Inorganic nano mental oxides used as anti-microorganism agents for pathogen control

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Abstract: Recently, inorganic antimicrobial agents are being increasingly for control of microorganism in various areas, especially in dentistry. Particle size of mental oxides had an impact on their anti-microorganism activity. There is growing interest in nanoscale particles since materials exhibit unique properties which offer considerably from those of macroscopic materials. Inorganic nano mental oxides including MgO, ZnO and CaO have been shown anti-microorganism activity. This chapter highlighted inorganic nano mental oxides used as anti-microorganism agents for pathogen control. Preparation of MgO, ZnO and CaO nanoparticles, effect of nanoparticles on anti-microorganism activity and antimicrobial mechanism of nanoparticles were discussed. Study of MgO, ZnO and CaO nanoparticles as anti-microorganism agents in our group was also introduced.

Keywords nano metal oxdies; anti-microorganism; pathogen control

Introduction

Fruit juices have a low pH that inhibits most bacteria, which are mainly acid tolerant bacteria such as Gram positive lactobacilli and leuconostocs. The lactobacilli are important in the spoilage of fruit juices. Some strains are quite acid tolerant and can metabolize citric and malic acids. This reduces the acidity, resulting in a bland flavour and loss in astringency. The production of diacetyl, hydroxyl butanone and dihydroxybutane by these organisms results in buttermilk like flavour in fruit juice. *Leuconostoc mesenteroides* produces dextranes, conferring an unpleasant slimy texture to juices [1]. *Alicyclobacillus* spp. is increasing being acknowledged as causative agents of spoilage of fruit juices. Major characteristics of *Alicycobacillus* spp. are their ability to survive commercial pasteurization processes, and produce off-flavors in juices [2]. Yeasts and fungi also can contaminate and ferment fruit juices. The most frequently occurring yeasts are: *Saccharomyces cerevisiae*, *Candida stellata* and *Zygosaccharomyces rouxii* [3]. If the sugar concentration is high, the osmophilic yeasts, *Saccharomyces rouxii* et al *S. mellis* can ferment the sugars to alcohol, and *Acetobacter* can convert the alcohol to acetic acid giving the fruit juice the vinegar flavour. Species of fungi identified in the spoilage of fruit juices include *Penicillium*, *Aspergillus*, *Paecilomyces* et al *Fusarium*. It has been shown that some anamorphic fungi (*Paecylomyces variotii* et al *Fusarium sp*.) could cause spoilage of pasteurized fruit juices [4]. Fungal biomass suspensions of *P. variotii* strains were able to survive higher temperatures for a longer time than spores.

Although potential spoilage organisms in fruit juices are heat sensitive and can be eliminated by pasteurization process, the juice industry faces potential economic losses caused by microbial production of off-flavors in pasteurized fruit juices. Heat resistance of thermophilic and acidophilic charcteristics of *Alicyclobacillus* species and anamorphic fungi, *Paecilomyces variotti* and *Fusarium* sp., enable them to survive current pasteurization process. In addition, post contamination of pasteurized juices can limit their shelf life. To guarantee longer shelf life and reduce losses, pasteurized juices must be aseptically packaged, or stored and distributed under refrigeration conditions. Because of high costs associated with aseptic processing as well as refrigeration, processors use chemical preservatives such as benzoate and sorbate to control spoilage organisms in pasteurized juices. Addition of chemical agents is an effective low-cost solution, which enables the processor to distribute price competitive fruit juices and drinks under ambient conditions. Since consumers are reticent towards chemical additives, there is great interest in the use of nature products as antimicrobial compounds in fruit juices. A variety of substances have been investigated in an effort to replace benzoate and sorbate: bacteriocins, lysozyme, propolis, chitosan, polylysine, isothiocyanates isolated from white mustard seeds, limoid glucosides, flavonoids, vanillin et calcium lactate [5-10]. Although these substances are effective antimicrobial agents, their use in juices is limited because they either alter the sensory attributes of fruit juices or they are costly. Juice processors need alternative substances that are functionally effective without altering organolepic quality of the juices, and which also price competitive. Heath beneficial agents would be all the more attractive for both consumers and the processors.

