

The Antioxidant Activity Comparison of *Malus sylvestris* Mill and Its Processed Products

Engrid Juni Astuti^{1*}, Agustin Rafikayanti¹, SeptianaTita Purwanto², Chenchen Puspitasari², Wenni Fista Rika², Yeni Yesica²

¹ Junior Research, Department of Pharmacy University of Muhammadiyah Malang, East Java, Indonesia

² Bachelor Pharma Student, Department of Pharmacy University of Muhammadiyah Malang, East Java, Indonesia
Jl. Bendungan Sutami 188-A Malang, 65145, Indonesia

Email: engridjuni81@umm.ac.id

ABSTRACT

Chemical compounds that can donor one or more electrons to free radicals to inhibit free radical reactions are called antioxidants. Manalagi apple ((Malus sylvestris Mill) is a fruit with high antioxidant activity. Manalagi apple is a typical fruit of Batu City. Processed apple products are apple cider, vinegar, dodol, and chips. These products are sold in Batu city from the same home industry. This study aimed to determine the antioxidant activity comparison between Manalagi apple and four processed apple products based on IC50 values. This study used the ABTS method (2,2-azino bis (3-ethyl benzothiazoline-6-sulfonic acid) and analysis used spectrophotometric visible. IC50 value as apples and their processed products were analyzed using a one-way ANOVA. The study results indicated that the IC50 value of Manalagi apple was insignificantly different from the processed apple product of dodol and chips. However, the IC50 value of manalagi apple was significantly different from apple cider and vinegar.

Keywords: Manalagi Apple; Apple cider; Apple Vinegar; Apple Dodol; Apple Chips

INTRODUCTION

Batu City is located in East Java, Indonesia, as one of the cities with a high quantity of apple production (Sa'adah, LIN; Estiasih, 2018). Statistical data of Batu City in 2018 on apple production in Batu City was 15.90 tons compared to 2017 with 19.10 tons. That has decreased by 16.75% (Anonim, 2020). Based on the high productivity of apples, people have diversified their apple products into various processed products such as apple *cider* beverages, vinegar, chips, and *dodol*. One of the most well-known apple varieties in Batu City is Manalagi apple. Manalagi apples have a different color when raw. They are green, while when ripe, they are yellowish-green, so many people find it difficult to tell the difference (Ciputra et al., 2018).

Apples are one of the subtropical plants that people like to eat. Apple skin contains flavonoid compounds beneficial for human health as antihypertensive properties (Balasuriya & Rupasinghe, 2012). Apple skin also contains high concentrations of quercetin (Cempaka, Anggun. Rindang., Sanarto Santoso, 2015). Previous studies found that apple peels were proven to have higher antioxidant activity than flesh and flesh-skin determined by the ORAC (Oxygen Radical Absorbance Capacity) method (Sutrisno, 2019).

Antioxidants can attack free radicals found in the body due to body metabolism, air pollution, food contamination, and sunlight (Asri Werdhasari, 2014). Antioxidants are very beneficial for health to prevent aging and degenerative like cancer, diabetes mellitus complications, and atherosclerosis which underlies heart disease and stroke (Sidor & Gramza-Michałowska, 2015).

Several factors affecting the concentration of bioactive compounds in fruit are various flavonoid types, tissue, varieties, storage time, conditions, and processing (Barreira et al., 2019). Besides, decreased antioxidant activity can also be caused by heating, light, and direct contact with metal materials. Based on research (Mikulic-Petkovsek et al., 2020), it is stated that processing techniques carried out on fruit, such as juicing or drying, hurt the content of

bioactive chemical compounds. Some drying techniques, such as ovens, can change the most bioactive chemical compounds (Lo Piccolo et al., 2020).

Several studies have reported that thermal treatment can reduce or increase its phenolic content and antioxidant activity, depending on the heat treatment's severity and exposure time. Fermentation can reduce the ability to fight the effects of radicals in the DPPH test. Hydrothermal treatment and decortication can reduce the polyphenol content. It has been reported that hydrothermal treatment can also reduce the total phenolic content (TPC). Processing can increase the bioavailability of bioactive compounds, but processing can reduce their levels (Chandrasekara et al., 2012).

