Studies on stability and distribution of polyunsaturated fatty acids in rat tissues

Studien zur Stabilität und zur Verteilung von polyungesättigten Fettsäuren in Rattengewebe

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II SUMMARY

In the wider field of nutrition, intensive efforts are under way to determine the effects of various nutrients on growth and development. The object of the present study is to demonstrate the effects of different dietary levels of DHA and/ or GLA from different oils in the presence of constant amount of LCPs n-3 (EPA) and AA as LCPs n-6 on the fatty acid patterns of brain, liver, plasma and their phospholipids during experiment at two stage (the first one after four weeks and the second after eight weeks). Rates with omega 6 diets were present various metabolic fatty acids pathway, liver and plasma fatty acids were significantly related after four weeks. Liver DHA was positively correlated with plasma DHA ($r = 0.81$, $P< 0.05$) and liver AA was positively correlated with plasma AA ($r = 0.86$, $P< 0.05$). Plasma and liver AA were not correlated with brain and kidney AA. However, DHA in liver and plasma DHA were significantly associated with DHA in brain ($r = 0.81$ and 0.92, $P< 0.05$), respectively. Brain DHA had not related with the increase with DHA in diets. In all groups, there was trend for α-tocopherol to decrease with time, especially between 4 week and 8 weeks of feeding with significance difference between the low and high level of ω-3 as well as low ω-6 and high concentration. The extracted liver and brain oils from low ω-3 as or ω-6 fed rats had the strongest radical scavenging activity compared to high levels with positive correlation between RSA and the levels of tocopherols in detected oils. The storage test existed correlations between peroxide value (PV) and conjugated dienes (CD) ($P< 0.05$) as well as 2-thiobarbituric acid-reactive substances (TBARS) and headspace volatiles (hexanal and propanal) content ($P< 0.001$) for most oils and extracted oils. Besides, it was found that a negative correlation was demonstrated between the TBRAS formation and the total phenolic contents. We can conclude that, high levels of DHA not contribute to increase of DHA in brain, liver or plasma beside reduction of plasma AA by adding EPA+ DHA to the diet with different amounts of GLA may be important to use for infant fed formula with a family history of inflammatory condition such as rheumatoid arthritis or AD diseases.

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VI ABBERVIATIONS USED

AA	Arachidonic acid
ADHD	Attention hyperactivity disorder
AI	Acceptable Intake
AMDR	Acceptable Macronutrient Distribution Range
AV	*p*-anisidine Value
CC	Column Chromatography
CD	Conjugated Diene
CHD	Coronary heart disease
COX	Cyclogenase
DHA	Docosahexanoic acid
DGLA	Dihomo-gamma-linolenic
DPPH	1, 1-diphenyl-2-picrylhydrazyl
EPA	Eicosapentaenoic acid
FAME	Fatty acids methyl esters
FAO	Food and Agriculture Organization
FID	Flame ionization detector
GC	Gas Chromatography
GLA	Gamma linolenic acid
GL	Glycolipids
HS-GC	Head space gas chromatography
IQ	Intelligence quotient tests
α-LA	Alpha-linolenic acid
LA	Linoleic acid
LCPs	Long-chain polyunsaturated fatty acids
LOX	Lipoxygenase
MAD	Malondialdehyde
MUSFA	Monounsaturated fatty acids

NL	Neutral lipids
NP-HPLC	Normal phase-high performance liquid chromatography
PL	Phospholipids
PUFAs	Polyunsaturated fatty acids.
PV	Peroxide value
RSA	Radical scavenging activity
TBARS	Thiobarbituric acid reactive substances
TEP	1, 1, 3, 3-tetraethoxypropane
TMSH	N-trimethylsulfoniumhydroxide
TPCs	Total phenolic compounds
ω-3	Omega 3
ω-6	Omega 6
WHO	World Health Organization

Studien zur Stabilität und zur Verteilung von polyungesättigten Fettsäuren in Rattengewebe

Einleitung

Muttermilch ist in ihrer Zusammensetzung ideal auf die biologischen Bedürfnisse des Babys abgestimmt. Das Fett der Muttermilch und der Nahrung sind der wichtigste Energieträger für den Säugling. Es liefert ca. 40 - 55 % der zugeführten Energie. Die wichtigsten Bestandteile der Nahrungslipide (Triglyceride, Phospholipide und Cholesterinester), nämlich die langkettigen polyungesättigten Fettsäuren (LCPs) sind essenziell für das normale Wachstum und die Entwicklung des Säuglings (3, 4). Die beiden Gruppen der LCPs, die omega-3- (ω-3) und omega-6-Fettsäuren (ω-6) haben besonders wichtige Funktionen: Docosahexaensäure (DHA; C22:6n-3) in Netzhaut und Gehirn, und Arachidonsäure (AA; C20:4n-6) als Vorläufer der Eicosanoide, die bei einer ganzen Reihe zellulärer Prozesse wichtig sind. Muttermilch wird als beste Quelle für essentielle Fettsäuren betrachtet; neben anderen Fettsäuren enthält sie die essentielle Linolsäure (LA; C18:2n-6) und alpha-Linolensäure (α-LA; C18:3n-3), und die LCPs: Arachidonsäure (AA) und Docosahexaensäure (DHA) (6-8).

Die Untersuchungen zeigen, dass das Neugeborene im Stande ist AA aus LA und DHA aus alpha-LA zu synthetisieren. Jedoch ist der Betrag der LCPs, besonders der an DHA, der erzeugt werden kann, infolge einer verminderten Desaturasekapazität nicht ausreichend um den Entwicklungsanforderungen das Säuglings gerecht zu werden, was eine exogene Versorgung mit LCPs während der ersten Lebensmonate erfordert (6, 10). Der Bedarf von Neugeborenen an LCPs ist ein Forschungsschwerpunkt im letzten Jahrzehnt gewesen. Ein Grund ist die Beobachtung, dass bei zu früh Geborenen die

Blutplasmawerte an LCPs nach der Geburt in mit Frühchenmilch gefütterten Säuglingen deutlich abnehmen verglichen mit fast unveränderlichen Niveaus in mit Muttermilch genährten Frühchen (12).

Diese Studie wurde mit Fütterungsversuchen an Ratten durchgeführt (Tabelle 1). Es wurden zwei Diätgruppen gebildet: eine omega-3-Gruppe mit DHA (1, 2, 3%) und eine omega-6-Gruppe mit GLA (1, 3, 5%). Ziel der Studie war es (a) die Wirkung der zunehmenden DHA-Menge auf die Fettsäurespektren in Gehirn, Leber und Blutplasma (Gesamt- und Phospholipide) innerhalb der ersten acht Wochen zu studieren; und (b) die Wirkung von diätetischer GLA in Gegenwart vom omega-3-Fettsäuren auf die Fettsäuremustern (Eicosanoid-Vorgänger) zu bewerten. Der Tocopherolgehalt (c) und die antioxidative Wirksamkeit (DPPH, d) *in vivo* wurde bestimmt. Ausgehend von den Ergebnissen beider Gruppen wurden drei verschiedene (Verhältnis %DHA zu %GLA; 1:1, 1:3, 3:0) pulverförmige Babynahrungspräparate hergestellt (Öl-Emulsion/AIG93 Diät-Trocknung-Lagerung) und deren Lagerfähigkeit bei 60℃ überprü ft.

