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Antioxidant capacity and phytochemical analysis of broccoli *(Brassica oleracea L. var italica)* powder with sun drying technology

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KEYWORDS

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ABSTRACT

Broccoli *(Brassica oleacea L. var italica)* is classified as a valueable agricultural product that contains high quality active compounds. Broccoli can be damaged easily without proper handling and post-harvest processing. The purpose of this study was to determine the phytochemical content and antioxidant capacity of broccoli stems, leaves and flowers by sun drying method. The treated samples then analyzed for the phytochemical content and antioxidant capacity. Furthermore, the data obtained were analyzed using ANOVA and DMRT. The results showed that broccoli leaf powder had the highest content of vitamin C (7,673mg/100g), chlorophyll (1746,198mg/kg), vitamin K (226,423 µg/g), phenolics (24210,120 mg/kg), flavonoids (15239,947 mg/kg) and antioxidant capacity (IC_{50}= 25,215 ppm) compared to flowers and stems. This study shows that sun drying process maintains the quality of the phytochemical content and antioxidant capacity of broccoli plants. It can be concluded that broccoli leaf powder has the highest phytochemical content and antioxidant capacity so it can be used as a food ingredient in food processing such as noodles and bread.

Introduction

Broccoli (*Brasicca oleraceae L. Var. Italica*) is one of the horticulture sectors in Malang city. Broccoli is a vegetable that contains the rich active components such as antioxidants, vitamin C, phenols, and other phytochemicals (Jalasena and Anjani, 2016). The Covid-19 pandemic has caused PPKM and influenced on the community activities, especially the agricultural sector on delivery of agricultural products to specified groups of markets to be hampered. This causes agricultural items, particularly vegetables, to decay and become ruined. According to Wadhani et al., (2018) one of the most common problems that farmers face is agricultural products that are easily damaged and rotten, such as broccoli, which is a perishable vegetable, the shelf life of vegetables, particularly broccoli, without post-harvest handling such as cooling/drying can last up to two days.

People in general processing broccoli only for flowers to cooked as vegetables as a complement to the menu, so the leaves and stems of the broccoli is wasted. According to Asgar, (2017) the flower section of broccoli may be processed to be cut to size and ingested, while the weevil part and the leaves can be removed. According to Jannah, (2014) broccoli leaves have more antioxidant activity than weevils. According to Nurlaili, (2017) broccoli leaves contains chlorophyll and antioxidant activity. With the phenomenon and also the benefits of unused parts of leaves and stems/weevils, it is a pity if thrown away just like that and underutilized. Therefore, it is necessary to carry out a follow-up handling broccoli process to be used as a dry powders product that can be durable and useful.

The approach and action are to process all parts of broccoli to be used as powder by drying it. The purpose of drying is to reduce the moisture content of the material to the extent where microorganisms and enzyme activities that can cause decay will stop, thus the dried material can have a long shelf life (Riansyah et al., 2013). The advantage of this preservation is that the material becomes durable and the volume of food ingredients decreases, making it easier to transport. Various drying methods namely natural and artificial drying. In this study uses natural drying with the sun drying process. The drying process is one that everyone can undertake and does not require a lot of money (Huriawati et al., 2016). According to Nafisah et al. (2018), sun drying process also preserve and create the maximum total

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phenol content in the material. Sun drying at low temperatures protect polyphenol components from damage (Nafisah et al., 2018). Sun-dried broccoli powder, ground to the appropriate particle size, and carefully packed is intended to provide high-quality, long-lasting, and easy-to-use vegetable powder products that may be a nutritious food element for the community.

Research Methods

Research design

This research is an experimental research, the research design uses a Completely Randomized Design (CRD). The treatment in this study was the sun drying treatment of the types of broccoli stems, leaves and flowers and with 2 repetitions of the experiment.

Research on the manufacture of broccoli powder products was conducted from January 2022 - May 2022 housed in the Industrial Laboratory of the Department of Industrial Technology, Faculty of Engineering, Universitas Negeri Malang. Phytochemical content analysis was carried out at the Universitas Muhammadiyah Malang (UMM).

