



Original Research Article

Effect of broken cell percentage in powdered citrus peel tissue on elution of constituent materials to a solvent

Yidi Cai and Yukiharu Ogawa*

Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo 271-8510, Japan

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ABSTRACT

Unlike the animal cell, the plant cell is framed by a cell wall as an overlayer of the cell membrane. Organelle which in cytoplasm such as vacuole is involved by the cell membrane and cell wall. Antioxidant materials like flavonoids are mainly stored in the vacuole. Therefore, these structural attributes would relate to the elution of intracellular antioxidant materials during processing or the enzyme accessibility during digestion. In this study, the effect of cell disruption percentages of citrus peel powder as a simulated model of plant-based materials on the elution of intracellular constituents was investigated. The flavedo layer of citrus peel was flaked using grater, freeze dried, pulverized using stone mill and sieved to separate particle size. These various size powders were regarded as a simulated tissue model which consist of various percentages of disrupted cell. The diameter of cells and powdered particles was measured by extrapolation of the microscopic image and averaged. The various size powders were soaked in distill water, ethanol and hexane, respectively, for 30 minutes. The absorbance spectra of soaked liquid were measured to examine the degree of elution from powders. The result showed that the absorbance at all range of visible light spectra decreased with increase in powder diameter. This tends to follow the approximate exponential decrease as shown by the changes in cell disruption percentage. Therefore, this also figured out that an increase of disrupted cell ratio would indicate comparatively higher elution from the powder, since the percentage of the disrupted cell connected to powder diameter. Thus, it was suggested that the structural attribute of cell-based plant tissue could connect to the elution property of intracellular materials. This would relate with postprandial nutritional properties of plant-based foods.

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* Corresponding author: Tel.: +81-473-088-848; fax: +81-473-088-848

E-mail address: ogwy@faculty.chiba-u.jp



INTRODUCTION

Plant cells consist of cell walls which envelope protoplasm, and it determines structural factors such as a cell strength, shape, and size (Bidhendi et al, 2016). A fiber as a main component of the cell wall is involved in elongation, biological defense, regulation of growth and differentiation of plant, etc. (Cosgrove, 2005). In general, the fiber, normally called as a dietary fiber, is regarded as an indigestible material for human digestion system, except for at large intestine in which intestinal microflora can partly digest the dietary fiber (Mudgil et al, 2013). Most of ingredients contained in plant-based foods are involved in the cell matrix surrounded by such cell wall materials (Cosgrove, 2005). Therefore, it could affect a digestibility of intracellular components if the cell matrix structure is existed.

Nutrients of plant-based food are mostly present in cells protected by a cell wall matrix. To absorb such nutrients by the human digestion system, physical destruction or chemical denaturation of the cell wall matrix is necessary for releasing them. A processing and cooking including cutting and crushing is one of the most useful solution for the increase in digestibility of plant-based food. Lots of studies reported that the structural changes in food matrix with cell destruction influenced on the human digestibility (Tamura et al, 2015).

Fruits and vegetables, a main plant-based food, generally contain flavonoids, vitamins, phenolics and antioxidants which support to maintain human health (Hur et al, 2014). The main digestive organ of human for antioxidant absorption is small intestine (Bouayed et al, 2011). Thus, the cell wall attribute concerning with nutrient absorption at the small intestine must be important. However, few reports could be found for the digestive properties of antioxidant materials in fruits and vegetables.

To examine such properties regarding antioxidant digestion, various size particles from citrus peel tissue were selected as a plant tissue model and elution properties from the particles to three types of solvent were investigated in this study. The relationships between particle size and color changes of its elution were also evaluated.

MATERIALS AND METHODS

Materials

Citrus unshiu (*Citrus unshiu*, cv. Miyagawa-wase) was used as a source of peel tissue in this study. The citrus fruit was harvested in the experimental field of Ehime University, Ehime, Japan in 2013 and transported to Matsudo, where was experimental place within 3 days after harvest.

Sample preparations

The citrus fruits were roughly washed by a tap water and then grated from the surface of peel using a grater (Microplane, Ikesyo, Tokyo, Japan) to correct tissues of flavedo layer. The corrected peel tissue was frozen using a freezer (NW 101953, Haier Japan, Osaka, Japan) at -30°C and stored. The frozen tissue was freeze-dried using a freeze dryer (FDU-100, Eyela, Tokyo, Japan). The freeze-dried tissue was roughly pulverized using a manual grinder (GM-45GT, Kyocera, Kyoto, Japan) or finely pulverized using an electric grinder (EU6820P, Panasonic, Osaka, Japan). The pulverized tissue was sieved and separated to various particle size distribution groups by 75, 150 and 300 µm mesh sieves. Thus, the sieved particles were separated to the particle size of 75 ~ 150 µm, 150 ~ 300 µm, and > 300 µm.

