



# Biofortification in Palm Oil Based on Saponin

## Nanotechnology: Innovation in Nutritional Stability,

## Antioxidants and for Functional Quality

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### ABSTRACT

Palm oil is the world's main vegetable oil commodity and significantly contributes to the food industry. However, challenges related to the low content of certain nutrients and susceptibility to lipid oxidation are major issues that affect product quality. Fortification technology is an innovative solution to improve palm oil's nutritional value and oxidative stability. This article aims to evaluate the development of modern technology in palm oil fortification, including the addition of vitamin A, vitamin E, bioactive compounds, and the use of natural and synthetic antioxidants. Various methods, such as microencapsulation, nanotechnology, and biofortification techniques, are discussed in the context of their effectiveness in maintaining nutritional stability, preventing lipid oxidation, and extending product shelf life. This review highlights the challenges of implementing fortification technology, including technical and regulatory aspects in the food industry. The results of the review indicate that fortification technology improves nutritional quality and the oxidative stability of palm oil, thus supporting the development of healthier and more value-added food products. The implementation of vitamin A and E fortification with nanotechnology is expected to be able to answer global nutritional challenges and increase the competitiveness of the palm oil industry in the international market.

**Keywords:** Antioxidants, Fortification, Nutritional Value, Oxidative Stability, Palm Oil.

### 1. INTRODUCTION

Indonesia is strategically located on the equator, so its climate is very supportive of the optimal growth of oil palm plants, tropical plants that produce vegetable oil. Indonesia is not only the largest producer, but also a major exporter of palm oil and its derivatives. The demand for vegetable oil is expected to continue to increase in the coming decades, along with global population and economic growth. Based on oil market research (2020), the palm oil sector is projected to continue to grow, with total production estimated to reach 98.82 million tons in 2024, with a growth rate of around 5.9% during the period 2019 to 2024 (Hariyadi, 2021).

Palm oil is one of the most widely used vegetable oils in the world due to its high productivity, year-round availability, and relatively low production costs. This oil is a major component in various food products, such as margarine, cooking oil, dairy products, and other processed foods. However, behind its popularity, palm oil has several challenges related to





nutritional quality and oxidative stability (Mba et al., 2015). The content of micronutrients such as vitamins A, E, and natural bioactive compounds tends to decrease due to processing, while the high content of unsaturated fatty acids makes this oil susceptible to lipid oxidation. Lipid oxidation can produce peroxide compounds and free radicals that have a negative impact on product quality and consumer health (Pignitter et al., 2016).

Fortification is one strategy to improve the nutritional quality and extend the shelf life of palm oil. The addition of vitamin A is the main focus because palm oil naturally contains  $\beta$ -carotene precursors that can be optimized as a source of vitamin A to overcome deficiency. The addition of vitamin E in the form of tocopherols and tocotrienols acts as a natural antioxidant, can protect the oil from oxidative damage, maintain stability during storage, and provide health benefits as an antioxidant compound in the body (Sen et al., 2010), (Aleman et al., 2010). Various fortification methods, such as the direct addition of vitamins A and E, microencapsulation to protect bioactive compounds, and the application of nanotechnology, have been developed to improve the stability, bioavailability, and effectiveness of added vitamins (C. Zhu et al., 2013), (Pignitter et al., 2016).

Palm oil fortification can be done by adding important components such as vitamin A, vitamin E, natural antioxidants, and other bioactive compounds. Modern technologies such as microencapsulation, nanotechnology, and biofortification are used to ensure the effectiveness of adding these components, considering stability, bioavailability, and ease of application on an industrial scale. Each of these fortification technologies has its own advantages and disadvantages that need to be further understood (Silalahi et al., 2017).

This article aims to provide a comprehensive review of palm oil fortification technology with a focus on the addition of vitamins A and E. With the right fortification technology, palm oil has great potential to become a more nutritious and high-quality food product, while supporting global nutrition improvement efforts. Vitamin A is important for eye health, the immune system, and cell growth. Palm oil contains a precursor of vitamin A in the form of  $\beta$ -carotene, but most of it will be reduced during the oil refining process. Fortification with retinyl palmitate or retinyl acetate is needed to overcome vitamin A deficiency, and vitamin A stability is better against heat and light.

### **Composition and nutritional potential of palm oil**

Palm oil is the most dominant vegetable oil in the global market due to its high productivity, competitive price, and wide application in the food and non-food industries. However, palm oil products have two main weaknesses, namely the low content of certain





nutrients such as vitamin A and E due to the processing process and its susceptibility to lipid oxidation (Silalahi et al., 2017). Lipid oxidation can reduce oil quality by forming free radicals and peroxide compounds that have a negative impact on taste, aroma, shelf life, and consumer health. The application of palm oil fortification technology is a strategic step to increase nutritional content, add added value to products, and prevent oxidative damage (Yeh et al., 2017).

Crude palm oil obtained from the mesocarp of oil palm fruit (*Elaeis guineensis*) is mainly composed of glycerides with small amounts of non-glyceride components. These non-glyceride components include free fatty acids, trace metals, water content, impurities, and other minor components. Minor components in crude palm oil include carotenoids, tocopherols, tocotrienols, sterols, phospholipids, squalene, and triterpenic and aliphatic hydrocarbons. Among these minor components, carotenoids, tocopherols, and tocotrienols are the most important because of their role in the stability and nutritional value of palm oil (Mba et al., 2015). Carotenoids give crude palm oil its characteristic reddish orange color. In addition, carotenoids also provide oxidative protection by being oxidized before triglycerides are oxidized. Carotenoids, especially  $\alpha$ - and  $\beta$ -carotene, function as precursors of vitamin A which can be converted into vitamin A in the body (Martianto et al., 2018). Meanwhile, tocopherol and tocotrienol are isomers of vitamin E which have strong antioxidant properties, thus providing oxidative stability to palm oil (Aleman et al., 2010), (Miyazawa et al., 2019).

Palm oil is one of the vegetable oils rich in bioactive compounds, especially carotenoids and vitamin E (tocopherol and tocotrienol), which contribute to its color, stability, and nutritional value. The composition of the three in palm oil can vary depending on the type of product and the level of processing carried out. Processes such as refining, fractionation, and further processing can affect the content of carotenoids, tocopherols, and tocotrienols which are indicators of the quality and nutritional function of palm oil (Nagendran et al., 2000).