Sample

Samples were obtained from local farmers in Malang. The broccoli used was obtained directly from the garden and was still fresh, no part was wasted in this study. All parts are used both leaves, flowers and stems of broccoli.

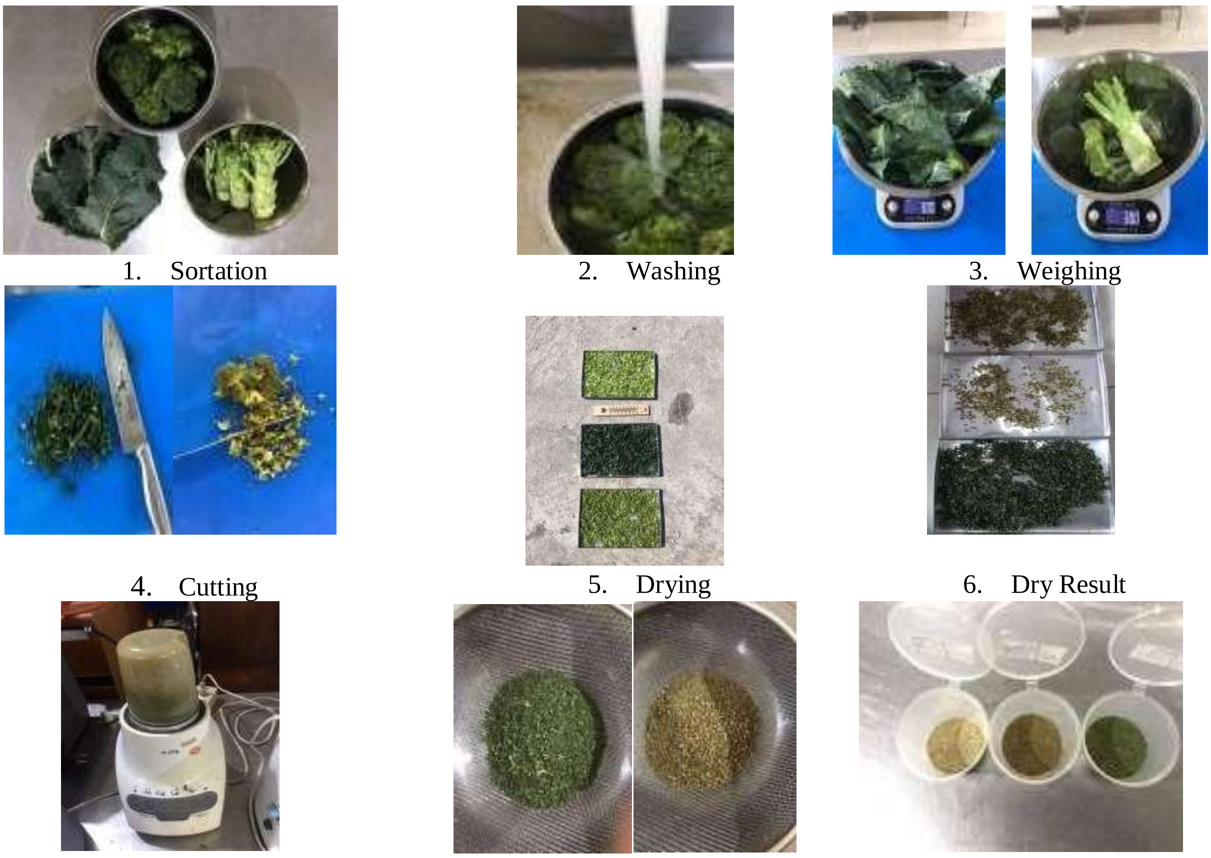
Production of Broccoli Powder

a. Ingredients

300 grams of stems, 300 grams of flowers, 300 grams of broccoli leaves.

b. Materials

Bowl, knife, digital scale, blender, aluminum pan, 80 mesh filter, packaging cup, and thermometer. The following procedure for making broccoli powder can be seen in Figure 1.



