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EFFECT OF DOUM PALM FRUIT (*HYPHAENE THEBAICA*) ON CERTAIN DAIRY STARTER CULTURES AND UNDESIRABLE MICROORGANISMS

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ABSTRACT

Aqueous extract of the doum palm fruits (*Hyphaene thebaica*) was screened for antimicrobial activity against some pathogenic and food spoilage bacteria and fungi. Also, the invigorating effect on viability and activity of some dairy probiotic cultures was examined. Antimicrobial activity gradually increased with increasing the amount of the aqueous doum palm extract. Also, there was a remarkable change (plus or minus) of heat treatment and autoclaving on the antimicrobial effect of aqueous doum palm extract. Presence of 1% of aqueous doum palm extract clearly revealed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* O157:H7. While, *Bacillus* and *Pseudomonas strains* found to be least susceptible up to 3% aqueous doum palm extracts. The highest antifungal activity of all aqueous doum palm extract was against *Asperagillus niger*, *Asp. fumigatus* followed by *Penicillium expansum*. However, *Fusarium moniliforum*, *Fus. oxysporium* and *Penicillium roqueforti* showed some more resistance for the presence of the aqueous doum extract. The use of 3% and 5% of crude or heated aqueous doum palm extracts gave the highest invigorating effect on the starter cultures. Then, mesophilic starter strain *Bifidobacterium longum* showed the highest response as compared with the other starter strains, up to 5% doum extracts on the contrary, the use of 8% of aqueous doum palm extracts caused a slight decrease in viability of starter cultures as compared with the other treatments. It could be concluded that, heat treated and autoclaved aqueous doum palm extracts had remarkable antibacterial and antifungal activities against the tested undesirable microorganisms. This would enhance the safety and increase the shelf life of some fermented dairy products.

Key words: Antibacterial activity, Aqueous extract of doum palm, Pathogenic, Spoilage bacteria, Antifungal, *Lactobacillus delbreuckii* ss. *bulgaricus*, *Str. thermophilus*, *Bifidobacteria*, *Lactococci*, and *Lactobacilli*.

INTRODUCTION

Incidences of foodborne illnesses are still a major problem, even in developed countries. It has been estimated that 6-81 million cases of illnesses and up to 9000 deaths annually were attributed to food borne pathogens in the USA alone **Mead**

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et al (1999). *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter jejuni* were the main pathogens incriminated in poisoning cases **Mead *et al* (1999).** In fact, food poisoning is still a threat for both consumers and the food industry despite the use of preserving processes. Meanwhile, consumers are concerned about the safety of food containing preservatives. Therefore, there has been growing interest in new and effective techniques to reduce case of food borne illnesses. Antimicrobial substances from natural sources like plants have been investigated to achieve higher levels of food safety. Also, yeasts and moulds play an important role in the spoilage of dairy products especially fermented milks and cheeses **(Welthagen & Viljoen, 1998 and Jackobsen & Narvhus, 1996)**.

Hyphaene thebaica Mart, a member of the Palmate, is known to grow extensively in the drier parts of Africa extending to Middle East. The plant produces ovoid fruits (3-lobed and measuring 3 inches long) which do mature in the month of March and may be dispersed thereafter or may persist on the fruit stalk until the appearance of the next season's flowers.

In the past 50 years, there has been a gradual increase in the consumption of fermented milks and yoghurt allover the world. This growth can be attributed to the addition of some additives and the dramatic increase in addition of probiotic bacteria. Production of functional and probiotic fermented milks had probiotic actions has been raising steadily, partially because of its good nutritional value and healthy effects. In response these growing demands for health-promoting cultures or food, these have been attracted the attention of microbiologist, nutritionist, medical world, food manufacturer and consumers **Kailasapathy and Rybka (1997).**

Gibson and Wang (1994) defined prebiotics as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacteria in colon that can improve the host health. While, "synbiotic" designates the synergistic combination of pre- and probiotics, a concept which looks most promising but still remains in its infancy. Functional foods referred to foods containing non-digestible ingredients that beneficially affect the host health.

The current terminology "probiotics" is used to describe foods that are produced by or contain live microorganisms that possess therapeutic or health benefits **Farnsworth, (2001).** The most recent consensus requires that probiotics are live and capable of surviving passage through the digestive tract and have the capability to proliferate in the gut **FAO/WHO, (2001)**, where they have been redefined as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host". *Lactobacillus* and *Bifidobacterium* are the principal bacterial genera centered to both probiotics and prebiotics (nondigestible food ingredients that stimulate the growth or activity of certain colonic bacteria) approaches to dietary modulation of the intestinal microflora **Schrezenmeir and De Vrese (2001).**

The main objectives of the present study was to evaluate the antibacterial and antifungal activities of aqueous doum palm extracts, and determine the effect of aqueous doum palm extracts on certain dairy starter cultures which used in the manufacture of some dairy products especially probiotics.

MATERIAL AND METHODS

Materials

Preparation of Doum powder

The Doum palm (*Hyphaene thebaica mart.*) samples were prepared in a powder form by cleaning and milling in a blender laboratory mill type (Broun, Germany). A 100g Doum sample was placed on the top of a graded set of sieve of 0.1 mm. The stake of sieve with the attached collecting pan was mechanically shaken by vibrator (Veb Mlw laboratechnik II. Menau Mlw, Germany) until the weight of the material on the smallest screen (0.1mm) had reached equilibrium. When sieving was completed, the residual material of the sieve was weighed **(Phillips *et al* 1988)**.

Doum palm powder contained 13.67% crude fibers (digestible and non-digestible), carbohydrates 67.64%, protein 2.9% and Ash 6.1%.

