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Healthy karish Cheese Flavored with Alliums sativum and Cyminum Cuminum Extract

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Abstract

Background and objective: The use of plants and spices in foods, as prebiotic and phytochemical constituent's resources, and lactic acid bacteria, as probiotics, is receiving a global increasing attention which is the aim of this study. Materials and methods: The chemical and microbiological standard methods and agar well diffusion method were followed. **Results:** The Preliminary phytochemical screening assay of methanol extracts of cumin **(***Cyminum L***)** and garlic **(***Alliums sativum L.*) were carried, and the results revealed that they contained Sterol, terpene, glycosides, and flavonoids and fixed & volatile oils, additionally only garlic contained**,** reducing sugars. Moreover, antimicrobial activity of Cumin and Garlic was measured in different extracts**,** separately or in combinations with *B. bifidum* and *L. acidophilus***,** against food borne pathogenic bacteria and A. *flavus*. The results also showed that alcoholic extract indicated an increase in antimicrobial activity in garlic and cumin, as it showed wider inhibition areas for pathogenic bacteria under testing. Furthermore, the results indicated that the combination of spices and LAB inhibited the growth of pathogenic strains to a greater extent than the use of garlic or cumin, individually. For application purposes, the extract of the cumin and garlic have been used in preparing karish cheese in combination with *L. acidophilus, Lactococcus lactis, and B. bifidum*, in different treatments. Conclusion: the prepared karish cheese in the different treatments gave high chemical, antimicrobial and sensory quality, especially the treatment that contained all the ingredients.

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Keywords: lactic acid bacteria, karish cheese Cyminum, Alliums sativum – photochemical - constituents- antimicrobial activity

1. Introduction

Nowadays, the increasing interest in the role of plant additives and the probiotics in food area to influence the composition and activity of the intestinal microbiota made more attention of the scientific community toward this point. Therefore, exploration of naturally occurring plant additives and its phytochemical compounds to prepare new functional foods of high health value is receiving increasing attention by the researchers and consumer awareness for functional foods [1,2]. To reach the phytochemical compounds of these plants, its aqueous solutions or oily extracts must be made to deal with these plants [3,4,5]. Consequently, it is possible to know the extent and quality of adding these natural compounds to any food product as flavor or functional value. Accordingly, synergistic effects of using some plant extracts with probiotic bacteria in foods has gained much attention nowadays, to obtain new functional food-dairy products [6,7,8]. Among these spices and plants, which are used in the preparation of many foods are Cumin (*Cuminum cyminum L.*), which was one of the oldest cultivated medicinal food herbs in Asia, Africa, Europe, and was used to flavor foods, and for medical preparations [9, 10]. Moreover, cumin seeds have been found to be rich in phenols, flavonoids, and other phytochemical compounds that increase the health of food added in addition to being a source of good taste [11,12]. Also, Alliums vegetables, especially garlic (*Alliums sativum* L) exhibited a broad antibiotic activity against both of Gram-

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positive and Gram-negative bacteria [13]. Garlic oil and water-soluble compounds, were responsible for the typical odor and flavor of garlic and play an important role in its antibiotic activity [14]. The phytochemical screening of garlic revealed the presence of chemical compounds such as saponin, tannin, carbohydrates, cardio glycoside, alkaloids, flavonoid, phlobatannin and glycoside, which is responsible for many of the garlic properties [15]. On the other hand, Cheese is a common dairy product consumed worldwide and its production from different types of milk [16]. karish is an Egyptian variety of soft cheese produced from skim milk by using acidification coagulants, as LAB, and the product contain about 70% moisture and not more than 10% fat, [17,18]. Production of Natural flavored cheese made in short time with highly nutritive value was a new trend for the last years [19]. In previous studies, most spices such as cumin, onions, garlic, turmeric and others were added to different types of cheese to add unique flavors that affected the chemical and microbiological quality of the cheese [6,20] Therefore, the aim of this study was to explore the chemically and microbiologically properties and synergistic effects of using the extracts of garlic and cumin, and in combination with lactic acid bacteria, to make a new type of probiotic dairy product "Garlic-Cumin karish cheese" which has undergone chemical, microbiological and sensory tests to ensure its general quality and specifications.

2-Material and methods.

Chemicals

All solvents and chemicals were HPLC grade, Fisher Scientific, USA. Di-methyl sulfoxide (DMSO) from SERVA Electrophoresis GmbH, Germany. Tween-80®, Food quality, Panreac, Spain, lithium chloride was from Sigma Chemical Co., USA.

