

## INHIBITION OF MOULD GROWTH ON THE SURFACE OF RAS CHEESE BY PLANT WATER EXTRACTS OR pH CONTROL

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### ABSTRACT

Moulds isolated from visibly moulded Ras cheese collected from different rural and urban localities at Kafr- El-Sheikh, Tanta and Damietta were identified and used in this study. The isolated fungi strains were *Penicillium spp.*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Geotrichum candidum*, *Cladosporium herbarum*, *Fusarium roseom*, *Alternaria tenuis*, *Aspergillus candidus* and *Steamphylium spp.* The isolated yeast strains were *Saecharomyces cervisiae*, *Trichosporon brassicae* and *Debaryomyces hansenii* In order to control the undesirable mould growth on cheese surface, aqueous extracts of different 22 plants were examined for their antifungal activity. Extracts of galangal, cina, samolia, red pepper, cinnomon, turmeric, cumin, migmorette and sage were more effective to inhibit the growth of 73-82% of the isolated strains. However, extracts of damssissa, aloe, cumin, fenugreek, thyme and anise were more effective to inhibit the growth of the isolated yeasts. Treatment of cheese surface with sodium carbonate completely suppressed the growth of moulds by controlling the pH value however it does not penetrate the cheese. The results demonstrated the inhibitory effect of different plant extracts on the growth of moulds on cheese surface during ripening and storage without affecting the cheese quality.

**Key words:** Ras cheese, Fungicide, Herbs, Spices.

### INTRODUCTION

Ras cheese is a popular dairy product in Egypt. It has two common public names, Roumy and Turkey cheese. As any hard cheese, Ras cheese usually ripened for several months in relatively low temperature and high humidity rooms. Under such conditions

moulds may grow on the cheese surface and may penetrate to the cheese producing off flavors leading to severe economic losses. In addition, the contaminated cheese with moulds is a health hazard because some moulds are capable of producing toxic metabolites in cheese. Level of mould contamination depends on the hygienic quality of the environment during cheese production, ripening and storage. The moulds are widely spread in the air, walls and shelves surfaces of cheese ripening rooms and cause spoilage of Ras cheese, especially when general good cheese manufacturing practices are not fully followed. Our previous study on the incidence of moulds in samples from Ras cheese surface and in air of their corresponding dairy factories in rural and urban areas showed that the air of Ras cheese factories especially that from the rural areas were highly contaminated with moulds that inflected on the moulds count of cheese (Zommara & Rashed, 2004). Therefore, controlling or preventing of moulds growth on cheese surface is of great importance either from the economic or public health view.

Several studies have been done to control the growth of moulds in dairy products. Currently, many methods based in synthetic and natural mycostatic agents and commercial coating materials are widely used to control growth of moulds on hard and semi hard cheese. However, these methods are expensive that increase the production cost. Therefore, the small cheese factories use some traditional methods such as washing the cheese after ripening or storage with salted water or scraping the cheese surface for removing moulds. Removing the moulds from the cheese surface may enhance its appearance for marketing but does not remove the mycotoxins produced by the moulds in the later stages of mycelial growth and absorbed to the cheese.

Plants produce a great deal of secondary metabolites, many of them with anti-fungal activity. Well known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Gomez Garibay *et al.*, 1990; Bennett and Wallsgrove, 1994; Grayer and Harborne 1994; Osbourane 1996). Many studies have been focused in the use of herbs and spices for preventing undesirable mould growth of cheese. Hassan and El-Deeb (1988) suggested the use of water or

acetone extracts of caraway (*Carum Carvi*) and red pepper (*Capsicum frutescense*) to inhibit mould growth and aflatoxins production by *Aspergillus* species. Abdel-Kader *et. al.* (2001) recommended the addition of 0.1% of the seeds of black pepper, black cumin or anise to the curd of Ras cheese to improve the organoleptic and microbial properties of the resultant cheese. Also, Hassan *et. al.* (2001) examined 14 kinds of spices and herbs for their antimycotic and antimycotoxigenic activities on *Aspergillus flavus* and *Penicillium roqueforti*. They found that *P. roqueforti* is more sensitive than *A. flavus* to the inhibitory effect of the used plants.