Dietary antioxidants have an essential role in the oxidation-reduction balance in the body, preventing oxidative stress-related disorders. Flavonoids in foods and beverages consumed regularly (tea, onions, apples) can reduce the risk of death from coronary heart disease in older adults. Several studies have demonstrated the preventive effect of antioxidants against oxidative-related diseases, although there is not enough evidence to prove the therapeutic effect of antioxidants in sick people. Understanding the relationship between human metabolic processes and the intake of antioxidants from food will provide opportunities to change diet to improve health. For an individual consumption of 2500 kcal per day, the antioxidant capacity requirement was estimated at 11.5 mmol TE. It calculated the antioxidant capacity intake per serving of various types of cereals, fruits, and vegetables. And by researching the antioxidant activity of food, it is hoped that it can increase the intake of the food consumed (Cömert & Gökmen, 2018).

This study aimed to identify the antioxidant comparison between manalagi apples and their processed products, namely apple *cider*, vinegar, *dodol*, and chips, besides determining whether the processing on manalagi apples could cause a decrease in antioxidant activity or not. The novelty of this result is no research has ever been conducted on samples used from Batu City.

METHODS

Materials and Tools

Manalagi apples and their processed products, namely apple *cider*, vinegar, *dodol*, and chips, were obtained from Home Industry "X" in Batu City. ABTS (2,2-Azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) (TCI), Vitamin C (pharmaceutical grade), K2S208 (*Sigma Aldrich*), aqua dest, ethanol. Equipment for this research is UV-Vis spectrophotometer (*UV mini-1240 Shimadzu*), Hellma Analytics cuvette, analytical balance scale (*Scout Pro 400g*), pycnometer (*Blau Brand*), juicer (*Philips*).

Preparation of ABTS Reagent Solution

In a dark room, seven mM ABTS and 2.45 mM K2S208 solution (1:1) were mixed and incubated for 12-16 hours. ABTS solution was dissolved into aqua dest and ethanol (1:1) until it reached absorbance of 0.700 ± 0.05 at the maximum wavelength. All of these methods are modified for Shalaby & Shanab researchers. (Shalaby & Shanab, 2013).

Measurement of Incubation Time

Incubation time was determined from operating time, from 2 to 65 minutes after adding ABTS adhesion to a positive control solution of vitamin C.

Preparation of Vitamin C Solution

The concentrations of vitamin C used were 3.14; 15.65; 31.39; 62.78, and 125.55 ppm. The test solution was measured in three replications. Each solution was pipetted 100 μ l, added

with 6.0 ml ABTS reagent solution. The mixture was incubated for 60 minutes after adding reagent solution, then measured by a UV-Vis spectrophotometer.

Preparation of Manalagi apple juice Solution

Apple juice was tested in various concentrations of 520; 5,200; 15,600; 26,000, and 52,000 ppm. The test solution was measured in three replications. Each solution was pipetted 100 µl, added with 6 ml ABTS reagent solution, then incubated for 60 minutes and measured by a UV-Vis spectrophotometer.

Preparation of Apple Cider Solution

Apple *cider* was tested in various concentrations of 25,750; 51,500; 103,000; 206,000 and 824,000 ppm of the test solution and measured in three replications. Each solution was pipetted 100 µl, added 6.0 ml ABTS reagent solution, incubated for 60 minutes, and measured by a UV-Vis spectrophotometer.

Preparation of Apple Vinegar Solution

Apple vinegar was tested in various concentrations of 12,375; 24,750; 99,000; 792,000 and 990,000 ppm of the test solution and measured in three replications. Each solution was pipetted 100 µl, added 6.0 ml ABTS reagent solution, incubated for 60 minutes, and measured by a UV-Vis spectrophotometer.

Preparation of Apple Dodol Solution

Apple *dodol* was tested in various concentrations of 502.74; 2,513.7; 5,027.39; 50,273.87, and 100,547.8 ppm of the test solution and measured in three replication. Each solution was pipetted 100 µl added, with 6.0 ml ABTS reagent solution, incubated for 60 minutes, and measured by a UV-Vis spectrophotometer.

Preparation of Apple Chips Solution

Apple chips were tested in various concentrations of 50.11; 2,505.52; 5,011.03; 7.516.55 and 12.527.58 ppm of the test solution and measured in three replication. Each solution was pipetted 100 µl, added 6.0 ml ABTS reagent solution, incubated for 60 minutes, and measured by a UV-Vis spectrophotometer.

Data Analysis

The absorbance data of each test solution in various concentrations were calculated the inhibition percentage using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{ABTS Solution Absorbance} - \text{Test Solution Absorbance})}{\text{ABTS Solution Absorbance}} \times 100\%$$

A regression equation was made between each test solution and the inhibition percentage of each test solution to calculate the IC50 value. The IC50 value results were compared using statistical analysis.

