

# EFFECT OF ZWIEBACK, A CRISP BREAD CONTAINING RED PALM OIL, ON SERUM ANTIOXIDANT AND INFLAMMATORY BIOMARKERS IN WOMEN WITH BENIGN BREAST TUMOURS

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## ABSTRACT

*This study investigated the effect of replacing margarine with red palm oil (RPO) in Zwieback on serum antioxidant and inflammation biomarkers in women with benign breast tumours. Using a double-blind, randomised controlled trial, 15 participants were divided into a control group (n = 8) and a treatment group (n = 7). The treatment group consumed Zwieback with RPO, providing 5.04 mg/day of  $\beta$ -carotene for eight weeks, while the control group received Zwieback without RPO. Results showed a significant increase ( $p < 0.05$ ) in SOD activity in both groups. A reduction of TNF- $\alpha$ , 8-isoprostane levels, and tumour size ( $p < 0.05$ ) was observed in the treatment group. However, the between-group difference in tumour size was not statistically significant after adjustment. The control group experienced a significant decline ( $p < 0.05$ ) in  $\beta$ -carotene levels. These findings suggest that adequate antioxidant intake, particularly  $\beta$ -carotene, may help maintain serum  $\beta$ -carotene levels and enhance antioxidant capacity. The consumption of Zwieback substituted with RPO may have beneficial effects on oxidative stress and inflammation, which are factors linked to cancer risk.*

**Keywords:** antioxidant, benign breast tumour, functional food, nutraceutical, red palm oil.

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## INTRODUCTION

Breast disease represents a complex area of medicine, predominantly affecting women and includes conditions ranging from infections and cysts to growths, some of which are painful (Stachs et al., 2019). While many growths are benign,

malignant ones can spread and lead to breast cancer, the most common cancer in women globally (International Agency for Research on Cancer, 2020). In 2022, there were 2.3 million new cases of breast cancer and 670,000 deaths worldwide (World Health Organization [WHO], 2024). Notably, women diagnosed with benign breast tumours remain at elevated risk of developing breast cancer, a risk that may persist for decades (Román et al., 2022). Early detection and intervention are critical in managing breast health and preventing cancer progression.

Reactive oxygen species (ROS), highly reactive molecules that can damage cells, play a key role in breast disease and cancer development (Subramaniam et al., 2022). Factors such as radiation exposure, environmental carcinogens and chronic inflammation increase ROS levels, leading to the oxidation of vital cellular components like

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DNA, proteins and lipids (Hong et al., 2024). Lipid peroxidation, in particular, generates 8-isoprostane, a recognised biomarker of oxidative damage (Valgimigli, 2023). Additionally, inflammation-related markers like TNF- $\alpha$  have been linked to an increased risk of breast cancer, suggesting that targeting oxidative stress and inflammation could be an effective strategy for cancer prevention (Fontvieille et al., 2022).

One promising approach involves enhancing the body's antioxidant capacity to neutralise free radicals and limit cellular damage (Robbins & Zhao, 2014). Red palm oil (RPO), rich in  $\beta$ -carotene, offers a natural antioxidant source. Unlike palm oil products, commonly found on the market like cooking oil, RPO undergoes minimal processing, preserving its vibrant red colour and  $\beta$ -carotene content. This  $\beta$ -carotene has been linked to a reduced risk of coronary heart disease and cancer (Vrolijk et al., 2015).

Red palm oil, derived from the fruit of the oil palm tree (*Elaeis guineensis*), is rich in antioxidant compounds such as tocotrienols, tocopherols and carotenoids, which are known for their potential anticancer properties (Hertanti et al., 2023; Marjan et al., 2016; Zhu et al., 2015). The high levels of carotenoids, particularly  $\beta$ -carotene and  $\alpha$ -carotene, offer potent antioxidant effects that can reduce oxidative stress and prevent cellular damage, potentially lowering the risk of cancer development. Additionally, RPO contains phytosterols, squalene and coenzyme Q10, which contribute to its anticancer and immune-supporting properties (Loganathan et al., 2015).

