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The potential of lemon juice-*ogi* steep liquor mixtures in the reduction of *Listeria monocytogenes* contamination of ready-to-eat vegetables

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ABSTRACT

This study evaluated the inhibitory effect of lemon juice *ogi* steep liquor mixtures (10–50% v/v) made from white and yellow maize, white and red sorghum and Pearl millet grains on three antibiotic resistant isolates (LM 29, 33 and 44) and a susceptible isolate (LM 50) of *Listeria monocytogenes*. The isolates were surface-inoculated onto ready-to eat-vegetables (cucumber-*Cucumis sativas*, cabbage-*Brassica olerecea*, carrot-*Daucus carota*, tomato-*Solanum lycopersicum*, lettuce-*Lactuca sativa)*. The most effective concentration (50% v/v, lemon juice-white sorghum) of the mixtures was further used for bioassay of vegetables contaminated with isolate with the lowest zone of inhibition (LM 29) to the treatments at dipping times of 0–20 min. The major active compounds in the mixtures were also elucidated with Fourier Transform Infra-Red spectroscopy. There were significant differences ($p \leq 0.05$) in the responses of the isolates to the treatments which varied with types of isolates, the cereal sources and contact times. There were reductions in the colonies of the isolates as the dip time increased and the isolates inoculated onto carrots; cucumbers and lettuce were completely inhibited at 10, 15 and 20 min treatment times. Alcohol and carboxylic acids were identified as the major active compounds responsible for reduction and inhibition of the isolates.

1. Introduction

Indigenous food fermentation is one of the oldest food biotechnological processes that is dependent on the biological activities of microorganisms such as moulds, bacteria and yeasts (Karki & Kharel, 2011). In Nigeria, fermented foods and beverages with traditional and cultural values include *Gari*, *Burukutu*, *Ogi*, *Pito*, *Iru*, *Ogiri* (Adesulu & Awojobi, 2014). *Ogi* is an indigenous fermented food that can be prepared from steeped maize, sorghum or millet. It is consumed by all age groups but the steep liquor is usually decanted and wasted. However, *Ogi* steep liquor had been used as an alternative raw material in yoghurt production (Farinde, Obatolu, Fasoyiro, Adeniran, & Agboola, 2008). It had also been used to control some pathogenic microorganisms such as *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Enterobacter* spp (Abdus-Salaam, Adepoju, Olaleye, & Adeoye, 2014; Ojokoh, 2011). Adebolu, Ihunweze, and Onifade (2012) also reported that *Ogi* steep liquor has metabolites like organic acids, hydrogen peroxide and bacteriocins which have antimicrobial activities.

The lemon (*Citrus limon*) tree is an important medicinal plant that belongs to the family . It is cultivated mainly for its alkaloids, flavanones, flavones and vitamin constituents and has anticancer potentials (Dhanavade, Jalkute, Ghosh, & Sonawane, 2011). It can also act as an acidulant. Lemon juice has been used in combination with other antimicrobial sources to inhibit some microorganisms such as *Salmonella, Shigella, E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Achi & Akomas, 2006; Dhanavade et al., 2011).

Listeria monocytogenes is a Gram positive facultative pathogenic saprophyte that can be found on raw and processed foods. It can grow over a temperature range of −0.4 °C to 45 °C and a pH 4.0 to

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9.6 (optimum 6–8), a water activity as high as 0.90 and under aerobic and anaerobic conditions (Valimaa, Tilsala-Timisjarvi, & Virtanen, 2015). It is ubiquitous and has been recovered from dusts, soils, water, sewage, decaying vegetation, animal feed and silage, food and food processing plants, including abattoirs and smokehouses from where it enters the food chain (Ikeh, Obi, Ezeasor, Ezeonu, & Moneke, 2010; Valimaa et al., 2015). It causes a disease known as listeriosis with a fatality rate of 12.7% (European Food Safety Authority, 2013).

Ready-to-eat vegetables (RTE) are rich sources of minerals and dietary fibres. The level of consumption of ready-to-eat vegetables has increased based on their proven medical and nutritional benefits. They have been strongly associated with improvement of gastrointestinal health, good vision, reduced risk of heart diseases, stroke and chronic diseases (Dias, 2012; Ieren, Bello, & Kwaga, 2013). They have also been identified as major vehicles for listeriosis due to direct contamination from decaying vegetation, animal feces, soil surfaces, rivers and canal waters, effluents from sewage treatment operations, improper harvesting and handling procedures, improper sanitary conditions of equipment and transportation practices (Ajayeoba, Atanda, Obadina, Bankole, & Adelowo, 2015).

