ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(1):38-48

REVIEW ARTICLE

Microbial Spoilage of Bakery Products and Its Control by Preservatives

P.Saranraj* and M.Geetha

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002

Received 11 Sep 2011; Revised 08 Dec 2011; Accepted 13 Dec 2011

ABSTRACT

Spoiled food may be defined as a food that has been damaged or injured so as to make it undesirable for human consumption. Bakery products are an important part of a balanced diet and a wide variety of such products can be found on supermarket shelves. However, bakery products, like many processed foods, are subject to physical, chemical and microbiological spoilage. While physical and chemical spoilage limits the shelf life of low and intermediate moisture bakery products, microbiological spoilage by bacteria, yeast and molds is the concern in high moisture products. Many industrially produced baked goods emerge from the baking process with a surface that is essentially sterile but post bake handling can quickly lead to fungal, microbial surface contamination as a result of exposure to airborne contaminants as well as equipment contact. This present review is focused on the microbial spoilage of bakery products and its control by preservatives. This review assesses the following topics: economical importance of bakery products, microbial spoilage of bakery products, physical factors affecting microbial growth, control of microbial growth in bakery products by using chemical preservatives and biopreservatives.

Key words: Bakery products, Microbial spoilage, Bacteria, Fungi and Preservatives.

1. INTRODUCTION

Bakery products are the important staple foods in most country and cultures. Bakery products and cereals are a valuable source of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements. Cereals have been a basic food of man since prehistoric times and were consumed long before bread making was developed. Variety breads and other bakery products have increased in sales volume within the past decades. The nutrients in bakery products are carbohydrates, proteins, lipids, vitamins and minerals. Bakery industry in India is the largest of the food industries with an annual turnover of about B 3000 crores. India is the second largest producer of biscuits after USA. The biscuit industry in India comprises of organized and unorganised sectors. Breads and biscuits form the major baked foods accounting for over 80 per cent of total bakery products produced in the country. The quantities of bread and biscuits produced are more or less the same.

Bakery products once considered as sick man's diet have now become essential food items of the vast majority of population. Though bakery industry in India has been in existence since long, real fillip came only in the later part of 20th century. The contributing factors were urbanization, resulting in increased demand for ready to eat products at reasonable costs etc. Importance of bakery products has expanded especially the use of whole and natural grains and other natural ingredients. Furthermore, bakery products are considered as a source of carbohydrates because starch is the main chemical constituent [1]. Bakery products are subjected to spoilage problems. These include physical, chemical and microbial spoilage. Since the most common factor of bakery products is water activity, microbiological spoilage, in particular mould growth is the major economical importance of bakery products. Mould spoilage is a serious and costly problem for bakeries.

Commercially produced and properly handled bread generally lacks sufficient amounts of moisture to slow growth of any microorganisms except moulds. As normal cooking temperature destroy fungal spores, post-process contamination from airborne spores and contact with contamination surfaces must be prevented. Filamentous fungi involved in spoilage of bread include *Rhizopus* sp., and *Mucor* sp., *Penicillium* sp., *Eurotium* sp., *Aspergillus* sp. and *Monilia sitophilia*. One of the most common is

***Corresponding Author:** P.Saranraj**, Email:** microsaranraj@gmail.com, **Phone No:** +91- 9994146964

Rhizopus stolonifer, often referred to as the 'bread mould'. Storage of bread under conditions of low humidity retards mould growth. In addition to the economic losses associated with bakery products, another concern is the possibility of mycotoxins production. *Eurotium* species are usually the first fungi to colonize improperly water allowing other species. *Aspergillus* and *Penicillium* which can produce toxins to thrive. Losses of bakery products due to mould spoilage vary between 1-5 per cent depending on seasons, type of products and methods of processing.

Members of the genus *Bacillus* bring about bacterial spoilage of bread known as rope. This is of major economic to the baking industry. Ropiness which is the most important spoilage of bread after moldiness occurs particularly in summer when the climatic conditions favour growth of bacteria. It is mainly caused by *Bacillus subtilis* but *Bacillus licheniformis*, *Bacillus magaterium* and *Bacillus cereus* have also been associated with ropy bread. The incidence of wheat bread spoilage caused by *Bacillus* has increased during the last few years presumably because more bread is produced without preservatives and often raw materials such as bran and seeds are added. Spoilage of bread by rope formation may constitute a health risk, high numbers of *Bacillus subtilis* and *Bacillus licheniformis* in foods may cause a mild form of food illness. Consumption of ropy bread has been association with food-borne illness in reports from Canada and the United Kingdom.

The stability of bakery products against the attack by fungi is mainly due to preservatives. Preservatives help to reduce or prevent wastage of food through spoilage caused by microorganisms. Longer shelf life enables a greater variety of products to be kept in store and in the home. Sofos and Busta (1991)[2] reported that chemical preservatives can control the growth of molds by preventing the metabolism, by denaturing the protein of the cell, or by causing physical damage to the cell membrane. Among these preservatives are propionic and sorbic acid or their salts which have been show to increase the shelf life of bakery products. Propionic acid and calcium propionate are usually employed at concentrations of 0.1 and 0.2 per cent respectively. At these levels, moulds can be inhibited for 2 days or more and the formation of rope can be prevented [3]. Sorbic acid is effective to control mold growth in bakery products at level of 0.125% to 0.3 per cent.

