# Fatty Acid–Based Formulations for Wood Protection against Mold and Sapstain

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**Abstract**

Safer, highly effective biocides providing long-term protection of mold growth on wood-based materials is of interest to the wood protection industry. Moldicide formulations containing synergistic combinations of ingredients derived from natural sources are commonly recognized as a promising approach for the next generation of wood protectants. Although fatty acid (FA)–based chemistry has had some development in food sanitation and agriculture, little exploration relating to new mold inhibitors for wood and wood products has occurred. Low molecular weight, saturated monocarboxylic acids combined with selected adjuvants can effectively inhibit mold spore germination. Specifically, formulations containing valeric or pentanoic (C5), hexanoic or caproic (C6), heptanoic (C7), caprylic or octanoic (C8), pelargonic or nonanoic (C9), and/or decanoic or capric (C10) saturated acid demonstrated efficacy against mold growth for up to 12 weeks in the ASTM D4445 standard laboratory test for mold. Pressure-treated wood was more resistant to mold growth than wood dip treated with FA formulations.

Finding a single synthetic or natural antimicrobial compound, either newly recognized or already registered, to inhibit the varied biological agents capable of colonizing wood and wood products is improbable. Likewise, biocide resistance that occurs frequently and to varying degrees in wood-inhabiting fungi increases the importance of cobiocide interaction for successful wood protection. Thus, developing multiaction synergistic combinations of selected compounds, preferably those derived from natural sources, is recognized as a promising approach for obtaining better antimicrobials (Dillon and Cook 1994, El-Ghaouth et al. 2000, Brul et al. 2002, Green and Schultz 2003, Ippolito and Nigro 2003, Marshall 2003). A number of effective combinations have been developed as general biocides, such as nisin and garlic or phenolic compounds (Adams 2003), sorbates with vanillin or citral (Alzamora and Guerrero 2003), chitosan with an antagonistic yeast (El-Ghaouth et al. 2000), antioxidants and/or metal chelators with an organic biocide (Schultz and Nicholas 2001), borates and quats with an azole (Clausen and Yang 2007), lactic and acetic acids (Adams 2003), and caprylic acid and glycolic acid (Coleman 2004).

Schmidt (1984) evaluated the influence of saturated fatty acids on spore germination of brown- and white-rot basidiomycetes and determined that caprylic or ocanoic (C8), pelargonic or nonanoic (C9), and decanoic (C10) acids (100 ppm) destroyed spores of test fungi, whereas dodecanoic acid (C12) was effective against brown-rot but not white-rot fungal spores. Schmidt's findings suggested a high degree of specificity dependent on the fatty acid concentration tested and test fungus. For example, all concentrations of hexadecanoic acid (C16) tested were totally ineffective against spores of all test fungi, while pentanoic and hexanoic acid were effective against all test fungi but only at a concentration of $10^3$ ppm. Designations such as C8, C9, C10, etc. refer to the carbon chain length in a fatty acid as shown in Table 1, and will be used throughout this article to differentiate the fatty acids in this study. Other researchers found that fungal spore germination is stimulated or inhibited depending on the particular fatty acid and

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*Table 1.—Classification of fatty acids, emulsifiers, and adjuvants.*

| Fatty acid | Emulsifier | Adjuvant |
|---|---|---|
| Propionic (C3) | Sorbitan | L-Lactic acid |
| Butyric (C4) | Phosphate ester | Methylated seed oil |
| Pentanoic (C5) | | Organosilicone |
| Caproic (C6) | | |
| Heptanoic (C7) | | |
| Caprylic (C8) | | |
| Pelargonic (C9) | | |
| Capric (C10) | | |

its concentration (Harman et al. 1980). Thus, some fatty acids at specific concentrations have the potential to protect wood from wood-inhabiting fungi; however, conditions relating to efficacy of a given fatty acid against a wide variety of ascomycetes, deuteromycetes, and basidiomycetes remains to be identified. One approach to enhance the performance of fatty acids as mold inhibitors involves the use of adjuvants such as organic acids. To date, multifactorial systems have not focused on combined organic and fatty acid chemistries applied specifically to control mold fungi for protection of wood.

Many nonfatty organic acids, including acetic to decanoic acid and L-lactic, citric, malic, and glycolic acids, are classified as generally recognized as safe (GRAS) compounds by the US Food and Drug Administration (FDA) and have common acceptance for use in the food industry as acidulants and flavor enhancers. The safety record of these compounds is a positive feature that addresses the need for development of antimicrobials that are based on green chemistries. Certain organic acids, such as acetic, citric, and tartaric, which are known to inhibit mold (Barbosa-Canovas et al. 1998), function as chelating agents and are used to inhibit lipid oxidation and deter browning in food products (Doores 1993). Chelation may play a beneficial role in biocide function by altering availability of micronutrients or metallic cofactors necessary for germination and hyphal development.