In the present study we studied the anti-fungal effect of 22 plant extracts on 9 fungi and 3 yeast strains isolated from visibly moulded Ras cheese samples collected from several sources. Also, the effect of controlling cheese surface pH, in the alkali side using sodium carbonate, on moulds growth was investigated.

## MATERIALS AND METHODS

### 1) Cheese samples

Ras cheese samples were collected from shops at Kafr El-Sheikh city, small cheese plants in rural areas at Abo Mandor and Qtoor, modern dairy products company (Misr Milk and Food Company, Damietta) and from Ras cheese produced in our department.

### 2) Isolation and identification of moulds

Yeasts and fungus were isolated on a potato dextrose agar (PDA) medium amended with 25mg/ml streptomycin sulfate to eliminate bacterial contamination. Fungi and yeast plates were incubated at  $21^{\circ}\text{C} \pm 2$  for 7 days and 48 h, respectively. The isolated fungi and yeast were purified by hyphal tip or single spore technique. The isolated colonies were identified to genus level and sometimes to species level as described by Barnett & Hunter (1979) and Booth (1971). Isolates of each strain transferred to PDA slants and kept in a refrigerator at  $4^{\circ}\text{C}$  as a stock culture. The examined mould strains are shown in Table 1.

### 3) Plant materials

Table 1. Mould and yeast strains used in the study

Strain name	% of isolates
<u>Moulds</u>	
<i>Penicillium spp</i>	41.7
<i>Aspergillus flavus</i>	31.9
<i>Aspergillus parasiticus</i>	4.17
<i>Cladosporium herbarum</i>	4.17
<i>Fusarium roseom</i>	2.78
<i>Alternaria tenius</i>	1.38
<i>Aspergillus candidus</i>	1.38
<i>Geotrichum candidum</i>	1.38
<i>Steamphylium spp.</i>	1.38
<u>Yeast</u>	
<i>Saccharomyces cervisiae</i>	5.56
<i>Trichosporon barassicae</i>	2.78
<i>Debaryomyces hansenii e</i>	1.38
Total	100

Twenty-two plants were collected from the local market and from our faculty botany farm. Different plant parts include leaves, rhizomes, seed, stems, roots, barks, gum, flesh and peels were used. A list of the tested plants used throughout these study are shown in Table (2).

#### **4) Preparation of plant aqueous extracts**

Dry plant materials (50g) were grounded into a fine powder in a high-speed micro mill. The powder of plant parts were soaked in distilled water at the rate of 1:3 (w/w) and allowed to stand overnight under shaking (Arjunan *et. al.* 1994). The mixture was filtered through cheese cloth and filter paper to remove plant parts. The resultant extracts were subjected to a Sartorius nitrate filter with a pore size of 0.22 $\mu$ m (Nalgen American filter) for sterilization.

#### **5) Measurement of the anti-fungal activity of plant extracts *in vitro***

The anti-fungal activity of plant extracts was carried out by paper disk diffusion assays as previously described by Quiroga *et. al.* (2001). Sterile paper disk (Whatman No. 4 paper, 5 mm) were impregnated with 10  $\mu$ l of different plant extracts of known concentrations. Six disks were spaced on the agar surface of each Petri dish (9 cm, diameter) contains 10 ml PDA medium mixed with 1 ml activated fungal spores or yeast cells contains 10<sup>8</sup> cell/ml. A disk negative control (10  $\mu$ l water) was included. The diameter of the inhibition zone around the disks was measured after incubation at 21°C  $\pm$  2 for 7 days and 48 h for fungus and yeasts, respectively. The values were the average (mm) of six disks per treatment.

