ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 14, Issue, 04, pp. 12470-12472, April, 2023

RESEARCH ARTICLE

INHIBITORY EFFECTS OF GRAPES, ORANGE, LEMON AND MANGO PEEL HYDROSOLS IN CONTRADICTION OF FOOD-BORNE PATHOGENS AND SPOILAGE BACTERIA IN VITRO AND ON FISH SKIN

***Dr. Peer Mohamed, S.**

Assistant professor, Department of Zoology, Sadakathulla Appa Collage, Tirunelveli-11, Tamilnadu, India

ARTICLE INFO

Article History:
Received 27th February, 2023
Received in revised form
22nd March, 2023
Accepted 30th March, 2023
Published online 27th April, 2023

Keywords:
Hydrosols, Peel extract, Antimicrobial activity, Foodborne pathogens, Spoilage bacterium, Fish skin.

ABSTRACT

In this study, improvement in antibacterial properties of grapes (*Citrus paradisi* Macfad), orange (Citrus siensis) and lemon (Limona taris), Mango (*Mangifera indica* L.) peel after the Hydrosols procedure was investigated. The antibacterial properties against common food-borne pathogens (Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia and Salmonella Paratyphi A) as well as 6 fishs poilage bacteria isolated from 4 spoiled fish species: mackerel, sardine, anchovy and rainbow trout, have been evaluated using paper disc diffusion, MIC and MBC methods. The grapes Orange, Lemon and Mango fruits peel Hydrosols showed bacteriostatic properties against the majority of tested bacterial strains, however, only the growth of Salmonella parathypi A, Vibrio vulnificus and Seratialique facies was inhibited by the essential oil in the concentration upto 25 mg/ml. The antimicrobial activity of all extracts, except the pomegranate ethanol extract, were dependent on the concentration of extract that the bacteria were exposed to during the trials. The antimicrobial activity of peel extracts of Pomegranate, Orange and Lemon indicates that these extracts maybe used as sanitizers to reduce microbial contamination of some foods and processing.

Citation: ***Dr. Peer Mohamed, S.*** **2023.** "Inhibitory effects of grapes, orange, lemon and mango peel hydrosols in contradiction of food-borne pathogens and spoilage bacteria in vitro and on fish skin", *Asian Journal of Science and Technology*, 14, (04), 12470-12472.

INTRODUCTION

Contaminated food products, with microbiological contamination being the main cause, are responsible for over 600 million disease incidences and 4,20 000 death every year (WHO, 2020). Moreover, microbiological decay is one of the main causes for the generation of food waste (Saucier, 2016). Therefore, one of the main aims of the current food technology is to find new and active means to inhibit and eradicate the microbiological contamination. Although many different compounds with antimicrobial properties have been exposed, recently there has been rising interest in the use of natural food stabilizers with antimicrobial properties. Such natural antimicrobials are often measured safer than their synthetic counterparts, which are more widely acknowledged by consumers and may demonstrate effective against pathogens which have advanced resistance to various synthetic antibiotics (Pisoschi *et al*., 2018). The prevalence of foodborne, bacterial illnesses has led to recommendations for the use of natural antimicrobial substances (e.g., bacteriocins) in combination with novel technologies for controlling microbial contamination of foods, thereby improving both food quality and food safety (Galvez *et al*., 2010).

The use of natural antimicrobial compounds in food has gained much attention by consumers and the food industry extracts of plants and fruits have been documented to process antibacterial, antifungal and anti-viral activities although there or reports on the antibacterial activity of fields of grapes, orange, lemon and mango peels using the disc diffusion method, there is little information of the growth kinetics of bacteria exposed to these plant extracts. Furthermore, little electron microscopy has been conducted to examine the form of plant extracts against bacteria or treated food surfaces stop the incorporation of these plant extra into foot packaging films challenge for food industry

MATERIALS AND METHODS

Collection and Authentication of Fruit: Fresh fruits of grapes, Orange, Lemon, Mango with no bruises were purchased from a local market. Fruit surfaces were thoroughly washed with distilled water and then dried with disposable paper towels. Fruit skin was peeled from the three fruits using a peeler and peels were collected in separate containers. Peels were freeze-dried for 48 h and a food grinder was used to pulverize the dried skins. Twenty grams each of powdered sample where Plants were collected from local markets. peels of fruits were

peeled manually with a knife and dried with sunlight under sterile laboratory conditions. On an average around 200 g of peel was obtained from every 1 Kg of fruits.