10. Laboratory analysis of phytochemical content and antioxidant capacity of broccoli powder

Figure 1. Process of making broccoli powder

c. ***Sun drying process***

Broccoli (*Brassica oleracea L. var italica*) with the sun drying process at 28-36 °C starting at 8-4 pm until the particle size decreas and the broccoli becomes dry. Sun drying process on the broccoli needs temperatures 28-30°C at 08.00-10.00 hours, 31-35 °C at 11.00-14.00 hours, and 32-33°C 15.00-16.00 hours. The temperature on sun drying process measured by placing a thermometer during the drying process and checking it once an hour, it aims to determine the stability of the temperature in the drying process in the sun. In this study, broccoli leaves dried within 8 hours, broccoli flowers dried within 17 hours, and stems dried within 27 hours. In research (Widarta and Wiadnyani, 2019) explains drying avocado leaves on sun drying method with a temperature of 27-35°C for 2 days.

Methods of analysis of phytochemicals and antioxidant capacity

Phytochemical analysis methods and antioxidant capacity of broccoli powder can be seen in Table 1. The total chlorophyll of broccoli powder will be determined using a spectrophotometer, according to the method of the Association of Official Analytical Chemists (AOAC). Briefly, the pigment will be extracted by shaking in 30mL heptane/ethanol (3:1) for one hour, away from sunlight in the shaker. The homogenate will be centrifuged at 20,000 rpm for 15 minutes at 15 °C, the pellet will be re-extracted, the supernatant will be combined and dehydrated with anhydrous sodium sulfate, will be dried in a rotary evaporator and the residue will be filled to 10-25 mL with diethyl ether. The pigment will be quantified by spectrophotometer at 660 and 642.50 nm.

Quantitative analysis of vitamin C includes acid-base titration methods, iodimetric methods. 25 ml of filtrate solution, then add 0.5 ml of 1% starch indicator. Titrate with 0.01 N I2 standard solution until the color changes to blue which does not disappear for 10-15 seconds. Record the titration volume (b).

Quantification by HPLC. Analysis was based on the methodology by Jakob and Elmadfa (2000; 1996) with an adjustment in the extract purification stage; after 5 minutes centrifuging at 3000 rpm and 4 °C, the supernatant was collected, filtered through a membrane, and transferred to a 50 mL evaporation flask and evaporated at 40 °C for 15 minutes using a rotary evaporator and vacuum system. The residue was dissolved in 200 µL of mobile phase and 50 µL was injected. A Shimadzu HPLC included a CBM-20A system (SCL-10AVP), LC-Solution Software, a SIL-20A auto sampler, an LC-20AT isocratic pump, and an RF-10AXL fluorescence detector. The separation was in reverse phase with a LiChrospher RP-18 5 µm endcapped LiChroCART 250-4.6 column, with a pre-column from Merck and a mobile phase consisting of dichloromethane/ methanol (10:90 v/v) with the addition of 5ml of methanol solution with zinc chloride (1.37 g), sodium acetate (0.41g) and acetic acid (0.30 g) per litre of mobile phase and was pumped at a flow rate 1.00 mL min -1 with isocratic elution. The post-column reduction (20 x 4.0 mm id) was filled manually with zinc dust p.a. grade from Merck with particles <45 µ, kept in a furnace (Shimadzu-CTO-6A) at 40 °C, with fluorescence detector excitation 243 nm and emission 430 nm.

Determination of phenolics A total of 1 ml of sample or standard plus 0.2 ml of folin c, 0.6 ml of Na2CO3 0.2 mM homogenized by vortex for 5 minutes and allowed to stand for 120 minutes. Measure the absorbance at 765 nm. The standard phenolic absorbance results that have been obtained are processed with the help of statistical programs to determine a simple linear regression equation y = a + b(x), where y = absorbance and x = phenolic concentration The phenolic content was determined with the help of standard regression equations taking into account the weight of the sample used and the dilutions carried out. (Harborne,1973).

