Bf}±2.12	24.38^{Bf}±0.55	28.03^{Bf}±2.83	39.25^{Be}±0.39
SP	23.48^{Bf}±0.21	24.30^{Bf}±1.64	27.70^{Bf}±0.11	38.78^{Be}±1.79
FO	27.73^{Bf}±0.31	29.11^{Bf}±1.15	33.48^{Bf}±1.10	46.87^{Be}±1.79
PC	35.28^{Af}±1.74	36.34^{Af}±1.70	41.79^{Af}±2.40	58.50^{Ae}±2.19
PSP	36.60^{Af}±0.34	37.84^{Af}±1.73	43.00^{Af}±0.33	59.80^{Ae}±1.20
PFO	38.50^{Af}±1.60	39.38^{Af}±1.15	46.28^{Af}±0.34	63.39^{Ae}±1.79

^{A,B} letters within the same column differ significantly at $P<0.05$; ^{e,f} letters within the same row differ significantly at $P<0.05$; C, yoghurt made from ewes' milk fed a control diet; SP, yoghurt made from ewes' milk fed *Spirulina platensis*; FO, yoghurt made from ewes' milk fed fish oil; PC, probiotic yoghurt made from ewes' milk fed a control diet; PSP, probiotic yoghurt made from ewes' milk fed *Spirulina platensis*; PFO, probiotic yoghurt made from ewes' milk fed fish oil

on this strain's ability to produce bioactive peptides with antioxidant activities co-cultured with yoghurt starters for probiotic yoghurt production. In vitro, hydroxyl radicals and superoxide anion were scavenged by the probiotic supernatant, intact cells, and intracellular cell-free extracts of *Bifidobacterium* (Shen et al. 2011). Probiotics can improve the antioxidant system and minimize free radical formation, according to Wang et al. (2017). However, the antioxidant activity against the DPPH• radicals was lower than that against the ABTS• radicals. This difference could be related to DPPH's solubility, which is limited to organic solutions. Furthermore, DPPH acts as an oxidizing substrate and a reaction indicator, causing considerable interference (Sah et al. 2014).

FO yoghurt has greater antioxidant activity against ABTS• radicals ($P<0.05$) than SP and C yoghurts. However, the DPPH• radical scavenging activity of C, SP, and FO yoghurts did not differ significantly ($p>0.05$). This suggests that feeding ewes on fish oil can improve the antioxidant activity of the resulting yoghurt, especially against the ABTS• radicals ($P<0.05$). Such an effect has been found in soft cheese made from milk of lactating animals fed on flaxseed or soybean oils (Hassan et al. 2020). Throughout the storage period, the antioxidant activity of all yoghurt samples increases at the same rate, with the difference being significant only at day 15 against ABTS• radicals. Protein hydrolysis may be correlated to an increase in antioxidant activity during storage. A high positive correlation ($r^2 = 0.75$) was found between the antioxidant activity of cheese and the degree of proteolysis (Hassan et al. 2020). Shazly et al. (2019) reported that the high antioxidant capacity of casein is related to the degree of hydrolysis. Similarly, Sah et al. (2014) discovered a strong positive correlation between the degree of hydrolysis and ABTS• radical scavenging activity (Table 5). Shazly et al. (2019) reported that the high antioxidant capacity of casein is related to the degree of hydrolysis.

Physical properties

As shown in Fig 2, the SP and FO yoghurts had better water-holding capacity (WHC) than the C yoghurt, but the differences were not significant ($P>0.05$). This suggests that consuming SP or FO did not influence the yoghurts' WHC. When *B. longum* was added, all yoghurt samples showed a small increase ($P>0.05$) in the WHC at the same rate. This is since *B. longum* produces a lot of capsular polysaccharides and

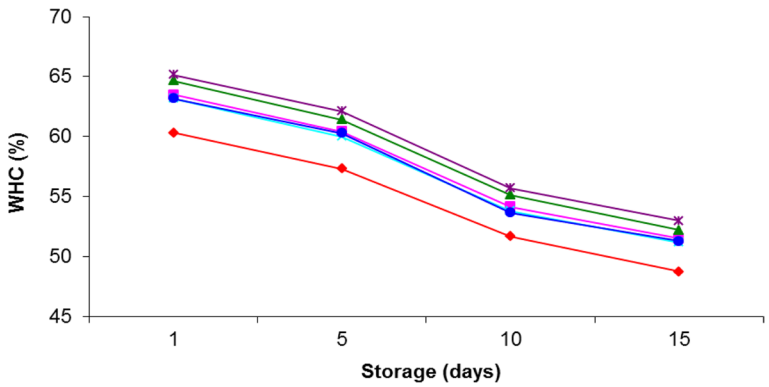

Fig. 2 Water-holding capacity of probiotic and non-probiotic yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2 °C for 15 days. —◆—, C yoghurt; —■—, SP yoghurt; —▲—, FO yoghurt; —×—, probiotic C yoghurt; —✳—, probiotic SP yoghurt; —●—, probiotic FO yoghurt