Zusammenfassung

Die Gehalte von DHA (omega-3-Gruppe) in Plasma und Gehirn korrelieren nach vier Entwicklungswochen miteinander ($r = 0.82$, $P < 0.05$), gleiches gilt für AA ($r = 0.89$, $P < 0.05$). Nach acht Wochen ergeben sich im Gehirn vergleichbare DHA-Werte, unabhängig von der Fütterungsmenge (1-3%). In den omega-6-Fütterungsversuchen korrelieren Leber-DHA und Plasma-DHA ($r = 0.81$, $P < 0.05$), als auch Leber- und Plasma-AA ($r = 0.86$, $P < 0.05$). Leber- und Plasma-DHA korrelieren nach vier Wochen auch mit Gehirn ($r = 0.81$, $P < 0.05$) ,dies gilt aber nicht für AA. Als Grund wird die Blockierung der ∆5-Desaturase durch die Konkurrenzreaktion EPA zu DHA angenommen (Figure 1). Zusammenfassend lässt sich sagen, dass die

Verhältnisse zwischen Nahrungsfetten und Körperfetten über den Fettstoffwechsel sehr komplex sind. Die über die Fütterung verursachte Verschiebung der Fettsäurezusammensetzung ist in der Leber am größten. Hier findet der Stoffwechsel statt und über das Blut gelangen die Fettsäuren ins Gehirn. Von dem DHA-Gehalt im Blutplasma kann man direkt auf den Gehalt in den Geweben schließen. Die zur Entwicklung des Gehirns, welche bei Ratten nach 28 Tagen beim Menschen nach dem 2. Lebensjahr abgeschlossen ist, notwendige DHA wird bereits mit 1% im Nahrungsfett ausreichend zugeführt. Die Verabreichung größerer Mengen führt zu keinem weiteren Anstieg im Gehirn.

Im Gegensatz zu anderen Studien konnte gezeigt werden, dass die Gabe von GLA, AA, EPA und DHA zu einer Senkung der Plasma-AA und einer Steigerung der Gehirn-DHA führt. Diese Beobachtung könnte eine neue Therapiemöglichkeit für Menschen eröffnen, die an Beschwerden leiden, welche aus dem Arachidonsäure-Stoffwechsel (Eicosanoide) herrühren, z.B. Neurodermitis und Multiple Sklerose.

Die untersuchten Proben enthalten α-Tocopherol und Spuren von γ-Tocopherol. Innerhalb der ersten acht Entwicklungswochen nimmt die Tocopherolgehalt stark ab, der Effekt wird durch steigende LCPs-Verabreichung, sowohl in der omega-3- als auch in der omega-6- Gruppe noch verstärkt (Tabelle 13). Dieses Ergebnis entspricht dem anderer Studien an Ratten bzw. Mäusen (99, 100) die zeigten, dass höhere Gehalte an omega-3-FA kleinere Tocopherolgehalte verursachen. Zwischen der gemessenen antioxidativen Wirksamkeit (RSA) und der Tocopherolkonzentration besteht ein direkter Zusammenhang.

Die Lagerstabilität der drei pulverförmigen Babynahrungspräparate beträgt bei 60 ℃ unabhängig von der Formulierungsf orm (Öl/Pulver) etwa

sechs Tage, dies entspricht etwa drei Monaten bei 20 ℃. Die unterschiedlichen Verhältnisse von DHA zu GLA haben keinen signifikanten Einfluss. PV, AV und CD korrelieren miteinander, ebenso TBARS und flüchtige Verbindungen. Die Aldehyde des Fettverderbs korrespondieren mit den anderen Verderbsparametern und steigen nach etwa 4-6 Tagen an. Der Verderb ist gleichermaßen über Propanal, Pentanal, Hexanal und Nonanal verfolgbar. Der Zusatz von 150ppm Tocopherol verzögert die Entstehung der Verderbsaldehyde signifikant (Tabelle 15) und ermöglicht eine bessere Lagerstabilität der Formulierungen.

1 INTRODUCTION

Dietary lipids are the major energy source for infants and young children. Lipid tissue accretion in growing infants is very high and consists of 90% of all energy deposited in the body during the first six months of infant's life (1, 2). The most important functional components of dietary lipids (triglycerides, phospholipids and cholesterol esters), practically long-chain polyunsaturated fatty acids (LCPs) are essential to normal growth and the infant development (3, 4). The two families of LCPs, the omega-3 (ω-3) and the omega-6 (ω-6) have specific functions: docosahexanoic acid (DHA; C22:6*n*-3) in retina and brain, whereas arachidonic acid (AA; C20:4*n*-6) is known to be a precursor of eicosanoids that are important for a number of cellular processes.

AA and DHA are deposited in large amounts in the nonmyelin membranes of the developing central nervous system. Adequate supplies of ω-6 and ω-3 fatty acids during nervous system development are of concern because of possible long-term changes in learning ability and reduce visual function (5). Human milk is considered to be the best source of fat and dietary essential fatty acids for infant feeding; as it provides a complex mixture of fatty acids, including the essential polyunsaturated linoleic acid (LA, C18:2*n*-6) and Alpha-linolenic acid (α-LA, C18:3*n*-3) and LCPs (AA, DHA)(6-8).

Current evidence suggests that the newborn is able to synthesize AA and DHA from LA and α-LA, respectively (9). However, the amount of LCPs being produced, particularly of DHA, may not be sufficient to meet the developmental requirements of the infant, because they have a limited desaturation capacity and depends on an exogenous supply of LCPs during the first months of life (6, 10). Moreover, a dietary lack of essential

fatty acids and their derivatives is evident also in weaned children during the second half of their first year of life (11).

Newborn requirements of the LCPs have been a major focus of research for the past decade. The reason for this intense interest was based on several observations such as: (a) plasma levels of LCPs decrease markedly after birth in formula-fed preterm infants as compared to almost constant levels in breast-fed infants of comparable gestational age; (b) the level of LCPs in human milk is constant as a function of length of lactation or geographical distribution (i.e., nutrition) and therefore to what extend can human milk be the gold standard (12); and (c) studies that evaluate the effect of formula supplementation with LCPs provide only partial answers as to benefit of such supplementation to full-term and pre-term infants (13). One important question of relevance to infant nutrition is whether there are critical neurodevelopmental stages in which DHA is required for optimal development (14). However, docosahexanoic acid and arachidonic acid are commonly added to infant formula worldwide, but dietary concentrations needed to obtain optimal tissue levels have not been established.