The objective of this study was to evaluate combinations of selected fatty acids and specific nonfatty organic acids and other adjuvants against mold growth on wood. Research findings demonstrate that certain adjuvants, even at low amounts, greatly enhance fatty acid bioactivity against mold growth on southern pine.

## Experimental Methods

### Test chemicals

Test chemicals evaluated are summarized in Table 1. Chemicals were supplied by Summerdale, Inc., Verona, Wisconsin. Experimental formulations consisted of a combination of one or more fatty acids, proprietary emulsifiers, and/or proprietary adjuvants. Above 3.0 percent (vol/vol), C6 through C9 require addition of an emulsifier for aqueous stability. Fatty acid concentrations in treating solutions ranged from 2.12 to 8 percent (vol/vol), emulsifier concentrations ranged from 0.3 to 2.2 percent (vol/vol), and adjuvant concentrations ranged from 0.24 to 1.0 percent (vol/vol). Emulsifier and adjuvant concentration ranges were selected for compatibility with fatty acid formulations to ensure concentrated formulation and microemulsion stabilities and to achieve adjuvant enhancement of fatty acid. Because of the variation in properties of the test emulsifiers, a dual emulsification system was evaluated to assess the potential benefit of incorporating both emulsifiers. Fatty acid formulations were compared at relatively low application rates (<3.0%, vol/vol) to distinguish treatment effects as mold inhibitors.

The pH values of the caprylic acid (C8) formulations with and without an organic acid supplement were considered as follows. $pK_a$ values of each acid and the Henderson–Hasselbach equation were used to calculate the degree of ionization and percent protonation for C8 with and without an organic acid supplement. Based on this calculation, the most effective organic acids with C8 were L-lactic, glycolic, succinic, and propionic. The level of protonated C8 was elevated from 56 percent without an organic acid supplement to 97 to 98 percent with an organic acid supplement.

### Wood treatment

*Dip treatment*.—Southern pine (*Pinus* spp.) specimens (7 by 20-mm cross section by 7 cm long) were cut from kiln-dried pine and submerged for 24 hours in deionized (DI) water before testing. The average moisture content of the specimens was 26.4 percent ($n = 5$) at the time of treatment. Prewetted specimens were dip treated for ~15 seconds in individual biocide formulations and held for 24 hours in a covered container before testing according to ASTM standard test method D4445-91 (American Society for Testing and Materials [ASTM] 1998). Prewetted untreated specimens served as controls.

*Vacuum treatment*.—Ten southern pine sapwood specimens (7 by 20-mm cross section by 7 cm long), prewetted as described in the previous section (26.4% moisture content) were vacuum treated (40 min at 550 mm Hg) with aqueous solutions of individual test formulations. Treated specimens were held at 25°C overnight in a closed container prior to testing according to ASTM D4445-91. Untreated specimens vacuum treated with water served as controls.

### Mold and sapstain tests

Treated specimens were arranged over four layers of blotting paper saturated with 35 mL of DI water and a polyethylene mesh spacer in sterile disposable petri dishes (150 by 25 mm; B-D Falcon, Los Angeles, California) according to ASTM D4445-91. Spore inoculum was prepared by washing spores from the surface of 2-week-old cultures of the test fungi. Mold fungi were grown on 2 percent malt extract agar, and the sapstain fungus was grown on 2 percent potato dextrose agar (Difco, BD, Sparks, Maryland). For the mold test, a mixed mold spore inoculum consisting of *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 was sprayed on specimens at a rate of 1 mL per plate (approximately $3 \times 10^7$ spores per mL). For the sapstain test, *Aureobasidium pullulans* spores were sprayed on specimens at a rate of 1 mL per plate (approximately $3 \times 10^7$ spores per mL). Plates were sealed in polyethylene bags to prevent drying and incubated at 27°C and 70 percent relative humidity up to 12 weeks. During incubation, individual specimens were periodically rated for mold growth on the following scale: 0 = no growth, 1 = 20 percent, 2 = 40 percent, 3 = 60 percent, 4 = 80 percent, 5 = 100 percent coverage with mold.

