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Anju Mariam Johnson
Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Akhila Etikala
Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Dr. Suresh Kumar Kalakandan
Professor and Head, Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Suman Thamburaj
Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Priya Subramanian Kalaimani
Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Rajendran Palanivel
Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Corresponding Author
Dr. Suresh Kumar Kalakandan
Professor and Head, Department of Food Biotechnology, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Tamil Nadu, India

Effect of chitosan edible coating with plant extracts on shelf life of chicken during refrigeration condition

Anju Mariam Johnson, Akhila Etikala, Dr. Suresh Kumar Kalakandan, Suman Thamburaj, Priya Subramanian Kalaimani and Rajendran Palanivel

Abstract
Chitosan is known for its functional packaging component used to maintain the quality and enhance the shelf life of perishable foods. This study was performed to investigate the effect of chitosan with *M. piperita* and *P. amboinicus* on physicochemical and microbial quality of chicken when stored at 4℃. The treatments were stored in the refrigeration condition and analysed for three-day interval. In microbial analysis, a significant lowering growth of bacteria was observed in chitosan with *M. piperita* and *P. amboinicus* (ECMO) when compared to control samples during 15 days at 4℃. Besides in increase in thiobarbituric acid reactive substances (TBARS), pH and exudate loss, the samples coated by ECMO was less than control samples. This finding suggests that chitosan with *M. piperita* and *P. amboinicus* can preserve the quality of chicken sample in refrigeration condition.

Keywords: ECMO, TBARS, exudate loss, physicochemical, microbial quality

1. Introduction
According to (FAO, 2020) [12] in total meat production, poultry meat contributes around 137 million tonnes. The increase in production is primarily due to consumer's efforts to substitute alternatives for meat products. Poultry meat has got a higher nutrient density, and a healthy source of protein with a high biological value (20-22%) and is also considered a valuable food due to its moderate energy content, B-group vitamins mainly thiamine, vitamin B6, and pantothenic acid, and minerals like iron, zinc, and copper (Barroeta, 2007; Marangoni *et al.*, 2015) [4, 19]. In terms of composition and nutritional value, chicken is similar to beef, pork, and lamb. Chicken protein is easily digestible, as it accounts for around one-fourth of the edible component. When compared to other meat substitutes, it contains all of the essential amino acids that humans need for good health (Demby & Cunningham, 1980) [10]. Due to the nutrient content of poultry meat, it has become a mass consumer commodity throughout the world (Magdelaine, Spiess, & Valceschini, 2008) [18].
The global consumption of chicken meat is continuously expanding. The developed countries that are the highest producers of chicken meat have the largest share of consumption. America has the highest share consumption followed by Asia and Europe (Belova, Smutka, & Rosochatecká, 2012) [6]. Despite having the highest consumption share, several Physico-chemical changes occur when it is stored for a long period such as lipid oxidation which is a source of meat and meat product quality deterioration because it causes color change, off-flavor, and nutrient loss, all of which are important determinants of meat quality and other factor is food-borne illnesses associated with poultry meat which have been reported throughout (Tan *et al.*, 2013) [28]. There are various food-borne pathogens associated with poultry products such as *Campylobacter*, *E. coli* and *Salmonella spp.* which causes illnesses such as diarrhoea, typhoid, haemorrhagic colitis and food poisoning (Akbar, Sitara, Khan, & Ali, 2014) [1]. The previous research used culture-based assays to assess individual pathogens and antimicrobial treatment to reduce pathogens on chicken carcasses (H. E. Kim, Lee, Lee, & Kim, 2019) [16].
Alternative method to control Physico-chemical and microbiological deterioration could be the utilization of edible coating and films. According to (Fang, Zhao, Warner, & Johnson, 2017) [11] reported that edible coatings in particular chitosan, carrageenan, sodium alginate and starch coatings were utilised to prevent microbiological proliferation and oxidative deterioration, thereby extending its shelf-life. In edible coatings and films, synthetic substances are added to increase the antioxidant and antimicrobial activity to prevent deterioration, although they have side effects as it can be toxic and carcinogenic.

