J. Trop. Agric. and Fd. Sc. 26(1)(1998): 79–84

The effects of chemical preservatives on the microbial population and shelf-life of chilli slurry

(Kesan bahan pengawet kimia terhadap populasi mikrob dan tempoh simpan cili bo)

M. A. Nor Azni* and Q. L. Yeoh*

Key words: chilli slurry, *cili bo*, chemical preservatives, microbiology

Abstrak

Kesan natrium benzoat, kalium sorbat, natrium laktat dan kalsium propionat terhadap tempoh simpan cili bo pada suhu bilik telah dikaji. Hasil daripada kajian menunjukkan bahawa penggunaan natrium benzoat dan kalium sorbat dengan tambahan asid asetik dapat memanjangkan masa simpan cili bo sehingga 10 minggu pada suhu bilik. Dengan penggunaan natrium laktat dan kalsium propionat, tempoh simpan cili bo masing-masing hanya 4 minggu dan 2 hari. Semasa penyimpanan, perubahan kandungan mikroorganisma dalam sampel yang mengandungi natrium benzoat + asid asetik dan kalium sorbat + asid asetik juga diawasi. Jumlah hitungan plat bagi kedua-dua sampel tidak banyak berubah.

Abstract

The effects of sodium benzoate, potassium sorbate, sodium lactate and calcium propionate on the shelf-life of chilli slurry stored at room temperature were investigated. The results indicated that the use of sodium benzoate and potassium sorbate with the addition of acetic acid was able to preserve the chilli slurry up to 10 weeks at room temperature, while sodium lactate and calcium propionate gave a storage life of 4 weeks and 2 days respectively. The changes in microbiological population during the storage period were also monitored for the samples treated with benzoate + acetic acid and sorbate + acetic acid. There were no marked changes in the total plate counts for both treatments.

Introduction

Chilli slurry, a food ingredient widely used in Malaysian households and food outlets, is prepared from dried chillies which have been soaked in water and ground into a fine paste (Sharifah Norhidayat and Abu Othman n.d.). It is a popular ready-to-use ingredient that makes cooking less tedious.

According to the Food Regulations 1985, 'Chilli slurry or commonly known as *cili bo* shall be the slurry obtained by grinding the clean, wholesome, fresh or dried chillies with clean potable water. It shall contain not less than 15% of chilli. It may contain salt and permitted preservative. It shall not contain any other added substance' (Regulation 299). The maximum permitted level of sodium benzoate allowed in *cili bo* is 750 mg/kg (Regulation 20).

Cili bo has a short shelf-life at normal room temperature. Its moist nature provides favourable conditions for growth of microorganisms and thus it is susceptible to microbial spoilage. Although its shelf-life may be prolonged with preservation techniques such as refrigeration and

*Food Technology Centre, MARDI Headquarters, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
Authors' full names: Nor Azni Md. Adnan and Yeoh Quee Lan

freezing, these techniques are costly and sometimes not available, especially in remote areas. Another technique for preserving *cili bo* is the use of chemical preservatives.

Generally, bacteria grow best at pH 6.5–7.5 while yeasts and moulds which have a wider pH range, can grow in foods with pH of less than 3.5. One of the effective ways of controlling microbial growth is, therefore, by pH control. A number of organic acids can be used as antimicrobials or acidulants. The main organic acids used as antimicrobials are benzoic, propionic and sorbic acids, and their salts, besides acetic, lactic, malic, citric and tartaric acids. All these acids have 'Generally Regarded As Safe' (GRAS) status if used at levels below 1 000 ppm (Giese 1994), and are more effective at low pH when they are in their undissociated forms (Doores 1983).

Currently, benzoic acid is the only chemical preservative permitted for use in *cili bo* under the Food Regulations. Benzoic acid is generally more effective as an antimycotic agent and it is only effective in its undissociated form. Thus, benzoic acid and sodium benzoate are more suitable for foods with pH 4.5 and below (Chipley 1983). They are effective against yeasts and moulds at levels of 500–1 000 ppm but concentrations greater than 2 000 ppm are required to be effective against spoilage bacteria (Chipley 1983).