Table 1. Carotene content (%) in various types of palm oil.

Palm Oil Types	$\alpha$ -carotene	$\beta$ -carotene	$\gamma$ -carotene	$\delta$ -carotene	$\zeta$ -carotene	cis- $\alpha$ -carotene	cis- $\beta$ -carotene
Crude palm oil	35,1	56,0	0,3	0,8	0,7	2,5	0,7
Red palm oil	37,0	47,4	0,5	0,6	1,3	6,9	0,8
Crude palm olein	36,9	49,4	0,4	0,7	0,8	5,0	0,7
Red palm olein	44,2	33,3	0,6	3,3	0,6	7,5	0,7
Commercial red palm olein	40,6-41,9	40,0-42,0	0,45-1,07	0,72-0,83	0,5-0,72	9,0-11,4	NA

Source: (Nagendran et al., 2000).

- *Crude palm oil*: palm oil extracted directly from the palm fruit without further refining or modification.





- *Red palm oil*: palm oil that has been processed to remove some impurities but retains most of the natural carotene content, the characteristic red-orange color.
- *Crude palm olein*: liquid fraction obtained from the fractionation process of crude palm oil.
- *Red palm olein*: liquid fraction obtained from the fractionation process of red palm oil.
- *Commercial red palm olein*: liquid fraction obtained from the fractionation process of red palm oil, produced for the market and usually through additional processes to improve stability and shelf life.
- NA: not available.

## Vitamin E

### Types, structures, and benefits of vitamin E

Four main types of vitamin E are found in palm oil:  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol,  $\gamma$ -tocotrienol, and  $\delta$ -tocotrienol. Their respective concentrations in crude palm oil, RBD (refined, bleached, and deodorized) palm olein, and commercial red palm olein are shown in Table 2.

Table 2. Vitamin E content (%) in various types of palm oil.

Palm Oil Types	$\alpha$ -tocopherol	$\alpha$ -tocotrienol	$\gamma$ -tocotrienol	$\delta$ -tocotrienol
Crude palm oil	21	24	43	11
Commercial red palm olein	19	29	41	10
RBD palm olein	25	29	36	11
Palm oil*	51.91 $\pm$ 1.07	NA	34.12 $\pm$ 1.07	5.37 $\pm$ 0.1

Source: (Nagendran et al., 2000); \* (Azlan et al., 2010) - (mg g<sup>-1</sup>)

- RBD: refined, bleached, and deodorized
- NA: not available.

More than 80% of the vitamin E contained in crude palm oil is maintained in red palm olein, compared to only about 65% in refined, bleached, and deodorized (RBD) palm olein. Vitamin E plays an important role as an antioxidant that provides protection against oxidative damage to the oil. In addition, the presence of carotene and vitamin E together creates synergistic protection against auto-oxidation and photo-oxidation of unsaturated triglycerides. Tocotrienols derived from palm oil have been reported to lower plasma cholesterol levels by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme that regulates cholesterol synthesis in the liver. Tocotrienols play an important role in inhibiting the development of several types of cancer, especially with their inhibitory effects on cancer cells, (Nagendran et al., 2000), and can significantly reduce ROS levels in breast cancer tissue (Diao et al., 2016).





Vitamin E is useful for maintaining body health and beautiful skin: a) as a powerful antioxidant, protecting body cells from free radicals that damage genetic material, helping to maintain cell integrity that can cause disease and premature aging, b) supporting heart health, namely protecting cells in the artery walls from atherosclerosis damage (plaque buildup), c) keeping the skin moist and elastic, not wrinkled and protecting against damage from exposure to sunlight and pollution, d) strengthening the immune system to fight infection, e) reducing the risk of prostate and breast cancer (Tresno, 2023).

The following is the structure of vitamin E related to antioxidant activity (Figure 1), where (Shahidi & De Camargo, 2016), explains that tocotrienol has a hydrophobic chain consisting of three double bonds. Differences in the structure of the hydrophobic chain affect: the

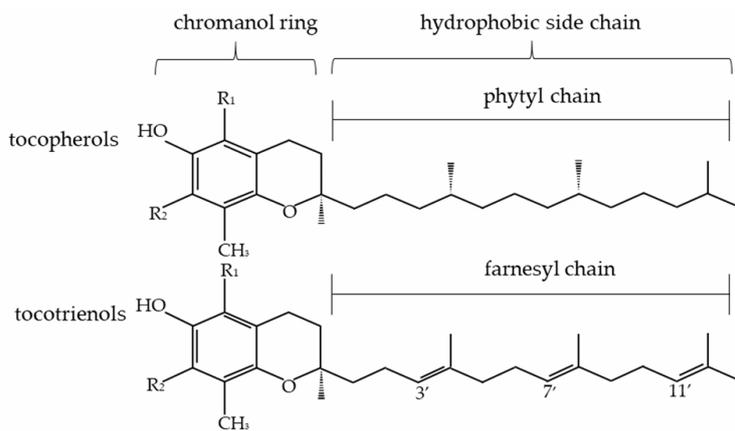


Figure 1. Structure of vitamin E

properties and biological activities of tocopherol and tocotrienol. Tocotrienol has: stronger antioxidant activity, greater anti-inflammatory, anti-cancer potential, and protective effects against cardiovascular disease. The hydrophobic chain of tocotrienol (farnesyl) provides lipophilic properties that allow tocotrienol molecules to cross cell membranes consisting of lipid layers and

more easily penetrate cell membranes. The ability to protect cells from oxidative damage in the cell membrane. The difference between tocopherols and tocotrienols (Figure 1) lies in the presence of three double bonds at positions 3', 7', and 11' in the tocotrienol side chain (Shahidi & De Camargo, 2016). The chemical structure of tocopherols and tocotrienols, there are three unsaturations at carbon positions 3, 7, and 11 in the isoprenoid side chain. This seemingly small difference has major implications for the bioavailability and specific biological functions of vitamin E isomers (Sen et al., 2010). In addition, the presence of three chiral centers causes tocopherols to have eight stereoisomers, while each tocotrienol has only two stereoisomers due to the absence of a chiral center in its side chain. Studies of the relationship between structure and activity indicate that some



isomers may be more active or more sensitive than others. The antioxidant activity of tocopherols and tocotrienols plays an important role in protecting monounsaturated and polyunsaturated fatty acids (PUFAs) from oxidation (Shahidi & De Camargo, 2016).