## Results and Discussion

### Fatty acid efficacy

Initially, fatty acids C3 through C5 were evaluated alone at 6 percent for efficacy against the test fungi. Propionic (C3) and pentanoic (C5) acids alone were effective inhibitors of test fungi at 6 percent concentration. Sorbitan and phosphate ester emulsifiers were compared for their ability to provide emulsion stability to fatty acids C5 to C9 in water. Emulsion stability is particularly important for saturated fatty acid formulations used at relatively high concentrations (>3.0%, vol/vol) in aqueous solutions. Emulsifiers may possess some ability to independently inhibit mold fungi and/or enhance the bioactivity of a particular fatty acid as shown in Figure 1. While both C6 emulsions were equally effective at inhibiting test fungi, C7 through C9 fatty acids emulsified with sorbitan emulsifiers provided greater mold inhibition than formulations containing a phosphate ester. However, phosphate ester provided superior emulsification properties compared with sorbitan emulsifiers, i.e., good stability of water emulsions after storage for 30 minutes (data not shown).

### Single versus dual emulsification

Formulations including both sorbitan and phosphate ester emulsifiers, compared with formulations containing a single emulsifier, suggest that dual emulsification improves both emulsification and mold-inhibitory activity (Fig. 2). The sole exception was pelargonic acid; all other dual-emulsified fatty acid formulations exhibited better antimold properties than single emulsified formulations. All dual-emulsified fatty acid formulations diluted in water were very stable, whereas pentanoic, hexanoic, and heptanoic formulations emulsified only with sorbitan emulsifiers were unstable at 3.0 percent (vol/vol) in water.

### Adjuvant effect

Fatty acid formulations complemented with selected adjuvants as candidate mold inhibitors were evaluated (Table 2). Southern pine dip treated with formulations with and without adjuvants were exposed to test fungi and rated periodically for 8 to 12 weeks ($n = 12$). Early test results using L-lactic acid and/or two adjuvants showed that selected pentanoic, heptanoic, and caprylic/capric formulations including adjuvant amendments maintained a high degree of mold inhibition for 12 weeks (Table 2). All fatty acid formulations benefitted substantially by the presence of an adjuvant.

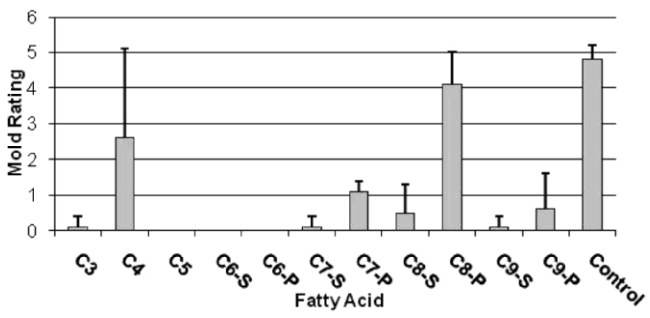


*Figure 1.—Effectiveness of 6 percent fatty acids against mold fungi on southern pine for 6 weeks;* n = *10. S and P designate sorbitan and phosphate ester emulsifiers, respectively.*

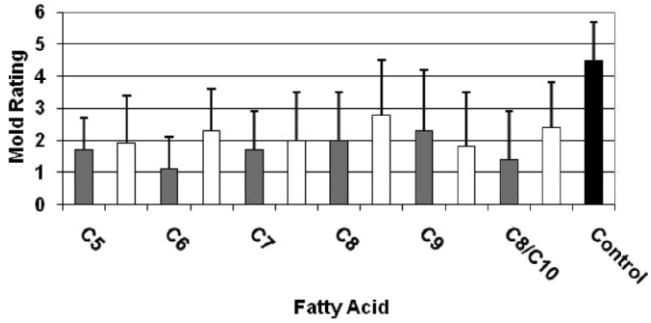


*Figure 2.—Effect of 2.1 percent fatty acid on southern pine comparing single with dual emulsification systems. Gray bars represent dual emulsification (0.6% sorbitan, 0.3% phosphate ester); white bars represent 0.9 percent sorbitan;* n = *12.*

### Pelargonic acid–based formulation

A fatty acid formulation containing pelargonic acid (C9), dual emulsifiers, and L-lactic acid was compared with and without additional adjuvants as a dip treatment (Table 3). Data represent three tests with 12 specimens per test ($n = 36$). Mold inhibition of the C9 formulation at both 6.0 and 8.0 percent (vol/vol) application rates was clearly enhanced by supplementation with either adjuvant.

