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EFFECT OF CALCIUM PROPIONATE ON THE INHIBITION OF FUNGAL GROWTH IN BAKERY PRODUCTS

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Abstract

Bakery products are valuable source of nutrients. Bakery products are considered as a source of carbohydrates because starch is the main chemical constituent. Bakery products are subjected to spoilage problems. Mould spoilage is a serious and costly problem for bakeries. The stability of bakery products against the attack by fungi is mainly due to preservatives. Propionates were effective in controlling mold growth on the surface of bakery products. In the present study, the effect of chemical preservative Calcium propionate against the bakery food spoilage fungi was tested in different concentrations, pH and temperature respectively. Among the various concentrations 0.25%, pH 7.5 and 20 °C was highly effective in the control of *Rhizopus stolonifer, Aspergillus niger, Penicillium chrysogenum* and *Mucor* sp.

Key words: Food spoilage fungi, pH, Temperature and Calcium propionates.

1. Introduction

Bakery products are an important source of nutrients *viz*., energy, protein, iron, calcium and several vitamins. Fibers enrichment of several bakery products has recently been tested using an ingredient containing 95 per cent short chain fructo-oligosaccharides (Hocking, 1998; Geetha *et al*., 2012; Jageethadevi *et al*., 2012).

Physical spoilage of bakery products usually involves moisture loss or gain resulting in a loss of texture or mold growth. Microbiological spoilage is often the major factors limiting the shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer (Hickey, 1998). This problem usually occurs in the summer season when the climate is warm and humid (Smith, 1993). Physical factors are the important factor governing mold free shelf life of bakery products. It plays a decisive role when molds compete with bacteria to spoil high moisture foods (Ponte *et al*., 1993). Preservatives are most commonly used to control mold growth in baked goods (CFR, 1990). Propionic acid, an aminocarbolic acid (CH_3CH_2-COOH), is a naturally occurring organic acid and is an oily liquid with a slightly pungent, disagreeable rancid odor. Its salts are white, free-flowing powders with a slight cheese like flavor (Doores, 1993). Propionates were selected on the basis that higher MW fatty acids had a higher antimicrobial effect. This acid or its salt can be used to prevent the bacterial spoilage of bread known as rope caused by certain *Bacillus* sp. (Legan, 1993). Several studies have also reported the effects of propionic acid and its salt on mold growth. Concentrations of propionate ranging from 8 to 12 per cent have been reported effective in controlling mold growth on the surface of bakery products (Doores, 1993).

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2. Materials and methods

Collection of bakery foods

Five different spoiled bakery foods were collected from the bakery in Chidambaram, Tamil Nadu, India. The collected spoiled bakery foods were;

a) Wheat bread.
b) Sponge cake.
c) Plum cake.
d) Chocolate cake.
e) Putting cake.

Isolation and identification of fungi from bakery foods

The samples were processed for the estimation of fungal population by dilution plating method by suspending 1g of each sample in 100 ml sterile water in conical flask. The flask was kept in mechanical shaker for 5 minutes to prepare the suspension. One ml of aliquots was pipetted out to 10 ml sterile water and dispersed uniformly by shaking. One ml of sample was transferred repeatedly till a dilution of 10^{-4} or 1/10,000 was obtained. Aliquots of 1 ml of the serial dilution 10^{-3} and 10^{-4} were pipetted out to sterile petridishes and the plates were poured with SDA medium. The medium was allowed to set and incubated in inverted position. All plates were incubated at room temperature for 3 days. The colonies were counted and expressed per gram of bakery products.

The Lactophenol cotton blue mount was especially used to identify the fungus. In this a drop of Lactophenol cotton blue was placed on a clean slide. With the help of the teasing needle small portion of the colony was picked and it was spread on Lactophenol cotton blue by using another teasing needle. Then, the cover slip was placed over that without any air bubbles. Then, the slide was examined under 45x objective lens.