In recent years, inorganic antimicrobial agents are being increasingly for control of microorganisms in various areas, especially in dentistry. The key advantages of inorganic agents are improved safety and stability compared with organic antimicrobial agents [11]. The antibacterial activity of metal oxides including MgO and ZnO was shown by a Japanese group [12]. There is growing interest in nanoscale particles since materials exhibit unique properties which differ considerably from those of macroscopic materials. The finding that nanosized silver exhibits a strong antimicrobial activity has inspired investigations on the antimicrobial activity of nanoscale metal oxides, in particular MgO. From the

standpoint of nutrition and health, magnesium, zinc and calcium are essential to human health, which are needed for more than 300 biochemical reactions in the body.

In this chapter, inorganic nano mental oxides used as anti-microorganism agents for pathogen control were reviewed. Preparation of nano mental oxides, effect of nanoparticles on anti-microorganism activity and antimicrobial mechanism of nanoparticles were discussed. Study of MgO, ZnO and CaO nanoparticles as anti-microorganism agents in our group was also introduced.

Preparation of nano metal oxides (MgO, ZnO and CaO)

As so many other nano particles, various chemical synthesis methods for nano MgO and ZnO have been employed by several researchers, such as PVD, CVD, solvothermal, hydrothermal, co-precipitation, homogenous precipitation, sonication, emulsion and sol-gel et al [13-17]. The morphology and size of the particles are greatly affected by the conditions used for fabrication: reaction condition including temperature, pH, reactant concentration, reactant ratio et al., calcination condition, drying method and gel preparation conditions such as, gelling agents, heating rate for gel formation and calcination temperature of the gels in sol-gel method. More detailed information is shown in Table1 and 2.

Table 1 Preparation of nano MgO.

Preparation method	Main Reactant	Particle Size/nm	References
PVD	Mg, O_2	10-100	18
Direct precipatitation	$NH_3.H_2O$, $MgCl_2$	80	19
Direct precipatitaion	$NH_3.H_2O$, $Mg(NO_3)_2$	50-100	20
Direct precipatitation	Na_2CO_3, $Mg(NO_3)_2$	30	21
Direct precipation	$NH_3.H_2O$, $MgCl_2$	62	22
Direct precipation	$MgCl_2$, NaOH	15	23
Homogenous precipitation	Urea, $MgCl_2$	10	24
Homogenous precipitation	Urea, $MgCl_2$	25	25
Homogenous precipitation	Urea, $MgCl_2$	15-20	26
Sol-gel	$Mg(OC_2H_6)_2$, H_2O	30	27
Sol-gel	$Mg(NO_3)_2$, stearic acid	20-50	28

Table 2 Preparation of nano ZnO.

Preparation method	Main Reactant	Particle Size/nm	References
CVD	Zinc acetate	20-30	29
Direct precipatitation	$NH_3.H_2O$, $ZnSO_4$	50	30
Direct precipatitaion	NH_4HCO_3, $ZnSO_4$	15	31
Direct precipatitation	$NaCO_3$, $ZnSO_4$	20	32
Direct precipation	NaOH, $ZnCl_2$	25	33
Homogenous precipitation	Urea, $Zn(NO_3)_2$	18	34
Homogenous precipitation	Urea, $Zn(NO_3)_2$	25	35
Sol-gel	$Zn(OC_2H_6)_2$, H_2O	20	36
Sol-gel	Ethanol, Acetate acid, $Zn(NO_3)_2$	17	37

