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Original Research Article

Analysis of Antioxidant Content of Fortified Moringa Salt during Storage

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Abstract

Fortified moringa salt was a form of food salt. Moringa has contains 46 healthy antioxidant compounds. These antioxidant compounds can neutralize free radicals that damage cells in the body. The addition of Moringa in salt expected to be one source of antioxidants so that salt does not only have NaCl content. The study aims to identify antioxidant activity in moringa fortification salts during storage. Packaging of Fortified moringa salt was aluminum foil and PET plastic bottles. The salt analysis of antioxidant content in 0 days, 10 days, 20 days and 30 days of storage. The temperature and packaging difference had significantly affected the antioxidant activity of the moringa fortification salts, but material packaging significant effect the antioxidant activity. On the 30th day of observation, the IC50 decreased from 27,766 ppm to 46,713 ppm in aluminum foil packaging at a temperature of 27°C. The storage temperature from 27,766 ppm to 34,086 ppm is likewise 45°C. Unlike fortified moringa salt with plastic bottle packaging, the antioxidant activity increased at a storage temperature of 45°C from 27,766 ppm to 10,194 ppm. The antioxidant content in moringa fortified salt included in the extreme antioxidant strength band, with a value below 50 ppm.

Keywords: Antioxidan, fortified moringa salt, storage.

INTRODUCTION

Fortification of foodstuffs is the application of one or more nutrients to food. The main aim is to increase the intake of added nutrients to enhance the nutritional status and the quality of the food. Food fortification's primary function is to prevent shortages, thus preventing disruptions leading to human deprivation and socioeconomic loss. Food fortification, however, has also been used to remove and control nutritional deficiencies and resulting disorders (Martianto *et al.*, 2009; Siagian, 2003).

One form of food fortification is salt made from iodine. Iodized salt helps to reduce iodine deficiency (GAKI) disturbances. The most popular process used for salt is salt iodization (Siagian, 2003) (Howson *et al.*, 1998). Fortification bases belong to UU No. 36 of 2009 on health, Food Law No. 18 of 2012, Presidential Decree No. 42 of 2013 on the National Campaign for the Acceleration of Nutrition Change, PP. 28 of 2004 on Food Safety, Quality and Nutrition.

Salt fortification actually does not only use iodine; other ingredients may have been added to supplement human nutritional needs. Additional components that may have been used in fortification include soybeans, known to increase iron in food products (Amin *et al.*, 2017), seaweed (Gracillaria sp.) as an iodine source, which may have been used in food products as an additional ingredient (Amin *et al.*, 2017). Carry out food fortification *(Rahayu et al.*, 2003), skin dragon fruit as a source of vitamin C (Maflahah & Asfan, 2020), and leaves of Moringa oleifera (Astutik *et al.*, 2019).

Moringa may have been used as a salt-fortification ingredient. Moringa is an arboreal. The container was flavonoids, saponins of cytokinine, caffeoylquinic acid, and it contains unsaturated fatty acids. Traditionally, Moringa leaves have used to treat hyperglycemia, inflammation, bacterial infections and cancer (Tiloke *et al.*, 2018). Moringa leaves' high antioxidant activity can be a source of external antioxidants that can prevent illness (Toripah *et al.*, 2014). The leaves of Moringa oleifera contain high antioxidant levels and bioactive compounds that can prevent oxidative stress and cancer (Kusmardika, 2020). Adding 5 ml of Moringa leaf extract to salt will produce salt with a content of 0,98 μg/g of vitamin A, whereas adding 10 ml may produce salt with a content of 1,37 μg/g of vitamin A. Moringa oleifera leaf extract, with the greatest antioxidant activity, is ethanol with an IC_{50} value of 103,98 μg/mL, containing alkaloids, flavonoids , tannins, steroids and saponins (Tutik *et al.,* 2018).

Flavonoid compounds are heat sensitive, causing chemical degradation during the heating or storage process (Mrmmosanin *et al.,* 2015). Temperature and storage time have a major effect on chemical degradation such as oxidation or hydrolysis, which may occur when the temperature rises. The higher the temperature , the higher the overall activity of phenols, antioxidants and antimicrobials (Soehendro *et al.,* 2015).

