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Effect of micronization process on the functional component content and anti-inflammatory activity of *Luffa cylindrical* peel

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ABSTRACT

The objectives of this study were to compare the anti-inflammatory effect of ethyl extract from micronized and nonmicronized luffa peels in BALB/c mice, and to analyse the functional components in both extracts. Results showed that the concentrations of nitric oxide, cytokines and prostaglandin E_2 (PGE_2) were reduced by feeding both of extracts for 20 days at a dose of 50 mg/kg BW. Also, the liver function index, number of white blood cell (WBC) as well as B cell and T cell, was inhibited after feeding of extract. Micronized sample showed more efficiency in reducing the level of WBC, glutamic pyruvic transaminase, interleukin-1β, tumour necrosis factor-α and PGE_2 than nonmicronized extract. Among the functional components, oleanolic acid and chlorophylls present more abundant amounts in micronized luffa peel. Overall, by means of size reduction, micronization process potentially increased the extraction of functional component, thus improved anti-inflammatory activity of luffa peels.

1. Introduction

In recent years, the reuse of the byproducts of fruit and vegetable processing has received growing attention. Most products available on the market are essential oils and detergents made from the fruit peels of citruses. However, *Luffa cylindrica* is another common vegetable worldwide, but its peel is generally processed into compost. This study investigated the functional ingredients and anti-inflammatory activity of the fruit peels of *L. cylindrica*.

Luffa (*L. cylindrica* Roem) is a subtropical vegetable widely cultivated in Asia, India, Brazil, and the United States. Studies have shown that luffa contains multiple functional ingredients, such as polyphenols, flavonoids, anthocyanins, vitamin C, saponin, carotenoids, and chlorophylls (Bor, Chen, & Yen, 2006; Kao, Huang, & Chen, 2012), and exhibits physiological activity such as antioxidation, antibacterial function, blood lipid and blood sugar regulation, and immune regulation (Bulbul, Zulfiker, Hamid, Khatun, & Begum, 2011; Hazra et al., 2011; Khajuria, Gupta, Garaib, & Wakhloob, 2007; Muthumani et al., 2010). In addition, our published study (Kao et al., 2012)

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revealed that luffa peel extracts can suppress the inflammatory response to RAW264.7 cells.

Micronization has been applied to functional and fortified foods. When food particles reach micron or nanometre scales, the increase in their surface area enhances water absorption, flavour release, and bioavailability, thereby strengthening the physiological functions of the foods, facilitating nutrient transportation to and absorption by the body (Sanguansri & Augustin, 2006). In recent years, studies on micronization have focused on powder properties; few studies have emphasized the effect on physiological activity.

Our previously published study indicated that luffa peel contains phenolic compounds, flavonoids, triterpene acid, carotenoids, and chlorophyll. In a cell model, both the peel and pulp can suppress inflammatory responses. In particular, the ethyl acetate extract of peel exerts the optimal effect (Kao et al., 2012). Consequently, the present study adopted an animal model to further examine the effect of luffa peel. Specifically, the anti-inflammatory effects of luffa peels before and after micronization were compared, and the functional components in the extracts were analysed to clarify how the anti-inflammatory activity of luffa peel is related to micronization and functional components.

2. Materials and methods

2.1. Materials

Edible *L. cylindrica* (L.) Roem was procured from Nan-Tou county, Taiwan. A total of 24 kg of luffa was cleaned, followed by removing peel with a knife. Then luffa peel was collected and freeze-dried under 60 millitorr at –50 °C and vacuum packed for storage at –20 °C until use.