Despite of using synthetic substances previous studies have shown that natural substances like essential oils from thyme, rosemary, basil, lemon leaf and cardamomum in coatings and films is a natural way to preserve and control meat and meat products from deterioration (Khorshidi, Mehdizadeh, & Ghorbani, 2020; Oussalah *et al.*, 2007; Sánchez-Ortega *et al.*, 2014; Valdés, Ramos, Beltrán, Jiménez, & Garrigós, 2017) [15, 22, 24, 30].

The medicinal plant extracts have positive effects as they have antioxidant, antibacterial, anti-inflammatory, and antimicrobial properties. The properties of the plant extracts are incorporated into films which can achieve as coating functions (Alexandre *et al.*, 2020; Han & Aristippos, 2005; S. J. Kim *et al.*, 2013) [2, 13, 17]. Aforementioned by (Alexandre *et al.*, 2020) [2] alginate-based coating with basil extract reduced the oxidative rancidity and increased antioxidant in meat. Medicinal plants such as *Mentha piperita* and *Plectranthus amboinicus* has compounds with pharmacological properties Therefore, the use of these extracts can be a natural additive to control pathogens and preservation of meat and meat products. (M. P. Singh & Singh, 2010.; R. Singh *et al.*, 2015.) [26, 25].

Therefore, this study shows the effects of a chitosan-based edible coating containing *M. piperita* and *P. amboinicus* on physicochemical and microbiological properties (pH, exudate loss, lipid oxidation and microbiological analysis) were investigated for 15 days under refrigeration condition.

2. Materials and Methods

2.1 Collection of meat

Fresh chicken breast fillets (500gm) were obtained from a local meat shop in Thanjavur, Tamil Nadu, India. Each chicken breast fillet weighing 20 grams was randomly used for experimental analysis.

2.2 Collection of leaves

The leaves of *P. amboinicus* and *M. piperita L.* were collected from the local market in Thanjavur, Tamil Nadu, India. Collected leaves was washed to remove the adhered external dusts. Subsequently, the leaves were dried in a hot air oven at 55℃ for 24 hours (Alexandre *et al.*, 2020) [2]. The dried sample was finely powdered and used for further analysis.

2.3 Preparation of leaf extracts

The finely pulverised leaf powders were soaked in 70% of ethanol (v/v) at the ratio of 1:10 and was subjected to maceration process for 120 rpm for 72 hours at 35℃. The extracts were segregated from filtrate through Whatman no.1 filter paper in which the filtrate is concentrated in a rotary evaporator at 55℃ under reduced pressure. The concentrated filtrate is dried and the amount of dried recovered crude extracts was stored at -20℃ which was used for further analysis (Biswas, Chatli, & Sahoo, 2012) [8].

2.4 Coatings and treatments

2.4.1 Preparation of coating solutions

Chitosan solution is prepared by dissolving 2% of chitosan in 100ml of 1% acetic acid and 1% of glycerol was added as a plasticizer to the solution. The chitosan solution was dissolved by using magnetic stirrer. Once the solution is clear, add each leaf extract to it. The fillets were dipped into the following treatment solutions for 5 minutes and was allowed to dry. The samples were packed and placed in refrigeration conditions at 4℃ (Zhang, He, Kang, & Li, 2018) [33].

2.4.2 Treatments

Samples were divided and distributed for five different treatments: CON (only meat without any coating), EC (meat with only chitosan coating), ECM (meat with chitosan solution and 1% of *M. piperita*), ECO (meat with chitosan solution and 1% of *P. amboinicus)* and ECOM (meat with chitosan solution and with 1% of *M. piperita* and *P. amboinicus*). The analysis was done for 1st, 3rd, 6th, 9th, 12th, and 15Th days respectively.

