Antimicrobial effects of curcumin against *L. monocytogenes, S. aureus, S.* Typhimurium and *E. coli* O157:H7 pathogens in minced meat

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ABSTRACT: The aim of this study was to determine the antimicrobial efficacy of curcumin, one of the active components of the *Curcuma longa* (turmeric) plant, against food pathogens in a minced meat medium. *Salmonella* Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O157:H7 ATCC 33150 and *S. aureus* ATCC 25923 strains were used as food pathogens. Minimum inhibitory concentrations (MICs) were determined using the macrodilution method. MIC values for curcumin were found to be 125 µg/ml for *L. monocytogenes* and *S. aureus*, and 250 µg/ml for *S.* Typhimurium and *E. coli* O157:H7. Food pathogens were added to the minced meat at 10^4 CFU/g (including the control group) and curcumin at doses of 0.5%, 1% and 2% (except the control). The curcumin-supplemented minced meat and control were analysed 0–7 days later. At the end of seven days, it was seen that the 2% dose of curcumin had lowered *L. monocytogenes* and *S. aureus* counts by approximately 3 log CFU/g, and *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; the 1% dose had lowered *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; and that the 0.5% curcumin dose had lowered *L. monocytogenes* and *S. aureus* count by approximately 2 log CFU/g, and *E. coli* O157:H7 and *S.* Typhimurium count by approximately 1 log CFU/g. Changes in bacterial counts were found to be statistically significant ($P \leq 0.05$). It was observed that antibacterial effect increased in direct proportion to dose, while sensory approval decreased. In this study, 0.5% and 1% curcumin doses were determined to be sensorily acceptable. It was concluded that, in view of the scientific benefits and antimicrobial efficacy of curcumin, it may be used instead of, or in smaller doses together with preservative additives in foods where colour change is not important.

Keywords: curcumin; antimicrobial effect; foodborne pathogen; minced meat

The use of antimicrobial substances is one of the most common methods to preserve and increase the durability of foods. Rising levels health problems are linked to various food additives, with antimicrobial and preservative substances topping the list (Kurt and Zorba 2005; Gokce 2011). The demand of consumers for safe food means that obtaining natural and reliable additives is an important issue (Kurt and Zorba 2005; Koyuncu et al. 2008).

For millennia, people have been consuming plants as tea and spices, as well as using them to treat various diseases. Extracts and volatile oils obtained from plants rich in components (alkaloids, volatile oils, glycosides, flavonoids, tannins, phenols, colour substances and resins) are

known to have antimicrobial actions (Koyuncu et al. 2008).

Turmeric is a spice obtained from the *Curcuma longa* L. plant (Stankovic 2014). It has an important place in Indian medicine (Aggarwal et al. 2007).

Curcumin is a phenolic component obtained from turmeric (Sharma et al. 2005; Stankovic 2014). Curcumin, which gives the yellow colour to turmeric, was first isolated almost two centuries ago (Aggarwal et al. 2007). As well as being a natural colourant, it has been reported that curcumin has antioxidant, antimicrobial, anti-cancer, analgesic, anti-ulcer and anti-inflammatory effects (Aggarwal et al. 2007; Akpolat et al. 2010; Stankovic 2014). The antimicrobial effect of curcumin is stated to be effective both in wound therapy and against food pathogens (Pattaratanawadee et al. 2006; Wang et al. 2009).

Curcumin is a food additive (E100) used as a colourant. Curcumin has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF). Curcumin is widely used to colour many foods. The Draft Codex General Standard for Food Additives provides an extensive list of such foods. Curcumin is listed for use in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and egg products, spices, soups, sauces and protein products, foodstuffs intended for particular nutritional uses, beverages, ready-to-eat savouries and composite foods. Used levels of curcumin are in the range from 5 to 500 mg/kg depending on the food category. JECFA specifications define only curcumin extracted from natural source materials. It can also be produced by chemical synthesis but synthetic curcumin is not used as a food additive (Stankovic 2014).