Preparation of Hydrosols: The Grapes, Orange, Lemon and Mango peel oil were separated by hydro distillation. After hydrosols were put into sterile glass jars. The hydrosols were allowed to cool at room temperature and then kept in the refrigerator until investigation Studies.

Tested Bacteria: The isolation and identification of fish spoilage bacteria (*P. damselae, E. faecalis, V. vulnificus, P. mirabilis, S. liquefaciens, P. luteola*) was performed in the same manner as described by Yazgan, Ozogul, and Kuley (2019). The bacteria were isolated from three spoiled fish species caught in the Mediterranean Sea: mackerel (*Scombers combrus*), anchovy (*Engraulis enrasicolus*) and sardine (*Sardinella autira*) and from rainbow trout Oncorhynchus mykiss) obtained from a local farm *S. pneumonia* (ATCC700603), *S. aureus* (ATCC29213) and *E. faecalis* (ATCC29212) were obtained from the Indian Type Culture Collection, while Salmonella Paratyphi A (NCTC13) was obtained from the National Collection of Type Cultures.

Disc Diffusion Method : The antimicrobial activity of four hydrosols was determined using the disc diffusion method of Murray *et al.* (1995). Nutrient agar was employed as the standard test medium for bacterial growth. The agar plate was spread with the inoculum having 108 cfu/ml pathogenic bacteria. Fifty microliters of undiluted hydrosols were pipetted on sterile filter paper discs (diameter 6 mm), which were permitted to dry in an open sterile petri dish in a biological safety cabinet with vertical laminar flow. Paper discs were set on the inoculated agar surfaces. After incubation at 37 °C for 18-24 h, the diameters (mm) of the zones of bacterial inhibition were determined. Each test was carried out in triplicate and the results were assessed for statistical significance. Vancomycin and Tetracycline antibiotics with positive responses were utilized as the control of Gram-positive and Gram-negative bacteria.

Determination of minimum inhibitory and bactericidal concentrations: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of hydrosols against bacteria were determined according to the Clinical and Laboratory Standards Institute's methods (2008). One milliliter of plant hydrosols (with a stock solution of 50 mg/ml) was added to the first tube in each series and subsequently, two-fold serially diluted with Mueller Hinton Broth (MHB). The inoculum suspension (1 ml) of each bacterial strain (106 cfu/ml) was then added in each tube containing hydrosols and MHB. The final concentrations of the extract were 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19 mg/mL. The tubes were incubated at 35°C for 18–24 hours after which the MIC was recorded. MBC was determined by subculturing the contents of tubes of MIC showing no growth.

RESULT DISCUSSION

The antibacterial properties of grapes, Orange, Lemon, Mango fruit peel hydrosols were analyzed using 3 analyses: the paper disc diffusion method, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). All microbiological analyses were performed on pure fruit peel hydrosols (100%) and their emulsions which contained 10%. The disc diffusion method was performed as described by Yazgan *et al.* (2019). Three positive (30 µg tetracycline, 10 µg streptomycin and 5 µg neomycin) and one negative (Tween 80) controls were used. The nutrient agar was used as a standard test medium. The amount of both grapefruit peel hydrosols used in the assay was 50 µL. The incubation of bacterial plates was carried out for 18–24 h at 37°C. The results were calculated as an average of four measurements. The MIC and MBC analyses were performed according to the the initial stock solution was adjusted to contain 50 mg/mL of fruit peel hydrosols, while the inoculum suspension used contained 6 log CFU of each bacterial strain. Two-fold dilution with Mueller Hinton Broth was used to obtain the final concentrations of the hydrosols within the range of 0.19–50 mg/ml. Positive tube with Mueller Hinton Broth and bacterial suspension and negative tube with Tween 80 control samples were also prepared. Incubation was carried out at 35 °C for 18–24h. Tubes without any visible growth were marked as MIC, while MBC was determined by subculturing the MIC tubes onto the Mueller Hinton Agar. The plates without any visible growth were marked as MBC.

