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Evaluation and predictive modeling the effects of spice extracts on raw chicken meat stored at different temperatures

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ABSTRACT

In the present study, the anti-microbial and anti-oxidant effects of *Syzygium aromaticum* (SA), *Cinnamomum cassia* (CC) and *Origanum vulgare* (OV) on the shelf life of raw chicken meat stored at different temperatures (4, 10, 15 and 20 °C ± 1) were studied. Gompertz model was used to model the microbial growth using the data from microbial analysis of meat samples. Arrhenius equation was applied to understand the effect of storage temperature on the specific growth rate (μ) and lag phase duration. Highest μ_{max} and LPD (lag phase duration) values were obtained for *Enterobacteriaceae* in T-SA (Treatment with 1% *S. aromaticum* extract) samples stored at 4 °C. The μ_{max} values of T-SA–CC–OV (Treatment with 0.33% *S. aromaticum* extract + 0.33% *C. cassia* extract + 0.33% *O. vulgare* extract) samples were found to be low at all the tested temperatures and especially at 4 °C with better color values and lower TBARS (Thiobarbituric acid reactive substances) values than the other samples. The best preservative effects were achieved with the combination of spice extracts.

1. Introduction

Meat is a very popular food commodity around the world due to its low cost of production, low fat content, high nutritional value and distinct flavor (Barbut, 2002; Patsias et al., 2008). The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens (Zhou et al., 2010). It is therefore essential that adequate preservation technologies are needed to extend the shelf life of perishable meat products which is a major concern for the meat industries (Wang et al., 2004).

Lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the meat products, leading to a retardation of spoilage, an extension of shelf-life, and a maintenance of quality and safety (Devatkal and Naveena, 2010). Therefore, there has been increasing interest in alternative additives from natural sources (Sebranek et al., 2005) which has gradually provided impetus to eliminating synthetic preservatives in food (McCarthy et al., 2001).

Naturally occurring antimicrobial compounds have good potential to be applied as food preservatives. Essential oils and other extracts from plants, herbs and spices and some of their constituents, have shown antimicrobial activity against different food pathogens and spoilage microorganisms (Bakkali et al., 2008; Burt, 2004; Holley and Patel, 2005). Spices have been employed since ancient times as flavoring and preservative agents for food, but the research on the spice extracts has been initiated in the last decade for their compounds exerting antimicrobial and antioxidant activities (Sagdic et al., 2003). The clove, cinnamon and oregano are considered as the most common spices and herbs with strong antimicrobial activity. Their essential oils containing chemical compounds such as eugenol, cinnamaldehyde and carvacrol are identified as the major chemical components responsible for exerting antimicrobial activity (Wei and Shibamoto, 2010; El-Massry et al., 2008; Kordali et al., 2008; Zawirska-Wojtasiak and Wasowicz, 2009). Some studies reported that there is a highly positive linear relationship between antioxidant activity, antibacterial activity and total phenolic content in some spices and herbs (Shan et al., 2007, 2005).

Determination of shelf life with traditional microbiological tests is expensive and time-consuming. An alternative is the concept of predictive microbiology, which uses mathematical models to predict the bacterial growth as a function of environmental factors such as temperature, pH and a_w (Cayre et al., 2005; McMeekin et al., 1987). It allows us to quantify and to predict the rate of growth of microorganisms under environmental conditions with the intention of assuring the hygienic quality of food, thus determining its storage life. Mathematical models fulfill the research gap on the inactivation kinetics of natural antimicrobial extracts

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on microorganisms inoculated in food products. One of the more frequently used models is that of Gompertz with parameters such as lag phase duration (LPD), maximum population density (MPD), growth rate (μ) and the activation energy (E_μ).

The objective of the present work was to model the shelf life of raw chicken meat based on the microbiological analysis and to determine the effect of temperature on the kinetic parameters such as LPD, MPD, μ and E_μ.

2. Materials and methods

2.1. Materials

Dried spices of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum cassia*) and oregano (*Origanum vulgare*) were obtained from Nuts and Spices Super market, Chennai, India.

2.2. Chemicals and reagents

Butylated hydroxytoluene (BHT), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were supplied by Sigma-Aldrich Chemicals, Germany. Methanol, Plate Count Agar (PCA), Violet Red Bile Glucose (VRBG) agar, Buffered Peptone Water, de Man Rogosa and Sharpe (MRS) agar was purchased from Merck, Darmstadt, Germany.

2.3. Preparation of extracts

Spices were grounded using mixer grinder (Preethi ChefPro model, Indian make) and sieved well using vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau) for 20 min in order to obtain particles of same size. Extraction was performed in soxhlet extractor by contacting solvent and sample at a constant temperature, which could ensure solvent reflux (78–80 °C). Samples of 50 g were placed into a round-bottomed flask filled with 2500 mL ethanol (solid–solvent ratio of 1:50) connected at the top to a cooler and extraction was carried out for 5–6 h. The extracts were filtered through Whatman filter paper No. 1 (Whatman International, Ltd.,) and concentrated using a rotary evaporator. Finally spice extracts were dissolved in water in the ratio of 1:10 (w/v) for further studies.

