ANTIOXIDANT TEST OF GRANOLA BAR FUNCTIONAL FOOD WITH CORK FISH AND BEETROOT (*BETA VULGARIS L*) AS SUPPLEMENTARY FOOD TO PREVENT *STUNTING*

Riska Chandra Pradana*, Septiana Laksmi Ramayani
Pharmacy Departement, Poltekkes Kemenkes Surakarta, Letjend Sutoyo, Mojosongo Surakarta 57127
Email: pradanachan@gmail.com

Abstract

Stunting generally occurs in children who have not received nutritious food intake. Sources of nutrition are not only sourced from animal protein but can also be sourced from plants. Combining high-protein foods from animal and vegetable sources will reduce stunting rates in growing children. Snack bars are a beneficial food product that provides all the essential nutrients for the body. In addition, probar consumption in Indonesia is still relatively low, and some people don't even know it exists. These snacks are high in usable energy and contain essential nutrients. This study aims to determine the free radical-catching activity of Granola Bar Functional Food with Cork Fish and Beetroot Fruit as Supplementary Food to Prevent *Stunting.* Free radical scavenging activity test was conducted using the DPPH (1,1- diphenyl-2-picrylhydrazyl*)* method using quercetin as the comparative compound. Preparation methods form formula I, which consists of 20% cork fishmeal and 80% beetroot flour, displayed the lowest IC50 value at 173 ppm. This result indicates that it has the strongest antioxidant activity among the three formulas, though it still falls within the weak antioxidant activity category. In comparison, Formula II, containing 40% cork fishmeal and 60% beetroot flour, had an IC50 value of 298 ppm, showing weaker antioxidant activity. Formula III, which is made up of 60% cork fishmeal and 40% beetroot flour, had the highest IC50 value of 339 ppm, indicating the weakest antioxidant activity of the three. Antioxidant activity analysis was performed using a spectrophotometer at a wavelength of 512 nm. The study concluded that the combination of cork fishmeal and beetroot flour in granola bars showed potential for antioxidant activity, with Formula I performing the best in terms of antioxidant strength.

Keywords: cork fish, beetroot, DPPH, free radical scavenger

1. INTRODUCTION

The prevalence of stunting in Indonesia has decreased since 2017 and is expected to continue until 2019. According to WHO, the prevalence of stunting in Indonesia was 20% in 2019; the national prevalence of stunting is 27.67% or 7 million Indonesian children; one in every three Indonesians is affected by stunting. Stunting occurs when a toddler becomes more disabled than usual. Stunting generally occurs due to a lack of nutritional intake in a child's first 1,000 days. The 1,000-day count here starts from the fetus and continues until the child is

two years old (Direktorat Promkes, 2018). Stunting is associated with a slight increase in brain growth and a slow rise.

The Ministry of Health has launched a national strategy to reduce stunting, including a specific nutritional intervention strategy during the first 1000 days of birth (HPK). Efforts are made to provide drugs or food for pregnant women or infants aged 0-23 months. Nutrition-sensitive interventions are also carried out through various health development initiatives, such as providing clean water or sanitation, nutrition education, and nutrition health promotion. The problem of stunting that occurs in children under five years of age has a terrible impact on morbidity, impaired physical growth, and mental health and can cause death (Rahayuwati et al., 2022).

The need to raise awareness of all parties on the importance of stunting due to the non-natural disaster of the Covid-19 pandemic. This creates a solid impetus to achieve long-term development goals, also known as the Sustainable Development Goals (S.D.G.s), through improved health and well-being. Optimizing the handling of nutritional problems in children under five can be done by diversifying the development of additional food formulas by considering aspects of nutrition, health, acceptability, durability, and the advantages of local food resources. Child malnutrition is still a global problem that contributes to the cause of mortality (Fitriyani & Aisyah, 2022).

Stunting generally occurs in children who have not received nutritious food intake. Sources of nutrition are not only sourced from animal protein but can also be sourced from plants. Combining high-protein foods from animal and vegetable sources will reduce stunting rates in growing children. Protein is needed by the human body to build new tissues and maintain existing tissues (Hamidah et al., 2017). This combination can be done by using cork fish and beetroot, which are known to be high in protein content in the form of probars that children favour.