In this study, the antimicrobial effect of curcumin on minced meat contaminated with pathogen organisms (*Salmonella* Typhimurium, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *S. aureus*) has been investigated.

MATERIAL AND METHODS

Bacterial cultures. Food pathogen strains *Salmonella* Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O157:H7 ATCC 33150 and *S. aureus* ATCC 25923 were used.

Preparation of minced meat. In this study, minced meat freshly prepared from veal suitable for consumption was purchased from the fresh meat section of a hypermarket. It was transported to the laboratory in the cold chain and tested for the presence of food pathogens. *Salmonella* Typhimurium, *Listeria monocytogenes*, *Escherichia coli* O157:H7 analysis was performed on a 25 g minced meat sample using the standard present/absent test. Pre-enrichment (Buffered Peptone Water, Oxoid CM1049, 37 °C, 24 h), selective enrichment (Rappaport Vassiliadis Soy Broth, Oxoid CM0866 41 °C, 24 h) and spread inoculation on selective solid medium (Xylose Lysine Deoxycholate Agar, Oxoid CM0469 37 °C, 24 h) was performed for *Salmonella* Typhimurium (ISO 6579-2002). *Listeria monocytogenes* analysis was carried out using the spread inoculation technique on Palcam Agar (Oxoid M0877, SR0150, 37 °C, 24 h) media following Fraser broth (½ strength, 225 ml, 30 °C, 24 h) enrichment. At the same time, 0.1 ml of pre-enrichment culture was added to 10 ml (full strength) Fraser Broth (Oxoid, CM0895). Following incubation (37 °C, 48 h), Palcam Agar was used again and the results were evaluated. For *E.coli* O157:H7 analysis, Modified Tryptone Soya Broth (Oxoid CM0989) containing novobiocin was used for enrichment (41 °C, 18 h). For selective isolation, a special streaking technique was used for transfer to sorbitol MacConkey agar (Oxoid CM0813) with cefixime tellurite (37 °C, 24 h). *S. aureus* analysis was performed on a 5 g minced meat sample using Baird-Parker Agar (Oxoid, CM0275) (Bennett and Lancette 2001; Chapman et al. 2001; Goncalves et al. 2005; Sireli et al. 2008).

The minced meat was preserved at −18 °C until microbiological results were obtained. The pathogen-free minced meat was defrosted in the refrigerator (Bosh, Turkey, +6 °C) prior to the study. The defrosted minced meat was divided into the following groups; no curcumin with pathogen, 0.5% curcumin and pathogen, 1% curcumin and pathogen and 2% curcumin and pathogen. For each group, the *E. coli*, *S.* Typhimurium, *L. monocytogenes* and *S. aureus* subgroups were established and 500 g of minced meat were used for each subgroup. For sensory assessment, samples of minced meat with no added curcumin and minced meat prepared with 0.5%, 1% and 2% curcumin were used.

Preparation of curcumin. Curcumin was obtained from Sigma Chemicals Co. (CAS No. 458-37-7,

C1386, St Louis, Missouri, USA) and was stored at –20 °C until use. To prepare the stock solution of curcumin, microbiologically-tested ethanol and dimethyl sulphoxide (DMSO) were used. Ethanol was prepared made up to a 9% solution (Gulcubuk et al. 2006). Curcumin was used at doses of 0.5%, 1% and 2%. The used curcumin concentrations were determined following preliminary tests. Experiments were started with data obtained from MIC results. Doses were gradually increased.

Addition of pathogen to minced meat. Following the revitalisation procedure, strains were regulated in the McFarland machine according to the 0.5 McFarland turbidity value (0.5 McFarland approximately 1.5×10^8 CFU/ml) (McFarland 1907; Natta et al. 2008). The final microorganism concentration minced meat was calculated as 10^4 CFU/g and a dilution process was carried out (Abdollahzadeh et al. 2014), which was confirmed using the petri dish method.