Like benzoic acid, sorbic acid is not very soluble in water at room temperature, and the sodium, potassium or calcium salts are more commonly used as preservatives. They are more effective against yeasts and fungi, and have a selective action against certain bacteria. The pH-dependant sorbates which are effective up to pH 6.5 (Sofos and Busta 1983), have been used at 500–3 000 ppm to prevent microbial growth in different types of food (Giese 1994).

The antimicrobial action of propionates is mainly directed towards fungi though they also retard the growth of certain bacteria, especially those causing rope spoilage in bakery products. Generally, they have an upper effective pH limit of 5.5. Levels used in foods range from 1 000 ppm to more than 3 000 ppm (Doores 1983). Currently under the Food Regulations 1985, propionic acid and its salts are only allowed in bread.

Lactic acid has been well accepted as a naturally occurring preservative, especially in fermented foods such as pickles and yoghurts. It lowers pH and displays inhibitory properties against spore-forming bacteria at pH 5.0 and against psychrotrophic gram negative bacteria, especially in meat (Daeschel 1989). In recent years, there has been increased interest in the use of sodium lactate as a preservative. It is reported to be effective against various *Bacillus* spp., and can reduce or delay the growth of aerobes, psychrotrophs and coliforms in various products stored at refrigeration temperatures. It is normally used as a 60% solution at 2–3% (Schefel 1994).

Acetic acid has been found to be more effective against yeast and bacterial growth than mould growth (Doores 1983). Although it has a strong odour and taste, acetic acid is commonly used as an acidulant as it is inexpensive.

This paper compares the effectiveness of some GRAS chemical preservatives for extending the shelf-life of *cili bo* at room temperature.

Materials and methods

Preparation of cili bo

Dried chillies were used in the preparation of the *cili bo (Figure 1)*. It was prepared according to the procedure outlined by Sharifah and Abu Othman (n.d.).

Treatments

Four food grade chemical preservatives, namely sodium benzoate, calcium propionate, sodium lactate and potassium sorbate, were evaluated for the preservation of *cili bo*. The preservatives were used individually and with addition of glacial acetic acid to reduce the pH to 4.1–4.3. The preservatives were added at 750 mg/kg. The

Dried chillies
↓
Sort and remove stem
↓
Weigh and wash
↓
Soak in hot water for 10 min
↓
Drain and grind with a little water till fine
↓
Add more water, acetic acid, preservative and salt
↓
Mix well and pack into containers
↓
Cili bo

Figure 1. Production of ***cili bo***

products were packed into plastic cups with lids in 80 g lots, similar to those used for commercial sale, and stored at room temperature. The experiments were replicated and the results are the mean average of duplicate samples.

Sampling

All the samples for each experiment were prepared as one lot. As such, it was assumed that the chilli samples were homogenous and the microbial load was uniform among the samples. Thus, less samples are needed than for non-homogeneous samples (ICMSF 1978a). At each storage interval of one week, the samples to be examined were randomly selected and the contents thoroughly mixed aseptically before the sub-samples for examination were aseptically taken. One of the ways to ensure that the microbiological analyses would more accurately estimate the true microbiological status of the products is to homogenise a larger sample prior to taking sub-samples for examination (ICMSF 1978b; Brown and Baird-Parker 1982). This method was, therefore, used to ensure that the samples used were representative.

Microbiological analyses

The samples for analysis were prepared based on the methods recommended by the International Commission on Microbiological Specifications of Food (ICMSF 1978a). Ten gram sample of *cili bo* was added to 90 mL quarter-strength Ringer's solution, which gave a 10^{-1} dilution. The mixture was then blended using the Stomacher Lab-blender 400 for 2 min. Further 10-fold serial dilutions, ranging from 10^{-2} to 10^{-6} were prepared from the 10^{-1} homogenate using 9 mL quarter-strength Ringer's solution as diluent. Total plate, spore-former and mould counts were determined according to the methods described by the ICMSF (1978a) and Speck (1976).

pH determination

The pH of *cili bo* was determined using the Orion pH meter.

