The Effect of *Ocimum basilicum, Thymus vulgaris, Origanum vulgare* Essential Oils on *Bacillus cereus* in Rice-Based Foods

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ABSTRACT

Food poisoning remains a major health problem, affecting both industrialized and developing countries. Here, we show that essential oils from *Ocimum basilicum* (basil), *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano) exhibit bactericidal properties against *Bacillus cereus* in rice-based foods. The optimal bactericidal activities were observed at room temperature in a concentration-dependent manner.

TEXT

Food poisoning remains a major health problem, affecting both industrialized and developing countries. For example, in the United Kingdom, the number of cases has risen from 14,253 in 1982 to 70,311 in 2004 (Health Protection Agency, 2005). In a recent survey by World Health Organization, it was reported that more than 2 million people died from food borne diarrhoeal diseases, worldwide (WHO 2002). Among bacteria, *Bacillus cereus*, *Escherichia coli* 0157, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Helicobacter pylori* and *Campylobacter jejuni* are major bacterial pathogens that contribute to food-borne diseases (Hyytiä-Trees *et al.,* 2007), with *B. cereus* as a key contaminant of rice-based foods (MMWR, 1994). *Bacillus cereus* is a spore-forming, Gram-positive anaerobe that causes two different forms of food poisoning: the emetic illness, and the diarrhoeal illness (Drobniewski, 1993; Ehling-Schulz *et al.,* 2004; Schoeni and Wong, 2005).

Recent studies have highlighted the use of essential oils as effective additives for food safety and preservation (Burt, 2004; Cowan, 1999; Dorman and Deans, 1999; Fisher and Phillips 2006). Essential oils have been shown to possess antibacterial properties against *Bacillus cereus*, *Escherichia coli* 0157, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella typhimurium* (Cosentino *et al.,* 1999). So far, essential oils from cinnamon, sage, rosemary and sassafras exhibited optimum inhibitory effects on *B. cereus* (Valero and Salmeron, 2003). However, there is limited research on the use of essential oils from *Ocimum basilicum* (basil), *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), as antimicrobials in the food industry. Rice is the main source of food for about half of the world's population. It is cultivated in more than 100 countries on every continent except for Antarctica, although 91 percent of the world's rice is grown and consumed in Asia. The aim of the present study was to determine the potential bactericidal properties of the aforementioned essential oils on *B. cereus* in rice-based foods. A variety of rice were used including: (1) Weight Watchers chicken tikka and coriander rice; (2) Waitrose food doctor Thai chicken with sesame seed wholegrain rice; (3) Sainsbury's chili con carne with rice; (4) Marks & Spencer chicken balti; (5) Chinese take-away with boiled rice; and (6) Chinese take-away with egg-fried rice.

Essential Oils Exhibited Bactericidal Effects in a Concentration-Dependent Manner

To determine the effects of the essential oils (i.e., basil, thyme, and oregano) on the growth of *B. cereus*, bacterial cultures were incubated with rice in the presence or absence of various concentrations of oils in an eppendorf tube as previously described (Fisher and Phillips, 2006). For each rice type, 10 grains of rice were used. The final volumes of the tubes were adjusted to 100 µl using phosphate buffer saline (PBS). The tubes were incubated at 37ºC for 2h

and bacterial counts determined by plating as previously described (Fisher and Phillips, 2006). The results revealed that all oils tested exhibited bactericidal effects in a concentration-dependent manner (Table 1). The results are representative of three independent experiments performed in duplicate and expressed as the mean ± standard error. The optimum bactericidal activities were observed with 80µl of oil (Table 1). Interestingly, essential oil from basil was most effective against *B. cereus* in all types of rice (Table 1).

Next we determined whether the mixtures of three oils would have additive effects. For this, the assays were performed using equal portions of all three oils (6µl, 13µl, and 25µl) as described above. The results revealed that mixtures of the aforementioned oils exhibit additive and/or synergistic bactericidal effects (Table 1). This was shown by findings that only 25µl essential oil mixtures was sufficient to produce bactericidal effects, similar to effects observed with 80µl for each oil. However, we are cautious in these findings as these effects may simply be due to the total amount of essential oil. For example, in the 25µl essential oil mixtures, the total amount of essential oil was 75µl (25µl of each essential oil). Future studies will determine whether these effects are additive and/or due to the amount of essential oils.

