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The Effect of Sorbic, Propionic and Benzoic Acids on Three Strains of Sporulating Yeasts

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Abstract

Four cultures of Sporulating yeast strains: Kluyveromyces bulgaricusSG 120, Saccharomyces bayanusPS8, Saccharomyces uvarum PS22and Saccharomyces sp. A7. The strains were isolated from spoiled canned apple, pasteurized beer and canned apple pieces respectively. The 4cultures of yeast was used. They were tested for their ability to produce ascospores. Techniques for inducing sporulation were used. A 48 hrs. culture suspension for each yeast was prepared. Adequate 1ml was inoculated into screw capped disposable test tubes containing 10 mls. Y M broth consisting of tested levels of 250, 500 1000 ppm of Sorbic, Propionic and Benzoic acids. Culture growth was measured each hour using a Nephelometer. The results showed that Sorbic cid inhibited the growth of yeast SG 120 at the three levels tested. It delayed the growth of strains PS22, PS 8 and A7 at higher levels of 500 and 1000 ppm. Both propionic and benzoic acid failed to inhibit the growth of the four strains SG120, PS8, PS22 and A7 at the three levels.

Keywords: Preservatives; Sorbic; Propionic; Benzoic; Sporulating yeast; inhibition.

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* Corresponding Reference .

1. Introduction

Sorbic, Propionic, Benzoic and their salts are food preservatives. Reference [1] stated that acetic, propionic Sorbic and benzoic acids are weak acids. They are the more common preservatives encountered in food. There are more potent inhibitors of spoilage at acid pHs because the proportion of acid molecules rises exponentially as the pH decreases. Reference [2] reported that the major weak organic food preservatives include benzoic, acetic, Sorbic and propionic acids. They constitute the most widely used acid preservatives in industrial food and beverages production. They function through disruption of membrane organization as well as oxidative stress. Cell of S. cerevisiae that are exposed to weak organic acid tend to form petites by losing their mitochondria Reference [3]. Reference s [4 and 5] reported a concerted loss of mitochondria through autophagy.

- ***Sorbic acid:***

The Reference [7] reported that Sorbic acid occurs naturally in fruits. It is used as a preservative. It inhibits fungi growth but allows the growth of bacterial activity. Its sodium, potassium or calcium sorbate are widely used s inhibitors of yeast, molds and bacteria. It is in use since 1945. The level of sorbate necessary for preservation of specific product depends on a number of factors, including the product composition (pH, moisture and etc). The initial contamination level packaging and storage temperature. It affords protection against recontamination by yeast heated, filtered or sterilized. It is used in table wine to prevent secondary fermentation of reduced sugar, dairy products, sea food, vegetables products and bakery products. Sorbic is effective at 5-6 pH. The permitted level juice and soft drinks 1000 ppm. The maximum acceptable daily intake is 25 mg/kg body weight.

- ***Propionic acid:***

Reference [6] stated that propionic acid is used as an antifungal in food. It is naturally occurring as one of the products of cellulose digestion in ruminants. It is commercially synthesized. Sodium propionate is used as an antimicrobial in bread to prevent some types of bacteria. Its permitted levels in baked food 2000 ppm up to 3000 ppm. No limit had been suggested as acceptable daily intake mg/kg body weight.

- ***Benzoic acid:***

Reference [6] reported that benzoic acid occurs naturally in varieties of products and synthesized industrially. It is an antiseptic, antifungal and antipyretic. Typical products preserve by it include jams, beer, and squash and fruit juice. Sodium and calcium benzoate are antifungal. Reference [8] stated that benzoic acid is more effective against yeast than molds. Its level used in fruit juices is 1000 ppm or as little 100 ppm. It appears that it is more effective in certain foods. The propyl ester has been found to be more effective against yeast. Reference [9] showed that benzoic acid and parbens have antibacterial and fungal properties for preservation of food they may cause urticaria, asthma, rhinitis and angioedema. Reference [10] stated that sodium benzoate may be used as preservative in soft drinks fruit juice and jams. Parbens as a class of benzoic cid derivatives as sometimes used as preservatives in food. Reference

[11] reported that potassium metabisulphite, benzoic acid and Sorbic acid had been widely suggested for maintaining quality of squashes. The limit level of benzoic acid in fruit juice is 1000 ppm. The acceptable level of daily intake of benzoates preservatives is 5mg per kg body weight.

The yeast:

Reference [12] stated that yeast is eukaryotics microorganisms classified as member of fungus Kingdom Phyla Ascomycota and sub Phyla Saccharomycotina (true yeast). They are microscopic unicellular fungi, which typically don't form mycelium and hence exist as single cells. The cells may be round, egg shape or elongated. The shape is usually constant for a given species. Reference [13] reported that yeast cellular organization is similar to organisms (humans). Comparisons of genetics indicated that 31% of yeast genes are similar to human genes and 20% of human diseases genes have counterpart in yeast. Yeast is a model organism for study. Yeast is widely distributed in nature. They are found in plant leaves, flowers, fruits as well as soil. They are found on surface of skin.