Water activity determination

The water activity (a_w) of *cili bo* was determined using the Rotronic water activity meter.

Titratable acidity

Titratable acidity was determined according to Speck's method (1976).

Results and discussion

Gas production and slime formation in the samples were taken as indicators of spoilage. Visual observation showed that the control sample, which did not contain any preservatives and had an average pH of 5.67, spoiled after 24 h of storage at room temperature. The pH of *cili bo* samples with the addition of only benzoate, sorbate, propionate and lactate was 5.18–5.93 *(Figure 2)*. All samples spoiled after 48 h at room temperature. The rapid spoilage was probably due to the high pH values of the *cili bo* samples which permitted microbial growth that consequently caused spoilage. Spoilage was mainly due to bacteria as no yeast or mould growth was detected in all samples analysed.

At the pH range of the above samples, none of the preservatives tested was effective in preserving *cili bo* at room temperature. This was because the pH values

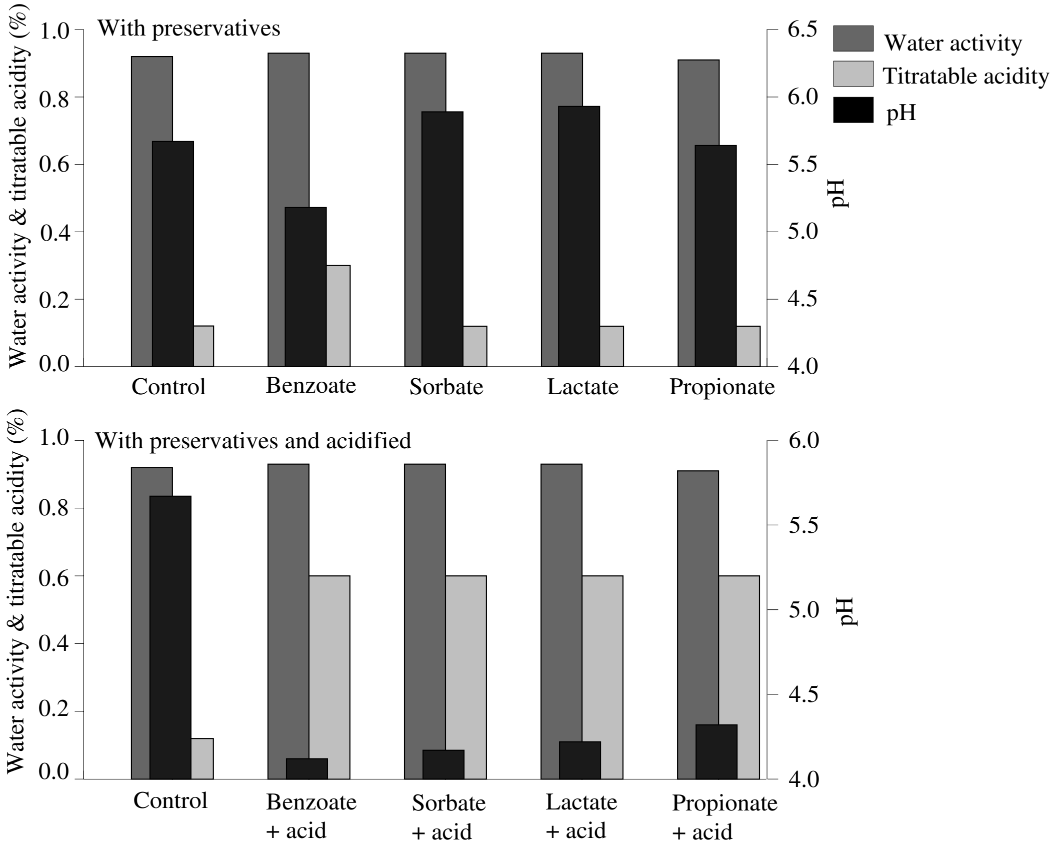

Figure 2. Water activity, titratable acidity and pH of ***cili bo*** *samples treated with various preservatives and acetic acid*

were not within the effective pH range for maximum activity of the preservatives tested. These preservatives are effective at lower pH values when they are in their undissociated forms (Chipley 1983; Doores 1983; Sofos and Busta 1983; Daeschel 1989).