The increase in the incidences of *L. monocytogenes* in food products and environmental samples (Abdus-Salaam et al., 2014; Ieren et al., 2013; Ikeh et al., 2010) in Nigeria and the fact that pure forms of organic acids (potassium sorbate, sodium diacetate, lactic acid) and antimicrobials used in inhibiting the growth of *L. monocytogenes* (Lloyd, Alvarado, & Berrang, 2012) are not readily accessible to the local populace necessitates the development of additional techniques to control the bacterium in foods. Thus there is need for development of a cheaper, simpler and more readily accessible method for inhibition of *L. monocytogenes* isolates.

The objective of the study was to determine the additive effect of lemon juice and *ogi*-steep liquor mixtures on *L. monocytogenes* contamination of RTE vegetables.

2. Materials and methods

2.1. Samples

Maize-*Zea mays* (white and yellow variety), sorghum-*Sorghum bicolor* (white and red variety), Pearl millet (*Pennisetum glaucum* L.) and lemon (*Citrus limon*) fruits were purchased from Bodija market, Ibadan and Igbona market, Osogbo, Nigeria.

2.2. Chemicals and cultures

All solvents and reagents used were of analytical grade and were purchased from Sigma Aldrich (USA). The media and antibiotic sensitivity discs were purchased from Oxoid (Thermo Scientific, UK). The 0.5 McFarland standards and primers were purchased from Invitrogen (Thermo Fisher Scientific, Massachusetts, USA). Antibiotic resistant isolates of *L. monocytogenes* (LM 29, LM 33 & LM 44) and a susceptible isolate (LM50) previously isolated and characterized from RTE vegetables in Nigeria (Ajayeoba et al.., unpublished) and stored in the Culture Collection Centre of the Department of Microbiology, University of Ibadan were used for the study.

2.3. Preparation of lemon juice-ogi steep liquors

Ogi was prepared according to the modified method of Oluwafemi & Adetunji, (2011) from white and yellow maize, white and red sorghum and Pearl millet grains. Briefly, 500 g cereal grains were cleaned of extraneous materials and steeped in sterile water at room temperature (25 °C 102 ± 2 °C) for 72 h in a 5 L loosely

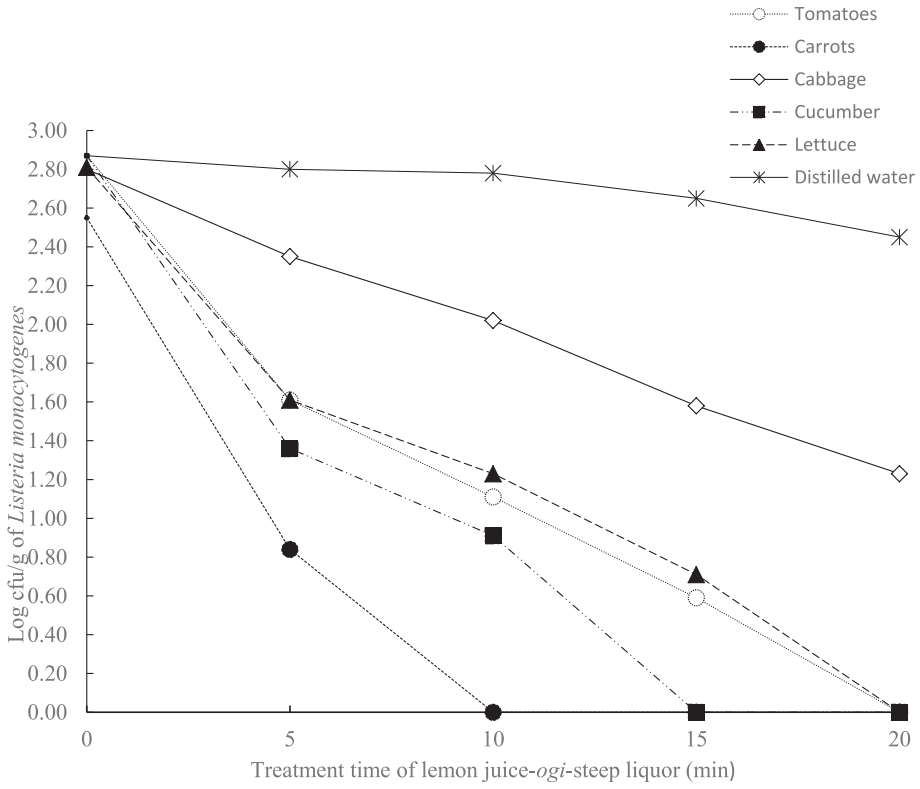

Fig. 1. Effect of 50% (v/v) lemon juice-white sorghum steep liquor mixture on artificially contaminated (LM 29) ready to-eat vegetables.