#### **6) Cheese treatment with plant extracts**

Ras cheese (2 blocks/treatment) was surface-treated, during ripening and salting period (50 days), with diluted water extracts (0.1%) of galangal, damssissa, cina, red paper, cinnamon, cumin, anise, sage and fenugreek. A treatment with sodium bicarbonate in wheat flour (10% w/w) and a treatment by dusting the cheese with sodium bicarbonate powder were used. A control group without any treatment was also included. At the end of this period, growth

Table 2. Plants and parts used in the study

No.	Scientific name	Arabic name	Family	Common name	Used Part
1	<i>Aloe vera</i>	الصبار	Liliaceae	Aloe	Leaves
2	<i>Alpinia galaga</i> Willd.	خلنجان	Zingibaraceae	Galangal	Rhizome
3	<i>Ambrosia maritime</i>	دمسيه	Asteraceae	Damssissa	Leaves & stems
4	<i>Ammi visnaga</i> L.	خله	Umbelliferae	Pick teeth	Leaves
5	<i>Artemisia cina</i>	شبح	Compositae	Cina	Seeds
6	<i>Boltus edulis</i>	عيش الغراب	Boletaceae	King boletus	Flesh
7	<i>Boswellia carterii</i>	لبان مر	Labiatae	Samolia	Gum
8	<i>Capsicum frutescense</i> L.	شطة حمراء	Solanaceae	Red pepper	Peels
9	<i>Cinnomomum zeylanicum</i>	قرقه	Leuraceae	Cinnomon	Bark
10	<i>Curcuma longa</i>	كركم	Zingibaraceae	Turmeric	Seeds
11	<i>Cuminum cyminum</i>	كمون	Umbelliferae	Cumin	Seeds
12	<i>Glycyrrhiza glabra</i> L.	عرقسوس	Leguminasae	Liquarice	Roots
13	<i>Lawsonia inermis</i>	حناء	Lythraceae	Migmorette	Leaves
14	<i>Majorana hortensis</i>	بردقوش	Labiatae	Majorarn	Leaves
15	<i>Nigella sativa</i> L.	حبة البركة	Ranunculaceae	Nigella	Seeds
16	<i>Pimpinello anisum</i> L.	يلسون	Umbelliferae	Anise	Seeds
17	<i>Rosmarinus officinalis</i> L.	حصالبان	Labiatae	Rosemary	Leaves
18	<i>Salvia officinalis</i> L.	مرمرية	Labiatae	Sage	Leaves
19	<i>Thymus vulgaris</i> L.	زعتر	Labiatae	Thyme	Leaves
20	<i>Trigonella foenumgraecum</i> L.	حلبه	Leguminasae	Fenugreek	Seeds
21	<i>Piper nigrum</i> L.	فلفل ابيض	Piperaceae	Black pepper	Seeds
22	<i>Zingiber officinale</i> Roscoe	زنجبيل	Zingibaraceae	Ginger	Rhizome

of mould on cheese surface was visually measured, and then the cheese was retreated with the above-mentioned materials and cold-stored for two months. A group coated with the commercially available polyvinyl acetate (PVA) plastics containing 0.05% natamycin (Ceskawl 500, Kaasdek middel, Sweeden), already used in Ras cheese factories, were included for comparison. At the end of the storage period growth of mould on cheese surface was visually measured then, the cheese was washed by tape water to remove the residual treatment materials, dried on the open air for an hour and sensory evaluation was carried out.

## RESULTS AND DISCUSSION

As shown in Table 1, morphological and microscopical examinations showed that the isolated fungus from samples of Ras cheese surfaces were of the genus *Geotrichum*, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Steamphylium* and *Alternaria*, and the isolated yeasts were of the genus *Saccharomyces*, *Trichosporon* and *Debaryomyces*.

