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Microbial decontamination of powdered black pepper (*Piper nigrum* L.) by using microwave

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Powdered black pepper with varying moisture contents were subjected to intermittent or continuous microwave treatments (2450±50 MHz, 450 Watt) for 50 or 150 sec. Microwave treatment reduced microbial load in all treated samples. Higher product moisture and prolonged treatment time resulted in higher microbial reductions. The highest microbial reduction was in the groups, to which microwave treatment was applied continuously for 150 sec. Compared to the untreated samples in this group, the counts of total aerobic mesophilic microorganisms, *Enterobacteriaceae*, and yeast and mould were reduced by 87.8, 94.9 and 90.7%, respectively. However, in all the treated groups, the losses of volatile oil were between 3.9 and 18.7%. The results indicated that microwave treatment caused microbial reductions in the powdered black pepper, although the reductions were unsatisfactory. The levels of the reduction depended on the moisture content of the samples and exposure time. The losses of volatile oils occurred during the process were in acceptable levels.

Keywords: Black pepper powder, *Piper nigrum*, Microorganisms, Decontamination, Microwave, Volatile oil

Spices and herbs, which are used ver thousands of years by many civilizaions to enhance the flavour and aroma of oods, may be contaminated with undesirble substances depending on their growth nd storage conditions. Many spices suplied by dealers contain a number of miroorganisms. The microbial contaminaion level may be as high as 10^8 cfu/g. Aicroflora of spices mostly consist of erobic and anaerobic spore-forming bacria, such as *Micrococcus* spp., coliforms, easts and moulds. Food poisoning bacria like *Salmonella* spp., *Staphylococus aureus*, *Clostridium perfringens*, *Baillus cereus* and *Escherichia coli* have lso been reported in spices and herbs Aksu et al 2000, Banerjee and Sarkar 003, Buckenhüskes 1996, Fehlhaber and anetschke 1992, McKee 1995, Pafumi 986, Rosenberger and Weber 1993). ince microbiological content of spices in eir natural state is high, it is often necssary to decontaminate them before usg in food products. Heat treatments, ommonly used to reduce the microbial ounts in many foodstuffs, have not been eferred to decontaminate spices due to sses in volatile oil as well as flavour egradation (Gerhardt 1994). Treatment ith ethylene oxide is another effective ethod which can reduce the microbial ounts by 90% in spices (Gerhardt 1994, afumi 1986, Vajdi and Pereira 1973). his gas, however, has been classified as carcinogen and it can react with chlorides in foods to produce toxic and persistent chlorhydrins (Their and Bolt 2000), as traces of ethylene oxide may remain on spices after treatment (Gerhardt and Effio 1983). For these reasons, the use of this gas for microbial reduction in spices has been prohibited. One of the most effective treatments to reduce or eliminate bacterial and fungal populations in spices is by ionizing radiation (Weber 1983, Zaied et al 1996, Zehnder and Ettel 1981). But this method is not commonly used in food industry because of the public reaction against irradiated foods. And it also alters the flavour (Piggott and Othman 1993, Emam et al 1995).

Microwave treatment, therefore, could be an alternative method to decontaminate spices. Microwaves are widely used in food industry and also possess destructive effects on microorganisms in foods (Daniel et al 1980, Decerau 1986, Fujikawa and Ohta 1994, Reuters 1995, Rosenberg and Bögl 1987, Stolle and Schalch 2000).

Some workers investigated the effects of microwave heating on microbiological quality of spice and herb and reported that the reduction of microorganisms varied for different applications (Gerhardt and Rommer 1985, Dehne and Bögl 1993, Dehne et al 1991, Emam et al 1995, Legnani et al 2001). However, Vajdi and Pereira (1973) reported that microwave treatment of spice could not reduce microbial population effectively. The aim of this study was to evaluate the effect of microwave treatment on the microbial load and volatile oil level of powdered black pepper.

