Antioxidant and Anti-Inflammatory Effects of Ethanol Extract from Whole Onion (*Allium cepa* L.) with Leaves

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Abstract: This study was conducted to evaluate and to increase the usage of the whole onion (*Allium cepa* L.), which is composed of a small bulb and many leaves that are discarded as by-products before the bulbs grow. Whole onions are harvested early in immature condition, which allows the other onion bulbs to grow well. We compared its functional activities with those of quercetin, which is one of its major components. The antioxidant activities of ethanol extract from the whole onion (WOEE) were measured by DPPH and ABTS radical scavenging activities, and superoxide dismutase (SOD) and catalase (CAT) activities. The anti-inflammatory effects of WOEE were investigated in RAW 264.7 macrophages treated with LPS by analyzing cytokine levels and expressions using ELISA kits and RT-PCR assays, respectively. WOEE showed high antioxidant effects on DPPH and ABTS radical scavenging activities, and SOD and CAT activities. WOEE significantly reduced the production of nitric oxide, IL-1β, IL-6, and TNF-α, and/or their mRNA expressions in a dose-dependent manner. The results indicated that whole onions had antioxidant and anti-inflammatory effects, which were comparable with quercetin and may be used as a novel potential therapeutic candidate.

Keywords: whole onion; by-product; antioxidant; anti-inflammatory

1. Introduction

The living body produces substances that induce oxidative stress in the metabolic process caused by oxygen respiration [1]. Our body also produces antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) to protect living organs from reactive oxygen species, that work against oxidative stress substances [2]. Reactive oxygen species and inflammatory cytokines are related to the mechanisms that induce this oxidative stress [3]. Several substances inhibit or alleviate the inflammatory response.

Inflammatory reactions refer to the essential defense mechanism that protects the living body during an external invasion or tissue damage [4,5]. Based on the action and the period of development, it is classified as an acute inflammatory reaction or chronic inflammatory reaction. In an acute inflammatory reaction, the antigen activates the macrophages to secrete several inflammatory mediators such as cytokine and nitric oxide (NO) and to remove the foreign substances, and tissues are regenerated [6]. In the inflammatory responses, macrophages involved in host defense and homeostasis are activated by lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, to express several inflammatory mediators [7].

Onions (*Allium cepa* L.) are mostly consumed in the form of seasoning, and about 10% of domestic production is used for processed products [8]. Due to consumers’ preference for fresh foods, onions are mainly distributed in a primary processed form, such as cut onions.
without the skins and roots. The skins and roots generated after processing are either used in feed or, more commonly, thrown away. Many scientists have studied anti-oxidant and anti-inflammatory effects of fresh onion bulbs and the skins to increase their usage [9,10]. Early harvested whole onions (WOs) are composed of a small bulb with many leaves, discarded to grow other onion roots better, and considered as another by-product. WOs are harvested more than 1 month earlier than the matured onions (end of April) and discarded, even though the by-products can be used as an alternative to matured onions before they come out to markets (early of June) and increase farmers’ income. WOs also can contribute to improving carbon neutrality by their usage as a food or functional supplement due to their antioxidant and anti-inflammatory activities, which are lately of much interest to people.

Onions are considered to be excellent antioxidant additives. Antioxidant compounds (flavonoids and phenols) of onions protect against a variety of degenerative pathologies [9]. Onions contain flavonoids such as polyphenolic compounds that have a strong antioxidant reaction. Quercetin and its glycosylated derivatives in onions are expected to have a chemical preventive action [11] and polyphenols have a high antioxidant capacity as they effectively remove reactive oxygen species [12] and have anti-inflammatory effects [5].

Onion by-products such as onion peels can play an important role in certain diseases due to their bioactive compounds [10]. Most research has been conducted using onion peel and onion flesh [13,14]. However, little is known about the functional effects of the whole onion (WO) which is harvested early, often at the end of April as compared to early June for matured onions, because the WO is considered as a by-product. Therefore, we have evaluated the functional effects of WOs to reduce the by-products and improve their usability by verifying their antioxidant and anti-inflammatory activities which can prevent many clinical disease symptoms such as cardiovascular failure and cancer [2,15].

Quercetin is considered as one of major compounds with high functional activity in onion [11]. Thus, we compared the functionalities of WOs, which were harvested at the end of April, with those of quercetin. The antioxidant and anti-inflammatory effects were mainly measured in the RAW 264.7 cells stimulated by LPS. The final purpose of this study was to investigate their functional values, focusing on the antioxidant and anti-inflammatory effects of WOs, and to reveal their potential as a natural anti-inflammatory agent. Therefore, the research in this paper suggests that WOs can be used as an antioxidant and anti-inflammatory substance to prevent related diseases and improve people’s health status.

