ANTIOXIDANT ACTIVITY OF DIFFERENT DRIED INDONESIAN CITRUS PEEL VARIETIES

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Abstract: The high production and market demand of citrus fruits for household and industrial consumption make it peels one of the most abundant wastes found in the environment. On the other hand, citrus peel is well known for bioactive compounds that beneficial to human health, including phenolic, flavonoids, and antioxidants. Recently, citrus peel waste is starting to be used for food ingredients. The drying technique is one of the solutions that can be used for preserving citrus peel waste which has high water content. This study was objected to identifying the effect of the drying type (tray dryer and freeze dryer) on the antioxidant activity alteration of three citrus varieties that commonly cultivated in Indonesia, i.e. pummelo/jeruk Bali (*C. maxima* Herr.), mandarine citrus/jeruk keprok (*Citrus reticulate*) and sweet orange/tangerine/jeruk Medan (*C. microcarpa* L. and *C. sinensis* L.). The antioxidant activity was measured by calculating the IC_{50} value. The results showed ethanol extract of fresh mandarin citrus peels showed the lowest IC_{50} (14.46 ± 3.63 mg/mL) compared to fresh pummelo peels (26.48 ± 5.17 mg/mL) and fresh tangerine peels (16.94 ± 1.51 mg/mL). Tray dryer technique reduced the IC_{50} value of the peel extracts of pummelo, mandarine citrus and tangerine were 78.92%, 72.34%, and 79.69%, respectively. Whereas Freeze dryer drying reduced the IC_{50} value of pummelo, mandarine citrus and tangerine peel extracts were 59.21%, 69.43%, and 80.46%, respectively.

Key words: freeze dryer, pummelo, mandarin citrus, tangerine, tray dryer

1. INTRODUCTION

Citrus fruits are the fruits plants cultivated throughout the world, from tropical to subtropical countries. In 2016, the world's citrus production reached 124.24 million tons. Meanwhile, citrus production in Indonesia reaches 1.57 million tons (FAO 2017). In general, there are three varieties of local citrus cultivated in Indonesia, i.e. the pummelo/jeruk Bali (C. maxima Herr.), mandarin citrus/jeruk keprok (Citrus reticulate), and the sweet orange/ tangerine/jeruk Medan (C. microcarpa L. and C. sinensis L.) (Kemenristek 2002).

The citrus processing industry, such as juice, jam, and marmalade, utilizes approximately 23.54 million tons of citrus and produces large amounts of citrus peel waste (FAO 2017). The citrus processing industry produces at least 50-60% of organic waste which is mostly dominated by citrus peels. This high level of organic waste becomes a source of economic and environmental problems. At the same time, efforts to reduce the environmental negative impact of citrus peel waste and its processing management were need (Satari and Karimi 2018).

Recently, many kinds of research have focused on increasing the capacity and sophistication of waste treatment systems, such as conversion to provide added value and the isolation of bioactive compounds or high-value chemicals from citrus peel waste (Puri et al. 2012). The high content of fiber, pectin, and polyphenol compounds (flavonoids and phenolic acids), carotenoids, and essential oils in citrus peel waste has health benefitting potentials. The newly “not peer-reviewed” article also shows the potential for hesperidin compounds in citrus as anti-virus compounds against Covid-19 (Utomo et al. 2020).

Citrus peel waste is now used in the design of functional foods, supplements, flavoring agents in processed food, preservatives, health drinks, and power drinks. The addition of citrus peel is known to improve the taste and aroma of food. In the world of cosmetics, the phytochemical compounds of citrus peel are used as antifungal, antibacterial, soap, perfume, and toiletries. Currently, in European countries, there has been a growing industry that uses citrus peel as one of the raw materials for their products, which are found in infusion products, confits (sweets), chips, bakery products, sweets and candy, jams, and marmalade (Bordas 2020). CP Kelco is one of the ingredient companies in Atlanta in 2019, has also launched fiber ingredient products made from citrus peels (Siegner 2019). Nowadays consumers are increasingly aware of the need for healthy nutrition, so the demand for natural ingredients as food ingredients encourages research to identify bioactive compounds derived from plants to replace expensive synthetic ingredients. Several studies have shown that citrus peel waste has a source of functional compounds and its use in food formulations is becoming a promising prospect (Mahato et al. 2018).