Problems due to spoilage yeasts in bread usually result from post-baking contamination, slicing machines, bread coolers, conveyor belts and racks have been identified as sources. Yeast spoilage is characterized by visible growth on the surface of products. The most frequent and troublesome yeast is *Pichia butonii*, which is known as "chalk mould". This yeast can multiply rapidly on bread, with visible growth often apparent some time before mould occurs. Filamentous fungi are more common than yeast on British breads. Since, filamentous fungi are more easily recognized than yeast, because they generate the majority of complaints. The stability of bakery products against the attack by fungi is mainly due to preservatives. Preservatives help to reduce or prevent wastage of food through spoilage caused by microorganisms. Longer shelf life enables a greater variety of products to be kept in store and in the home.

2.ECONOMICAL IMPORTANCE OF BAKERY PRODUCTS:

Bakery products are an important source of nutrients *viz*., energy, protein, iron, calcium and several vitamins. Commercial bread and biscuits contain around 7.5 per cent to 7.8 per cent protein respectively. Bakery products are good targets for fiber enrichment, as the decline of fiber consumption in the European diet is partially due to the refining of cereals. Most claims concerning fiber content refer to the inherent fibers from wholegrain flour. Fibers enrichment of several bakery products has recently been tested using an ingredient containing 95 per cent short chain fructo-oligosaccharides. These soluble fibers are naturally found in many vegetables including wheat, rye, onion, Jerusalem artichoke, and are structurally close to sucrose, therefore behaving like sugar regarding theology.

The economic losses associated with bakery products, another concern is the possibility of mycotoxins production. *Eurotium* species are usually the first fungi to colonize improperly dried, stored commodities, and when they grow, they increase the level of available water allowing other species (e.g. *Aspergillus* and *Penicillium*) to thrive. *Eurotium* sp. do not produce any significant mycotoxins [4]. Hunt and Robbins (2009)[5], told that bakery products accounted for approximately 9 per cent of total food expenditure, with bread king the most important, accounting for 27 per cents of each dollar spent. However, the consumption of white bread has decreased in the last two decades in western societies while sales of whole wheat bran bread have increased due to health concerns. Sales of flat breads, especially pita bread, have been

IJPBA, Jan - Feb, 2012, Vol. 3, Issue, 1

increasing in western societies due to migration of cultures and societies. Several methods can be used to classified bread products including methods of fermentation, bread volume, and water activity.

In the last few years, the bakery products and flour confectionary sector has witnessed particularly intense technological progress which has brought clear and tangible changes, not only in terms of commercial and qualitatively characteristics of the products, but also in terms of process innovation. Usually bakery products are packaged in plastic films after baking and cooling and they consumed within 1or 2 months post process contamination is unavoidable. Bakery products are classified as products of intermediate moisture content on the other hand the nutritional composition of different bakery products will differ and influence fungal growth. Contamination by xerophilic organisms in these kind of products usually comes from the post baking cooling period, as the cooking temperature is normally enough to eliminate previous contamination [6].

Bakery products are an important part of food expenditure. Consumption of bakery products have been falling since the end of world war two in some industrialized countries such as the USA, Canada, the UK and Australia. According to Hunt and Robbins (2009)[7] bakery products accounted for 9 per cent of the average weekly food consumption. Anon (2000)[8] estimated the consumption of bread in the UK was still 41.5 kg per person in 1990. Baur (2001)[9] estimated the western European bread market to be 23.000 million French francs. For several thousands of years, man has used wheat and other cereals to produce bread with an average consumption of about 65 kg of bread per capita per year in Europe, it remains and important constituent of a balanced healthy diet [10] convenience, taste and freshness determine to a great extent the appeal of bread products and are expected to remain the driving factors for purchases of bakery products.

3. MICROBIAL SPOILAGE OF BAKERY PRODUCTS:

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. These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover. Rachel Needham *et al*. (2004)[11] tested the microbial spoilage caused by bacteria, yeast and fungi and enzymic spoilage caused by lipoxygenase can be differentiated from one another and from unspoiled bread analogues after 48 hours using Cluster analysis, prior to signs of visible spoilage. Analysis of the bread analogues with gas chromatography mass spectrometry identified volatiles produced by the different spoilage types and unspoiled bread analogues. Microbial analysis showed that the levels of each microorganism used increased with time.

Francesca Valerio *et al*. (2009)[12] characterized 125 presumptive LAB isolates by repetitive extra genic palindromic – PCR (REP-PCR) and sequence analysis of the 16s rRNA gene, leading to the identification of the following species: *Weissella confusa*, *Weissella cibaria*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lactobacillus rossiae* and *Lactobacillus plantarum*. The REP-PCR results delineated 17 different patterns whose cluster analysis clearly differentiated *Weissella cibaria* from *Weissella confusa* isolates.

3.1. Bacterial spoilage

Bacteria also have a potential to contaminate baked products although their growth is more restricted by low water activity and low pH. The spores of *Bacillus subtilis* for examples are heat resistant; 55 per cent remain active in amylase after 20 minutes at 65°C. This microorganism, which is present in raw ingredients, e.g., flour, sugar, and yeast, causes rope in bread [13]. Ropey bread is characterized by discoloration from brown to black, the release of a rotten fruit odor and having an extremely moist, stringy bread crumb [14]. This problem usually occurs in the summer season when the climate is warm and humid [13]. Ropiness can develop very rapidly under warm and humid conditions. So, it is a common problem in the warm climates of Mediterranean countries, Africa and Australia [15].