Studies on animal models and human cells suggest that RPO's bioactive compounds may inhibit the growth of cancer cells, promote antioxidant activity and boost immune responses. Specifically, carotenoids in RPO have shown promise in antiproliferative effects on both human cancer cell lines by inhibiting the activation of NF- $\kappa$ B. Furthermore, its compounds may reduce the negative impacts of chemotherapy, supporting overall cellular health and resilience during cancer treatments (Loganathan et al., 2015).

Given these findings, RPO is gaining attention as a supplementary dietary option for cancer prevention, including breast cancer, due to its unique nutrient profile that supports antioxidative and immune defences. Despite Indonesia's status as one of the largest palm oil producers, the development of RPO-based functional foods remains limited. Currently, the availability of certain products, including biscuits and RPO encapsulation forms, is still limited. Similarly, clinical trials associated with these products are also in their early stages and have a limited scope (Hertanti et al., 2023; Marjan et al., 2016; Zhu et al., 2015). Based on its significant antioxidant properties, the integration of RPO

into everyday food products offers a distinctive opportunity to enhance health and potentially mitigate cancer risk by providing clinical evidence for a locally producible functional food.

Zwieback is a type of crisp bread that offers excellent shelf stability and microbial safety, making it a practical vehicle for delivering functional ingredients in clinical and community settings. Its structure allows uniform incorporation of lipid-based bioactives such as RPO, which is rich in provitamin A carotenoids, tocopherols and tocotrienols – compounds known for their antioxidant and anti-inflammatory properties (Khan et al., 2022). These bioactives remain relatively stable during the baking process, preserving their functional integrity under dry-heat conditions (Sambanthamurthi et al., 2000). Zwieback's mild flavour and soft texture also make it acceptable to individuals undergoing medical treatment, such as women with breast tumours, who may experience taste or gastrointestinal disturbances (De Vries et al., 2017). Its familiarity and convenience further support adherence and scalability in dietary interventions. This study aims to investigate the effect of Zwieback enriched with RPO on serum antioxidant and inflammation biomarkers in women with breast tumours, exploring its potential role in reducing breast cancer risk.

## MATERIALS AND METHODS

### Materials and Calculation of Carotene Content

The Zwieback products were produced by Fitria Cookery, a small-scale food enterprise specialising in Zwieback products located in Bogor, Indonesia. The control group received Zwieback, a sweetened, egg-enriched bread that is baked, sliced and toasted until dry and crisp, made with wheat flour, milk, sugar, margarine, eggs, yeast and salt. The intervention group received a similar Zwieback, with RPO substituted for margarine. The RPO used is the Salmira brand, manufactured by PT Nutri Palma Nabati, Bogor, West Java, Indonesia. This substitution of margarine with RPO provided 600 RE of vitamin A to fulfil the Indonesian Nutrition Reference Value (National Agency of Drug and Food Control of Indonesia, 2016). This amount of vitamin A in RPO is equivalent to 7.2 mg of  $\beta$ -carotene.

### Research Design

The research used a randomised controlled trial (RCT) with a double-blind pre-post study design consisting of two groups: A control group and a treatment group. The study was conducted in Sukabumi Regency, West Java, Indonesia, for eight weeks.

Based on sample size calculation using a two-sided hypothesis test for two population means (Levy & Lemeshow, 1999), and considering the value of standard deviation and effect size or clinically meaningful difference (Althubaiti, 2022), a minimum of seven subjects is required for each group, considering inclusion and exclusion criteria. This study also has a limited sample size, which is appropriate for the specific case of benign breast disease. Subject inclusion criteria included adult women aged 20-55 with a BMI of 18.5-27.0 kg/m<sup>2</sup>, diagnosed with a benign breast tumour, and willing to provide informed consent. Subject exclusion criteria included having congenital abnormalities, participating in other studies, not being willing to comply with the research procedures, taking antibiotics or laxatives (four weeks before the study) and having allergies to eggs and gluten. A total of 18 people who met the inclusion criteria participated in this study. They were randomly divided into the control group (n = 9) and the treatment group (n = 9); only 15 people participated in the study until the end (n = 8 in the control group and n = 7 in the treatment group).

