Prevention of fungal growth on Ras cheese surface using some plant extracts under different hygienic conditions

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Abstract

Ras cheese was subjected to surface treatment with ethanolic (EE) or water extracts (WE) of cinnamon, cumin. ambrosia and wheat or their mixture with or without 5% of sodium bicarbonate or calcium carbonate. The cheeses were stored for two months for ripening under bad or good hygienic conditions and the fungal growth rate on cheese surface was visually evaluated during storage period. The results showed that the EE and its mixture had significant antifungal effect than that of the WE. Among all plant extracts cinnamon extract was the most effective one. The mixture of EE with 5% of sodium bicarbonate appeared ability to prevent the fungal growth on cheese surface stored under uncontrolled hygienic conditions. On the other hand, the controlled hygienic conditions resulted in less fungal growth on cheese surface which inhibited by the mixture of EE or WE with sodium bicarbonate.

The *in vitro* inhibitory effect of acetonic extract (AE) of different plants on growth of some predominant fungal strains (isolated from Ras cheese surface) was also examined. The AE of cinnamon and cumin were more effective in inhibition of fungal growth followed by galangal and sage. Furthermore, The AE of ambrosia, cinnamon, cina, and cumin completely inhibited the growth of *Saccharomyces cervisiae*. Finally the commercial oils of anise, cina, cinnamon, cumin, nigella and sage showed no *in vitro* antimycotic activities.

Key words: Ras cheese, herbs, spices, fungicides

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Introduction

Ras or so called Romi or Turkey cheese is the best-known national hard cheese in Egypt. It is widely manufactured either in simple dairy pilots or in modern factories. However, the best quality of Ras cheese is produced in Damietta Governorate. Ras cheese was early investigated by Youssef (1966) and subsequently by Hofi *et al.* (1970). This cheese type is ripened for several months at low temperature and high relative humidity. During the ripening period, fungus may grow on cheese surface causing severe economic losses through the development of off-flavours and poor appearance. Also, mould growing on Ras cheese surface may be potentially hazardous to the health of consumers (Marquad & Frolich, 1992; and Tournas, 1994) by secreting some mycotoxins.

Fungal growth on cheese surface may be reduced by applying strict hygienic standards, use of pure starter cultures, control of cheese smear contamination and packaging under modified atmospheric condition. However, the mechanical removal of mould from cheese surface is not preferable as moulds and yeasts may penetrate deeply into cheese curd and spread inside. Removing the visible mould from cheese does not eliminate its toxic metabolites and therefore gives no guarantee of safety to the consumer. Although, Bulleman, 1986 suggested the remove of 1.3 cm of cheese around and beneath the mould to overcome the production of mycotoxins, which results in a considerable loss in cheese yield.

Therefore the inhibition of mould and yeast growth on cheese surface is a matter of challenge to the cheese producers; they commonly used either antifungal preservatives such as weak acids (sorbic acid) or antibiotics (natamycin). Sorbates tended to have an adverse effect on the appearance of the cheese as well as their limited effects against all mold strains. On the other hand, objections were raised against the use of natamycin these were either to the development of resistance strains against the permitted level of natamycin (Brul and Coote, 1997) or to its high price. Therefore, the use of natural preservatives to control mould spoilage in cheese has been subjected to research in recent years.

Spices and herbs have played an important role in human life prehistoric times. They are used in medical treatments and may fulfill more than one function in food to which they are added. They used as flavouring materials and also as antimicrobial and/or antioxidative substances (Abou Dawood, 1996). According to the International Organization for Standardization (IOS), there are over 70 spices and herbs officially recognized. However, there are more than 350-400 spices and herbs are used in different countries by people with different religion and climates (Nobuji, 1994). The present study was designed to explore the effect of some water and ethanol extracts from some selected plants on growth of fungi which contaminate Ras cheese surface during ripening under different hygienic conditions. The *in vitro* antifungal effect of some plants acetone extracts and some commercial herbs and spices oils were also investigated.

Materials and methods

Plant materials

Anise (*Pimpinella anisum L.*) seeds, cina (*Artemisia cina*) seeds, cinnamon (*Cinnamomum zeylanicum*) barks, cumin (*Cuminum cyminum*) seeds, galangal (*Alpinia galanga*) rhizomes, sage (*Salvia officinalis L.*) leaves and wheat (*Triticum aestivum*) flour were obtained from the local market at Alexandria city however, ambrosia (*Ambrosia maritima*) leaves were obtained from our faculty botany farm. The commercially available oils of anise, cina, cinnamon, cumin, nigella (*Nigella sativa L.*) and sage, were purchased from a supermarket at Alexandria city.