Determination of minimum inhibitory concentration values. Minimum inhibitory concentration (MIC) values were determined according to the Clinical and Laboratory Standard Institute guidelines (CLSI 2000), using the Mueller-Hinton Broth serial 2-fold dilution with the macrodilution (tube) liquid method.

Each trial was performed in parallel and was repeated three times. The point at which bacterial growth was completely inhibited was determined to be the MIC value.

Microbiological analysis. Microbiological analyses were performed in minced meat groups with pathogens and curcumin, as well as in minced meat groups with pathogens but no curcumin.

The dilution prepared for *S.* Typhimurium counts was transferred to XLD Agar and the petri dishes were incubated at 37 °C for 20–24 h (Kotzekidou et al. 2008; Andrews et al. 2011).

The dilution prepared for *L. monocytogenes* counts was inoculated onto Palcam Agar using the spread plate technique and incubated at 37 °C for 24 h (Hitchins and Jinneman 2011).

For *E. coli* O157:H7 counts, inoculation was done onto Sorbitol MacConkey Agar with cefixime and tellurite (SMAC) and incubated at 37 °C for 24 hours (Solomakos et al. 2008).

For *S. aureus*, inoculation was made onto Baird-Parker Agar and the petri dishes were incubated at 37 °C for 24 h (Bennett and Lancette 2001). Analyses were performed in parallel.

Sensory assessment. Minced meat samples containing curcumin (0.5%, 1% and 2%) and the control (no curcumin) sample were randomly numbered in 100 g batches. These were wrapped in aluminium foil and cooked in the oven (Bosh, Turkey) to an internal temperature of 72 °C as measured with a temperature probe (Testo 106). A panel of ten people (10 trained females, aged between 18–40 years) assessed the samples according to the 9-point hedonic scale. The scale is verbally anchored with nine categories, as follows: like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely (Inglet et al. 2005). The panellists were asked to assign nine points for their favourite samples and one point for their least favourite, as well as to make an overall assessment regarding the suitability of the curcumin-added samples for consumption.

Assessment of microbiological and sensory findings. Differences in the data obtained from the results of analyses were assessed statistically using the JMP IN 7.0.0 (Statistical Discovery from SAS 2007. Institute Inc.) programme. To determine statistically significant differences between the mean values of the different groups, the LSD (Least Significant Difference) test was used at the $P \leq 0.05$ probability level and this was repeated twice according to the randomized block trial design.

RESULTS

Minimum inhibitor concentration (MIC) values for curcumin were found to be 125 µg/ml for *L. monocytogenes* and *S. aureus;* and 250 µg/ml for *S.* Typhimurium and *E. coli.* No microbial growth was determined in DMSO.

Using 0.5%, 1% and 2% doses in minced meat medium, the effect of curcumin against the food pathogens *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus* was examined. At the end of seven days, it was seen that the 2% dose of curcumin had lowered *L. monocytogenes* and *S. aureus* counts by approximately 3 log CFU/g, and *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; the 1% dose had lowered *L. monocytogenes, S. aureus, E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; the 0.5% curcumin dose had lowered *L. monocytogenes* and *S. aureus* counts by approximately 2 log CFU/g,

Table 1. Antimicrobial effect of curcumin on *S. aureus* , *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in minced meat ($\log_{10}$ CFU/g)*