On the light of the fore going observations the objectives of this study were; (a) to study the effect of feeding various levels of DHA were (0, 2, 4, and 6 times those used in infant formulas) on rats growth ; brain, liver and plasma fatty acids and phospholipids, (b) to evaluate the effect of dietary gamma-linolenic acid (GLA, C18:3*n*-6) as an intermediate of AA synthesis at three levels in the presence of DHA, EPA plus AA (eicosanoid precursors) on the fatty acid patterns of tissues, plasma and their phospholipids of rats tissues, (c) to report the relationship between mixed oils and their antioxidant properties *in vivo,* (d) to report the effect of mixed omega 3 and omega 6 feed oils on their radical scavenging activity of brain and liver, (e) to assess oxidative stability of both of oils and

formulas by monitoring oxidative products and (f) to examine the effect of storage on the evolution as volatile secondary products of the oils and fractions.

2 REVIEW OF LITERATURE

2.1 Essential fatty acids and functions

The 'essential' fatty acids were given their name when researchers found that they were essential to normal growth in young children and animals, whereas mammalian cells lack the Δ -12 and Δ-15 desaturase enzymes (found in most plants) for insertion of a double bond at the ω-6 or ω-3 position, but able to synthesize (from non-fat precursors) saturated fatty acids and unsaturated fatty acids of the ω-9 and ω-7 series (9, 15). Thus, mammalian cells cannot synthesize ω-6 or ω-3 PUFAs *de novo* and must obtain them from the diet. Omega 3 polyunsaturated fatty acids are α-LA, EPA and DHA have 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms, respectively. All double bonds are in the *cis*-configuration. Linoleic acid and arachidonic acid are essential fatty acids (5, 16). Omega 3 and omega 6 essential fatty acids are needed for the membranes of all body cells as their role in health is wide reaching: encompassing not only healthy heart and brain good function, but also playing an important role in the normal function of the eyes, the nervous system, the kidney, and the liver, in fact all body systems. Other functions also include the contraction of muscles, blood clotting, and inflammatory processes (6-8, 17).

2.2 Essential omega 3 and omega 6 fatty acids requirements

As macronutrients, fats are not assigned recommended daily allowances, it have AI (Acceptable Intake) and AMDR (Acceptable Macronutrient Distribution Range) instead of RDAs. The AI for ω-3 is 1.6 grams/day for men and 1.1 grams/day for women, while the AMDR is 0.6% to 1.2% of total energy. The National Institutes of Health recently published recommended daily intakes of fatty acids (18); specific recommendations

include 650 mg of EPA and DHA, (because both have much greater physiological potency than α-LA for protection against coronary heart disease), 2.22 g/day of α-LA and 4.44 g/day of LA. This means that the ratio of ω-6 to ω-3 is 1.5:1.

2.2.1 Pregnancy and lactation

An adequate intake of DHA and EPA is particularly important during pregnancy and lactation periods. During this time the mother must supply all the baby's requirements of DHA and EPA because babies are unable to synthesize these essential fatty acids themselves. DHA makes up 15 to 20% of the cerebral cortex and 30 to 60% of the retina, so it is absolutely necessary for normal development of the fetus and baby as well (19, 20). During pregnancy, the fetus completely depends on maternal sources of DHA from lipid stores, maternal diet and nutritional supplements. During fetal life, placenta selectively and substantially transports AA and DHA from the mother to the fetus. During the third trimester of pregnancy, there is an avid accretion of DHA in the liver, brain and retina of the fetus at a rate of 4.13 g of EFA per week i.e. 0.59 g/day (18, 21). Pregnancy leads to a progressive depletion of maternal plasma DHA, presumably due to the increased supply of this critical nutrient to the developing fetal nervous system (22, 23). According to WHO and FAO the pregnant woman should take at least 2.6 g of ω-3 and 100-300 mg of DHA daily to meet the requirements of her fetus (24). In countries where pregnant women consume large amounts of fish, it seems to be having beneficial effects for the mother: a slightly longer pregnancy (by 1-3 days), slightly larger birth weight and also a possible decrease in the risk of pre-maturity (25-27). On the other hand, there is some evidence that an insufficient intake of ω-3 fatty acids may increase the risk of premature birth and an abnormally low

birth weight. Also, there is emerging evidence that low levels of ω-3 are associated with hyperactivity in children (28-34).

2.2.2 Infancy and Childhood

Once the baby is born, the mother needs a good supply of essential omega 3 fatty acids; through eating more oil-rich fish to increase the amount of DHA in the breast-milk infants, in order to improve the available amounts available for brain, eyes and the nervous system development. It is known that, infant and children are generally thought to require 1-2% of total dietary energy as LA to prevent EFA deficiency, but pre-term infants require at least 10% of this energy (18, 24, and 25). Babies who are born pre-maturely and miss out on the umbilical DHA supply during the last stage of development are born with low stores of DHA in the brain and liver. This can lead to visual impairment and abnormal retina function. Thus, pre-mature babies are given extra fatty acids in their feeds to avoid this miss function of retina. As these fatty acids are needed for eyesight and brain function, there is an evidence, which shows that a lack of DHA in the pre-term infant can lead to neurological deficits, such as learning disabilities, behavioral problems and perhaps lower scores on IQ (intelligence quotient) tests (35, 36). The weaning foods and diets of preschool children should contain DHA because metabolic conversion of α-LA to DHA is limited to less than 0.2% in children. There is evidence to suggest that, 40% of children with attention hyperactivity disorder (ADHD) have significantly low levels of plasma ω-3 and DHA (37). Forethere, it is recommended that weaning foods should be rich in DHA (fish oil, sea food and dry fruits) or supplemented with nutritional supplements containing DHA.

2.3 Essential omega 3 and omega 6 metabolism

2.3.1 Conversion of linoleic acid and linolenic acids up to eicosanoid

The commonly consumed polyunsaturated fatty acids are LA and α-LA. Once consumed, these fatty acids can be converted to the longer-chain, more unsaturated derivatives. Thus, linoleic acid is converted via GLA and dihomo-gamma-linolenic (DGLA; 20:3n-6) acids to AA. Likewise, α-linolenic acid is converted to eicosapentaenoic acid (EPA; 20:5n-3) (Figure 1). There is some controversy about the extent to which DHA can be synthesized from EPA in humans (15-17, 38). There are two steps in which dietary fatty acids can modulate eicosanoids biosynthesis from AA; the first step is a desaturation and elongation and the second step in which dietary fatty acids can modulate the biosynthesis of eicosanoids (prostaglandins, leucotrienes and thromboxanes by cyclooxygenase and lipoxygenase) is at the formations of endoperoxide intermediate (Figure 1). EPA as ω-3 can be metabolized by both cyclooxygenase and 5-lipoxygenase similarly to AA, leading to the formation of trienes prostaglandins and the leukotriene 5 series. So, it acts as a competitive inhibitor. Thus, increasing ω-3 in diets may reduce AA levels in tissue lipids and may decrease the formation of eicosanoid derived from AA through inhibiting the oxygenation of AA by cyclooxygenase. (15-17, 38-39).

