A Potential Source of Vitamin E Extracted from Palm Oil for Aquaculture Feeds

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INTRODUCTION

The aquaculture industry is currently the fastest growing food production sector in the world. World aquaculture produces about 60 million tonnes of seafood worth more than USD 70 billion annually (FAO, 2006). Farmed fish accounts for about 50% of all consumed fish in the world, and this percentage is expected to continue to increase due to dwindling catches from capture fisheries. In recent years, technological advances in the aquafeed manufacturing industry have made possible the incorporation of high levels of dietary oils in fish feeds to produce energy-dense diets. Improvements in growth and feed utilization efficiency have been reported in fish due to the protein-sparing effect of dietary lipids. However, feeding high levels of dietary fish oils, which contain a high proportion of polyunsaturated fatty acids (PUFA) which are highly susceptible to oxidation, can lead to increase oxidative stress for the fish that can result in pathological conditions (Sakai *et al.*, 1998) and deterioration of fillet quality (Scaife *et al.*, 2000). Farmed fish quality deteriorates rapidly after slaughtering and this affects the shelf-life, storage properties and quality of seafood and surimi-based products. Increases in the lipid content of commercial fish feeds are usually not followed by appropriate antioxidant supplementation in order to maintain normal antioxidant status which further exacerbates the deleterious effects of lipid peroxidation, especially in cellular biomembranes which contain high amounts of PUFA.

Vitamin E is a potent antioxidant that inhibits lipid peroxidation in cell membranes. Vitamin E is a generic descriptor attributed to a group of lipid-soluble, structure-related compounds, which occurs naturally as α-, β-, γ-, or δ-tocopherols (T), and the four corresponding tocotrienols (T3). Among them, α-T has the highest vitamin E activity. For dietary purposes, vitamin E activity is expressed as the α-tocopherol equivalent (α-TE) which is the activity of 1 mg RRR-α-tocopherol (Papas, 1999). So based on this, each of the natural vitamin E isoforms is assigned a biopotency factor (α-T, 1.0; β-T, 0.5; γ-T, 0.1; δ-T, 0.03; α-T3, 0.3; β-T3, 0.05; γ -T3, 0.01) according to the amount of vitamin E necessary to prevent fetal resorption in pregnant and vitamin E-deficient rats (Sheppard and Pennington, 1993; Drotleff and Ternes, 1999). The biopotency factor for δ-T3 is presently unknown. It is therefore not surprising that almost all vitamin E research in fish nutrition has focused on α-T, commonly supplied as the synthetic *all-rac*-α-tocopheryl acetate, as it is believed to be the most potent of all the isoforms. The synthetic *all-rac*-α-tocopheryl acetate is used worldwide in commercial fish feeds, and is a multi-million dollar industry based mainly in Europe. About 70% of all synthetic vitamin E produced ends up in vitamin premixes of animal feeds, including aquafeeds.

Recent research seems to indicate that the antioxidant activities among the various vitamin E isoforms are not necessarily correlated with their assigned biological activities. Serbinova *et al.* (1991) reported that *in vitro* α-T3 possesses 40-60 times higher antioxidant activity against lipid peroxidation and 6.5 times better protection of cytochrome P450 against oxidative damage than α-T in rat liver microsomal membranes. Ikeda *et al.* (2003) reported that in some tissues in rats fed the equivalent dietary levels of α-T or α-T3, both isoforms provided equal protection against lipid peroxidation. The protective ability of tocotrienols from the tocotrienols-rich fraction (TRF) extracted from palm oil was reported to be significantly higher

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compared to α-T as effective inhibitors of protein oxidation and lipid peroxidation in rat liver microsomes (Kamat *et al*., 1997), with γ-T3 being the most effective.

The Fish Nutrition Laboratory at Universiti Sains Malaysia has successfully introduced palm oil as an alternative source of lipid and energy in aquaculture feeds (Ng, 2006; Bahurmiz and Ng, 2007; Ng *et al*., 2007). Crude palm oil (CPO) is also one of the richest sources of natural vitamin E (600-1000 mg kg^{-1}), namely a unique mixture of tocopherols (18%-22%) and tocotrienols (78%-82%). Therefore, we conducted a series of feeding trials to investigate the use of palm vitamin E as a novel source of antioxidants for farmed fish.