The addition of acetic acid alone to reduce the pH to below pH 4.5 (about 4.1–4.2) was effective in giving a slightly longer shelf-life at room temperature (2–3 days) as this lowered the pH range to below that for the optimum growth for bacteria, thus delaying spoilage. However, further addition of acid to lower the pH gave a product that was too sour and was not acceptable to the taste panel. Thus, the use of acetic acid alone is not suitable for prolonging the shelf-life of *cili bo.*

The incorporation of acetic acid together with the chemical preservatives showed a positive effect on extending the shelf-life of *cili bo* at room temperature. Samples treated with benzoate + acetic acid and sorbate + acetic acid lasted for more than 10 weeks. While for lactate and propionate with the addition of acetic acid, the sample shelf-life was 4 weeks and 2 days respectively. With the addition of acetic acid, the pH of samples containing benzoate or sorbate were reduced to 4.12–4.17 *(Figure 2).* At this pH range, the benzoate and sorbate were found to be effective in preserving the *cili bo.* However, this was not so for samples with lactate and propionate which had a higher pH range of 4.22–4.32 *(Figure 2).* Furthermore, much higher levels of both propionate and lactate are required

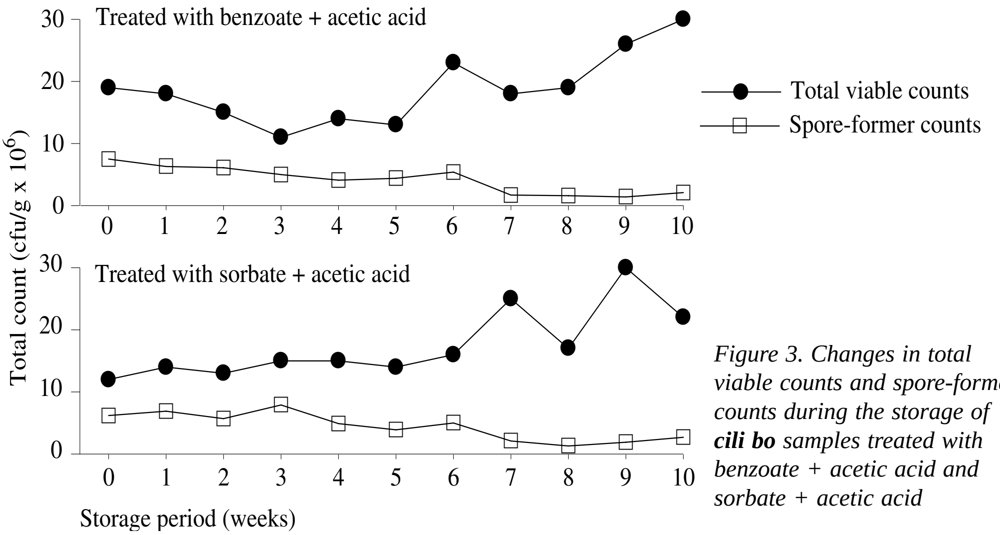

*Figure 3. Changes in total viable counts and spore-former counts during the storage of **cili bo** samples treated with benzoate + acetic acid and sorbate + acetic acid*

to be effective against the spoilage organisms (Giese 1994).

The microbiological analysis of the *cili bo* samples treated with benzoate + acetic acid as well as sorbate + acetic acid is shown in *Figure 3*. There was no distinct change in the total plate count during the 10-week storage period. At zero day, the total plate count was found to be 1.9 x 10^6 cfu/g (colony forming units/g) of sample. After 10 weeks of storage, the count increased slightly to 3.0 x 10^6 cfu/g. In the case of spore-formers, there was a slight decrease in counts with an initial count of 7.5 x 10^5 cfu/g decreasing to 2.5 x 10^5 cfu/g at the end of the 10-week storage period. Thus, the effect is more bacteriostatic than bactericidal. For the mould counts, no growth was detected during the 10-week storage period. A similar trend was observed in the samples treated with sorbate + acetic acid. There was no distinct change in the total plate count, which had an initial count of 1.2 x 10^6 cfu/g and a final count of 2.2 x 10^6 cfu/g. For the spore-formers, there was a slight decrease in the count from 6.2 x10^5 to 2.7 x 10^5 cfu/g *(Figure 3)*. Moulds were also not detected during the storage period. As sorbate is effective against selected bacteria, the preservative action against bacterial spoilage is probably due to the combined effect of the preservative and the pH lowering effect.