Variations in the chemical structure of the four main types of tocopherols and tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) are based on the position of the methyl group ( $\text{CH}_3$ ) on the chromanol ring.  $R_1$  and  $R_2$  refer to the position of the substituent group (methyl group or hydrogen) on the aromatic ring of the tocopherol and tocotrienol structure. The methyl group ( $\text{CH}_3$ ) or hydrogen (H) in positions  $R_1$  and  $R_2$  determine the type of tocopherol or tocotrienol.  $\alpha$ - type:  $\text{CH}_3$  is present in both  $R_1$  and  $R_2$  positions,  $\beta$ - type:  $\text{CH}_3$  is present only in  $R_1$  position, while  $R_2$  position is H,  $\gamma$ - type:  $\text{CH}_3$  is present only in  $R_2$  position, while  $R_1$  position is H, and  $\delta$ - type: both positions ( $R_1$  and  $R_2$ ) have only H. The difference in the position and number of methyl groups affects the biological activity and antioxidant capacity of each isomer. Generally,  $\alpha$ -tocopherol has the highest antioxidant activity among other tocopherols. Variations in substituents at  $R_1$  and  $R_2$  positions create differences between  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - tocopherols or tocotrienols, affecting the oxidative stability of the oil, vitamin E activity, and overall health effects (Szewczyk et al., 2021).

This could also explain the high concentration of these phenolic antioxidants in highly unsaturated vegetable oils. Some vegetable oils also contain tocotrienols, the lipid fractions of oilseeds and nuts being the main source of tocopherols and tocotrienols in the natural diet. The presence of these compounds in fruits and vegetables is generally very small due to their low lipid content; however, grains and other crop processing by-products can be alternative sources of vegetable oils with significant tocopherol and tocotrienol content (Szewczyk et al., 2021). The stability of tocopherols and tocotrienols can be influenced by the system in which they are present, as well as in the food by the fatty acid composition of their different lipid fractions, the storage and processing of the raw material, and by the cooking procedures used (Shahidi & De Camargo, 2016). The phytyl chains at positions 4 and 8 undergo structural modifications by oxidation. This oxidation can cause the bonds at these positions to become more reactive and susceptible to cleavage, the farnesyl chain has a longer and more complex structure compared to the phytyl chain. The farnesyl chain does not undergo oxidation at positions 4 and 8 as occurs in the phytyl chain, the bonds in the farnesyl chain tend to be more stable and are not easily broken (Szewczyk et al., 2021).

### **Mechanism of vitamin E in neutralizing free radicals**

Vitamin E acts as a natural antioxidant that can prevent lipid oxidation and has health benefits such as protection against oxidative stress and inflammation. Palm oil naturally contains





tocopherols and tocotrienols, but their levels can be increased through fortification. Vitamin E nanotechnology in the form of solid lipid nanoparticles (solid lipid nanoparticles/SLN) is used to increase bioavailability. The advantages are more even distribution in oil, higher antioxidant stability (Szewczyk et al., 2021).

Natural vitamin E derived from palm oil is able to reduce the arachidonic acid (AA) pathway in brain injury or inflammation, namely after being released from the lipid membrane bilayer by the enzyme phospholipase A2 (PLA2). Polyunsaturated fatty acids AA undergo oxidative metabolism through non-enzymatic and enzymatic pathways. As antioxidant compounds, natural vitamin E isomers  $\alpha$ -tocotrienol ( $\alpha$ TCT) and  $\alpha$ -tocopherol ( $\alpha$ TOC) can inhibit non-enzymatic oxidative metabolism of AA lipids. The antioxidant function possessed by all vitamin E isomers,  $\alpha$ TCT is specifically a strong inhibitor of cytosolic phospholipase A2 (cPLA2), c-Src kinase enzyme (c-Src), and 12-lipoxygenase (12-LOX) at nanomolar concentrations (Sen et al., 2010). The content of tocotrienols, especially  $\alpha$ - and  $\gamma$ -, is a unique characteristic of palm oil. The total levels of tocopherols and tocotrienols were recorded at 466 mg/kg (total tocopherols: 146 mg kg<sup>-1</sup> and total tocotrienols: 320 mg kg<sup>-1</sup>), indicating that all samples contained a number of these antioxidants. The content of heated tocopherol and tocotrienol analogues decreased by 20.2% compared to unheated palm oil. The mechanism of the role of arachidonic acid (AA) in the cell membrane and how  $\alpha$ -tocotrienol ( $\alpha$ TCT) affects lipid oxidative metabolism through non-enzymatic and enzymatic pathways. AA is an unsaturated fatty acid found in the membrane phosphatidylcholine, the release from the membrane is catalyzed by phospholipase A2 (cPLA2). The  $\alpha$ -tocotrienol functions as an inhibitor of cPLA2 activity, thereby preventing excessive release of AA (Silalahi et al., 2017).

Non-enzymatic oxidative metabolism of lipids:

- Free AA can undergo non-enzymatic oxidative metabolism triggered by free radicals (Fe<sup>2+</sup>, LOO<sup>-</sup>, LO<sup>•</sup>, <sup>•</sup>OH, and ONOO<sup>-</sup>).
- This process produces lipid radicals (LO<sup>•</sup>), which then turn into lipid peroxy radicals (LOO<sup>•</sup>), lipid hydroperoxides, and alkoxy radicals.
- $\alpha$ TCT and  $\alpha$ TOC ( $\alpha$ -tocopherol) inhibit this process by preventing the formation of lipid radicals (Sen et al., 2010).

Lipid radical formation reaction:

Lipid (LH) + O<sub>2</sub> → LOOH (lipid hydroperoxide)

L<sup>•</sup> + O<sub>2</sub> → LOO<sup>•</sup> (peroxy radical)

LOOH → LO<sup>•</sup> (alkoxy radical) + OH<sup>•</sup> (hydroxyl radical) (Sen et al., 2010).