RESULT AND DISCUSSIONS

The maximum ABTS solution wavelength was firstly determined to identify the produced maximum absorption of ABTS solution at 737 nm wavelength with an absorbance value of 0.631.

The operating time of vitamin C solution as a positive control was aimed to determine the reaction period perfectly run between the antioxidant compound (test solution) and ABTS reagent solution as indicated from a stable absorbance. The results obtained were at 60

minutes after the addition of ABTS solution. Another function of vitamin C was to validate the method used because it can inhibit free radicals and not compare it with the test solution as vitamin C is a natural antioxidant with a high IC50 value and is easy to obtain (Ramadhan, 2015).

An antioxidant activity test was carried out to determine the IC50 value of the sample. The IC50 value expressed the antioxidant activity. The IC50 is the concentration of the test solution that can soak at least 50% ABTS free radicals obtained through the regression equation.

Based on the results, the IC50 of apple *cider* solution, vinegar, *dodol*, chip, apple fruit, and vitamin C is 3,691,278.89 ppm; 2,224,333.33 ppm; 123,939.94 ppm, 13,871.63 ppm; 36,722.36 ppm, and 96.71 ppm, respectively. A smaller IC50 value means that the activity of the test solution is more effective as an antidote to free radicals (Ureta et al., 2018).

Table 1 Regression Equations and IC50 Vitamin C, Apple Fruit, and Its Processed Products

material	Replication	Regression Equation	r	IC50(ppm)	Average IC50
Vitamin C	1	y=0.3743X+10.627	1	105.19	96.71
	2	y=0.3836X+12.114	0.9994	98.76	
	3	y=0.4163X+14.131	0.9989	86.16	
Apple	1	y=0.0009X+13.672	0.9998	40364.44	36722.36
	2	y=0.0008X+19.501	0.9933	38123.75	
	3	y=0.0009X+21.489	0.9951	31678.89	
Apple <i>cider</i>	1	y=0.00001X+9.8423	0.9974	4015770.00	3691278.89
	2	y=0.000009X+16.343	0.9983	3739666.67	
	3	y=0.00001X+16.816	0.9917	3318400.00	
Apple vinegar	1	y=0.00002X+15.141	0.9997	1742950.00	2224333.33
	2	y=0.00002X+17.037	0.9921	1648150.00	
	3	y=0.00001X+17.181	0.9991	3281900.00	
Apple <i>dodol</i>	1	y=0.0003X+10.551	0.9923	131496.67	123939.94
	2	y=0.0004X+9.2718	0.9960	101820.50	
	3	y=0.0003X+8.4492	0.9996	138502.67	
Apple chips	1	y=0.0031X+4.1964	0.9992	14775.35	13871.63
	2	y=0.0037X+3.4915	0.9979	12569.86	
	3	y=0.003X+7.191	0.9998	14269.67	

The results of statistical tests using ANOVA SPSS 19 to compare the IC50 results from apple *cider*, apple vinegar, apple *dodol*, apple chips, and manalagi apple found that the value of P-value (0,000) \leq 0.05, therefore there was a significant difference in IC50 value among the test solutions. Based on the LSD test results, it was found that manalagi apple, apple *cider*, and apple vinegar had P-value (0,000) \leq 0.05, which means the IC50 value of manalagi apple, apple *cider*, and apple vinegar were significantly different. Meanwhile, manalagi apple and apple *dodol* had P-value (0.813) \geq 0.05. The IC50 values of manalagi apple and apple *dodol* were insignificantly different. Manalagi apples and apple chips had P-value (0.950) \geq 0.05. Thus the IC50 values of manalagi apples and apple chips were insignificantly different. Probability Value (P-Value) can be interpreted as the magnitude of the test statistic observed probability (probability). The researcher obtained from the error value of the statistical calculation results (Statistical Test Results). Here the researcher uses the value of = 5% or Ho. There is no significant difference if the P-value is not more than 0.05.

Several processing steps are carried out during the production process, cleaning, stripping, squeezing, drying, and heating. These are processing steps that require extended time to be conducted. It should be noted that the antioxidants in fresh fruit experience severe chemical alteration during processing. Processing has a positive effect, such as increasing the antioxidant activity, adverse effects, such as destroying phenolic compounds and triggering the formation of new compounds with antioxidant properties. The Maillard reaction product results from amino acids and saccharide condensation (Tarko et al., 2010).