Particle and cell size measurement

The separated particles were observed using a scanning electron microscope (SU1510, Hitachi high-technologies, Tokyo, Japan) to obtain an individual particle image. The diameter of a particle was measured as a circumscribed circle equivalent diameter using a graphic software (Photoshop CS6; Adobe, San Jose, CA, USA), and the averaged diameter was calculated from approximately 100 particles, which were randomly selected.

The separated particles were also observed using simple fluorescent observation mode of the confocal laser scanning microscope (LSM510, Carl Zeiss, Oberkochen, Germany) equipped with UV filter. The cell size in the particle was measured from the microscopic image and averaged by approximately 100 cell images.

Absorbance spectrum

The separated particles were soaked in a distilled water, an ethanol, or an n-hexane as a solvent to evaluate a degree of elution from cell matrices of the particle. 500 mg of each separated particles was added in 50 mL of each solvent and stirred for 30 sec. The particle-solvent mixtures were allowed to stand for 30 minutes, and then filtered through Whatman no. 1 filter paper (GE Healthcare UK, Buckinghamshire, UK) to separate the insoluble components. The filtered medium was measured the absorbance spectrum using a spectrophotometer (V-630BIO, Jasco, Tokyo, Japan) for the wavelength ranging from 190 to 1100 nm with 1 nm resolution.

Statistical analysis

Results were calculated as means ± standard deviations of three replicates. Subsequently, Tukey's test, in conjunction with an analysis of variance (ANOVA) was used to determine significant differences among means, at an a priori significance level of $P < 0.05$ using the Statistical Package for Social Sciences (SPSS for Windows, SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Particle size distribution

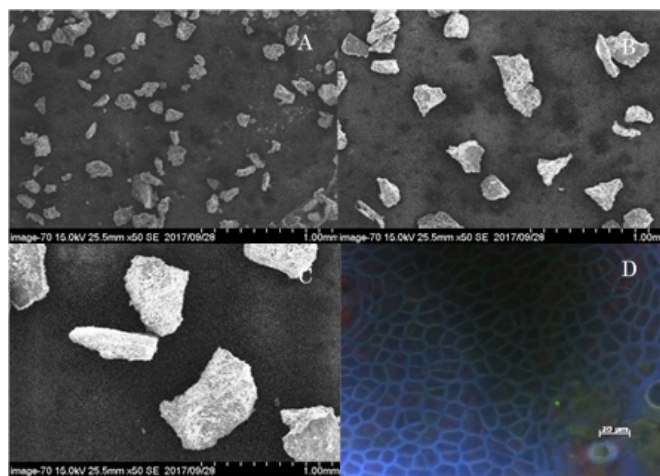


Figure 1. Microscopic images of each separated particle and cells in a particle (A: 75 ~ 150 µm, B: 150 ~ 300 µm, C: > 300 µm, D: Fluorescent image of a particle).

Fig. 1 shows images of each separated particle observed by a scanning electron microscope (SEM) and cells in a particle by a fluorescent microscope. The cell matrix can clearly be recognized in the fluorescent image (Fig. 1D). The particle size shown in each SEM image (Fig. 1A, B, C) was mostly similar, however the shape was not uniform. Thus, the particle size was measured as a circumscribed circle equivalent diameter and determined as means ± standard deviations (S.D.) of approximately 100 particles which were randomly selected in several SEM images. The calculated results are shown in Table 1. The mean diameter of separated particles which were sieved from 75 to 150 μm and from 150 to 300 μm closely approximated to upper sieve size. This meant the particle size distribution would not form the normal distribution. Therefore, the results of particle size were described not using distribution parameter but calculated means in this experiment.

