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Research Article

Optimization of Chemical Disinfection Conditions to Reduce Microbes in Spice Products

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Abstract: Due to the environment in which they are grown, spices and herbs often harbor large numbers of bacteria and fungi, including potential spoilage organisms and occasionally organisms of public health significance. Although some microorganisms are killed due to the inbuilt antimicrobial compounds of spices and drying in the production process, many bacteria and mold survive if product is not properly process and stored. As such, disinfection of spices is one of the main concerns in spice industry to produce quality products. Disinfection can be performed by using physical or chemical methods. In most of the cases 2 % NaOCl treatment is used as a chemical treatment in spice industry. However, this gives only limited reduction in microbial count. Commercial NaOCl which used in these industries does not come in pure form. To increase its stability NaOCl solution it is mixed with NaOH (1 %) which reduce the efficiency of NaOCl against microbes. According to the findings of the present study, efficiency of NaOCl can be increased by adding with an acid (acetic acid) to increase the formation of hypochlorous acid. In this study three different concentration of NaOCl (200 ppm,250 ppm, 300 ppm) were combined with acetic acid concentration (1:0.5, 1:1, 1:2) and treated for three different soaking time (5 min, 10 min, 15 min). As per results 10 min soaking using 300 ppm NaOCl incorporated with acetic acid (1:1) was selected as the most suitable treatment to reduce microbes in spice industry. Total microbial reduction of 300 ppm NaOCl treatment at 10 min. soaking was increased from 10^3 to 10^5 by 1:1 acetic acid incorporation. Total coliform level was reduced to undetectable level after drying.
Keywords: Spice, NaOCl, acetic acid, chemical disinfection

INTRODUCTION

Spices are vegetable products used for flavouring, seasoning and imparting aroma in foods [1]. Those are extremely important in consumer acceptance of some foods as those are responsible for their taste and flavour profiles [2]. Most of these spices contain natural antimicrobial compounds. Microbes are controlled naturally in these spices by those natural antimicrobial compounds [3, 4]. However, due to the environment in which they are grown, spices often harbor large numbers of microbes which can not be controlled by natural antimicrobial compounds alone [5]. Further, some amount of microorganisms also killed during the drying of spices. However, many bacteria and molds survive, if the products are not stored and shipped properly. In addition, when spices are incorporated into various food products, such as processes meats or dairy, the food are capable of supporting growth of the microorganisms. Therefore to prevent from getting higher microbial level at the end product it is important to disinfect all the raw material use in spice industry [1].

Disinfection of raw materials to reduce the amount of microorganisms to an acceptable level, chemical and physical methods can be used. In general, physical methods are preferred as they are very reliable and leave no residues behind. However, physical methods cannot always be applied owing to restrictions such as temperature, safety of personnel and design of the process flow [1]. When considering on spice composition, spices are enriched in essential oil which gives the value of spices. Therefore when considering a disinfection method it is always important not to remove the essential oil during the disinfection process. So, it is better to conduct chemical disinfection rather than going for physical methods.

When it comes to chemical disinfectants there are so many chemical disinfectants which are used in food industries. Each of the disinfectant has its own applications and its own restrictions in use. Spices are naturally inbuilt with many antimicrobial characters therefore most of the microbes present in spices grow on the surface area of spice. The main objective in disinfection of spices is to remove the microbes which grow on the surface area. In most of the cases 2 % NaocI treatment is used as a chemical treatment in spice industry. However, this gives only limited reduction in microbial count. Hence, this research was carried out to find out the reasons for this limited efficiency of NaOCl, and to develop an appropriate chemical disinfecting system to minimize the total microbial level of raw materials used in the spice industry to an acceptable level

MATERIALS AND METHODS

Sample preparation

Representative samples (5 kg) of each spice (Black pepper, White pepper, Chilly, Coriander, Turmeric, Cumin and Fennel) were purchased from local spice suppliers. Then, 1kg of each spice sample was sort out using corning and quartering method. Out of those 20 g samples were taken for disinfectant treatments followed by microbial tests namely, total plate count and total coliform count.

Disinfection of spices using different concentrations of NaOCl

Ten different concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm) of NaOCl were prepared as disinfectant solutions. Then, each spice samples of 20 g were separately soaked in each prepared disinfection solutions for 15 min. Soaked samples were drained and added separately to 180 ml of maximum recovery diluents solutions. From each of those solutions 1ml sample were used for microbial tests as explained bellow.

Disinfection of spices using different concentrations of NaOCl and acetic acid

All possible combinations (9) of three NaOCl concentrations (200 ppm, 250 ppm, 300 ppm), three acetic acid concentrations (NaOCl : Acetic acid, 1 : 0.5, 1 : 1, and 1 : 2) were prepared as disinfectant solutions. Then 20 g each of all spices were soaked in all prepared solutions for 5 min, 10 min, and 15 min separately (altogether 27 treatments). Soaked samples were drained and added separately to 180 ml of maximum recovery diluents solutions. From each of those solutions 1ml sample were used for microbial tests as explained bellow.

