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ANTIFUNGAL Citrus hystrix EXTRACT AS NATURAL FOOD PRESERVATIVE

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ABSTRACT

During their storage, the traditional ready-to-eat food, such as sticky rice cake, are easily contaminated by spoilage pathogens. Hence, this study aims to evaluate the effect of *Citrus hystrix* extract in reducing spoilage pathogens in sticky rice cake during storage. The experimental sticky rice cake was prepared and formulated with *Citrus hystrix* extract at varied level of concentrations of 0.65%, 1.26% and 1.82% (w/w). Treated samples were stored at room temperature for 28 days and evaluated periodically for their microbial activity (total plate count), thiobarbituric acid reactive substances (TBARS), and sensory analysis. For its antifungal activity, the *Citrus hystrix* extract was also compared against *Penicillium* sp. and *Aspergillus nidulans* prior to formulation. Results exhibited a significant advantage of the added extracts to the sticky rice cake. All extract levels effectively eliminated the spoilage microorganism and significantly lowered the TBARS values. The physico-chemical properties of sticky rice cake including pH, water activity, and moisture content were equal among all the formulated samples and slightly different at 1.82% (w/w) extract level. Moreover, the addition of *Citrus hystrix* extract up to 1.82% did not affect the acceptability sensory attributes of the sticky rice cake as compared to the control which has no *Citrus hystrix* extracts ($p > 0.05$).

Keywords: Antifungal, *Citrus hystrix*, natural preservative, sticky rice cake

INTRODUCTION

Food safety issues have recently become one of the main public health concerns. The spoiled food becomes unacceptable for human consumption due to its altered sensory attributes such as taste, color, appearance, odor and texture (Holley & Patel 2005). Addition of synthetic or natural preservatives directly to the food or incorporated in the food packages are widely used to protect microbial spoilage of food commodities (Brul & Coote 1999). Recently, there has been a worldwide effort to minimize the use of chemical preservatives since consumer preferences are inclined toward more natural and healthier products. Consequently, the consumer's inclination for foods deprived of chemical preservatives has led to the discovery of new natural antimicrobial and antioxidant preventing agents (Serra *et al*. 2008).

Natural preservatives such as essential oils, flavonoids, phenolic compounds and microbial metabolites are chemical agents derived from plants, animals and microbes that acts as food preservatives by fighting against fungi and food borne bacteria (Prakash *et al*. 2014). They prevent the decomposition of products by inhibiting microbial growth, oxidation and certain enzymatic reactions occurring in the foodstuffs (Singh *et al*. 2010). *Citrus hystrix* of the Rutaceae family, commonly known as kaffir lime, is a tropical herb distributed throughout Southeast Asia. The useful parts of *C. hystrix* are the fruit, leaves and peel. Its leaves are aromatic and used as a spice and for various flavoring purposes. The essential oil of *C. hystrix* was characterized by high contents of terpinen-4-ol (13.0%), a-terpineol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). *C. hystrix* oil was effective as antioxidant, repellent and antiviral agent (Waikedre *et al.* 2010). Its oil has antibacterial activity against 20 serotypes of *Salmonella* and

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five species of other enterobacteria (Nanasombat & Lohasupthawee 2005).

Sticky rice cakes are popular dessert in Asian countries, particularly in Indonesia. These cakes can be made from different base matters including sticky or glutinous rice, sugar (palm, brown and cane), and some other ingredients such as variety of beans, coconut and sesame seeds. The most popular methods to prepare these foods are by way of steaming, frying and boiling (Lee *et al*. 2009). Sticky rice cake is one of ready-to-eat foods which is commonly stored at room temperature. In California, USA, the Korean rice cakes are allowed for sale at room temperature for up to 24 hours after production by marking the date and time of manufacturing (California State Legislature 2016). Most literatures reported that sticky rice cakes can be kept for up to a few days of storage at room temperature. In addition, there have been many investigations on how to maintain the microbiological quality of these products. Hence, this study aimed to evaluate the potential of *C. hystrix* extract in reducing spoilage pathogens during storage of a ready-to-eat food, such as the sticky rice cake.

MATERIALS AND METHODS

Preparation of Leaves Extracts

C. hystrix leaves were purchased from Beringharjo market, Yogyakarta, Indonesia. The leaves were washed, dried and ground into powder. The powdered leaves were then turned into aqueous extract by homogenizing 250 g of the material with 2500 mL of sterile distilled water, followed by three times sonication at 60 min. each. The resulting liquid was filtered and allowed to evaporate to produce a concentrated extract. The extract was later used in the formulation for food preservative.