Apparatus: PH values were measured using a digital laboratory pH meter equipped with a combined electrode (Hanna, Germany).

Plant materials: Cumin (*Cuminum cyminum* L.) seeds and fresh garlic (*Allium sativum* L.) bulbs were purchased from a local supermarket of Egypt. Which were kindly identified by, Prof. Dr. Mona Marzok, Researcher at the Herbarium of National Research Center (NRC), Cairo, Egypt.

Skim milk retentate (SMR): Skimmed UF-retentate was procured from Animal Production Research Institute, Agriculture Research Center, and Dokki, Egypt.

Reference microorganisms:

Pathogenic Microorganisms were obtained from the research laboratory of Dairy Microbiology department, National research center. The microorganisms used in this study were: - *Bacillus cereus* (*ATCC 33018*), *Staphylococcus aureus* (*ATCC 2023*), *Escherichia coli 0157:H7* (*ATCC 6933*), *Salmonella typhimurium (ATCC* 14028), *Yersinia enterocolitica subsp. enterocolitica* (ATCC9610), *Listeria monocytogens*V7, *Streptococcus mutans*, *Pseudomonas aeruginosa* (*ATCC* 9027) and *Aspergillus flavus* (*ATCC 3357*)

Lactic acid bacterial (LAB) strains used in this study were as follows*: Lactobacillus acidophilus (N4495)*, *Lactococcus lactis sub spp. lactis* and *Bifidobacterium bifidum (*Bb-12) were obtained from Chr. Hansen's Lab., A/S Copenhagen- Denmark. All these strains were supplied in Clean Cultures from the Microbiology lab, Dairy department of the food industry and nutrition Division of National research center. LAB strains were cultured and enumerated onto MRS culture media according to [21, 22, 23]

Preparation of the extracts:

The dried seeds of cumin (*Cuminum cyminum* L.), and fresh garlic (*Allium sativum* L.) bulbs were washed with tap water, dried at room temperature in shad and seeds of cumin were grinded to fine powder whereas garlic bulbs chopped into pieces. The fine seeds of cumin powdered and pieces of garlic bulbs were successively extracted with water and methanol (HPLC grade) in percolator at room temperature till exhausted and concentrated under reduced pressure at 40ºC at rotary evaporator till dryness according and to the method adopted by [24], and then they were re-extracted with petroleum ether and stored at -20ºC until use. The yield of cumin and garlic were 8.78 and 15 %, respectively. All the extracts (were applied in the chemical and microbiological experiments.

Portion of each dried methanolic extract was suspended in distilled water with assistance of ultrasonic water bath and sequentially fractionated successively with petroleum ether. Petroleum ether was concentrated till dryness in rotor vapor at 40 ºC as described by [25]. The obtained extracts were stored at -20 ºC until used.

Preliminary phytochemical screening of the extracts

The phytochemical-constituents of garlic and cumin extracts were assayed qualitatively using standard methods as described by [26].

Microbial activity of garlic and cumin extracts with and without probiotic bacteria on pathogen:

Garlic and cumin extracts were dissolved in dimethyl sulfoxide (DMSO). The DMSO only was used as negative control. The antimicrobial activity of different garlic and cumin extracts (methanolic extract, Petroleum ether extract and aqueous extract) at a concentration (100µg/ml) was carried out against food borne pathogenic microorganisms using agar

well diffusion method. The pathogenic test microbial strains, *Escherichia coli 0157: H7 ATCC 6933, Bacillus cereus ATCC 33018, Staphylococcus aureus (ATCC 20231), Pseudomonas aeruginosa (ATCC 9027), streptococcus mutans, Listeria monocytogens V7, Yersinia enterocolitica subsp. enterocolitica (ATCC9610TM), Salmonella typhimurium (14028) and Aspergillus flavus (ATCC 3357*), were activated in Tryptone soy broth and then in plates, separately, at 37°C for 24h. After incubation the plates were examined for inhibition zones (mm) appeared around the wells containing the extracts and the results were expressed as average values. In the case of A. flavus, a spore suspension (10^6 spores/ml) was prepared and 100 µl of it was spread on potato dextrose agar (PDA) dishes. After absorption, the cork borer was used to bore. The dishes were incubated for 5 days at 25 °C. Visible inhibition zone around bore was used [27]. All the trails were carried out in triplicates.