Cork fish (Chana striata) and beetroot are local food ingredients that have the potential to be developed as food additives to help the government's efforts to reduce stunting. Cork fish is a type of freshwater fish that is commonly found in Southeast Asia, including Indonesia. Although scary, cork fish is rich in essential nutrients for children's growth and development. 150 grams of cork fish contains 4 grams of fat, 118 kcal of calories, 20 grams of protein, and 15 grams of carbohydrates. Cork fish also contains many vitamins and minerals that are very important to support the health of the body. These include vitamins A, B1, B2, and B3, zinc, copper, potassium, sodium, iron, phosphorus, calcium, and fibre, which are very good for the

digestive system.

Changes in eating behaviour in a negative direction can lead to the risk of weight gain and obesity as a risk factor for chronic diseases (Fitriyani & Aisyah, 2022). The combination of foods with high nutrition sourced from animals, when combined with animals, improves the concentration of protein and vitamin content in beetroot. Beetroot has long been used for natural medicine because it contains various nutrients, including carbohydrates, protein, fibre, and vitamins and minerals, such as vitamin C, folate, potassium, magnesium, sodium, and iron. In addition, beetroot is also low in fat and calories, contains no saturated fat or cholesterol, and is rich in antioxidants. Snack bars currently on the market are made from wheat flour and soy flour, imported commodities from Indonesia. Cork fish and beetroot are the food ingredients that do not need to be imported, so the state budget does not need to be hit because the possibility of local food ingredients is cheap. There is no need to import these food ingredients, as they are a well-known source of carbohydrates used in making staple foods.

Products made from nutritious and beneficial foods are a necessity of modern man. There is an urgent need to diversify food sources to meet the growing demand, considering health implications and the taste of the food itself. Snack bars are a wholesome food product that provides all the essential nutrients for the body. In addition, probar consumption in Indonesia is still relatively low, and some people are unaware of its existence. Snack bar products, or energy bars, are generally made from a mixture of several food ingredients such as cereal puffs, nuts, and dried fruits, that are held together by a binder (Mawarno & Putri, 2022). Snack bars are bar-shaped snacks made from various ingredients such as cereals and nuts; because they contain flour, sugar and fat as their main components, snack bars can be considered an energy source. Healthy snacks are foods high in usable energy and contain essential nutrients, including dietary fiber, protein, antioxidants, and various vitamins and minerals that benefit one's health.

2. METHODS

This study adopted a true experimental method to evaluate the antioxidant activity of granola bars containing cork fish and beetroot fruit. The aim was to assess the effectiveness of granola bars in preventing stunting in children through antioxidant treatment.

This research took place from January to December 2023 at the Integrated Laboratory of Campus III of the Poltekkes Kemenkes Surakarta. The primary variable analyzed was a functional granola bar using cork fish and beetroot as the main ingredients. This variable served

as a stimulus to assess its effect in the study.

In its operational definition, cork fish is a high protein source (25.2 g/100 g) and various other essential nutrients, while beetroot is rich in carbohydrates, protein, fiber, vitamins, and minerals. Antioxidant activity tests were conducted using the DPPH method, which measures the concentration of compounds that can reduce the intensity of the DPPH colour by 50%, or the IC50 value. The research instruments consisted of an oven, food processor, dough-making scales, a blender, beaker glass, test tubes, the antioxidant activity was measured using UV-Vis spectrophotometry with a wavelength of 516 nm, and other materials such as DPPH and quercetin for free radical activity tests.

The implementation of the study began with the preparation of cork fishmeal, which involved washing, cleaning, steaming, drying, and pulverizing the fish. Beetroot flour was made by washing, boiling, baking, and grinding the beetroot. Granola bars were then produced by mixing ingredients such as butter, milk powder, flour, coconut milk, syrup, and others, then baked until cooked and cooled.

For solution preparation, 6 mg of DPPH was dissolved in ethanol p.a. to 50 ml. Sample stock solution was prepared by dissolving 10 mg of extract in ethanol (e-merck brand) p.a. to 10 ml, and standard solution with 10 mg of quercetin in ethanol p.a. to 10 ml. The samples were processed as follows: Cork fish was washed, cleaned, steamed, dried, and pulverized. Beetroot was washed, boiled, baked, and ground to make beetroot flour

Quercetin was used as the standard compound because it is a known antioxidant and often used for comparison in free radical scavenging activity tests. The study did not mention directly that quercetin is naturally present in the samples but clarified that it was used to compare antioxidant activity.

An antioxidant activity test was performed by determining the maximum wavelength of DPPH (510-520 nm). After incubation for 60 minutes, the absorbance of the sample and control was measured. Percent free radical scavenging activity and IC50 values were calculated using linear regression.