Dairy starter cultures

A commercial freeze-dried yogurt starter culture (*Lactobacillus delbreuckii ss. bulgaricus, Str. thermophilus)* mesophilic starter culture (*Lactococcus lactis ss. lactis* and *Lactococcus lactis ss. cremoris*) containing *Bifidobacterium longum*, and AB starter culture (*Lactobacillus acidophilus* and *Bifidobacterium longum*) were obtained from Wiesby GmbH & Co. KG. Niebull, Germany. Also, *Bifidobacterium longum* ATCC 15707 and *Lactobacillus rhamnosus* DSM 20245 were obtained from the Egyptian Microbial Culture Collection [EMCC] at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Spoilage and pathogenic strains

Seven indicator bacterial strains were obtained from the Egyptian EMCC. These strains are: *Escherichia coli 0157*:H7 ATCC 35150, *Staphylococcus aureus* ATCC 13565, *Micrococcus luteus* ATCC 9341, two strains of *Bacillus* (*Bacillus subtilis* DSM 1088, *Bacillus cereus* ATCC 14579) and two strains of *Pseudomonas* (*Pseudomonas aeruginosa* ATCC 9027 and *Ps. flourescens* DSM 50090)

Mould or Fungi strains

Six food spoilage fungi were used for this study. Two strains belonging to the genus *Aspergillus* (*Aspergillus niger* DSM 737, and *Aspergillus fumigatus* DSM 819), two strains belonging to the genus *Fusarium* (*Fus. moniliforum* DSM 764 and *Fus. oxysporium* DSM 358), two strains of *Penicillium* (*Penicillium roqueforti* DSM 1079 and *Penicillium expansum* ATCC 28877) were used to study antifungal activity of aqueous doum palm fruit extract.

Experimental Procedures

Doum palm extracts

Crude doum palms powder was mixed with cold water with gentle agitation for 15 min then filtered by whatmann filter paper No.1 and used as crude aqueous doum palms extract (CCD) material. Heated water soluble doum palm extract (HCD) was prepared by dissolving crude doum palm powder into cold water with gentle agitation for 15 min and heated at 85°C for 15 min, then cooled and filtered by whatmann filter paper No.1.

Autoclaved water soluble doum palm extract (ACD) was prepared by dissolving crude doum palm powder into cold water with gentle agitation for 15 min and autoclaved at 121°C for 15 min then filtered by whatmann filter paper No.1.

Antibacterial activity

The disc diffusion method **Barry and Thornsberry (1985)** was used for detecting the antibacterial effect of doum palm and extracts on certain spoilage and pathogenic bacteria. 0.1 ml of activated test microorganisms were inoculated and spread on the surface of the media. A sterile disc filter paper was dipped in the appropriate CCD, HCD, and ACD extracts, and then placed onto the surface of inoculated plates. The inhibitory effect of the doum palm extracts were also tested by placing disc saturated with every extract in each inoculated plate. Within 15 min after the plates were inoculated, the blank disks were applied to the surface of the inoculated plates with a sterile forceps. All disks were gently pressed down onto the agar with forceps to insure complete contact with the agar surface. The plates were incubated at 32± 2°C for 24 h. The results were recorded by measuring the diameter of the inhibition zone (mm) around the discs. All tests conducted in triplicates with three discs per plate.

Antifungal activity

Oxytetracycline glucose yeast extract agar medium (Oxoid) was poured into plates to give a uniform depth of about 4-mm. The plates were allowed to cool to room temperature. Fungal spores on the potato dextrose agar slant were washed out with sterile saline containing 0.1% Tween 80 by brushing the surface of the slant with a sterile loop. Each suspension contained 10^6 cfu /ml was used directly as inoculums. After streaking the plates, they were allowed to dry for 20 minutes and incubated at 28°C for 24 to 36 h for mycelium growth **Barry and Thornsberry, (1985)**. A sterile standard Cork poorer was used to make disc from every fungus. Discs were applied onto the surface of plates containing the medium inoculated with CCD, HCD, and ACD in concentrations of 1, 3, 5 and 8% for every extract. Plates were incubated at 28°C. After 24, 48 and 72 h, and examined by measuring the diameters of the inhibition zones in millimeters.

Invigorating effect

Skim milk medium (Spray dried skim milk [VARIMEX Poland]) was prepared according to **Harrigan (1998)**. Skim milk medium were reconstituted to 10 % total solids with distilled water and sterilized at 121°C for 10 min, subsequently cooled to the incubation temperature. Doum palm extracts were added to obtain different concentrations (0, 1, 3, 5 and 8% v/v). Then Skim milk medium was inoculated at level of 1% (1:1) with different starter cultures. The incubation temperature was 32°C for mesophilic starter culture containing *Bifidobacterium longum*, and 37°C for yogurt, AB starter cultures and probioitic starter culture containing *Bifidobacterium longum* and *Lactobacillus rhamnosus*). Viable lactic acid bacterial count in MRS broth and on agar and M17 agar (Oxoide) was detected and titratable acidity was determined at zero time and after 2, 4 and 6 h of incubation

for each starter culture till the pH value reached 4.8.(**A. O. A. C., 1990).**

Microbiological examinations

Bifidobacteria count was enumerated according to **Dave and Shah (1996)** using modified MRS agar supplemented with 0.05% L-cystein and 0.3% lithium chloride. The plates were anaerobically incubated (using anaerobic jars and gas Pack BBL) at 37 °C for 48h. Lactobacilli count was determined using MRS agar according to **De Man *et al* (1960).** The plates were incubated at 37 °C for 48h. *Lactobacillus acidophilus* count was determined using modified MRS agar supplemented with 0.2% oxagal according to **Gilliland and Walker (1990).** The plates were incubated at 37 °C for 48h. Mesophilic starter culture (Lactococci) counts were determined using M17 agar **(Terzaghi and Sandine, 1975).** The plates were incubated at 30°C for 48h.