Fungi used for the *in vitro* experiment

The antimycotic effect of the plant AE and the commercial plant oils were examined by *Penicillium* spp. *Aspergillus flavus*, *Asp. parasiticus*, *Asp. candidus*, *Cladosporium herbarum*, *Fusarium roseom* and *Saccharomyces cervisiae*. Isolation and identification of these microorganisms are previously described (Zommara and Rashed, 2005).

Preparation of plant extracts

Dry plant materials (750g) were grounded into a fine powder in a high-speed micro mill. The powder of plant parts were divided into 3 portions of 250 g each and extracted with either distilled water, ethanol (80%) or acetone at the rate of 1:3 (w/w) and allowed to stand overnight under continuous shaking (Arjunan *et. al.*, 1994), The

mixture was filtered through cheese cloth and filter paper to remove plant parts. Acetone and ethanol was removed using rotary evaporator and dissolved in 5 ml distilled water to obtain the acetonic and ethanolic fractions, respectively. All fractions were sterilized by a sartorius nitrate filter with a pore size of 0.45 µm (Nalgen Aerican filter).

***In vitro* determination of the antifungal activity of plants acetonic fractions and the commercial oils.**

The anti-fungal effect of plant AE and the plant oils were carried out by paper disk diffusion assays as previously described by Quiroga *et al.*, (2001). Sterilized paper disk (Whatman No. 4 paper, 5 mm) were impregnated with 10 µl of different plant AE or oil. Six discs were spaced on the agar surface of each petri dish (9 cm, diameter) containing 15 ml PDA medium previously mixed with 1 ml of activated fungal spores (~10^8 spores/ml) or yeast cells (~10^8 cfu/ml). A negative control disc (10 µl sterilized water) was also included. The diameter of the inhibition zone around the disks was measured after incubation at 26°C ± 2 for48 h and 7 days for yeasts and moulds, respectively. The results were obtained as an average (mm) of six discs per treatment.

Treatment of cheese with plant extracts

Ras cheese (2 discs/treatment) was subjected to surface treatment with WE or EE of wheat flour, ambrosia, cumin, cinnamon, their mixture or their mixture mixed with 5% (w/w) of either sodium or calcium bicarbonate. These plants were selected according to their previously demonstrated antifungal activities (Zommara and Rashed, 2005). A negative control without treatment and two positive controls were performed. The positive control cheeses were treated with either 50 ppm of Delvocid or coated with the commercially available polyvinyl acetate (PVA) containing 0.05% natamycine (Ceskawl 500, Kaasdekmiddel, Sweeden. The experiment was carried out twice. The first experiment was carried out in Dameitta dairy plant (Misr Milk and Food Company). Ras cheese discs (one week old) were surface treated with one of the previously mentioned treatments and stored for 60 days for ripening under bad hygienic conditions. The bad hygienic condition was previously demonstrated by elevated moulds and yeasts counts in air of the ripening room (Zommara and Rashed, 2004). The

second experiment was carried out under relatively hygiene controlled conditions in the dairy pilot of the Department of Dairy Science, Kafr El-Sheikh University. The inhibition of fungal growth on cheese surface was visually evaluated by the staff members of Dameitta dairy plant (Misr Milk and Food Company) and by staff members of the Department of Dairy Science, Kafr El-Sheikh University. The growth of fungi on cheese surface was scored as a percent of the positive control cheeses coated with PVC.

Results and discussion

Data presented in Table (1) show the percent of mould growth on cheese surface treated with the EE or WE of the plants after 30 and 60 days of ripening under bad hygienic conditions. The data demonstrate that the plants EE or their mixtures were more effective in inhibition the fungi growth than that of WE after one or two months of ripening. In this respect, the EE of cinnamon were more effective than that of the other plants. The plants EE mixture inhibited moulds growth by 90% and 60% after 30 and 60 days of ripening, respectively. Addition of sodium bicarbonate by 5% to the plant mixtures increased its antimycotic effect to 95% and 80%, respectively. This may be attributed to change in the micro-environmental condition on cheese surface i.e. elevation of the pH to unsuitable value for fungi growth. In this respect, sodium bicarbonate salt was more effective than calcium carbonate salt which may be explained by the less solubility of calcium carbonate. Both of Delvocid or PVA treatments exhibited strong antifungal effect compared to the other treatments. However coating cheese with PVA was more effective as it prevented the contact between cheese and the surrounding environment. Also, it created anaerobic condition on cheese surface that prevented the growth of the obligatory aerobic fungi. The bad hygienic conditions presented in the ripening rooms of this experiment are inflected in the fungi growth on cheese surface. Table (1) also shows that the untreated cheese discs were highly contaminated with moulds that covered about 50% and 90% of the cheese surface after 30 and 60 days of ripening period, respectively. These findings leads us to examine the same treatments but under good and controlled hygienic conditions in our faculty milk pilot.