RESEARCHING THE USE OF PALM VITAMIN E

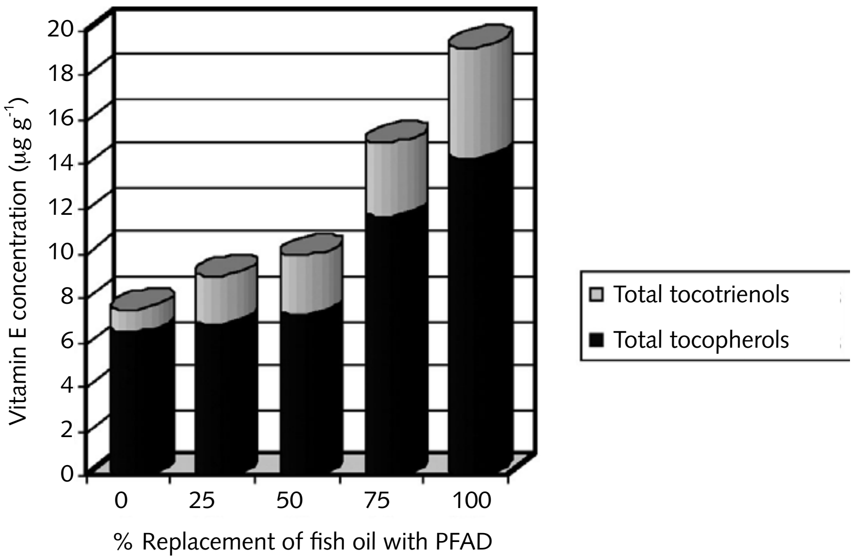
Tocotrienol Deposition in Fish Tissues

We first reported a linear increase in total vitamin E concentrations in the muscle of the African catfish fed practical diets with increasing levels of palm fatty acid distillate (PFAD) at the expense of fish oil (Ng *et al.*, 2004). As far as we know, these were the first reported data on the deposition of dietary palm tocotrienols in fish tissue. Muscle tocotrienol concentrations of the African catfish were observed to increase significantly concomitant with increasing dietary PFAD. However, when tocotrienol concentrations were expressed as a percentage of total vitamin E, it is interesting to note that despite an increasing percentage of tocotrienols in the diet (from 1.8% to 58.2%), tocotrienols constituted only 13.4% to 26.7% of the total vitamin E deposited in the catfish muscle (*Figure 1*). In catfish fed PFAD-supplemented diets, an equilibrium in T:T3 ratio of about 7.5:2.5 in the muscle was reached in eight weeks irrespective of dietary vitamin E composition. In the catfish muscle, 68.5% to 80.2% of the total vitamin E deposited was present as α-T.

When we fed red hybrid tilapia with diets supplemented with a TRF extracted from CPO, the concentrations of α-T, together with α- and γ-T3 were found to be deposited into the tilapia tissues, and their concentrations were observed to increase linearly in association with increasing levels of dietary TRF (Wang *et al*., 2006) (*Figure 2*). The predominant isoform (α-T) accumulated in all tissues and plasma. Results from this study indicated that palm tocotrienols supplementation in red hybrid tilapia diets could markedly enhance the tocotrienols concentration in the various tissues, but the deposition is very tissue-specific. Tocotrienols constituted equilibria of 46.7%-48.9%, 24.7%-33.1%, 21.6%-26.0%, 19.2%-22.2% and 8.0%-9.7% of the total vitamin E in adipose, liver, skin, muscle and plasma, respectively, of the tilapia fed with TRF-supplemented diets (E30 to E240), in spite of their high dietary compositions of about 80%. The adipose tissue of the tilapia had the largest capacity to take up palm tocotrienols, followed by the liver, skin, muscle and plasma.

Oxidative Stability of Fish Fillets

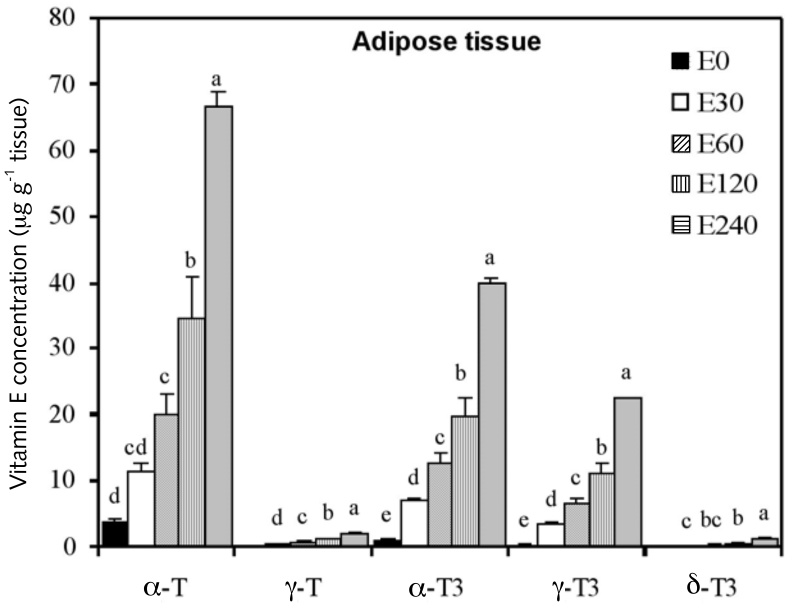
The role of elevated levels of dietary α-T in improving fish flesh quality by maintaining oxidative stability has been well recognized. All of these studies on the role of vitamin E in protecting membrane lipids from free radical attacks in fish tissues had relied on the application of synthetic *all*-*rac*-α-tocopheryl acetate as the sole dietary source of vitamin E. We were able to show that vitamin E concentrations in fish fillets increased in response to increasing dietary vitamin E originating from crude palm oil (Lim *et al*., 2001), PFAD (Ng *et al*.,