The subjects were randomly assigned to control and treatment groups using the RAND function in Microsoft Excel. The randomisation process was managed by independent personnel hired by the research team to maintain the confidentiality of the group assignments. These personnel were also responsible for preparing an intervention package for all groups. Furthermore, the design and packaging of the intervention product were intentionally made identical. This approach ensured that neither the research team nor the subjects were aware of the members of the control and intervention groups.

### Procedure of the Intervention Stage

This study has been approved by the Research Ethics Committee Involving Human Subjects of the IPB University, with the approval number 126/IT3.KEPMSM-IPB/SK/2018. In the intervention stage, the subjects were allowed to eat as usual. The dietary intake of the study subjects was assessed using a 2 x 24-hr food recall method at the pre-intervention, midpoint of the intervention and post-intervention periods. The study subjects in the control group received Zwieback without RPO, and the treatment group received margarine substitutes with RPO in Zwieback. The intervention packages were distributed to respondents weekly in as many as 14 packs (3 pieces/pack). Each subject received one jar to store the intervention product package, increasing food safety and reducing the risk of being damaged, scattered, or lost. Product packaging was collected, and a product

monitoring form was provided, which the subject must fill out.

Each subject was asked to consume six pieces of Zwieback (10 g/piece) daily as a snack. The intervention product contains 5.04 mg of  $\beta$ -carotene, so it follows the recommendations of the Institute of Medicine (IOM), which states that intake of  $\beta$ -carotene as much as 3.00-6.00 mg/day can maintain adequate serum levels of  $\beta$ -carotene and is associated with a reduced risk of chronic diseases such as cancer and heart disease (IOM, 2000).

Professional laboratory personnel conducted blood sampling at two points, approximately 10 mL each, before and after the intervention. In the intervention stage, the subjects were allowed to eat as usual.

### Biomarkers Analysis

The process of producing blood serum for analytical purposes involves coagulating blood at ambient temperature for 10-20 min. Subsequently, the blood undergoes a centrifugal process at speeds ranging from 2,000-3,000 RPM for 20 min. This process separates the blood components, leaving behind serum, which is then prepared for analysis. Serum  $\beta$ -carotene was determined using a high-performance liquid chromatography (HPLC) method using LC-2050C, Shimadzu, USA. This analysis was conducted at the SEAMEO REFCON Laboratory, University of Indonesia.

Superoxide dismutase (SOD) biomarker analysis was performed using an ELISA kit from Bioassay Technology Laboratory, China (Cat. No. E4502Hu), while TNF- $\alpha$  analysis was conducted with an ELISA kit from the same laboratory (Cat. No. E0082Hu). Serum 8-isoprostane levels were also measured using an ELISA kit from Bioassay Technology Laboratory, China (Cat. No. E6776Hu). All analyses were carried out at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, Indonesia, following the protocols provided by Bioassay Technology Laboratory. Furthermore, the size of the breast tumour was assessed using ultrasonography (USG) both pre- and post-intervention, with the same medical doctor and equipment employed for consistency.

### Data Analysis

Statistical analysis was performed using SPSS for Windows ver. 26 (IBM, USA). The significance level was set at  $p < 0.05$ . Data analysis used an independent t-test to see whether there was a difference in the average value of each variable between the control group and the treatment group. The difference in the average value of each variable before and after the

intervention in each group was analysed using the paired t-test. The ANCOVA test was used to analyse the differences in the changes of each variable.