Evaluation of Antifungal Activity

Three food spoilage microorganisms were isolated from sticky rice cake and identified as *Penicillium* sp., *Aspergillus nidulans* and *Rhizopus stolonifer*. Those fungi were then isolated and further used as the test organisms. The plates containing three extract concentrations, including control sample were inoculated in the plate core by spotting the 8 mm diameter of fungal species until round inoculums were formed. Inoculation was performed in four replications with two inoculums per plate. After inoculation, Petri plates were closed properly and incubated at 27 °C. The antifungal activity was evaluated by measuring the radial growth in three days of the mycelium (in diameter) in each plate for *Aspergillus nidulans* and *Rhizopus stolonifer* and in 7 days for *Penicillium* sp. in the presence of the extracts. The antifungal activity (AFA) was calculated by the following equation (Mori *et al*. 1997):

$$AFA\ (\%) = (GC - GT) / GC \times 100$$

where: AFA = antifungal activity (%);
GC = colony diameter on the control plate (mm);
GT = colony diameter on the test plate (mm)

Preparation of Extract Granule and Sticky Rice Cake

The concentrated extract of *C. hystrix* leaves was formulated with lactose through a granulation process in a ratio of 1 : 4 (w/w). The extracts were then homogeneously mixed with lactose fillers to produce a convenient wet mass. The wet mass was passed through a sieve with 30 mesh size. The obtained granules were dried at 60 °C for 36 h to attain less than 2% moisture content. Dried granules were then passed back through a sieve with a 30 mesh size and stored until it was used as the food preservative.

The dried granules were then mixed in the preparation of the sticky rice cake at various concentrations (0.65%, 1.26% and 1.82%). The mixing was carried out a few minutes before the cake was completely cooked. As the temperature was getting lower, small pieces of cakes were then packed in plastic containers.

Physico-chemical Properties of Sticky Rice Cake

Moisture content was determined using a moisture balance (AND MX50, Tokyo, Japan), the water activity by Kjeldahl steam distillation (JP Selecta Pro-Nitro S 4002851, Barcelona, ES) and the pH value was measured using the pH meter (Eutech PC700, NY, US).

Microbial Analysis of the Sticky Rice Cake

Four samples of the sticky rice cake, including control, were analyzed after 0, 7, 14, 21 and 28 days of storage. Total Plate Count (TPC) of colonies was determined following the incubation at 37 °C for up to 28 days, on Plate Count Agar (PCA, Merck). Twenty five grams of samples were dissolved in 225 mL of 0.85% NaCl solution, and then mashed using a stomacher. The prepared samples were serially diluted tenfold to minimize the number of microbes in the sample solutions. The bacteria were isolated using the pour plate method at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions. The colonies were counted (cfu/mL) using colony counter after 24 h and 48 h incubation.

Aerobic plate count was determined by the spread plate technique using standard methods with some modification. All yeast and mold counts were done in triplicate, using Potato Dextrose agar (PDA) medium (Merck, Germany), supplemented with 0.01% chloramphenicol (Merck, Germany). PDA plates were incubated for up to 5 days at 30 °C. The number of visible colonies on Yeast and Mold Count Plate was read after 3 days and 5 days.

TBARS Determination

The tested food products were analyzed after 0, 14 and 28 days of storage. Lipid stability was evaluated using the thiobarbituric acid reactive substances (TBARS) index according to Targladis *et al.* (1960) with slight modification. Ten grams of sticky rice cake sample in the distilled water (50 mL) were minced using a blender (Philips, England). The minced product was filtered and steam distilled with 4 N HCl (2.5 mL). The distillate (2.5 mL) was reacted with a 0.02 M thiobarbituric acid solution (2.5 mL), incubated in a boiled water bath for 35 min, and then cooled down until room temperature. Absorbance at 528 nm was measured using a UV Vis Spectrophotometer (Hitachi HALO RB-10). Thiobarbituric acid reactive substances values were calculated from a standard curve and expressed as mg MDA/kg sample.

Sensory Analysis of the Sticky Rice Cake

The sticky rice cake characteristics were frequently evaluated in terms of odor, tastes, color, texture and overall acceptance (Meilgaard *et al.* 1991). Twenty untrained taste panelists were asked to assess the sensorial properties every 7 days of the products storage duration. Sensory evaluation scores were determined using a 7-point Hedonic scale. The same superscript symbols in the sticky rice cake score indicate that samples do not significantly differ at a significance level of 95%.

