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Antifungal activity of octyl gallate

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Abstract

Antifungal activities of propyl (C_3), octyl (C_8) and dodecyl (C_{12}) gallates (3,4,5-trihydroxybenzoate) were tested against *Saccharomyces cerevisiae* ATCC7754 and *Zygosaccharomyces bailii* ATCC 60483. Octyl gallate was found to be the only active compound with the minimum fungicidal concentration of 25 μg/ml (89 μM) against *S. cerevisiae* and of 50 μg/ml (177 μM) against *Z. bailii*, respectively. The inactivation study showed that octyl gallate was fungicidal against both *S. cerevisiae* and *Z. bailii* at any stage of growth. These fungicidal activities were not influenced by pH values. Octyl gallate at 100 μg /ml reduced plasma membrane fluidity to 48% of control. On the other hand, dodecyl gallate at the same concentration reduced it to 76% of control. Only octyl gallate inhibited glucose-induced medium acidification, indicating direct or indirect inhibition of plasma membrane H^+-ATPase. The primary fungicidal activity of octyl gallate comes from its ability to act as a nonionic surface-active agent (surfactant), though it can not be inferred that membrane damage, such as a decrease in the membrane fluidity, is the only cause of the lethal effect.

Keywords: Antifungal activity; *Saccharomyces cerevisiae*; *Zygosaccharomyces bailii*; Octyl gallate; Surfactant property; Antioxidant activity

1. Introduction

Yeast fermentation is involved in the manufacturing of foods, such as bread, beer, wine, vinegar and surface-ripened cheese. Most yeasts of industrial importance are of the genus *Saccharomyces* and mostly of the species *S. cerevisiae*. These ascospore-forming yeasts are readily bred for desired characteristics. However, yeasts are undesirable when they cause spoilage to sauerkraut, fruit juice, syrup, molasses, honey, jelly, meat, wine, beer and other foods (Fleet, 1992). Finishing process of the fermentation is usually either through filtration or pasteurization. However, the use of the latter is limited to certain foods, since it is a heat treatment and, hence, denaturalizes proteins, and the former is also limited to clear liquids. Neither processes can be applicable to some foods, such as sauerkraut and ‘‘miso’’ (soy bean pastes).

Zygosaccharomyces bailii is a food spoilage yeast species. It is known for its capacity to survive in stress environments and, in particular, in acid media with ethanol, such as in wine (Fleet, 1992). In addition, spoilage of mayonnaise and salad dressing by this osmophilic yeast is well described (Smittle, 1977; Smittle and Flowers, 1982). Therefore, safe and effective fungicides are still needed to control yeasts.

Safety is a primary consideration for antifungal agents, especially for those in food products, which may be utilized in unregulated quantities on a regular basis. During our systematic structure and antimicro-

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bial activity relationship (SAR) study, we became aware that octyl gallate possesses a broad antimicrobial spectrum. The antifungal activities of octyl gallate against *S. cerevisiae* and *Z. bailii*, as well as *Candida albicans* and *Aspergillus niger*, was previously communicated in part (Kubo et al., 2001) and is now described in full.

2. Materials and methods

2.1. General

The procedures used for antimicrobial assay were the same as previously described (Kubo and Himejima, 1992; Kubo et al., 1995).

2.2. Chemicals

Gallic acid, propyl gallate, octyl gallate, dodecyl gallate, octanol, benzoic acid, sorbic acid, octanoic acid, 4-hydroxybezoic acid and 3,4-dihydroxyzenzoic acid were purchased from Aldrich Chemical (Milwaukee, WI). Octyl esters of benzoic acid, 2-hydroxybenzoic acid, 3-hydroxybenzoic acid, 4-hydroxy-benzoic acid, 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid were prepared as described previously (Kubo et al., 2001). Both octyl and dodecyl gallates were recrystallized prior to use. 3-(*N*-Morpholino) propane-sulfonic acid (MOPS) was purchased from Fisher Biotech (Fair Lawn, NJ). *N*,*N*-dimethylformamide (DMF) was obtained from EM Science (Gibbstown, NJ).