Microbial testes

Total plate count test and total coliform test were carried out for all samples treated by disinfectant following the procedure mentioned bellow.

Enumeration of micro organisms in spice raw materials by total plate count method

One milliliter of each maximum recovery solution was added in to Petri plates in three replicates. Then, nutrient agar media at 40 -45 ^{0}C were added to each Petri plate and circled 12 times clock wise and 12 times anti clock wise. Then the Petri plates were kept at laminar flow cabinet to solidify the agar medium. Then, those were incubated at 32 $\pm$ 1 ^{0}C temperature for 48 hours and the bacterial colonies were counted using a Colony Counter.

Enumeration of micro organisms in spice raw materials by total coliform test

In presumptive test, One milliliter of each recover solution was added in test tube which contained Brilliant Green Bile broth and Durham tubes filled with the same broth and kept in inverted position. Then, those were incubated at 30 $\pm$ 1 ^{0}C for 72 hours and were observed for gas formation. Then in the confirmed test, positive tubes from presumptive test were streaked onto selective agar mediums, Eosine Methylene Blue agar (EMB), Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar (Merck, Germany). Typical colonies in each agar were selected for further studies. In the completed test, selected typical colonies were inoculated into lactose broth containing Durham’s tube once again and incubated at 37ºC for 24h. Each positive tube was streaked on Trypticase Soy agar (TSA) plates for identification.

RESULTS AND DISCUSSION

Disinfection of spices using different concentrations of NaOCl

NaOCl has been used to control microbial pathogens in many different types of food [6-8] NaOCl produces HOCl when it is react with water. Due to this, P^{H} of the medium is expected to be decrease. Further, microbes are expected to be decreased gradually with the increase of NaOCl concentration.

$$NaOCl_{(aq)} + H_2O_{(aq)} \longrightarrow HOCl_{(aq)} + OH^-_{(aq)} + Na^+_{(aq)}$$

Results of microbiological test showed a positive reduction of microbes with the increase of NaOCl concentration. When the concentration increased to 150 ppm the reduction of microbes is high and most microbes are reduced at the concentration of 250 – 300 ppm. But when it comes to the reduction pattern it is not in a continuous dropping range as expected (Graph 1). Further, it was found an unexpected slight increase in P^{H} with the increase of NaOCl concentration (data not shown).

Disinfection of spices using different concentrations of NaOCl and acetic acid

Theoretically, with the increase of NaOCl concentration, P^{H} of the medium and microbial count of the treated samples should decrease gradually. However, results of the above study were not in agreement with this. Further, it was found that the commercial NaOCl generally used as disinfectant in spice industry has adulterated by 1 % NaOH to increase its stability under heat and sunlight. This NaOH adulteration has caused the reduction of the formation of hypochlorus acid.

$$NaOCl_{(aq)} \longrightarrow Na^{+}_{(aq)} + OCl^{-}_{(aq)}$$

$$OCl^{-}_{(aq)} + H_2O_{(aq)} \longrightarrow HOCl_{(aq)} + OH^{-}_{(aq)}$$

$$NaOH_{(aq)} \rightleftarrows Na^{+}_{(aq)} + OH^{-}_{(aq)}$$

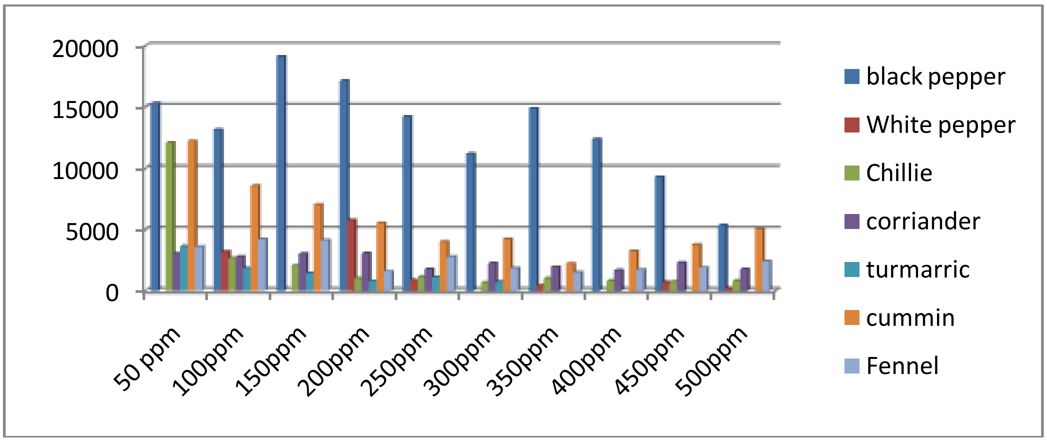

Fig. 1: Total plate count data of spice samples trated with different concentrations of NaOCl

This was found as the reason for non gradual reduction of microbes and slight increase of P^H with the increase of NaOCl concentration. As a solution to this, possibility of using commercial NaOCl combine with an acid was investigated. As this acid should not remove the chlorine from the disinfectant solution and should not damage the spice raw materials, acetic acid was selected for this purpose.

$$NaClO_{(aq)} + CH_3COOH_{(aq)} \longrightarrow HClO_{(aq)} + CH_3COONa_{(aq)}$$

As per the microbial test results of this study, shown in the table 1, gradual reduction of microbial count was observed with the increase of NaOCl concentration in all treatments when acetic acid was incorporated. Further, all treated samples were tested and found no residual chlorine.