Statistical Analyses

Data obtained from the triplicated experiments were computed and analyzed using the One-way Analysis of Variance (ANOVA) at 95% level of significance.

RESULTS AND DISCUSSION

Antifungal Activity of *C. hystrix*

The antifungal activity (AFA) of *C. hystrix* extract has been evaluated through the isolated spoilage fungi growth found in the sticky rice cake that were identified as *Penicillium* sp. and *Aspergillus* sp. The *in vitro* results showed that the selective antifungal activity of *C. hystrix* extract against *Penicillium* sp. and *Aspergillus nidulans* were 33.53 ± 31.67% and 40.22 ± 11.08%, respectively. However, no antifungal activity was observed against *Rhizopus stolonifer*. Since *C. hystrix* extract exhibited remarkable antifungal activity, the extract was further tested on a traditional sticky rice-based dessert, the sticky rice cake. Warm climate and lack of refrigeration are the main causes of high level contamination in sticky rice due to the activity of spoilage microorganism. However, little is known on the effect of *C. hystrix* leaf extracts on the microbiological stability of food products containing sticky rice. Therefore, this study was conducted to understand the potential of *C. hystrix* extract in reducing the effect of spoilage pathogens to a ready-to-eat food, such as the sticky rice cake.

Physico-chemical Properties of Sticky Rice Cake

Abundant nutrients, high water activity and nearly neutral pH make sticky rice cake a good medium for microbial growth and consequently, categorized as products that require temperature control. Since this product is normally stored under un-refrigerated method, a preservative additive is necessary. Water activity is one variable that contributes to the reduced shelf life of a food product. Hence, water that is retained and trapped inside the pores of food products should be removed. The water activity of all cake samples ranged from 0.68 to 0.78 (Table 1). This range is relatively lower than the minimum growth requirement for several pathogenic bacteria, such as *S. aureus* (0.86), *B. subtilis* (0.95), *E. coli* (0.96), *Salmonella* spp. (0.96), and *Psedomonas* spp. (0.97) (Molan 1992). Food spoilage is due to pathogenic species enduring in a lower water activity (0.95) (Filtenborg *et al.* 1996). High lactose concentration (1.82% extract level) can also lead to a reduced water activity (Mundo *et al.* 2004).

Total Plate Count (TPC)

After 28 days of storage, the average number of microbial colonies in the sticky rice cake has increased (Table 2). Most sticky rice-based desserts and snacks are soft and elastic, but during storage they become hard due to retrogradation in room temperature. Hence, it is not suggested to store them using refrigeration (Morris 1990). They are ready-to-eat food as they contain some components that are already cooked and do not need further heating prior to consumption, so the microbiological quality of sticky rice cake is very important. Microbiological purity is an important quality criterion in food products, with certain limits for the number of microbes as stipulated in applicable regional law.

Table 1 Physico-chemical properties of sticky rice cake formulated with *C. hystrix* extract

Physico-chemical properties	Level of extracts				
	Day	Control*	A (0.65%)	B (1.26%)	C (1.82%)
Water activity (a_w)	0	0.78	0.78	0.78	0.76
	14	0.76	0.70	0.70	0.68
	28	0.77	0.77	0.76	0.75
pH	0	6.21	6.07	5.56	6.01
	14	6.18	6.22	6.05	5.88
	28	6.27	6.26	5.93	5.76
Moisture content (%)	0	27.83	25.24	24.86	27.28
	14	20.15	19.14	21.05	20.77
	28	18.27	18.07	18.30	19.14

Notes: *Control = product without *C. hystrix* extract; A = product formulated with 0.65% *C. hystrix* extract; B = product formulated with 1.26% *C. hystrix* extract; C = product formulated with 1.82% *C. hystrix* extract.

