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Research Article

Onion Essential Oil-in-Water Emulsion as a Food Flavoring Agent: Effect of Environmental Stress on Physical Properties and Antibacterial Activity

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Plant essential oils (EOs), which are acknowledged as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), have the potential to be used as a flavoring agent. However, there are limitations to some EOs, such as low water solubility and high volatility, which limit their application in food technology. This study was conducted to develop onion (*Allium cepa*) EO as a flavoring agent and determine its stability against environmental stress via an emulsification technique, with different concentrations of sodium caseinate, as a delivery system. Emulsions containing onion EO were prepared using different concentrations of sodium caseinate (3, 5, and 7% *w*/*w*) via the solvent-displacement technique. The physical properties (average droplet size, color, turbidity, and stability measurement) and antibacterial activity (agar disk diffusion method) of emulsions were then determined. Results show that emulsion with 7% (*w*/*w*) sodium caseinate was the most desirable sample in terms of physical properties and antibacterial activity. Hence, it was selected for environmental stress studies (i.e., thermal processing, freeze-thaw cycles, and ultraviolet (UV) exposure). Results revealed that all types of environmental stresses had significant ($p < 0.05$) effects on droplet size, color, turbidity, and stability. Generally, the environmental stresses increased the droplet size except in the freeze-thaw cycle case, while all stresses decreased the stability and lightness. All types of environmental stress treatment did not show a significant ($p < 0.05$) effect on antibacterial activity enhancement against *Salmonella* Typhimurium and *Listeria monocytogenes* except in the case of UV treatment against *L. monocytogenes*. Therefore, the present work has demonstrated the potential use of emulsion as an encapsulation and delivery system of EO flavors for food applications.

1. Introduction

Flavors have an important role in the consumer acceptability, palatability, and quality of food in the food industry. A first judgment about the value of a food source is made on its appearance and smell [1]. On the other hand, due to increasing mankind's knowledge, people look for safe food with minimum side effects. Not surprisingly, consumers express considerable concern about the indiscriminate use of chemicals in foods (i.e., synthetic flavor materials) [2]. Essential oil (EO) is considered a natural flavoring [3] and has been used for centuries as perfume fragrances, in culinary as a flavoring, and in folk medicine [4]. For most purposes, EO is the preferred flavoring agent and is commercially available [2].

The use of EOs as flavoring agents is registered by the European Commission (EC) and by the FDA, and they are classified as GRAS (under section 201 (s) and 409 of the Act and FDA's implementing regulations in 21 CFR 182.20) and approved in the food additive status list [5, 6].

Onion oil is the non-water-soluble fraction from the steam distillation of macerated onions [7]. Also, onion flavors (EO) are important seasonings widely used in food processing [8]. Recent research has demonstrated that onions possess several properties, such as antibacterial [9], antimicrobial (bacteria, molds, and yeasts) [8] antimutagenic [10], and antioxidant activities [8, 11]. The most medicinally significant components of onion oil are the organosulfur-containing compounds [12, 13]. These compounds are reactive, volatile, odor producing, and lachrymatory [14]. The bioactive properties and characteristic flavor of onion have been attributed to sulfur-containing compounds, which are the main constituents of its EO (dipropyl disulphide (21.31-60.4%), dipropyl trisulphide (17.1-21.92%), methyl 5-methylfuryl sulphide (18.3%), methyl 3,4-dimethyl-2-thienyl disulphide (11.75%), methyl 1-propenyl disulphide (13.14%), methyl 1-propenyl trisulphide (13.02%), methyl propyl trisulphide (7.05-14.95%), methyl propyl disulphide (9.5%), propyl *trans*-propenyl disulphide (7.87%), allyl propyl disulphide (3.56%), dipropyl tetrasulphide (3.04%), dimethyl trisulphide (1.14-16.64%), propyl *cis*-propenyl disulphide (4.67-9.72%), dimethyl disulphide (1.31%), dimethyl tetrasulphide (0.46-7.24%), and isopropyl disulphide (0.31%)). The water-insoluble extractive obtained from onion EO consists of a complex mixture of volatile sulfur compounds, mostly mono-, di-, tri-, and tetrasulphides with different alkyl groups [15].