Day	Control	0.5%	1%	2%	Control	0.5%	1%	2%
		S. aureus				*E. coli* O157:H7		
0	4.92 ± 0.01[d]	4.70 ± 0.01[c]	4.51 ± 0.01[b]	4.51 ± 0.01[b]	4.96 ± 0.01[g]	4.85 ± 0.01[a]	4.62 ± 0.01[a]	4.57 ± 0.01[a]
1	6.51 ± 0.01[h]	5.49 ± 0.01[a]	4.96 ± 0.01[a]	4.20 ± 0.01[c]	7.34 ± 0.01[a]	3.72 ± 0.01[e]	3.81 ± 0.01[d]	2.79 ± 0.01[e]
2	6.23 ± 0.01[f]	4.72 ± 0.01[b]	4.51 ± 0.01[b]	5.81 ± 0.01[a]	6.00 ± 0.01[e]	4.85 ± 0.01[a]	4.38 ± 0.01[b]	3.45 ± 0.01[d]
3	7.91 ± 0.01[a]	3.74 ± 0.01[e]	3.80 ± 0.01[c]	3.43 ± 0.01[d]	6.51 ± 0.01[c]	4.73 ± 0.01[c]	3.91 ± 0.01[c]	3.54 ± 0.01[c]
4	7.45 ± 0.01[b]	2.92 ± 0.01[g]	3.58 ± 0.01[d]	2.88 ± 0.01[e]	6.64 ± 0.01[b]	4.76 ± 0.01[b]	3.81 ± 0.01[d]	3.62 ± 0.01[b]
5	6.74 ± 0.01[c]	3.52 ± 0.01[f]	3.48 ± 0.01[e]	2.64 ± 0.01[f]	6.43 ± 0.01[d]	3.72 ± 0.01[e]	3.62 ± 0.01[e]	2.49 ± 001[g]
6	6.36 ± 0.01[e]	3.88 ± 0.01[d]	2.71 ± 0.01[f]	1.72 ± 0.01[g]	5.80 ± 0.01[f]	3.65 ± 0.01[f]	2.43 ± 0.01[f]	2.53 ± 0.01[f]
7	5.51 ± 0.01[g]	2.62 ± 0.01[h]	2.67 ± 0.01[g]	1.36 ± 0.01[h]	4.85 ± 0.01[h]	3.78 ± 0.01[d]	2.38 ± 0.01[g]	2.26 ± 0.01[h]
		S. Typhimurium				*L. monocytogenes*		
0	4.51 ± 0.01[g]	4.45 ± 0.01[f]	4.40 ± 0.01[c]	4.85 ± 0.01[b]	4.90 ± 0.01[h]	4.86 ± 0.01[a]	4.11 ± 0.01[c]	4.23 ± 0.01[b]
1	5.80 ± 0.01[e]	5.86 ± 0.01[a]	5.86 ± 0.01[a]	5.00 ± 0.01[a]	5.41 ± 0.01[g]	4.73 ± 0.01[c]	4.40 ± 0.01[b]	4.15 ± 0.01[c]
2	6.65 ± 0.01[d]	4.82 ± 0.01[b]	4.73 ± 0.01[b]	4.50 ± 0.01[c]	6.34 ± 0.01[e]	4.79 ± 0.01[b]	4.61 ± 0.01[a]	4.51 ± 0.01[a]
3	7.85 ± 0.01[b]	4.73 ± 0.01[c]	3.68 ± 0.01[d]	4.11 ± 0.01[d]	6.79 ± 0.01[c]	3.94 ± 0.01[d]	3.89 ± 0.01[d]	3.20 ± 0.01[d]
4	8.52 ± 0.01[a]	3.86 ± 0.01[g]	3.61 ± 0.01[e]	2.91 ± 0.01[e]	7.08 ± 0.01[b]	2.70 ± 0.01[g]	3.71 ± 0.01[e]	2.65 ± 0.01[e]
5	7.57 ± 0.01[c]	4.50 ± 0.01[e]	2.79 ± 0.01[f]	2.79 ± 0.01[f]	7.53 ± 0.01[a]	3.61 ± 0.01[e]	2.85 ± 0.01[f]	2.53 ± 0.01[f]
6	6.65 ± 0.01[d]	4.55 ± 0.01[d]	2.62 ± 0.01[g]	2.72 ± 0.01[g]	6.40 ± 0.01[d]	3.49 ± 0.01[f]	2.81 ± 0.01[g]	1.85 ± 0.01[g]
7	5.71 ± 0.01[f]	3.72 ± 0.01[h]	2.53 ± 0.01[h]	2.43 ± 0.01[h]	5.86 ± 0.01[f]	2.51 ± 0.01[h]	2.63 ± 0.01[h]	1.26 ± 0.01[h]

*statistically significant difference at $P \leq 0.05$ is observed between mean values shown with different letters in the same column

and *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 1 log CFU/g.