2.3. Test strains

The test strains, *S. cerevisiae* ATCC 7754 and *Z. bailii* ATCC 60483, used in this study were purchased from American Type Culture Collection (Rockville, MD).

2.4. Media

Both *S. cerevisiae* and *Z. bailii* were maintained at − 80 °C in yeast nitrogen broth (YNB; Difco Lab., Detroit, MI) containing 25% glycerol and subcultured at 30 °C in Sabouraud's dextrose agar medium (Bactopeptone 1%, Dextrose 4%, Bacto-agar 1.8%). A fresh culture was incubated with shaking for 16 h at 30 °C in 2.5% malt extract (ME) broth (BBL) medium prior to the following experiments.

2.5. Antifungal assay

Minimum inhibitory concentrations (MICs) were determined as previously described (Kubo and Himejima, 1992). Serial 2-fold dilutions of the tested compounds were prepared in DMF. And then 30 μl of 100-fold concentration solution was added to 3 ml of ME broth for *S. cerevisiae* ATCC 7754 or YPD (1% yeast extract, 2% bactopeptone, 2% dextrose) broth for *Z. bailii* ATCC 60483 unless otherwise stated. These were inoculated with 30 μl of culture to give a final inoculum size of 10^5 colony forming units (CFU)/ml. The cultures were incubated without shaking at 30 °C for 48 h. MIC was the lowest concentration of test compound that showed no visible growth. Minimum fungicidal concentrations (MFCs) were examined as follows. After determining MIC, a 30-μl of aliquot was withdrawn from each culture and then added to 3 ml of a fresh ME or YPD broth. After 48-h incubation, MFC was determined as the lowest concentration of the test compounds in which no recovery of microorganism was observed.

2.6. Inactivation study

Exponentially growing cells were inoculated to 3 ml of ME and YPD broth supplemented with or without test chemicals for *S. cerevisiae* and *Z. bailii*, respectively. The cultures were incubated at 30 °C without shaking. At selected time intervals, the number of viable cells was determined by dilution plate counting on YPD agar. The plates were incubated at 30 °C for 48 h.

2.7. Adsorption test

The cells of *S. cerevisiae* were cultured with shaking in YPD broth at 30 °C overnight and then washed twice with 50 mM MOPS buffer (pH 6.0). After each gallate ester was mixed with or without the yeast cells (10^8 CFU/ml) in the same buffer at 30 °C, the suspension was vortexed for 5 s. Absorbance in the supernatants obtained by centrifugation for 2 min was measured at 272 nm.

2.8. Plasma membrane fluidity

Plasma membrane fluidity was detected by pyrene excimer fluorescence (Fujimoto et al., 1999). The exponentially grown *S. cerevisiae* cells in YPD broth were washed with phosphate buffered saline (PBS). The washed cells were incubated with shaking in PBS at 30 °C for 30 min. The cells were washed and then incubated in 20 mM potassium phosphate buffer (pH 7.4) containing 1.2 M D-sorbitol (lysis buffer). Zymolyase 20 T (Seikagaku, Japan) was added to the suspension to lyse the cell wall of the yeast cells. The spheroplast cells obtained were washed and then suspended in lysis buffer. Three milliliters of cell suspension (1×10^7 CFU/ml) was preincubated in lysis buffer with 10 mM pyrene at 30 °C for 1 min. After 100 μg/ml of each gallate ester was added to the suspension, the suspension was incubated for another 3 min. The intensities of monomer fluorescence of pyrene at 386 nm (I_M) and of excimer fluorescence at 480 nm (I_E) were collected with excitation at 340 nm, respectively. The ratio of intensities, I_E/I_M, is increased by pyrene collision related to the fluidity of the plasma membrane. Therefore, the membrane fluidity was evaluated by I_E/I_M. Values are means ± S.D. ($n = 3$).