One of the problems in the utilization of Citrus peel waste is the high water content leading to the ease to decomposition. One of the processing technologies that can be applied to increase its shelf life is drying. Drying also lower storage and transportation cost and produce materials with better storage ability (Shofinita, 2013). Several studies mentioned that the content of phenolic acids, flavonols, total flavonoids, polyphenols, and the antioxidant activity of citrus peels increased after the drying process (Singh et al. 2019). Chen et al. (2011) reported that methanol extract of dried orange peels (*C. sinensis)* contained around two-fold more total phenolic content than the fresh peel. Flavonoid as well as phenolic acids content also increased by drying at 90-100 ^{o}C before methanolic extraction of *C.sinensis*. The IC50 values for DPPH radical scavenging effect of citrus peel increased with heating due to the increade liberation of phenolic and flavonoids content (Chen et al, 2011). So far, no data is available regarding the effect of different drying techniques (tray dryer and freeze dryer) on the antioxidant activity of citrus peels, especially for three local Indonesian varieties (pummelo, mandarin citrus, and tangerine). This study was objected to identifying the effect of the type of drying (tray dryer and freeze dryer) on the antioxidant activity alteration of the citrus peel of its three varieties.

2. METHODS

The study was conducted from August to October 2020 at the Science Laboratory, and Food Processing Laboratory, Faculty of Halal Food Science, Djuanda University, Bogor.

2.1 Materials and Tools

This study used three citrus peels varieties, there were pummelo (*Citrur maxima* Merr.), tangerine (*Citrus reticulate*), and tangerine (*Citrus sinensis* L.), ethanol, DPPH, and methanol. The tools used in this study were tray dryer, freeze dryer, blender, sieve, knife, cutting board, scale, spectrophotometer, stirrer, and cuvette.

2.2 Method

Sample Preparation (Chen et al 2011, Dewi 2019 with modification)
Citrus fruits were firstly collected from the market then stored in the refrigerator to maintain their freshness. The citrus peels with a maturity level of 80% were selected uniformly based on smooth, shiny skin texture, firm color, fruit size and freshness. All fruits were used for good quality without blemishes or rot. After washing, the citrus peels were then separated from its fruits and the outer skin (exocarp) which is greenish-yellow in color were separated from the

inner skin which is white (mesocarp/pulp). The citrus peels were then cut into 1-2 cm sizes and dried using a tray dryer at 90 ^{o}C for 12 hours and by using a freeze dryer at -30 ^{o}C for 4 days. The dried citrus peels were then ground using a grinder to become a powder and filtered. The powder was then put into a vacuum plastic and stored in the freezer until the next extraction.

Citrus Peel Extraction (Paulpriya and Mohan 2012 with modification)
Citrus peel extraction was carried out by maceration using ethanol 96% (1:10). The maceration process was conducted in a dark place, avoiding direct light exposure. The solution was then agitated at a speed of 500 rpm at room temperature within 2 hours. The maceration process was then continued for 24 hours. The extract was filtered through Whatman filter paper and stored at -20˚C for further antioxidant activity analysis.