Disrupted cell percentage

Theoretically, cells at the fringe area of particles should mostly be disrupted by the pulverizing. However, cells inside the particle should be maintained their structure. As shown in Fig. 1D, the intact cell diameter is looked roughly similar. Thus, it can be averaged using the method of circumscribed circle equivalent diameter (Table 1). If the particle can be regarded as a spherical object, the particle volume can be calculated from the particle diameter. From the averaged cell size, the volume occupied by intact cells can also be calculated. Thus, the disrupted cell percentage can theoretically be calculated using following equation. The results are shown in Table 1 and a theoretical relationship between the particle diameter and the calculated disrupted cell percentage is shown in Fig. 2. As shown in the figure, the relationship could be approximated an exponential curve.

$$\text{Disrupted cell percentage} = \frac{V_p - V_i}{V_p} \times 100$$

where V_p is a volume of the spherical particle and V_i is a volume of all intact cells in the particle

Table 1. Averaged particle diameters and calculated cell disruption percentages (n=100)

averaged cell diameter (μm)	mean diameter of each separated particle (μm)	disrupted cell percentage (%)
	132 ± 47	31.43
15.6	279 ± 86	15.85
	591 ± 279	7.71

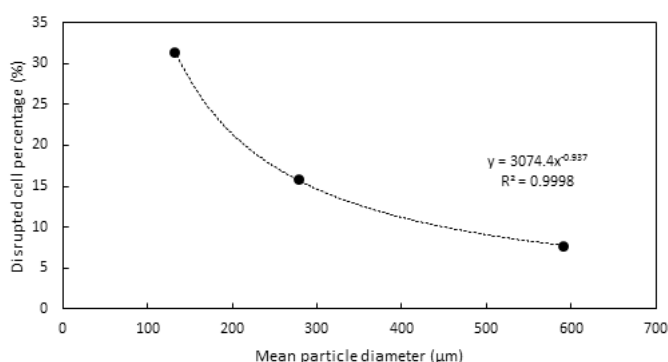


Figure 2. A theoretical relationship between the particle diameter and the calculated disrupted cell percentage

Relationships between particle diameter and elution properties to solvents

The separated particles were eluted to three types of solvent, such as distilled water, ethanol and hexane. The eluted solvents in which separated particles from citrus peel tissue was soaked and filtered showed yellowish variation due to changes in the concentration. Fig. 3 depicts the absorbance spectra of each eluted solvent. The absorbance spectra ranging from 580 ~ 630 nm were magnified in this figure.

The absorbance was varied by the particle size at all wavelength, although the particle was produced from the same source. It showed that the lower absorbance appeared to the solvent of the larger particle. Ethanol and hexane at the shorter wavelength ranging from 400 to 500 nm showed much clearer the relationships to the particle size. These results indicated that the disengaging degree of soluble compounds in the particle to the solvents was variable by physical particle size and solvent type, respectively. This also revealed that the soluble constituents in organic solvent were affected by the particle size. Since the particle size related to the cell disruption percentage, it should influence on the dissolution rate of intracellular substances like vitamin C.