## RESULTS AND DISCUSSION

The subjects ranged from 22-52 years old, with an average age of 36. A total of 73.3% of the subjects had a normal BMI, and 26.7% were classified as overweight. All subjects exercised less than three times a week, and 46.7% never exercised. There was no significant difference between the two groups on  $\beta$ -carotene serum, SOD activity, TNF- $\alpha$ , 8-isoprostane serum and tumour size before intervention (Table 1).

### Serum $\beta$ -carotene

Table 1 shows that there was no significant difference in serum  $\beta$ -carotene levels between the control and treatment groups before the intervention ( $p = 0.65$ ). On the other hand, after the intervention, a significant difference was observed between the two groups ( $p = 0.02$ ), with the treatment group showing an increase in serum  $\beta$ -carotene levels ( $0.275 \pm 0.227$  to  $0.309 \pm 0.157 \mu\text{mol/L}$ ) and the control group experiencing a significant reduction ( $0.294 \pm 0.206$  to  $0.131 \pm 0.046 \mu\text{mol/L}$ ). The increase in serum  $\beta$ -carotene in the treatment group may be attributed to the intervention, which likely enhanced the bioavailability of  $\beta$ -carotene. In contrast, the control group demonstrated a marked decrease.

These findings suggest that the intervention had a beneficial effect on  $\beta$ -carotene levels in the treatment group. This result contrasts with a previous study that reported no significant changes in serum  $\beta$ -carotene levels following an intervention with dry bread containing RPO (Harianti et al., 2022). The significant reduction in  $\beta$ -carotene in the control group underscores the importance of the intervention in maintaining or improving  $\beta$ -carotene status.

### Superoxide Dismutase (SOD) Activity

The results of the SOD activity in Table 1 indicate that there was no statistically significant difference ( $p > 0.05$ ) in SOD activity between the control and treatment groups both before and after the intervention. Meanwhile, both groups showed a significant increase in SOD activity after the intervention. The treatment group had a better significance value which was 51.7% increased after intervention.

The results show that the administration of substituted margarine with RPO Zwieback increased the activity of SOD. The content of  $\beta$ -carotene in Zwieback provides exogenous antioxidant intake, so it is possible to help SOD as an endogenous antioxidant to ward off free radicals formed in the body. It aligns with a study showing an increase in SOD activity from consuming dry bread with RPO (Harianti et al., 2022). Several studies have shown that increased levels of SOD in the body can decrease tumour growth from various types of cancer, including breast cancer (Morry et al., 2017).

TABLE 1. THE AVERAGE VALUE OF SERUM  $\beta$ -CAROTENE LEVELS, SOD ACTIVITY, 8-ISOPROSTANE SERUM, TNF- $\alpha$  AND TUMOUR SIZE BY SUBJECT GROUP BEFORE AND AFTER INTERVENTION

Variables	Subject group	Before intervention	After intervention	Difference	p-value
$\beta$ -carotene ( $\mu\text{mol/L}$ )	Control	$0.294 \pm 0.206$	$0.131 \pm 0.046$	-0.163	0.00 <sup>c*</sup>
	Treatment	$0.275 \pm 0.227$	$0.309 \pm 0.157$	0.034	0.66 <sup>c</sup>
	p-value	0.65 <sup>a</sup>	0.02 <sup>a*</sup>	0.87 <sup>b</sup>	
SOD ( $\mu\text{g/mL}$ )	Control	$10.07 \pm 2.11$	$17.31 \pm 7.21$	7.24	0.04 <sup>c*</sup>
	Treatment	$12.05 \pm 5.39$	$18.28 \pm 5.07$	6.23	0.02 <sup>c*</sup>
	p-value	0.74 <sup>a</sup>	0.77 <sup>a</sup>	0.67 <sup>b</sup>	
8-isoprostane (ng/L)	Control	$218.43 \pm 95.62$	$175.63 \pm 48.19$	-42.80	0.07 <sup>c</sup>
	Treatment	$384.18 \pm 299.62$	$184.29 \pm 118.48$	-199.90	0.02 <sup>c*</sup>
	p-value	0.05 <sup>a</sup>	0.17 <sup>a</sup>	0.04 <sup>b*</sup>	
TNF- $\alpha$ (ng/L)	Control	$215.00 \pm 74.18$	$149.08 \pm 34.68$	-65.92	0.02 <sup>c*</sup>
	Treatment	$345.66 \pm 264.11$	$186.96 \pm 146.92$	-158.70	0.02 <sup>c*</sup>
	p-value	0.08 <sup>a</sup>	0.73 <sup>a</sup>	0.01 <sup>b*</sup>	
Tumours size (mm)	Control	$5.50 \pm 1.51$	$4.63 \pm 2.26$	-0.86	0.20 <sup>c</sup>
	Treatment	$8.00 \pm 3.96$	$4.43 \pm 1.76$	-3.57	0.02 <sup>c*</sup>
	p-value	0.15 <sup>a</sup>	0.86 <sup>a</sup>	0.36 <sup>b</sup>	