Effect of calcium propionate on the inhibition of fungal growth

The inhibitory effects of different concentrations of calcium propionate on the fungal growth were studied in the laboratory (Grundy, 1996).

Sabouraud's dextrose broth was prepared and distributed at 50 ml quantities in 100 ml Erlenmeyer flasks in different concentration. The calcium propionate (0.0%, 0.5%, 0.10%, 0.15%, 0.20%, and 0.25%) were prepared and added to the broth and inoculated with 1 ml of fungal inoculums separately and incubated at room temperature for 3 days. After the incubation period the growths were measured at 420 nm using Spectrophotometer.

Effect of calcium propionate at different pH on the inhibition of fungal growth

Sabouraud's dextrose broth was prepared and distributed at 50ml quantities in 100 ml Erlenmeyer flasks in different concentration. The preservative (0.0%, 0.5%, 0.10%, 0.15%, 0.20%, and 0.25%) were added to the broth and the pH was adjusted to various level from 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 in each flask by adding 0.1 N NaOH or 0.1N HCl or and pH in each broth was tested with the help of glass electrode pH meter. The flasks were inoculated with 1 ml of fungal inoculums separately and incubated at room temperature for 3 days. After the incubation period the growths were measured at 420 nm using Spectrophotometer.

Effect of calcium propionate at different temperature on the inhibition of fungal growth

Sabouraud's dextrose broth was prepared and distributed at 50 ml quantities in 100 ml Erlenmeyer flasks in different concentration. The preservative (0.0%, 0.5%, 0.10%, 0.15%, 0.20%, and 0.25%) were prepared and added to the broth and inoculated with 1 ml of fungal inoculums separately and incubated for 3 days at different temperature *viz*., 10 °C, 15°C, 20 °C, 30 °C, 35 °C, 40 °C, and 45 °C in an incubator. After the incubation period the growths were measured at 420 nm using Spectrophotometer.

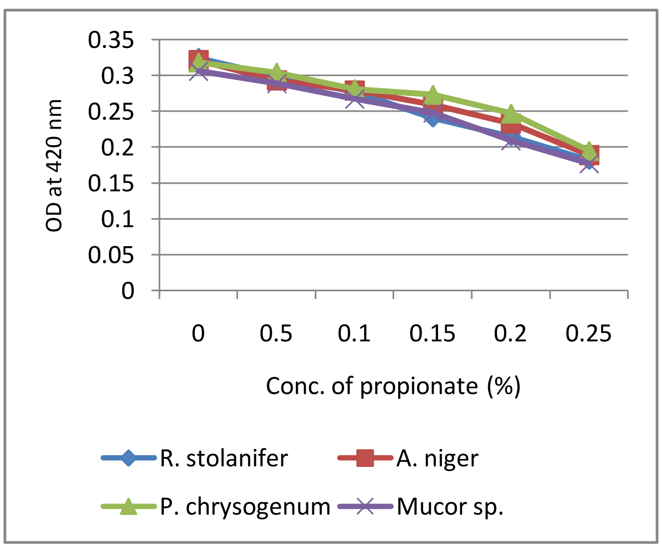
3. Results and Discussion

Spoilage by mold is one of the major problems in bakery food and these results will cause more economic losses. In this present study, five different bakery products *viz.,* wheat bread, sponge cake, plum cake, chocolate cake and putting cake were collected from the bakery in Chidambaram. The isolated cultures were identified as *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium chrysogenum* and *Mucor* sp.

Vytrasova *et al*. (2002) detected, isolated and identified xerophilic fungi *Eurotium amstelodami*, *Eurotium chevalieri*, *Eurotium herbariorum*, *Eurotium rubrum* and *Wallemia sebi*. The resistance of these fungi against elevated temperature and preserving agents was investigated. It was found that *Eurotium* sp. were more resistant than *Wallemia sebi*. Preservation against xerophilic fungi was more effective with the use of propionic acid than with potassium sorbates.