2. Materials and Methods

2.1. Antioxidant Effects of WOEE

2.1.1. Plant Material and Sample Preparation for Functional Analysis

The seeds were sown directly in the farm field (Wanju, Chunbuk, Korea) in the middle of September. The variety of onion used in this experiment was Top Red. WOs were harvested at the end of April, when they were discarded for other bulbs to grow better in farms. They were freeze-dried (PVTFD 10R, Ilsin Lab, Yangju, Korea), ground by a grinder (Hanil, Gwangju, Korea) to make powder, and sieved through a 100-mesh screen. The ethanol extract of whole onion (WOEE) was extracted with 10 volumes of 50% ethanol at room temperature for 24 h and concentrated by a rotary evaporator (EYELA N-1000, Riakikai Co., Ltd., Tokyo, Japan) at 50 °C. Then, the WOEE was frozen and lyophilized (PVTFD 10R) and stored at −70 °C for experimental use (RDA-2021-ACH-01).

2.1.2. Total Polyphenol Content

Total polyphenol contents (TPCs) in WOEE and quercetin were measured using modified methods [16]. Twenty microliters of sample were mixed with 200 µL of 90% diethylene glycol and 20 µL of 4 N NaOH solution. After the treatment for 30 min, the absorbance of the sample was measured at 700 nm using a microplate reader (Spectramax M5, Molecular Devices, Silicon Valley, CA, USA). Gallic acid (Sigma-Aldrich Co., St. Louis,
MO, USA) was used as a standard, and the results are shown as mg gallic acid equivalent (GAE)/g.

2.1.3. DPPH Radical-Scavenging Activity

The DPPH assay was performed using the modified method [17]. An amount of 50 μL of sample and standard solution were dispensed in a 96-well plate by concentration and 13 μM 1,1-diphenyl-2-picrylhydrazine (DPPH, Sigma-Aldrich Co.) was added to each well. The absorbance was measured at 517 nm using the microplate reader.

2.1.4. ABTS Radical-Scavenging Activity

ABTS analysis was conducted using a modified method [18]. After mixing 7.4 mM ABTS solution (Sigma-Aldrich Co.) and 2.6 mM potassium persulfate solution (Sigma-Aldrich Co.) in a 1:1 ratio, the WOEE and the ABTS solution were left at a ratio of 1:1 (100 μL:100 μL) for 10 min and then measured at 700 nm using the microplate reader.

2.1.5. Measurement of Superoxide Dismutase Activity

RAW 264.7 cells, which are mouse-derived macrophages (KCLB No. 40071), were received from Korea Cell Line Bank (Seoul, Korea). Cells were cultured using Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and 100 unit/mL of penicillin streptomycin (Gibco) while maintaining the conditions at 37 °C and 5% CO₂ (Forma Direct Heat CO₂ Incubator, Thermo Scientific, MA, USA). They were cultured in a 96-well plate at a concentration of 1 × 10⁶ cells/well. WOEE was treated at 50, 100, and 250 μg/mL for 4 h, added with LPS at a concentration of 2 μg/mL, and incubated for 24 h. The supernatant was used in the experiment and SOD activity was measured by the SOD Colorimetric Activity Kit (Invitrogen, Waltham, MA, USA) at 450 nm using the microplate reader.

2.1.6. Measurement of Catalase Activity

CAT activity was evaluated using the Catalase Colorimetric Activity Kit (Invitrogen Corporation, Carlsbad, CA, USA). RAW 264.7 cells were cultured in a 96-well plate at a concentration of 5 × 10⁶ cells/well. WOEE was treated at 50, 100, and 250 μg/mL for 4 h, added with LPS at a concentration of 2 μg/mL, and incubated for 24 h, and then the supernatant was used in the experiment. The absorbance was measured at 560 nm using the microplate reader and quantified by the catalase standard.

2.2. Anti-Inflammatory Effects of WOEE

2.2.1. Cell Viability Assay

RAW 264.7 cells were dispensed in a 96-well plate at a concentration of 1 × 10⁶ cells/well. One hundred μg/mL of extract (50, 100, 250 μg/mL) and 1 μg/mL of LPS (Sigma-Aldrich Co.) were added to the 96-well plate and cultured at 37 °C and 5% CO₂ in the incubator for 24 h. After incubation, 5 mg/mL of MTS reagent (Promega Corporation, Fitchburg, WI, USA) was added and cultured for 2 h. The absorbance was measured at 490 nm using the microplate reader.