Statistical analysis

The general linear models procedure of SAS (Statistical Analysis System User's Guide SAS (1994) (SAS Institute, Inc, U.S.A.) was used for analyzing the data. Separation among means ($p< 0.05$ and $p< 0.01$) was carried out by using Duncan multiple range tests.

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial effect of crude doum palm (CCD), heated water soluble doum palm extract (HCD), and autoclaved water soluble doum palm extract (ACD) on certain pathogenic and dairy spoilage bacteria are presented in Table (1) and illustrated in Fig (1). The inhibition zone diameters (mm) of all tested strains gradually increased with increasing of the concentration of doum extract. Also, there was remarkable effect of heat treatment and autoclaving doum extract on the antibacterial activity against all tested pathogenic and spoilage strains. Autoclaving caused a slight increase in antibacterial activity against all tested strains. The lowest antibacterial effect was recorded against *B. subtiles* and *B. cereus*.

On the other hand, the highest effect was detected against *Staphylococcus aureus* and *Escherichia coli* O157:H7. Heated water soluble doum palm extract (HCD), and autoclaved water soluble doum palm extract (ACD) resulted in antibacterial activity slightly higher than Crude extract. This increase might be due to the positive effect of heat treatment on doum palm extract to release (liberate) slightly higher some compounds of antibacterial activity. Aqueous doum palm extract resulted in antibacterial activity against *Pseudomonas flourescens* than *Pseudomonas aeruginosa*. Also, antibacterial activity against *B. cereus* was higher than *B. subtilus*. The antibacterial activity of aqueous doum palm extract was remarkable only with the use of soluble extracts and were not materialized in non soluble extracts (pilot experiments).

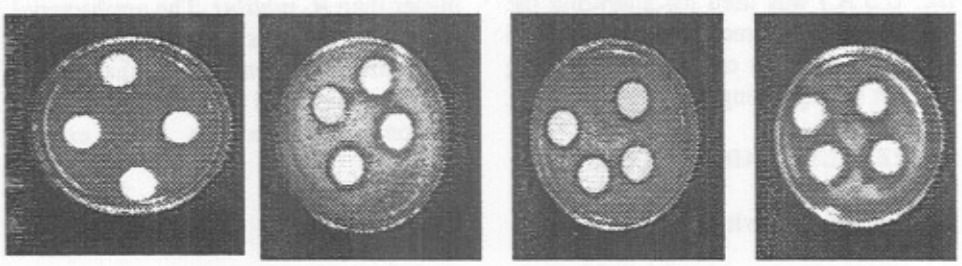
Antifungal effect

Table (2) and Fig. (2) illustrate the antifungal effect of aqueous doum palm extracts (Zone of inhibition as mm) on the viability of the most common undesirable fungi. Aqueous solutions of

Table 1. Antibacterial effect of aqueous doum palm extracts as a zone of inhibition, on the viability of some pathogenic and dairy spoilage bacteria.

Doum palm concentrations	pathogenic and dairy spoilage bacteria						
	Bacillus subtilis	*Bacillus cereus*	*Micrococcus luteus*	*E. coli* O157:H7	*Staph. aureus*	*Pseudomonas aeruginosa*	*Pseudomonas flourescens*
Crude extract	(zone of inhibition in mm)						
1%	*	*	*	6±0.16	7±0.13	*	*
3%	*	4±0.21	7±0.11	9±0.19	11±0.16	5±0.15	6±0.33
5%	5±0.2	6±0.28	8±0.13	11±0.22	14±0.21	8±0.21	11±0.32
8%	6±0.33	8±0.33	12±0.25	15±0.31	17±0.26	10±0.25	14±0.14
Heated extract							
1%	*	4±0.17	*	6±0.14	6±0.17	*	*
3%	*	6±0.29	7±0.14	8±0.17	8±0.19	*	4±0.17
5%	4±0.41	7±0.45	9±0.32	14±0.29	12±0.22	6±0.11	7±0.17
8%	6±0.28	8±0.55	11±0.44	18±0.42	16±0.29	8±0.14	10±0.22
Autoclaved extract							
1%	*	*	*	8±0.12	7±0.12	*	*
3%	*	7±0.33	6±0.21	11±0.18	9±0.11	5±0.16	7±0.13
5%	5±0.52	9±0.56	9±0.32	15±0.19	13±0.19	7±0.18	10±0.21
8%	7±0.35	13±0.46	13±0.41	17±0.36	18±0.33	9±0.32	11±0.36

* Inhibition zone is less than 4 mm.
Data represents Means of three replicates ± standard deviation



B. cereus *B. subtilis* *Pseudomonas aeruginosa* *E. coli* O157:H7

Fig. 1. Antibacterial activity of aqueous doum palm extracts on some pathogenic and dairy spoilage bacteria.

Table 2. Antifungal activity of aqueous doum palm extracts as a zone of inhibition, on the viability of some undesirable fungi.

Doum palm concentrations	Undesirable fungi					
	Asp. niger	*Asp. fumigatus*	*Fusarium moniliforum*	*Fusarium oxysporium*	*Pen. roqueforti*	*Penicillium expansum*
Crude extract	(zone of inhibition in mm)					
1%	*	*	*	*	*	*
3%	*	6±0.11	*	6±0.18	*	6±0.15
5%	7±0.31	8±0.17	5±0.16	7±0.24	7±0.19	9±0.27
8%	10±0.29	11±0.31	6±0.22	8±0.31	9±0.26	12±0.33
Heated extract						
1%	*	*	*	*	*	*
3%	*	6±0.12	*	*	*	5±0.15
5%	8±0.31	8±0.18	6±0.29	6±0.17	4±0.14	8±0.33
8%	10±0.29	10±0.11	6±0.35	7±0.24	7±0.29	10±0.39
Autoclaved extract						
1%	*	*	*	5±0.19	*	5±0.27
3%	5±0.12	7±0.17	6±0.11	7±0.33	*	8±0.32
5%	8±0.19	10±0.33	7±0.19	8±0.41	7±0.22	11±0.36
8%	12±0.26	12±0.29	9±0.22	10±0.38	9±0.27	13±0.41