For synergistic effects, garlic and cumin extracts in combination with *B. bifidum and L. acidophilus* were tested against the test pathogenic strains. For studying LAB in combination with the extracts, MRS broth medium of each the probiotic bacteria (*B. bifidum and L. acidophilus* at 2% broth) was added to the (Cumin- MRS), (Garlic- MRS) separately and the broths were incubated at 37°C for 48h under anaerobic condition. After incubation, each (Cumin, Garlic -MRS-Probiotic) mixture was centrifuged at 4000 rpm for 15 min at 4ºC. The supernatant was discarded and the antimicrobial activity of the resulted precipitate was tested against pathogenic bacteria using agar well diffusion method as previously indicated by [28].

Preparation of karish cheese with garlic and cumin extracts:

The current study was directed towards the using of garlic and cumin aqueous extracts and LAB in karish cheese making, in different treatments as shown in Table (1), due to the previous preliminary chemical-microbial evaluation. Garlic -cumin karish cheese was made by using skimmed UF-retentate heated to 85°C/5min, then cooled to 42ºC, and divided into two batches. The batches were supplemented, separately, with 50 and 100 ug of garlic and cumin aqueous extract / 100 ml of skimmed UF-retentate. Then, the two batches were inoculated individually with 2% of active mixture starter of *Lactococcus lactis subsp. lactis, Bifidobacterium bifidum* and *L. acidophilus* in different treatments as mentioned by [29].

Chemical analysis of Garlic-cumin karish cheese:

Cheese was homogenized well and analysed for moisture, fat, acidity, total solids (T.S), total nitrogen (T.N), ash content and pH values, Diacetyl and acetaldehyde were determined according to [30]

Microbiological analysis of Garlic-cumin karish cheese:

Garlic and cumin karish cheese samples, during cold storage were examined microbiologically for viable count of *Lactobacillus acidophilus* was determined according to [31] using MRS-sorbitol agar, and *Bifid bacterium bifidum* was enumerated on MRS agar (Oxoid) supplement with L-cysteine and lithium chloride [31] *Lactococcus lactis* sub spp. *Lactis*, using M17 agar plates 18, Total bacteria count was determined by aerobic plate count method on plate count agar (Oxoid); and Yeast & Molds using potato dextrose agar according to [32,33].

Sensory evaluation of Garlic-cumin karish cheese:

Cheese samples were sensory evaluated freshly and after 15 days of storage by ten panellists of staff members of Dairy Department at Food Industries and Nutrition Division, National Research Center, using the score sheet according to [34]. The scores of judging were 60 for flavour, 30 for body & texture and 10 for appearance.

Results and discussion:

Phytochemical analysis of Garlic and Cumin:

Results in table 2 clarifies the phytochemical screening of Garlic (*Allium sativum L.*) bulbs, as the results revealed the presence of essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, glycosides, steroids and terpenoids as the major phytochemical groups. Moreover, phytochemical screening analysis of garlic also revealed the presence of chemical compounds such as saponin, tannin, carbohydrates, cardio glycoside, flavonoid, [35]. The typical odor and flavor of garlic are mostly organosulfur compounds [36]. In addition, thiosulfates play an important role in the antibiotic activity of garlic [14]. Moreover, Garlic bulbs was also considered a rich source of other non-volatile compounds, similar to that was found in the current study, with important medicinal and therapeutic properties, [37].

Also results in table (2), illustrate the phytochemical screening analysis of cumin (*Cuminum cyminum L.*) seeds, as the results revealed that cumin contains a large phytochemical group and wide variety of biologically active compounds as essential oils, Lipids, fatty acids, phenols, flavonoids, flavonoid glycosides, terpenoids, monoterpenoid glucosides, steroidal, and carbohydrates. The literature indicated that the seeds contain mono terpenoid, glucosides [38], essential oils [9], fatty acid ester, terpenic and steroidal constituents [39]. Moreover, cumin seeds have been reported to be rich in phenolic acids and flavonoids [11]. In fact, they have been reported to contain a wide spectrum of sesquiterpenoid glucosides [40] and glycosides [41].

All these phytochemical components of garlic and cumin extracts raise the nutritional and health values as additives to any food product, as well as add a new

flavor. Also, these components may play an important role as inhibitors of pathogenic microbes or conversely, as stimulants prebiotic for probiotic bacteria if used as in karish cheese in the present study.

