

Anti-Yeast Effects of Some Plant Extracts on Yeasts Contaminating Processed Poultry Products in Egypt

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Abstract

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A total of 60 random samples of fresh chicken burger, fillet, and luncheon (20 of each) were collected from markets at Tanta city. The average total yeast counts (cfu/g) in burger, fillet, and luncheon samples were $2.7 \times 10^6 \pm 1.1 \times 10^6$, $2.1 \times 10^5 \pm 0.9 \times 10^5$, and $1.4 \times 10^7 \pm 0.7 \times 10^7$, respectively. A total of 158 yeast isolates of 23 species were isolated and identified. *Candida*, *Cryptococcus*, *Debaromyces*, *Issatchenkia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Trichosporon* and *Yarrowia* species were recovered from the examined samples of fresh chicken meat products in varying percentages ranging from 5% to 50%. The tested plant extracts of cinnamon, clove and thyme revealed a potent anti-yeast activity against *C. albicans*, *D. hansenii* and *S. cerevisiae* at 20% concentration, and a moderate inhibitory activity against these yeast strains at 10% concentration, while garlic extract had a lesser inhibitory effect on the yeast strains tested at the same concentration. Moreover, thyme, cinnamon and clove extracts had a complete inhibitory effect on chicken fillet inoculated with *Candida albicans* when incubated at 5°C and 25°C.

Keywords: chicken products; yeasts; plant extracts

Yeast enumeration in food is useful for the evaluation of the foods quality and the degree of deterioration and is becoming an essential component for the microbiological assurance programs (MARTA *et al.* 2001). Yeasts are generally not considered to be of major importance in the spoilage of meat products since their numbers in these products are highly variable compared to bacterial numbers (JAY & MARGITIC 1981).

From the economic point of view, yeasts may grow on the surfaces of meat and meat products causing sliminess, off odours, off tastes, and discolouration due to white, creamy, pink and brown pigments (FRAIZER & WEST HOFF 1978).

Yeasts have been isolated from the air and soil coming from poultry breeding and rearing houses,

old litter and litter-containing water, wet feed and bird droppings (BRYAN 1980). At the time of slaughtering, the feathers, feed and bodies of the birds have been found to be contaminated with yeasts (BARNES 1976). Yeasts make a significant contribution to the overall microbial ecology of poultry and may also contribute to the changes leading to spoilage (VILJOEN *et al.* 1998).

Most health authorities have considered the significance of yeasts in foods, including meat products, in view of the public health. The problem of food preservation has grown in complexity as new products are frequently introduced on the markets that require longer shelf life and greater assurance of protection from microbial spoilage (FARAG *et al.* 1989). Plant extracts used as flavour-

ing and seasoning agents in foods and beverages have been used therapeutically for centuries, the antimicrobial activity of garlic, cinnamon and cloves has been studied since the end of the last century (ELGAYYAR *et al.* 2001).

Natural antimicrobial compounds from plants are collectively called green chemicals for food preservation. On the toxicological basis, many herbs and spices are often known to be food grade or even GRAS (generally recognised as safe) (RAHMAN 1999).

The antimicrobial activities of extracts from several types of plants used as seasonings and flavouring agents for foods and beverages have been recognised for many years. Antimicrobial compounds in plant materials are contained in the essential oil fraction (CONNER 1994). However, most studies on the antimycotic activity of plant extracts have been conducted *in vitro*; consequently, little information exists regarding the practical use of such antimicrobial extracts in foods.

This work was designed to cover the following items:

(1) Study of the yeast populations contaminating the samples of chicken products examined.

(2) Screening of anti-yeast activity of some plant extracts against *Candida albicans*, *Debaromyces hansenii* and *Saccharomyces cerevisiae*.

(3) Study of the inhibitory activity of thyme, cinnamon and clove against *Candida albicans* in chicken fillets.

In this study, *Candida albicans* was chosen as an example of pathogenic yeasts causing health hazards for humans; further, both *Debaromyces hansenii* and *Saccharomyces cerevisiae* were selected for their spoilage effects in foods.

MATERIAL AND METHODS

Collection of samples. A total of 60 random samples of fresh chicken products, represented by chicken fillets, chicken burgers and chicken luncheons (20 of each), were collected from different markets at Tanta city, Gharbia governorate, where they were kept at room temperature. The samples were transferred to the laboratory in ice-boxes without undue delay.

Preparation of samples. 25 grams of each sample were mixed with 225 ml of sterile peptone water 0.1% and thoroughly homogenised under aseptic conditions, and then serially diluted to give a dilution of 10^6 (A.P.H.A. 1976).