Materials and methods

Powdered black pepper (PBP) (*Piper nigrum* L.) samples were collected from various retail points in Istanbul, Turkey. Microbiological and chemical analysis (to determine the initial microbial count, moisture content and volatile oil level), was performed and each batch of PBP were divided into 3 equal portions of about 800 g each, as group A, B and C. Each group sample was then divided into 4 subgroups (approx 200 g) each according to process types and processing times, and were aseptically put into sterile polyethylene bags, suitable for microwave heating. The moisture content of Groups B and C were adjusted to 15 and 17.5%, respectively, by spraying sterile tap water (Gerhardt and Romer 1985), and A was a natural control.

Microwave treatment: A specially designed industrial type microwave oven (2450±50 MHz) (Machine and Chemistry Company, Turkey) was used during trials. The samples in plastic bags were placed on to running band of the oven and flattened to approximately 5 mm. For each trial, a total of 12 samples were individually (one by one following each other) subjected to intermittent or continuous microwave heating (2450±50 MHz, 450 W) for various holding times (50 or 150 sec). At the intermittent application, a 5 sec standing time was given for each 10 sec exposure time. Standing times were not added to the total application time. Microwave treatment trials were replicated for three times by using distinct PBP.

Analysis: Treated and untreated black pepper samples were mixed thoroughly in their respective bags, before drawing samples for analysis.

For microbiological analysis, 10 g of samples were placed in sterile Stomacher bags and homogenized for 2 min in 90 ml of sterile 0.1% peptone water (Oxoid, CM 9) using Stomacher Lab Blender (Seward, England). Serial decimal dilutions were prepared in same diluents. Total aerobic mesophilic microorganisms were enumerated on plate count agar (Oxoid, CM 325) after incubating the plates at 30°C for 48 h, *Enterobacteriaceae* on violet red bile dextrose agar (Oxoid, CM 485) and incubated with a double layer for 24 h at 37°C, and yeasts and molds on potato dextrose agar (Oxoid, CM 139) containing 75 g/l NaCl (Sigma, S 7653) and 4 ml/l chlortetracycline. HCl (Biolife, 420000) at 25°C for 5 days (Andrews 1992, Harrigan 1998).

To determine the moisture contents, the samples were dried at 103±2°C up to the dry matter amount reached to a constant value or the differences in weight becomes < 1 mg between the last two measurements (Staesche 1970). Volatile oil contents of PBP were determined according to the AOAC (1997). Ground spice samples were boiled in H_2O solution. Released volatile oil (vapour form) was condensed and collected in trap, and then measured through trap calibration.

Statistical analysis: Each experiment was replicated 3 times. Microbial reduction and volatile oil losses were calculated on the absolute values. Colony counts were converted to logarithmic values. ANOVA and Duncan's multiple range test were used to analyze the microbiological log counts and volatile oil amounts. Statistical estimations were performed using the SPSS (8.0 package programme).

Results and discussion

The mean colony counts of total aerobic mesophilic microorganisms, *Enterobacteriaceae* spp. and yeast-mould in untreated PBP samples were 6.3, 4.4, and 3.9 $\log_{10}$ cfu/g, respectively (Table 1). Microwave treatment resulted in higher reduction from 49.5 to 87.8% for total aerobic mesophilic microorganism count from 7.5 to 94.9% for *Enterobacteriaceae* spp. count and from 33.9 to 90.7% for yeast and mould count. The significant difference ($p \le 0.05$) was obtained only between the control (untreated) group and group C for total aerobic mesophilic microorganism count and *Enterobacteriaceae* spp. count. The differences in yeast and mould counts between the treatments were not significant. Similar results were reported by other authors who worked on the microbial decontamination of PBP microwave. Emam et al (1995) reported that the number of total aerobic mesophilic microorganisms (6.9 $\log_{10}$ cfu/g) decreased to < 10^4 cfu/g after microwave treatment (2450 MHz, 750 W) for 75 sec; and the reduction with microwave exposure was similar to the reduction that was obtained with 10 kGy g- irradiation. They also reported that number of yeast and mould (4.9 $\log_{10}$ cfu/g) was decreased to < 10^2 cfu/g depending on the time of microwave exposure. In another study, Gerhardt and Romer (1985) reported that microwave applications (2450 MHz, 650 W) for various periods of time resulted in reductions in bacterial count of PBP ranging between 13.7 and 99.7% and decreased the yeast and mould to <10^2 cfu/g. Dehne and Bögl (1993) determined that the number of aerobic mesophilic microorganisms in the powdered white pepper was reduced from 6.1 to 4 $\log_{10}$ cfu/g by using microwave (2450 MHz, 0.6 kW). Legnani et al (2001) also obtained a successful result on the reductions of moulds and faecal indicators using microwave heating. Contrary to above results, Vajdi and Pereira (1973) reported that microwave treatment of black pepper and other spices was not effective in reducing the microbial population.