Peroxynitrite (ONOO<sup>-</sup>) formation reaction:



Nitric oxide (NO<sup>•</sup>) is a reactive gas containing one unpaired electron, and is produced by the enzyme nitric oxide synthase from arginine, while ONOO<sup>-</sup> is a highly reactive molecule that can cause oxidation and nitration of biomolecules (lipids, proteins, and DNA), causing cell and tissue damage (Aicardo et al., 2016). Peroxynitrite (ONOO<sup>-</sup>) is a reactive nitrogen species (RNS), and can isomerize to nitrate (NO<sub>3</sub><sup>-</sup>) or react with protons (H<sup>+</sup>) to produce highly reactive peroxynitrite acid (ONOOH) (Ahmad et al., 2019).

Enzymatic oxidative metabolism of lipids:

- AA undergoes oxidative metabolism through enzymatic pathways, namely 12-lipoxygenase (12-LOX) produces 12-HPETE (12-hydroperoxyeicosatetraenoic acid), cytochrome P450 produces epoxyeicosatetraenoic acid, and cyclooxygenase (COX) produces prostaglandins and thromboxanes.
- αTCT inhibits the activity of 12-LOX and the associated c-Src kinase enzyme, thereby reducing the production of oxidative radicals. αTCT's membrane lipid protection by preventing oxidative damage to lipids through both non-enzymatic and enzymatic pathways, and the combination of antioxidant roles and direct inhibition of specific enzymes makes αTCT an important compound in preventing membrane damage and impaired cell function (Sen et al., 2010).

Free radicals attack biologically important molecules (lipids, proteins, carbohydrates, and DNA) through hydrogen atom abstraction, double bond addition, and electron transfer reactions. Free radical-scavenging antioxidants (IH) react with free radicals through one of the following three reaction mechanisms. The following reactions depend on the radical, antioxidant, and microenvironment (Niki, 2014).

- Hydrogen abstraction:  $\text{X}^{\cdot} + \text{IH} \rightarrow \text{XH} + \text{I}^{\cdot}$
- Addition:  $\text{X}^{\cdot} + \text{C}=\text{C} \rightarrow \text{X}-\text{C}-\text{C}^{\cdot}$
- Electron transfer,  $\text{X}^{\cdot} + \text{IH} \rightarrow \text{X}^- + \text{IH}^{\cdot+} \rightarrow \text{X}^- + \text{I}^{\cdot} + \text{H}^+$

The mechanism of α-tocopherol as a free radical scavenging antioxidant against lipid peroxidation (Figure 2).



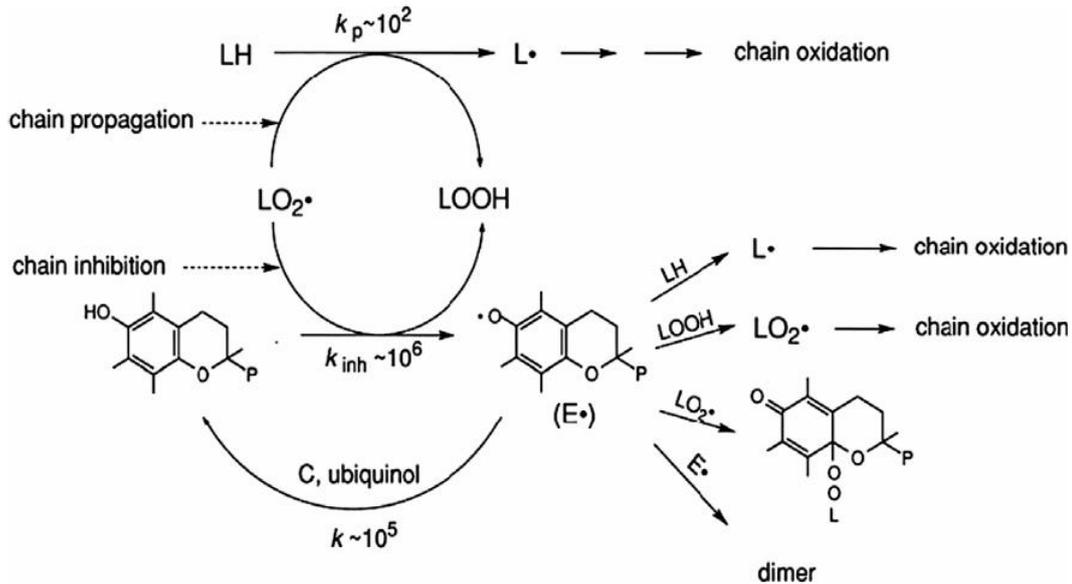
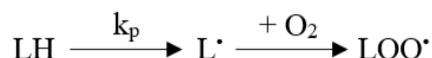


Figure 2. Inhibition of lipid oxidation chain by vitamin E.

Vitamin  $\alpha$ -tocopherol acts as a scavenging antioxidant/radical reducer against lipid peroxidation. Vitamin E captures lipid peroxy radicals ( $\text{LO}_2^\cdot$ ) before they attack lipids (LH), producing lipid hydroperoxides (LOOH) and lipid radicals ( $\text{L}^\cdot$ ), which then continue the chain oxidation process. The vitamin E radical ( $\text{E}^\cdot$ ) formed can: 1) be reduced by ascorbic acid (C) or ubiquinol to regenerate vitamin E, 2) react with LH or LOOH, 3) react with  $\text{LO}_2^\cdot$  to form adducts, or 4) react with other vitamin E radicals to produce dimers, which are stable products that are not radicals. The reaction rate constant ( $k$ ) with P is the phytyl side chain. The reaction rate constant in chain propagation reaches  $10^2$ , and in chain inhibition reaches  $10^6$ , while the regeneration of vitamin E by vitamins C and E reaches  $10^5 \text{ M}^{-1} \text{ s}^{-1}$  (Niki, 2014).

Mechanism of vitamin E (tocopherol) in neutralizing free radicals during lipid oxidation:

- Chain propagation, is the initial reaction where lipid molecules (LH) are oxidized by free radicals ( $\text{L}^\cdot$ ) into lipid radicals ( $\text{L}^\cdot$ ), this reaction produces lipid peroxy radicals ( $\text{LOO}^\cdot$ ) after reacting with molecular oxygen. The reaction occurs in a chain and damages the lipid structure of the membrane.