Tabel 1. Phytochemical analysis method and antioxidant capacity

Parameters	Methods	Ingredients
Chlorophyll	Spectrophotometry	Aceton 85%, filter paper
Vitamin C	Iodine Titration Method	Aquades lodin 0,01 N, filter paper
Vitamin K	HPLC	20 g/mL total phylloquinone in hexane
Phenolic	Spectrophotometry	Gallic acid standard, Folin C p.a, Na_2CO_3 0,2 mM, Aquadest Aquadest
Flavonoids	Spectrophotometry	Quercetin standard, NaNO2 5%, Al2Cl3 10%, NaOH 1 M, filter paper
Antioxidant capacity	DPPH	Methanol p.a. DPPH 0,2 mM Dissolve as much as 0.078 g in methanol p.a. to a volume of 1000 ml

Flavonoids were determined by taking 0.1 ml of sample or standard solution, adding 0.1 ml of 2% Al2Cl3, homogenizing with a vortex, and letting stand for 60 minutes at room temperature. Add distilled water to a volume of 1 ml. A red solution will form if there are flavonoids. Measure the absorbance with a spectrophotometer at λ 420 nm.

50% of DPPH radicals in a certain time period (IC50). The antioxidant content of broccoli powder will be determined using the aluminum chloride test. Volume of extract 1mL, blank or standard solution of quecertin (0-200μg mL-1) will be mixed with aluminum chloride solution (2%, 2mL). The mixture will be shaken thoroughly and incubated at room temperature for 30 minutes. The total chlorophyll of broccoli powder will be determined using a spectrophotometer, according to the method of the Association of Official Analytical Chemists (AOAC). Briefly, the pigment will be extracted by shaking in 30mL heptane/ethanol (3:1) for one hour, away from sunlight in the shaker. The homogenate will be centrifuged at 20,000 rpm for 15 minutes at 15 °C, the pellet will be re-extracted, the supernatant will be combined and dehydrated with anhydrous sodium sulfate, will be dried in a rotary evaporator and the residue will be filled to 10-25 mL with diethyl ether. The pigment will be quantified by spectrophotometer at 660 and 642.50 nm.

Data collection and analysis

Broccoli powder was analyzed for the phytochemical content and antioxidant capacity. Furthermore, the resulting data were analyzed using ANOVA analysis of variance (Analysis of Variance). DMRT (Duncan's Multiple Range Test) using SPSS IBM Statistics Version 22.

Results and Discussion

The results of the phytochemical content and antioxidant capacity of broccoli powder can be seen in Table 2.

***Chlorophyll content of broccoli powder* (*Brassica oleracea* L.)**

Chlorophyll is a pigment that gives a green color to plants, algae, and photosynthetic microorganisms (Hosikian et al., 2010). This pigment helps the process of photosynthesis in plants by absorbing and converting light energy into chemical energy. Higher plants contain two different types of chlorophyll, namely chlorophyll a ($C_{55}H_{72}O_5N_4Mg$), which is dark green, and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$), which is light green (Nio Song and Banyo, 2011).

Sun drying process on the broccoli stems, leaves, and flowers showed the highest chlorophyll content results on broccoli leaf powder with an average of 1746,198 mg / kg, compared to broccoli stem and flower powder. This study resulted in a higher chlorophyll content of broccoli leaf powder than Dian's research, (2017) which stated that the chlorophyll content of dried broccoli leaves was 538,061 mg / kg, but the results of the research on the chlorophyll content of broccoli leaves were lower in a study that had been conducted by Devi et al., (2022) obtained the highest dry broccoli powder chlorophyll content by drying using a cabinet dryer found in samples of broccoli leaf powder with a value of 1816,034 mg/kg compared to samples of stems and flowers.

The factor that causes the chlorophyll content of broccoli leaf powder to be higher than that of stems and flowers is that broccoli leaves require a relatively short drying time, which is 8 hours so that they are not overly exposed to the heat of the sun for an extended period, and the drying process is stopped until the leaves are easily broken (Rachmawati et al., 2006). This is supported by the findings of Madalena et al., (2010), who studied the process of warming cassava leaves at various temperatures and found that the longer the heating time, the lower the chlorophyll concentration.

The drying process might cause broccoli powder's chlorophyll level to decline and decrease. This is supported by Bjorn et al (2009) Heat, oxygen, light, and pH all make chlorophyll unstable. Budiari's (2011) study indicated that drying and heating purut orange leaves can degrade pigments and diminish chlorophyll concentration. Based on this, it is possible to deduce that the chlorophyll concentration of broccoli powder leaf samples can be lowered owing to the drying process. (Severini and colleagues, 2016).