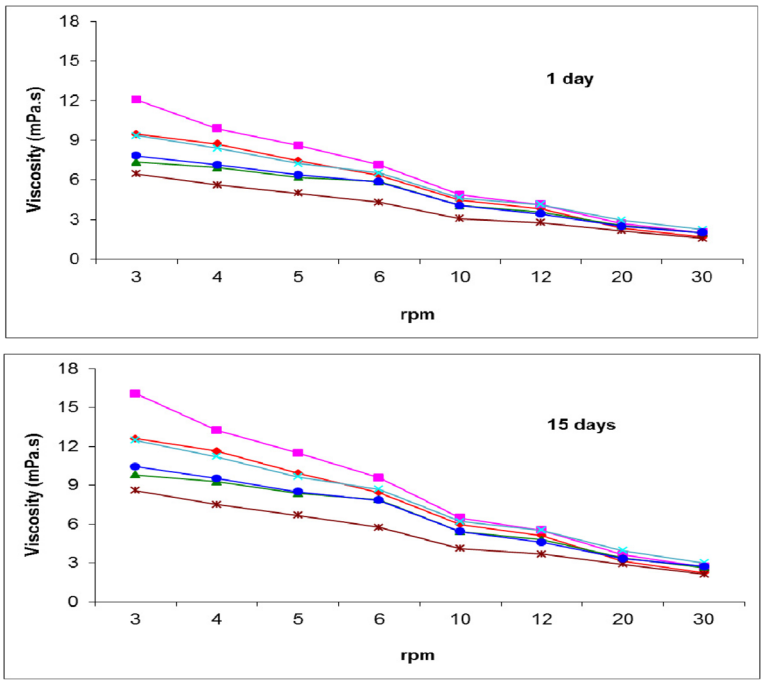

Fig. 3 Structure viscosity of probiotic and non-probiotic yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2°C for 15 days. —◆—, C yoghurt; —■—, SP yoghurt; —▲—, FO yoghurt; —✕—, probiotic C yoghurt; —✱—, probiotic SP yoghurt; —●—, probiotic FO yoghurt

exopolysaccharides (Tahoun et al. 2017). The production of exopolysaccharides by *Bifidobacteria* is one of the hypothesized mechanisms for their probiotic activities (Yan et al. 2017).

Also, SP yoghurt had a higher viscosity than both FO and C yoghurts at low rpm (up to 6 rpm) ($P<0.05$), while C yoghurt had higher viscosity than FO yoghurt; after that, the difference was not significant (Fig 3). According to Abd El-Aziz et al. (2015), a high amount of USFA in the emulsion produces small fat droplets, which increase the rheological properties; consequently, the high viscosity of SP yoghurt is due to its high level of SCFA rather than its USFA content. On day 15, all yoghurt samples had a higher viscosity than those on day 1 ($P<0.05$). The linkages between the gel particles are stronger, and their numbers are greater at a lower temperature throughout the storage time. The particles are more swollen and attached over a longer area, increasing viscosity (Walstra et al. 1999). Other researchers have reported such an effect (Doleyres et al. 2005; Hussein et al. 2017). When *B. longum* was added, the viscosity of SP yoghurt significantly decreased ($P<0.05$), while the viscosity of both C and FO yoghurt was not affected compared to non-probiotic yoghurts. In comparison to non-probiotic yoghurts, the viscosity of SP yoghurt dropped significantly ($P<0.05$) when *B. longum* was added, while the viscosity of C and FO yoghurts did not change.

Sensory properties

Table 6 displays the sensory scores of ewes' yoghurts during the storage period at 5±2°C for 15 days. There was no significant change in sensory attributes such as appearance, flavor, and body & texture during varied storage periods among all ewes' yoghurt samples ($P>0.05$). The previous finding suggests that the addition of probiotic bacteria (*B. longum*) or the type of animal feed (*Spirulina platensis* or fish oil) did not affect the yoghurt's sensory characteristics. Soft cheese prepared from the milk of nursing animals fed a diet enriched with soybean or flaxseed oils showed a similar tendency (Hassan et al. 2020.). Throughout the storage period, no significant changes ($P>0.05$) in the sensory characteristics scores of all yoghurt samples were seen after storage until 10 days, after which a significant decrease ($P<0.05$) was noted.