In Propionates, 0.25% was highly effective for the control of the growth of *Rhizopus stolonifer* (0.182 at 420nm), *Aspergillus niger* (0.189 at 420nm), *Penicillium chrysogenum* (0.195 at 420nm) and *Mucor* sp. (0.177 at 420nm) in Fig-1.

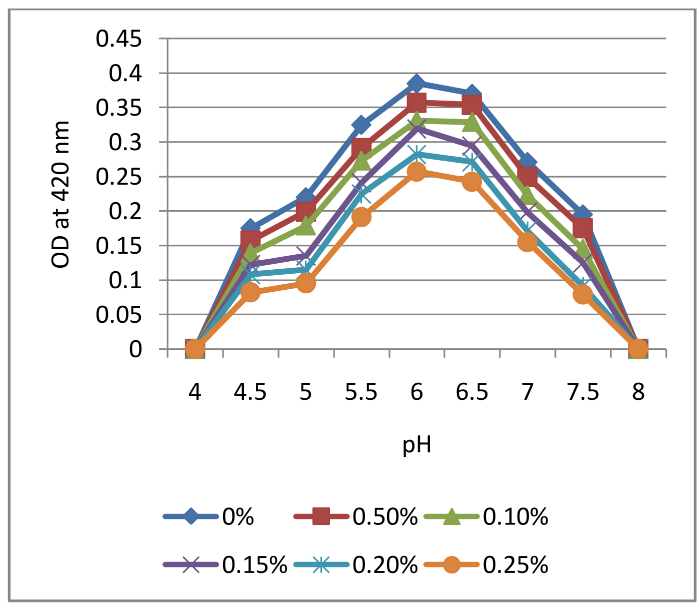
Fig – 1: Effect of Calcium propionate on the inhibition of fungal growth



The growth of the *Mucor* sp. was highly controlled when compared to *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium chrysogenum.* The results clearly showed that the Calcium propionate was effective in the control of bakery product spoilage fungi (Grundy, 1996).

In the present study, the effect of the chemical preservative, Calcium propionates against bakery food spoilage causing fungi was tested under different pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and the results were showed in Fig - 2, Fig - 3, Fig - 4 and Fig - 5. The preservative were used in different concentrations *viz*., 0.0 %, 0.5 %, 0.10 %, 0.15 %, 0.20 % and 0.25 %. The growth of the fungi *Rhizopus stolonifer* (0.079 at 420 nm), *Aspergillus niger* (0.045 at 420 nm), *Penicillium chrysogenum* (0.054 at 420 nm) was highly inhibited at pH 7.5 whereas, in *Mucor* sp. (0.078 at 420 nm) was highest at pH 7.0. For all the fungal isolates, the growth was completely arrested at pH 4.0 and 8.0 (Doores, 1993).

Fig – 2: Effect of Calcium propionate at different pH on the inhibition of *Rhizopus stolonifer* growth



The effect of chemical preservative Calcium propionate against bakery food spoilage fungi was tested under different temperature (10°C, 15°C, 20°C, 30°C, 35°C, 40°C, and 45°C) and the results were showed in Fig - 6, Fig - 7, Fig - 8 and Fig - 9. The preservative were used in different concentrations *viz.*, 0.0 %, 0.5 %, 0.10 %, 0.15 %, 0.20 % and 0.25 %. The growth of the fungi *Rhizopus stolonifer* (0.075 at 420 nm), *Penicillium chrysogenum* (0.087 at 420 nm) and

Mucor sp. (0.031 at 420 nm) was highly inhibited at 15 °C. Whereas, the growth of the fungi *Aspergillus niger* (0.115 at 420 nm) was highest at 20 °C. For all the fungal isolates, the growth was completely inhibited at 10 °C, 40 °C and 45 °C.