- Chain inhibition, vitamin E acts as an antioxidant by stopping the propagation of the oxidation chain. Tocopherol (E) donates a hydrogen atom to the lipid peroxy radical ( $\text{LOO}^\cdot$ ) to form a more stable lipid hydroperoxide (LOOH). The formed tocopherol radical ( $\text{E}^\cdot$ ) is relatively unreactive and can be further neutralized by other antioxidants, such as vitamin C or ubiquinol.



Inhibitory effect, tocopherol radical ( $\text{E}^{\bullet}$ ) tends not to continue the chain reaction, thus stopping further lipid damage.

- Formation of tocopherol dimer, if tocopherol radical ( $\text{E}^{\bullet}$ ) is not neutralized, it can react with other radicals ( $\text{LOO}^{\bullet}$  or  $\text{L}^{\bullet}$ ), forming unreactive products such as tocopherol dimer.



- Regeneration of vitamin E, tocopherol radical ( $\text{E}^{\bullet}$ ) Regeneration of vitamin E, tocopherol radical ( $\text{E}^{\bullet}$ ) can be regenerated into its active form by other antioxidants (vitamin C or ubiquinol/coenzyme Q10).



(Niki, 2014), (Diao et al., 2016), (Miyazawa et al., 2019).

## Vitamin A

### Benefits of vitamin A

Vitamin A is needed for normal growth, vision, reproduction, and differentiation of epithelial cells. Vitamin A deficiency can cause night blindness, xerophthalmia (progressive blindness due to drying of the cornea), keratinization (accumulation of keratin in the tissues of the digestive tract, respiratory tract, and urogenital tract), to fatigue and death. Vitamin A is a group of compounds that include retinoids and carotenoids. Vitamin A from plant sources is in the form of carotenoids that can be converted by the body into retinol. Fortification of vitamin A in palm oil products is one strategy to overcome deficiency. For most of the world's population, provitamin A carotenoids are the main source of vitamin A that can be relied on. Several provitamin A compounds, including  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, and as many as 50 other carotenoids, are found in the human diet, especially in orange, red, yellow, and leafy fruits and vegetables.  $\beta$ -carotene ( $\beta\text{C}$ ), As a lipophilic molecule,  $\beta\text{C}$  is most easily absorbed in oils,  $\beta\text{C}$  is more easily absorbed from fruits than from vegetables, and its bioavailability in oils is generally greater than in plants (Spiegler et al., 2012).

Vitamin A deficiency remains a serious public health problem in many developing countries due to low intake of vitamin A-rich foods and limited availability of micronutrients. Vitamin A deficiency can cause blindness, impaired growth, increased susceptibility to severe





infections, and higher risk of death in childhood and lactating mothers (WHO & FAO, 2006). Various strategies have been implemented to improve vitamin A status in the community, including through food diversification, vitamin A supplementation, and fortification. Fortification of staple foods or spices with micronutrients is considered an appropriate, logical, and recommended intervention program to be implemented in developing countries with large populations (Malau et al., 2019).

### **Biochemical breakdown mechanism of vitamin A**

Carotenoids are broad-spectrum pigments consisting of C40 main chains. Fruits and vegetables, animal products, marine algae, and certain seaweeds are good sources of carotenoids. Carotenoids are classified as carotenes consisting of carbon and hydrogen ( $\beta$ -carotene and lycopene) and xanthophylls (oxygenated carotenoids), which contain epoxy- (violaxanthin, neoxanthin, fucoxanthin), hydroxy- (lutein and zeaxanthin), keto- (astaxanthin, canthaxanthin) and methoxy- (spirilloxanthin) functional groups. In terms of nutritional approach, carotenoids are considered as important bioactive compounds, and food consumption is the only source to meet carotenoid needs in humans (Arathi et al., 2015).

The process of  $\beta$ -carotene breakdown and its conversion to retinoids. When  $\beta$ -carotene is symmetrically cleaved by the enzyme 15,15'-oxygenase (CMOI), into two molecules of retinaldehyde. Oxidation of retinaldehyde forms retinoic acid or is reduced to retinol, which is then converted to retinyl ester as a storage form of vitamin A in tissues. In the body, about 95% of retinoids derived from  $\beta$ -carotene are produced through this pathway. However,  $\beta$ -carotene can also be asymmetrically cleaved by the enzyme 9',10'-oxygenase (CMOII), producing apocarotenal which can be converted to retinaldehyde through chain shortening. Apocarotenal can also function as a ligand for peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) receptors, one molecule of  $\beta$ -carotene produces only one molecule of retinaldehyde (Spiegler et al., 2012).

Peroxisome proliferator-activated receptor (PPAR) forms a heterodimer with RXR after being activated by ligand. This complex binds to a response element on DNA called PPRE (peroxisome proliferator response element) to regulate gene transcription (Domínguez et al., 2018). RXR is activated by retinoids such as 9-cis retinoic acid. Activation of RXR enhances the function of partner receptors, including PPAR, in regulating gene transcription. Apocarotenal is a carotenoid compound with an aldehyde group (-CHO) at the end of its isoprenoid chain. The -CHO functional group in apocarotenal allows this compound to bind to the ligand domain on the PPAR





and RXR receptors. Biological effects, namely PPAR activation by apocarotenal contribute to the regulation of lipid metabolism, adipocyte differentiation, and reduction of inflammation. Overall, apocarotenal acts as a bioactive molecule that influences gene expression through its interaction with PPAR and RXR receptors, supporting its role in metabolic health and antioxidants (Amber-Vitos et al., 2016).

Vitamin A (retinol) is considered essential to support normal growth (FAO/WHO, 2004). Since 2012, various countries have adopted fortification strategies, including using vegetable oils as a nutrient delivery medium. Fortification of vegetable oils is an effective way to supply fat-soluble vitamins, such as vitamin A. Fortification of palm oil with vitamin A (retinyl palmitate) has been shown to be able to meet approximately 94% of the nutritional fortification needs of Indonesian families, where women receive 54% and children receive approximately 51%-57% of their average needs (Silalahi et al., 2017). The success of vegetable oil fortification with vitamin A is greatly influenced by the initial quality of the oil. The peroxide value (PV) is the main indicator of oil quality. Low PV ( $< 2 \text{ meq kg}^{-1}$ ) is considered an important parameter during production. In addition, the quality of vegetable oil before the fortification process (assessed based on PV) has a significant effect on the stability of retinyl palmitate (FAO/WHO, 2011), (Malau et al., 2019). Vegetable oil that is oxidized due to high peroxide levels will experience a significant decrease in vitamin A content (Pignitter et al., 2016). Hydroperoxides formed during the oil oxidation process are very unstable and will decompose into various compounds, such as aldehydes, ketones, hydrocarbons, alcohols, and other reaction products. This process continues during product storage, which can cause the oil to autooxidize and produce a rancid taste (C. H. Tan et al., 2017), (Loganathan et al., 2022).