Essential Oils Exhibited Optimal Bactericidal Effects at Room Temperature

Next, to determine the effects of varying temperatures on the bactericidal properties of essential oils, assays were performed at various temperatures including, room temperature (~20ºC), 25ºC and at 30ºC. At the room-temperature, no bacterial colonies were observed suggesting that essential oil mix exhibited bactericidal effects (Table 2). When incubated at 25ºC, only 5 and 6 (Chinese take-away boiled rice and Chinese take-away egg-fried rice, respectively) showed bacterial growth (Table 2). Again, at 30ºC, 5 and 6 had a higher number of bacterial cfu (Table 2). In addition, the ready-meals also showed some bacterial growth, with Marks and Spencer Chicken Balti and Rice giving the lowest bacterial counts (Table 2).

Previous studies have shown that *Ocimum basilicum* (basil) is a fragrant leave which is used as a seasoning herb with unique health protection effects as a flavonoids and volatile oil, while *Thymus vulgaris* (thyme) has a long history of use in natural medicine as a volatile oil and *Origanum vulgare* (oregano) is a volatile oil and contains numerous phytonutrients. These findings suggest that anti-bacterial properties of these herbs are most likely associated with their volatile oils, which contain *borneol, carvacolo, cineole, estragole, eugenol, geraniol, limonene, myrcene, linalool, sabinene,* and *thymol.* The findings that essential oils and/or herbs can have anti-bacterial effects are significant, especially in view of the fact that ready meals represent the ultimate convenience food and require no preparation, which seems to be important when looking at our working and family patterns.

In conclusion, these studies suggest the potential use of fresh herbs, *Ocimum basilicum* (basil), *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano) and/or their essential oils as antimicrobials in the food industry. The antibacterial properties of essential oils can be exploited in diverse ways such as dental root canal sealers (Manabe *et al.*, 1987), antiseptics (Bauer and Garbe, 1985; Cox et al., 2000), food preservatives (Mendoza-Yepes *et al.*, 1997) or insect repellents (Carson and Riley, 1993). Our studies support these findings and clearly showed bactericidal properties of essential oils from basil, thyme and oregano against *B. cereus* in rice-based foods.

Table 1: Bactericidal effects of different amounts of essential oils (basil, thyme, oregano) on *Bacillus cereus* at room temperature (~20°C) on various kinds of rice

Type of rice	Bacterial cfu in absence of oil	Bacterial cfu + 10µl oil (total vol. 100µl)			Bacterial cfu + 20µl oil (total vol. 100µl)			Bacterial cfu + 40µl oil (total vol. 100µl)			Bacterial cfu + 80µl oil (total vol. 100µl)			Bacterial cfu + mixture of three oils		
	without oil	basil	thyme	oregano	basil	thyme	oregano	basil	thyme	oregano	basil	thyme	oregano	6µl	13µl	25µl
1	2580 ± 61	1200± 11	1750± 45	1500± 29	1000± 17	1700± 28	1500± 39	700± 11	950± 27	1100± 33	0	50± 3	0	1200± 19	800± 29	50± 8
2	2944 ± 53	1700± 68	1900± 35	1450± 54	1300± 19	1800± 42	1100± 28	700± 18	1100± 36	1100± 58	0	150± 8	100± 8	1250± 25	900± 18	0
3	2542 ± 40	1100± 44	1900± 29	1500± 62	700± 35	1350± 62	1350± 48	750± 14	850± 49	950± 18	0	100± 6	0	1500± 62	1000± 29	100± 6
4	2512 ± 90	1400± 25	1600± 81	1800± 65	750± 57	1200± 28	1500± 39	800± 29	1000± 49	1500± 36	0	50± 5	0	1000± 45	800± 15	50± 4
5	3115 ± 26	2250± 78	3100± 69	3000± 83	1900± 28	2350± 56	2350± 60	1200± 31	1800± 28	2000± 40	100± 13	150± 9	200± 10	1700± 29	1200± 19	350± 7
6	3187 ± 55	2400± 76	2950± 41	3200± 90	2000± 84	2450± 76	2950± 41	1300± 25	1500± 19	2150± 98	100± 8	150± 12	150± 17	2000± 34	1450± 36	150± 10

Rice Code: 1 - Weight Watchers Chicken Tikka and Coriander Rice; 2 - Waitrose-Food Doctor Thai Marinated Chicken with Sesame Seed Wholegrain Rice; 3 - Sainsbury's Chili Con Carne With Rice; 4 - Marks and Spencer Chicken Balti and Rice; 5 – Chinese Take-Away, Boiled; 6 – Chinese Take-Away, Egg-Fried rice.

