

Vitamin C, total titrated acid and antioxidant activity of Oximata® jelly mix

¹Banin, M.M., ¹Nurdiana, S., ¹Emmawati, A., ¹Rohmah, M. and ^{1,2,*}Rahmadi, A.

¹Department Agricultural Products Technology, Faculty of Agriculture, University of Mulawarman, Jl. Tanah Grogot Kampus Gunung Kelua, Samarinda, East Kalimantan, Indonesia

²Research Center for Medicine and Cosmetics from Tropical Rain Forests (PUI-PT Oktal), University of Mulawarman, Jl Kerayan Kampus Gunung Kelua, Samarinda, East Kalimantan, Indonesia

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Abstract

Oximata® jelly mix is a functional food rich in carotenoids and tocopherols from the formulation of red palm oil, pumpkin juice, and red dragon fruit juice. This study was conducted to determine the effect of increasing red palm oil concentration, temperature, and storage time on vitamin C, total titrated acid, and antioxidant activity using the ABTS method ((2,2-Azinobis (3-ethyl benzothiazoline) -6-sulfonic acid) and FRAP (Ferric Reducing Antioxidant Power). The study used three factors with a combination of treatments consisting of observation day (day 1, 7 and 14), storage temperature (28°C and 7°C) and red palm oil concentration (0; 0.15%; 0.30% and 0.45%). The results showed that Oximata® jelly mix powder added with 0.45% red palm oil and stored at 7°C for seven days was the best treatment with vitamin C levels 5.83 ± 0.24 µg/mL, antioxidant activity $74.29 \pm 3.00\%$ (ABTS) and 22.17 ± 0.24 µg/mL (FRAP). The total titrated acid value increased in the addition of Red Palm Oil by 0.15 and 0.3% and storage for 14 days at 28°C, namely 1.54%. The concentration of red palm oil, temperature, and storage time affect vitamin C, total titrated acid, and antioxidant activity.

1. Introduction

Oximata® is a functional food in an emulsion drink made from red palm oil (RPO), pumpkin juice, and red dragon fruit juice. The raw material for making the emulsion is a superior product in East Kalimantan. The enhancement of the Oximata® product prototype in the form of jelly is designed to have a long shelf life and practical presentation by dissolving it again with hot water (Rahmadi *et al.*, 2020)

In previous research, Oximata products contain trans-β-carotene, α-tocopherol, vitamin E, lycopene, lutein, sterols, unsaturated fatty acids, and ubiquinone. Oximata is acidic with a pH of 3.6 and a vitamin C content of 13.2 mg/100 g of product (Rahmadi *et al.*, 2020). Pumpkin fruit (*Cucurbita moschata*) is one of the Oximata® raw materials, which contains protein, vitamins, minerals, antioxidants such as tocopherols, and carotenoids 2.7 mg β-carotene/g fruit, 19.1% fibre, and 7.3% pectin (Noelia *et al.*, 2011; Kim *et al.*, 2012). Red dragon fruit also contains fibre, vitamin B3 (niacin), vitamin C, and antioxidant compounds such as phenols, flavonoids, and betacyanin (Putriningtyas *et al.*, 2020). The addition of red dragon fruit significantly improves the emulsion's

vitamin C content and titratable acid content (Rahmadi *et al.*, 2017; Bohari *et al.*, 2018; Rohmah *et al.*, 2021). Oximata® can be used as a vitamin A supplement because RPO is rich in α- and β-carotene. The use of RPO with higher concentrations can increase the antioxidant activity of the product (Nnaji *et al.*, 2012). Temperature and storage time influenced the quality of Oximata® jelly mix (OJM) powder. These factors can cause changes in the nutritional value of food products, whether damaged or lost due to oxygen, pH, light, and storage temperature (Sarungallo *et al.*, 2018). Low-temperature storage inhibits antioxidant damage because it can stop or inhibit microbial growth, chemical reactions, and enzyme activity (Wulansari *et al.*, 2020).

Antioxidant activity, vitamin C, and total titrated acid (TTA) were factors determining the quality of OJM powder (as a marker). Researchers quantify antioxidant activity in food samples using methods based on free radical scavenging, such as ABTS (2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate)), and on iron ion reduction by phenol, such as FRAP (Ferric Reducing Antioxidant Power) (Biskup *et al.*, 2013). The ABTS and FRAP procedures have various advantages, including

*Corresponding author.

Email: arahmadi@unmul.ac.id

their ease of use, speed, and ability to determine the overall antioxidant capacity of a food sample, which allows for comparisons between samples with the same antioxidant content (Nilsson *et al.*, 2005).