Fig – 3: Effect of Calcium propionate at different pH on the inhibition of *Aspergillus niger* growth

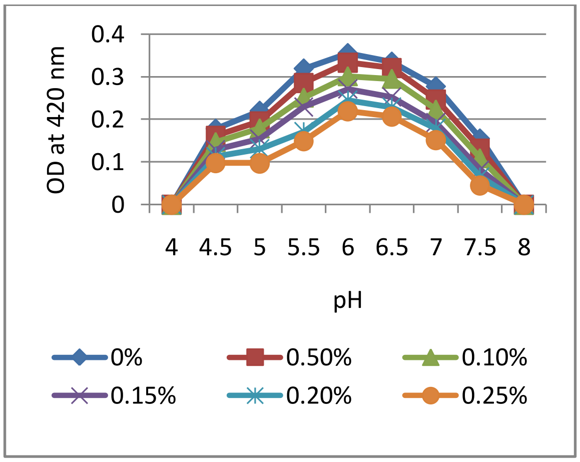
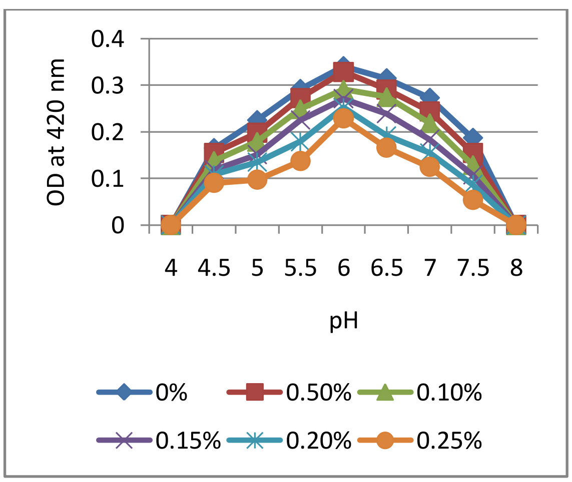


Fig – 4: Effect of Calcium propionate at different pH on the inhibition of *Penicillium chrysogenum* growth



Guynot *et al*. (2003) used a sponge cake analogue to study pH, water activity (a_w) and carbon dioxide (CO_2) levels on the growth of seven fungal species commonly causing bakery product spoilage (*Eurotium amstelodami*, *Eurotium herbariorum*, *Eurotium repens*, *Eurotium rubrum*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium corylophilum*).

Fig – 5: Effect of Calcium propionate at different pH on the inhibition of *Mucor* sp. growth