A major source of *Bacillus* contamination is from the raw ingredients so ideally it would be profitable for bakeries to use only ingredients with low level of contamination. Ropey bread is caused mainly by *Bacillus subtilis* but other species of *bacillus* are capable of causing rope and these include *Bacillus licheniformis, Bacillus megaterium* and *Bacillus cereus* [15]. Ropy spoilage in bread is first detected by an odour similar to that of pineapple. Later, the crumb becomes discoloured, soft and sticky to the touch, which makes the bread inedible. The deterioration of bread texture is due to slime being formed as a result of the combined effect of the proteolytic and amylolytic enzymes produced by some *Bacillus* strains that results in slime formation.

IJPBA, Jan - Feb, 2012, Vol. 3, Issue, 1

This prevention of rope problems require strict sanitary as well as good manufacturing practices designed to control the spores of *Bacillus* species. Preservatives, such as propionate, can be usually used to eliminate this problem [16].

Staphylococcus aureus is one type of bacteria known to contaminate pie fillings. This microorganism has also been implicated in food poisoning outbreaks from cream filled bakery products [17]. He also noted that of the 323 outbreaks of food poisoning which occurred in Britain between 1969 and 1972, cakes and desserts contributed 3 per cent. Other bakery ingredients, such as chocolate, desiccated coconut and cocoa powder were found to be contaminated with *Salmonella* [17]. For example, frozen pizza was significantly affected by *Salmonella typhimurium* [18].

3.2. Yeast spoilage

Yeast problems occur in bakery products. Wild yeast include *Trichosporon variable*, *Saccharomyces*, *Pichia* and *Zygosaccharomyces*. *Saccharomyces* sp. produce white spots in bread can be leading to the term chalk bread. Legan and Voysey (1981)[19] studied that the yeast problems in bakery products can be divided into two types: (a) visible yeast which grows on the surface of the bread in white or pinkish patches and, (b) fermentative spoilage associated with alcoholic and essence odors and hence osmophilic yeasts. Yeasts, which cause surface spoilage of bread, are mainly *Pichia burtonii* ("Chalk mold"). Contamination of products by osmophilic yeasts normally results from unclean utensils and equipment. Therefore, maintaining good manufacturing practices will minimize the contamination by osmophilic yeasts.

3.3. Mold spoilage

Mould spoilage is a serious and costly problem for bakeries and use of preservatives is therefore an attractive means to diminishes the spoilage and insure the food safety. However, consumers today are not in favor of additives as preservatives and an urge to reduce the quantities used exists within the bakery industry [20]. Mold growth is by far the major factor limiting shelf life of high and intermediate bakery products. In general, most molds prefer high a_w values (>0.8) while a few xerophilic molds prefer to grow at a_w values as low as 0.65. Mold growth on bakery products is a serious problem that results in economic losses. Furthermore, losses of products due to mold spoilage are between 1 and 5 per cent depending on the type of product, season, and the method of processing [21]. According to Hickey (1998)[22], losses due to mold spoilage in the bakery industry average about 200 million pounds of product each year.

Mold spores are generally killed by the baking process in fresh bread and other baked products [23]. Therefore, for bread to become moldy, it must be contaminated either from the air, bakery surfaces, equipment, food handlers or raw ingredients after baking during the cooling, slicing or wrapping operations. This means that all spoilage problems caused by molds must occur after baking. The mold spore counts are higher in the summer months than in the winter due to airborne contamination in the warmer weather and more humid storage conditions. Furthermore, moisture condensation on a product's surface, due to packaging prior to being completely cooled, may be conductive to mold growth. Jarvis (2001)[24] found that mold spoilage caused undesirable odors and is often found on the surface of the product. The most common molds found in bakery products are: *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Monilia* sp., *Mucor* sp. and *Eurotium* sp.

4. PHYSICAL FACTORS INFLUENCE THE MICROBIAL GROWTH

4.1 Effect of temperature, pH and water activity

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 [25]. Molds tend to be less fastidious in their relationships to pH than bacteria. Generally, molds are tolerant of acid conditions and favour an acidic pH (3.5-5.5). Therefore, foods with pH value <4.5 are not usually spoiled by bacteria but are more susceptible to mold spoilage.

Abellana *et al.* (1999)[26] to obtained a method for studying the growth of xerophilic fungi on bakery products, and to determine the effect of water activity (a_w), temperature, isolates and their interaction on mycelial growth of *Eurotium* sp. The results showed that there were intra-isolate differences (P, 0.001) due to water activity (a_w), temperature, isolate, and two-and three way interaction. Optimum growth of all isolates over water activity (a_w) temperature range tested showed optimum at 0.90 a_w and 30 °C, with an interval of growth rate of 3.8-5.1mm d^{-1} at 0.75 a_w, growth was less than 0.15 mm. d^{-1}.

Temperature plays a dominant role in mold growth and in the germination of spores. The majority of molds grow within a temperature range of 18.3-29.4 °C [27] when the temperature of bakery product is reduced from that for optimum temperature. Chamberlain (1993)[28] reported that the reduction in the storage temperature from 27 °C to 21 °C doubled the mold free shelf life of cake and emphasized the need for care during distribution and storage.

Abellana *et al*. (2001)[29] compared the effect of temperature and water activity and their interactions on the rate of mycelia growth of *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium corylophilum* and *Aspergillus flavus* on a sponge cake analogue. As expected, growth rates showed dependence on a_w and temperature. However, no significant differences were observed in the growth rates of different isolates. The minimum a_w values for growth of the *Penicillium* sp. was 0.85 – 0.90. *Aspergillus flavus* was able to grow at 0.90 a_w when the temperature was above 15.8 °C. They showed that fungal growth by these species on a sponge cake analogue, with a composition similar to usual bakery products, was prevented if the a_w is kept at 0.85.