Table 2. Phytochemical Content results and antioxidant capacity of broccoli powder with sun drying.

Sample	Chlorophyll (mg/kg)	Vitamin C (mg/100gr)	Vitamin K (μg/g)	Phenolics (mg/kg)	Flavonoids (mg/kg)	Antioxidant Capacity (IC^{50})
Steam	1167.116±6.898^a	5.933±0.316^a	182.056±4.921^a	18892.171±42.599^a	10839.586±15.323^a	30.726±0.207^a
Leaf	1746.198±5.754^b	7.673±0.304^b	226.423±2.553^b	24210.120±27.399^b	15239.947±235.038^b	25.215±0.150^b
Flowers	836.051±6.437^c	3.729±0.312^c	101.719±5.958^c	14063.365±185.161^c	8509.318±132.105^c	35.145±0.176^c

According to Murcia, the chlorophyll concentration of broccoli leaves is 1145.342 g/g (Murcia et al., 2000). Broccoli blooms have 852 g/g chlorophyll, broccoli stems contain 144 g/g chlorophyll, and broccoli leaves contain 4478 g/g chlorophyll, according to previous research (Liu et al, 2018).

Vitamin C content of broccoli powder (*Brassica oleracea* L.)

Vitamin C is a vitamin that can be synthesized by various plants and animals from carbohydrate precursors (Paciolla et al., 2019). Vitamin C does not match alkaline solutions but is relatively stable in acidic solutions (Zhang et al., 2018). Vitamin C is a water-soluble vitamin and moderately soluble in alcohol and glycerol but insoluble in non-polar solvents such as ether, benzene, and chloroform. In dry form, white crystals are odorless, sour, and stable. Vitamin C is a powerful reducing agent due to its susceptibility to oxidation (Padayatty et al., 2003). Vitamin C is a coenzyme or cofactor that performs various actions in the body (Carr and Maggini, 2017).

The content of vitamin C in dried broccoli stems, leaves, and flowers (*Brassica oleracea L.*) dried with sunlight varies greatly. The content of vitamin C in broccoli leaves was found to be higher than in the stems and flowers, which was 7,673mg / 100gr. This is to other studies, in particular the investigation of the IDN team which found that the vitamin C concentration of broccoli leaves is very high, which is 93.2 mg in 100 grams of raw broccoli leaves. In the study conducted, the highest vitamin C content of dry broccoli powder was obtained by drying using a cabinet dryer found in leaf powder samples with a value of 8.106 mg / 100 grams compared to samples of stems and flowers. (Devi et al., 2022).

Vitamin C is a transparent crystal that dissolves easily in water. While vitamin C is very durable in dry circumstances, it degrades rapidly when exposed to oxygen (oxidation), particularly when heated (Padayatty et al., 2003). Vitamin C is easily damaged by oxidation, particularly at high temperatures, and this vitamin is easily lost during processing and storage; thus, the content of vitamin C can be reduced if exposed to heat; vitamin C reduction can also be caused by various processing methods such as cutting, washing, and turning (Safaryani et al., 2007).

Vitamin C is abundant in fruits and vegetables, the most well-known of which being broccoli. Broccoli leaves contain the maximum vitamin C in broccoli dry powder (7,673 mg/100g). Broccoli leaves are dried quickly compared to blossoms and stems, therefore the vitamin C content is not greatly oxidized by heat, allowing vitamin C to be preserved in leaf samples. This is consistent with the findings of previous research. Cruciferous vegetables are also a rich source of vitamin C, with broccoli having a vitamin C concentration of more than 50 mg / 100 g of fresh weight. One broccoli accession, NS-50, is reported to contain up to 82 mg of vitamin C/100 g based on fresh weight (Manchali dkk.,2012).

Vitamin K content of broccoli powder (*Brassica oleracea* L.)

Based on the examination of the sun-dried broccoli stem, leaf, and flower powder, the vitamin K content of broccoli powder varies substantially. The vitamin K content of broccoli leaf powder was found to be greater than that of the stems and flowers, with an average of 226.4238 µg / g. This is due to the rapid drying period of broccoli leaves, which is 8 hours before the leaves dry and the texture is readily flimsy, as opposed to the stems, which are not exposed to the heat of the sun for as long.