Total yeast counts. The total yeast counts were determined using duplicate plates containing solidified malt extract agar; these were inoculated with 0.1 ml of food homogenate sample from each of the dilutions mentioned. The Petri dishes were incubated at 25°C for 3 days after which the yeast colonies were enumerated (A.P.H.A. 1976).

Isolation and identification of yeasts. Yeast colonies were isolated on sabouraud dextrose agar (BEUCHAT 1987), then kept for further identification. The isolated yeasts were identified morphologically and biochemically according to KREGER-VAN RIJ (1984).

Morphological examination:

(a) Growth on Sabouraud dextrose agar (BEUCHAT 1987),

(b) Vegetative reproduction was studied on rice agar medium (ROHDE *et al.* 1980),

(c) Growth at 37°C. The isolates were transferred into Sabouraud dextrose agar slopes and incubated at 37°C for 3–5 days and examined for growth.

Physiological examination:

(a) Pellicle formation (HARRIGAN & MCCANCE 1976),

(b) Germ tube test (KONEMAN *et al.* 1978),

(c) Urease production (CRUICKSHANK *et al.* 1975),

(d) Sugar assimilation and sugar fermentation (CRUICKSHANK *et al.* 1975),

(e) Nitrate assimilation (TERRENCE 1971).

Screening of anti-yeast activity by plant extracts *in vitro*:

Cinnamon, clove, garlic, and thyme extracts were purchased from commercial chemical and flavour shops. These plant extracts were screened for their ability to inhibit the growth of *Candida albicans*, *Debaromyces hansenii* and *Saccharomyces cerevisiae* on yeast extract-malt extract-peptone-glucose agar (YMPG) (CONNER & BEUCHAT 1984). The YMPG medium: peptone, 10 g glucose, and 20 g agar per liter of distilled water. The pH was adjusted to 5.5 with 6M HCl.

After sterilisation, the plates were poured on with the medium to a thickness of 5–6 mm and allowed to set at room temperature for 24 h to enable the surface of the medium to dry. The yeast cultures were activated by successive transfers in YMPG broth (pH 5.5; 100 ml per 250-ml Erlenmeyer flask) at 30°C. After 44–48 h of incubation on a rotary shaker, the cultures were diluted with 0.1M potassium phosphate buffer (pH 7.0) and 0.1 ml of the

diluted suspension was deposited onto each plate and spread uniformly with a sterile bent glass rod. Each of the plant extract tested was diluted with 95% ethanol to give concentrations of 5%, 10% and 20% (v/v). Sterile 6-mm diameter filter paper absorbent discs were dipped into the appropriate extract solution, blotted, and then placed on the surfaces of inoculated plates. The inhibitory effect of the 95% ethanol was also tested as control by placing discs saturated with 95% ethanol on each inoculated plate. The plates were incubated at 30°C for 4 days, after which the zones of the growth inhibition were measured. This method is recommended by CONNER and BEUCHAT (1984).

Efficacy of the plant extracts tested against *C. albicans* in fresh chicken fillets *in vivo*:

Fresh chicken fillet pieces were obtained from local markets at Tanta city, sliced to 2.5 cm thick pieces, and sterilised by U/V light (60 watt germicidal bulb; 51 cm distance from the fillet piece for 20 min). Each individual piece of fillet was attached to sterile jaw clips and inoculated by *C. albicans* strain by dipping or submerging it into 10 ml of a cell suspension, containing $9 \log_{10}$ cfu/ml, for 15 min at 25°C, hung in a covered sterile beaker and incubated at 5°C and 25°C for 1, 2, 4 and 7 days after which the yeast populations were enumerated (CUTTER & SIRAGUSA 1995). After treatment with cinnamon, clove and thyme extracts diluted 1:5 in 100% ethanol (DEANS & RITCHIE 1987), the fillet pieces were incubated at 5°C and 25°C for 1, 2, 4 and 7 days and subsequently homogenised in 0.1% sterile peptone water. Serial dilutions were made and samples were plated in malt extract agar. The yeast populations on the plates were enumerated after incubation for 1, 2, 4 and 7 days according to (A.P.H.A. 1976).

RESULTS AND DISCUSSION

The determination of the contamination of food ingredients and of processed foods with yeasts is

an essential part of any quality assurance or quality control program in the food industry (DEAK & BEUCHAT 1996).