The purpose of this study was to determine the concentration of RPO in OJM powder against changes in levels of vitamin C, TTA, and antioxidant activity using the ABTS and FRAP methods. OJM was stored at room temperature and in the refrigerator for a long observation for two weeks.

2. Materials and methods

2.1 Material

Materials used were red palm oil (RPO) (ExcelVite, Malaysia) with 10% α -tocopherol and 5% β -carotene, pumpkin, red dragon fruit, granulated sugar, citric acid, and sodium benzoate. The chemicals consist of ABTS (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), Phenolphthalein (Merck), NaOH (Merck), absolute ethanol (Fulltime China), $K_2S_2O_8$ (Merck), and $FeSO_4 \cdot 7H_2O$ (Merck).

2.2 Research design

This study used three factors with a combination of treatments consisting of (1) observation day (day 1, 7, and 14); (2) storage temperature (28°C and 7°C); and (3) RPO concentrations (0, 0.15%, 0.30% and 0.45%). The data obtained were analyzed using Two-way ANOVA, and further tests were carried out using Fisher's LSD test.

2.3 Pumpkin powder preparation

About 500 g of pumpkin (approximately three months old) was dried at 50°C for 18 hrs, then mashed and sieved (80 mesh). The resulting pumpkin powder was packaged and stored in dry conditions.

2.4 Red dragon fruit and red palm oil mixture powder production

A total of 100 g of pureed dragon fruit was added to 50 mL of water, and then filtered with a 120 mesh sieve. Red dragon fruit juice was then added with 100 g of sugar and RPO with several concentrations, namely 0.00%, 0.15%, 0.30%, and 0.45%. Then, heated at a temperature of 40-50°C for 30 mins to form granules, then cooled accompanied by stirring. The granules that were formed were then either reduced in size or crushed.

2.5 Preparation of Oximata® jelly mix powder

The Oximata® jelly mix formula is produced from 3.3 g pumpkin powder; red dragon fruit powder + RPO 6.7 g; citric acid 0.3 g; sodium benzoate 0.01 g for each RPO concentration. All the ingredients were mixed by

stirring until they were homogeneous to produce OJM powder. OJM was then stored at different temperatures, namely 28°C and 7°C, and then observed on days 1 (control), 7, and 14 each at different RPO concentrations of 0.00% (control), 0.15%, 0.30%, and 0.45%.

2.6 Vitamin C analysis

Analysis of vitamin C was conducted based on Setiawan *et al.* (2014) with modifications on the concentration of samples and standards. First, the determination of vitamin C was carried out based on a standard curve of ascorbic acid with 0.025 g of ascorbic acid mixed with 25 mL of 96% ethanol. Concentrations of 1 μ g/mL, 10 μ g/mL, 20 μ g/mL, 30 μ g/mL and 40 μ g/mL were made. Then, the sample was dried in the oven for 3 to 7 days, blended and sieved. A total of 0.025 g of sample was added with 25 mL of 96% ethanol. The absorbance value was measured at a wavelength of 270 nm.

2.7 Analysis of total titrated acid

The total titrated acid (TTA) was determined by titrimetry (Sudarmaji *et al.*, 2007) based on the levels of citric acid.

2.8 Antioxidant activity analysis

2.8.1 ABTS method

The analysis of antioxidant activity via the ABTS method (2,2-Azinobis (3-ethyl benzothiazoline) -6-sulfonic acid) was performed based on Sami and Rahimah (2016) with modifications. Firstly, the two stock ABTS solutions namely (A) 7.1015 mg ABTS and (B) 3.500 mg $K_2S_2O_8$ were prepared. The solutions were dissolved in 5 mL of distilled water and incubated for 12 hrs. After incubation, solutions A and B were mixed in a dark room and added with absolute ethanol until the total volume reached 25 mL. The ABTS blank solution was prepared by adding 1 mL of ABTS stock solution with absolute ethanol to make a 5 mL solution. A total of 500 μ L of 1000 μ g/mL sample solution was added with 1 mL of ABTS solution and absolute ethanol to make up the volume to 5 mL with a concentration of 100 μ g/mL. The absorption value of the samples and the blank was measured at the wavelength range of 745-755 nm and calculated. Vitamin C (5 μ g/mL) was used as a standard. The percentage of antioxidant activity in the sample was compared with the antioxidant activity of Vitamin C.