Antimicrobial properties: The results of the antimicrobial activity measured via the paper disc diffusion method are shown in Table 1.

Table 1.

Bacterial strains		Grapes	Orange	Lemon	Mango
		Inhibition Zone Diameter (mm)			
Pathogen bacteria	*Staphylococcus aureus*	0.00±0.00	7.50±0.50	0.00±0.00	6.63±0.41
	Klebsiella pneumonia	9.55±0.55	11.25±0.83	15.50±0.50	7.00±0.58
	Salmonella paratyphi	5.50±0.50	6.25±0.43	9.50±0.50	0.00±0.00
	Enterococcus feacalis	5.50±0.50	6.88±0.54	0.00±0.00	6.00±0.00
Spoilage bacteria	*Photobacterium damselae*	7.00±0.00	9.38±0.41	8.50±0.50	5.75±0.439
	Enterococcus Vibrio vulnificus	8.50±0.50	0.00±0.00	6.00±0.00	15.50±0.50
	Proteus mirabils	6.50±0.50	7.50±0.58	5.75±0.43	6.63±0.41
	Serratia liquefaciens	9.55±0.55	8.50±0.50	0.00±0.00	5.50±0.50
	Pseudomonas luteola	6.63±0.41	15.50±0.50	7.50±0.58	6.88±0.54

As expected, Tween 80, as a negative control, did not show an inhibition zone against any of the tested bacteria. The fruit peel hydrosols exhibited antioxidant activity against most of the tested bacterial strains, aside *from E. feaecalis and P.mirabilis,* both isolated from spoiled fish. On the other hand, the demonstrated hydrosols form lower antimicrobial activity against all of the tested pathogenic and fish-spoilage bacteria, with the exception of *P. damselae*. Moreover, the Fruit hydrosols did not exhibit any activity against *E. faecalis*, *V. vulnificus*, *P. mirabils*, or *S. liquefaciens*. Both hydrosol forms

had lower antibacterial activity than all compared antibiotics apart from *E. faecalis*, which was more susceptible to four fruit hydrosols than to streptomycin.

REFERENCES

Deng, W., Liu, K., Cao, S., Sun, J., Zhong, B., & Chun, J. (2020). Chemical composition, antimicrobial, antioxidant, and antiproliferative properties of grapefruit essential oil prepared by molecular distillation. Molecules, 25(1), 217. Retrieved from https://www.mdpi.com /1420-3049/25/1/217.

Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. Journal of Biotechnology, 233, 106–120. https://doi.org/10.1016/j. jbiotec.2016.07. 005

Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012). Design of Nano emulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. Journal of Biotechnology, 159(4), 342–350. https://doi.org/10.1016/ j.jbiotec.2011.07.001

Durmus, M. (2020). The effects of nanoemulsions based on citrus essential oils (orange, Mandarin, grapefruit, and lemon) on the shelf life of rainbow trout (Oncorhynchus mykiss) fillets at 4 ± 2◦C. Journal of Food Safety, 40(1), Article e12718. https://doi. org/10.1111/jfs.12718

FAO. (2017). Citrus fruit. Fresh and processed. Statistical Bulletin. http://www.fao. org/3/a-i8092e.pdfI.