The data in Table (2) shows the growth inhibition of fungi on Ras cheese surface during 60 days of ripening under controlled hygienic condition. It is clear that the plant EE exhibited high antimycotic activity during 30 and 60 days of ripening period. These extracts completely suppressed the growth of moulds and yeasts on cheese surface except for that treated with ambrosia extract which showed some spots of mould growth after 60 days of storage. Under such hygienic conditions, the negative control cheese (without treatment) had a little mould growth (10%) after 30 days increased to 20% after 60 days of ripening. Also, cheese coating by PVA or by 50 ppm of delvocid completely

Table (1). Fungi growth on Ras cheese surface treated with different plant extracts and stored under bad hygienic conditions for 30 and 60 days.

Cheese treatments	Mould growth (%)	
	30 days	60 days
Positive control		
PVC coating	0	0
Delvocid (50 ppm)	5	10
Negative control	50	90
Ethanolic extracts		
Cinnamon	10	40
Cumin	20	50
Ambrosia		
Wheat flour	20	50
Extracts mixture	20	50
Extracts mixture with 5% NaHCO3	10	40
Extracts mixture with 5% CaCO3	5	20
Water extracts		
Cinnamon	10	40
Cumin	30	70
Ambrosia	30	70
Wheat flour	30	70
Extracts mixture	10	40
Extracts mixture with 5% NaHCO3	10	30
Extracts mixture with 5% CaCO3	40	60

2 cheese discs per treatment

prevented the growth of fungi on cheese surface during the ripening period. Addition of 5% sodium bicarbonate to the WE mixture completely inhibited the mould growth during 30 and 60 days of ripening. However such effect was not found when the cheese was treated with 5% calcium carbonate, which had some mould growth spots after 60 days of ripening. Table (2) also shows that cinnamon and wheat flour extracts were more effective to prevent moulds growth than cumin and ambrosia extracts. It is obvious from the results illustrated in Tables (1) and (2) that the hygienic conditions of Ras cheese ripening rooms is of a great importance.

Table (2). Fungi growth on Ras cheese surface treated with different plant extracts and stored under good hygienic conditions for 30 and 60 days.

cheese treatments	mould growth (%)	
	30 days	60 days
positive control		
pvc coating	non	non
delvocid (50 ppm)	non	non
negative control		
ethanolic extracts	10	20
cinnamon	non	non
cumin	non	5
ambrosia	non	10
wheat flour	non	non
extracts mixture	non	non
extracts mixture with 5% nahco3	non	non
extracts mixture with 5% caco3	non	non
water extracts		
cinnamon	non	non
cumin	5	10
ambrosia	5	10
wheat flour	non	5
extracts mixture	non	5
extracts mixture with 5% nahco3	non	non
extracts mixture with 5% caco3	non	5

2 cheese discs per treatment

Effect of some plants acetone extracts or commercially available herbs and spices oils on moulds and yeasts growth *in vitro*.

The antimycotic effect of the essential oils extracted by acetone of anise, cina, cinnamon, cumin, ambrosia, galangal and sage were examined on the most predominant moulds and yeasts isolated from Ras cheese (Zommara and Rashed, 2005). The paper disk diffusion assay was adapted as previously mentioned. The results are shown in Table (3). The essential oils extracted from cinnamon and cumin were very effective for inhibition of moulds growth followed by galangal and sage. As seen in figure (1), the AE of cinnamon was effective to inhibit the

Table (3). *In vitro* growth inhibition zone diameter (cm) of some isolated moulds and yeasts by plants acetone extracts.