The vitamin A content in BASF vegetable oil sets a content of  $1.0 \text{ million IUg}^{-1}$  with a vitamin A equivalent value of 300,000 retinol equivalents (RE) per gram, while DSM products set a vitamin A content range of  $1.0 - 1.1 \text{ million IUg}^{-1}$ , in accordance with high vitamin A specifications. This parameter is important to ensure the quality, purity, and effectiveness of vitamin A as an active ingredient in food, supplement, or pharmaceutical applications. The stability of vitamin A content in RBDPOL was tested through a heat stability test. Storage at low temperatures ( $16 - 20 \text{ }^{\circ}\text{C}$ ) was most effective in maintaining vitamin A retention for 24 months. Storage at moderate temperatures ( $24 - 29 \text{ }^{\circ}\text{C}$ ) significantly slowed down the decline in vitamin A concentration in both packages. To maintain vitamin A stability, opaque nylon packaging is preferred, especially when storage temperature is difficult to control. Vitamin A Loss: Heating at  $180 \text{ }^{\circ}\text{C}$  for 5 min caused a significant decrease in vitamin A concentration in both samples, with





greater losses at higher initial vitamin A concentrations (Silalahi et al., 2017). Oil Oxidation (PV): Heating significantly increased the peroxide value (Malau et al., 2019). This indicates oil oxidation due to heat treatment. Oil damage free fatty acid (FFA) increased slightly after heating, indicating that fatty acid degradation was still low despite heating (B. A. Tan et al., 2023). Heating at high temperatures significantly affected vitamin A stability and oil quality, especially in terms of lipid oxidation. The composition of fatty acid (FA) concentrations is in accordance with the typical fatty acid profile of palm oil:  $50.52 \pm 0.26\%$  saturated fatty acid (SFA),  $39.87 \pm 0.20\%$  monounsaturated fatty acid (MUFA),  $9.62 \pm 0.05\%$  polyunsaturated fatty acids (PUFA), and  $0.09 \pm 0.01\%$  trans FA, (Silalahi et al., 2017). According to (Mancini et al., 2015) fatty acid composition of palm oil and palm kernel oil for total SFA 49.9 and 82.1; total MUFA 39.2 and 15.4; PUFA 10.5 dan 2.4% respectively.

The tocotrienol content in palm oil, especially  $\alpha$ - and  $\gamma$ -, is a unique characteristic of this oil. The total levels of tocopherol and tocotrienol were recorded at 466 mg/kg (total tocopherol: 146 mg/kg and total tocotrienol: 320 mg/kg). The content of heated tocopherol and tocotrienol analogs decreased by 20.2% compared to unheated palm oil (Silalahi et al., 2017).

#### **Rationale for fortification technology with saponin**

Saponin has a unique chemical structure in the form of glycoside groups (hydrophilic) and aglycones (hydrophobic), so that it can form micelles or emulsions in oil-water systems. With nanoemulsion technology, saponin can help disperse bioactive components ( $\beta$ -carotene, tocopherol, tocotrienol, flavonoids) into palm oil with better stability. The role of saponin as a natural emulsifier, in nanotechnology, saponin functions as a stabilizer to produce nano-sized particles (10–200 nm). This increases the dispersibility, bioavailability, and stability of bioactive components added to palm oil (McClements, 2018).

The stability of the bioactive component  $\beta$ -carotene, has sensitive properties to oxygen, light, and heat. The use of saponin as a nanoemulsion former can protect  $\beta$ -carotene from oxidative degradation during storage and heating processes. Tocopherols and tocotrienols act as antioxidants, but their stability can be compromised by exposure to high heat. Nanotechnology helps slow the degradation of these compounds by forming a protective layer (Y. Tan & McClements, 2021).

Saponins as emulsifiers, flavonoids can be more stable in oil, and their antioxidant bioactivity can be maintained. In addition to being emulsifiers, saponins also have natural antioxidant, antimicrobial, and antioxidant activities, which can support the role of tocopherols, tocotrienols,  $\beta$ -carotene, and flavonoids in preventing lipid oxidation. This shows that saponins provide double protection against palm oil degradation (Kregiel et al., 2017).





The fortification process with palm oil nanotechnology uses natural saponins, added bioactive components ( $\beta$ -carotene extract, tocopherol, and tocotrienol). The production methods used: High Energy Ultrasonication or High-Pressure Homogenization or Low-Energy Method (nanoemulsion: using a combination of saponins, temperature, and stirring to produce spontaneous emulsification). Development of parameters as independent variables: 1) stabilization and storage (nanoparticles or nanoemulsions formed are stored under controlled conditions to ensure the stability of bioactive components), 2) stability and effectiveness tests, namely stability testing against heat, oxygen, light, and evaluation of the bioavailability of components in the oil system (Alemán et al., 2014).

Advantages and challenges of using saponins in nanotechnology: its advantages are natural and safe, effective emulsifier (because the amphiphilic nature makes saponins efficient in forming stable nanoemulsions), bioactive protection (helps protect  $\beta$ -carotene, tocopherol, and tocotrienol from degradation, synergistic activity (because saponins have antioxidant properties that complement the function of other bioactive components). Fortification of palm oil with  $\beta$ -carotene, tocopherol, and tocotrienol using saponin-based nanotechnology is an innovative and potential approach (Mba et al., 2015). Saponin as a natural emulsifier supports the stability, bioavailability, and protection of bioactive components, while helping to overcome lipid oxidation in oil. With further research and optimization, this technology has the potential to be applied on an industrial scale to produce more nutritious, stable, and high-quality palm oil products (Y. Tan & McClements, 2021).