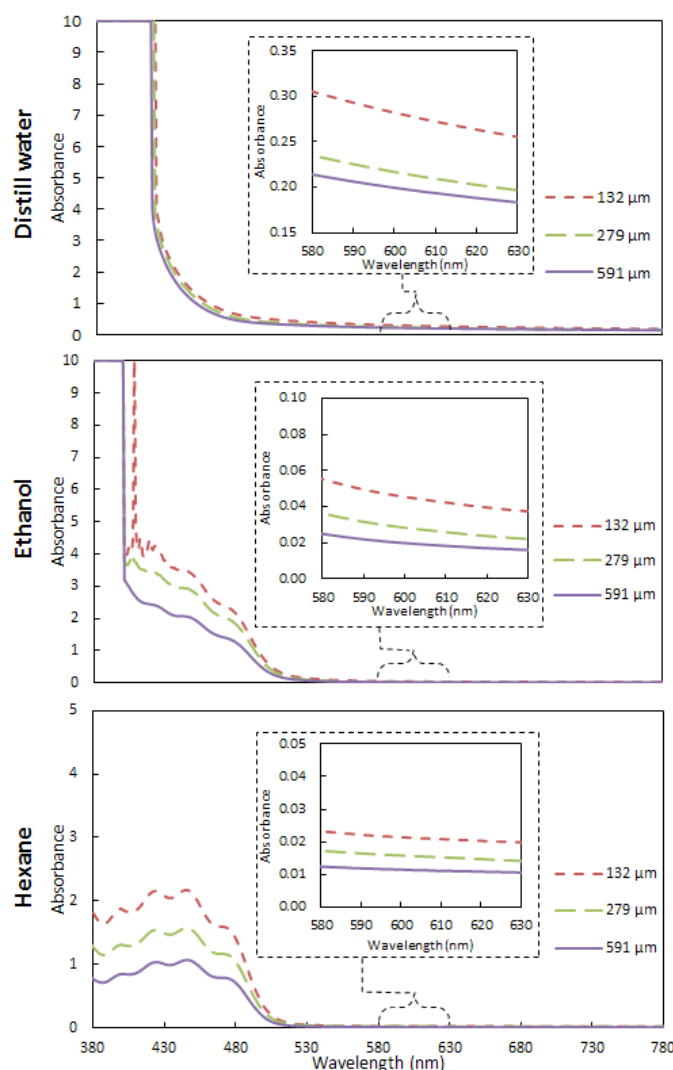


Figure 3. Absorbance spectra of eluted solvents for various size particles soaked in different types of solvent

From some previous reports regarding rice digestion study, changes in cell structural attributes influenced on the starch digestibility which increased in the increase of cell disruption percentage. Therefore, it can be suggested that the dissolution rate of intracellular substances in plant-based materials would be related with the cell disruption percentage.

Relationships between particle diameter and absorbance of eluted solvent

Fig. 4 shows relationships between particle diameter and absorbance at several wavelengths corresponding to yellow region. These results represented exponential decreasing with increase in particle diameter that is similar with a theoretical relationship for disrupted cell percentage. Thus, it was suggested that the eluted substances might mainly be disengaged from the disrupted cells at the conditions employed in this study.

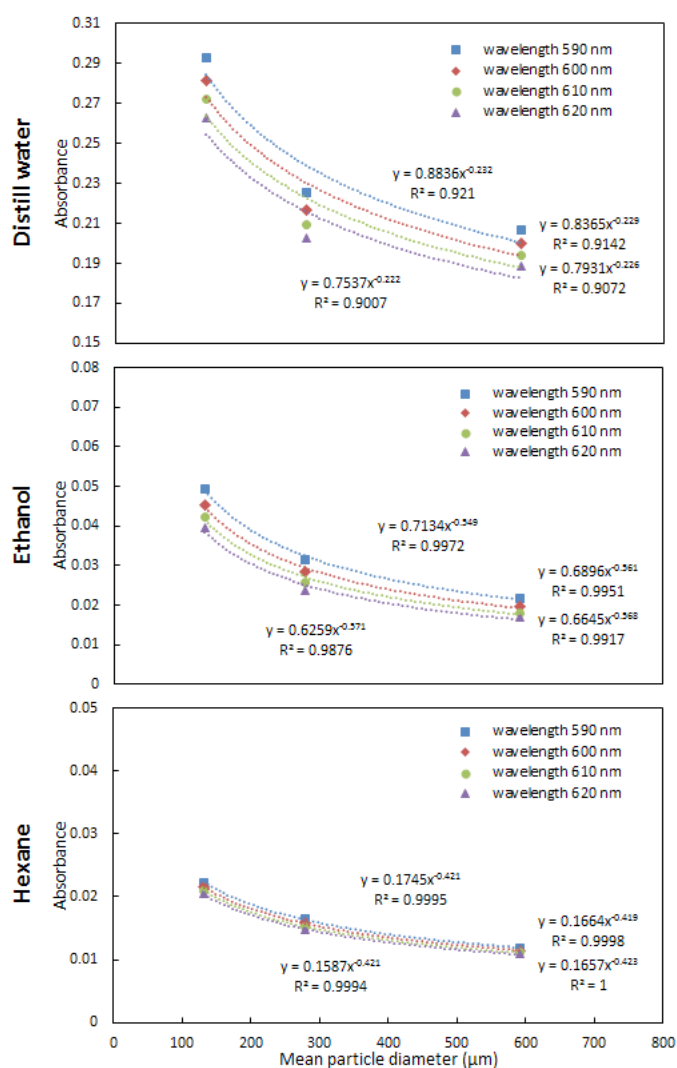


Figure 4. Relationships between particle diameter and absorbance of eluted solvent

CONCLUSIONS

In general, a plant-based material is constructed by cell wall matrices, which can prohibit the elution of intracellular substances. Mechanically cell disruptions could allow the elution of such intracellular compounds. This also suggested that the bioavailability of various nutrients in a plant-based food could be varied by a degree of mechanical disruptions which relates to structural cell and/or tissue attributes such as rheological properties.

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