Salt may cause the antioxidant content of moringa leaves to suffer degradation at this stage of making moringa fortification. In addition, the process of processing salt may also influence the antioxidant material. The product would evaporate in the salt storage process, thereby causing high temperatures in the surrounding atmosphere to affect the product quality. The form of packaging used by the company also affects antioxidant activity, in addition to temperature and storage time (Saputra *et al.,* 2018). This work will use the packaging of aluminum foil and plastic PET bottles (Polyethylene terephthalate) and storage at room temperature (27^{o}C) and 45^{o}C. The food product used aluminum foil packaging and PET bottle. Aluminum foil has properties that are not affected by sunlight, cannot burn, does not absorb other materials or substances, does not show a change in size by changing humidity, so this type of packaging is suitable for salt. Likewise, the type of PET bottle packaging is exact in color to increase consumer attractiveness to the product. Usually, salt storage is carried out at room temperature, so this study uses a temperature parameter of 27^o C. The choice of temperature is 45^{o}C because during the salt storage process in storage with extreme temperatures. This research aimed to establish the antioxidant activity during storage of fortified moringa salt.

RESEARCH METHODS

Tools and Materials

The materials used ethanol, DPPH, BHT, moringa fortification salt, aluminum foil, and plastic bottles PET. The instruments for this study: a spectrophotometer with centrifuge, blender, vortex, and Uv-Vis. Fortified salt packaging by Moringa using aluminum foil and plastic bottles made from PET.

Storage Conditions

One hundred grams of moringa fortified salt has been putting in aluminum foil (standing pouch) and a PET bottle. For control, we used moringa fortification salt without treatment. The sample was a store at 27^{o}C and 45^{o}C temperature for 30 days. Antioxidant activity was measured every ten days using the DPPH method. It's repeated all samples triplicate.

Antioxidant Activity

The sample processing step was weighing and mixing 100 grams of moringa fortification salt. Each sample is then filtered with filter paper. The filter results were centrifuged for 20 minutes at a speed of 5.000 rpm, separated by a pipette between the liquid and the dregs which settle beneath it. Then weigh each sample with 100 mg each and dissolve it with 100 ml of ethanol to obtain 1.000 ppm. Using aluminum foil to cover the pot and store it in the refrigerator at 4°C.

DPPH solution was prepared to weigh DPPH powder (BM 394, 32) as much as 19.7 mg and dissolve it in a volumetric flask with 500 ml of ethanol. Using aluminum foil, cover the volumetric flask then swirl the DPPH until homogeneous. DPPH with a concentration of 0.1 mM will be obtained from those samples. BHT Antioxidant Activity measurement Weigh BHT with a weight of 100 mg, then dissolve it with 100 ml of ethanol to obtain a concentration of 1000 ppm. 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm taken from this solution. Instead substitute with 1 ml of DPPH solution and pump up to 6 ml of methanol. The solution was then homogenized and incubated 30 minutes in a dark space, and the absorption was tested with a wavelength of 517 nm.

Measurement of Moringa Fortified Salt's Antioxidant Activity: Each sample took 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. To get 10 ppm to 1 ml of the sample concentration that has been dissolved in ethanol. Add 1 ml of DPPH to all five sample concentrations, add 6 ml of ethanol, then homogenize and incubate in a dark room for 30 minutes. And test the wavelength of 517 nm for absorption. The parameter used for the DPPH test is IC_{50} (inhibitory concentration), which is the number of concentrations of extract or test fractions required to capture 50 percent of DPPH radicals (Zou *et al.,* 2004) (Tristantini *et al.,* 2016). Percentage of inhibition was calculated as follows:

$$\%\ of\ Inhibition = \left(1 - \frac{B}{A}\right) x100$$

Where A is the antioxidant activity without inhibitor and B is the activity in the presence of inhibitor. Concentrations of extracts required for half maximal inhibitory concentration (IC_{50}) were also obtained.

Statistical Analysis

Analysis of variance (ANOVA) analyzed data, and Fisher's most significant difference test (LSD) was used to identify differences between means. Changes and significant differences were considered at P < 0,05. All experiments were in triplicate.

RESULTS AND DISCUSSION

The amount of DPPH inhibition (percent inhibition) will be obtained to assess the inhibitory concentration value (IC_{50}), based on the absorbance results of the sample. A linear regression equation can be used to measure IC_{50}, (Aksoy *et al.*, 2013). **Figure 1** shows that moringa fortification salt with the highest amount of antioxidant is salt packed using PET plastic bottles processed at 45°C with an IC_{50} value of 10,194 ppm to bind 0,1 mM of DPPH.