The results of the antioxidant activity of apple processed products, namely apple *cider*, vinegar, *dodol*, and chips, were shallow due to the long period required for the heating process. The food's manufacturing process significantly impacts its antioxidants in the final product (Koren et al., 2019). Processing can reduce antioxidants substantially due to high-temperature requirements and oxidation during processing (Techakanon & Sirimuangmoon, 2020).

The antioxidant activity of apple *cider* was significantly different from fresh apples as apple *cider* processing was exposed to air for two or three days at the pressing time. Therefore strong oxidation was produced. Flavan-3-ols and oligomeric procyanidins were quite sensitive to oxidation, leading to the formation of larger polymers. In the advanced stages of oxidation, increased polymerization degree reduced the polyphenol scavenging activity due to steric hindrance (Laaksonen et al., 2017).

From the results, it was found that the antioxidants content of apple vinegar decreased significantly compared to the antioxidant activity of fresh apples. This result follows (Tarko et al., 2020) that oxygenation that occurs during the fermentation process can reduce the concentration of volatile components. The electrochemical reduction current of oxygen increased due to the catalytic effect of the process. Phenolic compounds underwent enormous changes during the vinegar-making process, reducing the antioxidant activity (Bakir et al., 2016).

Dodol is a semi-wet food made from glutinous rice flour, coconut milk, and sugar with or without food additives. The result is in the solid elastic dough with light to dark brown color (Nuroso, 2013). The manufacturing process of *dodol* requires long heating period to ensure the dough produced is thick enough; therefore, this process could reduce the antioxidant activity of the apples used.

Apple chips were packaged with vacuum frying to maintain the antioxidant content of apple chips. The vacuum frying process, as the process of material heating under low pressure in a closed system, can reduce the boiling point of cooking oil and remove moisture content in fried food when the oil temperature reaches the water boiling point. Because food is heated at a lower temperature, its natural color and taste can be preserved (Dueik et al., 2010). By using vacuum frying in apple chips processing, the antioxidant activity can be maintained perfectly adequately.

CONCLUSION

Statistical analysis results of the IC50 values indicated that the antioxidant activity of manalagi apple was not significantly different from apple *dodol* and chips but was substantially different from apple *cider* and vinegar. The study results found that apple chips had the most significant antioxidant activity compared to other processed products. It may be possible to develop different antioxidant testing methods and other nutritional value tests for further research.

REFERENCES

- Anonim. (2020). Badan Pusat Statistik Kota Batu.
<http://batukota.bps.go.id/statictable/2019/12/03/549/produksi-buah-buahan-dan-sayuran-tahunan-menurut-jenis-tanaman-di-kota-batu-ton-2017-dan-2018>, Tanggal akses 1 september 2020
- Asri Werdhasari. (2014). Peran Antioksidan Bagi Kesehatan. *Jurnal Biomedik Medisiana Indonesia*, 3(2), 59–68.
- Bakir, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., & Capanoglu, E. (2016). Fruit antioxidants during vinegar processing: Changes in content and in vitro bio-accessibility. *International Journal of Molecular Sciences*, 17(10).
<https://doi.org/10.3390/ijms17101658>
- Balasuriya, N., & Rupasinghe, H. P. V. (2012). Antihypertensive properties of flavonoid-rich apple peel extract. *Food Chemistry*, 135(4), 2320–2325.
<https://doi.org/10.1016/j.foodchem.2012.07.023>
- Barreira, J. C. M., Arraibi, A. A., & Ferreira, I. C. F. R. (2019). Bioactive and functional compounds in apple pomace from juice and cider manufacturing: Potential use in dermal formulations. *Trends in Food Science & Technology*, 90, 76–87.
<https://doi.org/https://doi.org/10.1016/j.tifs.2019.05.014>
- Cempaka, Anggun. Rindang., sanarto santoso, laksmi karunia tanuwijaya. (2015). Indonesian Journal of Human Nutrition. *Indonesian Journal of Human Nutrition*, 2(1), 48–59. kalteng.litbang.pertanian.go.id
- Chandrasekara, A., Naczki, M., & Shahidi, F. (2012). Effect of processing on the antioxidant activity of millet grains. *Food Chemistry*, 133(1), 1–9.
<https://doi.org/https://doi.org/10.1016/j.foodchem.2011.09.043>
- Ciputra, A., Setiadi, D. R. I. M., Rachmawanto, E. H., & Susanto, A. (2018). Klasifikasi Tingkat Kematangan Buah Apel Manalagi Dengan Algoritma Naive Bayes Dan Ekstraksi Fitur Citra Digital. *Simetris: Jurnal Teknik Mesin, Elektro Dan Ilmu Komputer*, 9(1), 465–472. <https://doi.org/10.24176/simet.v9i1.2000>
- Cömert, E. D., & Gökmen, V. (2018). Evolution of food antioxidants as a core topic of food science for a century. *Food Research International*, 105, 76–93.
<https://doi.org/https://doi.org/10.1016/j.foodres.2017.10.056>
- Dueik, V., Robert, P., & Bouchon, P. (2010). Vacuum frying reduces oil uptake and improves the quality parameters of carrot crisps. *Food Chemistry*, 119(3), 1143–1149.
<https://doi.org/10.1016/j.foodchem.2009.08.027>
- Koren, D., Kun, S., Hegyesné Vecseri, B., & Kun-Farkas, G. (2019). Study of antioxidant activity during the malting and brewing process. *Journal of Food Science and Technology*, 56(8), 3801–3809. <https://doi.org/10.1007/s13197-019-03851-1>