Until now, few literatures have been mentioned on the preparation of nano-CaO. There are mainly three methods on the preparation of nano-CaO according to the literatures. One is thermal decomposition [38, 39]. The other is sol-gel [40]. Last one is thermal decomposition of metal hydroxide we used in our group [41]. Though CaO nano-particles can be obtained about 4 nm through sol-gel method, the cost is very high. What's more, the process is very complicated and time-consuming. So it is very difficult to apply sol-gel method into industry. Thermal decomposition method has some advantages such as simple process, low cost, high purity of product et al. So it is quite promising and facile to be applied into industry. But for thermal decomposition method, CaO is often obtained directly through calcining $CaCO_3$. High calcinations temperature is needed. It is very difficult to get nano-scale CaO, but Micrometer CaO (above 100 nm), directly through calcining $CaCO_3$ [42]. In our group, nanoparticle assemblies of metal oxides were prepared by thermal decomposition of metal carbonate or hydroxide or by sol-gel processes or by sonication (unpublished work). In the sol-gel procedure, several methods have been reported in inducing the formation of nanoparticles. Among them, the use of ultrasound is more common. Recent studies have shown sono-chemical effects on the acceleration of chemical reactions involved in the synthesis of novel nanomaterials in aqueous solution. Chemical effect of ultrasound originates from the formation of ultrasonic cavitation, the growth and collapse of microbubbles in the liquid phase generating very high temperatures and preparation of nanoparticulate metal oxides [43, 44].

Effect of nanoparticles on anti-microorganism activity

Recently, much attention has been given on the use of metal oxides as new inorganic antimicrobial agents. Sawai et al. [12] have shown that relatively basic metal oxides, including MgO, CaO and ZnO, have an antibacterial and antifungal activity. MgO and CaO in solution have a similar bactericidal activity against Gram positive and Gram-negative bacteria, while ZnO behaves as a growth inhibitor and has more ability against Gram positive. Moreover, these powders in suspension have shown some action against spores of *Bacillus subtilis*, which have considerable heat resistance and antimicrobial agents [45, 46]. Several factors related to the antimicrobial activity of metal oxides have been investigated such as the mixture concentration, pH, exposure time, the surface properties of the powder, the active oxygen generation and the size of particles of metal oxides [47, 48]. Combined with these many factors, the action of metal oxides against bacteria appears to be really close to the surface of the particle [12]. Contact between MgO particles and bacteria is also an important factor in their activity [49]. On the other hand, with regard CaO and MgO, the alkalinity of the surface is a major microbicidal effects against bacteria [50]. Yamamoto et al. [51] showed that when the suspension of the powdered metal oxide is concentrated, and the larger the specific surface, the better the antibacterial activity. Bae et al. [52] tested the bactericidal effect of calcium oxide against three pathogenic bacteria: *E. coli*, *L. monocytogenes* and *S. typhimurium*. Their observation indicated a high mortality of all three strains after exposure to 0.05 % CaO solution for 10 minutes, mainly due to alkaline pH. The surface charge of the particle and the bacteria can also interfere with the adhesion and thus prevent contact [53]. Furthermore, the existence of active oxygen, such as O^{2-}, on the surface of MgO and CaO has been observed [49]. When the particle comes into contact with a bacterial cell at neutral or slightly acid pH, the active oxygen formed would increase the antimicrobial activity of the powder. Also, increasing the speed of the agitator for the dispersion of MgO particles in suspension increased the death of E. coli, indicating that the frequency of contact between bacterial cells and particles directly affects the antimicrobial activity of MgO. Mg^{2+} and Ca^{2+} released by the particles in solution could also be involved in cell death. However, some studies indicated that these ions had no effect on the growth of *E. coli* and *S. aureus* [49, 54]. Moreover, contrary to CaO and MgO, ZnO is a metal oxide semiconductor and therefore does not display a high surface alkalinity in solution, but rather tends to neutral. This feature of zinc oxide makes it easy to use. In fact, it was reported that the surface of the particle generates hydrogen peroxide, H_2O_2, and, possibly, is the first factor to inhibit bacterial growth [55]. A difference in the antimicrobial activity of MgO, CaO and ZnO comes from active oxygen species generated by the powder in solution. Indeed, every bacterium responds unevenly to oxidative stress due to differences in the permeability of cell membranes [56]. Some microbial strains succumb to damage to cell walls by O_2^- and others, While, orthers shown greater sensitivity to H_2O_2, as is the case for *E. coli* [57].