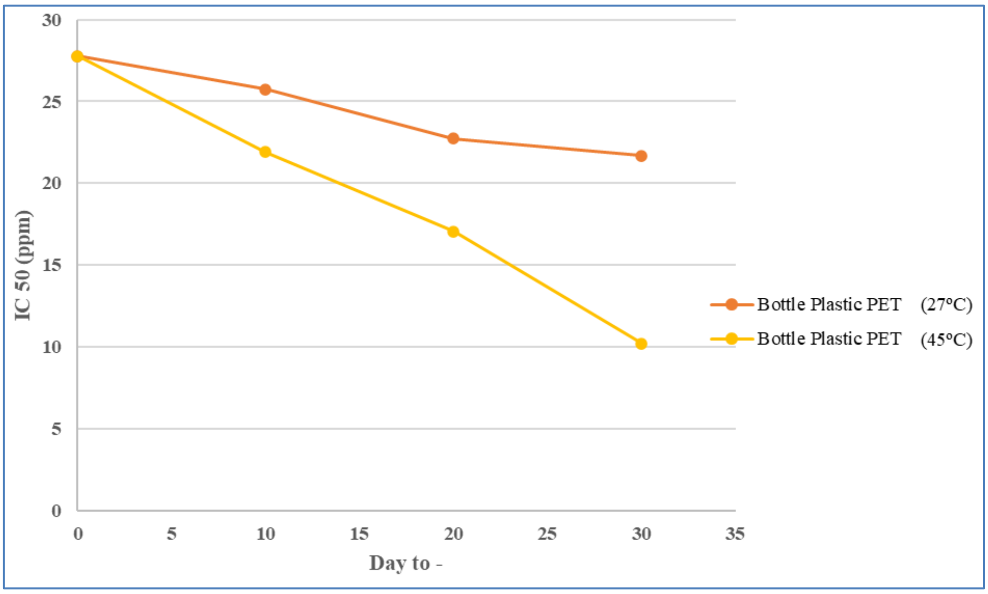

Figure 1: Comparation of IC_{50} During Storage fortified moringa salt using Bottle Plastic PET Packaging

Figure 2 shows that Moringa fortified salt with aluminum foil packaging with the lowest antioxidant (IC_{50} of 92,99 ppm), at normal temperatures storage. **Figure 1** and **Figure 2 s**hows that the four storage treatments have a value of IC_{50} which continues to change during storage. Based on the graph it can show that the moringa fortification salt has increased its antioxidant activity using PET plastic bottles. It has a faster decrease in antioxidant activity, as compared to the fortified moringa salt which uses aluminum foil packaging.

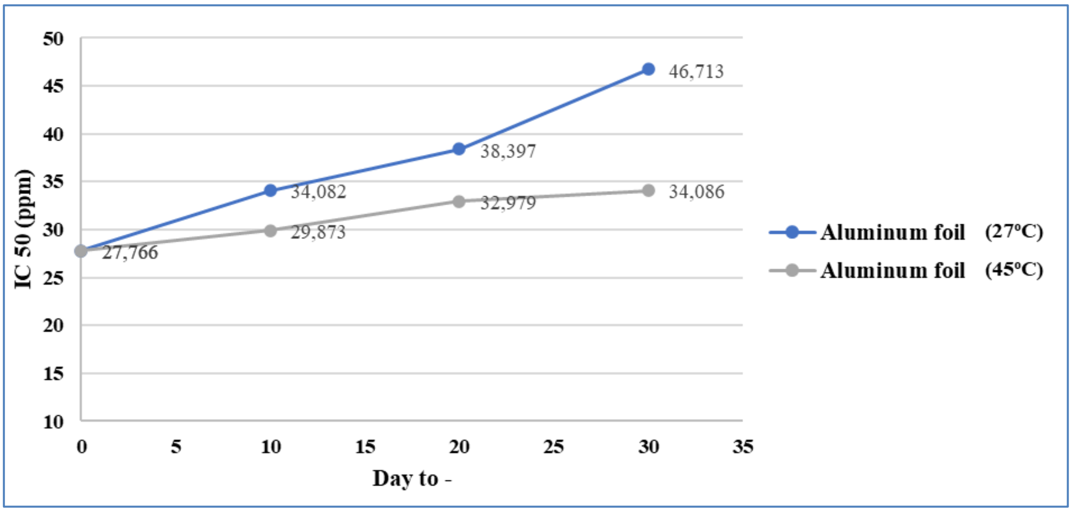

Figure 2: Comparation of IC_{50} During Storage fortified moringa salt using Aluminum Foil Packaging