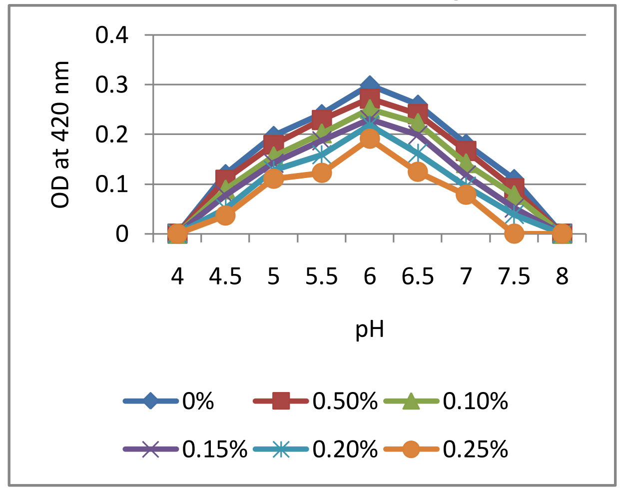
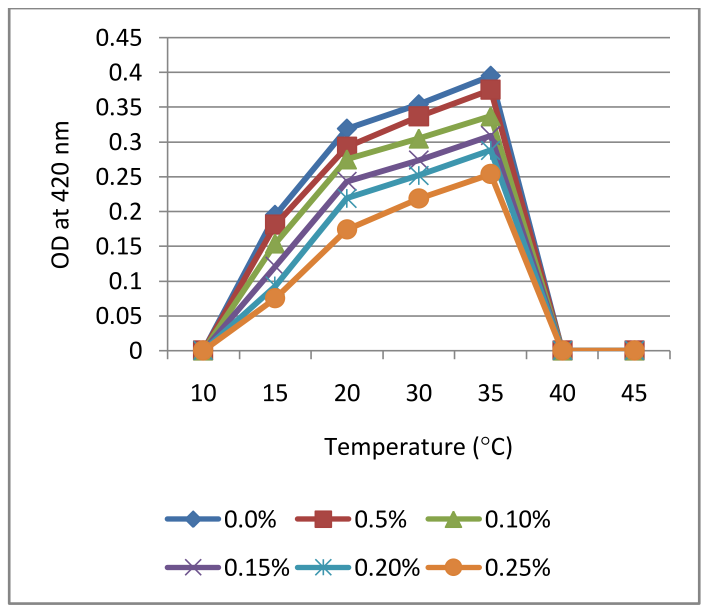
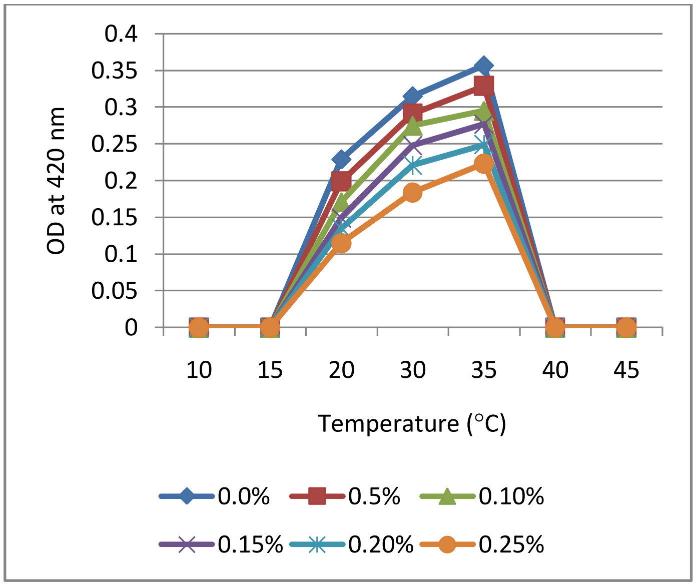


Fig – 6: Effect of Calcium propionate at different temperature on the inhibition of *Rhizopus stolonifer* growth



Water activity, CO_2, and their interaction were the main factors significantly affecting fungal growth. Water activity at levels of 0.80 to 0.90 had a significant influence on fungal growth and determined the concentration of CO_2 needed to prevent cake analogue spoilage. At an a_w level of 0.85, lag phases increased two fold when the level of CO_2 in the headspace increased from 0 to 70 per cent. In general, no fungal growth was observed for upto 28 days of incubation at 25 °C when samples were packaged with 100% CO_2, regardless of the a_w level.

Fig – 7: Effect of Calcium propionate at different temperature on the inhibition of *Aspergillus niger* growth



Sauer and Burrughs (1993) observed that the mold free shelf life of corn was extended for 1 week by 0.5-l. per cent calcium propionate. In the United States, preservation of bread for a few days is generally accomplished by the addition of sodium or calcium propionate. However, some species of *PencilIium* are resistant and can grow in media containing 5 per cent propionic acid (Doores, 1993). The advantage of using propionate is that it has little effect on yeast hence, propionates can be added to bread dough to prevent rope bacteria and mold growth without interfering with leavening (Grundy, 1996). Moreover, propionic acid and propionate are used at levels of 0.1 and 0.2 per cent by flour weight, respectively (Seiler, 1994; Saranraj and Geetha, 2012; Saranraj and Naidu, 2014).