Vytrasova *et al*. (2002)[30] 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 sorbic acid than with calcium propionate.

Guynot *et al*. (2003)[31] used a sponge cake analogue was used 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*). 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 per cent CO_2, regardless of the a_w level.

Elena Guynot *et al*. (2005)[32] studied the mould growth on fermented bakery product analogues (FBPA) of two different pH (4.5 and 5.5), different water activity (a_w) levels (0.80 – 0.90) and potassium sorbate concentrations (0–0.3%) by using seven moulds commonly causing spoilage of bakery products (*Eurotium* sp., *Aspergillus* sp. and *Penicillium corylophilum*). For the description of fungal growth as a function of a_w, potassium sorbate concentration and pH, 10 terms polynomial models were developed. Modelling enables prediction of spoilage during storage as a function of the factors affecting fungal growth. At pH 4.5 the concentration of potassium sorbate could be reduced to some extent only at low levels of a_w, whereas at pH 5.5 fungal growths were observed even by adding 0.3 per cent of potassium sorbate.

Mariona Arroyo *et al*. (2008)[33] examined nutrient assimilation by two mycotoxigenic spoilage fungi (*Penicillium verrucosum*, *Aspergillus ochraceus*) and four other food spoilage fungi (*Penicillium coryolophilum*, *Penicillium roqueforti*, *Cladosporium herbarumi*, *Eurotium repens*), of C-sources in wheat bread in relation to a biotic factors of water availability, pH and temperature and the presence/absence of a preservative, potassium sorbate. These studies were to understand the relative potential co-existence, nutritional partitioning and niche exclusion in bread-based matrices. The niche size decreased significantly with decrease in water availability, temperature and pH. There were also significant interactive effects between pH and the preservative. The data were used to determine the niche overlap indices (NOI) of competing fungi relative to the two ochratoxigenic species. These showed that *Penicillium verrucosum* and *Aspergillus ochraceus* were nutritionally dominant over the other species.

4.2. Effect of salt tolerance:

Samapundo *et al*. (2010)[34] evaluated the effect of NaCl and various NaCl replacers ($CaCl_2$, $MgCl_2$, KCl and $MgSO_4$) on the growth of *Penicillium roqueforti* and *Aspergillus niger* at 22°C. In addition, challenge tests were performed on white bread to determine the consequences of NaCl reduction with or without partial replacement on the growth of *Penicillium roqueforti*. The results obtained concluded that at equivalent water phase concentrations the isolates exhibited differing sensitivities to the salts evaluated with NaCl and $MgCl_2$ having the greatest inhibitory action on the growth of *Aspergillus niger* and *Penicillium roqueforti*, respectively. $MgSO_4$ had the least antifungal activity. At equivalent molalities, $CaCl_2$ had in general the largest antifungal activity. Although the water activity (a_w) lowering effects of the compounds studied play a large role in explaining the trends observed, at equivalent water phase concentrations $MgCl_2$ was found to have a smaller inhibitory effect on *Aspergillus niger* than that expected from its a_w depressing effect. The challenge tests revealed that no difference occurred in the growth of *Penicillium roqueforti* on standard white bread, bread with 30 per cent less NaCl and bread in which 30 per cent of the NaCl has been partially replaced by a mixture of KCl and Sub-salt.

5. CONTROL OF MICROBIAL GROWTH IN BAKERY PRODUCTS

Several methods can be used to control mold growth on bakery products including reformulation, freezing, and most commonly, the use of preservatives.

5.1. Reformulation to reduce product a_w

Reformulation involves a reduction of available water e.g., a_w in bakery products to obtain a longer shelf life. Reduction in product a_w can be achieved by dehydration, either through evaporation or freeze-drying or by high osmotically active additives e.g., sugars and salts, incorporated directly into the food. The degree of a_w reduction is of practical significance in making a food non-perishable. The response to a given degree of a_w varies greatly among microorganisms in different environments [35].

Water contained in solutions of sugars and salt becomes unavailable to microbes due to the increased concentration of crystalloid. Furthermore, microbes are directly damaged osmotically by concentrations of these substances. This effect may be due to the adverse influence of lowered water availability on all metabolic activities, since all chemical reaction of cells require an aqueous environment. Control of mold growth in bakery products normally relies on maintaining a sufficiently low a_w. For example, an a_w of 0.75 can give a 6 month extension in mold free shelf life. Higher a_w levels e.g., above 0.77 will only result in a short extension of shelf life. However, since low a_w can adversely affect the quality of the product and cause changes in shape and texture, care must be taken when reducing product a_w [36].

5.2. Freezing

Freezing has been used for long term preservation of bakery products particularly, cream filled products. Quick freezing is important in controlling the formation of ice crystals. Large ice crystals are formed when the rate of freezing is slower; the large crystals can disrupt membranes and internal cellular structures [37]. Cakes, cookies, short cake, and pancakes are commonly frozen and marketed in the frozen form. Bread has been held fresh for many months by storage at -22°C [38]. In contrast to fresh bread, which stales in less than a week, frozen bread stales very slowly. Therefore, the lower the temperature, the more slowly it stales. Desrosier (2006)[38] reported that bread frozen quickly after baking and held for one year at -18°C, was equivalent in softness to fresh bread held for two days at 20°C.