2.2.2. Nitric Oxide Assay

RAW 264.7 cells were adjusted to 1 × 10⁶ cells/mL and seeded in 24-well plates. Each concentration of WOEE (50, 100, 250 μg/mL) was treated with LPS (1 μg/mL) and incubated for 24 h. Using the Griess reagent (Sigma-Aldrich Co.), the amount of NO in the form of NO₂ present in the cell culture was measured. The absorbance was measured at 540 nm using the microplate reader.

2.2.3. Analysis of Cytokine Concentration in the Supernatant of the Cells Cultured with WOEE

Cytokine (IL-1β, IL-6, TNF-α) concentrations in the supernatant, produced from the RAW 264.7 cells and cultured with WOEE or quercetin (Sigma-Aldrich Co.) at 50, 100,
and 250 μg/mL, were analyzed using an ELISA Kit (Abcam, London, UK). On a 96-well plate with a capture antibody, 50 μL of cell culture and 50 μL of cytokine antibody mixture were added. The absorbance was measured at 450 nm using the microplate reader. The concentration of each cytokine was measured and calculated using the curve calculated from the standard solution included in the ELISA Kit.

2.2.4. Measurement of Cytokine Gene Expression Using a Real-Time PCR

Total RNAs were extracted from the cells cultured with WOEE or quercetin using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). A one-step, real-time PCR reaction was performed according to the Quantitect SYBR Green RT-PCR Master Mix Kit method. The reaction solution was amplified according to the conditions using a Bio-Rad CFX96 Real-Time System (Bio-Rad, Hercules, CA, USA). Results were adjusted by the expression level of the housekeeping gene (GAPDH), and the analysis results were normalized by the CFX Manager Software (Bio-Rad) program. The Quantitect primers used in the experiment were GAPDH (QT01658692), IL-1β (QT01048355), and IL-12 (QT00112315).

2.3. Statistical Analysis

All data are presented as Mean ± SE. Statistical analysis was conducted using the SPSS program (Statistical Package for the Social Sciences ver. 24, IBM Corp, Armonk, NY, USA) for one-way ANOVA, which showed significance at the p < 0.05 level.

3. Results and Discussion

3.1. Antioxidant Activity of WOEE

3.1.1. Total Polyphenol Concentration

The polyphenols of onions are an important source of bioactive compounds and are a potentially beneficial component to human health [19]. The TPC of quercetin and WOEE is shown in Figure 1a. In 500 μg/mL of quercetin and WOEE, TPCs were 3.87 mg GAE/g and 5.40 mg GAE/g in a dry base, respectively (Figure 1a). TPC showed a tendency to increase with the concentration of quercetin and WOEE. It is thought that the content of TPC increased as the concentration of quercetin and WOEE increased. TPC values of onion varieties were reported [20] and the ranges were similar to our results. It was found that the TPC decreases toward the inner edible part of the bulb due to the aging of the outer layer cells in the bulbs rather than the inner layer cells [21]. Therefore, it is considered that using WO rather than mature onion flesh is effective to obtain more TPC.

![Figure 1.](image_url)  
Figure 1. Antioxidant effects of quercetin and WOEE. (a) Total polyphenol content, (b) DPPH radical scavenging activity, and (c) ABTS radical scavenging activity. The data were analyzed by one-way ANOVA using SPSS software and each bar presents the mean ± SE. (n = 3). a–c Mean values with different letters are significantly different (p < 0.05) among groups, determined by Duncan’s multiple range test.

3.1.2. DPPH and ABTS Radical Scavenging Activity

Results are shown in Figure 1b,c. DPPH and ABTS radical scavenging activities showed a tendency to increase with the concentration of quercetin and WOEE. In 1000 μg/mL of
quercetin and WOEE, the DPPH radical scavenging activity was 14.78% and 18.47%, and the ABTS radical scavenging activity was 28.25% and 60.07%, respectively. ABTS radical scavenging activity was higher than that of DPPH radical scavenging activity. It was reported previously that onion showed high ABTS radical scavenging ability, and similar results were found in WOEE [22]. In this study, the higher the concentration of onion extract, the higher the antioxidant activity was found in WO extract (Figure 1c), which was consistent with the previous study [23].

