Global Veterinaria 21 (3): 156-160, 2019
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2019.156.160

Effect of Lemon Fruits and Turmeric Extracts on Fungal Pathogens in Refrigerated Chicken Fillet Meat

[1]F.A. Shaltout, [2]R.M. Salem, [2]E.M. El-Diasty and [3]W.I.M. Hassan

[1]Department of Food Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt
[2]Department of Mycology, Animal Health Research Institute Dokki, Giza (ARC), Egypt
[3]Free Veterinary Doctor

Abstract: Debates about the possible negative effects of synthetic preservatives have renewed consumer interest towards natural agents for extension of the product viability and the protection from microbial spoilage. For these reasons, there are current studies on the application of plant extracts and combination with each other as alternative biopreservatives. These compounds may be useful in limiting or preventing the development of harmful fungi in food, as additives, as surface protection. The subject of this study is the use of turmeric extracts, Lemon fruit, Lemon peel and lemon juice in controlling the growth of food-borne fungi and their biological effects. While, results of the present study showed lemon fruits extract; turmeric +lemon peel+ lemon juice and turmeric+ lemon fruit extract led to reduction (%) of various treated chicken fillet meat at a rate 98.9 %, 99.7 % and 99.8 %, respectively. The information given by the achieved results proved that treatment of chicken fillet meat by addition of fruits extract; turmeric +lemon peel+ lemon juice and turmeric + lemon fruit extract inhibited the mould growth and extends the shelf-life of refrigerated treated chicken fillet meat. It can be concluded that these plant extracts have the potential to be used in food as flavoring and natural preservatives to control food spoilage.

Key words: Turmeric · Lemon · Refrigerated Chicken Fillet Meat

INTRODUCTION

Chicken meat is favoured by consumers around the world because of its desirable nutritional qualities, such as a low fat content and a relatively high concentration of polyunsaturated fatty acids. Fresh meat products are usually marketed at refrigerated temperatures (2–5°C). Lipid oxidation and microbial growth may occur during refrigeration storage. Spoilage of fresh poultry meat is a financial burden to producers and requires the development of new methods to extend the shelf-life and overall safety/quality of the meat, which is the main problem faced by the poultry processing industry [1].

The inadequate and indiscriminate use of synthetic antimicrobials is leading to the selection of multi-resistant strains, the antimicrobial potential of plant extracts and essential oils are intended to delay this process through the emergence of new antimicrobial substances [2]. In addition to the antimicrobial potential, the fact that some synthetic antioxidants widely used in the food industry can lead to the development of tumour cells has led to increase the demand for similar products of natural origin, among which essential oils and plant extracts that have phenolic compounds in their composition stand out for their important antioxidant activity [3].

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [4].

The turmeric is used as a spice and medicine. The active ingredients found in turmeric are curcumin, demethoxycurcumin, bisdemethoxycurcumin [5] and tetrahydrocurcuminoids [6]. Plant extracts were found to have antifungal [5] and antioxidant value [6].

Corresponding Author: Eman El-Diasty, Department of Mycology,
Animal Health Research Institute Dokki, Giza (ARC), Egypt.

Citrus species also contain a group of flavonoids including polymethoxy flavones, flavone glycosides and limonoids which enhance antimicrobial activity. A recent study focused on the composition of citrus jambhiri and researchers isolated seven different kinds of polymethoxy flavones and showed that lemon in was the most abundant natural product isolated from the plant [7].

Therefore, this investigation was planned for is to investigate the potency of effect of turmeric extract, lemon juice, lemon fruit extract and lemon peel extract on fungal contamination of fresh refrigerated chicken fillet meat.

MATERIALS AND METHODS

Raw Materials: Turmeric powder (*Curcuma longa)* which used in this study was obtained from Harraz Company, while lemon fruits from supermarkets.

Preparation of extraction of Lemon juice according to Abdul-Husin *et al.* [8].

Preparation of lemon peels extract according to Gupta *et al.* [9].

Preparation of lemon fruits extract according to Valtierra-Rodriguez *et al.* [10].

Preparation of turmeric (*Curcuma longa*) extract according to Weerasekera *et al.* [11].