The anti-fungal activities of crude water extracts of 22 plants contain spices used in food preparation and herbs used in the traditional public medicine were investigated against the mould strains isolated from Ras cheese. The growth inhibitory activities of the examined extracts against fungi are summarized in Table 3. The data show that most of the plant extracts positively inhibited the fungi growth. The most effective extracts were of the plants galangal, cina, samolia, red pepper, cinnomon, turmeric, cumin, migmorette and sage as they inhibited the growth of 73-82% of the isolated fungi. As shown in Table 1, more than 80% of fungi found on Ras cheese surface belong to the genus *Penicillium* and *Aspergillus*. Accordingly, a plant water extract capable to inhibit their growth would be of special interest.

Cinnomon, cina, samolia, turmeric, cumin, migmorette and sage were found to inhibit the growth of the mentioned genus. Many reports described the inhibitory effect against fungal growth by different plant extracts from, cina (Tan *et. al.* 1999), turmeric (Apisariyakul *et. al.* 1995), cumin (Garg & Siddiqui, 1992), cinnamon (El-Sharaky, 1998) and migmorette (Bansal & Sobti, 1990). Hassan & El-Deeb (1988) demonstrated the inhibitory effect

Table 3. The growth inhibition of mould strains isolated from Ras cheese by different plant extracts as determined by paper disk diffusion assay.

*Plants	Mould strains										
	A	B	C	D	E	F	G	H	I	J	K
1	+	-	-	++	-	-	++	++	+	-	-
2	-	+	+	+	+	+	-	+	+	-	+
3	+	++	+	-	++	-	-	+	-	+	++
4	+	-	-	++	-	-	+	++	-	+	-
5	+	++	++	++	++	++	+	+	-	-	+
6	-	-	+	-	-	-	+	+	+	+	-
7	+	+	+	++	-	++	++	+	+	+	-
8	+	+	++	-	++	-	+	++	-	+	+
9	+	+	+	++	+	+	+	+	-	+	-
10	++	-	-	+	++	++	++	++	+	+	-
11	+	-	-	++	++	-	++	++	+	+	++
12	+	-	-	+	-	++	-	-	++	+	-
13	+	-	++	+	++	+	+	-	+	+	+
14	++	+	+	+	-	-	-	-	+	+	+
15	-	-	-	-	+	+	+	-	+	+	-
16	+	+	+	-	-	-	+	+	-	+	+
17	-	-	++	++	-	++	++	+	+	+	-
18	-	++	++	-	++	++	++	+	++	+	-
19	-	-	+	++	-	++	++	-	-	+	-
20	++	-	-	++	+	++	++	-	+	-	+
21	+	-	-	++	++	-	++	++	+	++	-
22	+	-	-	++	++	+	++	++	-	+	-

A, *Geotrichum candidum*; B, *Cladosporium herbarum*; C, *Penicillium* spp.; D, *Aspergillus parasiticus*; E, *Aspergillus candidus*; F, *Aspergillus flavus*; G, *Penicillium* spp.; H, *Penicillium roqueforti*; I, *Alternaria tenuis*; J, *Fusarium roseom.* K, *Stemphylium* spp. \*Plants names are shown in Table 1. The extract concentration was about 0.2 mg dry matter /disk. The diameter of the inhibition zone is reported as (-) without inhibition, (+)= 4 – 6 mm and (++)= 7 - 10 mm. (n=6).



of a water extract of red pepper (*Capsicum frutescense* L.) against the growth and mycotoxin production by *Penicillium* spp., *Aspergillus parasiticus* and *Aspergillus flavus*.

Table 4. shows the growth inhibition of yeast strains isolated from Ras cheese by plant extracts. Among the investigated plants water extracts of damssissa, aloe, cumin, fenugreek, thyme and anise were more effective to inhibit the growth of the three yeast strains isolated from Ras cheese than the other plants. Damssissa water extract showed the highest inhibition effect on yeast growth.

Several equal combinations of the above mentioned plant extracts were investigated in yeast growth. As shown in Table.5, incorporating damssissa to anise, cumin or fenugreek greatly enhanced their anti-microbial effect against the isolated yeast. Data from Tables 3 and 4 therefore show that an anti-microbial mixture of plant water extracts against both of yeasts and fungus should include damssissa, anise, cumin and fenugreek to achieve a good protection of cheese against mould attack.