2.5 Physico-chemical analysis of samples

2.5.1 pH

For estimation of pH value, 10gm of sample was homogenized for 1 minute in 50ml of distilled water. Filter the homogenized solution with Whatman filter paper no.1. The filtrate was used further for determining the pH value. The pH values were obtained by using a standardized pH probe meter (Vargas, Albors, & Chiralt, 2011) [31].

2.5.2 Exudate loss

Every day of analysis, the meat samples were weighed, and the results were expressed as a percentage of weight loss relative to day 0 (Chaparro-Hernández *et al.*, 2019) [9].

2.5.3 Lipid oxidation

The lipid oxidation of the samples was determined by a modified method (Mohamed, Mansour, & Farag, 2011). 5 grams of the chicken sample with 15 mL of deionized distilled water were homogenized. One millilitre of meat homogenate was transferred to a test tube, and 50 L of butylated hydroxytoluene (7.2%) and 2 mL of thiobarbituric acid (TBA)–trichloroacetic acid (TCA) (15 mM TBA–15% TCA) were added. The liquid was vortexes and then incubated at 50℃ for 60 minutes in a hot water bath to develop color. The absorbance value was measured in UV spectrophotometer at 534nm. The TBARS value were calculated according to the equation:

TBARS value = absorbance value * 7.8 (conversion factor)
The amount of TBARS was measured as milligrams of MDA per kilogram of the sample.

2.5.4 Microbiological analysis

Chicken samples of 25gms were transferred into sterile bag with 225ml of sterile buffer peptone water (Hi-Media, Mumbai, India) and homogenised using stomacher blender (stomacher model-400, Seward, U.K). The homogenate was serially diluted to six-fold dilutions. For each dilution, 0.1 ml was plated on medium. The total plate count was performed on plate count agar and determined after 24hours of incubation at 37℃ (Radha Krishnan *et al.*, 2014) [23]. Microbiological data was converted into logarithms of the number of colony forming units (log cfu/g).

2.6 Statistical analysis

The results were represented in the form of Mean ± SD for pH, exudate loss and lipid oxidation. The microbiological count was converted into log CFU/g. The data were statistically analyzed using SPSS version 26.0 (IBM ltd. New York, USA) utilizing one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

3. Result and Discussion

3.1 Physico-chemical analysis of samples

3.1.1 pH: In table 1 depicts the influence of chitosan with leaf extracts on pH of chicken samples stored at refrigeration condition for 15 days. The pH of control samples increased inevitably from 5.89±.010 to 6.33± .032 at end of the storage. However, there were no much variations in pH values among the coated samples. When the control samples were compared to the pH value of treated samples, the treated samples were found to be within 5.72 to 6.14. In previous research outcomes the pH of coated chicken meat with natural compounds notably controlled the pH value in food systems especially in meat and meat products (Berizi, Hosseinzadeh, Shekarforoush, & Barbieri, 2018; Mehdizadeh & Mojaddar Langroodi, 2019; Vaithiyanathan, Naveena, Muthukumar, Girish, & Kondaiah, 2011) [7, 20, 29].The pH augmented in control samples during storage maybe due to the action of microbial or endogenous enzyme such as lipase and protease which can induce trim ethylamine and ammonia (Mehdizadeh & Mojaddar Langroodi, 2019) [20].

Table 1: pH of chicken sample under various treatments during refrigeration condition

Storage days						
Treatments	**Day 1**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
C	5.89±.010^{hijkl}	5.95±.015^{efghi}	6.03±.030de	6.13±.035^{c}	6.23± .030^{b}	6.33± .032^{a}
EC	5.77± .015^{nopq}	5.83± .015^{klmno}	5.89± .010^{hijkl}	5.96± .01^{efghi}	6.04± .045^{d}	6.14± .040^{c}
ECO	5.74± .010^{pq}	5.77± .011^{nopq}	5.81± .010^{lmnop}	5.85± .010^{ijklm}	5.92± .01^{fghij}	6± .100^{def}
ECM	5.74± .011^{pq}	5.79± .005^{opq}	5.80± .011^{lmnop}	5.88± .01^{klmn}	5.94± .01^{ghijk}	6.02± .020^{defg}
ECMO	5.72± .010^{q}	5.75± .015^{nopq}	5.79± .015^{mnopq}	5.86± .017^{jklmn}	5.91± .005^{hijk}	5.96± .015^{defgh}