A problem of the use of different metal oxides in a liquid medium, such as fruit juice, comes from the fact that their use can change the visual appearance of the medium. Indeed, when the oxide powder, slightly soluble in solution, it is visible to the eye and tends to precipitate. A solution to this problem is using nanoparticle powder. A nanoparticle is a body having a size of about 100 nm or less, which gives them unique properties. In this form, the scattering particle in solution would be greatly facilitated, resulting in a transparent medium. Metal oxide nanoparticles have a large surface area [58]. Moreover, the particle size of metal oxide influences the antimicrobial characteristics of these materials. Yamamoto [59] studied the effect of the size of ZnO on the antimicrobial activity against a strain of *Staphylococcus aureus* and a strain of *Escherichia coli*. His results showed that a particle of 0.1 μm showed better growth inhibition of bacteria. The higher the particle size, the more it has a bactericidal or bacteriostatic ability. While very little research has been done to date on the antimicrobial effects of nano CaO. nano MgO has been studied for its microbicidal activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [47, 60]. All demonstrated that MgO nanoparticle damages cell walls and kills bacteria. Moreover, the same results could be observed during the use of nano ZnO for *B. atropheus*, *E. coli*, *E. coli 0157: H7*, *Listeria monocytogenes*, *Samonella enterotidis, S. aureus* and *Vibrio fisheri* control [57, 61-64]. In fact, one can assume that the concentration of H_2O_2 generated by the surface of ZnO increases when particle size decreases because the number of particles of ZnO powder per unit volume of the mixture of the powder increases with decreasing size of the particle. This seems equally true for CaO and MgO with the generation of O^{2-}. On this basis, the increase of antibacterial activity is directly related to the increase of active oxygen generated on the surface of particles of metal oxide nanoparticle, reducing the size of the particle. Moreover, the nanoparticle of oxides in solution enhances the possibility of interaction between the particle and the bacterial cell due to its surface charge and surface energy [57, 59].

Antimicrobial mechanism of nanoparticles

The antibacterial activity metal oxide appeared on the surface. Huang *et al.* [60] and Makhluf *et al*. [47] proposed that active superoxide ions are generated on the surface of the oxide, which can react with the peptide linkages in the cell wall of bacteria and thus disrupt them. The bactericidal action of CaO and MgO may results from attack of these superoxide ions on carbonyl group in the peptide linkages, leading to degradation of the proteins. As the surface area of the particles increases, it leads to an increase of the O_2^- concentration in solution and results in a more effective destruction of the cell wall of the bacteria. Micro-aerophilic lactic bacteria do not have enzyme superoxide dismutase

(SOD) that neutralizes superoxide radical. Although, they have developed an alternative mechanism based on the accumulation of Mn^{2+} which can also neutralize superoxides [65]. The level of its accumulation can be different for different lactic bacteria and also be affected by culture media. This could explain the high sensibility of *L. helveticus* to all different assembled MgO. The cell wall of this bacteria compared with *L. plantarum* can also play a role on the sensibility of *L. helveticus* to MgO. The Gram-positive cell wall of lactic bacteria consists of peptidoglycan, teichoic acids, proteins and polysaccharides. In fact, the peptidoglycan of *L. plantarum* contains the peptide Gln-mDpm (meso-diaminopimelic acid). The cell wall peptitoglycan of *L. helveticus* is of peptide type (Lys-D Asp) [66]. The high mortality of *L. helveticus* by MgO may also occur by the release of Mg^{2+}, undesirable and potentially toxic ion. Since glutamic acid diaminopimelic acid peptide can chelate metal ions, more effectively than Lys-Arp peptide, it is reasonable to assume that Mg^{2+} ions have impact on the viability of *L. helveticus*. In contrast, the Ca^{2+} and Mg^{2+} ions were evaluated by Sawai [54] on *S. aureus* and *E. coli* and did not affect the bacteria growth. However, further investigation on the possibility of killing effect from the release Ca^{2+} and Mg^{2+} ions against lactic bacteria should be processed.

Due to very high surface energy of nanoparticles, the aggregation becomes very significant due to interparticle interaction from van Der Waals', electrostatic force [60]. Consequently, the interaction between oxide particle and bacterium is reduced and the bactericidal efficiency tends to be lower. Yamamoto *et al.*[51] indicated that the higher the concentration, the better the antibacterial activity will be. This experiment shown, there was no proportionality between the concentration of oxides and their antimicrobial effect. The metal oxide particles when used at a concentration of 1000 ppm appear to form aggregates [57]. However, Maklhulf *et al.* [47] believed that the nanoparticles tend to form agglomerates inside the bacterial cell. The intimate contact between the cell and the particle seems to be more important because metal oxide particles do not necessarily have to enter the cells [60].