Table 2 Total bacteria of sticky rice cake formulated with *C. hystrix* extract

Storage duration (days)		Bacterial colonies (cfu/mL)			
		Control	A (0.65%)	B (1.26%)	C (1.82%)
0	24 h	0.51×10^4	0.93×10^4	9.75×10^4	1.10×10^4
	48 h	0.36×10^4	0.66×10^4	10.45×10^4	0.71×10^4
7	24 h	7.90×10^4	0.64×10^4	1.02×10^4	1.29×10^4
	48 h	6.45×10^4	0.97×10^4	0.99×10^4	1.02×10^4
14	24 h	1.09×10^4	0.31×10^4	0.19×10^4	1.83×10^4
	48 h	1.04×10^4	0.46×10^4	0.22×10^4	2.89×10^4
21	24 h	0.34×10^4	0.20×10^4	0.10×10^4	0.20×10^4
	48 h	0.43×10^4	0.31×10^4	0.15×10^4	0.30×10^4
28	24 h	0.20×10^4	0.20×10^4	0.15×10^4	0.20×10^4
	48 h	0.41×10^4	0.25×10^4	0.20×10^4	0.60×10^4

Notes: Control = product without *C. hystrix* extract; A = product formulated with 0.65% *C. hystrix* extract; B = product formulated with 1.26% *C. hystrix* extract; C = product formulated with 1.82% *C. hystrix* extract.

Total plate count (TPC) value of sticky rice cakes formulated with *C. hystrix* extract ranged from 0.1 × 10^4 cfu/mL (Treatment B at 7 days) to 10.5 × 10^4 cfu/mL (Treatment B at 2 days). The addition of uneven extracts during the mixing process resulted in a significant increase in the TPC value during storage time. All samples were classified as good or in an acceptable level of microbiological quality, which are < 10^5 cfu/g as the guideline for ready-to-eat food (NSW Food Authority 2009). Moreover, based on weekly observation the tested products showed that the addition of *C. hystrix* extract was effective as antibacterial agent in reducing the microbial growth compared to the control. However, better results were exhibited by the 1.26% extract than 1.82% extract. In other study, the addition of 1% or 3% green tea or rosemary to rice cakes did not significantly affect the number of microbial properties. However, on three days of storage, the growth of *B. cereus* and *S. aureus* could be suppressed at room temperature compared with control (Lee *et al.* 2009). Microbial pathogens such as *S. aureus* are the main causes of ready-to-eat food poisoning (Huong *et al.* 2010).

Yeast and Mold Counts

C. hystrix extract improved the microbial quality of sticky rice cake particularly on the level of 1.82% extract over 14 days storage compared to the control (Table 3). However, after 28 days of storage, the sticky rice cake formulated with 1.26% extract gave a better shelf life toward yeast and mold growth with the number of 26 × 10^2 cfu/mL compared to control. Mold growth in this formula is an indication of high moisture content in the product. The higher extract formulated in the sticky rice cake, the higher amount of lactose was added. Consequently, the higher the lactose amount, the easier for the cake to absorb water, thus allowing the mold to grow as shown by the higher moisture content of product formulated with 1.82% than those of other formulas (19.14%).

Molds are filamentous fungi with branching hyphae, multi-cellular, generally aerobic and grow at a pH range of 3 to 8. The spores can tolerate harsh environmental conditions but are sensitive to heat (Pal 2007). The formulation of sticky rice cake with *C. hystrix* extract slightly decreased the pH value of product. Moreover, the highest acidity was reached during the 28-day storage of the sticky rice cake formulated with 1.82% extract (Table 1). High acidity favors the growth of molds (Loureiro & Querol 1999). The addition of extract effectively eliminated the spoilage microorganism until 28 days of storage compared with control. After 28 days, the product was fully contaminated by molds. Microorganisms grow significantly over time in rice cakes and the product would be completely spoiled as seen in the alteration of the color, texture and flavor after three days of storage at room temperature (Ji *et al.* 2007). Molds commonly grow on all kinds of food, such as cereals, meat, milk, fruit, vegetables, nuts and fats. Mycotoxins, the toxic secondary metabolites found in vertebrate animals, are produced during growth of molds on food products. Mycotoxins from Penicillia normally grow in cereals kept in tropical countries having warm climate and high moisture including viridicatumtoxin (*P. aethiopicum*), citrinin (*P. citrinum*), cyclopiazonic acid, patulin and roquefortine C (*P. griseofulcum*) and secalonic acid D (*P. oxalicum*) (Frisvad & Filtenborg 1989).