The data obtained (Table 1) showed that chicken luncheon and burger samples were the most contaminated samples by yeasts with an average of $1.4 \times 10^7 \pm 0.7 \times 10^7$ cfu/g and $2.7 \times 10^6 \pm 1.1 \times 10^6$ cfu/g, respectively, while the average in chicken fillet samples was $2.1 \times 10^5 \pm 0.9 \times 10^5$ cfu/g. Regardless of the type of the chicken product samples examined, the maximum yeast count was 11.3×10^7 cfu/g, while the minimum count was 1.5×10^2 cfu/g. Nearly similar results were obtained by JAY (1978), EDRIS *et al.* (1992) and MAHMOUD and EL-TAHER (2001). The highest yeast count in the examined samples may be attributed to the use of raw chicken meat of bad quality as well as unhygienic conditions during slaughtering, handling, processing, storage, or transport of these chicken products. WALKER (1977) mentioned that high numbers of yeasts lead to undesirable changes in foods, and that yeasts significantly contribute to lipolytic and proteolytic changes in foods even in small counts. On the contrary, ISMAIL (1995) found that the mean of the total mesophilic yeast count in luncheon samples was $5.7 \times 10^2 \pm 2.1 \times 10^2$ cfu/g while the average of the yeast counts recorded by SAMAHA and ABD EL-HAFEIZ (1997) was $1.4 \times 10^3 \pm 3.7 \times 10^2$ cfu/g and $2.2 \times 10^3 \pm 4.1 \times 10^2$ cfu/g in chicken fillets and luncheons, respectively. Yeast and mould counts in meat and meat products were used as a sanitary index for the quality of meat and processed meat products (HEFNAWY 1978).

The results in Table 2 revealed that 158 yeast isolates represented 23 species isolated from the samples examined in different numbers and percentages. The isolated yeast genera were *Candida*, *Saccharomyces*, *Trichosporon* and *Yarrowia Cryptococcus*, *Debaromyces*, *Issatchenkia*, *Pichia* and *Rhodotorula*.

The most prevalent and predominant yeast strains isolated from the fresh chicken burger samples were *S. exiguus* (50%), *Y. lipolytica* (45%), *C. albi-*

Table 1. Statistical analytical results of yeast counts (cfu/g) of examined fresh chicken product samples collected from different markets in Tanta city ($n = 20$)

Product	Minimum	Maximum	Mean \pm SE
Fillet	1.5×10^2	13.6×10^5	$2.1 \times 10^5 \pm 0.9 \times 10^5$
Burger	1.9×10^2	9.2×10^6	$2.7 \times 10^6 \pm 1.1 \times 10^6$
Luncheon	6.4×10^2	11.3×10^7	$1.4 \times 10^7 \pm 0.7 \times 10^7$

Table 2. Incidence of isolated yeasts from fresh chicken product samples collected from different markets in Tanta city

Yeast species	Fillet		Burger		Luncheon	
	No.	%	No.	%	No.	%
<i>Candida</i> species						
<i>C. albicans</i>	1	5	2	10	–	–
<i>C. glabrata</i>	–	–	–	–	6	30
<i>C. rugosa</i>	3	15	3	15	2	10
<i>C. parapsilosis</i>	1	5	–	–	1	5
<i>C. zeylanoides</i>	–	–	5	25	7	35
<i>C. sake</i>	1	5	6	30	–	–
<i>C. stellate</i>	–	–	–	–	2	10
<i>C. catenulata</i>	–	–	1	5	1	5
<i>Cryptococcus</i> species						
<i>C. albidus</i>	4	20	7	35	5	25
<i>C. humicolus</i>	1	5	3	15	1	5
<i>C. laurentii</i>	1	5	4	20	3	15
<i>C. intermedia</i>	4	20	2	10	1	5
<i>C. scottii</i>	2	10	–	–	–	–
<i>Debaromyces</i> species						
<i>D. hansenii</i>	1	5	4	20	8	40
<i>Issatchenkia</i> species						
<i>Issatchenkia orientalis</i>						
<i>I. orientalis</i>	–	–	4	20	1	5
<i>Pichia</i> species						
<i>P. anomalis</i>	–	–	3	15	–	–
<i>P. fermentans</i>	–	–	–	–	2	10
<i>Rhodotorula</i> species						
<i>R. glutinis</i>	1	5	2	10	1	5
<i>R. pallida</i>	1	5	4	20	2	10
<i>Saccharomyces</i> species						
<i>S. cerevisiae</i>	–	–	2	10	–	–
<i>S. exiguus</i>	–	–	10	50	–	–
<i>Trichosporon</i> species						
<i>T. pullulans</i>	2	10	1	5	8	40
<i>Yarrowia</i> species						
<i>Y. lipolytica</i>	5	25	9	45	7	35

dus (35%), then *C. sake* (30%), and *C. zeylanoides* (25%); and from the luncheon samples *T. pullulans* (40%), *Y. lipolytica* (35%), *D. hansenii* (40%),