Strain	Ambrosia	Cinnamon	Anise	Cina	Cumin	Galangal	Sage
Penicillium spp	0.0	3.4	0.0	0.0	1.2	0.5	1.1
Asp. flavus	0.0	2.3	0.0	0.0	1.2	0.0	0.0
Asp. parasiticus	0.0	2.[illegible]	0.0	0.6	1.7	0.4	0.0
Cl. herbarum	0.0	2.[illegible]	0.0	0.0	2.2	2.0	1.6
F. roseom	0.0	1.5	0.0	0.0	2.5	0.3	0.2
Asp. candidus	0.0	2.3	0.0	0.0	1.0	0.3	0.0
Sac. cervisiae	9.0	9.0	0.0	9.0	9.0	0.0	0.0

growth of different kinds of moulds as measured by the disk diffusion assay. Oils extracted from ambrosia, cinnamon, cina and cumin completely inhibited the growth of *Saccharomyces cervisiae*. Shetty *et al.* (1994) and Meena and Sethi (1994) stated that *Asp. niger, Asp. flavus, Asp. parasiticus, Pen. chrysogenum, S. cerevisiae* and *Candida utilis* were sensitive to cumin volatile oil. The antimycotic activities of oils extracted from cinnamon, galangal and sage are demonstrated in several studies (Morita and Itokawa, 1988: Farag *et al.*, 1989a and 1989b: Mehmood *et al.*, 1997; Ramesh and Tiwari, 1997: Kalemba, 1999 and Daferera *et al.*, 2000).

The commercial oils were found to have no antimycotic effect on the examined fungi strains. The extraction methods for these oils may be responsible for the loss of its antimycotic activity as the plant seeds always subjected to a frying process before pressing it. The severe heating of seeds during frying may affect the oil composition through the evaporation or decomposition of its volatile fatty acids and other active component. On the other hand, the strong odor of these oils limits its use in the treatment of Ras cheese surface as it may

adversely affect cheese sensory properties. In conclusion, the study demonstrated the importance of applying hygienic and sanitation procedures in Ras cheese production pilots, especially the huge production of Ras cheese is made from raw milk in a small cheese pilots located in the rural areas. However, under such bad hygienic conditions, the use of a

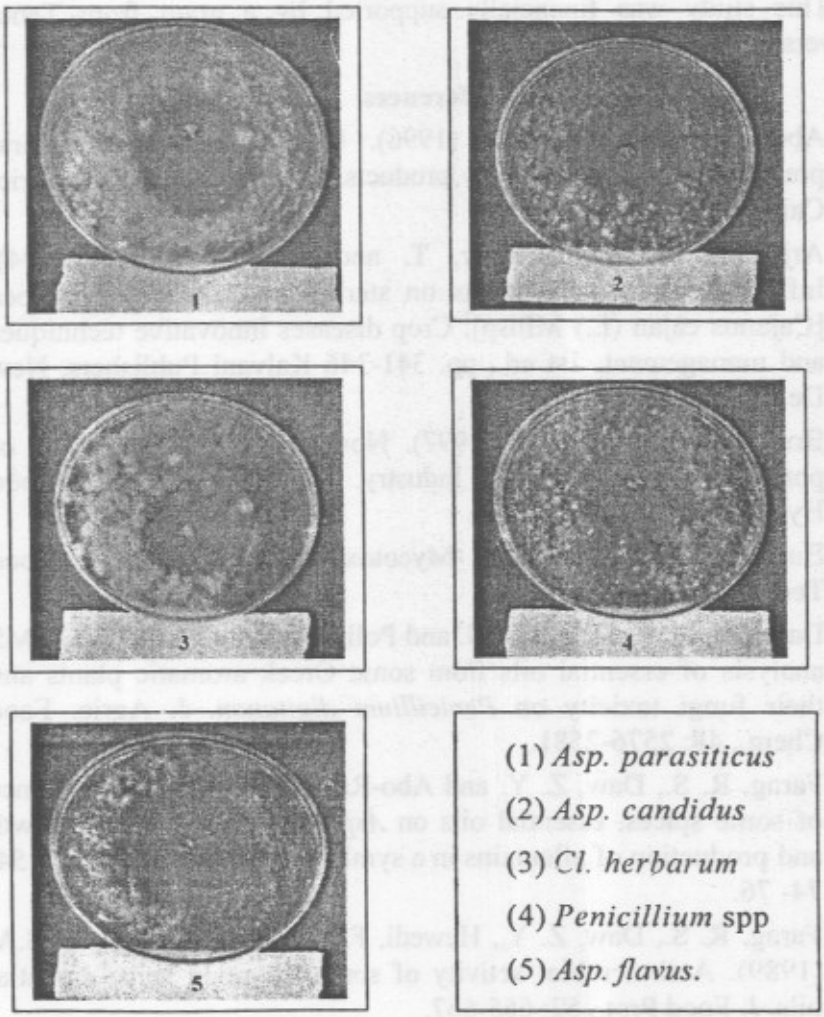

Fig. (1). The effect of cinnamon acetonic extract on the growth of different mould strains.