Moisture content (SNI 01-2891-1992)
The sample is weighed as much as 1-2 grams into a plate that has known its weight. The sample was then dried in an oven at 105 ^{o}C to obtain a constant weight. After the sample is dried into a desiccator, then it is weighed.
The water content is calculated by the formula:

$$\text{Water content (\%)} = \frac{w1}{w2} x100$$

W1 = weight sample before drying (in gram)
W2 = weight of sample after drying (in gram)

Yield

The yield of citrus peel powder was calculated by weighing the fresh citrus peel and the citrus peel powder produced from each drying. The yield of citrus peel powder was calculated by using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of citrus peel powder}}{\text{Weight of fresh peel powder}} \times 100\%$$

Antioxidant Activity (Munarko et al. 2020)
A total of 1.0 mL of the sample extract in each dilution series was inserted into a test tube, and then 3.0 mL of DPPH reagent was added in 140 µM methanol (Sigma Aldrich, Germany). As control was 80% ethanol to replace the sample and methanol as the blank. The solution was then vortexed, and allowed to stand for 60 minutes in the dark at room temperature, then the absorbance measured at a wavelength of 515 nm and the percent inhibition was calculated. The percentage of inhibition was calculated by the following equation:

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100\ \%$$

3. RESULTS AND DISCUSSION

The water content of the samples that have been dried using tray and freeze dryer was estimated, the primary goal of drying is to reach the minimum of moisture content and the lack of burning samples. This led to the differences in the endpoint of each drying method, so the moisture content in different drying method may differ from each other. Table 1 showed that there were significant differences moisture content in the dried sample that used different drying methods, especially in mandarin and tangerine. Freeze dried sample had lower moisture content than try dried sample in mandarin and tangerine. While, for pamelo there was no significant difference in moisture content on both dried samples.

Table 1 Moisture content of pummelo, mandarin citrus, tangerine

Citrus variety	Drying method	Water content (% w.b)
Pummelo	Fresh	76.09±0.12^{c}
	Tray Drying	10.76±0.07^{g}
	Freeze Drying	10.84±0.06^{g}
Mandarin citrus	Fresh	86.86±0.78^{a}
	Tray Drying	19.45±0.42^{d}
	Freeze Drying	18.47±0.83^{e}
Tangerine	Fresh	82.92±0.13^{b}
	Tray Drying	19.33±0.13^{d}
	Freeze Drying	15.38±0.18^{f}

Note: *Mean in a column with the same letters are not significantly different at $p \leq 0.05$ (n = 2).

Figure 1 shows the effect of different drying methods on the yield of dried citrus peel. Based on the data, the same drying methods shows different yields for each variety, which may due to the differences between the composition of each citrus peel. Shofinita et al. (2015) stated that different citrus variety shows different in citric acid and sugar content, the lowest content of citric acid and reducing sugar give the highest yield in the spray drying process. Cell structure materials also could significantly affect the drying process among its cultivar (Huang et al., 2017). Based on the data, freeze-dried mandarin citrus peel give the highest yield and freeze-dried pummelo give the lowest. Compare to the tray drying method, only freeze-drying of mandarin citrus peel give a lower yield value while the other two were vice versa.