Karioti, A., Skaltsa, H., & Gbolade, A. A. (2007). Constituents of the distilled essential oils of citrus reticulata and C. Paradisi from Nigeria. *Journal of Essential Oil Research*, 19 (6), 520–522. https://doi.org/10.1080/10412905 .2007.9699320

Kosker, A. R. (2020). The effects of nanoemulsions based on citrus essential oils on the formation of biogenic amines in trout fillets stored at 4 ± 2◦C. *Journal of Food Safety*, 40(1), Article e12762. https://doi.org/10.1111/jfs.12762

Li, P.-H., & Chiang, B.-H. (2012). Process optimization and stability of d-limonene-in water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. Ultrasonics Sonochemistry, 19(1), 192–197. https://doi.org/10.1016/j. ultsonch.2011.05.017

Li, W., Chen, H., He, Z., Han, C., Liu, S., & Li, Y. (2015). Influence of surfactant and oil composition on the stability and antibacterial activity of eugenol nanoemulsions. Lebensmittel-Wissenschaft und -Technologies- Food Science and Technology, 62(1, Part 1), 39–47. https://doi.org/10.1016/j.lwt.2015.01.012

Najafinejad, M. S., Mohammadi, P., Mehdi Afsahi, M., & Sheibani, H. (2018). Green synthesis of the Fe_3O_4@polythiophen-Ag magnetic nano catalyst using grapefruit peel extract: Application of the catalyst for reduction of organic dyes in water. *Journal of Molecular Liquids,* 262, 248–254. https://doi.org/10.1016/ j.molliq.2018.04.052

Njoroge, S. M., Koaze, H., Karanja, P. N., & Sawamura, M. (2005). Volatile constituents of red blush grapefruit (citrus paradisi) and pummelo (citrus grandis) peel essential oils from Kenya. Journal of Agricultural and Food Chemistry, 53(25), 9790–9794. https:// doi.org/10.1021/jf051373s

Okunowoa, W. O., Afolabia, L. O., Oyedejia, A. O., Matanmia, E., & Awodeleb, O. (2016). Larvicidal activity of essential oil from Citrus sinensis and Citrus paradisi against Anopheles gambiae. Biokemistri, 28(1), 16–23.

Ozogul, Y., Kuley Boga, ˘ E., Akyol, I., Durmus, M., Ucar, Y., Regenstein, J. M., et al. (2020). Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. Food Bioscience, 36, 100635. https://doi. org/10.1016/j.fbio.2020.100635

Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N., & Bajpai, V. K. (2017). Essential oils: Sources of antimicrobials and food preservatives. Frontiers in Microbiology, 7(2161). https://doi.org/10.3389/fmicb.2016.02161

Perez-Fonseca, A., Alcala-Canto, Y., Salem, A. Z. M., & Alberti-Navarro, A. B. (2016). Anticoccidial efficacy of naringenin and a grapefruit peel extract in growing lambs naturally-infected with Eimeria spp. Veterinary Parasitology, 232, 58–65. https://doi. org/10.1016/j.vetpar.2016.11.009

Pisoschi, A. M., Pop, A., Georgescu, C., Turcus¸, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. European Journal of Medicinal Chemistry, 143, 922–935. https://doi.org/10.1016/j.ejmech.2017.11.095

Prakash, A., Baskaran, R., Paramasivam, N., & Vadivel, V. (2018). Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: A review. Food Research International, 111, 509–523. https://doi.org/ 10.1016/j.foodres.2018.05.066

Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2014). Impact of microfluidization or ultrasound processing on the antimicrobial activity against Escherichia coli of lemongrass oil-loaded nanoemulsions. Food Control, 37, 292–297. https://doi.org/10.1016/ j.foodcont.2013.09.015

Saucier, L. (2016). Microbial spoilage, quality and safety within the context of meat sustainability. Meat Science, 120, 78–84. https://doi.org/10.1016/j. meatsci.2016.04.027

****