2.9. Medium acidification

The glucose-induced medium acidification of *S. cerevisiae* was measured with a modified procedure (Haworth et al., 1993). The test strain was cultured with shaking in YPD broth at 30 °C overnight and then washed twice with cold distilled water. The cells were diluted to 10^8 CFU/ml with cold distilled water and then kept on ice. The reaction mixture contained 2.7 ml of cell suspensions and 30 μl of the DMF sample solution, and was preincubated at 30 °C for 5 min. A 20% glucose solution of 0.3 ml was added (final 2%) to induce acidification. The pH value of external medium was checked.

3. Results

3.1. Antifungal action of octyl gallate

In our continuing search for antifungal agents, propyl, octyl and dodecyl gallates, which are currently permitted to use as antioxidant additives of foods in USA (Aruoma et al., 1993), together with their related compounds were tested against *S. cerevisiae* and *Z. bailii*. The results are listed in Table 1. Among the three tested gallates, octyl (C_8) gallate was found to be the only active compound with a MFC of 25 μg/ml (89 μM) against *S. cerevisiae* and of 50 μg/ml (177 μM) against *Z. bailii*, respectively. It also exhibited antifungal activity against *Aspergillus niger* with a MFC of 100 μg/ml (355 μM). Interestingly, no differences in MIC and MFC against both yeasts were noted, suggesting that no residual fungistatic activity was involved. In contrast, neither propyl nor dodecyl gallates showed any fungicidal activity up to 1600 μg/ml against both yeasts. The length of alkyl group in the gallates is associated with their antifungal activity, similar to those found in alkanols (Kubo et al., 1995). The antifungal activity disappeared after the alkyl length reached the maximum (the so-called "cutoff" phenomenon), and the dodecyl group in dodecyl gallate is beyond this point. On the other hand, propyl group in propyl gallate is not long enough to elicit the activity.

Among the food preservatives currently used, sorbic acid and its salts are some of the most commonly used as yeast inhibitors (Sofos and Busta, 1983). Hence, the activity of octyl gallate was compared with those of sorbic acid. Despite their wide application in various foods, especially as growth inhibitors of yeasts and filamentous fungi, sorbates are generally static (Robach and Sofos, 1982). In our preliminary screening, sorbic acid needs 3200 μg/ml to exhibit

Table 1
Antifungal activities (μg/ml) of gallic acid and its esters, and selected compounds against *Z. bailii* and *S. cerevisiae*

Gallates tested	*Z. bailii*		*S. cerevisiae*	
	MIC	MFC	MIC	MFC
Gallic acid	>3200	>3200	>3200	>3200
Propyl gallate	3200	>3200	3200	>3200
Octyl gallate	50	50	12.5	25
Dodecyl gallate	>1600	>1600	>1600	>1600
Geranyl gallate	25	25	50	50
Octanol	50	50	50	50
Benzoic acid	800	1600	800	1600
Octanoic acid	100	400	100	400
4-Hydroxybenzoic acid	>3200	–	1600	>3200
3,4-Dihydroxybenzoic acid	>3200	–	3200	>3200

The cells of *Z. bailii* and *S. cerevisiae* were grown in YPD and 2.5% ME broth, respectively, at pH 6.0. –, Not tested.

Table 2
pH Effect on antifungal activities (μg/ml) of octyl gallate and sorbic acid against *Z. baili* and *S. cerevisiae*

pH	*Z. baili*				*S. cerevisiae*			
	Octyl gallate		Sorbic acid		Octyl gallate		Sorbic acid	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
3	6.25	12.5	400	1600	6.25	12.5	200	3200
5	12.5	12.5	800	>1600	12.5	12.5	400	3200
7	12.5	25	>1600	–	12.5	25	1600	>3200
9	12.5	25	>1600	–	12.5	25	3200	>3200

The cells were grown in 2.5% ME broth. –, Not tested.

lethal activity (MFC) against *S. cerevisiae*. This lack of potency limits use as a food preservative. The fungicidal activity of octyl gallate against *S. cerevisiae* is 128-fold more potent than that of sorbic acid. More importantly, octyl gallate has another superior property. As a weak acid antifungal agent, the antifungal activity of sorbic acid increases as pH of the substrate decreases. Therefore, the activity is pH dependent (Sofos and Busta, 1983; Brul and Coote, 1999) as shown in Table 2. At higher pH values (>5), sorbic acid did not show any antifungal activity up to 1600 μg/ml. In contrast, octyl gallate was active at any pH values tested. Interestingly, octyl gallate also inhibited the growth of *Z. bailii* with a MFC of 25 μg/ml at any pH values as listed in Table 2.