2.8.2 FRAP method

The analysis of antioxidant activity via the Ferric Reducing Antioxidant Power (FRAP) method was performed based on Vichitphan *et al.* (2007) with modifications. A stock solution of 10,000 μ mol/L

FeSO₄·7H₂O was prepared by dissolving 2.78 g of FeSO₄·7H₂O in 1000 mL of distilled water. The stock solution (100 mL) was diluted to 1000 mL to obtain a concentration of 1000 μmol/L FeSO₄·7H₂O solution. Then, 1, 2, 3, 4 and 5 mL of the 1000 μmol/L FeSO₄·7H₂O solution was placed in different volumetric flasks and topped up with distilled water to 100 mL which made a concentration of 10, 20, 30, 40, and 50 μmol/L, respectively. Each aliquot (1 mL) of the different concentrations of FeSO₄·7H₂O solution was added with 3 mL of FRAP reagent and the absorbance was measured at 588-598 nm wavelength to form a FeSO₄·7H₂O calibration curve. Samples (0.1 mL) was mixed with 3 mL of the FRAP reagent and measured its absorbance at 588-598 nm wavelength. The total antioxidant in the sample via FRAP method was deduced using the FeSO₄·7H₂O calibration curve.

3. Results and discussion

3.1 Vitamin C

Vitamin C, also known as ascorbic acid, is one of the most reactive compounds to oxidation. Vitamin C has a chemical structure consisting of a chain of 6 C atoms.

Vitamin C is unstable (C₆H₈O₆) because it quickly reacts with oxygen in the air to become dehydroascorbic acid (Thuy *et al.*, 2020). Vitamin C in OJM is affected by the addition of different concentrations of RPO (Figure 1). The highest vitamin C in the addition of RPO 0.45% was 9.51±0.06 μg/mL and decreased with storage time.

The increase in RPO concentration was in line with the increase in vitamin C, where the addition of 0.45% RPO showed the highest significance of vitamin C. Red palm oil is rich in antioxidants and can be used to preserve food by reducing rates of damage, rancidity, and discoloration due to oxidation (Shahidi and Ambigaipalan, 2015). Increasing the concentration of RPO causes the levels of vitamin C in OJM to be higher because the content of RPO protects the emulsion from the formation of free fatty acids and other free radical components during product storage.

In addition, RPO is rich in α-tocopherol and β-carotene, which has a synergistic effect with vitamin C in red dragon fruit and pumpkin to increase free radical scavenging activity. Lipid peroxidation was also effectively inhibited by the combination of α-tocopherol