Note: [a] C- Control
EC- chitosan coating
ECO- chitosan coating with *P. ambonicus*
ECM- chitosan coating with *M. piperita*
ECMO- chitosan coating with *M. piperita* and *P. ambonicus*

3.1.2 Exudate loss: The chitosan edible coating with leaf extracts decreased the weight loss in the meat samples from day 1 to day 15 presented in table 2. The initial values for exudate loss on day 1 ranges from 2.23% to 1.10% (C, EC, ECO, ECM and ECMO). At the end of the storage period, there was an increase in exudate loss majorly in control samples 9%, whereas the exudate loss percentage were highly controlled in ECMO treated samples from day 1 to day 15 ranging about 1.1% to 5.13%. The presence of little exudate from the sample can be unappealing to the customers (Vital *et al.*, 2016). During the storage period, the chitosan coated samples subsided the weight loss in chicken samples. As a result, chitosan-based plant extracts may control the weight loss in chicken.

Table 2: Exudate loss of chicken sample under various treatments during refrigeration condition

Storage days						
Treatments	**Day 1**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
C	2.23± .028^{op}	3.41± .028^{k}	5.31± .251^{fg}	7.28± .028^{c}	8.1± .050^{b}	9.43± .057^{a}
EC	1.63 ± .028^{q}	2.21± .028^{op}	3.33± .057^{kl}	4.36± .028^{i}	5.3± .050^{fg}	6± .050^{d}
ECO	1.76± .028^{q}	2.05± .050^{p}	2.75± .010^{o}	3.95± .100^{lm}	4.16± .125^{h}	5.73± .076^{f}
ECM	1.60± .076^{q}	2.05± .028^{p}	2.46± .057^{n}	3.10± .050^{j}	4.65± .028^{ij}	5.43± .076^{e}
ECMO	1.10± .050^{r}	1.58± .028^{q}	2.13± .076^{p}	2.91± .076^{mn}	4.26± .152^{i}	5.13± .125^{g}

Note: [a] C- Control
EC- chitosan coating
ECO- chitosan coating with *P. ambonicus*
ECM- chitosan coating with *M. piperita*
ECMO- chitosan coating with *M. piperita* and *P. ambonicus*

3.1.3 Lipid oxidation

The lipid oxidation is an important parameter that determines the quality of the meat products. During the storage period, the TBARS levels of treated samples were substantially lower than those of untreated samples in table 3. On day 1, the TBARS values were significantly different among the C, EC, ECO and ECM. Whereas, on the 15th day, TBARS value of control sample and EC sample alone increased reaching to 2.28 mg and 1.28 mg of MDA/kg. There was an intermediate decrease in lipid oxidation in ECO and ECM. The ECMO had decreased the levels of TBARS values. At the end of the storage period, the TBARS values of C, EC, ECO, ECM and ECMO were nearly to 2.28, 2.02, 1.81, 1.31 and 1.28 mg MDA/kg of chicken. Oxidation, as well as the development of microbes, causes degradation in food quality, loss of quality during exhibition, and is also linked to customer rejection (Johnson & Decker, 2015) [14]. During the shelf-life process, it is important to maintain the oxidative stability among the products hence antioxidant substances are prime compounds (Alexandre *et al.*, 2021) [3]. As a result, this study revealed that the application of *P. ambonicus* and *M. piperita* into chitosan was beneficial in slowing the oxidation process in chicken over 15 days of storage period.