* Inhibition zone is less than 5 mm.
Data represents Means of three replicates ± standard deviation

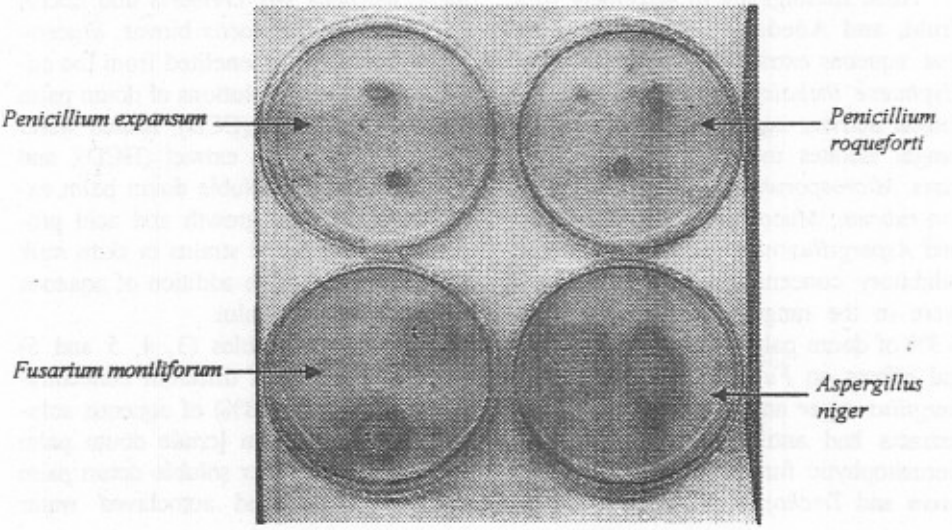


Fig. 2. Effect of different aqueous doum palm extracts concentrations on some spoilage fungi.

crude doum palm (CCD), heated water soluble doum palm extract (HCD), and autoclaved water soluble doum palm extract (ACD) had antifungal effect on the viability of all tested fungi. Also, antifungal activity of all aqueous solutions of doum palm gradually increased with the increase of aqueous solutions concentrations. Antifungal activity of heated aqueous solutions of doum palm was slightly lower than the aqueous solutions of autoclaved doum palm (CCD). While, aqueous solutions of autoclaved water soluble doum palm extract (ACD) resulted in slightly higher antifungal activity than the crude and heated aqueous solutions. Furthermore, the highest antifungal activity of all aqueous solutions of doum palm was against *Penicillium expansum*, *Aspergillus Fumigatus* and *Aspergillus niger* followed by *Fusarium oxysporium*. Crude and heated aqueous doum palm extract showed the least antifungal activity against *Fusarium moniliforum*, and *Pen. roqueforti*. The mechanism of antifungal action of the aqueous doum palm fruit extracts is not clear.

These findings are in agreement with **Irobi, and Adedayo (1999)** who found that, aqueous extract of dormant fruits of *Hyphaene thebaica* had significant antifungal activity against a wide range of fungal isolates including *Candida albicans*, *Microsporum gypseum*, *Trichophyton rubrum*, *Mucor* sp., *Fusarium solani* and *Aspergillus niger* and the minimum inhibitory concentrations of the extract were in the range 3.1-25% v/v.. Also, 6.3% of doum palm extracts had antifungal effects on *Fusarium solani* and *Aspergillus niger* and 3.1% of doum palm extracts had antifungal effects on The dermatophytic fungi (*Microsporum gypseum* and *Trichophyton rubrum*). While, 25% of doum palm extracts had effects on *Mucor* sp. and *Candida albicans*

It could be concluded that the aqueous doum palm fruit extracts proved to have significant antibacterial and antifungal activities against some undesirable (pathogenic and dairy spoilage) bacteria and fungi.

Invigorating effect

The determination of the best conditions for the preparation of a probiotic starter culture (composed solely of *Bifidobacterium longum* and *Lactobacillus acidophilus* or *Lactobacillus rhamnosus* or mesophilic starter cultures) which might be suitable for making fermented milk is critical if a consistently reliable acid production and the highest viability are to be achieved, especially because bifidobacteria have strict requirements for growth. Therefore, we determined whether *Bifidobacterium longum* and *Lactobacillus acidophilus* or *Lactobacillus rhamnosus* or Mesophilic starter cultures (*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*) required or benefited from the addition of aqueous solutions of doum palm [crude doum palm (CCD), heated water soluble doum palm extract (HCD), and autoclaved water soluble doum palm extract (ACD)] The growth and acid production of all tested strains in skim milk were affected by the addition of aqueous solutions of doum palm.

The data in Tables (3, 4, 5 and 6) show the effect of different concentrations (1, 3, 5, and 8%) of aqueous solutions of doum palm [crude doum palm (CCD), heated water soluble doum palm extract (HCD), and autoclaved water

Table 3. Effect of aqueous doum palm extracts on viability and activity of yoghurt starter culture along incubation period (up to 6 h).