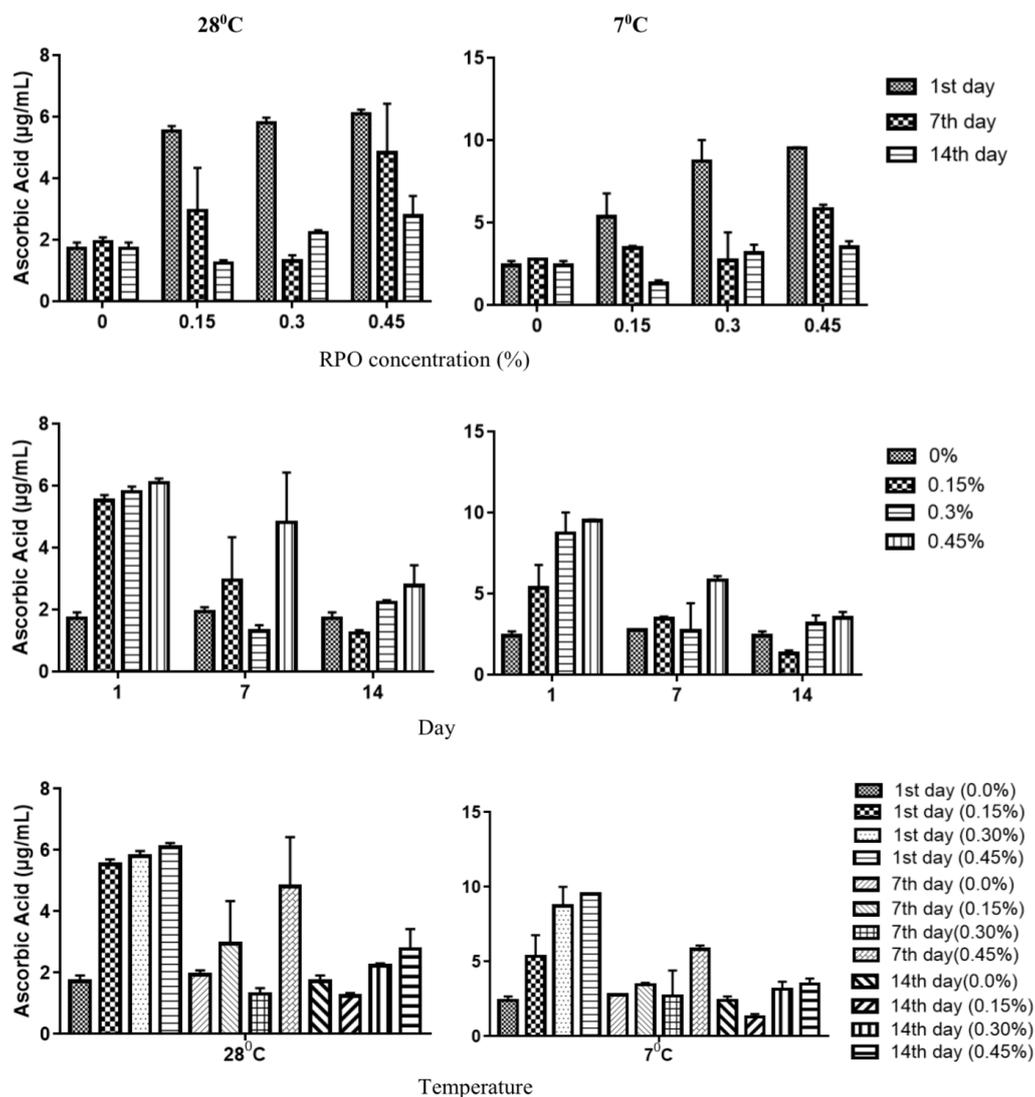


Figure 1. Vitamin C of Oximata® jelly mix with different red palm oil concentrations, storage time, and temperature.

and ascorbic acid. During lipid peroxidation, lipid membranes generate free radicals which are then absorbed by α -tocopherol and oxidized to produce α -tocopheroxyl radical. Furthermore, ascorbic acid captures the α -tocopheroxyl radicals which are included in the radicals that can sink in the water phase. The direct chemical reaction between ascorbic acid and α -tocopherol, in which electrons are transferred from one molecule to the other, is responsible for the synergy (Hazewindus et al., 2012; Widowati et al., 2017).

Storage plays an essential role in the quality of products, including vitamin C. After two weeks of storage at 28°C and 7°C, there was a decrease in the value of vitamin C in all samples. Vitamin C is a type of vitamin that is easily damaged and oxidized by heat, light, pH, and oxygen (Shahidi and Zhong, 2010). The highest vitamin C was found in OJM stored at 7°C and decreased slower than OJM stored at 28°C. Vitamin C is damaged due to oxidation, transformed into L-dehydroascorbic acid, and converted into L-diketogulonic acid, reducing its antioxidant activity. At room temperature, decreased levels of vitamin C happen at the earliest because of heat and oxygen (Rahayu and

Pribadi, 2012). A decrease in temperature can help prevent the oxidation of vitamin C (Spínola et al., 2013). Vitamin C has a functional role in product quality, such as increasing colour, shelf life, and acceptability of food products because of its role as an antioxidant (Astawan et al., 2017; Thuy et al., 2020)

OJM with an RPO concentration of 0.45% had the highest levels of vitamin C. Storage at 28°C for seven days was able to maintain the vitamin C content of $5.83 \pm 0.24 \mu\text{g/mL}$. OJM stored for 14 days showed a significant decrease in vitamin C levels, namely 42.34%. Storage at 7°C is better than storage at 28°C, which is 40%. Therefore, seven days of storage at 7°C with an RPO concentration of 0.45% was the best treatment. Carotene in RPO is one of the antioxidant compounds that have the ability to quench so that it can inhibit the oxidation process caused by the light (photo-oxidation) (Rahmadi et al., 2017)

3.2 Total titrated acid

The addition of RPO, storage temperature, and length of observation resulted in different TTA (Figure 2). The first-day storage at 28°C and 7°C and the