*Table 2.—Effect of adjuvant on mold-inhibitory properties of fatty acid formulations on dip-treated Southern pine after 12 weeks of exposure to mold fungi.*

| Fatty acid | % | Lactic acid | Supplement[a] | Mold rating, avg (SD) |
|---|---|---|---|---|
| Pentanoic | 6.0 | − | | 3.0 (2.5) |
| Pentanoic | 5.4 | + | | 0.3 (0.5) |
| Caprylic/capric | 4.2 | − | S | 2.6 (1.2) |
| Caprylic/capric | 4.2 | + | S | 1.3 (1.1) |
| Heptanoic | 5.6 | − | P | 3.1 (0.9) |
| Heptanoic | 5.6 | + | P | 1.3 (1.6) |
| Heptanoic | 5.6 | − | P/MSO | 2.1 (1.2) |
| Heptanoic | 5.6 | + | P/Org | 0.1 (0.2) |
| Control | — | — | — | 3.9 (1.2) |

[a] Emulsifiers: S = sorbitan; P = phosphate ester. Adjuvants: MSO = methylated seed oil; Org = organosilicone.

*Table 3.—Average mold rating after 12 weeks for southern pine treated with dual-emulsified pelargonic acid formulations.*[a]

| Concentration (%) | Adjuvant (%)[b] | Mold rating, avg (SD)[c] |
|---|---|---|
| 6 | — | 1.1 (1.4) |
| 6 | MSO (1.0) | 0.6 (1.2) |
| 6 | Org (0.25) | 0.3 (0.9) |
| 8 | — | 0.9 (1.1) |
| 8 | MSO (1.0) | 0.4 (0.9) |
| 8 | Org (0.25) | 0.2 (0.5) |
| — | Control | 2.4 (1.4) |

[a] Ratio of C9:P:S:lactic acid was 70:10:10:10.
[b] MSO = methylated seed oil; Org = organosilicone.
[c] Average of three replicate tests; $n = 36$.

*Table 4.—Average mold rating for southern pine vacuum treated with caprylic acid formulation and exposed to mold fungi or a sapstain fungus.*[a]

| Test fungi | Concentration (%) | Mold rating, avg (SD)[b] | | |
|---|---|---|---|---|
| | | 4 wk | 8 wk | 12 wk |
| Mold[c] | 6 | 0 | 0 | 0 |
| Control | — | 2.5 (1.3) | 2.9 (1.7) | 3.0 (1.5) |
| Sapstain[d] | 6 | 0 | 0 | 0 |
| Control | — | 4.4 (0.5) | 4.8 (0.4) | 5.0 (0) |

[a] Ratio of C8:P:lactic acid was 50:20:10.
[b] $n = 12$.
[c] Mixed mold inoculum: *Aspergillus niger, Trichoderma viride,* and *Penicillium chrysogenum*.
[d] *Aureobasidium pullulans*.

Lactic acid, produced by lactic acid bacteria (LAB), is well recognized as a biopreservative of food (Yang and Clausen 2005). The study by Yang and Clausen on mold inhibition by *Lactobacillus* reported that lactic acid, a major metabolite from LAB, together with other cell-free metabolites, caused 95 to 100 percent fungal biomass inhibition of mold fungi in vitro. Adding select organic acids, such as L-lactic acid, to the fatty acid emulsifications may increase the proportion of nonionized fatty acids over ionized species, thereby promoting greater fatty acid penetration through cell membranes. Intracellular proton pump activity is increased and more energy is required by the cell for electrolyte balance, thus placing more stress on the cell (Coleman and Penner 2006, 2008). Higher intracellular concentrations of free fatty acids may result in damage to organelle membranes and protein structure (Ecklund 1989, Barbosa-Canovas et al. 1998). This is one possible hypothesis for the mode of action on vegetative mold hypha, but it likely does not apply to spore coats.

## Caprylic acid–based formulation

A caprylic acid–based formulation (C8) with phosphate ester emulsifier and L-lactic acid was 100 percent effective at 6 percent as a pressure treatment against three mold fungi and one sapstain fungus (Table 4).

## Conclusions

Several fatty acid formulations and propionic acid inhibited mold test fungi. L-lactic acid and selected adjuvants appear to enhance the antifungal properties of the formulations. Multifactorial fatty acid emulsifications incorporating an appropriate adjuvant are promising for effective protection against numerous fungal species that affect wood products. With this strategy, new green wood protection formulations are being developed, and their application is likely to extend to other biodeteriorating agents such as termites and decay fungi. More work is needed to optimize efficacy and assess field performance of these new mold-inhibiting formulations.

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