5.3. Preservatives

Preservatives are most commonly used to control mold growth in baked goods. The Code of Federal Regulations (CFR) defines preservatives "as an antimicrobial agent used to preserve food by preventing growth of microorganisms and subsequent spoilage". There are two classifications of preservatives: chemical and natural permitted chemical mold inhibitors in bread include acetic, sorbic, propionic acids and their salts. Natural food preservatives, such as cultured products, raisins, vinegar, are identified by their common name on the ingredient statement.

6.EFFECT OF CHEMICAL PRESERVATIVES:

Marin *et al*. (2002)[39] used the hurdle technology approach to prevent fungal growth of common contaminants of bakery products including isolates belonging to the genera *Eurotium*, *Aspergillus* and *Penicillium*. Several levels (0.003%, 0.03% and 0.3%) of calcium propionate, potassium sorbate and sodium benzoate were assayed on a model agar system in a full-factorial experimental design in which the other factors assayed were pH (4.5, 6 and 7.5) and a_w (0.80, 085, 0.90 and 0.95). Potassium sorbate was found to be the more suitable preservative to be used in combination with the common levels of pH and $a_{w.}$

Guynot *et al*. (2004)[40] applied a hurdle technology approach to control common mold species causing spoilage of intermediate moisture bakery products (*Eurotium* sp., *Aspergillus* sp., and *Penicillium corylophilum*), growing on a fermented bakery product analogue (FBPA). The factors studied included a combination of different levels of weak acid preservatives (potassium sorbate, calcium propionate, and sodium benzoate; 0–0.3%), pH (4.5–5.5) and water activity (a_w; 0.80–0.90). Potassium sorbate was found to be the most effective in preventing fungal spoilage of this kind of products at the maximum concentration tested (0.3%) regardless of a_w. The same concentration of calcium propionate and sodium benzoate was effective only at low a_w levels. On the other hand, potassium sorbate activity was slightly reduced at pH 5.5, the 0.3 per cent being only effective at 0.80 a_w.

Francesca Valerio *et al*. (2009)[41] characterized seventeen strains by a different REP-PCR pattern, were screened for their antifungal properties. They were grown in a flour-based medium, comparable to a real food system, and the resulting fermentation products (FPs) were tested against fungal species generally contaminating bakery products, *Aspergillus niger*, *Penicillium roqueforti* and *Endomyces fibuliger*. The results of the study indicated a strong inhibitory activity comparable to that obtained with the common preservative calcium propionate (0.3%w/v) often Lactic acid bacterial strains against the most widespread contaminant of bakery products, *Penicillium roqueforti*. The screening also highlighted the unexplored antifungal activity of *Lactobacillus citreum*, *Lactobacillus rossiae* and *Weissella cibaria*, which inhibited all fungal strains to the same or a higher extent compared with calcium propionate.

6.1. Sorbic acid and sorbates

Sorbic acid (CH_3-CH=CH-CH=CH-COOH) and its potassium salt, are recognized as effective antimold agents, and have been considered historically safe for food use. Sorbic acid and potassium sorbate are “Generally Regarded As Safe” (GRAS) for their use in foods. This acid or its potassium salts, has been used to retard microbial degradation in a large variety of food items. Major groups of foods in which sorbate has been used commercially because of its antimicrobial activity include baked goods, cheese, cake, chocolate coatings, fish products, fruit, butter, salad, vegetables and wine. The usefulness of sorbic acid as a mold inhibitor in bakery products such as cakes, cake mixes, pies, pie filling, doughnuts, etc. has also been demonstrated by Gorton (1999)[42]. In general, sorbic acid is effective against bacteria, and especially molds and yeasts. The major commercial use of sorbate is as a fungistatic. Several studies have demonstrated the inhibitory effect of potassium sorbate on mold growth in food products. Ray and Bullerman (2001)[43] reported that potassium sorbate exhibited a great effect on the growth of *Aspergillus niger* and *Penicillium* species. Sauer and Burroughs (1993)[44] observed that mold was inhibited for 2 weeks by using 0.5 per cent potassium sorbate.

The levels of sorbate used in bakery products ranges from 0.001-0.3 per cent [45]. These concentrations have no major impact on food quality, but higher levels may cause undesirable changes in taste and flavor. Sorbates are more than twice as effective as propionates in inhibiting mold growth in bakery products, but have an adverse effect on yeast, reducing loaf volume and making dough sticky and difficult to process. This problem can be overcome by either spraying sorbate onto the product's surface after baking or mixing anhydrates of sorbic acid with fatty acids, such as palmitic. In addition, sorboyl palmitate has also been successful in controlling mold growth without interfering in the fermentation process. The heat of the baking process hydrolyses sorboyl palmitate and releases sorbic acid which inhibits molds during storage [45]. Sorbate acts synergistically with sodium chloride, calcium propionate, sodium propionate, citric acid and sucrose achieving a longer shelf life.

6.2. Propionic acids and its salts

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 [46]. 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. 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 [46]. However, not all molds were equally sensitive to the inhibitory effect of propionate. For example, at 0.3 per cent calcium propionate, growth of *Monilia sitophila* and *Pencillium virdiicatum* in bread was inhibited for 2 days and 0.5 day respectively.

Marin *et al*. (2002)[47] 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.

IJPBA, Jan - Feb, 2012, Vol. 3, Issue, 1

IJPBA, Jan - Feb, 2012, Vol. 3, Issue, 1

Marin *et al*. (2003)[48] studied the effects of sorbic acid and potassium sorbate on growth of different *Eurotium* isolates when added to a bakery product analogue were tested under different environmental conditions. Water activity of the products was adjusted to values in the range of 0.75-0.90, and storage temperatures were in the range of 15-30 °C. Preservatives were added in concentrations ranging from 0.025 per cent to 0.2 per cent. It was observed that 0.025 per cent and 0.05 per cent concentrations always enhanced the isolates growth, while 0.1 per cent had little preservative effect. Finally, even the highest concentration (0.2%) was not suitable as it only controlled fungal growth under certain water activity and temperature levels.