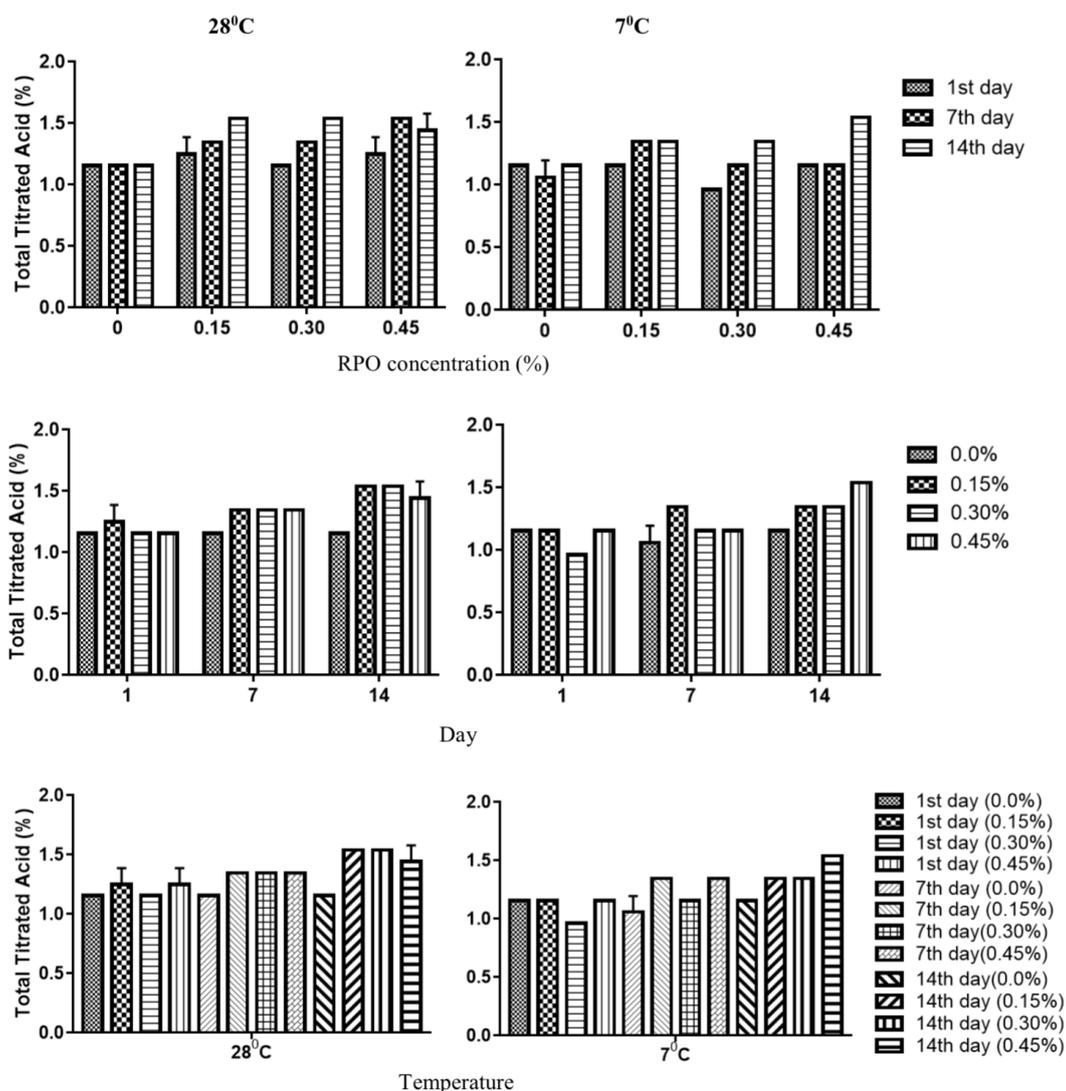


Figure 2. Total titrated acid of Oximata® jelly mix with different red palm oil concentrations, storage time, and temperature.

addition of varying RPO concentrations had nominal TTA values. The higher the RPO concentration, the higher the TTA, namely 1.54%. On test days 1, 7, and 14, the TTA values for all concentrations were significantly higher than those without the addition of RPO. The storage temperature of 7°C for seven days did not affect the TTA value of the product, namely 1.15%.

Over time the value of TTA increased in all samples. This is because the time observed and the high storage temperature causes an increase in TTA, where the total acid is closely related to the pH value. The higher the total acid, the smaller the pH value. The increase in total acid causes a decrease in pH (Rahmadi *et al.*, 2020). The increase in TTA from samples stored for 14 days at 28°C could be caused by the decomposition of the fermentable substrate, especially the carbohydrate component in the OJM powder, thereby increasing the acidity. In contrast, the samples stored at 7°C had a slight change in TTA, which indicated that low temperature slowed the growth rate of microorganisms (Chiabrando and Giacalone, 2015; De Leonardis *et al.*, 2016). So that storage at a temperature of 7°C for seven days can maintain the TTA value so that there is no significant increase.

3.3 Antioxidant activity

3.3.1 ABTS method

ABTS is a chemical compound 2,2'-azinobis (3-ethyl benzothiazoline-6- sulfonic acid) with properties as a cation radical. The radical has a nitrogen centre with a characteristic blue colour. If antioxidants reduce ABTS, they will turn into a non-radical and colourless form (Tai *et al.*, 2016). ABTS is a cation radical that is highly reactive to lipophilic antioxidants (i.e., α -tocopherol or β -carotene) (Martysiak-Zurowska and Went, 2012). The antioxidant activity of the sample can be determined by ABTS testing. The test results showed that the addition of different concentrations of RPO had a significant effect on antioxidant activity (Figure 3).