2.4. Application of spice extracts in meat samples

Raw chicken breast meat (70.1 g/100 g moisture, 22.9 g/100 g protein, 2.1 g/100 g fat content) were purchased from local meat market (Chennai, Tamil Nadu, India). Meat samples were transferred through insulated polystyrene boxes to the laboratory within 1 h of production. Fresh meat samples were obtained separately for each of the replications. The meat samples were cut into pieces of 25 g, thickness 0.8 cm and treatment was performed as follows: 1. NC (negative control – without any additive), 2. PC (positive control with 0.02% BHA – Butylated hydroxyanisole), 3. T-SA (Treatment with 1% *S. aromaticum* extract), 4. T-CC (Treatment with 1% *C. cassia* extract), 5. T-OV (Treatment with 1% *O. vulgare* extract), 6. T-SA–CC (Treatment with 0.5% *S. aromaticum* extract + 0.5% *C. cassia* extract), 7. T-SA–OV (Treatment with 0.5% *S. aromaticum* extract + 0.5% *O. vulgare* extract), 8. T-CC–OV (Treatment with 0.5% *C. cassia* extract + 0.5% *O. vulgare* extract), 9. T-SA–CC–OV (Treatment with 0.33% *S. aromaticum* extract + 0.33% *C. cassia* extract + 0.33% *O. vulgare* extract). Meat samples were stored at 4, 10, 15 and 20 °C ± 1 and microbial counts, color values and TBARS (Thiobarbituric acid reactive substances) values were determined during the storage period. Samples stored at 4 °C were analyzed after 1, 2, 4, 6, 10, 15 and 20 days; those stored at 10 °C after, 1, 2, 4, 6 and 10 days; the ones at 15 °C after 1, 2, 4, and 6 days of storage and samples stored at 20 °C were analyzed after 1, 2 and 4 days. At these two last temperatures analyzes were carried out for fewer days because of the greater rate of decay of the meat. All the analyzes were performed in triplicate.

2.5. Microbial analysis

For the microbiological assays, a representative of 10 g meat sample was withdrawn and homogenized (Model PT-MR-2100, Kinematica AG, Switzerland) aseptically using 90 mL 0.1% peptone water and serial dilutions were made using 0.1% sterile peptone water. Total Viable Count (TVC) was determined on PCA agar by incubating plates at 37 °C for 24 h. Lactic Acid Bacteria (LAB) were counted on MRS Agar plates and incubated at 30 °C for 72 h. Total *Enterobacteriaceae* were counted on VRBG plates and incubated at 37 °C for 24 h. After incubation, plates having 25–250 colony-forming units (CFU) were counted and the results expressed in logarithmic of colony-forming units per gram of meat (log CFU/g).

2.6. Mathematical modeling of bacterial growth

Modified Gompertz equation was used to generate the bacterial growth curves by data fitting (Zwietering et al., 1991) and Eq. (1)

Table 1
Maximal growth rate (μ_{max}), lag phase duration (LPD) and maximum population density (MPD) obtained by the Gompertz equation of Total Viable Count (TVC), Lactic Acid Bacteria (LAB) and *Enterobacteriaceae* counts for raw chicken meat samples stored at 4 °C.

Sample	Microorganisms	μ_{max}	LPD	MPD	R^2
NC	Total viable count	0.659	7.33	8.96	0.96
	Lactic acid bacteria	0.470	6.81	7.95	0.97
	Enterobacteriaceae	0.609	7.18	7.15	0.95
PC	Total viable count	0.579	7.27	8.68	0.94
	Lactic acid bacteria	0.380	6.72	7.59	0.95
	Enterobacteriaceae	0.515	7.06	6.78	0.98
T-SA	Total viable count	0.306	7.02	7.61	0.97
	Lactic acid bacteria	0.239	6.51	6.95	0.98
	Enterobacteriaceae	0.465	6.99	6.57	0.95
T-CC	Total viable count	0.389	7.11	7.95	0.97
	Lactic acid bacteria	0.251	6.56	7.01	0.96
	Enterobacteriaceae	0.391	6.88	6.24	0.98
T-OV	Total viable count	0.364	7.09	7.85	0.99
	Lactic acid bacteria	0.242	6.54	6.97	0.93
	Enterobacteriaceae	0.454	6.98	6.52	0.95
T-SA–CC	Total viable count	0.283	7.00	7.49	0.98
	Lactic acid bacteria	0.192	6.47	6.72	0.95
	Enterobacteriaceae	0.413	6.91	6.34	0.97
T-SA–OV	Total viable count	0.352	7.07	7.81	0.94
	Lactic acid bacteria	0.206	6.49	6.79	0.96
	Enterobacteriaceae	0.400	6.89	6.28	0.97
T-CC–OV	Total viable count	0.390	7.11	7.96	0.98
	Lactic acid bacteria	0.192	6.47	6.75	0.97
	Enterobacteriaceae	0.360	6.82	6.09	0.98
T-SA–CC–OV	Total viable count	0.251	6.96	7.35	0.97
	Lactic acid bacteria	0.137	6.36	6.42	0.97
	Enterobacteriaceae	0.350	6.79	6.04	0.98

μ_{max}: (Δlog (CFU/g)/day); LPD: days; MPD: (log (CFU g^{-1})).
NC – negative control; no extract; PC – positive control with 0.02% BHT; T-SA – Treatment with *Syzygium aromaticum* extract (1% v/w); T-CC – Treatment with *Cinnamomum cassia* (1% v/w); T-OV – Treatment with *Origanum vulgare* extract (1% v/w); T-SA–CC – Treatment with *Syzygium aromaticum* (0.5% v/w) + *Cinnamomum cassia* (0.5% v/w); T-SA–OV – Treatment with *Syzygium aromaticum* (0.5% v/w) + *Origanum vulgare* (0.5% v/w); T-CC–OV – Treatment with *Cinnamomum cassia* (0.5% v/w) + *Origanum vulgare* (0.5% v/w) and T-W-SA + T-W-CC + T-W-OV – Treatment with *Syzygium aromaticum* (0.33% v/w) + *Cinnamomum cassia* (0.33% v/w) + *Origanum vulgare* (0.33% v/w).