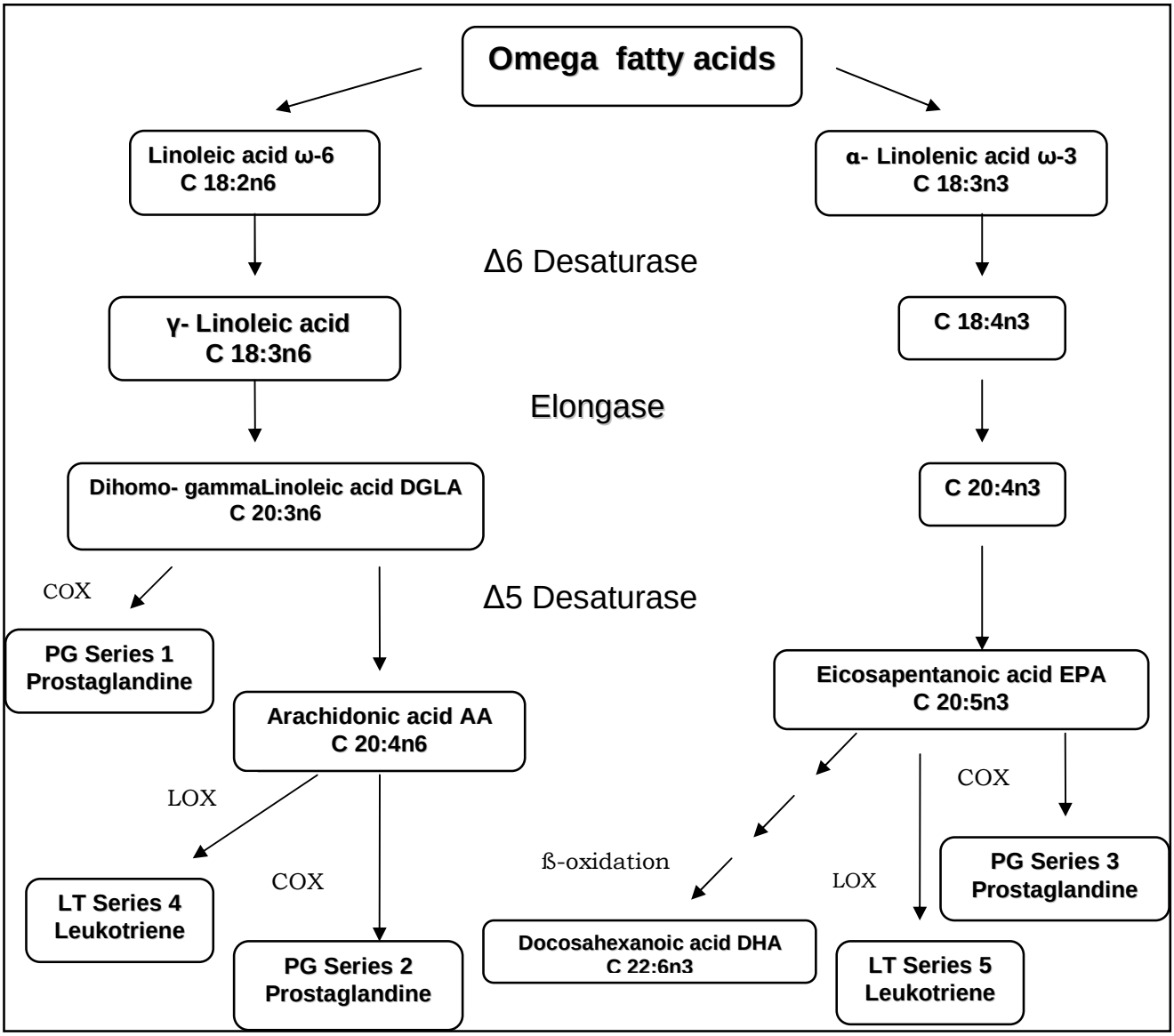


Figure 1: Metabolic pathway for conversion of the dietary essential fatty acids to eicosanoids via the cyclooxygenase (COX) and Lipoxygenase (LOX) pathways.

2.3.2 Competition between unsaturated fatty acids families

The actions of the essential omega 3 and omega 6 fatty acids are best characterized by their interactions; they cannot be understood separately. In the body, LA competes with α-LA, for Δ6-desaturase, and thereby eventually inhibits formation of anti-inflammatory EPA (15, 16). In contrast, GLA derives from LA and does not compete for Δ6-desaturase. GLA's

elongation product DGLA competes with 20:4n-3 for the Δ5-desaturase. Arachidonic acid was derived from DGLA pathway. It sits at the head of the "arachidonic acid cascade" more than twenty different signaling pathways that control a bewildering array of bodily functions, but especially those functions involving inflammation and the central nervous system (15, 40). DGLA and EPA compete with AA for accessing to the cyclooxygenase and lipoxygenase enzymes. So the presence of DGLA and EPA in tissues lowers the output of AA's eicosanoids. For example, dietary GLA increases tissue DGLA and lowers thromboxanes B_2 (16, 17, 41, and 42) .Likewise, EPA inhibits the production of prostaglandins series-2 and thromboxanes (40, 43).

This competition was recognized as important when it was found that leukotrienes were similarly found to be important in immune inflammatory system response, and therefore relevant to some diseases like arthritis and asthma. These discoveries led to greater interest in finding ways to control the synthesis of omega-6 eicosanoids. The simplest way would be by consuming more ω-3 and fewer ω-6 fatty acids (17, 44).

2.3.3 Omega 3, omega 6 and eicosanoids

Eicosanoids are a family of very potent biological signaling molecules that act as short-range messengers, affecting tissues near the cells that produce them. In response to hormonal or phospholipase A2, which are present in most types of mammalian cells, attacks membrane phospholipids, releasing arachidonate from the middle carbon of glycerol. Then arachidonate converted to prostaglandins, beginning with the formation of prostaglandin H2 (PGH2), the immediate precursor of many other prostaglandins and of thromboxanes (Figure. 1). The two reactions that lead to PGH2 are catalyzed by a bifunctional enzyme, cyclooxygenase (COX), also called prostaglandin H2 synthesis. In the first of the two steps,

the cyclooxygenase activity introduces molecular oxygen to convert arachidonate to PGG2. The second step, catalyzed by the peroxidase activity of COX, converts PGG2 to PGH2 (Figure. 1). Animal studies showed that increased dietary ω-3 resulted in decreased AA in brain and other tissues (15). Linolenic acid contributes to this by displacing LA from the elongase and desaturase enzymes that produce AA (17). EPA inhibits phospholipase A2's which releases AA from cell membrane. Other mechanisms involving the transport of essential fatty acids may also play a role (44, 45). Thus, eicosanoids are involved in reproductive function; in the inflammation, fever, and pain associated with injury or disease; in the formation of blood clots and the regulation of blood pressure; in gastric acid secretion; and in a variety of other processes important in human health or diseases (46-49).

2.3.4 Relationship between polyunsaturated fatty acids, lipid oxidation and antioxidant.

Vitamin E as an antioxidant prevents rancidity of fats in plant sources and in the animal's digestive tract, through inhibiting the oxidation of PUFAs in tissue membranes, especially at the cellular level in the membranes surround the cells, the sub-cellular particles, and the ercythrocytes (50, 51). It also stabilizes the lipid parts of cells and protects them from damage from toxic free radical formed from the oxidation of PUFAs as well as being an end-product of the oxidation of α-tocopherol, as the α-tocopherol quinone is the cofactor for the mitochondrial fatty acid desaturation/elongation pathway (51-53). It has been suggested that the severe neurological degeneration in patients with a genetic lack of *α*-tocopherol transfer protein or abetalipoproteinemia is caused by failure of synthesis of long-chain polyunsaturated fatty acids (50, 51).