These results showed that with the addition of benzoate + acetic acid or sorbate + acetic acid, there was no marked increase in the microbial population in the *cili bo* samples during the 10-week storage period at room temperature. The pH of the samples remained relatively constant throughout the study. The pH was in the range of 4.11–4.16 for samples treated with benzoate + acetic acid and 4.17–4.20 for samples with sorbate + acetic acid. As mentioned earlier, these pH values are within the effective pH range for maximum activity. This probably resulted in the inhibition of microbial development in the *cili bo* although the water activity values in both cases were in the range of 0.92–0.94 throughout the storage period and were favourable for growth of microorganisms. Titratable acidity in both cases also remained fairly constant throughout the study, ranging from 0.60% to 0.66% acetic acid.

Conclusion

From the experiments conducted, it can be seen that the addition of 750 ppm benzoate or sorbate in the presence of acetic acid (pH range: 4.1–4.2) has more potential in preserving *cili bo* stored at room temperature than lactate and propionate or acetic acid alone. However, as sorbate is currently not approved for use in *cili bo* under the Food Regulations, the problem of *cili bo* spoilage can be effectively overcome by using acetic acid to lower the pH to the effective range for benzoate and adding the permissible amount of benzoate. In view of the fact that sorbate is also effective in prolonging the shelf-life of *cili bo* and is effective to a higher pH level (pH 6.5 compared to pH 4.5), it may be appropriate to petition for its inclusion in the Food Regulations as an alternative to benzoate.

Acknowledgements
The authors wish to thank Ms Josephine Mary, Ms Fatimah Hassan and Ms Norrijah Osman for their assistance.

References

Brown, M. H. and Baird-Parker, A. C. (1982). The microbiological examination of meat. In *Meat microbiology* (Brown, M. H., ed.) p. 423–521. London and New York: Applied Science Publishers Ltd.

Chipley, J. R. (1983). Sodium benzoate and benzoic acid. In *Antimicrobials in foods* (Branen, A. L. and Davidson, P. P., ed.) p. 11–35. New York: Marcel Dekker Inc.

Daeschel, M. A. (1989). Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol*. ***3(1):*** 164–7

Doores, S. (1983). Organic acids, p. 75–108. *See* Chipley (1983)

Giese, J. (1994). Antimicrobials: Assuring food safety. *Food Technol*. ***June:*** 102–10

International Commission on Microbiological Specifications of Food (1978a). *Microorganisms in foods: Their significance and methods of enumeration* (Thatcher, F. S. and Clark, D. S., ed.) 2nd ed., p. 52–63. Toronto: University of Toronto Press

—— (1978b). *Microorganisms in foods 2. Sampling for microbiological analysis: Principles and specific applications* 2nd ed. p. 32–64. Toronto: University of Toronto Press

Schefel, L. A. (1994). Antimicrobial effects of lactates: A review. *J. Food Protection* ***57:*** 445–50

Sharifah Norhidayat, A. R. A. and Abu Othman, A. R. (n.d.). Perusahaan memproses cili boh. *Siri Panduan Untuk Usahawan* ***No. 22*** 7 p. Serdang: MARDI

Sofos, J. N. and Busta, F. F. (1983). Sorbates, p. 141–75. *See* Chipley (1983)

Speck, M. L., ed. (1976). *Compendium of methods for the microbiological examination of foods* p. 107–130, 223, 225–8, 235–8, 568–73. Washington: American Public Health Association

Accepted for publication on 16 April 1998