Note: \* - significantly different; <sup>a</sup> - independent t-test results between groups; <sup>b</sup> - ANCOVA test results that have been corrected for body mass index,  $\beta$ -carotene intake and nutritional adequacy levels (energy, protein, fat, carbohydrates); <sup>c</sup> - paired t-test results in one group.

### Serum 8-Isoprostane Levels

The analysis showed that the administration of Zwieback reduced serum 8-isoprostane levels. There was a significant difference in the value of 8-isoprostane serum levels before and after intervention between the treatment group ( $p = 0.02$ ). The ANCOVA test, which had been adjusted for body mass index, beta carotene intake and nutritional adequacy levels, also revealed a significant difference in serum 8-isoprostane levels between the control group and the treatment group ( $p = 0.04$ ). This aligns with study showing that consuming foods or supplements containing antioxidants like vitamin C, vitamin E,  $\beta$ -carotene and lycopene has been associated with reduced 8-isoprostane levels, indicating a decrease in the body's oxidative stress level (Milne et al., 2015).

### Serum TNF- $\alpha$

The results of the TNF- $\alpha$  analysis in *Table 1* also support the results of serum SOD and 8-isoprostane measurements, which show an increase in antioxidant activity and a decrease in oxidative stress. The results of the analysis show a significant reduction in TNF- $\alpha$  levels. These findings were further validated by an ANCOVA test, which controlled for body mass index,  $\beta$ -carotene intake and nutritional adequacy. This test confirmed a statistically significant difference in serum TNF- $\alpha$  levels between the control and treatment groups. The rise in TNF- $\alpha$  in cancer conditions is thought to increase the activation of NF- $\kappa$ B, which will activate the expression of anti-apoptosis genes so that cancer cells can survive and prevent the death of cancer cells due to the body's defences by genome and tumour-surveillance mechanisms (Cai et al., 2017; Sethi et al., 2008; Wu & Zhou, 2010). Park and Hong (2016) mentioned that antioxidants could inhibit the activation of NF- $\kappa$ B. This is further supported by study showing that antioxidants may suppress TNF- $\alpha$ , thereby inhibiting NF- $\kappa$ B signalling pathways (Assar et al., 2016).

### Breast Tumour Size

The findings in *Table 1* demonstrate a statistically significant difference ( $p < 0.05$ ) in breast tumour size before and after the intervention in the treatment group administered by Zwieback. In contrast, the control group showed no significant difference in tumour size before and after the intervention. These results are consistent with the measurements of SOD, 8-isoprostane serum and TNF- $\alpha$ , indicating an increase in antioxidant activity and a decrease in oxidative stress. Moreover, the reduction in TNF- $\alpha$  levels observed in the treatment group suggests its

potential role in inhibiting the growth of tumour cells. However, after adjusting for covariates, the difference in change between the groups was not statistically significant.