According to the presentation (DSM Nutritional, 2021), a sufficiently lengthy drying and heating process can cause harm to nutrients and content included in products; the nature of vitamin K itself is heat resistant but can be readily degraded when exposed to acids, bases, and sunshine. Based on this, it is possible to conclude that a lengthy drying process reduces the quantity of Vitamin K and minerals in broccoli powder.

Drying basil leaves by natural drying method is carried out with controlled room temperature at a temperature of 25 °C and microwave using microwave power of 100,300,500,700, and 900 W at a temperature of 50 °C, the drying period lasts between 16.5 hours 1620 minutes / 27 hours. The best grinding method regarding drying time, energy consumption, protein amount, and fat-soluble vitamins (beta-carotene, vitamin E, and vitamin K) is microwave-dried at 700 W (Alibas et al., 2021).

Phenolic content of broccoli powder (*Brassica oleracea* L.)

Phenolic substances and polyphenols are chemical components that have antioxidant properties (Santos and Magalhaes, 2020). They are widespread, especially in plants, and have the potential to scavenge beneficial free radicals (Panche et al., 2016). According to research conducted by Lin et al., (2016) the phenolic content in broccoli leaves is 4.14 mg GAE /g, the phenolic content in broccoli flowers is 2.51 mg GAE / g, and the phenolic content in broccoli stems is 1.41 mg GAE/g.

Powder of broccoli stems, flowers, and with sun drying process showed varying phenolic content results, the phenolate content in broccoli leaf powder was found to be higher than that of flowers and stems with an average of 24210.12 mg / kg (24.21g / kg, 2.4210%). This is corroborated by a study conducted by Devi et al., (2022) which found the highest phenolic content of broccoli powder by drying using a cabinet dryer found in leaf powder samples with a value of 2.3702% compared to samples of stems and flowers.

Samples of broccoli leaf powder were found to have a higher phenolic content, this is because the drying of broccoli leaves dries faster and is not exposed to heat for so long with a time of 8 hours, in contrast to broccoli flowers and stems that are dried in the sun with a time of 17 and 27 hours, therefore broccoli leaf samples can still maintain a fairly high polyphenol content. This is corroborated by the research of Nafisah et al., (2018) which states that the higher the phenol component that can be maintained during the drying process also affects the total amount of phenol that can be dissolved in foodstuffs.

According to Vazquez et al.,(2019) research, the longer the drying duration, the lower the content of phenolic compounds in the completed product. Roshanak et al., (2016) discovered that the samples included a high concentration of phenolic compounds, but that a lengthy, sufficient drying period caused the concentration of phenolic compounds to drop, as observed with the fading of green tea leaves (Camellia sinensis or C. assamica). The amount of phenols that can be dissolved in foodstuffs is affected by the number of phenol components that can be preserved during the drying process.

Flavonoid content of broccoli powder (*Brassica oleracea* L.)

Flavonoids have different sensitive properties in heat treatment depending on their structure (Putri et al., 2021). The number and position in the OH group in the molecule affect the strength of antioxidant activity in flavonoids. If the a more frequent substitution of hydroxy groups to flavonoids, the antiradical activity will be higher (Puspitasari et al., 2016).

Based on the results of the analysis of the flavonoid content of broccoli powder with solar heat drying, it was shown that the flavonoid content of dry powder in broccoli leaf samples was higher than the flavonoid content of dry powder samples of broccoli stems and flowers with an average of 15239,947 mg/kg (15.23g / kg, 1.523%), this shows that the flavonoid content of broccoli leaf samples is higher than that of stem and flower samples. Drying on broccoli leaves is the shortest drying with a drying time of 8 hours compared to the stems and flowers which take 17 and 27 hours to dry. According to suryaningrum research, bioactive substances that are sources of antioxidants are easily damaged when exposed to heat and sunlight when drying (Suryaningrum et al., 2006). Therefore broccoli leaf samples can still maintain the highest flavonoid content.