The difference in the sensitivity of lactic bacteria to the metal oxides (CaO, MgO and ZnO) may also be due to lipid oxidation arising from the generation of free radicals, and the ability of the peptides in the cell wall peptidoglycan to chelate or complex soluble metal ions, in addition to the pH effect. While lactic bacterial cell membrane lipids are composed of saturated fatty acids and cyclic fatty acid (cyclopropane fatty acid or lactobacillic acid), *L. helveticus* cell membrane contains linoleic acid and vernolic acid (an epoxy fatty acid) [67]; whereas *L. plantarum* and *L. mesenteroides* contain mainly mono-unsatured fatty acids which are less susceptible to oxidation. Also, *L. plantarum* and *L. mesenteroides* contain glutamic acid-meso-diaminopimelic acid peptide in their peptidoglycan. Among the lactic bacteria investigated, *L. helveticus* was more sensitive to alkalin pH compared with *L. plantarum* or *L. mesenteroides*.

The effect of ZnO on three lactic bacteria (*L. helveticus, L. plantarum* and *L. mesenteroides*) is opposite to alkali metal oxides. With ZnO, *L. helveticus* is more resistant than either *L. plantarum* or *L. mesenteroides*. The pH of ZnO suspension is near neutral and it is also less soluble. The slower action of ZnO on bacteria and then different sensitivities to ZnO compared with alkali metal oxides suggest that the mode of action of ZnO may be different from that of alkali metal oxides. ZnO is a semiconductor oxide. Semiconductor oxides have ionic crystals and are classed as n- or p- type semiconductors. ZnO is a n-type, characterized by free electrons associated with anion vacancies in the crystal lattice. A feature of these oxides is that oxygen is chemisorbed as O^{2-} taking electrons from the oxides [68-70].

$$O_2 + 2é \longrightarrow O^{2-}$$

Subsequently, H_2O_2 is formed:

$$O^{2-} + 2H^+ \longrightarrow H_2O_2$$

It is plausible that these active oxygen species react with cell surface enzymes and inactivating them. In ZnO-bacterium interaction, free electrons may also participate in redox reactions with cell surface components, they all leading to cell surface denaturation and inactivation of the cells. It would seem then *L. helveticus* is less sensitive to oxidation of cell surface components.

Sawai *et al.* [55] proposed that the generation of hydrogen peroxide be the main mechanism of its antibacterial activity. The same group reported that the concentration of H_2O_2 produced was linearly proportional to ZnO particle concentration in the slurry. On the other hand, Zn^{2+} ions present in ZnO slurry had no effect on the growth of *E. coli* and *S. aureus* [54]. Although, increasing concentration seems to increase the antibacterial effect, but the killing effect was not proportional to the increase of concentration. This suggests a large degree of aggregation in the slurries and it supports the hypothesis that the binding of the particles on the bacterias surface due to the electrostatic forces [46].

Study of nano metal oxides used as anti-organism agents in our group

The antibacterial activities of nano-assembled metallic oxides (CaO, MgO and ZnO) were evaluated on three strains of lactic bacteria and spores of *Alicyclobacillus acidoterrestris* involved in fruit juice spoilage. The effects of particle size, pH, concentration and exposure time on the viability were examined in physiological solution and growth in culture broth. The tests were performed by adding the bacterial or spore suspensions in flasks containing metal oxides. Our results shown clearly bactericidal activities of different type of ZnO of different sizes against lactic bacteria. This result support works done by Reddy *et al.* [63] on selective toxicity of zinc oxide nanoparticles to *E. coli* and *S. aureus*. Contrary to CaO and MgO, the pH of ZnO dispersed in physiological solution was between 7.7 and 8.6, irrespective of the particle size or concentration. This usually does not affect bacterial survival or growth [59].