mixture of cinnamon, cumin, ambrosia and wheat flour mixed with 5% sodium bicarbonate would be more or less effective for the inhibition of fungi growth and therefore, it

may be recommended for treatment of Ras cheese surface before ripening or storing. Providing good hygienic conditions during cheese making and in cheese ripening rooms is of great importance and highly recommended to decreases the chance of cheese contamination with fungi and other microorganisms.

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الملخص العربى

المقدرة التثبيطية لبعض المستخلصات النباتيه على نمو الفطريات الملوثة لسطح الجبن الراس المخزنه تحت ظروف صحيه مختلفة

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أجريت هذه الدراسة لأستكمال دراسات سابقة قمنا بإجرائها للتعرف على الفطريات والخمائر التى تنمو على سطح جبن الراس أثناء عملية التسوية والتخزين ومدى تأثير البيئة المحيطة بها أثناء التصنيع والتسوية والتخزين على درجة تلوثها بهذه الكائنات الدقيقة وكذلك إمكانية استخدام بعض المستخلصات المائية لبعض الأعشاب والتوابل فى مقاومة الفطريات والخمائر على سطح الجبن والتى تسبب خسائر إقتصادية فادحة للصناع بالإضافة لما قد تسببه من مخاطر صحية للمستهلكين نتيجة إفرازها للسموم الفطرية. فى هذه الدراسة تم معاملة سطح الجبن الراس بمستخلصات مائية أو بمستخلصات كحول الإيثايل للقرفة والكمون والدمسيسه ودقيق القمح أو خليط متجانس من هذه المستخلصات مع أو بدون إضافة ٥% بالوزن بيكربونات الصوديوم أو كربونات الكالسيوم. تم تخزين الجبن للتسوية تحت ظروف صحية سيئة وكذلك تحت ظروف صحية جيدة ومتحكم بها لحد كبير لمدة ٦٠ يوم وذلك للتعرف على مدى كفاءة المعاملات فى مقاومة نمو الأعفان تحت ظروف صحية مختلفة. وقد تم تقدير كفاءة المعاملات السابقة عن طريق مقارنة كمية النموات الفطرية السطحية على الجبن بأقراص من الجبن الغير معامل (مقارنة سلبية) وكذلك بأقراص من الجبن المعامل أما بالمضاد الفطرى التجارى دلفوسيد (٥٠ جزء فى المليون) أو بالطلاء بمادة PVC المحتوية على المضاد الفطرى ناتاميسين بتركيز ٠,٠٥%. لقد أوضحت النتائج أن المستخلصات الكحولية كانت أكثر فاعلية فى مقاومة الأعفان من المستخلصات المائية. وأظهر المستخلص الكحولى للقرفة كفاءة عالية عن باقى المستخلصات كما أن إضافة بيكربونات الصوديوم لمخلوط المستخلصات زاد من فاعليتها وكان أفضل من كربونات الكالسيوم. أوضحت النتائج أهمية أتباع إجراءات وأساليب صحية أثناء تصنيع وتسوية وتخزين الجبن الراس حيث إنخفض تأثير المستخلصات فى الظروف الصحية السيئة عن الأخرى المتحكم فيها. أوضحت نتائج استخدام المستخلصات الأسيتونية للينسون والشيح والقرفة والكمون والدمسيسة والخلنجان والمرمرية على الفطريات الشائعة على سطح الجبن الراس والنامية على بيئة أجار البطاطس والدكستروز فى أطباق بترى أن مستخلصات القرفة والكمون كانت الأكثر كفاءة تلاها الخلنجان والمرمرية، بينما منعت الدمسيسة والقرفة والشيح والكمون نمو الخميرة *Saccharomyces cervisiae* تماما. هذا ولم تظهر الزيوت النجارية للينسون والشيح والقرفة والكمون والحبة السوداء والمرمرية أى قدرة على مقاومة الفطريات والخمائر النامية على الأطباق وقد يرجع ذلك إلى فقد المواد الفعالة بها اثناء عملية الأستخلاص والتجهيز.