Conclusion

It can be concluded that supplementing the ewes' diets with *Spirulina platensis* or fish oil can change the fatty acid composition of the resulting yoghurt. Short and

Table 6 Sensory evaluation of probiotic and non-probiotic yoghurt made from ewes' milk fed a diet supplemented with *Spirulina platensis* or fish oil during storage at 5±2 °C for 15 days

Treatments	Storage periods (days)			
	1	5	10	15
Appearance and color				
C	8.77^{Be}±0.15	8.63^{Aef}±0.17	8.45^{Ae}±0.13	7.44^{Ag}±0.11
SP	8.90^{ABe}±0.10	8.74^{Aef}±0.10	8.55^{Ae}±0.09	7.25^{Ag}±0.13
FO	9.00^{Ae}±0.10	8.84^{Ae}±0.08	8.63^{Af}±0.08	7.62^{Ag}±0.10
PC	8.93^{ABe}±0.06	8.77^{Af}±0.07	8.59^{Ae}±0.04	7.55^{Ag}±0.09
PSP	8.87^{ABe}±0.06	8.75^{Ae}±0.05	8.53^{Af}±0.13	7.53^{Ae}±0.12
PFO	8.77^{Be}±0.15	8.63^{Aef}±0.17	8.45^{Af}±0.13	7.44^{Ag}±0.11
Flavor				
C	9.40^{De}±0.10	9.29^{De}±0.08	9.10^{Df}±0.10	8.20^{Dg}±0.10
SP	9.60^{BCe}±0.10	9.45^{BCe}±0.10	9.21^{BCf}±0.11	8.25^{BCg}±0.05
FO	9.70^{ABe}±0.10	9.52^{BCf}±0.08	9.32^{ABg}±0.07	8.36^{ABh}±0.05
PC	9.50^{CDe}±0.10	9.39^{CDf}±0.10	9.20^{BCf}±0.10	8.31^{BCg}±0.09
PSP	9.70^{ABe}±0.01	9.57^{ABf}±0.08	9.34^{ABg}±0.05	8.39^{ABh}±0.08
PFO	9.82^{Ae}±0.03	9.67^{Af}±0.03	9.44^{Ag}±0.05	8.45^{Ah}±0.05
Body and texture				
C	9.40^{De}±0.10	9.29^{De}±0.08	9.10^{Df}±0.10	8.2^{Dg}±0.10
SP	9.60^{BCe}±0.10	9.45^{BCe}±0.10	9.21^{BCf}±0.11	8.25^{BCg}±0.05
FO	9.70^{ABe}±0.10	9.52^{BCf}±0.08	9.32^{ABg}±0.07	8.36^{ABh}±0.05
PC	9.50^{CDe}±0.10	9.39^{CDf}±0.10	9.20^{BCf}±0.10	8.31^{BCg}±0.09
PSP	9.70^{ABe}±0.01	9.57^{ABf}±0.08	9.34^{ABf}±0.05	8.39^{ABg}±0.08
PFO	9.82^{Ae}±0.03	9.67^{Af}±0.03	9.44^{Ag}±0.05	8.45^{Ah}±0.05

^{A,B,C,D} letters within the same column differ significantly at $P<0.05$; ^{e,f,g,h} letters within the same row differ significantly at $P<0.05$; C, yoghurt made from ewes' milk fed a control diet; SP, yoghurt made from ewes' milk fed *Spirulina platensis*; FO, yoghurt made from ewes' milk fed fish oil; PC, probiotic yoghurt made from ewes' milk fed a control diet; PSP, probiotic yoghurt made from ewes' milk fed *Spirulina platensis*; PFO, probiotic yoghurt made from ewes' milk fed fish oil

medium-chain fatty acids and some unsaturated fatty acids like linolenic acid and CLA were all increased, but oleic acid was decreased. The starter culture's activity, flavor compounds, and some chemical, physical, and antioxidant properties of milk produced from these diets can be improved, particularly in yoghurt treated with probiotic bacteria (*B. longum*). Generally, *Spirulina platensis*, rather than fish oil, had a stronger impact on these modifications in ewes' milk. Propionic bacteria, on the other hand, remained more stable in FO yoghurt during storage.

Authors' contributions

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Consent for publication

All of the authors consent to the publication of this manuscript in Annals of Microbiology.

Competing interests

The authors declare no competing interests in publishing this manuscript.

Author details

[1]Dairy Department, Food Industries and Nutrition Research Institute, National Research Centre, Giza, Egypt. [2]Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

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