Data analysis involved comparing absorbance data to determine the percent free radical scavenging activity. Linear regression was used to obtain the IC50. Furthermore, normality and homogeneity tests were performed using Kolmogorov- Smirnov. Standard data were compared using One Way Anova, while non-normal data were analyzed by Kruskal-Wallis, followed by a post hoc test.

3. RESULT AND DISCUSSION

The study began by selecting samples of cork fish meal and beetroot fruit meal to manufacture snack bars. Samples must have a PIRT certificate from the seller showing that the product has received official permission from BPOM or the district/city Health Office.

Cork fishmeal is brownish-white, resulting from the white flesh of cork fish. The aroma of this flour is typically fishy, reflecting the typical smell of cork fish. The taste is similar to fish but not as sharp as tuna or stingray (Nadimin et al., 2018). On the other hand, beetroot meal has a distinctive colour, taste, and aroma, essential aspects both before and after processing.

Honey is used as a sweetener because its water content gives the snack bars a soft texture and has benefits for increasing stamina and endurance. Honey also has fructose, which provides 1.8 times greater sweetness than sucrose and is preferred over sugar because it makes the snack bar texture softer and more accessible to bite.

Margarine is added as fat to produce soft snack bars, provide savoury taste lubrication, and affect the texture and appearance of baked products. (Faustina, 2023). Making snack bars involves mixing, baking, cooling, and cutting. Roasting temperature affects the hardness and colour of snack bars; higher temperatures result in a darker colour and more complex texture (Azizaah et al., 2022) (Sarno et al., 2018). Cooling is essential to release steam and harden the texture before cutting (Falah et al., 2022).

Three snack bar formulations were tested with different main ingredientratios. Visually, all three looked similar, but the organoleptic results showed differences. Formula I, with more cork fish meal, produced a dark red colour and a less dominant cork fish flavour. Formula II, with less beetroot flour and more cork fish meal, had a lighter colour and more prominent cork fish flavour. Formula III, with more cork fish meal, produced a more pungent fishy taste and lighter color due to less beet fruit meal. This finding aligns with research by (Setyawati, Vilda Ana Veria dkk, 2018) regarding the effect of cork fish flour on the colour, aroma, and taste of biscuit products.

Table 1. Organoleptic Test

Parameters	Formula		
	FI (20% and 80%)	FII (40% and 60%)	FIII (60% and 40%)
Color	Dark red Brownish	Brownish Red	Brownish Red
Aroma	Savory and slightly fishy	Fishy savory	Fishy savory
Taste	Sweet	Sweet slightly corky flavor	Sweet cork flavor

Sensory observations showed that all formulas had similar sensory properties. All three formulas produced a standard aroma with no detectable aroma of fresh beetroot. The reddish-brown color of the snack bars was influenced by the dark red color of beetroot and the sweetness derived from carbohydrates. Snack bars from all three formulas had a soft texture and solid shape, similar to other snack bars. According to the study, adding cork fish meal significantly affected the product's colour, and color changes can affect the level of liking for the resulting product.

Research shows that adding cork fishmeal can reduce the level of liking for the product. Beetroot flour is intensely red due to betalain pigments, which consist of reddish-purple betasianin and yellowish betaxanthin. Betalains are water-soluble, rich in nitrogen, and give a reddish colour. The earthy aroma in beetroot flour is due to the compound geosmin, a secondary volatile aromatic metabolite that gives red beetroot its characteristic earthy flavour.

A qualitative test was performed by testing the sample for antioxidant compounds. The color change from lowest to highest only showed a shift from purple to faint purple. Although the colour change of DPPH from purple to faint purple shows a positive result, antioxidant compounds are characterized by a change in the colour of the DPPH solution from intense purple to faint purple or yellow. If all electrons on DPPH are paired, the colour of the solution will change from dark purple to bright yellow (Rowland et al., 2018).

Snack bars show free radical capture activity, following the research of Aklimah & Ekayanti (2022), which explains that changes in the colour of the test solution occur due to chemical reactions between antioxidant compounds and DPPH free radicals, which involve the donation of hydrogen atoms by antioxidant compounds. This causes a color change from purple to yellow or from intense purple to faint purple.

Quantitative antioxidant activity tests were conducted using a UV-Vis spectrophotometer to measure the absorbance of DPPH after adding samples. The free radical capture activity of

the sample is characterized by a decrease in DPPH absorbance and an increase in sample concentration (Rowland et al., 2018).