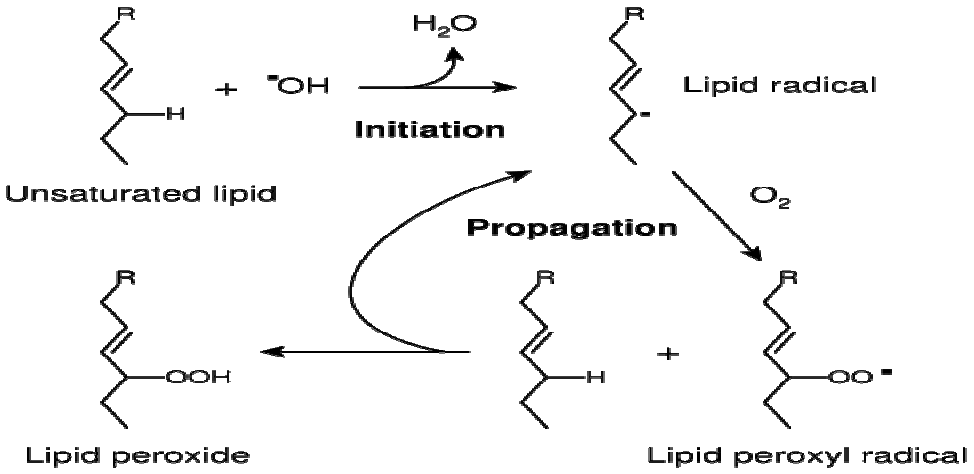


Figure 2a: Radical chain reaction mechanism of lipid peroxidation.

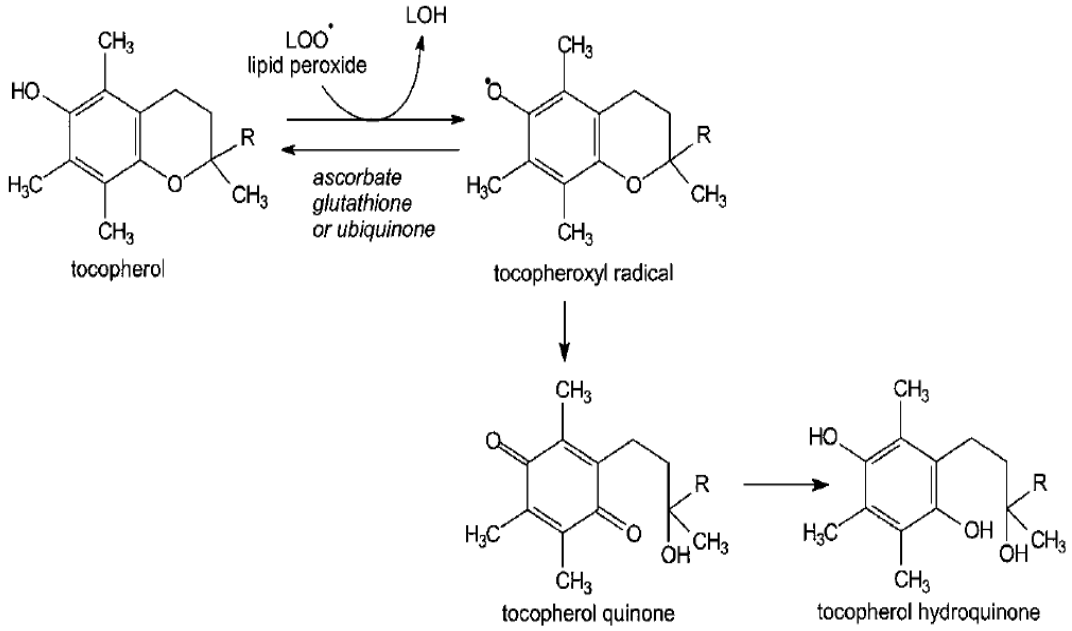


Figure 2b: Reaction of tocopherol with lipid peroxides; the tocopheroxyl radical can be reduced to tocopherol or undergo irreversible onward oxidation to tocopherol quinone.

So, the primary function of vitamin E is to help protect the integrity of cellular and intra-cellular structures. There is much controversy as to how this function is carried out catalytically as a chain-breaking lipophilic antioxidant in membranes and plasma lipoproteins? Because the tocopheroxyl radical formed by reaction of *α*-tocopherol with the lipid peroxide radical (Figure. 2) can be reduced to tocopherol in three main ways by reaction with: (a) Ascorbate to yield the monodehydroascorbate radical, this in turn can either be reduced to ascorbate or can undergo dismutation to yield dehydroascorbate and ascorbate. There is an integral membrane oxidoreductase that uses ascorbate as the preferred electron donor, linked either directly to reduction of tocopheroxyl radical or via an electron transport chain linked to the oxidation of NADH and succinate involving ubiquinone in mitochondria (51, 54). (b) Other lipid-soluble antioxidants in the membrane or lipoprotein, including ubiquinone, which is present in large amounts in all membranes as part of an electron transport chain, not just the mitochondrial inner membrane (53, 54, and 56-58). (c) Glutathione is catalyzed by glutathione peroxidase, which is a specific isoenzyme (selenoenzyme). Furthermore, selenium has a direct role in the recycling of tocopherol (51, 55).

It is noteworthy that when fat is added to the diet it will destroy the vitamin E in both of diet and digestive tract if rancidity occurs. For this reason, the quantitative relationship between vitamin E and the amount and kind of dietary fat is practical importance. The higher consumption of PUFA fat related with higher vitamin requirement (50, 51, 54, and 58).

3 MATERIALS AND METHODS

3.1 Materials

Oils were obtained from Henry Lamote GmbH, (Bremen, Germany). AIN-93G was obtained from SSNIFF Spezialdiäten GmbH, Soest, Germany. Vitamin E standard (α-, δ-, β-, γ-tocopherol) and Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). DPPH; 1, 1-diphenyl-2-picrylhydrazyl (approximately 90%) was obtained from Sigma (St. Louis, MO, Germany). *p*-Anisidine (4-Amino-anisol; 4-Methoxy-anilin) and caffeic acid were from Fluka (Buchs, Swityerland). TMSH; N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany). TEP; 1, 1, 3, 3-tetraethoxypropane from Sigma (approximately 97%, Steinheim, Germany), 2-thiobarbituric acid (TBA)**,** trichloroacetic acid (TCA) and butylated hydroxyltoluene (BHT) were from Sigma (St. Louis, MO, Germany). Propanal, pentanal, hexanal, octanal and neonanal were purchased from Fluka (Steinheim, Germany). Toluene of HPLC grade was used throughout the experiment. All other chemicals and solvents were from the highest commercial grade and were used without further purification.