Reproduction in yeast:

Yeast is able to reproduce asexually by budding. A daughter cell is formed on parent mother. It duplicates and segregates its DNA. The nucleus divides and migrates to daughter cell. Yeast can reproduce sexually conjugation of two opposite cells **A** cell and Alpha cell. Yeast can reproduce by simple vision. When yeast cell**s** are starved of nutrition, they undergo sporulation.

Phases of growth of yeast:

Growth of yeast is characterized by four phases. Lag phase, occurring immediately after inoculation, depending on the size, inoculum, time of recovery from shock and synthesis of essential enzymes for growth. The second phase is the logarithmic or exponential phase associated with rapid increase number of cells growing at geometric progression at constant rate depending on the composition of the medium and the condition of incubation. The third phase is the stationary phase where steady state equilibrium takes place. The rate of cell growth is exactly balanced by the rate of death. Population growth is limited by exhaustion of available nutrient accumulation of inhibitory metabolize or end products or lack of biological space. The last phase is a decline or death phase. During this phase the numbers of viable cells decrease exponentially.

Factors affecting yeast growth:

- pH: the hydrogen ion concentration has a marked effect on growth of yeast. All organisms had an optimum pH at which they grow best. Yeast and molds grow well in an acid environment pH 3.5- 4.5.
- Optimum temperature: Reference [14] stated that yeast and mold have been cultivated on a media of low pH and temperature of 20- 30^{o}C. the optimum temperature at which the organism grows best. Reference [15] showed that the optimum temperature ofthe yeast strains Kluyveromyces marxianus (SG120), Saccharomyces

cerevisiae (PS8), Saccharomyces cerevisiae (PS22), and Saccharomyces sp. (A7), 44.1^{o}C, 36^{o}C, 35.5^{o}C and 39^{o}C respectively (table1).

Table 1: Minimum, optimum and maximum temperatures for yeast

Yeast	min. temp. ^{o}C	opt. temp^{o}C	max. temp.^{o}C
Kluyveromyces marxianus (SG120)	20.1	44.1	> 47.9
Saccharomyces cerevisiae (PS8)	16.1	36.0	42.0
Saccharomyces cerevisiae (PS22)	17.0	35.5	40.3
Saccharomyces sp. (A7)	18.20	39.0	37.0

Source: Reference [15].

- Moisture: Reference [14] stated that water stated that water accounts 80- 90% of the total weight of the living cell. All organisms require it for growth. Organisms vary in their water requirement. The requirement is not the total water but is the available water. It is expressed as water activity (a_w), the a_w of pure water 1.0 and yeast 0.90.
- Oxygen requirement: organisms differ in their requirement of molecular oxygen (O_2) and atmospheric gases e.g. CO_2. Saccharomyces cerevisiae is a facultative n aerobe which can grow both under anaerobic conditions i.e. fermentation which is known as Crabtree effect. It can grow under aerobic conditions. Yeast are reducing and oxidizing organisms. They have redox potential.
- Nutrient requirement of yeast: all microorganisms require C, N, S, P and various trace elements. Reference [16] showed that Saccharomyces cerevisiaecells consist of 4.1µg/cell 10^4 of total N, 16.6µg/cell 10^4 of protein, 0.8µg/cell $10^4$of total phosphates and 13.8µg/cell 10^4 of total carbohydrates from a total dry mss of 50µg/cell 10^4.

Inhibitory effect of weak organic acids on yeast

Reference s [2] stated that the inhibition of a weak organic acid preservative e.g. benzoic acid requires a level near or greater than legal limits. They identified a synergistic effect of the chemical preservative benzoic acid and Nitrogen starvation: exposure S. cerevisiae to either benzoic acid or N starvation is cytostatic while the combination of the treatment is cytocidal and can be used beneficially in food preservation. Reference [1] reported that all of

weak-acid preservatives are potent inhibitors of spoilage yeast at acidic pHs because the proportion of acid molecules rises exponentially as the pH decreases. The antimicrobial action of the weak acid is initiated by the rapid diffusion of undissociated lipophylic cid molecules through the Cytoplasmic membrane and into cytosol. Because it is close to neutrality, the yeast Cytoplasmic pH causes lipophylic weak acid molecules to dissociate into anions and protons; being charged, these anions and protons are lipid insoluble and accumulate in the cytoplasm. The theory suggests that metabolism is inhibited though proton accumulation sufficient to cause catastrophic decline in intracellular pH. Such an acidification of cytoplasm had been demonstrated in yeast and is caused by acetic acid sulfite and benzoic acid.

Spoilage of yeast

Reference [12] stated that yeast of Zygosaccharomyces genus have a long history as spoilage yeast within food industry because they can grow in presence of high sucrose, ethanol, acetic acid, Sorbic, benzoic and SO_2 concentrations. These acids are common food preservatives. Reference s [2] reported that microbial spoilage of food causes up 40% of all food grown for human consumption worldwide. Yeast growth is a major factor in the spoilage of foods and beverages that are characterized by a high sugar content, low pH and low water activity (a_w). It is a significant economic problem.

Information of the effect of Sorbic, propionic and benzoic acids as preservatives on strains of Sporulating yeast is scarce.