### Saponins and biological activities

The term saponin comes from the Latin word *sapo* which means soap, because saponin molecules, when shaken with water, form soap-like foam. Saponins have lyobipolar properties that allow interaction with the plasma membrane, which in aqueous solutions causes a decrease in surface tension (Fekadu Gemedo, 2014), (Thakur et al., 2019). Saponins are synthesized by various plant species as secondary metabolites. In plant cells, saponins are stored in the form of inactive precursors. When plants encounter pathogens, saponins are converted into active antibiotics through the action of plant enzymes. Saponins are classified into three main types, namely: 1) triterpenoids, 2) steroids, and 3) glycoalkaloids, each of which has a different chemical structure and biomedical properties (Samtiya et al., 2020). Saponins, obtained from the plant *Saponaria officinalis* L., have been used for centuries as household detergents due to their amphiphilic





properties. The saponin structure contains a lipid-soluble aglycone and a water-soluble chain, which makes it function as a surfactant. The biological activities of saponins are not only limited to their traditional uses but also in pharmaceutical applications. Saponins have a variety of pharmaceutical properties, including hemolytic, molluscicidal, anti-inflammatory, antifungal, antibacterial, antimicrobial, antiparasitic, antitumor, and antiviral effects (Kregiel et al., 2017). The discovery of anticancer properties in saponins has encouraged efforts to improve saponin extraction from plants, possessing antioxidant properties (Juang & Liang, 2020), immunological adjuvant activity (Moghimpour & Handali, 2015), and hemolytic activity (Sharma et al., 2021).

Saponins extracted from plants exhibit biomedical properties (Cheok et al., 2014), in addition to having various pharmaceutical properties such as immunological adjuvant activity (Sharma et al., 2021), hemolytic activity and antioxidant properties (Soltani et al., 2015), (Juang & Liang, 2020), saponins have also been shown to have anticancer effects (Majnooni et al., 2023), thus attracting much attention as potential targets for the development of functional food research, and the following is the basic structure of saponin types. 1) Furostan saponins are steroidal type saponins that have an additional ring structure forming furan (five members), are found as precursors of spirostanes, and have important biological activities, including as anti-inflammatory and immunomodulatory molecules. 2) Spirostan is a type of steroidal saponin, but differs from furostan because it has an additional ring structure in the form of spiran (two rings that share a central oxygen atom), its bioactive activity is stronger compared to furostan, especially as an active ingredient in herbal medicine. 3) Triterpenoidal saponins are a type of saponin built from a triterpene framework (30 carbon atoms), this structure has varying functional groups R<sub>1</sub>, R<sub>2</sub>, and R<sub>4</sub>, which can affect its biological properties, and are widely found in plants and often show activities such as anticancer, antimicrobial, and anti-inflammatory (Liu et al., 2016), (Anggraeni Putri et al., 2023).

### **Nanotechnology extraction and biofortification**

The extraction and purification methods of saponins used: acid and base solvents, microwave assisted solvent extraction (MASE), maceration, soxhlet, heat reflux, and ultrasound assisted extraction (Liu et al., 2016), (Sharma et al., 2021). Factors affecting extraction: solid-liquid ratio, pH value of acid isolation, pH value of alkali solution, stirring, contact time, particle size, and extraction temperature on the rate of saponin extraction. The order of influence is solid-liquid ratio > pH of acid isolation > pH of alkali solution > extraction temperature. Optimized extraction conditions: extraction temperature 68°C, pH of alkali solution 9.1; pH of acid isolation 4.1; and solid-liquid ratio 15.9:1; produced a saponin yield of 76.12% (Liu et al., 2016).





Comparison of saponin extraction methods using the MASE method with conventional extraction (soxhlet, and maceration), showed significant saponin yields:  $5.11 \pm 0.3$ ;  $2.5 \pm 0.1$ ; and  $4 \pm 0.2\%$  with contact times of 4 minutes, 3 hours and 24 hours, respectively (Sharma et al., 2021).

In addition to pharmaceutical applications, saponins have been used in food as natural surfactants and function as preservatives in controlling microbial spoilage in food. The effectiveness of natural surfactants isolated from plant parts to form and stabilize emulsions with synthetic surfactants (Tween80) has been compared by (Z. Zhu et al., 2019), (Meshram et al., 2021). After comparing the effects of homogenization pressure, number of passes, and emulsifier concentration on the particle size produced from these two surfactants, they suggested that natural surfactants can effectively replace synthetic surfactants in food and beverage products. This natural surfactant has been further proven its stability and effectiveness in forming an edible vitamin E emulsion system, thus it is recommended for functional food and beverage encapsulation applications. The use of saponins as natural biosurfactants to improve the surface properties of foods, because they can provide a continuous hydrogen bonding pathway (Jiang et al., 2018), (Meshram et al., 2021).

In general, extraction techniques used in saponin extraction can be classified into two categories, namely conventional technology and green technology. Conventional extraction techniques are maceration, Soxhlet, and reflux extraction, while green technology is ultrasonic-assisted extraction, microwave-assisted extraction, and accelerated solvent extraction. The extraction uses a large amount of solvent to extract the desired solute, which is assisted by high temperatures through heating, mechanical stirring, or shaking (Cheok et al., 2014).

Fortification of  $\beta$ -carotene, tocopherol, and tocotrienol, in palm oil with nanotechnology using natural saponins is very possible, considering the natural amphiphilic nature of saponins which have hydrophobic (oil-soluble) and hydrophilic (water-soluble) parts. Saponins can act as natural emulsifiers that help form nanoparticles or nanoemulsions, so that the bioactive components can be dispersed more evenly and stably in palm oil (Kregiel et al., 2017).

The rapid development of nanotechnology has opened the door to innovation in many industrial sectors, including agricultural production, feed, and food processing. The expected benefits of nanotechnology-enabled products in these sectors are to improve the efficacy of agrochemicals, increase the bioavailability of nutrients or safer packaging materials (Amenta et al., 2015).

Applications of nanotechnology in the food sector: 1) Food packaging, nanotechnology is used to create smart and active packaging that can extend shelf life, monitor quality, and prevent





microbial contamination, 2) Food safety, nanosensors can detect toxins, pathogens, or changes in food quality in real time, improving food safety for consumers, 3) Biofortification and nutrition, in this context nanotechnology is applied to increase nutrient content through nanoformulations, for example nano-encapsulation of vitamins, minerals, or other bioactive compounds, nanoemulsions and 4) Food processing, this technology can be used to improve the texture, taste, or stability of products through manipulation at the molecular level (Tahir et al., 2021). Nanotechnology in food production, most of its applications involve food additives that improve food stability during processing and storage, enhance product characteristics, or increase the potency and/or bioavailability of nutrients in food products (Amenta et al., 2015), (Jafari & McClements, 2017).