3.2 Analytical Methods

3.2.1 Animals and experimental design

Long-Evans offspring pregnant rats were acquired at 10-12 day gestation from the commercial supplier of Animal House. Only males were used in this study to minimize possible effects of hormonal factors. Animals were housed and cared there, and then kept in individual cages in a thermo-neutral environment at room temperature (26 ℃) and 60% relative humidity with a cycle of 12 h light and 12 h dark. Each cage was equipped with a water bottle with metal lid, a cup for administration attached to a

stainless steel plate to avoid over throwing and spillage. Animals were placed on a semi-synthetic diet, which was based on the AIN-93G (59). In the first Phase, the newborn pups were selected on a random basis for each experiment, with mean body weight with no significantly differences. Each animal within a particular group was from a different litter and then this litter was used as a basis for the number of animals. Rat pups were dam-reared until 8 day of age. Dams were fed as described previously (60). The artificially hand rearing system was used for pup feeding until day 17 when rats were hand fed every 3-4 h. On the day 17-28 (after the opening eye), artificial rat milk was fed in a 50 ml water bottle. In the second Phase (from 4 weeks to 8 weeks), rats were fed a diet containing the similar fatty compositions (**Table 1,** p 71).

3.2.2 Diets and preparations

The diets used were modified from AIN-93 G rodent diet (60). The dietary oils were a mixture of hydrogenated coconut oil, flaxseed oil, rapeseed oil, borage oil and marine oil. The oils were obtained from Henry Lamote GmbH, (Bremen, Germany). Coriander oil was cold extracted by n-hexane. AIN-93G was obtained from SSNIFF Spezialdiäten GmbH (Soest, Germany). All formulas were prepared to provide about 15 % LA, 3 % α-LA, 0.58% AA and 0.89% EPA of dietary fat in omega-3 and omega-6 groups, with the exception of deficient group (control only contained 15 % LA and 3 % α-LA of dietary fat). The omega-3 group contained three levels of docosahexanoic acid (DHA): 1%, 2% and 3 % DHA, while the omega-6 group contained 1%, 3% and 5% gamma-linolenic acid (GLA). Compositions of dietary fat used oils in the experiment were determinate by gas chromatography analysis.

3.2.3 Lipid extraction and separation of phospholipids from the tissues and plasma

At four time points, rats were killed and the liver and brain were removed from the body and collected, washed three times in ice cold saline, blotted dry and weighed. Lipids were extracted according to Folch *et al. 1957* (61). The organs were homogenized with twenty fold volume of dichloromethane: methanol (2:1, v/v) containing 10 mg of buthylated hydroxytoulene (BHT)/L as an antioxidant, which was then flushed with nitrogen and left to be extracted overnight at 4 ℃. The homogenate was filtered and washed with additional 10 ml of solution mixed with 20 ml of 0.85% sodium chloride (v/v), shake and left to partition overnight at 4℃. The lower organic phase was transferred and evaporated. Extracts were combined and dried under a steam of nitrogen. Blood was collected, transferred to a plastic tube containing EDTA, centrifuged at 3000 rpm at 4℃. An aliquot of the upper-phase (plasma) was transferred to another tube. For lipid extraction from plasma 500 µL plasma was mixed with ethanol containing 0.005% BHT (500 µL) and extracted with hexane (2 ml) after manually shaking for 3 min (62). Phospholipids were obtained for application on silica gel plates (thickness = 0.25 mm; Merk, Darmstadt, Germany) activated at 120℃ for two hours immediately before use. Plates were developed with chloroform: methanol: acetic acid: water (25: 15: 4: 2, by volume) (63), after visualization with 2, 7-dichlorofluorescein, phospholipids band was isolated and transmethylated.

3.2.4 Lipids transmethylation and gas chromatography

A portion of total lipid extracts from each tissue was transmethylated using methanol (1% H_2SO_4 in methanol) for three hour at 70℃ (64). After cooling the resulting fatty acid methyl esters (FAME) were extracted with *n*-hexane, dried with anhydrous sodium sulfate and then concentrated to a

small volume with a steam of nitrogen and transferred to micro vials for gas chromatographic (GC) injection, whereas phospholipids were transmethylated, phospholipids band was isolated and transesterified into their methyl esters (FAME) using N-trimethylsulfoniumhydroxide (TMSH), methyl esters were extracted twice into one ml hexane and the combined extracts were dried with steam nitrogen.

The fatty acid methyl esters were then identified and quantified on a Shimadzu GC-14A equipped with flame ionization detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan).The samples were separated on a 30m SP™ -2380 capillary column (Supelco, Bellefonte, PA, USA: 0.25 mm diameter, 0.2 µm film thickness) using helium at a flow rate of 0.6 ml/ min with a spilt ratio of 1:40. The chromatographic run parameters included an oven starting temperature of 100℃ that was increased by the rate of 5℃/min to 175 ℃ and then were held for 10 min before increasing to 220℃ at 8℃/min, with a final hold of 10 min .T he injector and detector temperatures were both constant at 250℃. Peaks wer e identified by comparison of retention times with external standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC) on the same conditions.

3.2.5 Determination of tocopherols by NP-HPLC

3.2.5.1 Apparatus and chromatographic conditions

Separations were accomplished on a NP-HPLC with Lichrosorb Si 60, 5 µm (250 *4 mm, i.d.) analytical column from Knauer (Berlin, Germany). Isooctane: acetyl acetate 96:4 (v/v) were used as a mobile phase and delivered at a constant flow rate of 1.0 ml/ min with column back-pressure of about 65–70 bar. The used solvent delivery module was LC-9A from Shimadzu HPLC (Kyoto, Japan), while the chromatographic system included a Model 87.00 variable wavelength monitor detector from Knauer, Berlin, Germany. The solvent system selected, retention time, and

UV detection of eluting components are shown in **Table 2a**. The (20 µL) diluted oils solution (20-30 mg in 1ml) of the selected mobile phase) was directly injected onto the HPLC column. Tocopherols were identified by comparing their retention times with those of authentic standards.

3.2.5.2 Standard curves preparation

Standard solution of vitamin E (50 mg/L), were prepared in n-hexane. The standard solutions were stable at least one month at 4º C in an argon atmosphere. Fresh working standard solution was prepared daily by approximate dilutions of standards in mobile phase solution. Twenty µL were injected four times into the HPLC system and the peak areas were determined to generate standard curve data.

Table 2a: Linearity in studied vitamin E by NP-HPLC at 295 nm.

Compound	RT (min)*	R^{2**}	Equation curve***
α-Tocopherol	9	0.9989	y = 10436 x - 141.48
ß-Tocopherol	13	0.9996	y = 11396 x - 5152.4
γ-Tocopherol	15	0.9967	y = 12124 x - 118.88
Δ-Tocopherol	20	0.9935	y = 12224 x - 3532.6

* Rt, retention time
** Determination coefficient
*** X, concentration (µg/ml); y, peak area

Slope of standard curves (six concentrations levels) were obtained by linear regression. Shimadzu C-R6A chromatopac integrator was used for all quantization based on peak areas.

