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Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions

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ABSTRACT

Quantitative investigation and systematic studies of quercetin, total phenolics, flavonoids, antioxidants, antibacterial and antibiofilm or antibiofouling properties of methanolic extracts of onions obtained from six different varieties have been carried out to explore their relative merits in terms of biological activities of fresh and aging onions. Total phenolic content in the extracts was examined spectrophotometrically using Folin–Ciocalteau's phenol reagent and total antioxidant activity was studied by FRAP and DPPH methods. *In vitro* antibacterial activity of the extracts was investigated on Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) respectively, by using a modified Kirby–Bauer disc diffusion method. Antibiofilm activity was tested by crystal violet assay. The best results against biofilm formation were observed for the extracts obtained from onions stored for three months. The total phenolic and antioxidant content found to be increased upon aging in all the six varieties; red skinned onion (Happyhong) showed the highest level of total phenolics (5110.07 ± 196.56 μg $GAEg^{-1}$ FW) and total flavonoids (2254.00 ± 154.82 μg QEg^{-1} FW) after three months. The results showed that in all the varieties, quercetin content as well as biological activity increases with aging in the stored onions compared with the fresh ones.

1. Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown worldwide. During the last 10 years its production has increased by more than 25% of the total production [1]. In most countries, approximately 10% of the total annual harvest is either discarded or inadequately treated in the market as worthless onions because they fail to meet the quality standards required for marketing to the customers [2]. The current quality demand by customers and search for high quality levels lead to the rejection of even more numbers of onions as worthless during selection and calibration stages (irregular shape, injured parts, non-commercial sizes). Dealing with onion wastes from storage and processing industries has become a real and huge problem for the market suppliers. Firstly, it is neither suitable for cattle fodder because of its strong characteristic pungent smell, nor can be used as an organic fertilizer because of rapid development of

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phytopathogenic agents, such as *Sclerotium cepivorum* [3]. Therefore, the only option remains with their disposal is the landfill. And, the landfill disposal of onion waste results in high economic costs, as well as a bad impact on the environment. Furthermore, their removal by incineration does not seem appropriate because of high water content, rendering it expensive with additional fuel consumption, and environmental pollution with the release of carbon dioxide and other gases. Therefore, utilization of the worthless onions effectively becomes a challenging problem and a burning topic of waste reuse and environmental research. Moreover, if the recovery of valuable phytochemicals and production of novel compounds of nutraceutical and pharmaceutical importance from onion wastes are fruitful, the environmental problems could be successfully solved. Thus, the onion producers and breeders are interested in developing the alternative means for the valorization of onion wastes to promote its profitable usage, and its conversion to food grade products. Nowadays researchers are aiming to manage onion wastes in such a way that the undesirable leftover plant materials are eliminated and used for the production/isolation of some useful phytochemicals that may otherwise be lost with the waste. Ramos et al. (2006) carried out oxidation of onion waste consisting mainly dried onions and observed good antibacterial and antioxidant property by oxidized products [4]. Furthermore, high antimicrobial activity of the onion skin waste extracts has also been observed against bacteria *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus cereus*, and fungi *Aspergillus niger*, *Trichoderma viride and Penicillium cyclopium* [5]. Onion waste is reported to contain high content of phenolics and flavonoids, mainly quercetin which shows high antibacterial activity [6]. Recovering antioxidants from onion waste is very appealing in terms of low processing costs which allow its extensive use in the food industry [7]. Overall processing scheme for exploiting onion wastes has been proposed by Waldron [8].

Two flavonoid subgroups present in onions are anthocyanins, which impart a red/purple color to some varieties, and flavonols, such as quercetin and its derivatives, which play an important role in the production of yellow and brown compounds in the skins of many varieties [9]. In onions, quercetin aglycone accounts up to 10% of the total flavonoids, and the remaining is in the form of its glucosides. In addition, phenolics and polyphenols from skin and extracts from the edible part of onion (*A. cepa* L.) are also known for its antimicrobial and antibiofilm properties [10,11]. These compounds have increased the interest of the food and pharmaceutical industries in order to improve food stability against microbiological spoilage agents. The flavonol composition in deteriorated or aging onions which are either discarded in the landfills or stored under intermediate facilities (before final disposal) changes due to natural physiological processes as well as activities of parasitic microorganisms. Culled onions (*A. cepa*) are commonly fed to cattle. The safety of feeding depends upon acceptability, species susceptibility and toxic potential of the onions [11] as these might result in secondary organ damage or even death when onions are consumed in large quantities. Onion toxicities are consistently noted in animals that ingest onions more than 0.5% of their body weight at one time [12]. Spontaneous ingestion of onions (*A. cepa*) causes hemolytic anemia and methemoglobinemia, leading to cyanosis hemolytic anemia with the formation of Heinz bodies, and fatal consequences has been previously reported in cattle, sheep, dogs, cats, and horses [13]. The epidemiology of onion toxicosis differs among species. The clinical symptoms of onion toxicosis observed in the cattles include in appetence, staggering, abortion, onion odor of the breath and feces, elevated heart and respiratory rates, pale mucous membranes, jaundice, and elimination of brown urine [14]. Onion toxicosis in water buffalo causes pale mucous membranes, lethargy, constant vocalization, tenesmus, weakness, dyspnea, production of dark urine, and the presence of an onion odor in their breath [15]. It also causes gallbladder enlargement and secretion of clotted bile, and excretion of onion fragments with ruminal contents. Onions also have fungicidal and bactericidal properties that alter rumen microbial populations [16]. These activities, in conjunction with high water content, may alter patterns of ruminal digestion. One of the effective treatment recommendations for onion toxicosis is restriction of intake. Blood transfusion may be given to severely affected animals which may promote clinical recovery [17]. Administration of antibiotics to be taken orally may be beneficial in reducing ruminal anaerobic bacteria that promote formation of some oxidative substances [18]. Proper and systematic utilization of onion wastes will also help in protecting the small randomly roaming domestic animals, such as dogs, cats and other cattles from the toxicities arising from changes in the bioactive components which may bring fatal consequences. Therefore, it is important to utilize and study the content of onion waste produced during and after its storage period.

Here, in the present research work, our aim is to analyse the variations in chemical composition of onions during aging process under storage of certain period ranging between one to six months. The visible symptoms of chemical composition variation upon aging and deterioration begin to appear on the onion skin or surface which continue to progress until it becomes unsuitable for any domestic purpose. We found major differences in the patterns of contents of quercetin and its glucosides during aging. There was observed a major reduction in total flavonoids (Q + QMG + QDG), however, a drastic increase in the quercetin content was also observed. Further, we also evaluated the antibiofilm and antibacterial potential. This study hints for a plausible economic exploitation of aging and deteriorated otherwise worthless or waste onions in pharmaceutical and food industries due to its high quercetin content.

2. Methods and materials

2.1. Chemicals and microorganisms

All solvents used in this study were of high-performance liquid chromatography (HPLC) grade. Trifluoroacetic acid (extra-pure grade) was supplied by Alfa Aesar (Ward Hill, MA, USA). Quercetin-3,4′-O-diglucoside and quercetin-4′-O-monoglucoside were supplied by Polyphenols Laboratories AS (Sandnes, Norway). Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tris (1-pyridyl)-5-triazine