Regulatory challenges related to nanotechnology: 1) Safety and risks, nanoparticles can have potential risks to human health and the environment, related to regulations in the European Union and other countries addressing this issue, 2) Standards and testing, regulations are needed to ensure that nanotechnology products used in the food sector meet international safety standards, and 3) Labeling, whether products using nanotechnology must be specifically labeled to inform consumers (Amenta et al., 2015).

Indonesia has the potential to reach its entire population with cooking oil fortified with vitamin A, even the most vulnerable groups who need this important vitamin. Unbranded cooking oil with a high initial PV value has a very short shelf life and will soon become unfit for human consumption. Related to this and to protect human health, the role of the state should be to prohibit highly oxidized cooking oil from being sold on the market. In addition, it is important to promote and support fortification of cooking oil with low peroxide levels. The quality of vegetable oil before fortification is an important factor in ensuring the success of fortification in providing positive impacts on health (Andarwulan et al., 2014).

The success of cooking oil fortification is highly dependent on the quality of the oil used. Standards ensure that the oil used meets certain qualities, fortification can provide optimal health benefits and protect the public from the risk of consuming oxidized (C. Zhu et al., 2013), (Alemán et al., 2014), (Martianto et al., 2018).

According to (Malau et al., 2019) shows the relationship between peroxide value (PV) and decreased vitamin A activity in fortified palm cooking oil.

- Oil with high initial PV shows a faster increase in PV value during storage. This reflects the acceleration of fat oxidation, indicating that the oil has undergone oxidative degradation before the fortification process.





- Oil with low initial PV experiences a slower increase in PV compared to oil with high PV. This shows that fresher oil or oil with a low initial oxidation level is more stable against degradation during storage.
- Vitamin A degradation, oil with high initial PV causes vitamin A in the oil to degrade faster, because lipid oxidation produces free radicals that accelerate the damage of vitamin A. Conversely, oil with low initial PV, vitamin A degradation occurs more slowly, indicating that vitamin A stability is better in fresher or less oxidized oil.
- Storage at  $60 \pm 5$  °C accelerates the oxidation reaction, thus providing a simulation of how oil quality and vitamin A content will be degraded in hot environmental conditions. • The author's resume, emphasizes the importance of the initial quality of fortified cooking oil. Oil with a low initial peroxide value provides better stability for vitamin A during storage. Fortification of oil with a high PV is not only less effective, but also risky because oxidation products can be harmful to health. Fortification is effective if carried out on oil with good initial quality.

### **Codex palm oil standards**

Palm oil is one of the leading commodities in the global market, playing an important role in the food and non-food industries. As the main source of vegetable fat, palm oil is used in various products such as margarine, cooking oil, processed foods, cosmetics, and biofuels. The increasing popularity of palm oil demands international standards to ensure the quality, safety, and fairness of trade between countries (Andarwulan et al., 2014). CODEX Alimentarius, established by FAO and WHO, is the main reference in developing global food standards. The CODEX standard for palm oil aims to provide clear guidelines regarding the physicochemical characteristics, safety, and handling of palm oil throughout the supply chain, from production to consumption. This standard includes quality parameters such as relative density, apparent density, refractive index, saponification value, and iodine value (Hariyadi, 2021). The adoption of CODEX standards in the palm oil industry provides assurance that the products produced meet internationally recognized quality and safety requirements. Industry standards not only increase consumer confidence but also strengthen the competitiveness of producers in the global market (Andarwulan et al., 2014). CODEX standards also encourage the implementation of sustainable cultivation and processing practices that support environmental sustainability (Hariyadi, 2021). Through this document, it is hoped that readers can understand the essence and relevance of CODEX palm oil standards in supporting the development of strategic commodity-based industries. Strengthening standards is expected to answer global challenges related to the issue of sustainability and acceptability of palm oil products in various parts of the world (Sulaiman et al., 2022).





Globally, around 85% of palm oil produced is used for food purposes, as cooking oil, raw material in margarine, ice cream, and various ready-to-eat foods. The Food and Agriculture Organization (FAO, 2019) reminds us that if it is not safe, it is not food. This shows that palm oil used for food purposes must meet food safety standards. For Indonesia, international palm oil trade, and compliance with international food safety standards, such as the Codex Standards developed by the Codex Alimentarius Commission (CAC), are very important (Hariyadi, 2021).

Tabel 3. Chemical and physical characteristics of crude palm oil (Codex Standard for named vegetable oil – CXS 210-1999).

Keragaman	Relative density (°C/water at 20°C)	Apperent density (g/ml)	Refractive index (ND 0°C)	Saponification value (mg KOH/g oil)	Iodine value
Palm Oil	0,891 – 0,899 X=50°C	0,889 – 0,895 at 50°C	1,454 – 1,456 at 50°C	190 – 209	50 – 55
Palm Olein	0,899 – 0,920 X=40°C	0,896 -0,898 at 40°C	1,458 – 1,460	194 – 202	56
Palm Stearin	0,881 – 0,891 X=60°C	0,881 – 0,885 at 60°C	1,447 – 1,452 at 60°C	193 – 305	< 48
Palm Super Olein	0,900 – 0,925 X=40°C	0,886 – 0,900 at 40°C	1,459 – 1,460	180 – 205	≥ 60
Palm Kernel Oil	0,899 – 0,914 X=40°C	NA	1,448 – 1,452	230 – 254	14,1 – 21
Palm Kernel Olein	0,906 – 0,909 X=40°C	0,904 – 0,907	1,541 – 1,453	231 – 244	20 – 28
Palm Kernel Stearin	0,902 – 0,908 X=40°C	0,904 – 0,907	1,449 -1,451	244 – 255	4 – 8,5
Palm Oil with heigher oleic acid	0,896 – 0,910 X=50°C	0,904 – 0,906	1,459 – 1,462	189 – 199	58 – 75

Source: (Hariyadi, 2021).

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