3.2.6 Radical Scavenging Activity of total lipids from brain and liver against DPPH radicals

The reduction of toluenic solution of DPPH was examined radically in the presence of hydrogen donors in total lipids from brain and liver. Briefly, freshly made DPPH radical at a concentration of 0.0001 M in toluene was added into toluene solutions of total lipids from brain and liver to start the reaction. For determination, ten mg (in 100 µL toluene) of total lipids from brain or liver were mixed with 390 µL toluenic solution of DPPH radicals and the mixture was vortexed for twenty seconds at ambient temperature. The possible dilution in the cuvette was taken into account wherein the final volume in all of the assays was 400 µL. The absorbance at 515 nm was measured in 1-cm quartz cells using UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan) against a blank of pure toluene after the reaction was carried out at ambient temperature for 60 min (65). Radical DPPH scavenging activity was estimated from the difference in absorbance with or without sample and expressed as percent DPPH inhibiting. All tests were conducted in triplicates.

3.3. Oxidative stability of oils and formulas during storage

3.3.1 Chromatographic Purification.

Control oil (Table 1) was purified chromatographically to remove tocopherols and other antioxidants and carotenoids by the following procedure. A glass column was packed with a hexane slurry of 14 g of high-purity Merck silica gel (grade 60, 230-400 mesh, activated overnight at 120 ℃), followed by a hexane slurry of 4 g of a ctivated carbon (100-200 mesh, Sigma Chemical Co., St. Louis, MO), and then was washed with hexane. A 3.0 g of oil was passed through the column and eluted with 200 mL of hexane, followed by 100 mL of 10% ethyl ether in hexane (v/v). The

chromatographed oil recovered in 98% yield was not significantly changed in fatty acid composition. Another control was used containing 150 ppm α-tocopherol.

3.3.2 Preparation of the emulsions and samples

Oil-in-water emulsions contained ten percent oil and one percent lecithin and were prepared by a previously described procedure with a slight modification (66) using a phosphate buffer (25 mM, pH 3.8), and then were added to AIG 93G free fat diet, mixed for ten min as well as freeze-dried, packed and sealed under vacuum condition for omega 3 and omega 6 formula oils (1% DHA with1% GLA, 3% DHA and 3% GLA). All samples were placed in the oven at 60ºC for storage study.

3.3.3 Determination of Total Phenolic Compounds (TPCs)

TPCs were quantified colorimetrically using Folin-Ciocalteu reagent. Folin-Ciocalteu was diluted thirty fold in distilled water. Aliquots of oil (2 g) were dissolved in five ml n-hexane and mixed with ten ml methanol solution (80%) in glass tubes for two min in a vortex and then were centrifuged at 3000 rpm for 10 min. The extracts were combined and concentrated *in vacuo* at 30ºC until consistency was reached. After purification with acetonitrile, 0.2 ml and one ml diluted Folin-Ciocalteu was added, the flasks were shaken vigorously. After three min, 0.8 ml of 20% Na_2C0_3 were added and the mixtures were mixed thoroughly again. The mixtures were allowed to stand for one hour protected from light. The absorbance of the blue color produced was measured at 765 nm using a UV-260 visible spectrophotometer (Shimadzu, Kyoto, Japan).The concentration of the total phenolic compound for each extract was expressed as caffeic acid in parts per million based on the calibration of standard (six serial in triplicate).

3.3.4 Lipid Classification by column chromatography (CC)

Oils and recovered oils in chloroform were separated into the different classes neutral lipids (NL), glycolipids (GL) and phospholipids (PL) by passing through a glass column (20 mm dia * 30 cm) packed with a slurry of activated silicic acid (65- 230 mesh; Merck) in chloroform (20 %, w/v) according to Rouser el al. (67). NL was eluted with three times the column of chloroform, but GL and PL were eluted with five and four times of acetone and methanol, respectively.

3.3.5 Peroxide, anisidine and conjugated diene values

Measuring progress of oxidation levels of the oils during storage period was followed by determination of primary and secondary oxidation products through changes in fat peroxide (*AOCS Cd 8-53*), whereas was assayed by indirect titration with sodium thiosulfate, and the iodine resulting from the oxidation of iodide by peroxides was measured. The oil sample weight in the range of 0.2- 0.5 g was used. Also, a blank assay was carried out. Peroxide values (PV) are expressed as mill moles (mmol) of active oxygen per kg of oil; as well as p-anisidine (*AOCS Cd 18-90)* and conjugated dienes and trienes were expressed as absorptitivies of the one percent oils in isooctane at 232 and 270 nm. All tests were conducted in triplicate and averaged. No significantly statistic difference (P> 0.05) was found among the replicated experiments.

3.3.6 Characterization of thiobarbituric acid reactive substrate and free radical activity

Compounds' reacting with thiobarbituric acid (TBA) was determined by the described Pegg's method (68), with slight modification. Twenty µL butylated hydroxyltoluene (BHT) (to prevent oxidation during the assay) were added to 100-200 mg oil, two ml 10% (wt/v) trichloroacetic acid (TCA)

in water and two ml 0.6% (wt/v) TBA in 0.25 M HCL. The tubes were shaked and heated at 80ºC in a water bath for one hour, left to cool at room temperature and centrifuged 3000 rpm for 20 min. Calibration curve: 0, 20, 40, 100, 150 or 200 µL 0.1 nM 1, 1, 3, 3-tetraethoxypropane (TEP), an MDA precursor, was completed to 2ml with water. The procedure described for samples was then applied. According to color intensity, as an indicator of malondialdehyde (MDA) content, malondialdehyde was measured at 532 nm, using a Shimadzu UV-260 (Kyoto, Japan) spectrophotometer. Radical scavenging activity of oils and their fractions were examined by reduction of DPPH in toluene. (See details 3.2.6).

3.3.7 GC-HS analysis of chosen secondary oxidation products

Oils and oils fractions (NL, GL and PL) were carried into special headspace vials sealed with silicone rubber Teflon caps with a crimper. Oils were heated at 60ºC for 17 min and the head gas phase was injected for 60-90 seconds in HS-GC (a Hewlett–Packard 5890) with FID detector and split/splitless injector. Chromatographic separation of butanal, pentanal, hexanal, octanal and neonanal were carried out using a DB-1701 column (30 m length, 0.32 mm i.d., and 1µm film thickness; J & W Scientific, Folsom, CA). The oven temperature was 40ºC for 10 min, followed by temperature programming to 210 ºC at 4ºC/min and then was held for 10 min. The FID temperature was 300ºC and the injection port was held at 280ºC. Helium was used as the carrier gas. Peaks were identified by comparison of retention times with external standard mixture (Fulka, 99% purity specific for GLC) on the same conditions. Results were calculated in micro moles per kilogram of oil.

Table 3b: Linearity in studied propanal, pentanal, hexanal and nonanal by GC-HS.