The level of antioxidant activity in a substance is shown in **Table 1**. The IC_{50} values for aluminum foil packed moringa reinforced salt were 46,713 ppm (27°C temperature) and 34,086 ppm (45°C temperature). The value of IC_{50} fortified salt with PET plastic bottles in moringa, meanwhile, was 21,7 ppm (temperature 27°C) and 10,194 ppm (temperature 45°C). Overall, the antioxidant activity in moringa fortified salt has been placed in the group of extreme antioxidant strength with a value below 50 ppm. Moringa leaf extract contains very strong antioxidant activity (Yati *et al.*, 2018). This antioxidant content can be maintained in fortified moringa salt

Table 1: Antioxidant-Power Levels

Antioxidant Intensity	IC50 Value (ppm)
Very strong	< 50
Strong	50 – 100
Moderate	100 – 250
Weak	250 – 500
Not active	> 500

Source: Jun *et al.*, 2010

A statistical test performed to assess the effect on antioxidant activity of temperature and packaging materials during storage. Based on the results of statistical analyzes using the SPSS program, it showed that the treatment for storage temperature had significant effect on antioxidant activity ($p<0.05$), namely the p-value (0,029). Inline to research (Saputra *et al.*, 2018), which states that antioxidant activity is affected by temperature and type of packaging. The storage time affects the anti-oxidant activity of the product. The type of packaging treatment significantly impacted moringa fortification salt's antioxidant activity, namely the p-value (0,003)(Hartono *et al.*, 2019). In the treatment of aluminum foil packaging at a storage temperature of 27^o C for 30 days, i.e. 46,713 ppm, the highest average value of moringa fortification salt antioxidant activity occurred and the lowest was in the PET plastic bottle packaging with a storage temperature of 45^{o}C for 30 days, i.e. 10,194 ppm.

In every 10 days of observation, the content of antioxidant activity in moringa fortified salt with aluminum foil packaging and plastic PET bottles with storage temperatures of 27^{o}C and 45^{o}C differed. On the 30th day of testing, IC_{50} decreased from 27,766 ppm to 46,713 ppm in aluminum foil packaging at a temperature of 27^{o}C. The storage temperature from 27,766 ppm to 34,086 ppm is likewise 45^{o}C. The antioxidant activity increased from 27,766 ppm to 10,194 ppm at 45^{o}C storage, as opposed to moringa fortified salt with plastic bottle packaging. During storage, decreased antioxidant activity may be attributed to the cycle of oxidation. Packing of aluminum foil has properties as an excellent heat conductor to raise the product temperature in the bag. Raising the product temperature makes the process of oxidation easier.

Aluminum foil is an "alloy" sheet with 99,4 percent aluminum material. Aluminum foil is rendered in various shapes depending on the use or finish. Aluminum foil is brittle and is often plastic or paper laminated to make it more useful. Some of the aluminum foil properties that are very desirable to use as a packaging material are lightweight, flexible, easy to form according to the packaging purpose, attracting the attention of customers, airtightness, water and grease, hygiene, non-toxicity, without affecting taste and smell, and wrapping items or goods. Also, aluminum foil is a good heat conductor for electric power and heating. The downside is that it can hurt the acids, table salt, and heavy metals. This may also induce decreased antioxidant activity in moringa-fortified salt, based on the weaknesses of aluminum foil packaging, namely as a heat conductor and may be impaired by table salt.

Polyethylene Terephthalate (PET) plastic is clear, transparent, durable, solvent-resistant, gas-resistant and water-resistant 180^{o}C softener. It fully melts in 200^{o}C. The benefits of plastic packaging are insulators for heat and electricity, and are waterproof. Plastics also have an excellent permeability value so they are gas, water vapor, and microorganism resistant (Buckle *et al.*, 1987). The advantage is what allows the salt fortified by moringa to retain the antioxidant activity found in it. The antioxidant activity content may be reduced during processing and storage when there is a heating process.

CONCLUSIONS

The temperature and packaging difference had significantly affect the antioxidant activity of the moringa fortification salts. Using aluminum foil and PET plastic bottles with a room temperature and temperature of 45^{o}C, the packaging type has a significant effect on the antioxidant activity content. On the 30th day of observation, the IC_{50} decreased from 27,766 ppm to 46,713 ppm in aluminum foil packaging at a temperature of 27^{o}C. The storage temperature from 27,766 ppm to 34,086 ppm is likewise 45^{o}C. Unlike fortified moringa salt with plastic bottle packaging, the antioxidant activity increased at a storage temperature of 45^{o}C from 27,766 ppm to 10,194 ppm. The antioxidant content in moringa fortified salt included in the extreme antioxidant strength band, with a value below 50 ppm. Moringa fortified salt can serve as an antioxidant source.

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