Table 1: different treatments of Karish Cheese with LAB, garlic and cumin aqueous extract

Abbreviations	Treatments
C1	50 ug of garlic and 100 ug cumin aqueous (water) extract
C2	100 ug of garlic and 100 ug cumin aqueous extract
T1	2% *L. acidophilus* + 50 ug of garlic and 100 ug cumin aqueous extract
T2	2% *Bfido*. *bifidum* + 50 ug of garlic and 100mg cumin aqueous extract
T3	2% *L. acidophilus* + *2% Bfido. bifidum* +50 ug of garlic and 100 ug cumin aqueous extract
T4	2% *L. acidophilus* + 100 ug of garlic and 100mg cumin aqueous extract
T5	2% *Bfido.bifidum*+100 ug of garlic and 100 ug cumin aqueous extract
T6	2% *L. acidophilus*+ *2% Bfido. bifidum* +100 ug of garlic and 100 ug cumin aqueous extract

Table 2: phytochemical screening of methanol extracts of cumin and garlic

Test	Cumin (Cuminum cyminum L)	Garlic (Allium sativum)
Sterol and/or terpenes	+	+
Carbohydrates and/or glycosides	+	+
Reducing sugar	—	+
Alkaloids	—	—
Saponins	—	+
Phenolic	+	+
Tannins	—	+
Flavonoides	+	+
Coumarins	—	—
Amino acid and Protein	—	+
Anthraquinone Glycosides	+	+
Hydroxyanthraquinone	—	—
Quinones	—	—
Cardiac glycosides	—	+
Fixed oils	+	+
Volatile oils	+	+

(+) and (-) refers to present and absent of certain compounds, respectively.

Synergistic effects of cumin, garlic and microorganisms:

1- Against food borne pathogenic microorganisms:

Results, as shown in Figure (1), indicated the highest inhibition of cumin alcoholic (methanol) extract was shown against *S. aureus*, which is the major pathogen resistant toward most antibiotics [42] Whereas the other extracts of cumin showed lesser extent of inhibition zones against *Salmonella, B. cereus, E. coli, Yersinia, Listeria, Pseudomonas and Streptococcus*. Additionally, cumin aqueous residue

properties [45,46,47]. Phytochemical constituents of

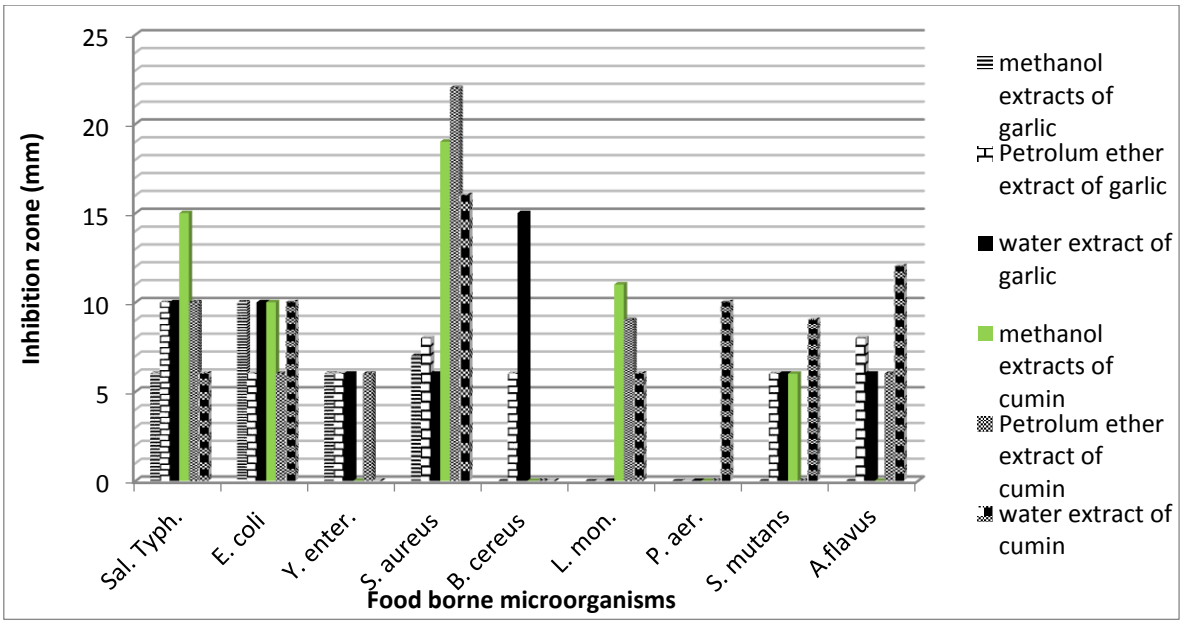

Figure (1) Effect of Garlic and Cumin extracts*, separately, in a concentration of 100ug/ml/each on Food borne microorganisms.

showed moderate inhibition zone against *Aspergillus flavus*.