Doum palm concentration	*Streptococcus* count				*Lactobacillus* count				Titratable acidity			
	0h	2h	4h	6h	0h	2h	4h	6h	0h	2h	4h	6h
Crude extract	(Log cfu/ml)								% Lactic acid			
Control	6.63^{a}	7.90^{a}	8.22^{b}	9.07^{bb}	7.10^{b}	8.60^{b}	9.07^{b}	8.60^{a}	0.16	0.45	0.68	0.87
1%	6.60^{ab}	7.80^{b}	8.29^{a}	8.82^{d}	7.10^{b}	8.61^{b}	8.82^{d}	8.61^{a}	0.17	0.43	0.69	0.85
3%	6.62^{a}	7.40^{d}	8.14^{c}	8.80^{d}	7.14^{b}	8.38^{d}	8.80^{d}	8.38^{c}	0.18	0.41	0.67	0.84
5%	6.54^{b}	7.26^{e}	8.08^{c}	8.71^{e}	7.04^{c}	8.24^{e}	8.71^{e}	8.24^{d}	0.19	0.38	0.63	0.82
8%	6.51^{b}	6.97^{e}	7.92^{d}	8.45^{f}	6.88^{d}	8.13^{f}	8.45^{f}	8.13^{e}	0.2	0.38	0.60	0.80
Heated extract												
1%	6.66^{a}	7.80^{b}	8.20^{b}	8.80^{d}	7.10^{b}	8.52^{d}	8.80^{d}	8.52^{b}	0.17	0.38	0.65	0.88
3%	6.61^{b}	7.49^{c}	8.14^{c}	8.83^{cd}	7.14^{b}	8.38^{d}	8.83^{d}	8.38^{c}	0.18	0.41	0.62	0.85
5%	6.64^{a}	7.32^{d}	8.24^{ab}	8.76^{e}	7.11^{b}	8.30^{de}	8.76^{de}	8.30^{cd}	0.19	0.42	0.61	0.82
8%	6.54^{b}	7.20^{e}	8.20^{bc}	8.59^{f}	6.95^{d}	8.02^{f}	8.59^{f}	8.02^{f}	0.2	0.43	0.59	0.81
Autoclaved extract												
1%	6.50^{b}	7.40^{d}	8.30^{a}	9.35^{a}	7.30^{a}	8.67^{a}	9.35^{a}	8.67^{a}	0.17	0.36	0.67	0.84
3%	6.31^{c}	7.52^{c}	8.22^{b}	9.11^{b}	7.34^{a}	8.50^{b}	9.11^{b}	8.50^{b}	0.18	0.38	0.63	0.81
5%	6.28^{c}	7.40^{d}	8.17^{c}	8.90^{c}	7.20^{ab}	8.31^{c}	8.90^{c}	8.31^{cd}	0.19	0.40	0.61	0.79
8%	6.25^{c}	7.15^{e}	8.05^{c}	8.75^{e}	6.99^{cd}	8.12^{e}	8.75^{e}	8.12^{e}	0.21	0.41	0.60	0.79

^{A,b,c,d,e} Means values in the same column within treatment bearing different superscription are not significantly ($P < 0.05$). different

Table 4. Effect of aqueous doum palm extracts on viability and activity of AB starter culture along incubation period (up to 6h).

Doum palm	*Bifidobacterium* count				*Lactobacillus acidophilus* count				Titratable acidity			
concentration	0h	2h	4h	6h	0h	2h	4h	6h	0h	2h	4h	6h
Crude extract	(Log cfu/ml)								% Lactic acid			
Control	6.03^{c}	6.4^{d}	7.0^{d}	7.85^{c}	6.0^{c}	6.48^{c}	7.24^{c}	7.89^{d}	0.16	0.34	0.50	0.72
1%	6.0^{c}	6.52^{c}	7.15^{c}	7.99^{b}	6.1^{bc}	6.64^{b}	7.43^{b}	8.3^{a}	0.16	0.36	0.53	0.74
3%	6.09^{b}	6.6^{b}	7.3^{d}	8.1^{b}	6.13^{b}	6.43^{c}	7.30^{c}	8.11^{c}	0.16	0.36	0.54	0.72
5%	6.1^{b}	6.43^{d}	6.9^{b}	7.71^{d}	6.02^{c}	6.4^{c}	7.17^{d}	8.02^{c}	0.17	0.38	0.54	0.75
8%	6.13^{b}	6.38^{d}	6.77^{e}	7.52^{d}	6.06^{c}	6.35^{d}	6.85^{e}	7.71^{d}	0.18	0.36	0.52	0.73
Heated extract												
1%	6.2^{a}	6.6^{b}	7.05^{cd}	7.8^{c}	6.21^{a}	6.8^{a}	7.40^{b}	8.10^{b}	0.15	0.33	0.61	0.72
3%	6.21^{a}	6.74^{ab}	7.1^{c}	8.0^{b}	6.25^{a}	6.65^{b}	7.54^{a}	8.32^{a}	0.16	0.35	0.62	0.74
5%	6.28^{a}	6.51^{c}	6.98^{d}	7.67^{d}	6.14^{b}	6.42^{c}	7.28^{c}	8.13^{b}	0.17	0.33	0.61	0.71
8%	6.15^{b}	6.4^{d}	6.84^{de}	7.56^{d}	6.1^{c}	6.3^{d}	7.0^{de}	7.90^{c}	0.19	0.32	0.59	0.70
Autoclaved extract												
1%	6.1^{b}	6.7^{ab}	7.3^{b}	8.06^{b}	6.15^{b}	6.78^{a}	7.56^{a}	8.16^{b}	0.16	0.36	0.55	0.74
3%	6.0^{c}	6.8^{a}	7.45^{a}	8.25^{a}	6.1^{bc}	6.7^{b}	7.6^{a}	8.25^{ab}	0.16	0.37	0.57	0.73
5%	6.03^{c}	6.52^{c}	7.1^{c}	8.0^{b}	6.2^{a}	6.6^{b}	7.41^{b}	8.09^{c}	0.17	0.35	0.53	0.71
8%	6.0^{c}	6.35^{d}	6.78^{e}	7.7^{d}	6.0^{c}	6.4^{c}	7.01^{e}	7.93^{c}	0.18	0.35	0.51	0.70

^{A,b,c,d,e} Means values in the same column within treatment bearing different superscription are not significantly ($p<0.05$) different

Table 5. Effect of aqueous doum palm extracts on viability and activity of (BR) starter culture (*Bifidobacterium* and *Lactobacillus rhamnosus*) along incubation period (up to 6h).