Microbiological and sensory assessment results are shown in Tables 1 and 2.

In the sensory assessment, the control sample was the most highly approved minced meat sample with a total of 364 points. Increasing the dose was seen to decrease total points. Statistical evaluation of the control and curcumin-supplemented groups revealed significant differences ($P < 0.05$). No statistically significant difference was found between the points scored for odour properties, and the control, 0.5% and 1% samples displayed similar statistical values regarding structure and aroma. During the scoring, panellists were assessed for their perception of features that would prevent consumption such as colour, taste, appearance, odour and structure. It was concluded that, for the panellists, there was no differences that would adversely affect consumption (bitterness, bad/sharp odour, sour, hot, strong pungent aroma etc) and that all samples could be consumed. However, when cooked, a colour formed on the surface of the 2% curcumin-supplemented samples, which was not favoured by the panellists.

DISCUSSION

According to MIC results, it was determined that the value achieved against Gram-positive bacteria (125 µg/ml), was lower than the value achieved against Gram-negative bacteria (250 µg/ml). The

Table 2. Sensory assessment results of curcumin in minced meat*

Dose (%)	Appearance	Colour	Smell	Structure	Flavour
Control	7.8 ± 1.5[a]	7.5 ± 1.30[a]	7.7 ± 1.30[a]	6.5 ± 1.8[a]	6.9 ± 1.29[a]
0.5	5.5 ± 1.6[b]	5.5 ± 1.78[b]	6.5 ± 1.78[a]	5.8 ± 2.10[a]	5.8 ± 1.88[a]
1	5.3 ± 1.5[b]	5.3 ± 1.89[b]	6.9 ± 1.60[a]	5.2 ± 1.32[ab]	6.2 ± 0.92[a]
2	4.3 ± 0.8[b]	4.4 ± 1.65[b]	6.1 ± 2.28[a]	3.9 ± 2.18[b]	3.9 ± 2.77[b]

*statistically significant difference at $P \leq 0.05$ is observed between mean values shown with different letters in the same column

reason for the higher antimicrobial effect on Gram-positive bacteria is explained by the bacteria cell wall structures (Wang et al. 2009; Bhawana et al. 2011).

Various studies have reported MIC values of curcumin. MIC values were reported by Tajbakhsh et al. (2008) to be 187.5 μg/ml for *S. aureus* and 93.8 μg/ml for *E. coli*; by Wang et al. (2009) using microcapsule curcumin, as 62.5 μg/ml for *S. aureus* and 250 μg/ml for *E. coli*; by Gunes et al. (2013) in a study using 67% pure curcumin, as 163 μg/ml for *E. coli*, 219 and 217 μg/ml for methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus*, respectively; and by Bhawana et al. (2011) in a study where curcumin prepared with DMSO was compared to nanocurcumin prepared with water, as 150 μg/ml for *S. aureus* and 300 μg/ml for *E. coli* with curcumin, and 100 μg/ml for *S. aureus* and 250 μg/ml for *E. coli* with nanocurcumin.

Curcumin doses determined according to MIC data were then used for the experiments on food pathogens in minced meat medium. However, successful results were not achieved. In this study, it was seen that, if the same effect is to be achieved in food medium then curcumin doses used need to be increased. In some studies where antimicrobial efficacy has been tested (Solomakos et al. 2008; Abdollahzadeh et al. 2014), it can be seen that experiments are carried out after cooking of the minced meat and elimination of the flora present in the food. The present study is important with regard to understanding the behaviour of pathogenic microorganisms and curcumin in minced meat medium, which possesses its own specific microbial flora.

