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Carvacrol nanoemulsion combined with acid electrolysed water to inactivate bacteria, yeast *in vitro* and native microflora on shredded cabbages

Li Cheng Sow [a, b], Felisa Tirtawinata [a, b], Hongshun Yang [a, b, *], Qingsong Shao [c], Shifei Wang [d]

[a] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China
[c] Department of Traditional Chinese Medicine, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, PR China
[d] Changzhou Qihui Management & Consulting Co., Ltd., Changzhou, Jiangsu 213000, PR China

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ABSTRACT

Carvacrol is an effective antimicrobial agent originated from essential oils, this natural antimicrobial agent has higher consumer acceptance compared to chemical agents. Due to the low solubility of carvacrol in water, carvacrol was delivered as a nanoemulsion. A carvacrol nanoemulsion contained 3.5% (w/w) oil phase (1% carvacrol and 2.5% corn oil, w/w) and 3.5% (w/w) Tween 80 was produced by ultrasonification at 10 min using 100% amplitude; the median particle size was 309 ± 19 nm. The nanoemulsion was shelf-life stable for 1 month without any significant changes in particle size. When applied against *Escherichia coli* ATCC 25922 and *Pichia pastoris* GS115 growth in nutrient broth, carvacrol nanoemulsion (0.5% w/w carvacrol) achieved 3 log reductions of microorganisms. When microorganisms were fixed and dried on stainless steel coupon surface, the carvacrol nanoemulsion treatment was more effective on *E. coli* than *P. pastoris* with about 5 and 0.3 log reduction of viable count, respectively. The native microflora on shredded cabbages was challenged by combining carvacrol nanoemulsion and acidic electrolysed water (AEW) that contained ≤ 4 mg/L free available chlorine (FAC). The treatment reduced about 0.5 log of aerobic mesophilic and psychrotropic bacteria counts and the antimicrobial activity of carvacrol nanoemulsion and AEW lasted up to 2 days. The results indicated that carvacrol nanoemulsion is promising in controlling the safety of fresh-cut vegetables.

1. Introduction

In recent years, there have been growing interest and potential of applying essential oils or the essential oil's active component in food as a natural antimicrobial agent (Chang, McLandsborough, & McClements, 2013). Essential oils possess broad spectrum antimicrobial activity, they also have the clean label status as they are generally recognised as safe (GRAS) (Chen, Davidson, & Zhong, 2014). Carvacrol is a phenolic compound that was proven to be a very active antimicrobial agent. It can disrupt the membrane of cell and mitochondria, causing damage of permeability barrier and leakages of ions, ATP, nucleic acids and amino acids (Donsì, Annunziata, Vincensi, & Ferrari, 2012).

Since the water solubility of carvacrol is as low as 0.11–0.83 g/L at 25 °C (Chen et al., 2014), it is difficult to directly apply carvacrol in food. An oil-in-water (O/W) nanoemulsion system can be used to deliver the active essential oils component. Nanoemulsion is a kinetically stable system which contains submicron size of dispersed particles and is mostly opaque (Ferreira et al., 2010). Nanoemulsion can provide protection to the active component against environmental stresses and increase the partition of the hydrophobic component to aqueous phase (Chang et al., 2013; Donsì et al., 2012), the small particle size of nanoemulsion also offers good physical stability and increased bioactivity (Donsì,

* Corresponding author. Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

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Cuomo, Marchese, & Ferrari, 2014; McClements & Rao, 2011). The antimicrobial activity of carvacrol was found to be dependent on the composition of nanoemulsion (type of surfactant, concentration of oil phase, ratio of carvacrol to carrier oil, etc.), the particle size and the solubility of nanoemulsion, as well as the type of food matrix where the carvacrol nanoemulsion is applied (Chang et al., 2013; Donsì et al., 2012). Therefore, there is a compelling need to test and explore the antimicrobial efficacy of the carvacrol nanoemulsion on more varieties of food.

Acidic electrolysed water (AEW) can be applied on food and food contact surface, the active oxidising components and chlorines in AEW make it an effective sanitiser against foodborne pathogens including *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* (Park, Alexander, Taylor, Costa, & Kang, 2008; Yang, Feirtag, & Diez-Gonzalez, 2013). When the free available chlorine concentration (FAC) of AEW is less than 5 mg/L, it complies with the regulation limit for treatment of drinking water and can be considered as safe (WHO, 2011), while up to 4 mg/L is permitted for organic food sanitisation. With the low surface tension property of nanoemulsion due to the incorporation of surfactants, the antimicrobial agents in nanoemulsion can have better contact onto the surface of fruits and vegetables that are mostly hydrophobic or non-uniform (Xiao et al., 2011). Previous reports by Zhang and Yang (2017) have studied the sanitising effect of electrolysed water with citric acid and H_2O_2. Zhang and Yang (2017) have also investigated the antimicrobial activity of the combined used of neutralised electrolysed water with ultrasonification. Liu, Tan, Yang, and Wang (2016) also explored the combination of low concentration AEW and mild heat to sanitise organic broccoli. It is, therefore, interesting to investigate the antimicrobial effect of combining the use of AEW and carvacrol nanoemulsion, especially on waxy fruits and vegetables.