Although EOs are available as natural flavoring, they have some limitations that must be overcome before applying them to food systems. The main properties that make EOs difficult to apply in the food system are low water solubility, high volatility [16, 17], and strong odor [16]. The hydrophobicity properties of EOs cause nonuniform distribution in food matrices and reduce their antimicrobial effectiveness when directly incorporated into foods due to their hydrophobic binding with food components [18]. Also, a high concentration of EOs affects the organoleptic properties because the concentration of essential oils required to cause a bacterial inhibitory effect in vitro is significantly higher than the concentrations required to cause similar effects in real foods [19]. Hence, the emulsification process can help to solve this problem [20].

Emulsions or dispersions are produced by homogenizing two immiscible phases together in the presence of stabilizer molecules [21, 22]. Emulsification of EOs not only able to overcome the limitations of EOs but also able to increase their antibacterial activity. The emulsion-based systems are the most desirable delivery systems for encapsulating, protecting, delivering, and releasing poorly oil- or water-soluble drugs and food-active ingredients [23]. Through the emulsification process, the dispersed phase is broken up into small droplets [24]. It might facilitate diffusion of the encapsulated antibacterial to reach the right site (i.e., membrane of bacteria). Additionally, the emulsions might cause the permeabilization of the cells and disrupt the bacteria's cell membrane integrity [25]. According to Topuz et al. [26], the emulsified EO of anise showed better and longer-term physicochemical stability and antimicrobial activity compared to bulk anise oil. Moreover, emulsified EOs also showed higher antimicrobial activity, even at far lower concentrations [27]. Furthermore, food products during storage and processing undergo various environmental stresses such as sunlight, heat, and freezing. Therefore, there is an increasing emphasis on developing a more fundamental understanding of the influence of conditions and environmental stresses on the functionality of the stabilizer system [22] and, as a result, on the stability of the emulsion system. To the best of our knowledge, the antibacterial activity and physical properties of onion oil in water emulsion as a food flavoring agent and the effect of environmental stress on the physical properties and antibacterial activity of onion oil in water emulsion have not been studied. For the stability of emulsions, sodium caseinate was selected as an emulsifier agent since not only it is frequently used as a natural emulsifier [28] but also, in the food industry, it is one of the proteins that is largely used as an ingredient [29]. Sodium caseinate is flexible, moderately highly soluble, and quickly adsorbed at the oil-water interface [28]. Thus, this study is aimed at determining the antibacterial activity against food-associated bacteria and physical properties of the onion essential oil in water emulsion and at evaluating the effect of environmental stress, namely, thermal, UV, and freeze-thaw treatment, on the physical properties and antibacterial activity of the best-produced onion oil emulsion.

2. Materials and Methods

2.1. Materials. The pure essential oil of onion (*Allium cepa* L.) was provided by BF1 Soul Brand, Malaysia. Sodium caseinate was obtained from R&M chemicals (Tamil Nadu, India). Acetone was purchased from ACME Chemicals (Malaysia, Selangor). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were supplied by Merck (Berlin, Germany). The blank paper disk and sterile swab used for antimicrobial properties were purchased from Bioeconomy Co. (Kuala Lumpur, Malaysia). *Listeria monocytogenes* ATCC 19114, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 19585, and *Escherichia coli* ATCC 25922 were the target bacteria.

2.2. Fabrication of Onion Essential Oil Emulsion. The onion EO emulsions were prepared according to the solvent displacement technique reported by Ribeiro et al. [30]. The organic phase was prepared by dissolving 5% (w/w) onion essential oil in 5% (w/w) acetone. The aqueous phase was prepared by dissolving sodium caseinate (3, 5, and 7% w/w) into distilled water (87, 85, and 83% w/w). The selected concentrations of sodium caseinate were based on our preliminary study. Sodium caseinate at concentrations of 1 and 2% (w/w) did not form stable emulsions (i.e., it showed creaming after a few hours). The organic phase was added to the aqueous phase that had been hydrated for 24 hours under moderate magnetic stirring (1500 rpm) and continuous magnetic stirring. Finally, the resulting emulsion was subjected to rotary evaporation (Eyela NE-1101, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at a temperature of 40°C under reduced pressure (0.25 bar) to remove the organic solvent (acetone), which