Source: Modified from Ng *et al.* (2004).

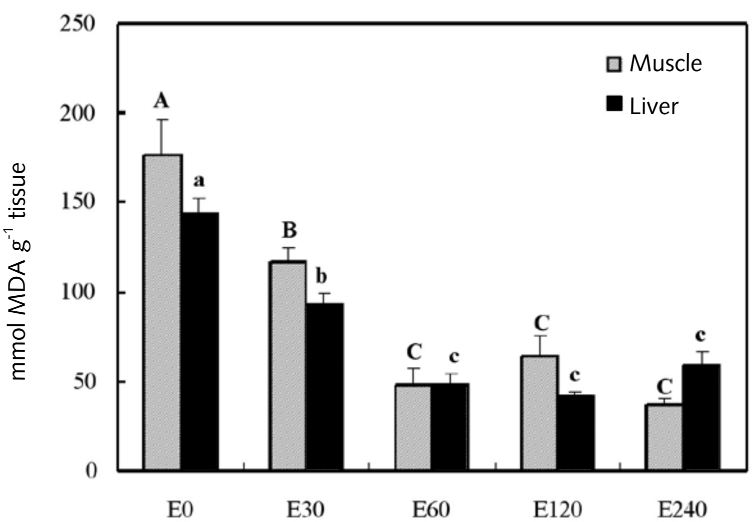
Figure 1. Deposition of palm tocopherols and tocotrienols in the muscle tissue (ug g^{-1}) of African catfish fed with palm fatty acid distillate (PFAD)-based diets.

2004) or palm TRF (Wang *et al.*, 2006), and we provided evidence to support the role of the accumulated palm vitamin E in enhancing the oxidative stability of fish fillets. Lipid peroxidation (measured as TBARS) in muscle, liver and plasma of red hybrid tilapia fed with low dietary TRF diets (E0 and E30) was significantly higher than those of fish fed high dietary TRF diets (E60 to E240) (*Figure 3*).



Source: Adapted from Wang *et al.* (2006).

Figure 2. Vitamin E concentrations in the adipose tissue of red hybrid tilapia fed a diet without added vitamin E (E0) and with diets supplemented with 30 to 240 (E30, E60, E120 or E240) mg kg^{-1} total vitamin E derived from a tocotrienols-rich fraction (TRF) extracted from crude palm oil (CPO).



Note: MDA = malondialdehyde.

Source: Adapted from Wang *et al.* (2006).

Figure 3. Effects of graded levels of total vitamin E (mg kg^{-1}) derived from palm tocotrienol-rich fraction (TRF) on lipid peroxidation in red hybrid tilapia fillet.