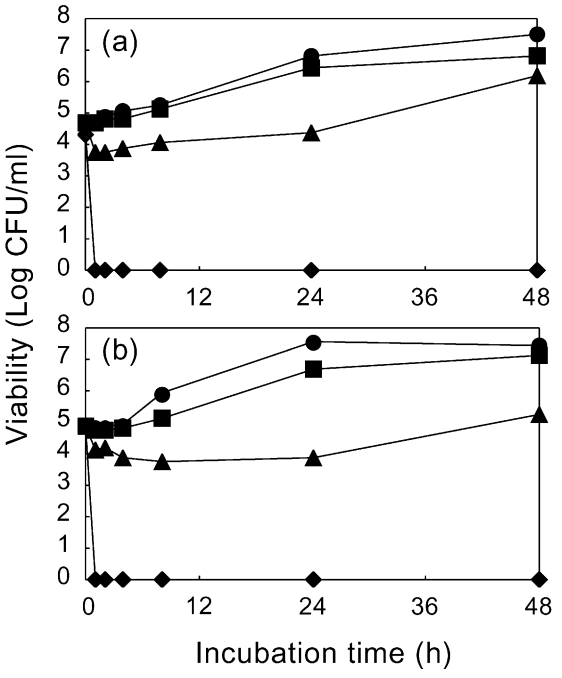

Fig. 1. Fungicidal effects of octyl gallate against (a) *Z. bailii* ATCC 60483 and (b) *S. cerevisiae* ATCC 7754. (a) Exponentially growing cells of *S. cerevisiae* were inoculated to ME broth and then cultured at 30 °C without shaking. Octyl gallate (μg/ml): 0 (●), 6.2 (■), 12.5 (▲) or 25 (◆). (b) Exponentially growing cells were inoculated to YPD broth and then cultured at 30 °C without shaking. Octyl gallate (μg/ml): 0 (●), 12.5 (■), 25 (▲) or 50 (◆). A representative result of at least two replicate experiments is shown.

3.2. Effect of growth phase on octyl gallate-induced cell death

The fungicidal effect of octyl gallate against *Z. bailii* was confirmed by the time kill study as shown in Fig. 1a. Cultures of *Z. bailii*, with a cell density of 5×10^4 CFU/ml, were exposed to three different concentrations of octyl gallate. The number of viable cells was determined following different periods of incubation with octyl gallate. The result verified that MICs and MFCs were the same. It shows that half MIC reduced the growth rate but the final cell count was not significantly different from the control. This

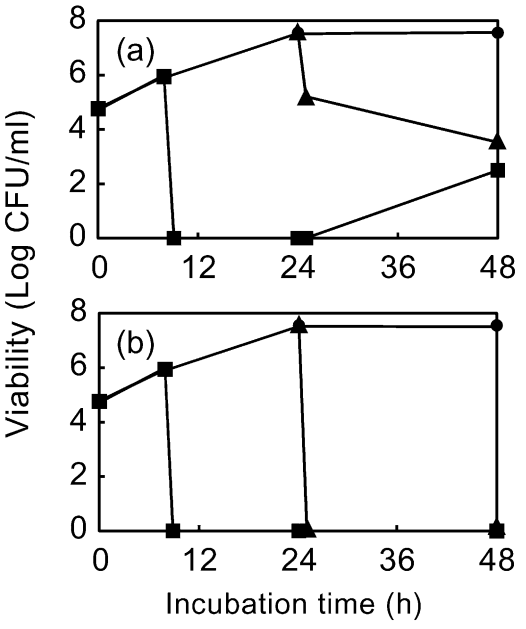

Fig. 2. Effect of growth phase on the octyl gallate-induced cell death of *S. cerevisiae* ATCC 7754. Octyl gallate was added at (a) 25 or (b) 50 μg/ml after 8-h (■) or 24-h (▲) cultivation. Control (●) indicates no addition of the compound.