Cabbage is a typical example of waxy vegatables. The cabbages are frequently subject to shredding and consumed raw as salad. This minimally processed product often has a shorter shelf-life than intact produce as the wounded tissues can undergo accelerated tissue softening and enzymatic browning and more prone to microbial contamination (Lin & Zhao, 2007). Organic cabbages have been studied due to the increasing demand of high quality organic produce, it is therefore important to ensure both chemical safety (Yu & Yang, 2017) and microbiological safety of organic produces (Zhang & Yang, 2017). Besides the food itself, food processing equipment could also be a carrier of spoilage microorganisms and foodborne pathogens. Hence, there is a need for sanitisation of food processing equipment surfaces during food processing and handling to ensure food safety.

The objectives of this study were to develop a stable carvacrol nanoemulsion and evaluate the antimicrobial activities of carvacrol nanoemulsion and AEW against *Escherichia coli* ATCC 25922 (*in vitro*) as a representative bacterium strain, *Pichia pastoris* GS115 (*in vitro*) as a representative yeast strain and the native microflora on shredded cabbages. The antimicrobial results could suggest suitable use of the carvacrol nanoemulsion. To the best of our knowledge, this is also the first study which combines the use of carvacrol nanoemulsion and AEW on food.

2. Materials and methods

2.1. Materials

Food grade carvacrol (99%) and propylene glycol ($\geq$ 99.5%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Polyoxyethylene (20) monooleate (OmniPur® Tween 80®) was purchased from Merck (Darmstadt, Germany), corn oil (100%) was purchased from local supermarket (FairPrice, Singapore). Organic cabbage (*Brassica oleracea L. var. capitata*) was purchased from a local market (GreenCircle, Singapore). Microbiological media including tryptic soy broth (TSB), malt extract broth (MEB), tryptic soy agar (TSA), potato dextrose agar (PDA), standard plate count agar (PCA) and peptone were from Oxoid (Hampshire, UK).

2.2. Preparation of nanoemulsion

The total oil phase (3.5–10%, w/w) consisted of carvacrol and corn oil as carrier oil. A coarse emulsion was first produced by mixing oil phase and Tween 80 (3.5%, w/w) together followed by combining with deionised water using a high shear mixer (8000 rpm, 10 min). The coarse emulsion was homogenised by high pressure homogenisation (HPH) or ultrasonification (USF). HPH method was employed in screening of nanoemulsion formulation due to the ability to produce samples of large batch size within a short time. In HPH method, the coarse emulsion was passed through APV-2000 high pressure homogeniser (SPX FLOW, North Carolina, U.S.) at 1000/100 bar (first stage/second stage pressure) twice to form nanoemulsion. In USF method, the coarse emulsion was immersed with a sonotrode (3.4 cm diameter) attached to a 20 kHz sonicator with a power output of 1000 W (UIP1000, Hielscher, Germany) and the amplitude used was 100%. The maximum temperature of samples during sonication was 25 °C.

Blank nanoemulsion without carvacrol was used to screen suitable formulations (oil phase concentration, surfactant to oil ratio (SOR)) and processing time of USF. The optimised formula and method was selected to produce antimicrobial nanoemulsion by adding carvacrol (1%, w/w) to replace corn oil while retaining the total oil phase concentration. To prevent contamination of the carvacrol nanoemulsion (CRV) as much as possible, all the containers, water, apparatus used were sterilised by autoclaving at 121 °C for 15 min before use. The sonotrode was sanitised with 75% (v/v) ethanol before contact with the nanoemulsion.

2.3. Characterisation of nanoemulsion

The particle size distribution of nanoemulsion was determined using the Horiba laser scattering particle size distribution analyser (LA-950 V2, Horiba Ltd., Kyoto, Japan). The refractive index of 1.33 was used for all sample measurements. The volumetric distribution of particles was considered and the result was reported as D10, D50, D90, which were the size of particles (in nm) where 10%, 50%, 90% of the particles lied below each number, respectively. For stability testing, the nanoemulsion was stored for one month and the particle size was re-evaluated and compared to the particle size of freshly produced nanoemulsion (Pan, Chen, Davidson, & Zhong, 2014).

The turbidity of nanoemulsion was determined by diluting nanoemulsion with DI water in the ratio of 1:3 (v/v) and its absorbance was measured using UV-VIS spectrophotometer (UVmini-1240, Shimadzu (Asia Pacific) Pte. Ltd., Singapore) at 600 nm wavelength (Rao & McClements, 2011). The viscosity of nanoemulsion was determined using a Brookfield DV II+ viscometer (Brookfield Engineering, Middleboro, MA, USA) with No. 1 spindle at 150 rpm rotation at 25 °C. The surface tension was determined using du Noüy ring tensiometer with a ring having a circumference of 4 cm at 25 °C.

2.4. Antimicrobial activity against E. coli and P. pastoris

2.4.1. Bacterial and yeast strain

Escherichia coli ATCC 25922 was obtained from Dr. Hyun-Gyun Yuk of National University of Singapore, Food Science and Technology program, while *Pichia pastoris* GS115 was isolated by