The DPPH method shows that as the sample's concentration increases, the sample's absorbance decreases and the inhibition level increases. The decrease in absorbance is caused by DPPH electrons pairing with electrons from the sample. The correlation between concentration and absorbance shows an inverse relationship; the higher the concentration, the lower the absorbance, indicating that fewer antioxidant particles are available to neutralize the radical properties of DPPH.

DPPH standard solution was made with a concentration of 0.075 mM, according to research by Wulan Yuliarti et al. (2022) DPPH powder was dissolved to have a concentration of 0.075 mM. The maximum wavelength is determined by scanning at a wavelength of 400-800 nm, where the wavelength that produces the highest absorbance is the maximum wavelength. This is important because to get maximum sensitivity on the same DPPH solution, the risk of error is smaller if repeated measurements are made.

Purple DPPH free radicals will produce maximum absorbance at 515-520 nm wavelength with ethanol (Rowland et al., 2018). In this study, a wavelength of 516 nm was obtained in 0.075 mM DPPH solution. The wavelength of 516 nm is in accordance with research (Tsvetkova et al., 2023) stated that the wavelength of DPPH is 516 nm. DPPH dilution is done in a closed container and avoided from light. This is because DPPH compounds are susceptible to light, which causes damage to the solution (Ramayani et al., 2021).

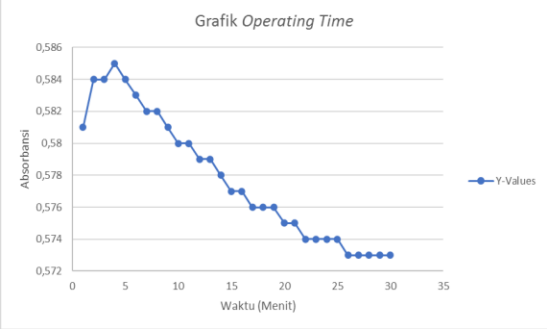

Figure 1. Operating time graph

Determination of operating time aims to determine the measurement time of a compound that provides stable absorbance and no decrease in absorbance. Operating time is measured by measuring the difference between the measurement time and the absorbance of the solution. Determination of operating time can also minimize the occurrence of measurement errors. If the measurement is carried out before the operating time, there is a possibility that the reaction

formed is not perfect. Compounds need time for the compounds formed to stabilize (Khorifatunnisa, 2023).

Determination of operating time was carried out using DPPH standard solution with a concentration of 0.075 mM at a maximum wavelength of 516 nm and sought stability time in the range of 0-30 minutes with an interval of 1 minute. The resulting absorbance value has been stable from the 26th minute to the 30th minute with an absorbance result of 0.573. The time range has a relatively stable absorbance of the compound measured; the stability of this absorbance indicates that the formation reaction is optimum. So it is determined that the operating time used in this research is 30 minutes (Arista & Siregar, 2023).

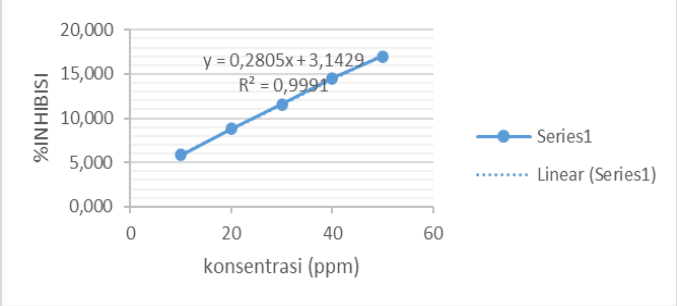

Figure 2. Sample Standard Curve

In this study, the strength of antioxidant activity is expressed by IC_{50} , which is the concentration of substances that can inhibit or reduce the absorbance of DPPH by 50%. The IC_{50} value is obtained based on linear regression analysis of *snack bar* concentration with the percentage of DPPH absorbance inhibition. (Oktavia & Sutoyo, 2021). Based on the data, a graph can be made between the concentration of the extract solution and the percentage of absorbance reduction or DPPH inhibition, which is presented in Figure 4.7. Based on Figure 4.7, the regression equation y = 0.2805x + 3.1429 is obtained, then entering the value of y with 50%, the value of x or IC_{50} is obtained at 167 ppm. Because the IC_{50} price is in the range of < 200 ppm, the antioxidant activity of the *snack bar* combination of cork fish flour and beet fruit flour is in the weak category. (Rowland et al., 2018). IC_{50} values are shown in Table 4.1.