The alkali metal oxides CaO and MgO exhibited somewhat similar trends in their lethal effects against lactic bacteria. *L. helveticus* was more susceptible and *L. plantarum* as well as *L. mesenteroides* were more resistant when exposed to them for 6 h. However, the latter were resistant to MgO even after 24 h of exposure. Both alkali metal oxides contribute to the alkalinity of the medium and they are also relatively more soluble compared to ZnO. Given the differences between *L. helveticus* and *L. plantarum* as well as *L. mesenteroides*, the susceptibility of *L. helveticus* to the alkali metal oxides can be understood from pH effect, lipid oxidation provoked by free radicals and by the effect of free ions.

The effects of calcium oxide, magnesium oxide and zinc oxide on spores of *Alicyclobacillus acidoterrestris* were also examined. The results shown no antibacterial effect against spore of *A. acidoterrestris* after 96 h of exposure. Spore cells are much more robust with their thick proteinous spore coat than vegetative cells. The CaO and MgO slurries were able to kill the spores of *B. subtilis* in physiological saline at a higher concentration of the metal oxide used in this study. This fact suggested that the concentrations used were not enough to promote the spore killing of *A. acidoterrestris*. The target of the oxidizing agent, such as peroxide, could be lipids or the proteins of the inner membrane. In the case of *Alicyclobacillus*, due to its unique fatty acid composition (essentially composed of saturated branched and cyclic fatty acids of this membrane[71], proteins could be the major target for antibacterial agents [72]. Yamazaki *et al.*[73] reported that under low pH condition, spores of *A. acidoterrestris* exhibited strong binding character of Ca^{2+} and they were not affected by Mg^{2+}. These two facts may explain the strong resistance ability of spores against metal oxide tested.

Conventional and nano-assembled metal oxides (CaO, MgO and ZnO) were evaluated for their lethal effect on yeasts (*S. cerevisiae* and *C. tropicalis*) and fungal spores (*A. niger*, *P. variotii* and *Byssochlamys spp.*) and growth inhibitory effect on yeasts and fungi, which are implicated in the spoilage of fruit juices and drinks. The effect of exposure time, pH, concentration and particle size of metal oxides were examined. The lethal effect was determined by exposing yeast cells or fungal spores to specified concentrations of metal oxides. The viable cells were counted on culture media.

Alkalinity effect is considered as a major factor in the antimicrobial action of CaO and MgO. The bactericidal actions of CaO and MgO were compared with alkaline (NaOH) solution and were found to be higher than those of NaOH solution at identical pH. The pH values of isotonic solution containing different metal oxide powder showed a very high alkalinity for CaO (10.7 to 11.2 at 100 ppm to 12.3 at 1000 ppm). At 100 ppm, both yeasts were killed by the high pH (pH control) of CaO. *S. cerevisiae* was more sensible to the pH variation than *C. tropicalis*. This suggests that the fungicide action of CaO is due to its surface alkalinity, which could lead to solubilisation of cell surface proteins and cell wall alkali-soluble polysaccharides [74]. The change in the pH of MgO with increase of its concentration in isotonic solution was small and stabilized around 10.5, as noted by Makhluf *et al.* [47] for MgO nanoparticles. At this pH, the viabilities of yeasts were reduced by about 1 log. Compared to this value, MgO nanoparticles had a higher killing effect at 1000 ppm against *S. cerevisiae*. In the growth medium, change of pH tends to reduce considerably the growth of both yeasts tested, because no growth was observed in the control pH 8.5. However, control pH corresponding to ZnO slurries pH in isotonic solution did not disturb the viability of yeasts and molds, whereas in nutrient broth, their growth was inhibited by change of pH. Praphailong and Fleet [75] showed that the effect of pH on the growth of *S. cerevisiae* and that its growth was inhibited at pH 8.0. CaO and MgO are also relatively soluble in water compared with ZnO, which are alkali metal oxides with high solubility constants (CaO, 5.02×10^{-6}; MgO, 5.61×10^{-12}; and ZnO, 3.0×10^{-17}). It suggests that CaO and MgO nanoparticles may kill or inhibit micro-organisms by other properties than their high alkalinity alone.