The objectives of this research work are:

- Study of effect of Sorbic propionic and benzoic acids on four strains of yeast at three levels of concentrations 250 ppm, 500 ppm and 1000 ppm. Assessment of Sorbic, propionic, benzoic acids as preservatives against four strains of sporulating yeast.

2. Material and Methods

The source, species, strains, reference code, source of isolation and cultures obtained are presented in table (2).

Table 2: shows four different strains of yeast and their sources.

Species strains reference code	source of original isolation	source of cultures obtained
Kluyveromyces marxianus	SG 120	spoiled canned apples	Reference [17]
Saccharomyces cerevisiae	PS8	Pasteurized beer	Reference [18]
Saccharomyces cerevisiae	PS22	Pasteurized beer	Reference [18]

Saccharomyces	*sp.*A7	Canned apple slices	Reference [19]

Sporulation media:

- Fowell's Acetate Medium [20]
- Kleyn's Acetate Medium [21]
- MacClary's Acetate Medium [22]
- Korodkowa Medium [23]
- V8 Juice Agar]24]
- Malt Extract Agar (Oxoid).

A 48 Hours culture of each yeast was used to inoculate plates of each of the above medium, which were incubated aerobically at 25^{o}C and 37^{o}C.the resultant cultures were examined microscopically on daily basis over 7 day period. Heat fixed smears were stained to show the presence of spores [24]. All the cells present in 10 randomly chosen fields of view under the microscope were examined for the presence of spores. The resulting data was analyzed statistically using student's test [25].

Aliquots (1m) of a 48 h culture suspension of each yeast inoculated into a screw capped disposable test tubes containing 10ml YM Broth consisting of the three different acids : sorbic, propionic and benzoic.

Culture growth was measured each hour using a Nephelometer (Corning EEl, Evans Electroselenium Ltd.). a series of histograms showing the change in optical of cultures over the time period was plotted for each yeast (Figures1-12).

3. Results

The results of the effect of sorbic, propionic and benzoic acids on yeast strains SG120, PS8, PS22and A7 at 1000ppm, 500ppm and 250ppm concentration are presented in Figures 1-12.

Sorbic acid:

The results of sorbic acid on strains SG120, PS8, PS22 and A7 at 1000, 500 and 250ppm are shown in (Figures 1-4). Sorbic acid is cytocidal to the yeast strain SG120. It stopped the growth of it at the end of the lag phase at the 3rd hour.It is the preservative of choice against spoilage yeast strain SG120. When 500ppm level was used the growth was retarded specially at 6th – 8th hours. Sorbic acid retarded the growth the strain specially at 8th h. Sorbic acid retarded the growth of strain PS8 (Figure2) at all levels used. The retardation was proportional to the concentration of sorbic acid. Similar results were observed for strain PS22 (Figure3) and A7 (Figure4). Although sorbic acid retarded the growth of strain PS8 at 1000ppm, it was able to reach the stationary phase. Similar behavior at 500 and 250 ppm compared with the control was observed. These results are in agreement with Reference [1] who stated that

S. cerevisiae degraded food preservatives to hydrocarbons. Hence; the failure of sorbic acid to inhibit the growth of strains PS8 and PS22. Both strains belong to S. cerevisiae.

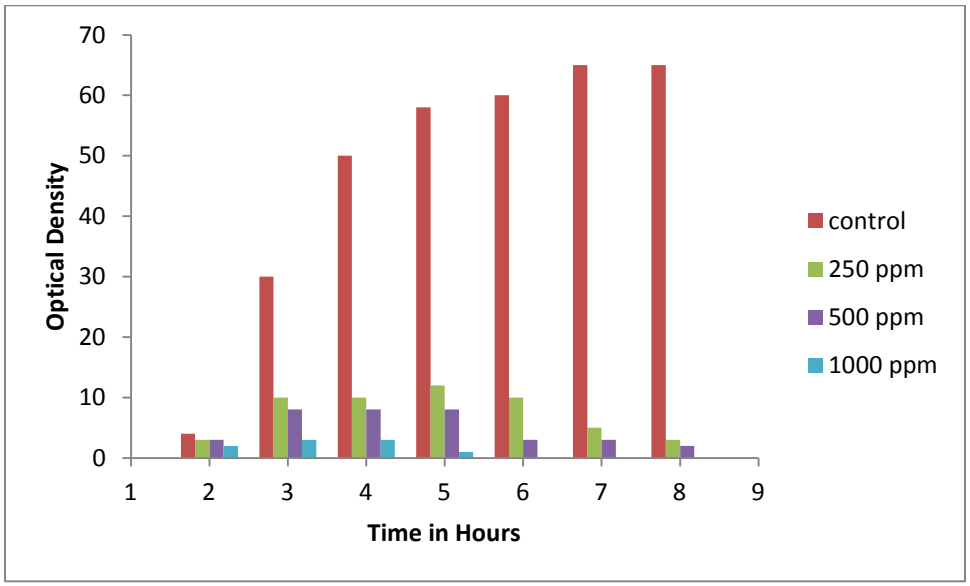

Figure 1: Optical Density of the effect of Sorbic Acid (ppm) on Kluyveromyces marxianus SG 120 in eight hours.