Table 3 Total yeast and mold counts of sticky rice cake formulated with *C. hystrix* extract

Storage duration (days)		Yeast and mold (cfu/mL)			
		Control	A (0.65%)	B (1.26%)	C (1.82%)
0	3 days	2.0 × 10^2	2.0 × 10^2	0.4 × 10^2	0.3 × 10^2
	5 days	3.0 × 10^2	4.0 × 10^2	3.0 × 10^2	2.0 × 10^2
7	3 days	3.8 × 10^2	3.2 × 10^2	1.4 × 10^2	1.4 × 10^2
	5 days	4.1 × 10^2	4.1 × 10^2	4.0 × 10^2	3.0 × 10^2
14	3 days	10 × 10^2	1.0 × 10^2	1.0 × 10^2	0.9 × 10^2
	5 days	4.0 × 10^2	5.0 × 10^2	2.0 × 10^2	1.8 × 10^2
21	3 days	9.0 × 10^2	8.0 × 10^2	1.0 × 10^2	4.0 × 10^2
	5 days	12 × 10^2	11 × 10^2	3.0 × 10^2	11 × 10^2
28	3 days	34 × 10^2	25 × 10^2	21 × 10^2	27 × 10^2
	5 days	39 × 10^2	28 × 10^2	26 × 10^2	32 × 10^2

Notes: Control = product without *C. hystrix* extract; A = product formulated with 0.65% *C. hystrix* extract; B = product formulated with 1.26% *C. hystrix* extract; C = product formulated with 1.82% *C. hystrix* extract.

TBARS analysis

TBARS values refer to the oxidative stability of the sticky rice cake. The addition of *C. hystrix* extract to the sticky rice cake has reduced the TBARS values during the 28-day storage with no significant difference among the three levels of extract formula (Table 4). In other studies, the addition of herbal extracts as antioxidant supplementation in some foods was effective in controlling lipid oxidation during storage (Formanek *et al.* 2001; Park *et al.* 2016). Moreover, the pH condition of the food influenced the activity of oils. At low pH, the hydrophobicity of some essential oils contained in *C. hystrix* will increase and is subjected to partition in the lipid phase of the food, thus reducing the oxidative stability.

Sticky rice cake, containing coconut milk, poses a major problem associated with the production of off-odors due to free radical-induced lipid oxidation. The *C. hystrix* extract was added to a processed sticky rice cake to improve sensory attributes and to control oxidation reactions.

Sensory Analysis

To evaluate the influence of *C. hystrix* extract on the acceptability of product by customer, the organoleptic analysis was carried out after a week of storage (Table 5).

Based on the sensory attributes, the addition of *C. hystrix* extract did not reduce the acceptability of sticky rice cake when compared with the control sample with no extract. Statistically, no significant differences were observed in the acceptance of products formulated with 0.65%, 1.26% and 1.82% extracts. However, the sensory test should be performed every week until 28 days storage in order to observe changes in the acceptance level. Most plant extracts do not affect the acceptability of sticky rice cake as long as the appropriate concentration is used. Irradiated and freeze-dried green tea leaf extracts formulated in raw and cooked pork patties had no influence on the physical and sensory properties of the patties (Jo *et al.* 2003). This study results indicated that sticky rice cake formulated with *C. hystrix* extracts exhibited sensory stability similar to that of the control based on a one-week storage.

Table 4 Effects of *C. hystrix* extract on the oxidative stability (TBARS) values of sticky rice cake

Storage duration (days)	TBARS index (mg MDA/kg)			
	Control	A (0.65%)	B (1.26%)	C (1.82%)
0	0.45	0.22	0.39	0.24
7	0.33	0.39	0.39	0.49
14	0.22	0.16	0.41	0.17
21	0.67	0.47	0.33	0.36
28	0.18	0.19	0.15	0.12

Notes: Control = product without *C. hystrix* extract; A = product formulated with 0.65% *C. hystrix* extract; B = product formulated with 1.26% *C. hystrix* extract; C = product formulated with 1.82% *C. hystrix* extract.

Table 5 Sensory evaluation of sticky rice cake formulated with different levels of *C. hystrix* extract

Level of extracts	Attributes				
	Color	Odor	Taste	Texture	Overall acceptability
Control (0%)	5.89^{ab}	6.33^{b}	6.67^{b}	5.94^{b}	6.56^{b}
A (0.65%)	5.50^{a}	5.50^{ab}	5.61^{a}	4.89^{a}	5.78^{a}
B (1.26%)	6.06^{ab}	5.39^{a}	5.67^{ab}	5.78^{ab}	5.94^{ab}
C (1.82%)	6.44^{b}	5.89^{ab}	5.83^{ab}	6.33^{b}	6.67^{b}

Note: ^{a,b,ab} Mean values with different superscripts within the same column are significantly different at $p < 0.05$.