Also, results in Figure (1) indicated that garlic extracts exhibited growth inhibition on *E. coli, Staphylococci, Salmonella,* while *E. coli* was sensitive to alcoholic (methanol) extract, and aqueous (water) residue extract. Petroleum ether extract exhibited moderately inhibition on the growth of *S. aureus* and *Aspergillus flavus,* respectively. However, garlic in aqueous (water) extracts showed the highest inhibition zone on *B. cereus.*

The present study suggests that cumin extracts exhibit higher activity against *S. aureus.* Also, very active against *salmonella* whereas, aqueous residue was pronounced on *Aspergillus* garlic extracts, on the other hand than, garlic aqueous residue showed the highest inhibition of *B. cereus* more than of aqueous residue cumin. The present study clearly shows that cumin extracts had marked positive effect on the growth of some of the bacteria and fungi tested, also garlic extracts have antimicrobial properties, and inactive against fungi. The current data were in consistent with [43]. The similar inhibitory effect on the growth of Gram positive bacteria and *E. coli* (Gram negative) has been reported by [44]. Also**,** Garlic and cumin are aromatic plants and characterized by volatile oil which have antimicrobial garlic and cuminas polyphenols, terpenoids, and alkaloids these components may play an important role as responsible for antimicrobial activities and inhibitors for pathogenic microbes or conversely, as stimulants prebiotic for probiotic bacteria if used as in karish cheese in the present studies [48, 49].

2-Incombination with B. bifidum and Lactobacillus acidophilus, against food borne microorganisms.

Data in Figure (3) illustrates the antimicrobial effects (inhibition zones mm) of garlic and cumin extracts in combinations with LAB (*B. bifidum* and *L.* acidophilus) against food borne microorganisms. Results reveal that adding *L. acidophilus* and *B. bifidum* to the cumin extract gave the highest effect against *Salmonella, E. coli, Y. enterocolitica and pseudomonas,* and less effective in inhibiting against *S. aureus, Streptococcus mutans, L. monocytogenes and B. cereus,* respectively. the lesser degree with similar trend results reveal that adding *L. acidophilus* and *B. bifidum* to the garlic extract gave the highest effect on the G-ve bacteria; *as Salmonella, E. coli, Y. enterocolitica and pseudomonas* and lesser degree on the G+ ve bacteria, such as *S. aureus, Streptococcus, Listeria spp* and *B. cereus,* respectively. Therefore, it can be observed that adding *B. bifidum* and *L. acidophilus* to cumin and garlic extracts increased their antibacterial effect on the pathogenic bacteria, especially G-ve, and to a lesser extent, on the G+ve. The mechanisms of action of the natural antimicrobials, as in cumin and garlic, include the rupture of the cell membrane, affect the nucleic acids