C. parapsilosis (5%), then *C. glabrata* (30%) and *C. albidus* (25%) while those isolated from fillet samples were *Y. lipolytica* (25%), *C. albidus* (20%),

C. intermedia (20%), *C. scottii* (10%), then *C. rugosa* (15%); furthermore, the fillet samples were free of *C. glabrata*, *C. zeylanoides*, *C. stellate*, *C. catenulata*, *I. orientalis*, *P. anomalis*, *P. fermentans*, *S. cerevisiae* and *S. exiguus*. While burger samples were free of *C. glabrata*, *C. parapsilosis*, *C. stellate*, *C. scottii*, luncheon samples were free of *C. albicans*, *C. sake*, *C. scottii*, *C. anomalis*, *S. cerevisiae* and *S. exiguus*. Both fillet and burger samples were free of contamination with *C. glabrata*, *C. stellate*, while mean burger and luncheon samples did not contain *C. scottii*.

Both *R. glutinis*, and *R. palida* were isolated from one fillet sample at a low percentage (5%). These results indicate that the fresh chicken burger and luncheon samples were more contaminated with various species of yeast strains than were the fillet samples; this may be due to the presence of yeasts and moulds in the meat products indicating bad hygienic measures during the processing steps and handling. The high proportion of the isolated *Candida*, *Debaromyces* and *Yarrowia* species were in agreement with those observed by VILJOEN *et al.* (1993, 1998), ABOU-ARAB (1995), ISMAIL (1995), MAHMOUD & EL-TAHER (2001) and BASYONI (2003). In view of the significance for public health, *Candida albicans* is one of the etiological agents of thrush and the pathological conditions of white patches in the mouth, throat and oesophagus (WILSON *et al.* 1981), while, meningitis pulmonary diseases and cutaneous infections were produced by *Cryptococcus albidus* and *Cryptococcus laurentii* (LYNCH *et al.* 1981). Moreover, yeast affections lead to certain defects that may change the flavours and quality of meat products rendering them unmarketable or inappropriate for human consumption (JAY 1978).

Anti-yeast activities of cinnamon, clove, garlic and thyme extracts against *C. albicans*, *D. hansenii* and *S. cerevisiae*, *in vitro* are shown in Table 3. These activities ranged from no inhibition to slight or mild and eventually strong inhibition of the ye-

ast cultures tested. CONNER and BEUCHAT (1984) classified the inhibitory effects of essential oils according to their activity as strongly active (inhibition zone, > 11 mm), moderately active (inhibition zone, < 6 to < 11 mm), or inactive (inhibition zone, < 6 mm). Cinnamon, clove and thyme were strong inhibitors against the yeasts strains tested at 20% level; overall, thyme extract was the most potent extract. *Candida albicans* resists the inhibitory effect of cinnamon at 5% but is slightly inhibited by clove, garlic and thyme at the same level 5%; garlic cannot inhibit *D. hansenii* and *S. cerevisiae* either while only *D. hansenii* resists clove at 5%. Thyme, clove and cinnamon have potent anti yeast effects on *C. albicans*, *D. hansenii* and *S. cerevisiae* at 20% levels. All extracts under test are moderate inhibitors of yeast strains at 10%. Garlic has a lesser inhibitory effect on *C. albicans*, *D. hansenii*, and *S. cerevisiae* than thyme, cinnamon and clove. Our results are nearly similar to those given by MOORE and ATKINS (1977), CONNER and BEUCHAT (1984) and FARAG *et al.* (1989). Garlic has been used as a folk medicine since ancient times for a variety of ills including snakebite, hemorrhoids, rheumatism, abdominal pains and parasitic infections (MOORE & ATKINS 1977). So, the antimicrobial activities of cinnamon, clove and thyme against the yeast cultures tested suggest that they may be useful in chicken products formulations as preservatives extending the shelf life of these chicken products. Among the compounds having wide spectra of antimicrobial activities are thymol from thyme and oregano, cinnamaldehyde from cinnamon, and eugenol from clove (NYCHAS 1995).

The food industry relies heavily upon the use of antimicrobial agents for the extension of shelf life and the preservation of the freshness of products. Because of governmental restrictions and consumer demands, it is desirable to optimise the use of antimicrobials in foods (DAVIDSON & PARISH 1989).