CONCLUSION

Addition of *C. hystrix* extract in food formulation had reduced the microbial growth and extended the shelf life of the sticky rice cake. This indicated that *C. hystrix* enhances the antifungal activity over time. The increasing level of *C. hystrix* extract up to 1.82% of sample (w/w) was still acceptable for all the sensory attributes. Thus, the blend of *C. histrix* extract in the sticky rice cake adds potential value of available good quality and healthy food options in Indonesia.

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REFERENCES

Brul S, Coote P. 1999. Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int J Food Microbiol 50, 1-17.

California State Legislature. 2016. Health and Safety Code-HSC. Division 104. Environmental Health. Part 5. Sherman Food, Drug, and Cosmetic Laws. Chapter 5. Food. Article 14. Asian rice noodles, Korean rice cakes and Vietnamese rice cakes.

Filtenborg O, Frisvad JC, Thrane U. 1996. Moulds in food spoilage. Int J Food Microbiol 33:85-102.

Frisvad JC, Filtenborg O. 1989. *Terverticillate penicillia*: chemotaxonomy and mycotoxinproduction. Mycologia 81:836-61.

Holley AH, Patel HM. 2005. Improvement in shelf life and safety of perishable food by plant essential oils and smoke antimicrobials. Int J Food Microbiol: 22:273-92.

Huong BTM, Mahmud ZH, Neogi SB, Kassu A, Nhien NV. 2010. Toxigenicity and genetic diversity of *Staphylococcus aureus* isolated form Vietnamese ready-to-eat foods. Food Control 21:166-71.

Ji Y, Zhu K, Qian H, Zhou H. 2007. Microbiological characteristics of cake prepared from rice flour and sticky rice flour. Food Control 18:1507-11.

Jo C, Son JH, Son CB, Byun MW. 2003. Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4 °C. Meat Sci. 64:17-33.

Lee SY, Gwon SY, Kim SJ, Moon BK. 2009. Inhibitory effect of commercial green tea and rosemary leaf powders on the growth of foodborne pathogens in laboratory media and oriental-style rice cakes. J Food Prot 72(5):1107-11.

Loureiro V, Querol AD. 1999. The prevalence and control of spoilage yeasts in foods and beverages. Trends Food Sci Technol 10:356-65.

Meilgaard MC, Civille GV Carr BT. 1991. Sensory evaluation techniques. 2nd Edition. Washington DC (US): CRC Press.

Molan PC. 1992. The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World 73(1):5-28.

Mori M, Aoyama M, Doi S, Kanetoshi A, Hayashi T. 1997. Antifungal activity of bark extract of deciduous trees. Holz als Roh und Werkstof 55: 130-2.

Morris VJ. 1990. Starch gelation and retrogradation. Trends Food Sci Technol 1:2-6.

Mundo MA, Padilla-Zakour OI, Worobo RW. 2004. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. Int J Food Microbiol 97:1-8.

NSW Food Authority. 2009. Microbiological quality guide for ready-to-eat foods. NSW/FA/CP028/0906, 6-7.

Pal M. 2007.Veterinary and Medical Mycology. 1st Edition. New Delhi (IN): Indian Council of Agricultural Research.

Prakash B, Mishra PK, Kedia A, Dubey NK. 2014. Antifungal, antiaflatoxin and antioxidant potential of chemically characterized *Boswellia carterii* Birdw essential oil and its *in vivo* practical applicability in preservation of *Piper nigrum* L. fruits. LWT– Food Sci Technol 56:240-7.

Serra AT, Ana AM, Ana VMN, Leitão MC, Brito D, Bronze R, SilvaS, Pires A, Crespo MT, San Romão MV, Duarte CM. 2008. *In vitro* evaluation of olive- and grape-based natural extracts as potential preservatives for food. Innov Food Sci Emerg 9: 311-9.

Singh A, Sharma PK, Garg G. 2010. Natural products as preservatives. IJPBJ2 1(4):601-12.

Targladis BG, Watts BM, Younathan MT, Duggan LR. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37, 44-48.

Nanasombat S, Lohasupthawee P. 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. KMITL Sci Tech J. 5, 527-538.

Waikedre J, Dugay A, Barrachina I, Herrenknecht C, Cabalion P, Fournet A. 2010. Chemical composition and antimicrobial activity of the essential oils from new Caledonian *Citrus macroptera* and *Citrus hystrix*. Chem Biodivers 7:871-7.