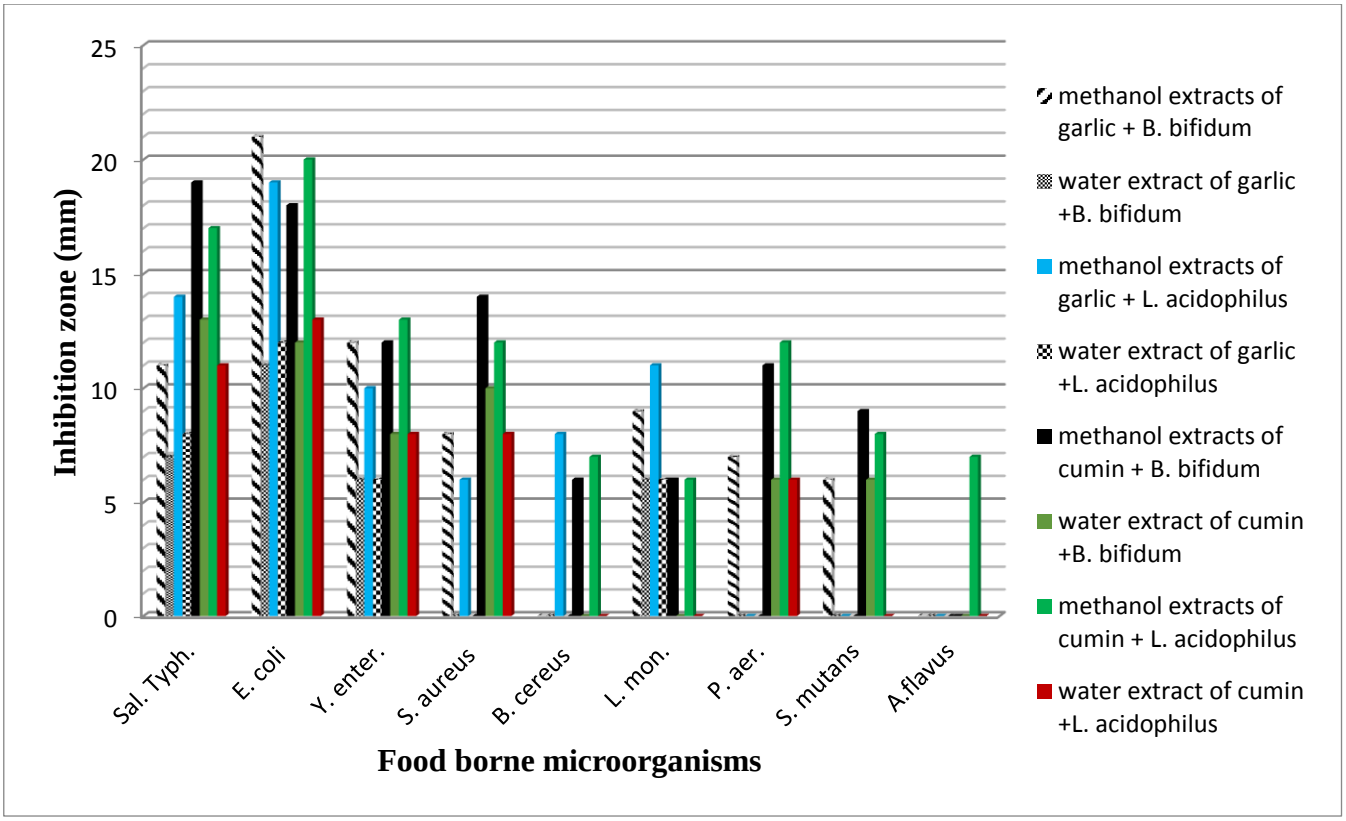

Figure (2) synergetic effect of garlic and cumin extracts in combinations with *B. bifidum and L. acidophilus* against foodborne microorganisms.

mechanisms, decay of the proton motive force and depletion of adenosine triphosphate (ATP) [50]. Also, Polyphenols and essential oils, as in cumin and garlic, with metabolites from LAB, as *L. acidophilus* and *B. bifidum*, had an action that plays an important role in the antimicrobial activity against food borne pathogenic bacteria [51]. Furthermore, synergism approaches of using natural antimicrobial compounds from plants and bacteria may be more useful for controlling foodborne bacterial pathogens and /or prolonging the shelf-life of food [52].

Chemical analysis of Garlic-Cumin karish cheese:
Chemical composition of the fresh karish Cheese with different treatments of garlic and cumin aqueous extractions as shown in Table (3) revealed that the moisture contents (78.88 to 78.11%), total solids (21.89 to 21.12 %), Protein (14.97 to 14.42) and ash (2.27 to 2.20), showed no significant differences between the treatments and the current results agree with [53; 54, 55, 56]. During cold storage of the cheese as shown in Table (4) illustrates that total solid (TS) of the cheese slightly increased during storage up to 15 days, especially T3 (22.88%), and this is might be due to the decrease in moisture contents and acidity development. These results were in agreed with the result obtained by [57].
In the fresh cheese, pH values were slightly decreased (5.49 to 5.8) after 7 and 15 days of cold storage for all the treatments, and this is due to the activity of the starter culture and the increase of TS. The same results obtained by the studies by [58, 59]. From the above, it can be concluded that the activity of starter bacteria are not discouraged or suppressed by adding garlic and cumin aqueous extractions to the cheese, which is consistent with [53,54]. On the other hand, the diacetyl content in the cheese was higher in all treatments (T1-T6) than control (C1, C2), this might be due to activity of the starter culture, probiotic bacteria and/or plant additives [60]. Additionally, the high content of acetaldehyde in different treatments of the cheese comparing to the control was recorded, which decreased with increasing storage time, that might be due to the starter bacterial cultures which may reduce acetaldehyde to ethanol [61, 62 and 63].