Preparation of spore suspension of fungal culture *(A. flavus)*:

The present study carried out by using a strain of *Aspergillus flavus* (GenBank accession number: KP137699) isolated previously at the Mycology Department - Animal Health Research Institute, Giza, Egypt. The *A. flavus* subcultured and grown for 7 days on Czapek yeast extract. The *A. flavus* culture was washed with 10 ml sterile distilled water in 2% Tween 80 with the aid of glass beads to help in the spore dispersion. The spore suspensions were standardized by calculated using haemocytometer with dilution to reach to$1.1x10^6$ spores/ml.

Preparation of Food Model: Raw fresh chicken breast fillets (Boneless) were purchased from a local poultry shop. The breast fillets were first washed and rinsed with sterial distilled water. The fillets then cut into pieces of approximately (5 cm x 10 cm size) using sterile knife. The pieces were kept in sterile open petri dishes and exposed to Ultra Violet rays (At 254 nm) for 30 minutes each side. The samples were divided into seven groups, each group weighted 200 g., then each group subdivided into two smaller portions (100g, for each).

Marinated Preparation: The first and second portion in six group were dipped in 5 ml of Lemon juice, lemon peels extract, lemon fruits extract, turmeric (*Curcuma longa*) extract, mixture of turmeric extract + lemon peel extract + Lemon juice with equal volumes and final group mixture of turmeric extract + lemon fruits extract with equal volumes and left for 30 minutes at room temperature.

Samples Inoculation: The first portion of each group for organoleptic evaluation. The second portion of each group was dipped in the prepared *A. flavus* (Count of about $1.1x10^6$). The incubated for 30 minutes at 25°C, then kept for another 30 minutes at room temperature to enhance the fungal spore attachment. All samples were stored at 4°C for 9 days and fungal counts were done every 3 days. The seven group are divided into two portion considered as control without any treatment and second portion was dipped only in the prepared *A. flavus* (Count of about $1.1x10^6$).

Sensorial Analyses: Sensorial analysis of marinated chicken fillet meat were carried out according to general sensory analysis guidance, given in SRPS ISO 6658 [12].

Determination of Total Mould Count: According to the technique recommended by ISO (ISO-21527-1) [13].

RSULTS AND DISCUSSION

Odour and flavour are important aspects of meat quality, sometime used as determining criteria in the acceptance or rejection of the products [14] while Miller [15] stated that texture or feel and colour of muscle food influence perceptions of acceptability and are the most reliable, rapid indicators of their quality.

In the present study chicken fillet meat was preserved using natural product in comparison to preservation in various combinations of turmeric extracts, lemon peel extract, lemon juice, lemon fruit extract, turmeric+ lemon peel+ lemon juice and turmeric + lemon fruit extract. The treated chicken fillet meat was stored at 4°C±1 temperature for a total period of 9 days to investigate the effect of various extracts on the shelf life extension.

In organoleptic examination the test revealed that, (As shown in Table 1 and 2) the colour, odour and texture of the control sample changed at third day, while the colour, odour and texture of chicken fillet treatments not changed in case treatments of turmeric + lemon peel+ lemon juice and turmeric + lemon fruit extract at ninth day and high scores organoleptically from the panel of judges at day 9 day. While the colour, odour and texture changed

Table 1: Organoleptic evaluation of examined samples using single type preservative

Preservation time	Control			Turmeric extract			Lemon peel extract			Lemon juice		
	Colour	Odour	Texture	Colour	Odour	Texture	Colour	Odour	Texture	Colour	Odour	Texture
0 day	4	4	4	4	4	4	4	4	4	4	4	4
3rd day	2	1	2	3	3	3	3	2	3	3	3	3
6th day		Spoiled		2	2	3	2	2	3	2	2	3
9th day		spoiled		1	1	2	1	1	2	2	1	2

Excellent =4 Very good =3 Good =2 Bad =1

Table 2: Organoleptic evaluation of chicken fillet meat samples treated with mixed type Turmeric and lemon

Preservation time	Control			Lemon fruit extract			Turmeric +Lemon peel+ lemon juice			Turmeric + Lemon fruit extract		
	Colour	Odour	Texture	Colour	Odour	Texture	Colour	Odour	Texture	Colour	Odour	Texture
0 day	4	4	4	4	4	4	4	4	4	4	4	4
3rd day	2	1	2	3	3	3	4	3	4	4	4	4
6th day		Spoiled		3	3	3	4	3	4	4	4	4
9th day		spoiled		3	3	3	4	3	4	4	3	4

Excellent =4 Very good =3 Good =2 Bad =1

in a treatment of turmeric extract, lemon peel extract and lemon juice after sixty and ninth days of preservation, these samples could not retain their quality due to the undesirable odour.