Table 1: Total plate count and total coliform count data of spice sample treated with different concentrations of NaOCl, NaoCl: acetic acid ratios and different soaking times

NaOCl: Acetic acid	Soaking time	NaOCl concentration					
		Total plate count			**Total coliform**		
		200 ppm	**250 ppm**	**300 ppm**	**200 ppm**	**250 ppm**	**300 ppm**
1 : 0.5	**5 min**	3.18×10^3	2.84×10^3	1.80×10^3	1.75×10^3	0.61×10^3	0.22×10^3
	10 min	2.56×10^3	2.20×10^3	1.64×10^3	1.27×10^3	0.17×10^3	0.17×10^3
	15 min	2.40×10^3	1.52×10^3	1.50×10^3	0.50×10^3	0.40×10^3	0.19×10^3
1 : 1	**5 min**	4.80×10^3	4.80×10^3	1.58×10^3	0.64×10^3	0.30×10^3	0.07×10^3
	10 min	4.8×10^3	2.00×10^3	0.76×10^3	1.12×10^3	0.18×10^3	0.04×10^3
	15 min	6.14×10^3	1.89×10^3	0.82×10^3	0.47×10^3	0.15×10^3	0.04×10^3
1 : 2	**5 min**	4.28×10^3	3.38×10^3	2.60×10^3	0.41×10^3	0.25×10^3	0.05×10^3
	10 min	8.68×10^3	2.00×10^3	1.22×10^3	0.37×10^3	0.22×10^3	0.04×10^3
	15 min	5.02×10^3	1.96×10^3	0.35×10^3	0.22×10^3	0.10×10^3	0.03×10^3

Better microbial reduction was observed in all samples treated with NaOCl : acetic acid ratios of 1 : 1 and 1 : 2 at 300 ppm NaOCl concentration. Out of these, NaOCl : acetic acid ratio 1 : 1 should be selected as it need less adulteration of acetic acid. In all treatments, 10 min. and 15 min. soaking time gave significantly higher microbial reduction. However, out of those, 10 min soaking time can be recommended as it reduces the time of spice production. As such, treatment by 300 ppm NaOCl with acetic acid in the ratio of 1 : 1

for 10 min. was selected as the best treatment to reduce microbes in raw materials used in spice industry.

CONCLUSION

Microbial quality of spices are always important to maintain its market value and for consumer safety. Optimum conditions for chemical disinfection of spice raw materials using sodium hypochlorite (NaOCl) was investigated in this study. Incorporation of acetic acid in disinfectant solution was found to be more effective than the commonly used NaOCl treatment. Out of the all treatments tested, 300 ppm NaOCl with acetic acid in the ratio of 1:1 for 10 min. treatment was selected as the best treatment considering less production time, less amount of acetic acid usage and higher microbe reduction.

REFERENCES

1. Peter KV; Handbook of herbs and spices. 1st edition, England: Woodhead Publishing Limited, 2000.
2. Singhal RS, Kulkarni PR, Rege DV, Handbook of indices of food quality and authenticity. 1st edition, England: Woodhead Publishing Limited, 1997.
3. Ozcan M, Erkmen O; Antibacterial activity of the essential oils of Turkish plant spices. European food research and technology, 2001; 212 (6): 658-660.
4. Bahramian S, Soleimanian-Zad S, Sheikh-Zeinoddin M, Kabir GH; Establishment of an Inexpensive Procedure to Reduce the Microbial Load of Spices Used in Mayonnaise Industry. Botany Research International, 2009; 2 (4): 235-238.
5. Sospedra I, Soriano JM, Manes J; Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant food for human nutrition, 2010; 65 (4): 364-368.
6. Ji-Hyoung H, Sang-Do H; Synergistic Effects of Sodium Hypochlorite and Ultraviolet Radiation in Reducing the Levels of Selected Food borne Pathogenic Bacteria. Food borne Pathogens and Disease. 2011; 8(5): 587-591.
7. Kim HJ, Ha JH, Kim SW, Jo C, Park J, Ha SD; Effects of Combined Treatment of Sodium Hypochlorite/Ionizing Radiation and Addition of Vitamin B_1 on Microbial Flora of Oyster and Short-Necked Clam. Food borne Pathogens and Disease. 2011; 8(7): 825-830.
8. Elano RR, Kitagawa T, Bari ML, Kawasaki S, Kawamoto S, Inatsu Y; Comparison of the Effectiveness of Acidified Sodium Chlorite and Sodium Hypochlorite in Reducing *Escherichia coli*. Foodborne Pathogens and Disease. 2010; 7 (12): 1481-1489.