Sawai and Yoshikawa [76] evaluated antifungal activity of metallic oxides (MgO, CaO and ZnO) by an indirect conductimetric assay. Their results indicated that CaO and MgO had antifungal activity above 1600 ppm against *S. cerevisiae* and other fungi. However, ZnO exhibited only a weak antifungal activity against *S. cerevisiae*, but some growth inhibition was observed at 100 ppm. Our results shown that there was a little or no effect of particle size on either the lethality or growth inhibition of metal oxides.

Our work shown for the first time a fungicidal effect of ZnO against yeasts and also a fungiostatic action against molds. Unlike CaO and MgO, pH of ZnO dispersed in physiological solution was between 7.7 and 8.6, irrespective of its particle size or concentration. It did not affect microbial survival or growth [59, 75]. The difference between yeasts and molds could be attributable to the difference in their cell wall and membrane structure. Chitin makes up to 45% of the cell wall of *A. niger*, however, it is present only 3% in *S. cerevisiae* (Roller and Covill, 1999). For almost all fungi, the central core of the cell wall is a branched β-1,3, 1,6 glucan that is linked to chitin via a β-1,4 linkage [74]. This prove that spore of molds are much more resistant to environment perturbation than yeast. However, S. *cerevisiae* is known to possess the enzymes catalase that catalyses the breakdown of H_2O_2 to O_2 and H_2O [77]. It suggests that metal oxides may inhibit fungi by other characteristics than their generation of superoxides at their surface. The binding of the oxides particles on the fungal cell surface through electrostatic interactions could be a possible mechanism [46]. Concerning ascospores of *Byssochlamys spp.*, the results shown no effect against them. This was expected because ascospores are more resistant to environmental stress than spore itself. Also, younger ascospores might succumb to stress more rapidly than older one because of their relatively 'weak' cell wall, whereas mature ascospores have a denser cell wall which may protect them from perturbation [78].

While the growth inhibition of fungi by CaO and MgO may be primarily due to pH effect in growth medium, ZnO inhibited their growth in culture media even at near neutral pH. This suggests that ZnO could possibly disturb fungal spore germination or upset the mycelia growth. Further study is needed to understand mechanisms of the growth inhibition of fungi by ZnO.

In summary, three metal oxides tested showed both lethal and inhibitory effects on the yeasts, but *S. cerevisiae* was more susceptible to the oxides than *C. tropicalis*. Exposure time was a factor in enhancing the lethal effects of oxides against yeasts, similar with particle size or concentration of metal oxides. There was almost no effect of metal oxides in killing *A. niger*. MgO and ZnO were able to inhibit its growth, whereas CaO was less effective. Metal oxides had modest lethal effects on the spores of *P. variotii* and ascospores (*Byssochlamys spp.*). All metal oxides were able to inhibit the growth of *P. variotii*. There was no effect of either particle size or concentration of metal oxide on their antifungal action.

Conclusions

The producers of fruit juice apply a combination of methods, physical and chemical, to ensure control over pathogenic micro-organisms that may contaminate the product and thus prolong the life of the latter. Among these methods, the addition of preservatives such as sorbate and benzoate in juices frequently occurs as additional protection to a product that pasteurization should be light. However, consumers, more and more health conscious, more demanding use of natural preservative. The control of micro-organisms and involved in the alteration of fruit juice seems possible by the application of various preparations of metal oxide nanoparticles.

For nano ZnO, it has been industrialized using many prepartion methods. However, for nano CaO and MgO, most of research limited into lab scale. Lots of work need to do before it is industrialized. In our work, we focused on investigating the antimicrobial effect of metal oxides (CaO, MgO and ZnO) with different characteristics and sizes on several bacteria, yeast and molds. Our results shown that all the microorganisms tested were subsceptible to one or more of metal oxides. Nanoscale particles has shown a good potential for pothogen control. However, it is still relatively unknown how the properties and activities of these metal oxides act upon microorganism. And, nanoparticles are easy to aggregate during the application process due to its high surface area. But, our work has developed a promising alternative to the use of synthetic agents in the preservation of fruit juices. This alternative is attractive for juice processors and consumers in terms of food security, health and cost, thus leading to a better product image, which will surely enhance the competitiveness of producers of juice fruit.

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