- Laaksonen, O., Kuldj  r  , R., Paalme, T., Virkki, M., & Yang, B. (2017). Impact of apple cultivar, ripening stage, fermentation type and yeast strain on phenolic composition of apple ciders. *Food Chemistry*, 233, 29–37.
<https://doi.org/https://doi.org/10.1016/j.foodchem.2017.04.067>
- Lo Piccolo, E., Garcia, L. M., Landi, M., Guidi, L., Massai, R., & Remorini, D. (2020). Influences of postharvest storage and processing techniques on antioxidant and nutraceutical properties of *rubus idaeus* L.: A mini-review. *Horticulturae*, 6(4), 1–13.
<https://doi.org/10.3390/horticulturae6040105>
- Mikulic-Petkovsek, M., Koron, D., & Rusjan, D. (2020). The impact of food processing on the phenolic content in products made from juneberry (*Amelanchier lamarckii*) fruits. *Journal of Food Science*, 85(2), 386–393. <https://doi.org/10.1111/1750-3841.15030>
- Nuroso, A. (2013). Pembuatan Dodol Susu. *Jurnal Teknologi Pertanian*, 2(2), 12–17.
<https://doi.org/10.32520/jtp.v2i2.52>
- Sa'adah, LIN; Estiasih, T. (2018). Karakterisasi minuman sari apel produksi skala mikro dan kecil di kota Batu: kajian pustaka. *Jurnal Pangan Dan Agriindustri*, 3(2), 175.
- Shalaby, E. A., & Shanab, S. M. M. (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian Journal of Marine Sciences*, 42(5), 556–564.
- Sidor, A., & Gramza-Micha  wska, A. (2015). Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. *Journal of Functional Foods*, 18, 941–958. <https://doi.org/https://doi.org/10.1016/j.jff.2014.07.012>
- Sutrisno, A. D. (2019). Identifikasi Kandungan (Antioksidan, Vitamin C Dan Serat Kasar) Pada Buah Lokal Dan Impor (Jeruk, Apel Dan Mangga). *Pasundan Food Technology Journal*, 6(1), 1. <https://doi.org/10.23969/pftj.v6i1.1502>
- Tarko, T., Duda-chodak, A., Sroka, P., & Januszek, M. (2020). Effect of musts oxygenation at various stages of cider production on oenological parameters, antioxidant activity, and profile of volatile cider compounds. *Biomolecules*, 10(6), 1–11.
<https://doi.org/10.3390/biom10060890>
- Tarko, T., Duda-Chodak, A., Sroka, P., Satora, P., & Tuszy  ski, T. (2010). Production of flavored apple chips of high antioxidant activity. *Journal of Food Processing and Preservation*, 34(4), 728–742. <https://doi.org/10.1111/j.1745-4549.2009.00389.x>
- Techakanon, C., & Sirimuangmoon, C. (2020). The effect of pasteurization and shelf life on the physicochemical, microbiological, antioxidant, and sensory properties of rose apple cider during cold storage. *Beverages*, 6(3), 1–18.
<https://doi.org/10.3390/beverages6030043>
- Ureta, R., Mejico, S., & Maranan, Y. (2018). Free Radical Scavenging Activity and Antioxidants of *Hydrocotyle vulgaris* L. (Pennywort): Baseline Study in Developing Biocosmetic-Antidote for Pathological Aging. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine*, 10, 1–12.
<https://doi.org/10.18052/www.scipress.com/ijppe.10.1>