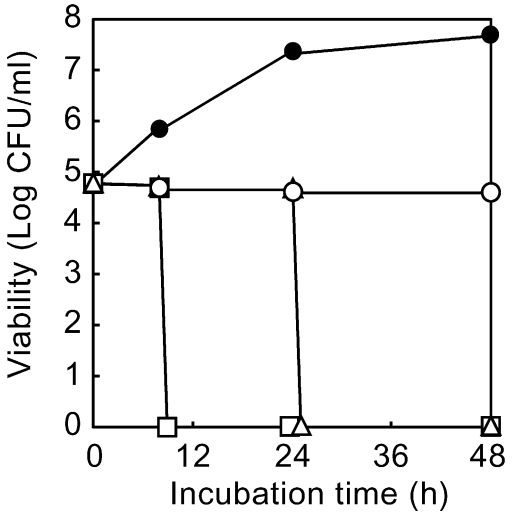

Fig. 3. Effect of octyl gallate on the cycloheximide-treated cells of *S. cerevisiae* ATCC 7754. The cells were grown with (open) or without (closed) 50 μg/ml of cycloheximide. After 8-h (□) or 24-h (△) cultivation, 25 μg/ml of octyl gallate was added to each culture.

result shows that lethality occurs remarkably quickly, within the first 1 h after adding octyl gallate. The similar result was also obtained in the test against *S. cerevisiae* as shown in Fig. 1b.

In Fig. 2, cultures of *S. cerevisiae* were exposed to two different concentrations of octyl gallate after 8- or 24-h incubation. It shows that octyl gallate at 1 × MFC rapidly reduced the number of viable cells within the first 2 h when added to the culture at the exponentially growing culture stage (10^6 CFU/ml), but the final cell count was not significantly different from the control when incubation was continued for 40 h as shown in Fig. 2a. In addition, octyl gallate at 1 × MFC was not fungicidal when added to the culture at a stationary growing culture (10^8 CFU/ml). Complete lethality occurred at 2 × MFC. In the case of 2 × MFC, no viable cells were detected after being exposed to 50 μg/ml of octyl gallate for 2 h when added to the culture at either the exponentially or stationary growing culture stage as shown in Fig. 2b. The result obtained indicates that although the concentration of octyl gallate needs to increase as the number of viable cells increases, octyl gallate was found to be fungicidal against *S. cerevisiae* at any stage of growth.

Further support for this postulate was also obtained in experiments that showed a rapid decline in the number of viable cells after the addition of octyl gallate in cycloheximide-treated *S. cerevisiae* cells as shown in Fig. 3. Cycoheximide restricts cell division by inhibition of cytoplasmic protein synthesis. This observation excludes several possible modes of action of octyl gallate, such as inhibition of DNA, RNA and protein synthesis in vivo.

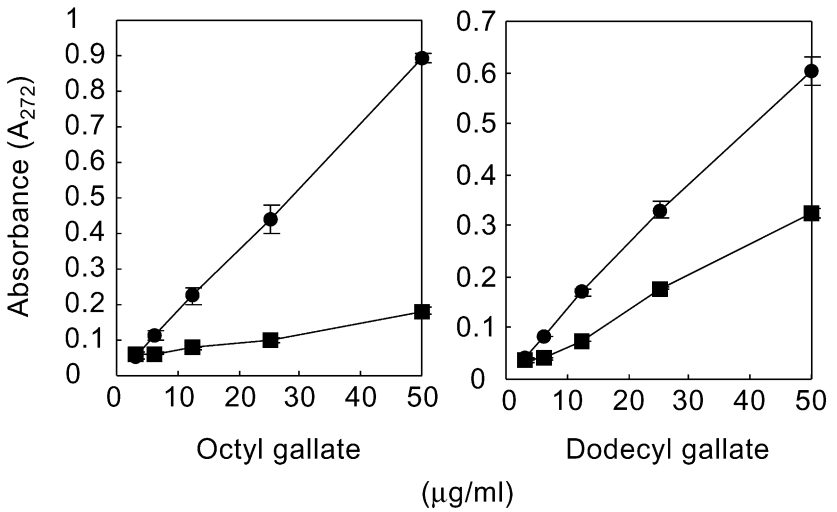

Fig. 4. Adsorption of gallate esters to the cells of *S. cerevisiae* ATCC 7754. After each gallate ester was mixed with (■) or without (●) yeast cells (10^8 CFU/ml), the suspension was vortexed for 5 s. Absorbance of the supernatant obtained by centrifugation for 2 min was measured. Values are means ± S.D. (n=3).