Table (3): Major chemical composition of fresh karish Cheese with different treatments*

Treatments	Moisture %	Total solids %	Protein %	Ash %
C1	78.11^{G}	21.89^{A}	14.61^{CD}	2.20^{D}
C2	78.42^{D}	21.58^{D}	14.97^{A}	2.22^{C}
T1	78.47^{C}	21.53^{E}	14.42^{E}	2.26^{AB}
T2	78.88^{A}	21.12^{G}	14.59^{BC}	2.25^{B}
T3	78.12^{G}	21.88^{A}	14.93^{A}	2.27^{A}
T4	78.35^{E}	21.65^{C}	14.85^{AB}	2.25^{B}
T5	78.18^{F}	21.82^{B}	14.47^{DE}	2.20^{D}
T6	78.74^{B}	21.26^{F}	14.50^{DE}	2.27^{A}
*(see Table 1)				

Microbiological analysis of Garlic-Cumin karish cheese:

Results as shown in Table 5 reveal the microbial examination of karish cheese made with different treatments of *L. acidophilus and B. bifidum* flavored with cumin and garlic, during refrigerated storage. The microbiological results showed a slight increase in the number of *Lactococcus lactis* over *Bifidobacterium bifidum* and *L. acidophilus.* Furthermore, it was observed that bacterial addition of garlic and cumin extracts to karish cheese did not show a significantly microbial effect on the total bacterial count or any of these bacteria separately, during the period of cheese storage. On the other hand, molds and yeasts were not detected in all treatments of karish cheese untie the 21st of storage, while it appeared in the controls, C1 and C2, after 15 days, indicating the efficacy of these additives for elongating the cheese shelf life. These findings are in agreement with reports by [8, 64, 65] for the antifungal effect of cumin extracts in cheese. Also, the results agree with the use of garlic and cumin in dairy products [8].

Table (4): Phys-chemical properties of karish Cheese with different treatments* during cold storage (days)

Treatments	Total solids %			pH			Diacetyl (µm/100g)			Acetaldehyde (µm/100g		
	Cold storage days											
	0	7	15	0	7	15	0	7	15	0	7	15
C1	35.7	40.20	45.38	4.77	4.89	5.70	294.0	162.0	90.0	22.49	21.92	21.89
C2	45.84	57.72	59.52	4.71	4.86	5.71	237.6	180.0	134.4	22.58	21.80	21.58
T1	48.36	59.40	61.32	4.73	4.82	5.73	255.6	210.0	147.6	22.53	21.74	21.53
T2	49.72	59.52	69.36	4.76	4.79	5.69	313.2	220.8	165.5	22.82	21.53	21.12
T3	50.46	65.88	68.40	4.63	4.71	5.49	374.4	241.2	199.2	22.88	21.96	21.88
T4	60.0	69.60	73.56	4.76	4.75	5.73	314.4	279.6	187.2	22.65	21.99	21.65
T5	61.8	68.64	74.04	4.89	5.04	5.81	423.6	297.6	186.0	22.82	21.92	21.82
T6	67.02	74.04	80.52	4.72	4.90	5.81	435.6	301.2	213.6	22.26	21.34	21.26

Table5: Microbial examination of karish soft cheese with different treatments** of cumin and garlic during refrigerated storage (log counts cfu/g) *

treatments	Total bacterial count				L. lactis sub spp. lactis				Bifid bacterium bifidum				L. acidophilus			
	Storage time / days															
	0	7	15	21	0	7	15	21	0	7	15	21	0	7	15	21
C1	7.25	7.8	7.9	7.3	8.1	8.32	8.45	7.4	7.12	7.5	7.0	6.51	7.25	7.33	7.31	7.12
C2	7.21	7.28	7.31	7.26	8.0	8.41	8.52	7.41	7.1	7.32	7.6	6.41	7.30	7.38	7.32	7.26
T1	7.10	7.16	7.23	7.15	8.12	8.32	8.41	7.23	7.13	7.4	7.49	6.81	7.28	7.41	7.50	7.11
T2	7.21	7.25	7.52	7.3	8.21	8.33	8.29	7.6	7.19	7.74	7.81	6.91	7.11	7.25	7.52	7.31
T3	7..9	8.1	8.0	7.77	8.01	8.21	8.32	7.6	7.22	7.51	7.62	7.1	7.19	7.8	8.0	7.45
T4	8.0	8.12	7.98	7.85	8.14	8.29	7.9	7.3	7.25	7.8	7.62	6.8	7.21	7.42	7.36	7.25
T5	7.9	7.98	8.0	7.8	8.13	8.5	8.1	7.8	7.19	7.8	7.71	7.3	7.31	7.81	8.1	7.78
T6	8.23	8.45	7.9	7.68	8.16	8.41	8.3	7.77	7.32	7.6	7.55	7.17	7.32	7.4	7.55	7.45

*molds and yeasts were not detected in all treatments untie the 21st of storage in only C1 and C2.