OJM powder stored at 28°C experienced a decrease in the inhibition value of ABTS during storage, from $66.45 \pm 5.35\%$ to $47.05 \pm 7.27\%$. This can occur due to oil oxidation affected by energy inputs such as light or heat, oxygen, free fatty acids, thermally oxidized materials, and antioxidants. Oil oxidation and hydroperoxide decomposition increase with increasing temperature, on the contrary, slowly at low temperatures (Choe and Min, 2006).

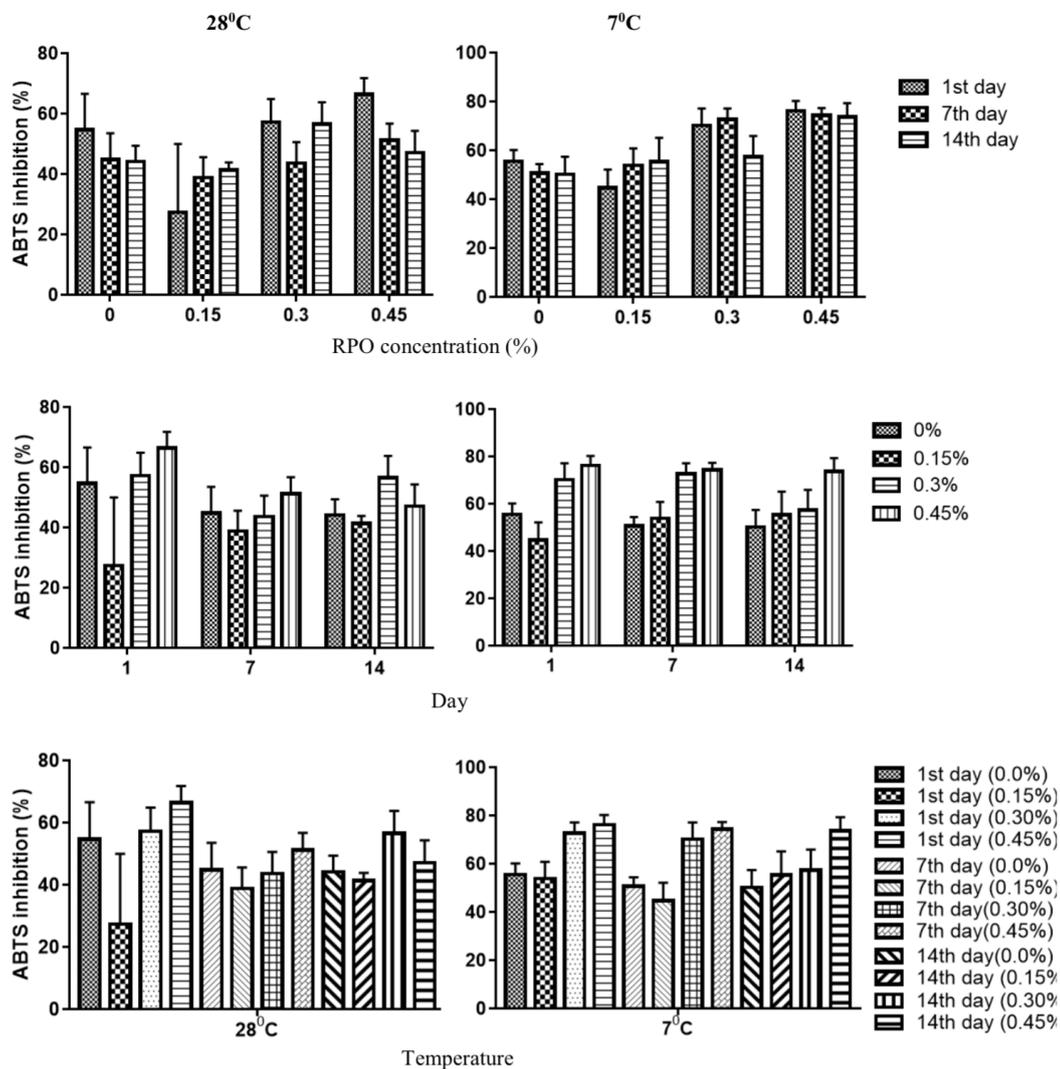


Figure 3. ABTS inhibition of Oximata® jelly mix with different red palm oil concentrations, storage time, and temperature.

One of the efforts to prevent a decrease in antioxidant capacity is to store it in cold temperatures so that the oxidation reaction can be slowed down (Wulansari *et al.*, 2020). Storage at 7°C showed a higher value than storage at 28°C, so it was able to retain the ABTS inhibition value over two weeks of storage. Storage temperature seven on days 7 and 14, the inhibition value of ABTS was not significant to storage on day 1, especially at the RPO concentration of 0.45%, which was able to maintain the inhibition value of ABTS for 14 days, from 76.19±4.00% to 73.63±5.68%.