Table 2. Antioxidant Activity (IC_{50}) from Snack bar

Parameter	Variation of Concentration of Combination of Materials		
	Cork Fish Meal 20% and Beet Flour 80% FI	**Cork Fish Flour 40% and Beetroot Flour 60% FII**	**Cork fish Flour 60% and Beetroot Flour 40% FIII**
Antioxidant Activity / IC_{50} (ppm)	$173^{(a)}$	$298^{(b)}$	$339^{(b)}$

Description: There is a significant difference in mining (a) on Formula I with formula II and II with symbol (b)

The formula I showed showed that the antioxidant activity of snack bars was weak, with the ratio of using less cork fishmeal to using more beetroot flour. The formula I has low antioxidant activity, but formulas II and II produce snack bars with high IC50 values when using more cork fish flour. The high IC_{50} value indicates that formulas II and III do not have antioxidant activity.

Data from the IC value$_{50}$ of antioxidant activity obtained were tested for normality with the *Saphiro-Wilk* test method, resulting in sig. > 0.05, they indicated that the data was normally distributed. The homogeneity of variance test resulted in sig. > 0.05, they indicated that the variance of several treatments carried out is homogeneous. The data were then statistically analyzed using *One Way ANOVA*, resulting in a sig value <0.05, indicating a significant difference in the results of the IC value$_{50}$ with different concentrations of 20%: 80%, 40%: 60%, and 60%: 40%.

The statistical data results show that formula I has weak antioxidant activity; this statement is supported by research (Prasetyo & Winardi, 2020). This statement is supported by research (Prasetyo & Winardi, 2020), which shows that antioxidant compounds obtained from anthocyanins cannot withstand heating of more than 70^{o} C. Antioxidant activity is lower when beet fruit flour is processed into snack bars. The level of anthocyanins contained in the ingredients influences the decrease in antioxidant activity. Although anthocyanins are water-soluble compounds, the relatively small contact between the ingredients and water causes the loss of anthocyanin compounds.

Quercetin and anthocyanins are different compounds. Quercetin is a flavonoid known for its antioxidant properties, while anthocyanins are water-soluble pigments that also possess antioxidant activity but are chemically distinct. The standard solution of quercetin was used to benchmark the antioxidant activity of the samples.

Anthocyanins are water-soluble pigments which cause red, violet, and blue colours. The process of making flour uses a temperature of more than 70°C; even when making snack bars, the oven cooks at a temperature of 120^0C. The results of antioxidant activity are influenced by the composition of more beets, which will affect the antioxidant activity possessed by *snack bars*, supported by the research of (Sukmawati et al., 2023), which states that beets are foods that have a relatively high antioxidant content with an antioxidant *inhibitor concentration* value of 50% (IC_{50}) of 21.89 ppm. The addition of cork fish flour makes the resulting *snack bar* have no antioxidant activity, supported by the research of Putri et al. (2020), which states that cork fish haruan has antioxidant activity with an IC value of 356.985 ppm.

4. CONCLUSION

Based on the results of research and discussion of the antioxidant activity of snack bars combined with cork fish flour and beet fruit flour, it can be concluded that The formula that has antioxidant activity with a weak range is formula I with an IC50 value of 173 ppm, Formula II has an IC50 value of 298 ppm, meaning it has no antioxidant activity and formula III has IC50 value of 339 ppm has no antioxidant activity. Formula I, with a 20% cork fishmeal and 80% beetroot flour ratio, showed the strongest antioxidant activity (IC50 of 173 ppm), though it is still in the weak category. Formulas II and III, with higher amounts of cork fish flour, had higher IC50 values (298 and 339 ppm), indicating weaker antioxidant activity. Therefore, Formula I, despite being in the weak antioxidant range, is the better formula because it showed the most promising antioxidant activity among the tested combinations.

5. REFERENCE

Arista, N., & Siregar, R. M. (2023). Uji Aktivitas Antioksidan Ekstrak Kulit Pisang Barangan (Musa Acuminata Linn) dengan Metode DPPH. *Ilmiah Multidisiplin*, *1*(12), 1477–1484.

Azizaah, N. E., Supriyanto, & Indarto, C. (2022). Profil Tekstur Snack Bar Tepung Jagung Talango ang Diperkaya Antioksidan Dari Tepung Kelor (Moringa oleifera L.) Textur Profile of Antioxidant Enriched Cornmeal Snack Bar From Moringa Flour (Moringa Oleifera L. *Jitipari*, *7*(2), 100–108.