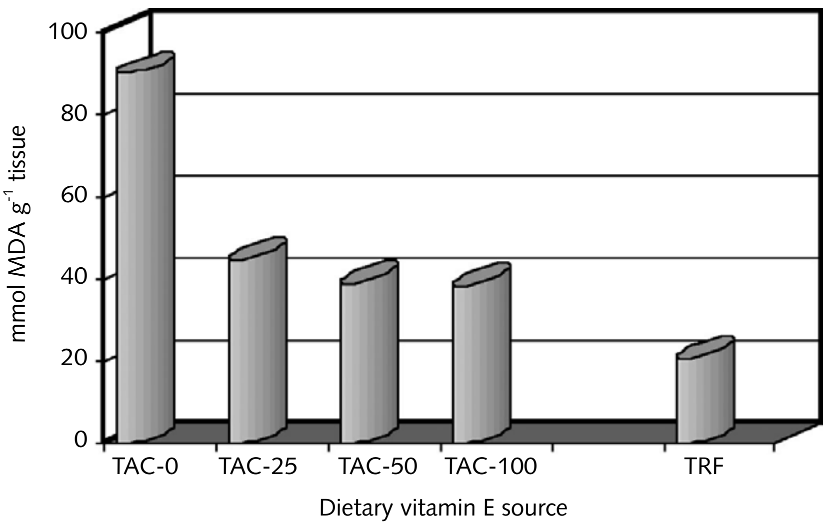
A Potent Antioxidant

A comparative study on the antioxidant potency of synthetic tocopheryl acetate compared to TRF was conducted in red hybrid tilapia. Our research showed that there was no significant decrease in lipid peroxidation products beyond 50 mg *all*-*rac*-α-tocopheryl acetate/kg diet, but the addition of dietary TRF at about 100 mg kg^{-1} diet caused a further decrease in lipid peroxidation as indicated by the concentrations of MDA in *Figure 4* (Ng, unpublished data). This showed that TRF extracted from CPO is a more potent antioxidant compared to the conventional synthetic vitamin E when used in tilapia feeds.

POTENTIAL APPLICATION OF RESEARCH RESULTS

Longer Shelf-life and Quality of Seafood Products

Elevated dietary levels of TRF resulted in marked increases in the deposition of vitamin E in fish tissues, improving oxidative stability which in turn can effectively prolong the storage duration or shelf-life of fresh and frozen fish fillets and surimi-based products. This will lead to increase profits for seafood processors. As a potent natural antioxidant, palm vitamin E may enhance the deposition of carotenoids in pigmented seafood such as salmon and shrimp enhancing flesh quality, consumer acceptance and marketability (*Figure 5*). The accumulated palm vitamin E in these seafood products would slow down the oxidation of these pigments, thereby maintaining colour for longer periods. The reddish colour in seafood products is often used as a



Source: Ng (unpublished data).

Figure 4. Effects of graded levels of all-rac-α-*tocopheryl acetate (TAC) at 0 to 100 mg kg*$^{-1}$ *diet on thiobarbituric acid-reactive substances from iron-vitamin C-induced lipid peroxidation in muscle of red hybrid tilapia compared to palm tocotrienol-rich fraction (TRF).*

Figure 5. The potential for improved shelf-life, fillet and nutritional quality in tocotrienol-enhanced seafood products.

quality parameter and may increase the market value.

Human Health Benefits

The deposition of tocotrienols (and other non-α-T isoforms) in red hybrid tilapia fillets also adds value to the product as the potential health benefits of tocotrienols in the human diet may include beneficial effects in preventing of cardiovascular diseases, cancer and stroke, among other degenerative diseases (Watkins *et al*., 1999).

Japanese restaurants are mushrooming in most large cities of the world and many urbanites are drawn to the nicely-packaged ready-to-eat foods such as sushi and sashimi sold in major supermarkets. The full health benefits of tocotrienols to the human consumer will be obtained when seafood products are consumed raw as sashimi. Seafood products are already known for their health benefits, and reputable organizations such as the American Heart Association strongly endorse the use of omega-3 fatty acids, found in fatty fish, for cardiovascular disease prevention. Combined with the health benefits of tocotrienols found in farmed fish fed with diets supplemented with palm TRF, the image of seafood as a healthy meat product will be further enhanced in the public's perception.

Fish Offal Oil

The perivisceral adipose tissue of the red hybrid tilapia was the major depot for vitamin E among the various tissues examined. At all dietary inclusion levels of TRF, total vitamin E concentrations found in the adipose tissue were the highest. Unlike other tissues, the adipose tissue of tilapia fed with palm TRF were rich in tocotrienols. Tocotrienols made up almost 50% of the total vitamin E deposited. This makes fish offal oil a very useful by-product from the processing factories of farmed fish, and can be marketed as a tocotrienol-enriched fish oil targeting the health foods sector.

Commercial Potential

Feed-grade TRF extracted and concentrated from CPO for use as a natural additive in aquaculture finishing feeds, especially for farmed fish with a high fat content, has great commercial potential (*Figure 6*). This innovation is a novel concept for delivering tocotrienols in a wider variety of consumer products. It is anticipated that such tocotrienol-enriched seafood products can be sold to niche markets, especially in developed countries and in large cities where health-conscious consumers are willing to pay a premium price for such products.

Figure 6. Numerous international awards won for the innovative concept of adding a tocotrienol-rich fraction from palm oil as an additive in aquafeeds formulated for farmed fish.

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