The addition of 0.3% and 0.45% of RPO showed a significant increase in ABTS inhibition compared to without the addition of RPO. This indicates that the antioxidant activity is closely related to the active components contained in it, such as carotenoids, tocopherols, and tocotrienols which are active components of red palm oil (Loganathan *et al.*, 2017). Tocopherols and tocotrienols are vitamin E isomers' potent antioxidants that provide oxidative stability to oil (Nagendran *et al.*, 2000). Therefore, the higher the

addition of RPO concentration, the higher the ABTS inhibition.

Vitamin C is an antioxidant that works to shield against radiation. Vitamin C (ascorbic acid) and α -tocopherol or β -carotene have a synergistic effect in increasing free radical scavenging activity (Swindells and Rhodes, 2004; Widowati *et al.*, 2017). In addition, the increase in antioxidant activity is in line with the high concentration of RPO. This can be caused by the pro-oxidation properties of carotene and other antioxidant compounds in red palm oil, which are thought to be vitamin E (tocopherols and tocotrienols) (Mukherjee and Mitra, 2009; Nnaji *et al.*, 2012). Therefore, the best antioxidant activity in OJM was 74.27±3.00%. It is a sample with the addition of RPO 0.45% with storage for seven days at 7°C.

3.3.2 FRAP method

Antioxidants are compounds that delay or inhibit the onset of oxidation of other compounds (Embuscado, 2015). In this study, changes in antioxidants were