3.3. Affinity of gallate esters to S. cerevisiae cells

The adsorbing sites may not be specific but need to be clarified in order to reveal the difference. After each ester was incubated with cell suspension of *S. cerevisiae*, absorbance in the supernatant from the suspension was measured at 272 nm. The absorbance in the treatment with 50 μg/ml of octyl gallate reduced to 19% of that without octyl gallate as shown in Fig. 4. On the other hand, in the case of dodecyl gallate the reduction was 53%. This result indicates that octyl gallate rapidly bind with *S. cerevisiae* cells but dodecyl gallate does not. Hydrophilic catechol or pyrogallol moiety binds with an intermolecular hydrogen bond like a "hook" in attaching itself to hydrophilic portion of the membrane surface. This creates, as a surfactant, a disorder in the fluid bilayer of the membrane. The binding sites may not be specific but need to be clarified. In this connection, most of the dodecyl gallates did not bind with *S. cerevisiae* cells remaining in the water based medium, probably in the form of insoluble monolayer or spread film (Jones and Chapman, 1994). Therefore the length of the alkyl chain is not a major contributor but plays an important role to elicit the antifungal activity.

3.4. Effect of gallate esters on plasma membrane fluidity

Rapid adsorption of octyl gallate to membranes of *S. cerevisiae* cells might affect physical nature of the membrane, resulting in a change in the three-dimensional structure of the proteins embedded in the membrane bilayer. Such changes would modulate enzyme activity of the proteins, such as plasma membrane H^+-ATPase, which is a major protein in the plasma membrane. Therefore, we examined the effect of octyl and dodecyl gallate on the membrane fluidity of the yeast cells. The membrane fluidity (IE/IM) of control, octyl gallate and dodecyl gallate were 0.33 ± 0.03, 0.16 ± 0.02 and 0.25 ± 0.02, respectively. Octyl gallate reduced the fluidity to 48% of control. Dodecyl gallate, which did not exhibit any antifungal activity against yeast cells, also reduced it to 76% of control. On the other hand, *n*-alkanols, which possess antifungal activity against *S. cerevisiae*, increased the fluidity of nervous cell membrane (Edelfors and Ravn-Jonsen, 1990). Our result indicates that higher affinity of octyl gallate to the membrane than that of dodecyl gallate might be associated with immediate decrease in the flexibility of the plasma membrane in *S. cerevisiae*.

3.5. Effect of gallate esters on glucose-induced medium acidification

It is known that *S. cerevisiae* produces acidification of the external medium during growth on glucose. The external acidification is closely associated with the metabolism of the sugar and also depends on the buffering capacity of the growth medium. The H^+-ATPase is important not only in the regulation of internal pH but also in the energy-dependent uptake of various metabolites (Coote et al., 1994). This glucose-induced medium acidification process was only inhibited by octyl gallate as shown in Fig. 5. Therefore, it is possible that the antifungal activity of octyl gallate is, at least in part, due to its inhibition of the plasma membrane H^+-ATPase.