Comparison of microbiological data obtained using curcumin against pathogen microorganisms in minced meat medium reveals the following antimicrobial effects; *L. monocytogenes* > *S. aureus* > *E. coli* O157:H7 > *S.* Typhimurium. These data mirror what was observed in the MIC assessment results.

In a study where Lourenco et al. (2013) examined the antimicrobial activity of a 1% dose of turmeric on *S. aureus* and *E. coli* in chicken breast, it was reported that the number of microorganisms did not show a great difference between the control group with no added turmeric and groups with added turmeric, all inoculated at a level of 10^4. While varying according to the method by which it is obtained, turmeric is comprised of approximately 3% (2–5) curcumin (Natta et al. 2008; Akpolat et al. 2010).

Abdollahzadeh et al. (2014) reported that turmeric has weak action according to the agar disc diffusion method.

Hosny et al. (2011) reported that, in Karishcum cheese prepared with 0.3% curcumin, at the end of 14 days of storage, there was an approximately 1 log decrease in the *S.* Typhimurium count and a 2 log decrease in the *E. coli* O157:H7 count, and also that *S. aureus* and *L. monocytogenes* counts were negative.

Despite increasing the curcumin doses, according to the MIC data, it was seen that the pathogenic microorganism count in minced meat medium could not be eliminated, but only lowered. Changes in the microbial flora, water activity value, oil ratio and pH value may affect curcumin activity (Negi 2012). Oil solubility also limits antimicrobial property. Curcumin is not water-soluble in acidic medium or neutral pH. However, it is soluble in alkali medium (Stankovic 2014).

In this study, it was seen that, as curcumin dose increased, antimicrobial action also increased. However, sensory approval decreased. It was observed that the 0.5% and 1% curcumin doses used in this study were acceptable. In the authors' opinion, a colour change is among the main reasons for a drop in sensory approval. Panellists stated that the curcumin-supplemented minced meat, and the 2% dose in particular, was disliked due to its very different appearance from natural minced meat. Therefore, we conclude that more positive results would be achieved when curcumin is used in other products prepared using minced meat (meat mixtures, meat products, soups etc.) where colour is not important. Since curcumin is partly soluble in hot water, it might be suitable to use in food undergoing any heating process (Ozcan and Akgul 1995) such as cooking. In their study performed using microcapsule curcumin, Wang et al. (2012) stated that the heat process increased activity.

It has been reported that curcumin turns bright yellow in an acidic environment and red in alkali (Ozcan and Akgul 1995; Sharma et al. 2005; Stankovic 2014). The antioxidant properties of curcumin have also been reported (Ozcan and Akgul 1995; Sharma et al. 2005; Stankovic 2014). In this study, putrefaction signs and darkening of colour were not seen over seven days in the curcumin-supplemented minced meat.

According to the data obtained at the end of seven days using 0.5%, 1% and 2% doses of curcumin in

food pathogen-supplemented minced meat; the 2% dose of curcumin lowered *L. monocytogenes* and *S. aureus* counts by approximately 3 log CFU/g and *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; the 1% dose lowered *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 2 log CFU/g; the 0.5% curcumin dose lowered *L. monocytogenes* and *S. aureus* counts by approximately 2 log CFU/g and *E. coli* O157:H7 and *S.* Typhimurium counts by approximately 1 log CFU/g. Changes in bacterial counts were found to be statistically significant ($P \leq 0.05$).

In conclusion, the 0.5% and 1% doses of curcumin used in this study were seen to be of sensorily acceptable quality. In the light of its strong antimicrobial action, it was concluded that curcumin may be used instead of preservatives or in decreased doses together with such substances in foods where colour change is not important. The legal limit should be determined for curcumin as a preservative food additive and curcumin extracted from natural source materials must be used.

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