Sorbic acid retarded the growth of yeast strain SG120at 1000ppm and its effect is marked at the8th h. When 500 pp was used. Sorbic acid has no effect on strain A7 at 250ppm level (Figure4). The results are not in agreement with Reference [7] who stated that sorbic acid had been shown to have inhibitory activity against a wide spectrum of yeast, mold and bacteria. No decarboxylation of sorbic acid spoilage yeast was observed as reported by Reference [1].

The present study showed that sorbic acid was cytocidal to yeast strain SG120 at 1000ppm. It retarded the growth at 500 and 250 ppm. It is the most suitable preservative for Yeast SG120 at the permitted level 1000ppm. The maximum acceptable daily intake of sorbic acid is 25ml/kg body weight.

Propionic acid:

The results of propionic acid on strains SG120, PS8, PS22 and A7 at 1000, 500 and 250ppm are shown in (Figures 5- 8). Propionic acid had no effect on yeast strain SG120. The strain was able to grow through the exponential phase to stationary phase at the three levels. This is noticed in yeast strain A7 Figure 5. The growth of the four strain of yeast may be due to their ability to decarboxylate propionic acid as noted by Reference [1] or to the level of concentration used. Reference [6] stated that the permitted level of propionic acid in baked food is 2000- 3000ppm. Propionic acid is not a good preservative for spoilage yeast. It is used as anti –microbial in bread to prevent some types of bacteria. Propionic acid is not a good preservative for spoilage strains SG120, PS8, PS22 and A7 at the three levels (Figures 5, 6, 7and 8).

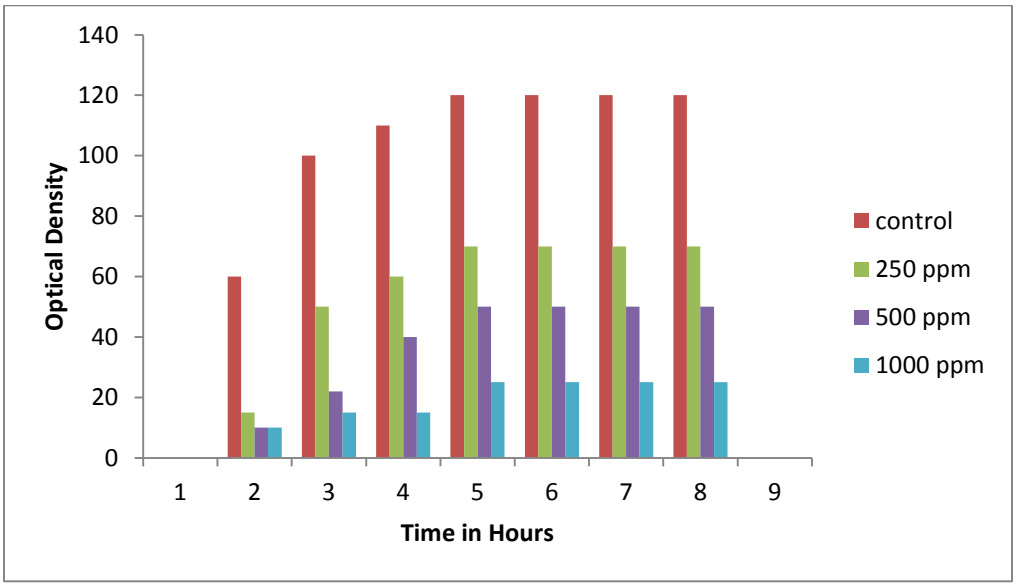

Figure2: Optical Density of the effect of Sorbic Acid (ppm) on *S. cerevisiae* PS 8 in eight hours

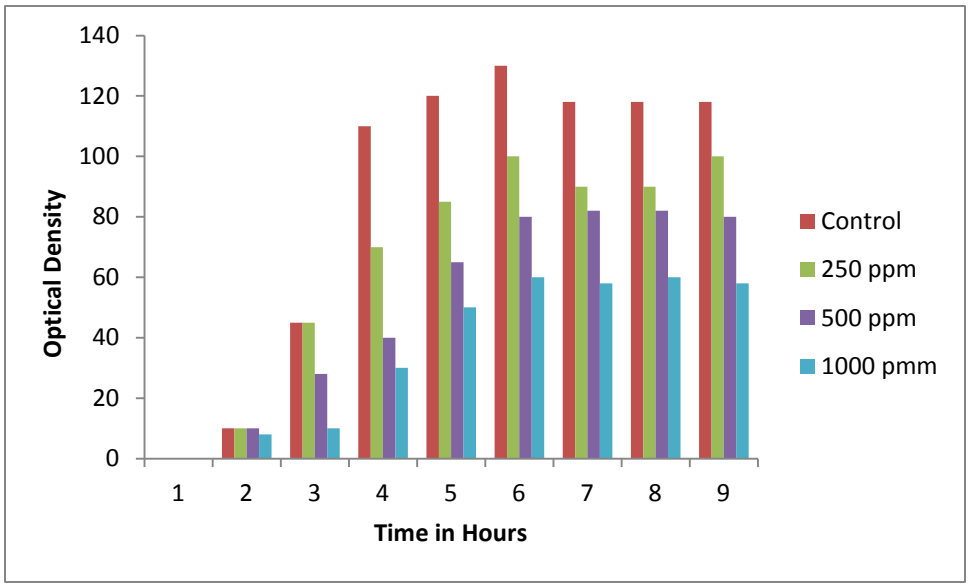

Figure 3: Optical Density of the effect of Sorbic Acid (ppm) on *Saccharomyces . cerevisiae* PS 22 in Nine hours