The substitution of margarine with RPO in Zwieback led to positive results in improving breast tumour biomarkers and a significant decrease in tumour size in the study subjects. This study also found improvements in biomarkers of oxidative stress, which is associated with breast tumour disease.

### Discussion

The present study investigated the effects of Zwieback containing RPO on women with benign breast tumours. We evaluated several biomarkers, including  $\beta$ -carotene serum levels, SOD activity, TNF- $\alpha$ , 8-isoprostane and breast tumour size. Our results indicate that the treatment group increased serum  $\beta$ -carotene levels, while the control group significantly decreased serum  $\beta$ -carotene levels.  $\beta$ -carotene can be converted into vitamin A, which helps stimulate cell communication, inhibit chemically induced neoplastic transformation and act as an antioxidant (Shete & Quadro, 2013; Sommerburg et al., 2013). The role of  $\beta$ -carotene as an antioxidant is particularly important given the high oxidative stress experienced by subjects due to their pathophysiological condition. Beyond its antioxidant properties,  $\beta$ -carotene has been widely studied for its capacity to arrest the cell cycle, induce apoptosis and promote differentiation in various tumour cells, including breast tumours (Dulińska et al., 2005). The breakdown of  $\beta$ -carotene into retinoic acid (vitamin A) can further inhibit tumour cell division by reducing the activation of extracellular signal-regulated protein kinase 1 (ERK1), which signals cell division (Crowe et al., 2003). Both  $\beta$ -carotene and retinoic acid play a crucial role in cell differentiation. This aspect was explored by Lim et al. (2014) in neuroblastoma tumour cells. Their study revealed that  $\beta$ -carotene supplementation can inhibit tumorigenesis by inducing neuronal differentiation, suppressing cancer stem cells and regulating hypoxia-related HIF-1 $\alpha$ , thereby inhibiting vascular endothelial growth factor (VEGF). VEGF is a growth factor for blood vessel endothelial cells that facilitates cell migration and proliferation. Considering these functions,  $\beta$ -carotene can be utilised by the body to address its pathological conditions.

Furthermore, our results show that substituting margarine with RPO Zwieback increased SOD enzyme activity. It is conceivable that the results observed were not exclusively due to a single factor, such as the administration of Zwieback containing RPO. Other variables, including an unrestricted diet, physical activity, behaviour and

other factors not examined in this study, may have also played a role. Nonetheless, the  $\beta$ -carotene present in Zwieback may serve as a source of exogenous antioxidants, which could enhance the function of SOD, an endogenous antioxidant, in neutralising free radicals in the body. This finding aligns with a study demonstrating increased SOD enzyme activity from consuming dry bread with RPO (Harianti et al., 2022). Another study has demonstrated that increased levels of SOD in the body can decrease tumour growth in various types of cancer, including breast cancer (Morry et al., 2017). These findings suggest that consuming Zwieback with RPO may have potential health benefits for women with benign breast tumours.

The results from testing serum 8-isoprostane levels in *Table 1* support the increase in SOD levels observed in the group given Zwieback. The analysis revealed a marked difference in serum 8-isoprostane levels before and after the intervention in the treatment group. In contrast, the control group showed no noticeable change in serum 8-isoprostane levels. These findings indicate that the administration of Zwieback effectively reduced serum 8-isoprostane levels. The difference in the decrease of 8-isoprostane serum levels before and after intervention was significant between the control and treatment groups. These results align with the study by Milne et al. (2015), which states that consuming foods or supplements that contain antioxidants, such as vitamin C, vitamin E,  $\beta$ -carotene, lycopene and others, can reduce 8-isoprostane levels. This decrease suggests reducing the body's oxidative stress levels.