Octyl gallate can be considered as a head and tail structure similar to long chain alkanols (Kubo et al., 1995) and hence its mode of antifungal action is expected to act as a surfactant. However, additional

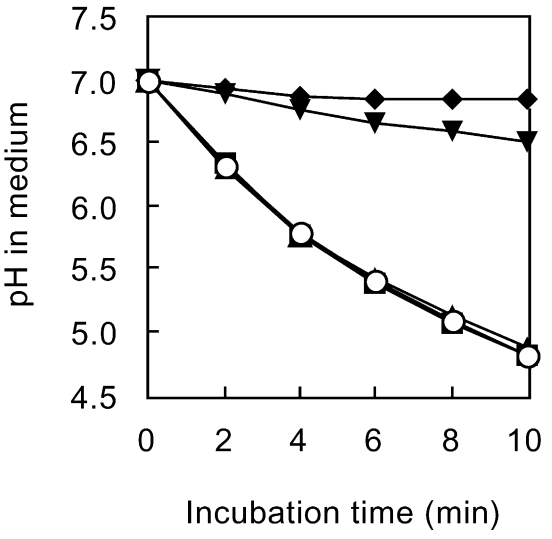

Fig. 5. Inhibition of glucose-induced medium acidification by gallate esters in *S. cerevisiae* ATCC 7754. The cell suspension (10^8 CFU/ml) was mixed with 2.5 mM propyl gallate (■), 0.313 (▼) or 2.5 (♦) mM octyl gallate or 2.5 mM dodecyl gallate (▲). Control (○) contained DMF only.

Table 3
Antifungal activities (μg/ml) of octyl esters of various phenolic acids against *Z. bailii* and *S. cerevisiae*

Octyl esters tested	*Z. bailii*		*S. cerevisiae*	
	MIC	MFC	MIC	MFC
3,4,5-Trihydroxybenzoate (gallate)	50	50	12.5	25
3,4-Dihydroxybenzoate	25	50	6.25	12.5
3,5-Dihydroxybenzoate	>400	>400	>200	>200
2,3-Dihydroxybenzoate	>400	>400	>200	>200
4-Hydroxybenzoate	>400	>400	>400	–
3-Hydroxybenzoate	>400	–	>400	–
2-Hydroxybenzoate	>400	–	>400	–
Benzoate	>400	>400	>400	–

The cells of *Z. bailii* and *S. cerevisiae* were grown in YPD and 2.5% ME broth, respectively, at pH 6.0. –, Not tested.

functions may need to be considered for this synthesized phenolic ester. For example, the ester group did not exist in the original alkanol structure and may be related to elicit the additional activity. As listed in Table 1, octanol itself exhibited the antifungal activity, therefore the possibility of secretion of an esterase that hydrolyzes octyl gallate to the original gallic acid and antifungal octanol in *S. cerevisiae*, was first taken into account. If this benzoate was hydrolyzed by esterase, one of the hydrolyzate, octanol should show some antifungal activity. However this possibility can be readily ruled out, since neither octyl benzoate nor octyl 3-hydroxybenzoate exhibited any antifungal activity against *S. cerevisiae* up to 400 μg/ml as shown in Table 3. The existence of the ester group is not directly related to the activity. It should be noted that the hydrolyzable ester group was selected in order to avoid undesired side effects, particularly endocrine disrupting activity of environmentally persistent estrogen mimics (White et al., 1994), such as alkylphenolic compounds (Soto et al., 1991).

4. Discussion

Propyl, octyl and dodecyl gallates are currently permitted to use as antioxidant additives of foods in USA. Therefore, they were tested for their antioxidant activities (Kubo, 1999). All the gallates tested here, regardless of their alkyl chain length, showed a potent scavenging activity on the 1,1-diphenyl-2-*p*-picryhydrazyl (DPPH) radical, indicating that the alkyl chain length was not directly related to this activity (Kubo, 1999). Therefore, all the gallate esters can be used as antioxidants in foods. It is evident that the potency of antifungal activity of the gallates against microorganisms depends on their alkyl chain length as described above but not their scavenging activity.