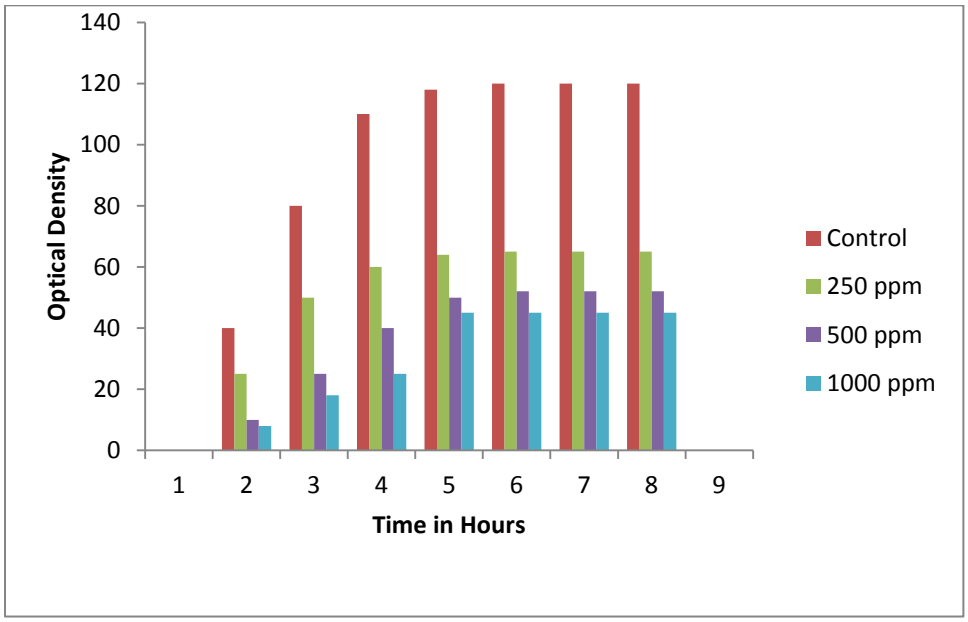

Figure 4: Optical Density of the effect of Sorbic Acid (ppm) in *Saccharomyces sp.*A7

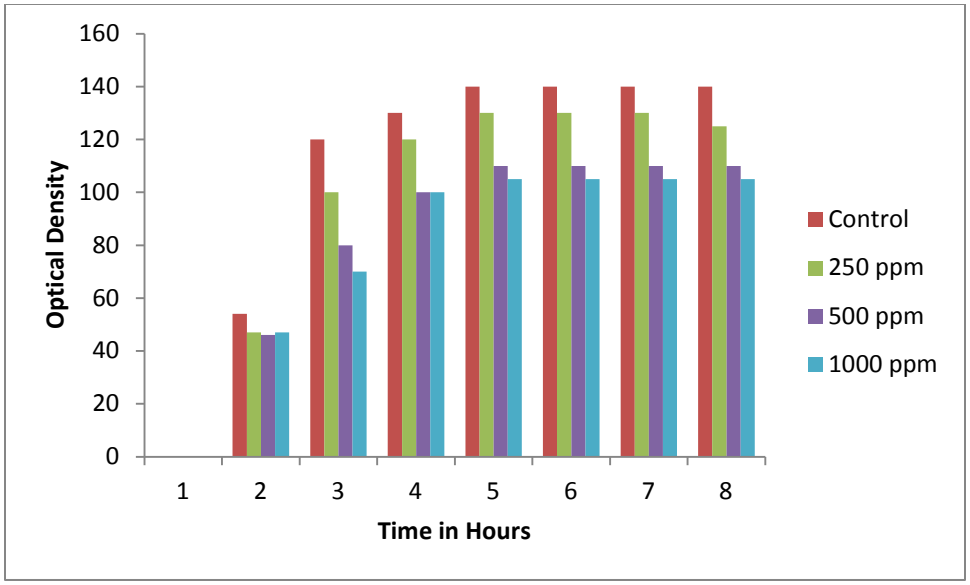

Figure5: Optical Density of the effect of Propionic Acid (ppm) on *Kluyveromyces marxianus* SG 120 in eight hours