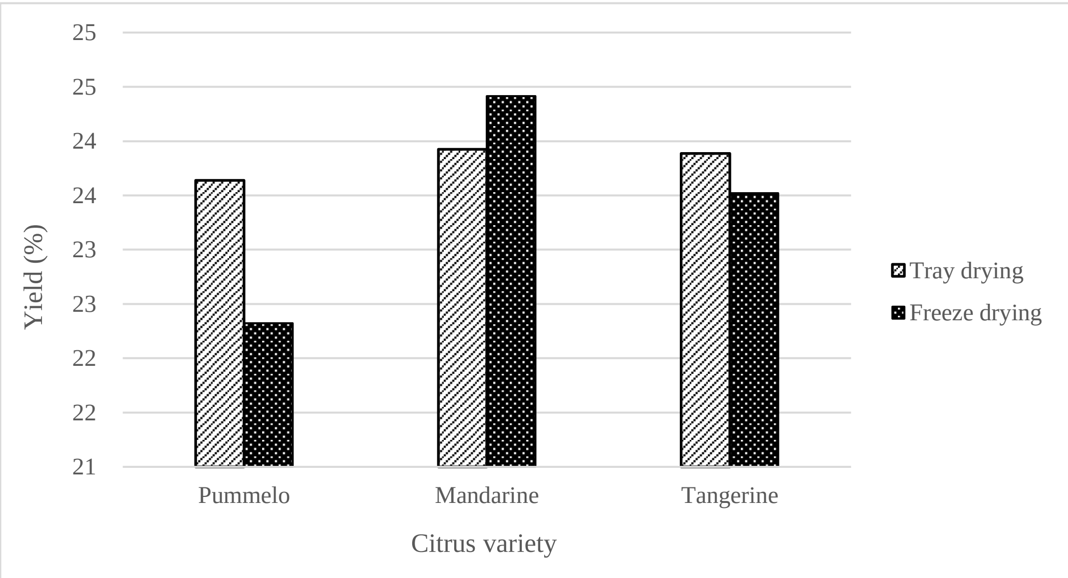

Figure 1 Yield (% d.b) of dried citru peel

Antioxidant activity test was carried out using the DPPH method (1,1-diphenyl-2-picrylhydrazyl). DPPH acts as a free radical compound, the antioxidant activity of the sample was measured based on its ability to capture DPPH compounds. The presence of antioxidant compounds in the sample will neutralize the DPPH radical by donating electrons to the DPPH compound then resulting in a change in the color of the test solution from purple to yellow (Parakash 2001). In the study present, the antioxidant activity of each sample was described by the IC_{50} value. The IC_{50} value showed the number of the most effective concentrations of the test compound in producing 50% free radical scavenging activity of DPPH compounds. The smaller IC_{50} value, the free radical scavenging activity becomes higher (Molyneux 2004). The IC_{50} value is presented in Table 2.

Table 2 The IC_{50} value of pummelo, mandarin citrus, tangerine

Citrus variety	Drying method	IC_{50} (mg/mL)
Pummelo	Fresh	26.48±5.17[a]
	Tray Drying	5.58±0.19[d]
	Freeze Drying	10.80±0.77[c]
Mandarin citrus	Fresh	14.46±3.63[c]
	Tray Drying	4.00±0.70[d]
	Freeze Drying	4.42±0.60[d]
Tangerine	Fresh	16.94±1.51[c]
	Tray Drying	3.44±0.34[d]
	Freeze Drying	3.31±0.44[d]

Note: *Mean in a column with the same letters are not significantly different at $p \leq 0.05$ (n = 2).

Based on the results, the ethanol extract of fresh mandarin citrus peel has the lowest IC_{50} value (14.46 ± 3.63 mg/mL) compared to fresh pummelo peel (26.48 ± 5.17 mg/mL) and fresh tangerine peel (16.94 ± 1.51 mg/mL). However, the IC_{50} value of fresh mandarin citrus peel did not differ significantly from tangerine peel. This indicated that tangerines had the highest oxidant activity of fresh citrus peel extract. The differences in the IC50 value related tothe nature nd characteistics of the diffret varieties of citrus fruit. Based on the literature, the methanol extract of fresh sweet orange (*C. sinensis*) peel originally from Taiwan has an IC_{50} value of 2.05 ± 0.10 mg/mL. This value is much smaller than the results in this study. The difference in the value of antioxidant activity is due to differences in varieties, geographic location, harvest time, the type of solvent that used for extraction and the extraction method (Singh et al. 2020). So far there are no data regarding the antioxidant activity of the extract of fresh pummelo and mandarine citrus which can be used as a comparison.

Drying technology is a popular processing technique for preserving various types of food because of its easy and cheap technique. This technique aims to reduce the moisture content in the material to a certain level that is acceptable for sales, storage and production purposes. One dryer type of the most used is the tray dryer. Samples with a certain thickness are spread out on a tray installed in layers so that a uniform drying rate is obtained. The uniformity of the rate of hot air flowed into tray dryers is a factor in the success of drying operations (Misha et al. 2013).