Table (6): Sensory evaluation of karish cheese with different treatments * during cold storage (days)

Treatments	Appearance (10 points)			Body & texture (30 points)			Flavor (60 points)			Total scores (100 point)		
	Storage time / days											
	0	7	15	0	7	15	0	7	15	0	7	15
C1	6.0^{Db}	7.2^{Ba}	$7.5^{Da}\pm0.091$	24.0^{Ec}	25.3^{Cb}	$26.5^{Ga}\pm0.12$	40.0^{Db}	40.6^{Gab}	$41.3^{Ha}\pm0.34$	70.0^{Dc}	73.1^{Eb}	$75.3^{Ga}\pm0.12$
C2	6.2^{Db}	7.5^{Ba}	$7.4^{Da}\pm0.21$	24.0^{Ec}	25.5^{Cb}	$26.8^{Fa}\pm0.032$	40.3^{Da}	40.9^{Fb}	$41.7^{Ga}\pm0.059$	70.5^{Dc}	73.9^{Eb}	$75.9^{Fa}\pm0.077$
T1	7.0^{Cc}	8.0^{Ab}	$8.3^{Ca}\pm0.022$	25.1^{Dc}	26.6^{Bb}	$27.4^{Ea}\pm0.041$	41.2^{Cc}	42.4^{Eb}	$43.5^{Fa}\pm0.032$	73.3^{Cc}	77.0^{Db}	$79.2^{Ea}\pm0.036$
T2	7.0^{Cc}	8.0^{Ab}	$8.3^{Ca}\pm0.044$	25.3^{DCc}	26.8^{Bb}	$27.6^{Da}\pm0.057$	41.4^{Cc}	42.7^{Db}	$43.7^{Ea}\pm0.066$	73.7^{Cc}	77.5^{DCb}	$79.6^{Da}\pm0.047$
T3	7.5^{Bb}	8.3^{Aa}	$8.5^{Ba}\pm0.11$	25.7^{BCDb}	27.0^{Aa}	$27.9^{Ba}\pm0.077$	42.7^{Bc}	43.4^{Bb}	$45.2^{Da}\pm0.005$	75.9^{Bc}	78.7^{Bb}	$81.6^{Ba}\pm0.051$
T4	7.0^{Cb}	8.0^{Aa}	$8.2^{Ca}\pm0.058$	26.2^{ABCc}	26.5^{Bb}	$27.7^{CDa}\pm0.04$	42.9^{Bc}	43.1^{Cb}	$45.4^{Ca}\pm0.09$	76.1^{Bc}	77.6^{Cb}	$81.3^{Ca}\pm0.07$
T5	7.0^{Cb}	8.0^{Aa}	$8.2^{Ca}\pm0.049$	26.4^{ABc}	26.7^{Bb}	$27.8^{BCa}\pm0.011$	43.1^{Bb}	43.3^{Bb}	$45.7^{Ba}\pm0.067$	76.5^{Bc}	78.0^{BCb}	$81.7^{Ba}\pm0.05$
T6	7.8^{Ac}	8.4^{Ab}	$8.7^{Aa}\pm0.057$	26.8^{Ac}	27.5^{Ab}	$28.2^{Aa}\pm0.006$	44.3^{Ac}	46.6^{Ab}	$47.3^{Aa}\pm0.058$	78.9^{Ac}	82.5^{Ab}	$84.3^{Aa}\pm0.058$

* (see table,1)

Sensory evaluation of acid curd cheese with different treatments:

Results in Tables 1 and 5 show the sensory evaluation of the karish cheese made with garlic & cumin extracts in combination with LAB starter culture and probiotic bacteria, in six treatments and two controls. All the treatments of the karish cheese were appreciated by the 10 panelists for sensory evaluation (average score), as for appearance (8.2-8.7), body & texture (27.4-28.2), flavor (43.5-47.3) and total scores (79.2-84.3), over the control, after 15 days of storage. The highest acceptability was noted with T6, (contained *L. acidophilus, Bifidobacterium bifidum* and equal ratio of garlic & cumin extracts), when fresh and during the storage period. It was also noticed an increase in scores of all sensory evaluation factors by increasing the storage period (15 days). This improvement in sensory properties may be due to the increased production of volatile fatty acids that are produced by probiotic and LAB bacteria during storage [53, 4]. Also, the results obtained agreed with [63, 6, and 66]. Additionally, there are several recommendations to use these additives to cheese and foods to improve its healthy nutritional, sensory quality and as natural preservatives [4, 20, 67].

Conclusions

The Produced karish cheese made from cumin and garlic extracts in combination with *Lactococcus lactis*, *L. acidophilus* and *Bifidobacterium bifidum* was of a high degree of nutritional, healthy, microbial and sensory quality and could be considered as a new functional dairy product, due to its contents of phytochemicals and the probiotic bacteria.

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