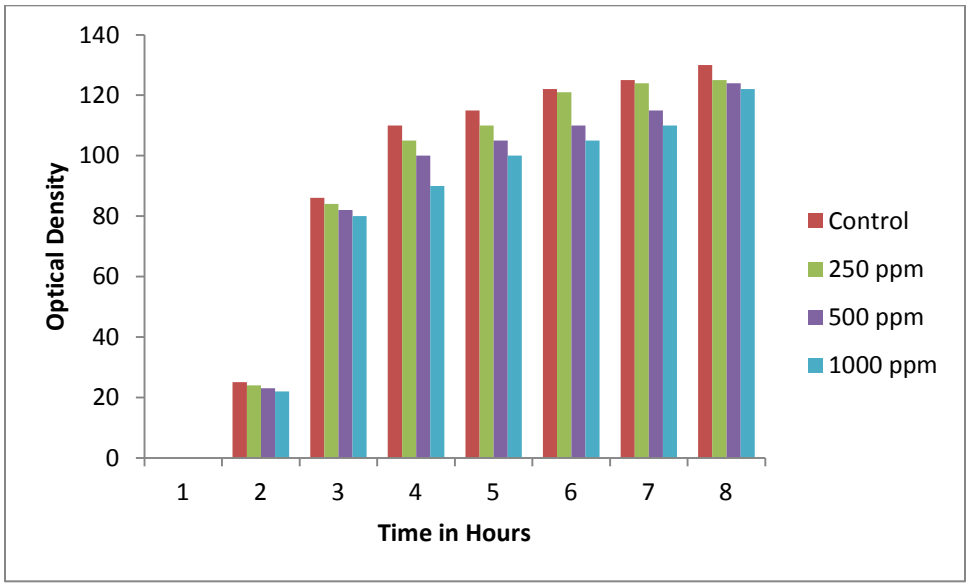

Figure6: Optical Density of the effect of Propionic Acid (ppm) on *S.cerevisiae*PS 8 in eight hours

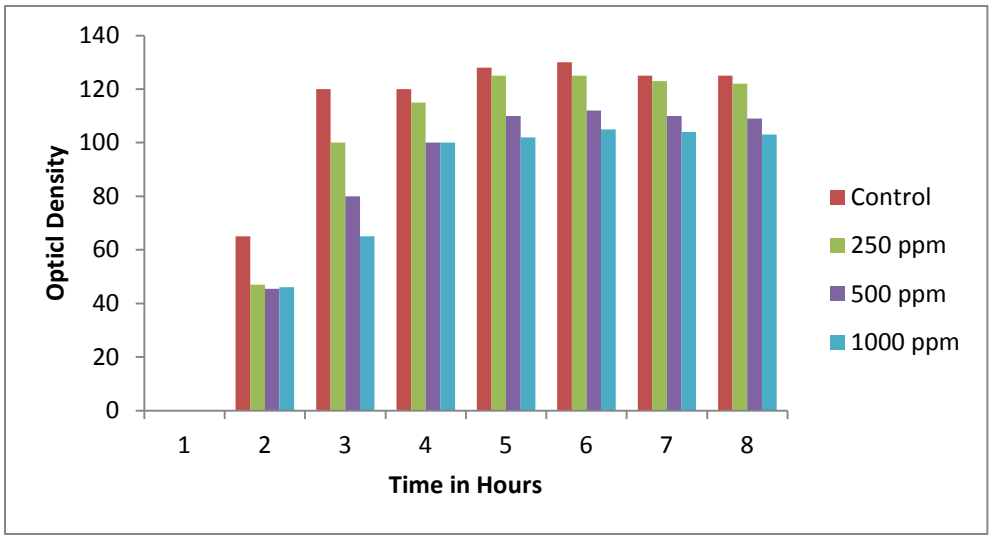

Figure 7: Optical Density of the effect of Propionic Acid (ppm) on *Saccharomyces cerevisiae* PS 22 in eight hours

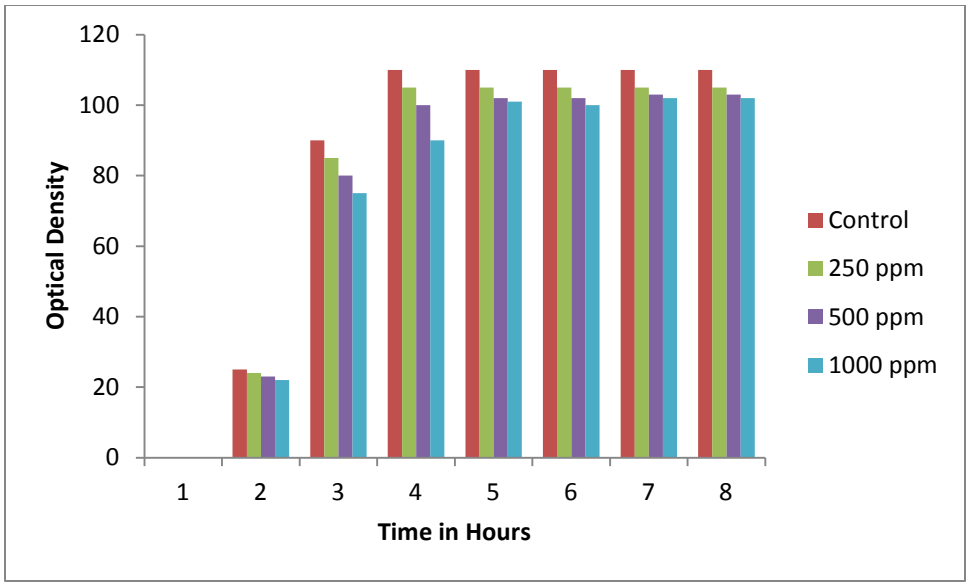

Figure 8: Optical Density of the effect of Propionic Acid (ppm) in *Saccharomyces sp.*A7in eight hours

Benzoic acid:

The results of benzoic acid on spoilage strains SG120, PS8, PS22 and A7 at 1000, 500 and 250ppm are shown in (Figures 9-1 2). The results of strain SG120 is shown in Figure 9.