Suhr and Nielsen (2004)[49] investigated the inhibition of spoilage organisms from bakery products by weak acid preservatives in concentrations of 0%, 0.003%, 0.03% and 0.3% (w/v) experimentally on a substrate media with water activity (a_w) and pH ranging from sourdough fermented acidic rye bread to alkaline intermediate moisture sponge cake types (a_w 0.80-0.95, pH 4.7-7.4). Initially, rye bread conditions (a_w 0.94-0.97 and pH 4.4-4.8) in combination with calcium propionate. Results showed that the highest concentration of propionate (0.3%) at all conditions apart from high a_w (0.97) and high pH (4.8) totally inhibited fungal growth for a 2 week period, with the exception of *Penicillium roqueforti*, *Penicillium commune* and *Eurotium rubrum*.

7. EFFECT OF BIOPRESERVATIVES:

In recent years, bio-preservative (The use of microorganisms and their metabolites to prevent spoilage and to extend the shelf life of foods) has gained increasing interest due to consumer's demands. Lactic acid bacteria (LAB) as bio-preservation organisms are of particular interest. They have been used for centuries as starter cultures in the food industry and are able to produce different kind of bioactive molecules, such as organic acids, fatty acids, hydrogen peroxide and bacteriocins. The antifungal activity of LAB is documented [50].

Ozay Mentes *et al.* (2005)[51] studied the effect of two different sourdoughs, produced with *Lactobacillus plantarum* and *Lactobacillus alimentarius,* with antimicrobial activities on inhibition of rope-forming *Bacillus* strains in wheat bread was studied. Addition of 15 per cent or 20 per cent low pH (pH 3.5–4.0) sourdough to bread dough, which were produced by using two strains (*Lactobacillus plantarum* and *Lactobacillus alimentarius*) separately, prevented the generation of visual rope caused by *Bacillus subtilis* and *Bacillus licheniformis*. However, adding 10 per cent sourdough was not enough to prevent the generation of visual rope. When repeated with sourdoughs with a higher pH (pH > 4), additives at 10 per cent or 15 per cent did not prevent the generation of rope, whereas additives at 20 per cent prevented the generation of visual rope caused by both *Bacillus subtilis* and *Bacillus licheniformis.*

Sana M'hir *et al*. (2007)[52] collected thirty samples of fermented wheat dough microflora from different Tunisian bakeries. Forty per cent of the samples contained approximately 10^6cfu/g of mesophilic aerobic bacteria (MAB). Lactic acid bacteria (LAB) and yeasts dominated the microflora of these samples. They varied from 10^5 to 10^8cfu/g. The LAB/yeasts ratio arising from microbial counts were varied between 1/1 and 200/1. More than 50 per cent of the analysed samples were deprived of *Enterococcus* sp. The content of contaminating microflora like coliforms and mesophilic *Bacillus* ranged from 10^2 to 10^4cfu/g. The ratio between LAB and coliforms were estimated to about 26 per cent of the analysed samples. This ratio is more important between LAB and mesophilic *Bacillus*. The LAB/mesophilic *Bacillus* ratio was about 10^4 for 45 per cent of the analysed samples. However, *Micrococcaceae* were absent in all samples.

Ryan *et al*. (2008)[53] investigated that sourdough fermented by antifungal *Lactobacillus plantarum* strains was investigated for the ability to inhibit growth of common bread spoilage fungi. In both *in vitro* and sourdough wheat bread system, the antifungal sourdoughs significantly affected the outgrowth of *Aspergillus niger, Fusarium culmorum* or *Penicillium expansum* spores. However on wheat bread outgrowth of *Penicillium roqueforti* spores was not affected. In an attempt to reduce the amounts of chemical additives in bread, the antifungal sourdoughs were used in combination with calcium propionate (CAP) and possible synergistic effects were evaluated. Presence of 3000 ppm CAP in the bread did not affect the outgrowth of *Penicillium roqueforti*, whereas outgrowth of the other fungi was retarded. A strong synergistic effect was observed when CAP and antifungal sourdoughs were combined into the bread formulation, and outgrowth of *Penicillium roqueforti* was affected.

Carla Luciana Gerez *et al*. (2009)[54] evaluated the ability of lactic acid bacteria to inhibit *Aspergillus*, *Fusarium* and *Penicillium*, the main contaminants in bread. Only four strains (*Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus brevis* from 95 strains tested displayed antifungal activity. The major antifungal compounds were acetic and phenyllactic acids. The fermentation quotient (FQ = 2.0) and the leaven volume (80 cm) of dough's with *Lactobacillus brevis* and yeasts were higher than dough's without *Lactobacillus brevis*. The inclusion of antifungal LAB strains in the starter culture allowed a reduction in the concentration of the chemical preservative calcium propionate by 50 per cent.

8. CONCLUSION

The present review concludes the predominant efficacy of preservatives in bakery foods. Mold spoilage is still a major problem limiting the shelf life of many high and intemediate moisture bakery products. Losses due to mold spoilage have been resulting in lost revenue to the baking industries. Therefore, methods to control mold growth and to extend the shelf life of bakery products is of great economic importance to the baking industry where an increased demand in global consumption exists. Other measures as good hygiene in the bakeries and if necessary complementary post packaging heat treatments or modified atmosphere packaging is the best alternatives.