Gul and Bakht [16] reported that treated meal prepared from boneless chicken meat was preserved using natural product from turmeric extracts in comparison to chemical preservation in various combinations. The treated meal prepared from boneless chicken meat was stored at ambient temperature for a total period of 90 days to investigate the effect of turmeric extract/oil on the shelf life extension. From our results, it is clear that samples treated with turmeric extract alone, received comparatively high scores organoleptically from the panel of judges at day zero, however, these samples could not retain their quality due to the undesirable odour and filling of gas inside the pouches.

Meat colour is the first condition that consumer use to judge meat quality and acceptability. Anwer *et al.* [17] stated that colour is an important factor in selection of meat products. Meat colour is one of the most important for consumers are indication of an originality and uprightness. Consumers will often refuse products in which the colour varies from the predictable appearance. Therefore, colour is frequently used to determine economic value of food.

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life. Because food products are now often sold in areas of the world far distant from their production sites, the need for extended safe shelf-life for these products has also expanded. The development of food preservation processes has been driven by the need to extend the shelf-life of foods. Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Several food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning; however, these techniques frequently have associated adverse changes in organoleptic characteristics and loss of nutrients. Firstly, consumers require more high quality, preservative-free, safe but mildly processed foods with extended shelf-life. Many of these ready-to-eat and novel food types represent new food systems with respect to health risks and spoilage association. Against this background and relying on improved understanding and knowledge of the complexity of microbial interactions, recent approaches are increasingly directed towards possibilities offered by biological preservation [18].

The results obtained from Table (3) showed that lemon fruits extract; turmeric +lemon peel+ lemon juice and turmeric+ lemon fruit extract could reduce mould count from $1.1x10^6$ to $1.2x10^4$, $3.8x10^3$ and $2.6x10^3$ cfu/g at 9 days, respectively. While, results of the present study showed that turmeric extract, lemon peel extract and lemon juice extracts could reduce mould count from $1.1x10^6$ to $9.4x10^4$, $3.7x10^5$ and $1.3x10^5$, respectively.

Regarding the results tabulated in Table (4) showed that reduction % at various treated chicken fillet meat of lemon fruits extract; turmeric +lemon peel+ lemon juice and turmeric+ lemon fruit extract were 98.9 %, 99.7 % and 99.8 %, respectively. While, turmeric extract; lemon peel extract and lemon juice the reduction % 91.5 %, 66 % and 87.5 %, respectively.

Table 3: Antifungal activity of different treated chicken fillet meat samples during stored at 4 ± 1°C

Treatment	0 day	3rd day	6th day	9th day
Turmeric extract	$1.1x10^6$	$4.2x10^5$	$9.5x10^4$	$9.4x10^4$
Lemon peel extract	$1.1x10^6$	$4.3x10^5$	$4x10^5$	$3.7x10^5$
Lemon juice	$1.1x10^6$	$1.6x10^5$	$1.4x10^5$	$1.3x10^5$
Lemon fruits extract	$1.1x10^6$	$1.6x10^4$	$1.3x10^4$	$1.2x10^4$
Turmeric +Lemon peel+ lemon juice.	$1.1x10^6$	$9.5x10^3$	$5.9x10^3$	$3.8x10^3$
Turmeric+ Lemon fruit extract	$1.1x10^6$	$8.4x10^3$	$3.1x10^3$	$2.6x10^3$

Table 4: Reduction % of total mould (*A. flavus*) count in treated chicken fillet meat samples during stored at 4 ± 1°C

	3rd day	6th day	9th day
Treatment	Reduction (%)	Reduction (%)	Reduction (%)
Turmeric extract	62	91.4	91.5
Lemon peel extract	61	63.6	66
Lemon juice	85.2	87.5	87.5
Lemon fruits extract	98.5	98.9	98.9
Turmeric +Lemon peel+ lemon juice.	99.1	99.5	99.7
Turmeric+ Lemon fruit extract	99.3	99.7	99.8

Upendra *et al.* [19] mentioned that the addition of turmeric powder in plant tissue culture showed that turmeric at the 0.8 and 1.0 g/l had appreciable inhibitory activity against fungal contaminations. Jing *et al.* [20] mentioned that Citrus essential oils (CEOs) are a mixture of volatile compounds consisting mainly of monoterpene hydrocarbons and are widely used in the food. Also mentioned that CEOs showed that *Aspergillus flavus* failure of growth at 250 µg/ml and 500µg/ml.