3.1.3. Superoxide Dismutase and Catalase Activities

SOD is an enzyme that breaks down superoxide (O$_2^-$) radicals into molecular oxygen (O$_2$) and hydrogen peroxide (H$_2$O$_2$). Hydrogen peroxide also damages cells and is generally catalyzed by catalase and peroxidase enzymes. Therefore, SOD is the enzyme responsible for the primary defense mechanism of all living cells that perform aerobic respiration [24]. Previous studies reported that SOD was an enzyme with a potential anti-inflammatory effect due to its ability to remove peroxide-free radicals [25]. It was reported that SOD acted as a second messenger in inflammatory cytokine production, and SOD mimetics are known to inhibit cytokine production, including production of IL-1β, IL-6, and TNF-α [26]. SOD activity increased with increasing concentrations of quercetin and WOEE (Figure 2a). SOD activity of quercetin ranged from 6.21 to 7.19 U/mL at 50, 100, and 250 µg/mL, and WOEE showed activity from 5.82 to 6.08 U/mL at the same concentration. Therefore, the results indicate that WOEE shows a similar tendency to quercetin, and WOEE may help remove reactive oxygen species from food and the body.

CAT is found to be a major antioxidant that reduces hydrogen peroxide in water and molecular oxygen [27]. CAT not only destroys hydrogen peroxide but also prevents the formation of other cytotoxic oxygen species and may have anti-inflammatory effects [28]. Figure 2b shows CAT activities of quercetin and WOEE in RAW 264.7 cells treated with LPS. As a result of CAT, both quercetin and WOEE showed higher activity than the untreated control group. CAT activities significantly increased in the supernatants of the cells treated with quercetin or WOEE. It was reported that high inhibitory activity of CAT was observed in LPS-stimulated cells when the quercetin concentration was increased [1]. Cells defended themselves from ROS and RNS with antioxidant enzymes such as CAT and SOD. The SOD enzyme neutralizes the peroxide and CAT enzymes and catalyzes the reaction of H$_2$O$_2$, which is considered as radical, to water and oxygen [27]. The total antioxidant capacity increased due to the increase in catalase enzyme activity, and the ethanol extract of A. cepa

Figure 2. Antioxidant effects of quercetin and WOEE. (a) SOD and (b) CAT activities. The data were analyzed by one-way ANOVA using SPSS software and each bar presents the mean ± SE. (n = 3). a–c Mean values with different letters are significantly different (p < 0.05) among groups, determined by Duncan’s multiple range test.
bark exhibited high antioxidant properties [28]. Therefore, as the CAT activity increased, it had an antioxidant effect, showing similar results to this study. WOEE can be considered as a useful material in preventing and inhibiting inflammation by inhibiting the CAT activity.

3.2. Anti-Inflammatory Effects of WOEE

3.2.1. Cell Viability

RAW 264.7 cells are mouse macrophages used in inflammatory models to assess anti-inflammatory effects. The cell proliferation increased depending on the treatment concentration of WOEE. The cell viability cultured with WOEE was higher than that of quercetin. Previous studies reported that onion peel extracted by hot water increased cell viability and was not cytotoxic [29]. After confirming that WOEE has no cytotoxicity to RAW 264.7 cells, the following experiment was conducted (Figure 3a).

![Figure 3a. Cell viability of RAW 264.7 macrophages](image)

3.2.2. Nitric Oxide Production

NO is a free radical with a single electron that is physiologically generated from L-arginine under the influence of the nitric oxide synthase (NOS) enzyme [30]. Inducible NOS (iNOS) isoforms produce large amounts of NO and are partially responsible for tumor and immunomodulatory activity [31]. Quercetin and WOEE were used to treat RAW 264.7 cells at various concentrations with LPS, and the effects of suppressing NO production were observed. As a result, the increased NO production in RAW 264.7 cells by LPS treatment was suppressed in the quercetin and WOEE (Figure 3b). A previous study reported that Allium cepa L. inhibited NO production [32]. Another study reported that leek, yellow onions, and green onions inhibited NO production with increasing concentrations, and each showed a high anti-inflammatory effect [33]. The suppression of NO production in RAW 264.7 cells by LPS treatment appeared to be more suppressed in WOEE than in quercetin. These results suggest that WOEE has effective anti-inflammatory potential.