Microbial reductions obtained in the present study were to a lesser degree than

Table 1. Mean total counts of microorganisms and volatile oil contents of powdered black pepper treated with microwave.

Groups[1]	Moisture content, %	Exposure time, sec	Treatment type[2]	Mesophilic aerobic		Enterobacteriaceae		Yeast and mould		Volatile oil	
				$\log_{10}$ cfu/g	Reduction, %	$\log_{10}$ cfu/g	Reduction, %	$\log_{10}$ cfu/g	Reduction, %	$\log_{10}$ cfu/g	Reduction, %
Untreated				6.3±0.26 [a]	0.0	4.4±0.30[a]	0.0	3.9±0.30[a]	0.0	1.56±0.187[a]	0.0
AA	Natural	50	C	6.1±0.21[ab]	49.5	4.3±0.21[ab]	7.5	3.8±0.27[a]	33.9	1.50±0.169 [a]	3.9
AB	"	50	I	6.0±0.21[ab]	56.0	4.3±0.29[ab]	44.6	3.6±0.40[a]	33.1	1.49±0.170 [a]	4.2
AC	"	150	C	6.0±0.20[ab]	57.1	4.1±0.17[abc]	40.0	3.5±0.31[a]	60.5	1.45±0.149 [a]	6.8
AD	"	150	I	5.9± 0.22[ab]	68.0	4.1±0.29[abc]	70.1	3.6±0.28[a]	49.3	1.46±0.155[a]	6.4
BA	15.00	50	C	5.9±0.16[ab]	69.2	4.0±0.21[abcd]	68.9	3.5±0.26[a]	63.0	1.43± 0.172[a]	8.1
BB	"	50	I	5.8±0.12 [ab]	75.1	4.0±0.29[abcd]	65.6	3.7±0.26 [a]	41.6	1.44±0.171[a]	7.6
BC	"	150	C	5.7±0.10 [ab]	81.8	3.7±0.11[abcd]	87.6	3.4±0.28[a]	69.7	1.36±0.203[a]	12.5
BD	"	150	I	5.7±0.07 [ab]	81.6	3.7±0.27[abcd]	83.8	3.4±0.42[a]	64.3	1.38±0.187[a]	11.6
CA	17.50	50	C	5.7±0.16 [b]	80.5	3.8±0.17[abcd]	84.4	3.4±0.23[a]	71.1	1.32±0.146[a]	15.5
CB	"	50	I	5.6± 0.22[b]	80.8	3.6±0.12[bcd]	90.8	3.3±0.25[a]	74.2	1.34±0.161[a]	14.2
CC	"	150	C	5.5± 0.21[b]	87.8	3.3±0.32[cd]	94.9	2.9±0.25[a]	90.7	1.27±0.199[a]	18.7
CD	"	150	I	5.6± 0.24[b]	86.7	3.3± 0.20[d]	92.4	3.2±0.26[a]	79.3	1.29±0.244[a]	17.2

[1]As in text; [2]At the intermittent application, a 5 sec standing time was given for each 10 sec exposure time; Means in a column with different superscripts are significantly ($p \le 0.05$) different from one another; n=3; C: Continuous; I: Intermittent

ıose mentioned above. The differences ı the reductions between the present study nd other studies may be due to the variaons in the applications such as frequency, istance from the source, shape and type f container, as well as the potential power f the microwave sources. The power of ıicrowave oven used in the present study ʼas lower (450 W) than those used in the bove studies, which might be the main ıctor for lower microbial reductions.