Ali *et al*. [21] studied the antibacterial and antifungal activity of the peel of the dried fruit of Citrus lemon was judged by using disk diffusion method. He found that the minimum inhibitory concentration of the plant extracts was less than 100 µg/ml and therefore the plant was considered to have a very good antimicrobial activity. Chen *et al*. [22] reported that the inhibition behaviour of *C. longa* (turmeric) on fungal growth (*Aspergillus flavus*) is involved in its ability to disrupt the integrity of plasma membrane and mitochondrial dysfunction, inducing metabolic stagnation. Curcumin and â-elemene have been reported to exert significant antifungal activity. The antifungal effect included fungal cell membrane disruption and inhibition of ergosterol synthesis, respiration, succinate dehydrogenase (SDH) and NADH oxidase.

Velázquez-Nuñez *et al.* [23] recorded that main compounds identified in the lemon peel EO were: limonene, β-myrcene, β-pinene, α-pinene, as well as citral Z and E; of which, limonene represented 96.62%. The minimum inhibitory concentration for the growth of *A.flavus* by direct addition was 16, 000 mg^{-1}, while for the vapour contact was 8000 mg of EO l-1 of air. For both studied methods *A. flavus* growth decreased when increasing EO concentration. Although the effect of peel EO direct addition was faster, peel EO vapors were more effective, since lower concentrations were required to achieve the same antifungal effect.

Mathur *et al*. [24] showed that the queous extracts from citrus fruit pulp had antimicrobial activity with significantly ratio. The microbicidal acids of the citrus juices are substances active-membrane which destroyed inner membrane in their unrelated form. They change the permeability of the microbial cell membrane and acidify the cytoplasm [25].

The information given by the achieved results proved that treatment of chicken fillet meat by addition of fruits extract; Turmeric +lemon peel+ lemon juice and turmeric + lemon fruit extract inhibited the mould growth and extends the shelf-life of refrigerated treated chicken fillet meat. It can be concluded that these plant extracts have the potential to be used in food as flavoring and natural preservatives to control food spoilage.

REFERENCES

1. Zhang, L., Z. Yang, J. Wei, P. Su, W. Pan, X. Zheng, K. Zhang, L. Lin, J. Tang and Y. Fang, 2017. Essential oil composition and bioactivity variation in wild-growing populations of *Curcuma phaeocaulis* Valeton collected from China. Ind. Crops Prod., 103: 274-282.
2. Weber, L.D., F.G.S. Pinto, M.C. Scur, J.G.L. Souza, W.F. Costa and C.W. Leite, 2014. Chemical composition and antimicrobial and antioxidant activity of essential oil and various plant extracts from *Prunus myrtifolia*. African Journal of Agricultural Research, 9(9): 846-853.

3. Sacchetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 91(4): 621-632.
4. Hammer, K.A., C.F. Carson and T.V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., 86: 985-990.
5. Wuthi-udomler, M., W. Grisanapan, O. Luanratana and W. Caichompoo, 2000. Anti-fungal activities of Turmeric as anti-aflatoxicosis plant extracts. Southeast Asian J. Trop Med. Public Health, 31: 178-82.
6. Osawa, T., Y. Sugiyama, M. Inayoshi and S. Kawakisi, 1995. Antioxidative activity of tetrahydrocurcuminoids. Biotechnol. Appl. Biochem., 59: 1609-1612.
7. Aibinu, I., T. Adenipekun, T. Adelowotan, T. Ogunsanya and T. Odugbemi, 2007. Evaluation of the antimicrobial properties of different parts of *citrus aurantifolia* (lime fruit) as used locally. Afr. J. Trad. CAM, 4(2): 185-190.
8. Abdul-Husin, I.F., S. Al-Musawi, A.N. Hindi and S. Abudl-Mahdi, 2018. Aqueous lemon extracts as antimicrobial agent against some pathogenic bacteria. Plant Archives, 18(1): 431-434.
9. Gupta, S., C. Gupta, D. Prakash and A.P. Garg, 2017. Comparative Study of Antimicrobial Effects of Lemon Oil and Peel Extract against Food-Spoilage Microbes. Journal of Nutritional Health & Food Science, 5(6): 1-5.
10. Valtierra-Rodriguez, D., N.L. Heredia, S. Garcia and E. Sanchez, 2010. Reduction of *Campylobacter jejune* and *Campylobacter coli* in poultry skin by fruit extract. J. Food Protection, 73: 477-482.
11. Weerasekera, D., N. Fernando, L.B.A.E. Bogahawatta, R. Rajapakse-Mallikahewa and D.J. Naulla, 2008. Bacterial effect of selected spices, medicinal plants and tea on *Helicobacter pylori* strains from Sri Lanka. J. Natl. Sci. Foundation Sri Lanka, 36: 91-94.
12. SRPS ISO 6658, 2002. Quantitative descriptive test, Sensory analysis, methodology, General Instructions., ISO 6658/2002; SRPS Sensory Analysis.
13. ISO (21527-1:2008) "International Standards Organization: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and mould. Part 1: Colony count technique in products with water activity greater than 0.95.
14. Mottram, D., 1994. Meat flavour, in: Understanding Natural Flavours, J. R. Piggott, A. Paterson, eds., Blackie Academic & Professional, New York, pp: 141-163.
15. Miller, R.K., 1994. Quality characteristics. In: Kinsman, K.M.: Kotula, A.W. and Breidenstein, B.C. (Eds.). Muscle Foods. Chapman & Hall New York. London.
16. Gul, P. and J. Bakht, 2015. Antimicrobial activity of turmeric extract and its potential use in food industry. J. Food Sci. Technol., 52(4): 2272-2279.
17. Anwer, M., M.I. Khan, I. Pasha, M.R. Tariq and M. Sohaib, 2013. Quality assessment of meat in relation to colour and muscle fiber. Pak. J. Food Sci., 23(2): 80-86.
18. Rasooli, I., 2007. Food Preservation - A Bio-preservative Approach. Food Global Science Books, 1(2): 111-136.
19. Upendra, R.S., P. Khandelwal and A.H.M. Reddy, 2011. Turmeric powder (*Curcuma longa* Linn.) as an antifungal agent in plant tissue culture studies. International Journal of Engineering Science, 3(11): 7899-7904.
20. Jing, L., Z. Lei, L. Li, R. Xie, W. Xi, Y. Guan, L.W. Sumner and Z. Zhou, 2014. Antifungal Activity of Citrus Essential Oils. J. Agric. Food Chem., 62(14): 3011-3033.
21. Ali, J., B. Das and T. Saikia, 2017. Antimicrobial activity of lemon peel (*Citrus limon*) extract. International Journal of Current Pharmaceutical Research, 9(4): 79-82.
22. Chen, C., L. Long, F. Zhang, Q. Chen, C. Chen, X. Yu, Q. Liu, J. Bao and Z. Long, 2018. Antifungal activity, main active components and mechanism of *Curcuma longa* extract against *Fusarium graminearum*. PLOS ONE https://doi.org/ 10.1371/ Journal. pone.0194284
23. Velázquez-Nuñez, M.J., R. Avila-Sosa, E. Palou and A. López-Malo, 2013. Antifungal activity of *Citrus sinensis* var. Valencia peels essential oil applied by direct addition or vapor contact. Food Control, 31(1): 1-4.
24. Mathur, A., S.K. Verma, R. Purohit, V. Gupta, V.K. Dua, D. Mathur, S.K. Singh and S. Singh, 2011. Evaluation of *in vitro* antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. IJPI'S J of Biotechnology and Biotherapeutics, 1(2): 1-17.
25. Puupponen-Pimia, R., L. Nohynek, H.L. Alakomi and K.M. Oksman-Caldentey, 2004. Bioactive berry compounds novel tools against human pathogens. App. Microbiol. Biotechnol., 67: 8-18.