2.2. Chemicals and reagents

The derivatization agent Sylon BTZ and standards including triterpene acids, chlorophylls and carotenoids as well as internal standard zinc-phthalocyanine and all-*trans*-β-apo-8′-carotenal were separately from Sigma-Aldrich (St. Louis, MO, USA), Extrasynthese (Genay Cedex, France), ChromaDex (Irvine, CA, USA), Fluka (Buchs, Switzerland) and Alfa Aesar (Ward Hill, MA, USA). HPLC and analytical grade solvents were from Merck (Darmstadt, Germany). Deionized water was obtained through a Milli-Q water purification system from Millipore (Billerica, MA, USA). The cell culture reagents were from Invitrogen (Carlsbad, CA, USA) and HyClone (Logan, UT, USA). Lipopolysaccharide (from *Escherichia coli* O26:B6), lipopolysaccharide (from *E. coli* O55:B5), concanavalin A (ConA), interferon-γ (IFN-γ) and ammonium pyrrolidinedithiocarbamate (PDTC) were from Sigma-Aldrich. The prostaglandin E_2 (PGE_2) EIA kit was from Enzo Life Sciences (Farmingdale, NY, USA). The DuoSet ELISA development system for determination of cytokine was from R&D System (Minneapolis, MN).

2.3. Instrumentation

The HPLC-DAD-MS system was from Agilent Co. (Palo Alto, CA, USA), being composed of G1311A pump, G1316A column temperature controller, G1315B photodiode-array detector and 6130 Quadrupole mass spectrometer with multiple ion source (APCI and ESI). The GC-MS system was also from Agilent, composed of a GC system (6890) and mass spectrometer (5973). The PM100 ball miller was from Retsch Co. (Haan, Germany). The freeze dryer (FD24) was from Gin-Ming Co. (Taipei, Taiwan). The Sorvall RC5C high-speed centrifuge was from Du Pont Co. (Wilmington, DE, USA). The VersaMax ELISA microplate reader was from Molecular Devices Co. (Sunnyvale, CA, USA).

2.4. Preparation of micronized luffa peel

Freeze-dried luffa peels were first pulverized and sieved to 40 mesh. Then 20 g luffa peel powder was micronized with 540 g zirconium oxide bead (5 mm diameter) in a 500 mL milling jar by planetary ball milling at 2.68 *g* for 6 h in every single run. Micronized luffa peel powder was vacuum packed for storage at –20 °C in dark until use.

2.5. Preparation of ethyl acetate extract from nonmicronized (NML) and micronized luffa (ML) peels

A method based on Kao et al. (2012) was modified to extract luffa peel. Every 2 g each of nonmicronized or micronized powder sample of luffa peel was put in 12 centrifuged flask with a volume of 250 mL separately, then every flask added 100 mL of ethyl acetate, after which each mixture was shaken at 25 °C for 1 h and then centrifuged at 100,621 *g* for 20 min. The supernatants were collected and filtered through Waterman No. 1 filter papers, followed by evaporating to dryness and weighing to obtain 410.4 and 625.6 mg for nonmicronized extract and micronized extract, respectively. Both extracts were dissolved in 95% EtOH for functional component analysis, and also dissolved in soybean oil with a final concentration of 5 mg/mL for animal test.

2.6. Experimental animals

This animal test was permitted by Institutional Animal Care and Use Committee established in Fu Jen Catholic University. A total of 50 SPF (specific pathogen free) male BALB/c mice (8 weeks old, body weight 20 ± 1 g) were purchased from BioLASCO (Taipei, Taiwan). They were housed in individual ventilation cages (IVC) in the animal centre of Fu Jen University with a temperature at 22 °C and relative humidity at 50–60%, and fed a laboratory rodent diet 5001 (LabDiet Co, St. Louis, MO, USA) *ad libitum*. The body weight was measured every week during the feeding period. After 1-week adaptation, mice were divided into 5 groups with 10 each and administered with the following solutions of *luffa* peel extract by tube feeding daily at a dose of 50 mg/kg BW: (1) Normal group – 0.2 mL of soybean oil (sample solvent) for each mouse; (2) Control group – 0.2 mL of soybean oil for each mouse; (3) PDTC group (positive control) – 0.2 mL of soybean oil for each mouse; (4) NML group – 0.2 mL of nonmicronized luffa peel extract dissolved in soybean oil for each mouse; (5) ML group – 0.2 mL of micronized luffa peel extract dissolved in soybean oil for each mouse. After feeding for 20 days, the PDTC group was first given an intraperitoneal injection (IP) of PDTC at a level of 100 mg/kg BW, followed by IP of 1 mg/kg BW of LPS to induce systemic inflammation