In the present study, the moisture ontent of the samples subjected to mirowave and exposure times were found) be very effective in reducing microbial opulation. Increasing exposure time from 0 to 150 sec improved the effectiveness f treatment, as expected. However, no orrelation was found between the reducon levels and heating products (intermitːnt or continuous). Increasing the moisıre content of PBP also increased the ːductions. The least microbial reduction ʼas obtained in group AA, non-moistned (12.4%) and treated with microwave ontinuously for 50 sec. On the other and, the most effective reduction was bserved in group CC, moistened to 17.5% nd subjected to microwave treatment ontinuously for 150 sec. Gerhardt and .omer (1985), who investigated the efːct of microwave treatments on the miroorganisms in moistened spices, also ːported that the reductions in the microial counts increased as the moisture ontent increased.

Heat generation by microwave ocurs by the movement of water molecule nd friction between the molecules (Daniel t al 1980, Mudgett 1989, Stolle and chalch 2000). Therefore, heat generation ıight be low, if the samples have low ʼater content. According to Vajdi and ereira (1973), spices are incapable of eing heated sufficiently by microwave ue to their low moisture content. In the resent study, the observation of higher ıicrobial destruction in samples containıg more moisture is apparently related to igh level of heat release.

The most important advantage of the ıicrowave heating is the ability to rapidly enerate heat in the product. And thus, ıis heating method may prevent the losses f volatile oil which is responsible for ʼpical flavour in spice, because the exposure time to decontaminate the microorganisms is shorter than that of conventional methods. In the present study, the mean volatile oil level in the PBP just before the applications was found to be 1.56±0.19 ml/100g (on dry weight basis). However, in the treated groups, the amount of volatile oils decreased to various degrees (3.9-18.7%). Although the volatile oil content of the treated samples were lower than that of the control, the differences were not significant statistically (< 0.05). Intermittent treatment did not affect the losses markedly compared to continuous treatment. Increasing exposure time and moisture content of black pepper also increased the losses in volatile oil contents. Emam et al (1995) reported that increasing exposure time from 40 to 75 sec caused higher losses in volatile oil contents. Gerhardt and Romer (1985) also determined that prolonged microwave treatments from 50 to 150 sec increased the loss of volatile oil in spices and continuous process resulted in more losses of volatile oil than the intermittent application. But, the losses of volatile oil obtained from the work of Gerhardt and Romer (1985) occurred at higher levels than the losses reported by the study presented here, and reached up to 78% in 22% moistened samples. In another study, Dehne and Bögl (1993) treated a mixture of spices with microwave and reported volatile oil losses as 6.0-10.5% for crushed white pepper, 8.7-18.2% for crushed black pepper, and 47.1% for ground black pepper. The authors recommended that it would, therefore, appear to be more beneficial to pasteurize the spices before grinding. Emam et al (1995) found low levels of volatile oil losses in non-moistened PBP samples treated with microwave. These researchers suggested the use of microwave treatment for decontamination of PBP without the loss of their flavour components. Plesi et al (2002) also investigated the effect of microwave treatment on volatile oil compounds of white and black pepper, and reported that the microwave treatment seems to preserve the principal aroma of compounds.

In conclusion, application of various microwave treatments reduced the levels of microorganisms in PBP samples; and spraying samples with water treatment before increased microbial reductions. There have also been some reductions in the volatile oil levels, as well with high moisture which may be considered acceptable.

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