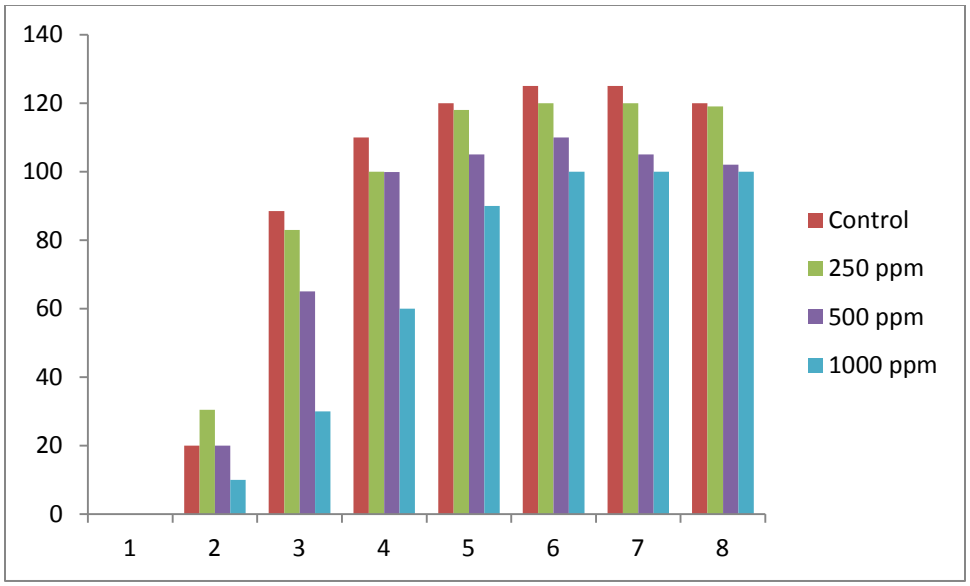

Figure 9: Optical Density of the effect of Benzoic Acid (ppm) on *Kluyveromyces marxianus* SG 120 in eight hours.

Benzoic acid failed to inhibit the growth of yeast strain SG120 until it reaches the stationary phase at the level 1000ppm. Retardation of the growth was noticed at 2nd- 4th hrs. While it was not noticed at 8th hr. As the level of 500ppm retardation was noticed at the 3rd hr. only. At 250ppm benzoic acid failed to retard the growth and the growth was equal to the control as from 5th- 8th hr. The effect of benzoic acid on spoilage yeast S. cerevisiae strain PS8 and PS22 is shown in Figure (10 and 11)

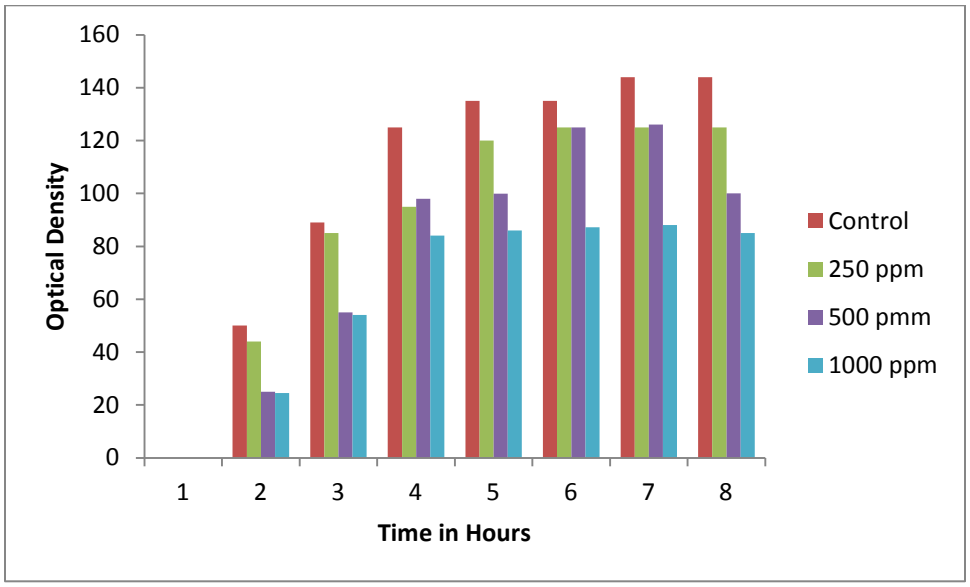

Figure10: Optical Density of the effect of Benzoic Acid (ppm) on *S.cerevisiae*PS8 in Eight hours

Benzoic acid alone failed to inhibit the growth of spoilage yeast *Saccharomyces sp*. Strain A7. The retardation of the four strains is proportional to levels of benzoic acid. The results are in agreement with Reference s [2] who stated that the growth of S. cerevisiae can be retarded by weak organic acid preservatives.