3.2.3. Inflammatory Cytokines Levels in the Supernatant of the RAW 264.7 Cells Cultured with WOEE

To analyze the anti-inflammatory efficacy of quercetin and WOEE, inflammation in RAW 264.7 cells was induced with LPS, and the samples were treated with various concentrations (50–250 µg/mL). As LPS is activated through TLR4 to express inflammatory mediators, pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are also generated [34]. Among these cytokines, IL-1β had a strong effect on pain and inflammatory processes in the peripheral and central regions [35]. IL-1β mediated the interaction between...
cells in the damaged area, such as glial and neuron cells, promoting synaptic activation and pain transmission, and may contribute to the pathogenesis of chronic pain. IL-6 plays an important role in the pathogenesis of neuropathy and inflammatory pain. In a previous study, the treatment that inhibited this interleukin was shown to attenuate allodynia and hyperalgesia [36]. However, it induces a variety of diseases through overproduction [37].

In this study, the secretion of each cytokine showed a concentration-dependent decrease upon the addition of quercetin and WOEE (Figure 4a–c). The LPS group treated with LPS alone showed 11.46, 111.8, and 7244.71 pg/mL in IL-1β, IL-6, and TNF-α levels, respectively, whereas quercetin and WOEE treated at concentrations of 50, 100, and 250 µg/mL decreased the cytokine levels in a dose-dependent manner. Quercetin inhibited the secretion of LPS-activated IL-1β, IL-6, and TNF-α proteins in the culture medium [38]. The hot water extraction of onions decreased cytokine secretions, as shown in [29]. WOEE also suppressed the secretion of LPS-induced pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in RAW 264.7 cells and the effects were comparable to quercetin as an anti-inflammatory agent.

**Figure 4.** Anti-inflammatory effects of quercetin and WOEE on LPS-stimulated RAW 264.7 macrophages. (a) IL-1β, (b) IL-6, and (c) TNF-α levels (pg/mL) in the supernatant. The data were analyzed by one-way ANOVA using SPSS software and each bar presents the mean ± SE. (n = 3). a–d Mean values with different letters are significantly different (p < 0.05) among groups, determined by Duncan’s multiple range test.

### 3.2.4. IL-1β, IL-6, and IL-12 mRNA Expression in the Cells Cultured with WOEE

Inflammatory cytokines are a type of glycoprotein released from cells that are used for signaling for immune responses. Among inflammatory cytokines, IL-1β and IL-6 are produced in the early stages of inflammation and act on endothelial cells and leukocytes, a substance that induces inflammation. The stronger the acute inflammatory response, the higher the expression [39]. It has been reported that inflammatory cells release pro-inflammatory cytokines such as IL-1β, IL-6, and IL-12 [40]. Therefore, the inflammatory response in the corresponding tissues or cells can be evaluated through the expression of IL-1β, IL-6, and IL-12, which are expressed when the inflammatory response is induced. Compared to the control induced with LPS only, significant reductions in IL-1β, IL-6, and IL-12 expressions were found in the cells treated with quercetin and WOEE (Figure 5a–c) in a dose-dependent manner. In a previous study, it was reported that quercetin inhibited the secretion of LPS-activated IL-1β, IL-6, and TNF-α proteins in the culture medium [37]. It was also reported that onion aqueous extract decreased TNF-α mRNA expression [41]. The chronic inflammatory conditions were highly related with cardiovascular risk [42]. Consumption of onions influences lipid blood chemistry and improves indices of cardiovascular health [43–45]. These results supported that the WOEE could suppress the inflammatory response of RAW 264.7 cells, and it could be used as a healthy food and not only as a by-product.
4. Conclusions

In this study, the antioxidant and anti-inflammatory effects of WOEE were examined. WOEE showed a high amount of polyphenol and high DPPH and ABTS radical scavenging activities, SOD, and CAT activities compared to the quercetin. WOEE decreased the secretions of NO and inflammatory cytokines (IL-1β, IL-6, and TNF-α), and mRNA expressions of IL-1β and IL-12. The effects were comparable to quercetin, which is well known for its antioxidant and anti-inflammatory activities. Therefore, WO may be used as antioxidant and anti-inflammatory material to prevent related diseases, such as cardiovascular disease, asthma, atopic dermatitis, and arthritis, and to improve related and highly possible health conditions. However, more studies on the anti-inflammatory effects of WOs should be conducted in in vivo models to verify its mechanism.

Author Contributions: S.-H.L. conceived and designed the study. S.-H.L., J.-H.K., J.-S.K., S.-H.K., S.-H.J., S.-K.L., U.-Y.J. and J.-E.J. conducted experiments and analyzed data. J.-H.K. and S.-H.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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