At these levels, the mold free shelf life can be increased by 1-2 d. Compared to sodium benzoate; propionates are generally more active against mold (King, 1981). However, propionic acid caused undesirable odors and flavors in baked goods. Therefore, the bakers prefer to use solid calcium propionate because it is easier to handle the solid salt than the corrosive liquid acid (Seiler, 1994). Sodium, potassium, and calcium propionate are the most widely used antimicrobial additives in the baking industry. They are active against molds and have little effect against bacteria (Smith, 1993). Both calcium propionate and sodium propionate have been affirmed as GRAS (Grundy, 1996).

Fig-8. Effect of propionate at different temperature on the inhibition of *Penicillium chrysogenum* growth

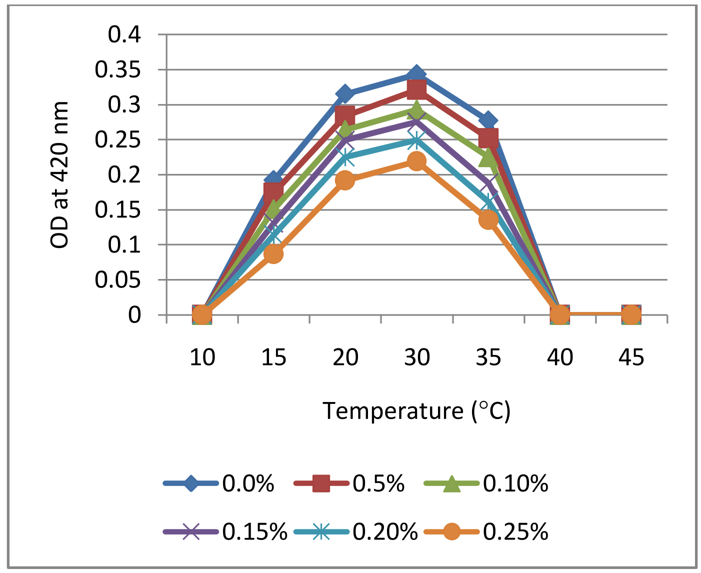
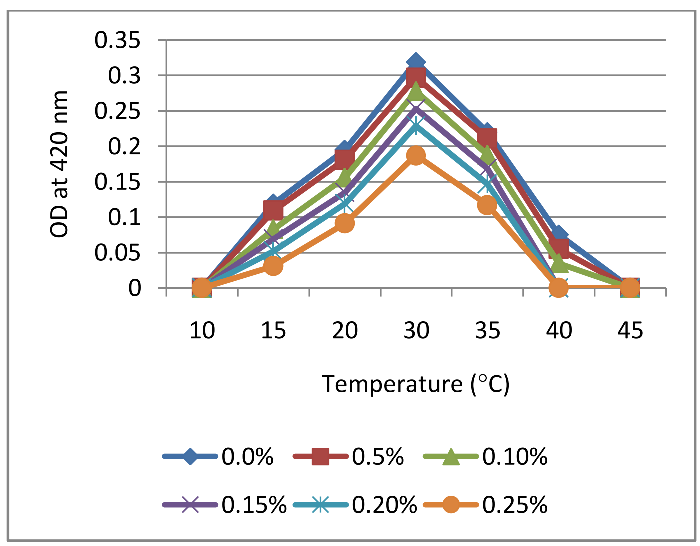


Fig-9. Effect of propionate at different temperature on the inhibition of *Mucor* sp. growth



Marin *et al*. (2002) tested the use of weak-acid preservatives (potassium sorbate, calcium propionate, and sodium benzoate) to prevent spoilage by *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium corylophilum* in analogs of a bakery product. A hurdle technology approach has been considered in which factors other than preservatives are pH and water activity. Potassium sorbate has been found to be the most effective in preventing fungal spoilage of this kind of products at the maximum concentration tested (0.3%). Suboptimal doses (0.03%) of all preservatives tested led to an enhancement of growth of *Aspergillus* and *Penicillium* isolates. The characteristics of the products involved must be carefully considered before making the decision of adding weak-acid preservatives; moreover, they must be added at the right concentrations. Sofas and Busta (1991), reported that the effect of sorbates is more inhibited mold growth than propionate and benzoates.

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