They identified synergistic effect of benzoic acid and N

starvation exposure to S. Cerevisiae: to either benzoic acid or N starvation is cytostatic. The combination between the two is cytocidal. Benzoic acid and N starvation are beneficial, can be used for food preservation. Reference [1] theory on weak acid preservatives on spoilage yeast was based on acidic pHs because the proportion of acid molecules rises exponentially as the pH decreases. The antimicrobial action weak acids is initiated by rapid diffusion of undissociated lipophylic acid molecules through the Cytoplasmic membrane to cytosol.

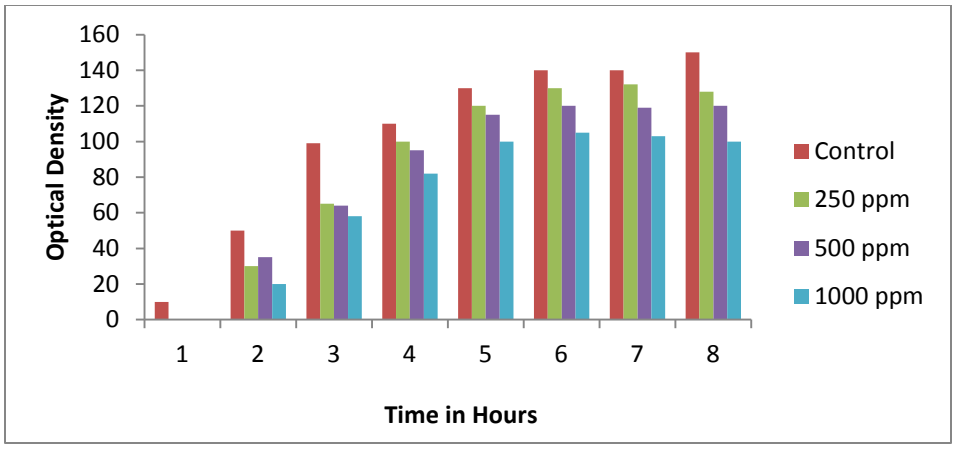

Figure 11: Optical Density of the effect of Benzoic Acid (ppm) on *Saccharomyces cerevisiae* PS 22 ineight hours requires levels near or greater than the legal limits.

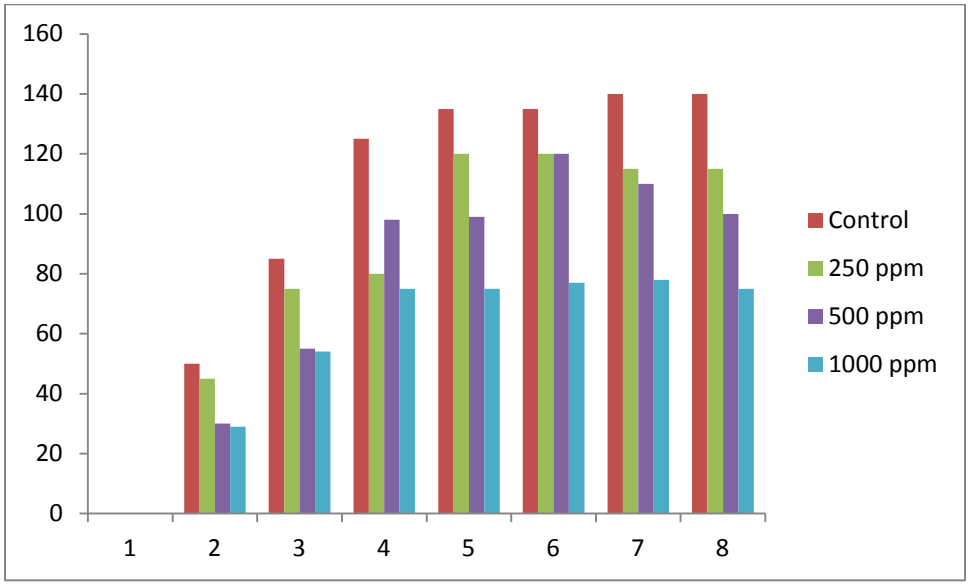

Figure 12: Optical Density of the effect of Benzoic Acid (ppm) in *Saccharomyces sp.*A7 in eight hour

The yeast Cytoplasmic pH causes lipophylic acids molecules to dissociate into anions and protons to sufficient accumulation to cause catastrophic decline intracellular pH. The results disagree with Reference s [6, 8, 9 and11] who stated that benzoic acid is typical product preservative, more effective against yeast. Similar pattern of retardation by benzoic acid was observed for spoilage yeast A7 (Figure 12).

4. Conclusion

- Sorbic acid was cytocidal to spoilage yeast strain SG120. It is the preservative of choice for food spoilage by the strain. It retarded the growth of yeast strains PS8, PS22 and A7 at all levels. Retardation of growth of strains was proportional to sorbic acid concentration.
- Propionic acid failed to inhibit the growth of the four strains of spoilage yeast. They were able to grow through the exponential phase to the stationary phase, especially spoilage yeast strain A7.
- Benzoic acid alone retarded the growth of the four strains at the three levels. It did not inhibit the growth of the four strains. Both propionic and benzoic acid alone are not good food preservatives of the four spoilage yeast strains studied.

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