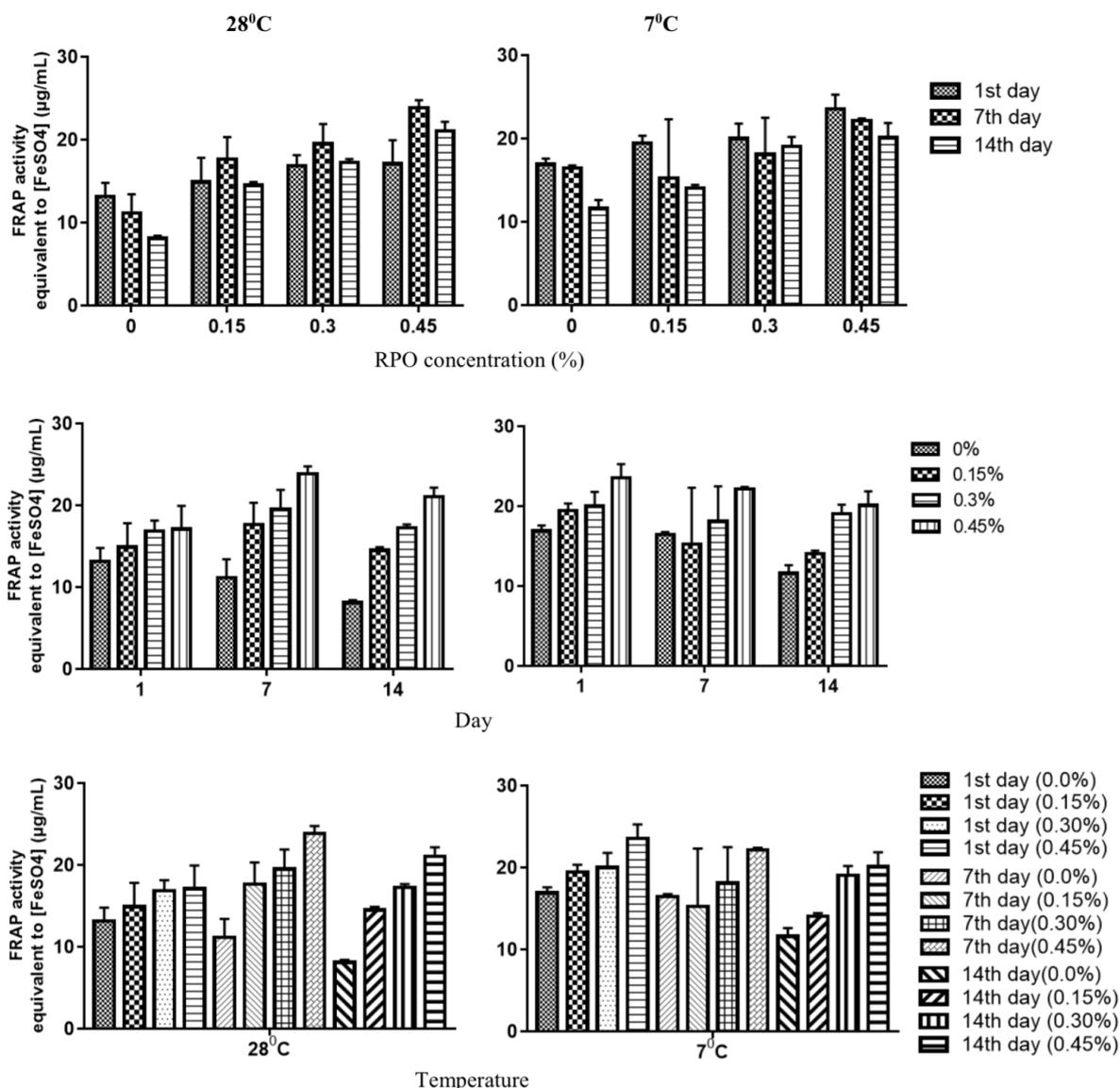


Figure 4. FRAP Activity of Oximata® jelly mix with different red palm oil concentrations, storage time, and temperature.

calculated using the FRAP method, as shown in Figure 4. Differences in storage temperature, observation days, and RPO concentrations in OJM significantly affected changes in antioxidant activity.

The increase in FRAP activity is directly proportional to the addition of the RPO concentration. The addition of 0.3% and 0.45% RPO significantly increased FRAP activity compared to samples that only added 0.15% RPO and samples without RPO. The antioxidant content of 10% tocopherol and 5% carotene plays a role in increasing antioxidant activity. Harianti *et al.* (2018) stated that the higher the tocopherol, tocotrienol, and β -carotene, the greater the antioxidant activity. In addition, ascorbic acid, better known as vitamin C in red dragon fruit and pumpkin, can ward off extracellular free radicals. This is because vitamin C has a free hydroxy group that acts as a free radical scavenger and a polyhydroxy group that increases antioxidant activity (Kusumawati and Haryoto, 2019).

The decrease in antioxidant activity was in line with the storage time of OJM powder, where storage for two weeks decreased the antioxidant activity in all treatments. The temperatures of 28°C and 7°C have an effect on antioxidant activity in OJM. OJM stored at 7°C had higher antioxidant activity than OJM stored at 28°C. Low temperature can maintain OJM antioxidant activity for up to 7 days of storage. Antioxidant activity decreased significantly on storage on day 14. OJM with 0.45% RPO concentration had relatively stable FRAP activity during storage when compared to other samples. The FRAP activity value on day 1 was 23.56 ± 1.71 $\mu\text{g}/\text{mL}$, then on day 7 was 22.17 ± 0.24 $\mu\text{g}/\text{mL}$ and on day 14 was 20.10 ± 1.75 $\mu\text{g}/\text{mL}$.

The protective effect of low temperature on oxidation is in line with the research of Tristanto *et al.* (2017), which tested the antioxidant activity of dry stevia leaf powder stored at refrigerators and room temperature. The results showed that dry stevia leaf powder stored at refrigerator temperature had a higher antioxidant activity value when compared to the powder stored at room temperature. This difference can be due to the high storage temperatures will accelerate the degradation of the antioxidant compounds in the dry stevia leaf powder.

Heat is one of the factors that can affect the rate of lipid oxidation. Oxidation can occur by photo-oxidation (singlet oxygen) or autoxidation (triplet oxygen). Autoxidation is a chain reaction through the triplet oxygen mechanism with radical free radicals. The response begins with radical components of foodstuffs, which then react with radical triplet oxygen (Choe and Min, 2006; Falade *et al.*, 2015). Autoxidation causes the loss of carotenoids, tocopherols, and the content of polar

phenolic compounds. Autoxidation is affected by light, temperature, fat composition, prooxidant, and antioxidants in various vegetable oils (Lee *et al.*, 2007).

The addition of 0.45% RPO gave the highest antioxidant activity. It could be maintained for seven days of storage at 7°C, namely 22.17 ± 0.24 $\mu\text{g}/\text{mL}$. These results are better when compared to holding at a temperature of 28°C. Low temperature can inhibit the degradation process of compounds and chemical reactions contained in a product.

4. Conclusion

RPO concentration, storage temperature, and length of observation affected vitamin C levels, total titrated acid, and antioxidant activity (ABTS and FRAP). OJM stored at 7°C with RPO 0.45% treatment had better levels of vitamin C, TTA, and antioxidant activity (ABTS and FRAP) compared to storage at 28°C. In all tests, 0.45% RPO treatment was the best treatment for OJM raw materials powder mixtures. OJM stored on the 7th day with 0.45% RPO was the best treatment compared to the 14th day. 0.45% RPO stored at 7°C had the best ABTS and FRAP values because the α -carotene and tocopherol components in RPO contributed to the antioxidant activity of OJM.

Conflict of interest

The authors declare no conflict of interest.

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