Doum palm concentration	*Bifidobacterium* count				*Lactobacillus* count				Titratable acidity			
	0h	2h	4h	6h	0h	2h	4h	6h	0h	2h	4h	6h
Crud extract	(Log cfu/ml)								% Lactic acid			
Control	6.1^{c}	6.4^{d}	7.2^{d}	7.9^{e}	6.61^{a}	7.02^{c}	7.81^{b}	8.67^{d}	0.16	0.36	0.52	0.81
1%	6.18^{c}	6.7^{b}	7.5^{a}	8.3^{a}	6.52^{b}	7.13^{b}	7.95^{b}	8.73^{cd}	0.16	0.42	0.55	0.77
3%	6.13^{c}	6.7^{b}	7.38^{b}	8.12^{c}	6.62^{a}	7.16^{b}	8.04^{ab}	8.84^{c}	0.17	0.43	0.58	0.76
5%	6.16^{c}	6.6^{c}	7.3^{c}	8.0^{d}	6.57^{a}	7.10^{b}	8.11^{a}	8.76^{cd}	0.17	0.41	0.57	0.80
8%	6.2^{b}	6.48^{d}	7.16^{d}	7.8^{e}	6.5^{b}	6.90^{ab}	8.0^{ab}	8.60^{d}	0.18	0.38	0.51	0.77
Heated extract												
1%	6.28^{b}	6.63^{c}	7.43^{b}	8.22^{b}	6.55^{ab}	6.84^{d}	7.98^{c}	8.6^{d}	0.16	0.34	0.62	0.74
3%	6.15^{c}	6.57^{c}	7.3^{c}	8.15^{c}	6.48^{b}	6.97^{c}	7.8^{ab}	8.8^{c}	0.17	0.36	0.64	0.76
5%	6.3^{ab}	6.47^{d}	7.22^{d}	8.03^{d}	6.35^{c}	6.85^{d}	8.0^{c}	8.9^{b}	0.17	0.38	0.66	0.75
8%	6.2^{b}	6.5^{d}	7.03^{e}	7.91^{e}	6.6^{a}	6.77^{d}	7.67^{c}	8.5^{e}	0.18	0.33	0.61	0.71
Autoclaved extract												
1%	6.36^{a}	6.87^{a}	7.51^{a}	8.11^{c}	6.52^{b}	7.1^{ab}	7.6^{b}	8.9^{b}	0.16	0.37	0.58	0.75
3%	6.18^{c}	6.71^{b}	7.40^{b}	8.29^{a}	6.48^{b}	7.3^{a}	7.8^{b}	8.97^{a}	0.17	0.39	0.61	0.72
5%	6.25^{b}	6.79^{ab}	7.3^{c}	8.02^{d}	6.38^{c}	7.11^{b}	8.0^{ab}	8.79^{c}	0.18	0.40	0.62	0.74
8%	6.27^{b}	6.58^{c}	7.11^{d}	7.86^{e}	6.40^{c}	7.03^{c}	7.6^{c}	8.6^{d}	0.19	0.35	0.60	0.70

^{A,b,c,d,e} Means values in the same column within treatment bearing different superscription are not significantly ($p<0.05$) different

Table 6. Effect of aqueous doum palm extracts on viability and activity of Mesophilic (Lactocccci) starter culture along incubation period (up to 6h).

Doum palm concentration	*Bifidobacterium* count				Lactococci count				Titratable acidity			
	0h	2h	4h	6h	0h	2h	4h	6h	0h	2h	4h	6h
Crud extract	(Log cfu/ml)								% Lactic acid			
Control	6.21^{bc}	6.48^{d}	7.03^{d}	7.78^{cd}	6.03^{c}	6.40^{d}	7.3^{d}	8.64^{bc}	0.16	0.3	0.56	0.70
1%	6.25^{b}	6.75^{b}	7.24^{c}	8.18^{a}	6.10^{b}	6.78^{a}	7.6^{b}	8.9^{a}	0.16	0.3	0.57	0.71
3%	6.30^{b}	6.79^{b}	7.31^{b}	7.98^{b}	6.17^{b}	6.82^{a}	7.72^{a}	8.72^{b}	0.18	0.31	0.60	0.74
5%	6.26^{b}	6.46^{d}	7.23^{c}	7.81^{c}	6.07^{b}	6.65^{c}	7.58^{b}	8.41^{d}	0.18	0.34	0.61	0.74
8%	6.18^{c}	6.43^{d}	7.02^{d}	7.60^{d}	6.12^{b}	6.50^{d}	7.10^{e}	8.20^{d}	0.19	0.31	0.62	0.72
Heated extract												
1%	6.26^{b}	6.63^{c}	7.33^{b}	8.01^{b}	6.18^{a}	6.60^{c}	7.29^{d}	8.72^{b}	0.15	0.33	0.65	0.72
3%	6.33^{ab}	6.57^{c}	7.21^{c}	8.02^{b}	6.15^{ab}	6.71^{b}	7.4^{cd}	8.75^{b}	0.17	0.36	0.63	0.75
5%	6.23^{b}	6.47^{c}	7.11^{cd}	7.89^{c}	6.01^{c}	6.60^{c}	7.65^{b}	8.57^{c}	0.17	0.34	0.65	0.73
8%	6.22^{bc}	6.5^{d}	7.06^{d}	7.77^{d}	6.11^{b}	6.42^{d}	7.32^{d}	8.40^{d}	0.19	0.32	0.62	0.71
Autoclaved extract												
1%	6.40^{a}	6.87^{a}	7.36^{a}	8.10^{a}	6.2^{a}	6.67^{bc}	7.4^{cd}	8.85^{a}	0.17	0.35	0.62	0.73
3%	6.38^{a}	6.71b	7.45^{a}	8.11^{a}	6.05^{c}	6.62^{c}	7.26^{d}	8.61^{c}	0.17	0.36	0.60	0.71
5%	6.35^{a}	6.79^{b}	7.19^{bc}	7.95^{bc}	6.14^{ab}	6.50^{d}	7.50^{c}	8.50^{c}	0.18	0.37	0.59	0.72
8%	6.31^{b}	6.58^{c}	7.03^{c}	7.81^{c}	6.0^{c}	6.39^{e}	7.30^{d}	8.33^{d}	0.18	0.34	0.57	0.70