Direktorat Promkes. (2018, January 26). *Mengenal Stunting dan Gizi Buruk. Penyebab, Gejala, Dan Mencegah*. Direktorat Promosi Kesehatan Kementerian Kesehatan RI. https://promkes.kemkes.go.id

Falah, M. S., Priyono, S., & Fadly, D. (2022). Formulasi Snack Bar Tepung Beras Merah (Oryza nivara) dan Edamame (Glycine max (L)merrill): Karakteristik Fisikokimia dan Sensori. *FoodTech: Jurnal Teknologi Pangan*, *5*(1), 25. https://doi.org/10.26418/jft.v5i1.57341

Fitriyani, F., & Aisyah, R. D. (2022). Edukasi Nutrisi dan Optimalisasi Tumbuh Kembang Balita Pada Masa Pandemi COVID19 di Desa Pekajangan Kabupaten Pekalongan. *Jurnal ABDINUS : Jurnal Pengabdian Nusantara*, *6*(1), 91–98. https://doi.org/10.29407/ja.v6i1.16100

Hamidah, S., Sartono, A., & Kusuma, H. S. (2017). *Perbedaan Pola Konsumsi Bahan Makanan Sumber Protein di Daerah Pantai, Dataran Rendah dan Dataran Tinggi*.

Khorifatunnisa, N. (2023). Uji Penangkapan Radikal Bebas Sediaan Lotion Minyak Atsiri Kulit Jeruk Lemon (Citrus Limon L.) Dengan Metode Dpph (2,2-Diphenyl-1-Picrylhydrazyl. *Journal of Engineering Research*.

Mawarno, B. A. S., & Putri, A. S. (2022). Karakteristik Fisikokimia dan Sensoris Snack Bar Tinggi Protein Bebas Gluten dengan Variasi Tepung Beras, Tepung Kedelai dan Tepung Tempe. *AgriHealth: Journal of Agri-food, Nutrition and Public Health*, *3*(1), 47. https://doi.org/10.20961/agrihealth.v3i1.60632

Nadimin, N., Nurjaya, N., & Lestari, R. S. (2018). Daya terima terhadap jajanan lokal Sulawesi Selatan subtitusi tepung ikan gabus (Channa striata. *AcTion: Aceh Nutrition Journal*, *3*(2), 141. https://doi.org/10.30867/action.v3i2.115

Oktavia, F. D., & Sutoyo, S. (2021). Skrining Fitokimia, Kandungan Flavonoid Total, dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan Selaginella Doederleinii. *Jurnal Kimia Riset*, *6*(2), 141. https://doi.org/10.20473/jkr.v6i2.30904

Rahayuwati, L., Ibrahim, K., Hendrawati, S., Sari, C. W. M., Yani, D. I., Pertiwi, A. S. P., & Fauziyyah, R. N. P. (2022). Pencegahan Stunting melalui Air Bersih, Sanitasi, dan Nutrisi. *Warta LPM*, 356–365. https://doi.org/10.23917/warta.v25i3.1031

Ramayani, S. L., Permatasari, E. A., & Novitasari, I. (2021). *Pengaruh Metode Ekstraksi Terhadap Kadar Total Fenolik , Kadar Total Flavonoid Dan Aktivitas Antioksidan Ekstrak Daun Mengkudu*, *18*(1), 40–46.

Rowland, R. G., Dong, J., & Migdal, C. A. (2018). Antioxidants. In *Lubricant Additives: Chemistry and Applications* (Third). https://doi.org/10.1201/9781315120621

Sarno, I. P. A., Wulandari, Y. W., & Suhartatik, N. (2018). Karakteristik Snack Bars Dengan Variasi Suhu Pemanggangan Dan Perbandingan Tepung Milet Kuning (Panicum Sp)

Dengan Tepung Pisang Raja Bandung (Musa Paradisiaca L. *Snack Bars Characteristics With Variation of Drying Temperature and Comparison of Yellow*. *Teknologi Pangan*, *12*(2), 47–53.

Sukmawati, A., Azzuhdiyah, Y., Marthadilla, C. C., & Risdiyanti, I. V. (2023). Aktivitas Antioksidan Mikropartikel Kitosan Dengan Kandungan Sari Umbi Bit (Beta Vulgaris Linn) Antioxidant Activity of Chitosan Microparticle Containing Beetroot (Beta Vulgaris Linn) Extract. *Open Journal Systems STF Muhammadiyah Cirebon*, *ammadiyahcirebon.ac.id (Vol. 8, Issue 3)*.