*Table 1* shows the results of the TNF- $\alpha$  analysis, which supports the findings of the serum SOD and 8-isoprostane measurements. These results indicate an increase in antioxidant activity and a decrease in oxidative stress. Notably, there was a significant decrease in TNF- $\alpha$  levels. In addition, the observed reduction in TNF- $\alpha$  levels in the treatment group indicates its potential to inhibit the growth of tumour cells. The increase of TNF- $\alpha$  in cancer conditions is thought to increase the activation of NF- $\kappa$ B, which will activate the expression of anti-apoptosis genes so that cancer cells can survive and prevent the death of cancer cells due to the body's defences by genome and tumour-surveillance mechanisms. Prior studies by Sethi et al. (2008), Wu and Zhou (2010) and Cai et al. (2017) support this finding. Antioxidants can inhibit NF- $\kappa$ B activation, as Park and Hong (2016) mentioned. Furthermore, Assar et al. (2016) found that antioxidant substances can suppress TNF- $\alpha$  and inhibit NF- $\kappa$ B signalling pathways.

The study revealed that substituting margarine with RPO in Zwieback significantly improved breast tumour biomarkers and reduced tumour size.  $\beta$ -carotene, a key antioxidant in Zwieback,

was found to alleviate oxidative stress, a condition closely linked to breast tumour development. These findings align with a systematic review and meta-analysis by Peraita-Costa et al. (2022), which demonstrated that consuming foods rich in  $\beta$ -carotene may lower the risk of breast cancer. Additionally, another study highlighted that the average daily intake of  $\beta$ -carotene was inadequate. It further showed a positive correlation between  $\beta$ -carotene intake and SOD activity, with SOD activity inversely associated with breast tumour size, suggesting an indirect relationship between  $\beta$ -carotene intake and reduced tumour size (Rizqiawan et al., 2021).

This study highlights the potential of phytonutrients present in RPO to reduce breast tumour size. The results indicate a statistically significant reduction in breast tumour size in the treatment group following the intervention, whereas no significant change was observed in the control group. However, after adjusting for covariates, the difference in changes between the groups was not statistically significant. These findings suggest that the observed effect is not solely attributable to Zwieback containing RPO, and other unobserved factors may have contributed to the outcome. Furthermore, the intervention implemented in this study was conducted over a period of only eight weeks, which is relatively brief for observing significant changes in tumour size or long-term modulation of biomarkers.

Nevertheless, RPO has the potential to play a significant role in the findings presented in this study. A review by Loganathan et al. (2015) found that tocotrienol, tocopherol, carotenoids, squalene and co-enzyme Q10 present in RPO exhibit anti-proliferative effects on breast cancer cells. Similarly, Subramaniam et al. (2022) demonstrated that tocotrienol in palm oil can reduce tumour growth and metastasis of breast cancer cells in a syngeneic mouse model. Additionally, the study by Sommerburg et al. (2013) suggested that when  $\beta$ -carotene and  $\alpha$ -tocopherol are present together in a homogeneous solution,  $\alpha$ -tocopherol is consumed predominantly and  $\beta$ -carotene is spared. On the contrary,  $\beta$ -carotene is consumed faster than  $\alpha$ -tocopherol when the radicals are generated within the lipophilic compartment of the membrane (scavenging lipophilic radicals within the membrane by  $\beta$ -carotene) (Shete & Quadro, 2013). Although our study only measured Zwieback's  $\beta$ -carotene content, we assume there may be synergistic effects of  $\beta$ -carotene, tocotrienol, tocopherol and other phytonutrients in RPO. The mechanisms underlying the anticancer activities of RPO phytonutrients are complex and multifactorial. Further comprehensive study is necessary to fully elucidate their potential for clinical application.

## CONCLUSION

This study revealed that giving substituted margarine with RPO in Zwieback containing 5.04 mg of  $\beta$ -carotene to the study subjects for eight weeks was associated with improvements in oxidative stress markers and potential reductions in tumour size. This suggests that developing products containing RPO may potentially have beneficial effects on oxidative stress and inflammation, which are factors linked to cancer risk. Further study involving larger sample sizes and extended study durations is necessary to elucidate the role of phytonutrients in RPO in reducing breast tumour size in women.

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