On the basis of the results obtained, it appears that the antifungal activity of octyl gallate is primarily due to its surface-active property, similar to alkanols (Kubo et al., 1995). Thus, the fungicidal activity of gallates was distinctly increased for every additional CH_2 group. In the inactivation study, (a) lethality occurs notably quickly, within the first 1 h after adding octyl gallate, (b) fungicidal activity was found at any growing stage, and (c) octyl gallate rapidly killed *S. cerevisiae* cells in which cell division was inhibited by cycloheximide, were observed. Octyl gallate also reduced plasma membrane fluidity of *S. cerevisiae* cells. These results support the surfactant concept. Although the molecular nature of general antifungal target sites remains unknown, the antifungal activity of octyl gallate could be due to disrupting or disorganizing the lipid bilayer–protein interface rather than the lipid–bilayer portions. The inhibition of sarcoplasmic reticulum calcium pumps, Ca^{2+}, Mg^{2+}-ATPase was due to changes in spatial conformation of the pump proteins and a decrease in the membrane fluidity in rabbit skeletal muscle (Chen et al., 2000). An altered lipid environment, such as enhanced membrane fluidity, affected the function of ABC transporter, Cdr1P in *Candida albicans* (Krishnamurthy and Prasad, 1999). It maybe the same case with octyl gallate's inhibition of the plasma membrane H^+-ATPase in *S. cerevisiae*. Further support of this postulate was obtained in experiments that showed a rapid decline in the number of viable cells after the addition of octyl gallate at the exponential growth phase. The mechanism of action of octyl gallate involves interaction with membrane with loss of membrane potential and leakage of intercellular materials (Brötz et al., 1998). The data obtained so far are consistent with an effect on the bulk membrane rather than a direct interaction of the specific target protein and octyl gallate's nonspecificity of antimicrobial activity also supports this assumption.

In general, octyl gallate acts as a multifunctional agent in foods—at least as an antifungal and an antioxidant agent. After this gallate is consumed

together with foods to which they are added as additives, these esters are hydrolyzed to the original gallic acid and the corresponding alcohols. The former still acts as a potent antioxidant, and the latter is a common plant component. More specifically, as an antioxidant, the free gallic acid scavenges superoxide anion generated enzymatically and nonenzymatically—even more potent than its ester form. In addition, octanol likely prevents generation of superoxide anion and hydrogen peroxide by mitochondria in the resting state, as an uncoupler (Hammond and Kubo, 2000), similar to fatty acids (Korshunov et al., 1998). On the basis of our previous SAR study with alcohols (Kubo et al., 1995), geranyl gallate can be expected to show similar activity as octyl gallate. The rationale for this idea is that the hydrophobic alkyl chain length from the hydrophilic hydroxy group as well as its volume is a key factor. It seems that geraniol nicely fits this assumption as a superior example. Hence, geranyl gallate was synthesized and assayed. As expected, this gallate exhibited an antifungal activity against *S. cerevisiae* with a MFC of 50 μg/ml, nearly equal to the MFC of octyl gallate. It should be noted that the alcohol moiety of this ester, geraniol, is known to increase glutathion *S*-transferase activity which is believed to be a major mechanism for chemical carcinogen detoxification (Zheng et al., 1993). Gallic acid is known in many plants, such as blackberry bark, henna, tea and uva ursi. Geraniol is reported in a large number (>160) of essential oils, such as lemon grass, coriander, lavender, carrot and geranium—and used as food flavor for baked goods, soft and hard candy, gelatin and pudding, and chewing gum. As aforementioned, the three gallates—propyl, octyl and dodecyl—are currently permitted to use as antioxidant additives in food, therefore, the octyl gallate should be safe to use as food preservatives. In addition, it is evident that the volume of the hydrophobic portion also contributes to the activity. This suggests that even more optimization is possible through the synthetic approach. Therefore, the most pertinent volume to elicit the maximum activity needs to be established.

Compared to sorbic acid, octyl gallate is odorless, and therefore can be a superior food additive, particularly to control *Z. bailii* and *S. cerevisiae* (Fleet, 1992). Its antifungal activity is not influenced by pH values. Different from sorbic acid, gallate esters, regardless of their alkyl chain length, have potent antioxidant activity, which is also an important property to protect foods. In addition, a broad antimicrobial spectrum of octyl gallate would appear to be great overall value. Binding all those together, octyl gallate should have wider application potential as a food additive. It also should be noted that this antifungal agent—as a nonionic surfactant—targets the extracytoplasmic region and thus does not need to enter the cell, thereby avoiding most resistance mechanisms, such as enzymes that degrade drugs to inactivate.

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