Based on the study results, the drying process with a tray dryer can significantly reduce the IC_{50} value of the citrus peel extract of the three varieties. The decrease in the IC_{50} value was the highest in tangerine peel extract, which was 79.69%. Meanwhile, pummelo and mandarin citrus decreased by 78.92% and 72.34%, respectively. Increased antioxidant activity of the citrus peel extract due to the drying process has also been previously reported by several researchers. Ho and Lin (2008) reported that the antioxidant activity of the Ponkan mandarin peel extract which was heated at 100^0C for 180 minutes increased up to 68% compared to those without heating.

A similar result was reported by Jeong et al. (2004) stated that the antioxidant activity of the ethanol extract and water from the peel of *C. unshiu* orange increased by 29.64-63.25% and 15.81-54.70%, respectively compared to the control after heating at 150 ^oC for 60 minutes. The increase in antioxidant activity due to this heating process is thought to be due to an increase in the release of phenolic and flavonoid compounds (Singh et al. 2020). Chen et al. (2011) reported that the higher the heating temperature, the IC_{50} value of the methanol extract of *C. sinensis* peel would decrease significantly. Tamanna and Mahmood (2015) showed that the heating process may cause the formation of new compounds that contribute to the antioxidant activity of dried citrus peel extract.

Based on literature studies, it is known that the IC_{50} value of 70% ethanol extract of dried pummelo peels is 574.02 ppm (Suryanita et al. 2019), while the IC_{50} of dry mandarine peel extract obtained by maceration in 96% ethanol solution for 3x24 hours is equal to 237.94 ppm (Sriarumtias et al. 2019), and the IC_{50} of the methanol extract of dry *C. sinensis* peel is 0.57 ± 0.01 mg/mL (Chen et al. 2011). This value is much smaller when compared to the results in this study. This difference in value is thought to be influenced by geographic location, harvest time, type of solvent and extraction method. The duration of maceration is known to have a significant effect on differences in antioxidant activity. Asendy et al. (2018) reported that the antioxidant activity of the ethanol extract of lemon peel increased at 18-36 hours of maceration time, but decreased at 48 and 72 hours.

Freeze dryer is a drying method by removing water from food through the sublimation technique of ice crystals. The drying process is carried out at a low temperature and vacuum conditions so that it can retain the nutritional components and bioactive compounds in foodstuffs (Bhatta et al. 2020). Based on the study results, the freeze dryer drying process significantly reduced the IC50 value of the citrus peel ethanol extract of the three varieties. Tangerine peel had the highest decrease of IC50 value (80.46%) than others. Meanwhile, the pummelo and mandarin citrus peels decreased by 59.21% and 69.43%, respectively. However, the IC50 value of pummelo and mandarin citrus peel extracts dried by tray dryer did not differ significantly from those dried using a freeze dryer. Papoutis et al. (2017) reported that drying lemon peels (C. limon) using hot air provided has a higher antioxidant activity value compared to freeze dryers. In contrast, Sun et al. (2015), reported that the antioxidant activity of various citrus varieties dried by freeze-drying is higher than that of hot air or sunlight. This difference is thought to be caused by differences in temperature, drying time and citrus varieties (Papoutis et al. 2017).

4. CONCLUSION

The moisture content in different drying method differ from each other. In general, freeze dried sample had lower moisture content than tray dried sample. same drying methods shows different yields for each variety, which may due to the differences between the composition of each citrus peel. The drying process, both tray dryer and freeze dryer, had a significant effect on the antioxidant activity of the peel extracts of pummelo, mandarine citrus and tangerine. Tray dryer tended to give the lower IC_{50} value than freeze dryer, except for tangerine samples. Extract sample of tangerine peel had the highest decrease (80.46%) in IC_{50} value which dried by freeze dryer, it was 3.31 ± 0.44 mg/mL. The drying process with tray dryer or freezes dryer can be used effectively to preserve citrus peel without reducing its antioxidant activity. Further studies are needed to determine the optimal drying conditions for each citrus peel variety and to identify compounds that contribute to its antioxidant activity.

ACKNOWLEDGMENT

This work was supported by Djuanda University Bogor under "The Beginner Lecturer Research Grant Program". Thanks to all those who have contributed in this study and preparation of manuscript.

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