^{A,b,c,d,e} Means values in the same column within treatment bearing different superscription are not significantly ($p<0.05$) different

soluble doum palm extract (ACD)] on the viability and activity of yoghurt, AB, probioitic starter culture containing *Bifidobacterium longum* and *Lactobacillus rhamnosus* (BR), and mesophilic starter cultures containing *Bifidobacterium longum* during incubation for 6 h. Table (3) presents the effect of different concentrations of aqueous solutions of doum palm on the viability and activity of yoghurt starter cultures. Aqueous solutions of doum palm had a slight effect on the viability and activity of starter culture. *L. delbrueckii ss. bulgaricus* count ranged from 6.88 to 7.34 log cfu / ml in the beginning of incubation and gradually increased along the incubation and reached the highest viability at the end of incubation.

The highest viability of *L. delbrueckii ss. bulgaricus* was found in yoghurt starter culture without adding of aqueous solutions of doum palm and the viability decreased with the increase of aqueous solutions of doum palm concentrations. Also, aqueous solutions of doum palm slightly affected on the viability of *Str. thermophilus*. Autoclaved aqueous solutions of doum palm caused a slight increase in the viability of *L. delbrueckii ss. bulgaricus* along incubation period compared with control treatment.

Effect of aqueous solutions of doum palm on the viability of probiotic starter culture containing *Bif. longum* and *Lactobacillus rhamnosus* presented in Table (4) shows that, *Bifidobacterium longum* and *Lactobacillus rhamnosus* counts slightly increased with the addition of 1, 3 and 5% aqueous solutions of doum palm, and the viability decreased with the increase of doum palm concentration. The titratable acidity development was affected with the increase of doum palm concentration to 8%. The addition of 1, 3, and 5% doum palm extract caused increase in the acidity, however it decreased with the addition of 8% of doum palm extract. Also, aqueous solutions of autoclaved doum palm extracts had a slight effect on increasing the viability of starter culture when compared with control treatment. Furthermore, Bifidobacteria and *Lactobacillus rhamnosus* counts were higher than those of Bifidobacteria and *Lactobacillus acidophilus* in the presence of 3 and 5% of Aqueous solutions of doum palm extracts.

Table (5) shows the effect of aqueous solutions of doum palm on the viability and activity of AB starter culture (*Bif. longum* and *Lactobacillus acidophilus*) along the incubation period.

The viability of *Bif. longum* and *Lactobacillus acidophilus* slightly increased with the addition of 1,3 and 5% aqueous solutions of doum palm, as compared with control treatment. On the other hand, the addition of 8% aqueous solutions of doum palm had a slight effect on the viability of *Bif. longum* and *Lactobacillus acidophilus*. Generally, the viability of *Bif. longum* and *Lactobacillus acidophilus* was higher than 7.7 log cfu / ml for all treatments.

Effect of aqueous solutions of doum palm on the viability of mesophilic starter culture containing *Bif. longum* and lactococci strains, presented in Table (6) which shows that the viability of lactococci count were higher than 8.4 log cfu / ml for all treatments. Also, the presence of 1, 3 and 5% aqueous solutions of doum palm caused a remarkable increase on the viability of Lactococci and Bifidobacterial counts. Furthermore, titratable acidity increased gradually with increase of different aqueous solutions of doum palm

concentrations till 5% of doum palm extract then decreased with the addition of 8% of aqueous doum palm extract.

Also, some of these strains are known to be of antibacterial and some of antifungal activity (**Effat, 2000 and Batish *et al* 1997**).

It could be concluded that, the viability of probiotic cultures *Bif. longum* and *Lactobacillus rhamnosus* in the presence of 1, 3 and 5% aqueous solutions of doum palm, was the highest among the other starter cultures. Also, mesophilic starter culture containing *Bif. longum* had higher viability and the activity was higher with the addition of 1, 3 and 5% aqueous solutions of doum palm than the control treatment.

In conclusion, for enhancement of safety and long shelf for the neutraceutical dairy products, adding aqueous doum palm extract at the rate of 3 - 5% could be recommended.

REFERENCES

A.O.A.C. (1990). ***Official methods of Official Analysis Chemists. 15th Ed.*** William Hornitz, (ed). Pub Association of Official Analytical Chemists, Washington, D.C., U.S.A.

Barry, A. and C. Thornsberry (1985). Susceptibility tests diffusion tests procedures. In Lennette, E.H; A. Balows, W. J. Hausler and H. J. Shadomy (ed), ***Manual of Clinical Microbiology 4th Ed, pp. 978- 987.*** American Socity for Microbiology, Washington , DC, USA.

Dave, R.I. and N.P. Shah (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. bulgaricus, *Lactobacillus acidophilus*, and Bifidobacteria. ***J. Dairy Sci. 79 (9): 1529-1536.***

De Man, J.C.; M. Rogosa and M.E. Sharp. (1960). A medium for the cultivation of lactobacilli. ***J. App. Bacteriol. 22: 130-136.***

Effat B. A. (2000). Antifungal substances from some lactic acid bacteria and probionibacteria for use as food preservatives. ***J. Agric. Sci. Mansura Univ. 25: 6291-6299.***

Farnsworth, E.R. (2001). Probiotics and prebiotics. ***In: Handbook of Nutraceutical and Functional Foods. pp.407-422.*** CRC Press LLC, Boca Raton, FL.