Table 2: Effects of essential oil mixtures (equal portions of 25µl) on *Bacillus cereus* on various kinds of rice at different temperatures

Type of rice	Bacterial cfu at room temp.		Bacterial cfu at 25°C		Bacterial cfu at 30°C	
	-ve	+ve	-ve	+ve	-ve	+ve
1	2580 ± 16	0	3145 ± 49	0	3718 ± 42	9 ± 5
2	2944 ± 53	0	3478 ± 87	0	4146 ± 64	39 ± 5
3	2542 ± 40	0	3217 ± 25	0	3709 ± 68	7.5 ± 6
4	2512 ± 90	0	3301 ± 48	0	3705 ± 22	3 ± 2
5	3115 ± 26	0	3273 ± 73	6 ± 2	3800 ± 90	48 ± 14
6	3187 ± 55	0	4014 ± 14	15 ± 6	4200 ± 18	108 ± 22

- indicates bacterial incubation with rice in the absence of essential oil mix
+ indicates bacterial incubation with rice in the presence of essential oil mix

REFERENCES

1. BAUER, K. and GARBE, D. 1985. In: Common Fragrance and Flavor Materials. Preparation, Properties and Uses, VCH Verlagsgesellschaft, Weinheim, p. 213.
2. BURT, S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 94, 223-253.
3. CARSON, C.F. and RILEY, T.V., 1993. Antimicrobial activity of the essential oil of *Melaleuca alternifolia*. Lett. Appl. Microbiol. 16, 49–55.
4. COWMAN, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564-582.
5. COSENTINO, S., TUBEROSO, C.I.G., PISANO, B., SATTA, M., MASCIA, V., ARZEDI, E. and PALMAS, F. 1999. *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett. Appl. Microbiol. 29, 130–135.

6. COX, S.D., MANN, C.M., MARKHAM, J.L., BELL, H.C., GUSTAFSON, J.E., WARMINGTON, J.R. and WYLLIE, S.G. 2000. The mode of antimicrobial action of essential oil of *Melaleuca alternifola* (tea tree oil). J. Appl. Microbiol. 88, 170–175.

7. DORMAN, H.J.D. and DEANS, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308-316.

8. DROBNIEWSKI, F.A. 1993. *Bacillus Cereus* and related species. Clin. Microbiol. Rev. 6, 324-338.

9. EHLING-SCHULZ M., FRICKER, M. and SCHERER S. 2004. *Bacillus cereus*, the causative agent of an emetic type of food-borne illness. Mol. Nutr. Food Res. 48, 479-487.

10. FISHER, K. and PHILLIPS, C.A. 2006. The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus in vitro* and in food systems. J. Appl. Microbiol. 101, 1232-1240.

11. HEALTH PROTECTION AGENCY. (2005). NOIDS Food Poisoning. Available from URL http://www.hpa.org.uk/infectious/noids/food_poisoning.htm, accessed on 6 March 2006.

12. HYYTIA-TREES, E.K., COOPER, K., RIBOT, E.M. and GERNER-SMIDT, P. 2007. Recent developments and future prospects in subtyping of foodborne bacterial pathogens. Future Microbiol. 2, 175-185.

13. MANABE, A., NAKAYAMA, S. and SAKAMOTO, K. 1987. Effects of essential oils on erythrocytes and hepatocytes from rats and dipalitoyl phophatidylcholine-liposomes. Jap. J. Pharmacol. 44, 77–84.

14. MENDOZA-YEPES, M.J., SANCHEZ-HIDALGO, L.E., MAERTENS, G. and MARIN-INIESTA, F. 1997. Inhibition of *Listeria monocytogenes* and other bacteria by a plant essential oil (DMC) en Spanish soft cheese. J. Food Saf. 17, 47–55.

15. MMWR – Morbidity and Mortality Weekly Report. 1994. *Bacillus cereus* Food Poisoning Associated with Fried Rice at Two Child Day Care Centers. 1994. U.S. Department of Health and Human Services. March, 18, 1994/Vol. 43/No.10.

16. SCHOENI, J.L. and WONG, A.C. 2005. *Bacillus cereus* food poisoning and its toxins. J. Food Prot. 68, 636-648.

17. VALERO, M. and SALMERO'N, M.C. 2003. Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. Int. J. Food Microbiol. 85, 73–81.

18. WORLD HEALTH ORGANIZATION (WHO). 2002. Food safety and foodborne illness. World Health Organization Fact sheet 237, revised January 2002. Geneva.

19. WORLD HEALTH ORGANIZATION (WHO). 2002. World health report 2002: Reducing risks, promoting healthy life. Geneva, World Health Organization, 30 October 2002. ISBN 92 4 156207 2 ISSN 1020-3311, p. 248.