Table 3: Lipid oxidation of chicken sample under various treatments during refrigeration condition

Storage days						
Treatments	**Day 1**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
C	.751±.004^{k}	.951±.007^{i}	1.25±.007^{g}	1.48±.011^{e}	1.79±.007^{c}	2.28±.011^{a}
EC	.582±.004^{m}	.656±.022^{l}	8.34±.020^{j}	1.35±.016^{f}	1.67±.007^{d}	2.02±.007^{b}

ECO	.361±.004^{n}	.561±.007^{m}	.748±.007^{k}	1.07±.025^{h}	1.52±.007^{e}	1.81±.011^{c}
ECM	.283±.003^{o}	.561±.007^{m}	.795±.007^{jk}	.994±.003^{i}	1.13±.003^{h}	1.34±.003^{f}
ECMO	.089±.003^{p}	.237±.003^{o}	.542±.003^{m}	.826±.007^{j}	.933±.083^{i}	1.28±.031^{g}

Note: [a] C- Control
EC- chitosan coating
ECO- chitosan coating with *P. ambonicus*
ECM- chitosan coating with *M. piperita*
ECMO- chitosan coating with *M. piperita* and *P. ambonicus*

3.2 Microbial analysis of samples

The changes in total bacterial count of chicken samples were shown in figure 1. Initially, the TPC value of control sample was 3.39 log cfu/g and substantially it increased to 7.49 log cfu/g during 15th day of the storage period. In contrast, lower TPC value were recorded in the range of 3.39 log cfu/g to 4.49 log cfu/g when chitosan with *M. piperita* and *P. ambonicus* was applied to the sample. Similar study was reported in (Bazargani-Gilani, Aliakbarlu, & Tajik, 2015) [5] where chitosan enriched with pomegranate juice and *Zataria multiflora* boiss essential oil effectively controlled foodborne pathogens throughout 15 days of storage. The standard acceptability limit for fresh meat is 7 log cfu/g, the coated samples were discerned to be within the limits whereas the control samples was above the limit at the end of storage period which was unacceptable (Taheri, Fazlara, Roomiani, & Taheri, 2018) [27].

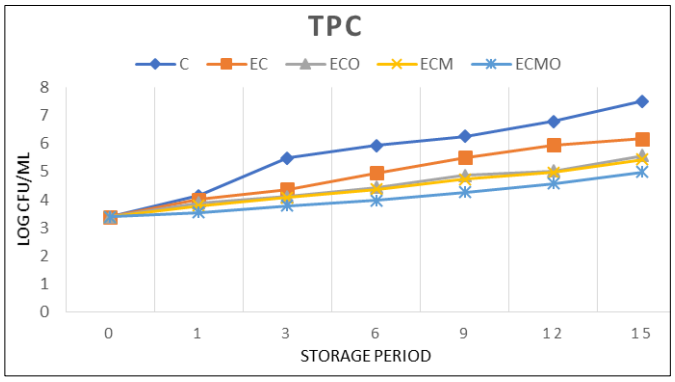

Fig 1: The TPC value of chicken during refrigeration condition

4. Conclusion

The present study showed that chitosan with *P. ambonicus* and *M. piperita* could effectively control the microbial load and physicochemical deterioration during 15 days of storage period of chicken. The shelf life of uncoated samples was maximum up to 3 days whereas coated samples could preserve for 12 to15 days. The samples treated with chitosan, chitosan with *M. piperita*, chitosan with *P. ambonicus* and chitosan with *P. ambonicus* and *M. piperita* restrained from deterioration. However, when compared to treated samples, the chitosan with *P. ambonicus* and *M. piperita* exhibited that it effectively lowered and controlled the pH, exudate loss, lipid oxidation and microbial load in chicken throughout the storage period. Therefore, chitosan with *P. ambonicus* and *M. piperita* extended the shelf life of chicken during refrigeration condition and it can be used as an alternative for synthetic substances.

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