FAO/WHO (2001). ***Report on joint FAO/WHO Expert Conclusion on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk With Live Lactic Acid Bacteria. pp. 1-11.*** http://www.Fao.org/es/ESN/Probio/Probio.htm.

Gibson G. R.; R. X. Wang (1994). Regulatory effects of bifidobacteria on the growth of other colonic bacteria. ***J. Appl. Bacteriol. 77: 4, 412-420.***

Gilliland, S.E. and D.K. Walker (1990). Factors consider when selection a culture *Lactobacillus acidophilus* as a dietary adjunct to produce hypocholesteromic effect in human. ***J. Dairy Sci. 73: 905-911.***

Harrigan W.F. (1998). ***Laboratory Methods in Food Microbiology. 3rd Ed. p. 66.*** Academic Press, London, UK.

Irobi, O. N. and O. Adedayo, (1999). Antifungal activity of aqueous extract of dormant fruits of *Hyphaene thebaica* (Palmae). ***Pharmaceutical Biology. 37 (2): 114-117.***

Jackobsen M. and T. Narvhus (1996). Yeasts and their possible beneficial and negative effects on the quality of dairy products. ***Int. Dairy J. 6: 755-761.***

Kailasapathy, K. and S. Rybka, (1997). *Lactobacillus acidophilus* and *Bifidobac-*

terium spp. - their therapeutic potential and survival in yogurt. Australian *J. Dairy Technol. 52(1): 28-35.*

Mead, P.S.; L.V. Slutsker; L.F. McCaigand and R.V. Tauxe. (1999). Food related illness and death in the United States. *Emerg. Infect. Dis. 5: 607-625.*

Philips, R.D.; M.S. Chinman; A.L. Branch; J. Miller and K.H. McWatters. (1988). Effects of pretreatment on functional and nutritional properties of Cowpea meal. *J. Food Sci. 53: 805-809.*

SAS Institute. (1994). *SAS/STAT User's Guide: Statistics. Ver 6.04,* Fourth Edition SAS Institute Inc., Cary, NC.

Schrezenmier J. and M. DeVrese (2001). Probiotics, Prebiotics and synbiotics to approaching a definition. *Am. J. Clin. Nutr. 73: (Suppl.). 361s-364s.*

Welthagen J.J. and B.C. Viljoen. (1998). Yeast profile in Gouda cheese during processing and ripening. *Int. J. Food Microbiol. 41: 185-193.*

Terzaghi, B.E. and W.E. Sandine. (1975). Improved medium for lactic streptococci and their bacteriophages. *Appl. Microbiol. 29: 807-811.*

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تأثير مستخلص ثمار الدوم على بعض مزارع بادئات الألبان وبعض البكتريا غير المرغوبة

[١٣]

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تمت دراسة تأثير المستخلص المائى للدوم(الخام والمعامل حراريا" والمعقم) بتركيزات ١و ٣و ٥و ٨% على حيوية بعض أنواع البكتريا المرضية والمسببة للفساد وهى:

Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli O157:H7, *Micrococcus luteus, Pseudomonas aeruginosa, Ps. flourescens*

كذلك دراسة التأثير المضاد للمستخلص المائى للدوم على نشاط بعض أنواع الفطريات المسببة للفساد وهى:

Asperagillus niger, Asp. fumigatus, Fusarium moniliforum, Fus. oxysporium, Penicillium roqueforti ,Penicillium expansum.

كما تمت دراسة تأثير إضافة المستخلص المائى لثمار نخيل الدوم المطحونة بتركيزات مختلفة (١% ، ٣ % ، ٥% و ٨ %) بثلاث صورهى الخام والمعامل حراريا" والمعقم على نمو ونشاط بعض بادئات الألبان.

وقد أشارت النتائج إلى أن المستخلص المائى للدوم ذو تأثير مثبط لنمو ونشاط

السلالات المختبرة كما إزداد التأثير المثبط بزيادة تركيز مستخلص الدوم. كما كان المستخلص المائى للدوم أكثر تثبيطا لسلالات كل من: *Escherichia coli* و *Staphylococcus aureus* O157:H7 و *Micrococcus luteus* على الترتيب. على العكس من ذلك كانت سلالات كل من *Bacillus subtilis, Bacillus cereus* هى أكثر السلات البكتيرية مقاومة لتأثير المستخلص المائى للدوم. كما كان للمستخلص المائى للدوم تأثير تثبيطى واضح على كل سلالات الفطريات المختبرة وكان أعلى تأثير تثبيطى على كل من فطريات *Asperagillus niger, Asp. fumigatus* ثم *Penicillium expansum* بينما كان أكثر الفطريات مقاومة هو *Fusarium moniliforum* و *Fus. oxysporium*.

ومن ناحية أخرى فقد لوحظ وجود تأثير منشط للمستخلص المائى لثمار الدوم على نوعى البكتريا ذات الصفات الصحية والعلاجية والوقائية *Bif. longum* و *Lactobacillus rhamnosus* فى وجود تركيزات ٣ و٥% من المستخلص المائى للدوم مقارنة بحيوية السلالات الأخرى بدون استخدام المستخلص المائى لثمار الدوم. إلا أن تركيز ٨% من المستخلص المائى للدوم كان له تأثير تثبيطى على حيوية ونشاط كل أنواع البادئات المختبرة.

لذلك يمكن التوصية بإستخدام تركيزات ٣ و٥% من المستخلص المائى للدوم كمادة مشجعة لنمو سلالات بادئات الألبان وكذلك لزيادة جودة بعض منتجات الألبان العلاجية.

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