The minimum and maximum total counts of *C. albicans* inoculated chicken fillet treated with

Table 3. Inhibition zones (mm) of yeast growth by plant extracts (in %, v/v)

Yeast species	Ethanol			Cinnamon			Clove			Garlic			Thyme		
	95	5	10	20	5	10	20	5	10	20	5	10	20		
<i>Candida albicans</i>	3	0	13	22	9	15	19	4	9	13	8	19	22		
<i>Debaromyces hansenii</i>	1	8	11	17	0	12	23	0	3	11	12	16	26		
<i>Saccharomyces cerevisiae</i>	2	7	13	19	7	11	16	0	13	19	13	21	29		

Table 4. *Candida albicans* populations in chicken fillet samples treated with cinnamon, clove and thyme extracts (20%) and incubated at 5°C and 25°C

Days	Control		Cinnamon				Clove				Thyme			
	5		5		25		5		25		5		25	
	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
1	4.1×10^6	5.1×10^6	1.6×10^2	1.4×10^6	1.1×10^2	1.8×10^6	2.3×10^2	3.3×10^5	2.6×10^2	9×10^5	3.6×10^2	11×10^6	2.1×10^2	5×10^5
2	3.9×10^6	5.5×10^6	1.3×10^2	7×10^5	8×10^1	2×10^6	1.7×10^2	1.8×10^5	1.3×10^2	5×10^5	2.5×10^2	3×10^4	1.9×10^2	2×10^5
4	3.7×10^5	6.1×10^6	3.4×10^2	1.8×10^6	1.2×10^2	1.6×10^6	1.3×10^2	5×10^5	1.1×10^2	2.9×10^5	1.4×10^2	1×10^4	1.4×10^2	1×10^4
7	3.1×10^6	6.3×10^6	6×10^1	2.2×10^5	5×10^1	7×10^5	0.0	0.0	3.6×10^2	2×10^5	1.8×10^1	2×10^4	9×10^1	2×10^4

cinnamon, clove and thyme plant extracts are presented in Table 4. The results obtained indicated that the application of clove extract at 20% resulted in the lowest count of *C. albicans* in inoculated chicken fillet samples after 7 days of incubation at 5°C when compared with cinnamon and thyme extracts. However, cinnamon extract at 25°C produced the lowest count, followed by thyme, and clove came in the last rank after 7 days of treated chicken fillet samples.

The results represented in Table 5 revealed that the clove extract was more effective in controlling *C. albicans* in inoculated fillet samples (91.95%), on 1st day of incubation at 5°C than cinnamon (65.85%) and thyme (73.17%), respectively. Clove and thyme extracts caused complete inactivation (100%) of *C. albicans* at the same temperature on 7th day of incubation.

It was reported that no clear differences exist in the inactivation of *C. albicans* by cinnamon, clove and thyme extracts when incubated between 5°C, 25°C, so the application of herbs and spices or their extracts on foods may provide a protection against improper storage temperature (EVERTING & DEIBEL 1992). The extracts from spi-

ces and herbs usually contain more than a single compound possessing antimicrobial activity. In addition, spice and herb extracts are widely used in the food industry and considered GRAS. Hence, both consumers and regulatory bodies would be likely comfortable with their use in foods (HAO *et al.* 1998).

The high contamination of the fresh chicken products examined may be attributed to the use of chicken meat of inferior quality in processing, to unhygienic measures during manufacture, handling and storage at ordinary temperatures (room temperatures), in addition to the use of contaminated food additives such as spices and herbs. To avoid this contamination, good quality raw chicken meat must be used in manufacture, furthermore, strict hygienic measures must be taken during slaughtering of chicken and the subsequent processing, handling and storage. Moreover, educational programs and training courses should be recommended to meat handlers and workers. In addition to the sanitary rules including personal hygiene, cleaning and disinfections of utensils and equipments should be adopted with periodical checks of the meat products. Our data suggest

Table 5. Inhibition percentages of *Candida albicans* under the effect of cinnamon, clove and thyme extracts at 20% (v/v) at 5°C and 25°C

Time (days)	Cinnamon		Clove		Thyme	
	5	25	5	25	5	25
1	65.85	47.06	91.95	82.35	73.17	90.20
2	82.05	63.64	95.35	90.91	97.95	96.36
4	81.08	73.78	86.49	95.25	99.46	99.84
7	92.90	88.89	100.00	96.83	100.00	99.68

that plant extracts under study, i.e. cinnamon, clove and thyme extracts, can be applied practically as anti-yeast agents in the food treatments that will inhibit the deterioration of fresh chicken products by yeasts.

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