REFERENCES

1. Kent, N.L. 1983. Technology of cereals. Third Edition. Pergamon Press, Oxford.
2. Sofos J. N and F. Busta. 1991. Antimicrobial activity of sorbate. *Journal of Food Protection*, 44: 614-621.
3. Seiler, D.A.L. 1994. Preservation of bakery products. Institute of Food Science and Technology Proceedings. 17: 35-40.
4. Hocking, A.D. 2008. Mould and yeast associated with foods of reduced water activity. Ecological interactions. In: Seow, C.C. (Ed), Food preservation by moisture control. Elsevier, London pp. 57-72.
5. Hunt, L. and Robbins, L. 2009. Food expenditure patterns of Canadian Consumers. *Food Market Commentary*, 11: 42-51.
6. Roessler, P.F. Ballenger, M.C. 2006. Contamination of and unpreserved semi soft baked cookie with a *Xerophikic*, and *Aspergillus* species. *J. Food Prof*., 59: 1055-1060.
7. Hunt, L. and Robbins, L. 2009. Food expenditure patterns of Canadian Consumers. *Food Market Commentary*, 11: 42-51.
8. Anon. 2000. House hold consumption and expenditure on cereal-based foods. Home Grown Cereals Authority Weekly Diges.18: 2-3.
9. Baur, J. 2001. La Boulangerie en Europe. Industries des Cereals. 73: 39-48.
10. Hoseney, R.C., Sievert, D and Delcour, J.A. 2008. Bread and other baked products. In Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag, Weinheim Germany, in the press. doi: 10.1002/14356007. 04_331.
11. Rachel Needham, James William, James Williams, Nikki, Beales, Phil Voysey, Naresh Magan. 2004. Early detection and differentiation of spoilage of bakery products. Sensors and Actuators B 106: 20-23.
12. Francesca Valerio, Mara Favilla, Palmira De Bellis, Angelo Sisto, Silvia de Candia, Paula Lavermicoca. 2009. Antifungal activity of strains of Lactic acid bacteria isolated from semolina ecosystem against *Penicillium roqueforti, Aspergillus niger* and *Endomyces fibuliger* contaminating bakery products. *Systematic and Applied Microbiology*, 32: 438-448.
13. Smith, J.P. 1993. Bakery products. In: Principles and Application of Modified Atmosphere Packaging of Food (ed. R. T. Parry). Blackie Academic and Professional, Glasgow, UK. pp. 134-169.
14. Rosenkvist, H. and Hansen, A. 1995. Contamination profiles and characterization of *Bacillus* species in wheat bread and raw materials for bread production. *International Journal of Food Microbiology*, 26: 353-363.
15. Voysey, P.A and Hommond, J.C., 1993. Reduced additive bread-making tech. In: Smith. J. (Ed). Tech. of reduced, additive foods. Blackie, Academic and professional, London, pp. 80-94.
16. Bailey, C.P. and Holy, A.V. 1993. *Bacillus* spore contamination associated with commercial bread manufacture. *Food Microbiology*, 10: 287-294.
17. Seiler, D.A.L. 2000. Modified atmosphere packaging of bakery products. In: Controlled/ Modified Atmosphere/Vaccum Packaging of Foods (ed A.L. Brody). Trumball. CT: Food and Nutrition Press. pp. 119-133.
18. Dickson, J.S. 2001. Survival of selected indicator and pathogenic bacteria in

IJPBA, Jan - Feb, 2012, Vol. 3, Issue, 1

refrigerator pizzas. *Journal of Food Protection*, 50: 59-86.