As an application of the obtained results, Ras cheese samples were subjected to surface treatment with some plant extracts showed *in vitro* anti-fungal effects or by sodium bicarbonate. Four cheese blocks were surface-treated with 0.1% of water extracts from galangal, damssissa, cina, red paper, cinnamon, cumin, anise, sage and fenugreek. Also, a 10% mixture of sodium bicarbonate in wheat flour and a treatment with sodium bicarbonate powder were used. The principle of using sodium bicarbonate is to keep the pH on cheese surface in the alkali side and create unsuitable condition for mould growth. The mould growth on cheese surface was measured visually during salting, ripening and cold storage with or without cheese coating. Table 6 shows the obtained results. During salting and ripening period (50 days) galangal, damssissa, cinnamon and 10% sodium bicarbonate were more effective than the other treatments and caused about 90% inhibition of mould growth. Table 6 also show that coating the cheese with PVA containing natamycine protected the cheese from mould attack during 2 months of cold storage at about 10 °C. Treatment cheese surface with sodium bicarbonate was more effective to inhibit mould growth during storage period. Cheese treated with sodium bicarbonate does not show any significance changes in its pH after

Table 4. The growth inhibition of yeast strains isolated from Ras cheese by different plant extracts as determined by paper disk diffusion assay.

Plant name	Yeast strains		
	<i>S. cerevisiae</i>	<i>T. brassicae</i>	<i>D. hansenii</i>
1 <i>Aloe vera</i>	+	+	+
2 <i>Alpinia galanga</i> Willd.	-	-	+
3 <i>Ambrosia maritima</i>	++	++	++
4 <i>Annona visnaga</i> L.	+	+	-
5 <i>Artemisia cina</i>	-	+	+
6 <i>Bolus edulis</i>	-	-	-
7 <i>Boswellia carterii</i>	+	-	-
8 <i>Capsicum minimum</i> L.	-	+	-
9 <i>Cinnamomum zeylanicum</i>	-	-	-
10 <i>Curcuma longa</i>	-	-	+
11 <i>Cuminum cyminum</i>	+	+	+
12 <i>Glycyrrhiza glabra</i> L.	+	-	+
13 <i>Lawsonia inermis</i>	+	-	-
14 <i>Majorana hortensis</i>	+	-	-
15 <i>Nigella sativa</i> L.	+	-	-
16 <i>Pimpinello anisum</i> L.	+	-	++
17 <i>Rosmarinus officinalis</i> L.	+	++	-
18 <i>Salvia officinalis</i> L.	-	-	+
19 <i>Thymus vulgaris</i> L.	+	+	+
20 <i>Trigonella foenumgraecum</i> L.	+	+	+
21 <i>Piper nigrum</i> L.	-	-	+
22 <i>Zingiber officinale</i> Roscoe	+	-	-
23 Control	-	-	-

The extract concentration was about 0.2 mg dry matter /disk. The diameter of the inhibition zone is reported as (-) without inhibition, (+)= 4 – 6 mm and (++)= 7 - 10 mm. (n=6).

Table 5. Effect of some plant water extracts mix on the growth of yeast isolated from Ras cheese

Plant mixtures	Yeast strains		
	<i>S. cerevisiae</i>	<i>T. brassicae</i>	<i>D. hansenii</i>
Damssissa + Anise	++	++	++
Damssissa + Cumin	++	++	++
Damssissa + Fenugreek	++	++	++
Anise + Fenugreek	+	+	+
Anise + Cumin	+	+	+
Control	-	-	-

The extract concentration was about 0.2 mg dry matter /desk.

The diameter of the inhibition zone is reported as (-) without inhibition, (+)= 4 – 6 mm and (++)= 7 - 10 mm. (n=6).

Table 6. Inhibition of moulds and yeasts growth on Ras cheese surface during salting, ripening and storage periods by some selected plant water extracts and sodium bicarbonate.