When the broccoli dry powder research was done, the results revealed that the leaf samples were more valuable than the blossom and stem samples. This is supported by a research done by Devi et al., (2022), which discovered that the greatest flavonoid concentration of broccoli powder dried using a cabinet drier was observed in leaf powder samples with a value of 1.405 percent when compared to stem and blossom samples. The findings of determining total flavonoid levels in freeze-dried leaves were greater than those of weevils in the previous study, with an average percent value of total flavonoid levels in leaves of 1.716 percent and weevils of 1.073 percent (Jannah, 2014). This is further confirmed by Jannah's (2014) findings, which discovered that total flavonoid levels in broccoli leaves were greater than in broccoli weevils.

The results of the research conducted by Janah and the results of the analysis carried out on broccoli drying extract did not differ markedly. It is related to temperature and drying time. The longer of the drying time, the less flavonoid components are present in the product (Bjorn et al., 2009). Similarly, Zhang's findings found that samples had high concentrations of flavonoid compounds, but long drying times could cause the concentration of flavonoid compounds to decrease, which was indicated by the loss of orange hue in foodstuffs (Zhang et al., 2018).

Antioxidant capacity of broccoli powder (*Brassica oleracea* L.)

Antioxidants are needed to prevent oxidative stress, which functions importantly in the etiology of the occurrence of various degenerative diseases (Werdhasari, 2014). Antioxidants can fight free radicals in the human body, which are found in the results of air pollution, body metabolism, polluted food, sunlight, and so on.

Antioxidant capacity is expressed by ic50 rated. IC_{50} (Inhibitory Concentration 50) is a number that shows a sample concentration (ppm) that is able to inhibit free radicals by 50%, The smaller the IC_{50} value means the stronger the antioxidant capacity. In the study, broccoli powder with sun drying process, the antioxidant capacity showed antioxidant capacity with the smallest IC_{50} value, namely in leaf samples with an average IC_{50} value of 25,215 ppm, compared to broccoli stem and flower powder with an average of 30,721 ppm, 35,145 ppm. Specifically, a compound is said to be a very strong antioxidant when the value IC_{50} (< 50 ppm), strong when the IC_{50} value (50-100 ppm), medium when the IC_{50} value (100-150 ppm), and weak when the IC_{50} value (151-200 ppm) (Molyneux, 2004). Thus, the antioxidant capacity of the broccoli stem, leaf, and flower powder may all be stated to be extremely powerful because the IC_{50} value is 50 ppm, but broccoli leaf powder retains the highest antioxidant capacity because the IC_{50} value is lower than stem and flower powder.

In comparison to stem and blossom powder The findings of this study were also supported by a study done by Devi et al., (2022), who discovered that the maximum antioxidant capacity of broccoli powder dried using a cabinet drier was observed in leaf powder samples with an IC_{50} value of 25,917 ppm when compared to stem and blossom samples. According to Sari and Ayustaningwarno (2014), the antioxidant activity discovered in fresh broccoli blooms and broccoli scions is 78.20 percent, whereas the antioxidant activity found in fruit meat and broccoli scions is 8.18 percent. According to suryaningrum studies, bioactive compounds that are antioxidant sources are rapidly degraded when dried and exposed to heat and sunshine (Suryaningrum et al., 2006). The reduced content of antioxidant activity in broccoli dry powder can be attributed to various factors, some of which are related to the process of cutting, washing, temperature, and drying time of broccoli (Leos et al., 2021), (Xiao et al., 2017). Following the findings of a study conducted by Ayu et al., (2017) which found that drying is influenced by various factors, including temperature and drying time. When dried at high temperatures for a long time, the antioxidant activity of dry matter may decrease.

Conclusion

Based on the research results, it was concluded that broccoli leaf powder has the highest content of vitamin C (7.673 mg/100 g), chlorophyll (1746.198 mg/kg), vitamin K (226.423 ug/g), phenolics (24210.120 mg/kg), flavonoids (15239.947 mg/kg), and the strongest antioxidant capacity (IC_{50} = 25.215 ppm) compared to powdered broccoli flowers and stems. Broccoli leaf powder shows the highest content of active compounds and phytochemicals compared to flowers and stems.

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Declarations

Conflict of interests the authors declare no competing interests.

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