19. Legan, J.D. and Voysey, P.A. 1981. Yeast spoilage of bakery products and ingredients. *Journal of Applied Bacteriology*, 70: 361-371.
20. Membre, J.M., Kubaczka, M and Chene, C. 2001. Growth rate and growth-no-growth interface of *Penicillium brevicompactum* as functions of pH and preservative acids. *Food Microbiology,* 18: 531-538.
21. Malkki, Y. and Rauha, O. 2000. Mould inhibition by aerosols. *Baker's Digest*., 52: 47-50.
22. Hickey, C.S. 1998. Sorbate spray application for protecting yeast-raised bakery products. *Baker's Digest*, 54: 4-7.
23. Knight, R.A. and Menlove, E.M. 2006. Effect of the bread baking process on destruction of certain mould spores. *Journal of the Science of Food and Agriculture*, 10: 653-660.
24. Jarvis, B. 2001. Mould spoilage of food. *Process Biochemistry*, 7:11-14.
25. Ponte, J.G., J.D. Payne and M.E. Ingelin. 1993. The shelf life of bakery foods. In: Shelf Life of Foods and Beverages (ed G. Charalambous). Elsevier Science Publishers. pp.1143-1197.
26. Abellana, M., X. Magri, V. Sanchis and A.J. Ramos. 1999. Water activity and temperature effects on growth of *Eurotium amstelodami*, *E. chevalier* and *E. herbaviorum* on a sponge cake analogue. International Journal of Microbiology, 52: 97-103.
27. Frazier, W.C. and Westhoff, D.C. 1978. Food Microbiology. Third edition. Hill Book Co., New York.
28. Chamberlain, N. 1993. Mould growth on cake. *Biscuit Maker and Plant Baker,* 14: 961- 964.
29. Abellana, M., V. Sanchis, A.J. Ramos. 2001. Effect of water activity and Temperature on growth of three *Penicillium species* and *Aspergillus flavus* on a sponge cake analogue. *International Journal of Food Microbiology*, 71: 151-157.
30. Vytrasova, J., P. Pribanova and L. Mar vanova. 2002. Occurrence of *Xerophilic* fungi in bakery production. *International Journal of Food Microbiology*, 72: 91-96.
31. Guynot, M.E., S. Marin, V. Sanchis and A.J. Ramos. 2003. Modified atmosphere packaging for prevention of mold spoilage of Bakery products with different pH and water activity levels. *Journal of Food Production*, 10: 1864-1872.
32. Elena Guynot, M., Sonia Marin, Vicente Sanchis and J. Antonio Ramos. 2005. Low intermediate moisture bakery product by Mudelling *Eurotium* sp. *Aspergillus* sp. and *Penicillium corylophilum* growth. *International Journal of Food Microbiology*, 101: 169-177.
33. Mariona Arroyo, David Aldred and Naresh Magan. 2008. Environmental factors and weak organic acid interactions have differential effects on control of growth and ochratoxin A production by *Penicillium verrucosum* isolates in bread. *International Journal of Food Microbiology*, 98: 223-231.
34. Samapundo, S., N. Deschuyfteleer and D. Van Laere. 2010. Effect of NaCl reduction and replacement on the growth of fungi important to the spoilage of bread. *Journal of Food Microbiology*, 27: 749-756.
35. Kyzlink, V. 2001. Principles of food preservation. Elsevier Publ., Amsterdam. pp. 247- 370.
36. Seiler, D.A.L. 2000. Modified atmosphere packaging of bakery products. In: Controlled/ Modified Atmosphere/Vaccum Packaging of Foods (ed A.L. Brody). Trumball. CT: Food and Nutrition Press. pp. 119-133.
37. Banwart, G. J. 2004. Basic Food Microbiology. A VI Publ. Inc., Westport. pp. 505-544.
38. Desrosier, N.W. 2006. The Technology of Food Preservation. Avi Publ., Westport. pp. 110-148.
39. Marin, S. Guynot, M.E. Sanchis, V. Arbones, J. and Ramos, A.J. 2003. *Aspergillus flavus*, *Aspergillus niger* and *Penicillium coryophilum* spoilage prevention of bakery product by means of weak-acid preservatives. *Journal of Food Science*, 64: 2271.
40. Guynot, M.E. Ramos, A.J. Sanchis V. and Marin, S. 2004. Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5–5.5). *International Journal of Food Microbiology*, 101: 161-168.

41. Francesca Valerio, Mara Favilla, Palmira De Bellis, Angelo Sisto, Silvia de Candia, Paula Lavermicoca. 2009. Antifungal activity of strains of Lactic acid bacteria isolated from semolina ecosystem against *Penicillium roqueforti*, *Aspergillus niger* and *Endomyces fibuliger* contaminating bakery products. *Systematic and Applied Microbiology*, 32: 438-448.
42. Gorton, L.A. 1999. Sorbate solution triples bread's shelf life. *Baking Industry*, 144: 20-21.
43. Ray, L. and Bullerman, L.B. 2001. Preventing growth of potentially toxic molds using antifungal agents. *Journal of Food Protection*, 45: 953-963.
44. Sauer, D.B. and Burroughs, R. 1993. Efficacy of various chemicals as grain mold inhibitors. *Tram American Society Agriculture*, 17: 357-559.
45. Sofos J. N. and Busta, F. 1991. Antimicrobial activity of sorbate. *Journal of Food Protection*, 44: 614-621.
46. Doores, S. 1993. Organic acids. In: Antimicrobials in Foods (eh. P. M. Davidson und A. L. Branen). Marcel Dekker, Inc., New York pp. 117-119.
47. Marin, S. Guynot, M.E. Sanchis, V. Arbones, J. and Ramos, A.J. 2002. *Aspergillus flavus, Aspergillus niger* and *Penicillium coryophilum* spoilage prevention of bakery product by means of weak-acid preservatives. *Journal of Food Science*, 64: 2271.
48. Marin, S. Abellana, M. Rubinal, M. Sanchis, V and Ramos, A.J. 2003. Efficacy of sorbates on the control of the growth of *Eurotium* species in bakery products with near neutral pH. *International Journal of Food Microbiology*, 87: 251-258.
49. Suhr, K.I and Nielsen, P.V. 2004. Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activity and pH values. International of *Food Microbiology*, 95: 67-78.
50. Hassan, Y.I. and Bullerman L.B. 2008. Antifungal activity of *Lactobacillus, Paracasei* sp., tolerans isolated from a sourdough bread culture. *International Journal of Food Microbiology*, 121: 112-115.
51. Ozay mentes, Recai Ercan and Mustata Ajcekuj. 2005. Inhibitory activity of two *Lactobacillus* strain, isolated from sourdough, against rope-forming *Bacillus* strain. *Journal of Food Microbiology,* 18: 359-363.
52. Sana M'hir, Mondher, Mejri and Moktar Hamd. 2007. Microflora distribution and species ratio of Tunision Fermented doughs for bakery industry. *African Journal of Food Microbiology*, 6 (18) : 2122-2129.
53. Ryan L.A.M. Dal Bello, F and Arendt, E.K. 2008. The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. *International Journal of Food Microbiology*, 125: 274-278.
54. Carla Luciana Gerez, Maria ines Torino. 2009. Prevention of bread mold spoilage by using lactic acid bacteria with antifungal properties. *Journal of Food Science*, 20: 144-148.