Treatment	Salting & ripening	Cold storage
<i>Alpinia galaga</i> Willd.	++	+
<i>Ambrosia maritime</i>	++	++
<i>Artemisia cina</i>	+/-	+
<i>Capsicum minimum</i> L.	+	+
<i>Cinnomomum zeylanicum</i>	++	+
<i>Cuminum cyminum</i>	+	+
<i>Pimpinello anisum</i> L.	+	+
<i>Salvia officinalis</i> L.	+/-	+
<i>Trigonella foenumgraecum</i> L.	-	+
10% Na <sub>2</sub> HCO <sub>3</sub> in wheat flour	++	+
Na <sub>2</sub> HCO <sub>3</sub> (powder)	+	++
Control	-	-

\*Moulds growth inhibition was reported as (++) 90% inhibition, (+), 80% inhibition, (+/-), 50% inhibition and (-) no inhibition. n=4 cheese blocks/ treatment.

ripening or storage period. Cheese samples were taken every 5 mm from cheese surface showed a slight increase in the pH (0.01) within the first 5 mm of cheese surface only when compared with the control one. The sensory evaluation of all cheese after ripening and storage periods does not show any off flavours and were almost comparable to the control.

As a conclusion, the treatment of Ras cheese surface with a mixture of water extracts of galangal, damssissa, cinnomon and cumin may protect it from mould growth without affecting its sensory properties. Treating cheese surface with sodium bicarbonate or coating it with PVA obtained comparable results however, the later method is more expensive.

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### الملخص العربي

تنشيط نمو الفطريات على سطح الجبن الراس بواسطة مستخلصات مائية نباتية أو التحكم في الأس الهيدروجيني

محسن عبد العزيز زماره ومصطفى على راشد  
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فى هذه الدراسة تم عزل الفطريات والخمائر النامية على سطح عينات من الجبن الراس المجمعة من مناطق ريفية وأخرى حضرية فى مدن كفر الشيخ وطنطا ودمياط وتم التعرف على الفطريات والخمائر الملونة لها وكانت أنواع الفطريات:

*Penicillium spp.*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Geotrichum candidum*, *Cladosporium herbarum*, *Fusarium Steamphylium spp. roseom*, *Alternaria tenius*, *Aspergillus candidus*  
بينما كانت الخمائر من الأنواع

*Saccharomyces cervisiae*, *Trichosporon brassicae*, *Debaryomyces hansenii*

ومن أجل السيطرة على النمو الغير مرغوب للفطريات والخمائر على سطح الجبن تم استخدام المستخلص المائى لـ ٢٢ نبات مختلف وأختبر فاعليتها فى مقاومة هذه النموات. شملت هذه النباتات الصبار، الخنجان، الدمسيمة، الخلة، الشيح، عيش الغراب، اللبان المر، الفلفل الأحمر، القرفة، الكركم، الكمون، العرقسوس، الحناء، البردقوش، حبة البركة، اليانسون، الحصابان، المرمرية، الزعتر، الحلبة، الفلفل الأبيض والزنجبيل.

كانت مستخلصات نباتات الخنجان، الشيح، اللبان المر، الفلفل الأحمر، القرفة، الكركم، الكمون، الحناء والمرمرية. أكثر تأثيرا فى منع نمو الفطريات حيث منعت نمو ٧٢-٨٢% من الفطريات المعزولة. بينما كانت مستخلصات الدمسيمة، الصبار، الكمون، الحلبة والزعتر أكثر تأثيرا فى القضاء على الخمائر.

أدت معاملة سطح الجبن بمسحوق كربونات الصوديوم الى المنع التام لنمو الفطريات والخمائر على سطح الجبن وذلك عن طريق التحكم فى قيمة الـ pH بدون نفاذ الكربونات الى داخل الجبن. لوضحت النتائج أن مستخلصات النباتات المذكورة أدت الى منع نمو الفطريات والخمائر على سطح الجبن أثناء فترة التسوية والتخزين بدون أى تأثيرات سلبية على خواص الجبن