Compound	RT (min)*	R^{2**}	Equation curve***
Propanal	4.9	0.994	y = 66233 x - 12490
Pentanal	7.7	0.998	y = 52444 x - 19676
Hexanal	11.3	0.998	y = 36956x + 2730
Nonanal	24.1	0.997	y = 2900.6 x + 848.12

* Rt, retention time
** Determination coefficient
*** X, concentration (µg/g); y, peak area

All rats fatty acids compositions are expressed as mean ± SD. Least significance Difference was used to compare the different of means between samples. Differences were analysis with the Correlation coefficient and SPSS 12.0 for windows. Statistical were considered significantly at a value of $p < 0.05$.

RESULTS AND DISCUSSION

Part I Animal experiment

4.1 Effect of feeding on the weight and fatty acid composition

4.1.1 Impact of feeding on rat's growth

Experimental rats' growth showed a slight increase in body weight of the 3% DHA group than another 1% DHA and 2% DHA groups at days 3, 6 until 56. Body weights at day 56 were 178, 183 and 196 g for the 1% DHA, 2% DHA and 3% DHA omega 3 groups, respectively. On the other hand, there were no significant differences in rat weight gains in omega-6 group (Figure 4, p 85). Hence, it could be concluded that high levels of DHA in the omega-3 diet may result in an increase in body weight of the experimental animals (69).

4.1.2 Impact of feeding on liver weight and fatty acids composition

4.1.2.1 Omega-3 group

Liver fatty acids composition (Table 3, p 72), as affected by different levels of GLA along with eight weeks, reflected more biological variability in the liver. It has been reported that, liver cells are the main site for the biosynthesis of LCPs from 18 carbon precursors, and for the formation of lipoproteins that transport fatty acids in the plasma and uptake by most other cells (70 and 71)**.** LA was found to be higher (11.6%- 16.2%) than that obtained in brain of all studied groups (0.8%- 1.53%) after 4 weeks and 8 weeks. However, as dietary DHA increased, LA was increased after 8 weeks (11.6%, 14.4% and 16.2% in 1%, 2% and 3% DHA groups, respectively) and AA decreased (10.5%, 9.88% and 9.72% in the same order). These findings were supported by Laidlaw and Holub (72) and Jensen et al. (73) that high levels of dietary DHA may inhibit ∆5- and ∆6-desaturase activity and thereby decrease the formation of AA from LA. α-LA was found to be 0.12% at birth , then significantly increased to 0.45%

and 0.52% after 4 and 8 weeks, respectively in control group, but this amount was insufficient to meet the developmental requirements. This can be emphasized by the low level of DHA (5.86%) at birth and after 4 and 8 weeks (4.93% and 6.03%, respectively). On the other hand, although α-LA was not detected in all omega-3 groups after 4 weeks (may be due to the complete conversion to another omega-3 metabolic product or DHA, it was detected in decreased levels about 58.4, 66.7% and 33.3% in 1%, 2% and 3% DHA, respectively after 8 weeks compared to that obtained at birth 0.12%). However, it reflects high ∆6- and ∆5- desaturase activity to the formation of C20:4n3, EPA and DHA from α-LA.

In regard to C18:4n3 and EPA which intermediate DHA synthesis, the results illustrated that C18:4n3 was not detected in the profile of liver fatty acids at birth and after 4 and 8 weeks in control group, may be due to the formation of EPA from C18:4n3, particularly in the presence insufficient amount of DGLA. It was reported that, DGLA competes with C20:4n3 (C18:4n3 elongation product) for the ∆5- desaturase and inhibit formation of EPA, DHA and eicosanoids from EPA. In the other interpretation in the body, LA ca competes with α-LA for ∆6- desaturase and thereby eventually inhibits formation of C18:4n3, C20:4n3, EPA, DHA and eicosanoids (15 and 16). After 8 weeks, C18:4n3 was detected with insignificantly differences between omega-3 groups (0.29%- 0.46%), which may be attributed to the conversion of α-LA into C18:4n3.

EPA significantly showed lower level at birth (0.31%), compared to those obtained after 4 weeks (1.49%- 5.52%) and (0.45%- 2.19%) after 8 weeks in all groups. After 8 weeks, 3%DHA group illustrated the highest significantly EPA level (2.19%) and followed by 2%DHA group (1.39%), 1%DHA group (0.76%) and control group (0.45%). However, the increasing of C18:4n3 and EPA compared to that obtained at birth can be

referred to the metabolism of α-LA, and this is in agreement with Sastry (74), Lands (75) and Salem (76).

Liver DHA level was found to be 5.86% at birth and then significantly increased to 6.03% in the control group and to 13.9%-14.8% in omega-3 groups after 8 weeks, with significantly differences between groups. The data also showed that 3%DHA group had the highest DHA level (14.8%) followed by 2%DHA group (14.3%) and 1%DHA group (13.9%). On the other hand, the lowest DHA level and the increase in n-6 PUFA in the control group reflects the low available supply of DHA to the nervous system, but by contrast after 8 weeks, DHA was increased with increasing LCPn-6 to 23.8%, 26.1% and 27.6% in 1%, 2% and 3% DHA, respectively depending on the biological behavior in liver and dietary supplementation. As previously mentioned, depending on inhibition of ∆6- and ∆5-desaturase activity, the results showed that , as DHA level increased in the diet, AA levels significantly decreased. Wherefore in 1%, 2% and 3% DHA groups after 8 weeks, compared to that obtained after birth (19.8%).

In general, all groups showed very similar profile of liver fatty acids after 8 weeks, which composed of 38.6%- 40.7% SFA, 12.4%- 22.7% MUFA and 38.7%- 46.9% PUFA of total fatty acids; and 8.24%- 19.2% as oleic acid which represents 53.1%- 84.6% of total MUFA. AA/ LA, DGLA/ AA and n-3/n-6 ratios reflect the change in enzymatic activities. The high ratios of n-3/ n-6 after 8 weeks in omega-3 groups compared to that obtained from control group, but with comparatively higher levels in 3%DHA group.

AA/ LA and DGLA/ AA ratios reflect the change in the ∆6- and ∆5-desaturase activity in all groups. After 8 weeks, control group had higher levels of AA/ LA and DGLA/ AA (1.23% and 423.3%, respectively) compared to that obtained after 4 weeks. The higher ratios of AA/ LA and DGLA/ AA can be due to EPA, which acts as competitive inhibitor that

decreases the formation of eicosanoids by inhibiting oxygenation of AA (cyclooxygenase and lipoxygenase pathways). On the other hand, AA/ LA and DGLA/ AA were decreased about 31.1% and 66.6%, respectively in 1%DHA group; 16.7% and 37.5%, respectively in 2%DHA group; and 25.9%- 24.6%, respectively in 3%DHA group after 8 weeks compared to the ratios at 4 weeks, but with comparatively higher AA/ LA (0.9) and DGLA/ AA (7.0) ratios after 8 weeks in 1%DHA group. This finding reflects the effect of added EPA to diet that inhibits ∆5- desaturase activity and thereby the formation of AA from DGLA (77). A DHA/EPA ratio reflects the changes in the DHA status in all groups. After 8 weeks, the ratio was increased by about zero, 191.7%, 81.8% and 273.5% compared to that obtained after 4 weeks in control, 1%, 2% and 3% DHA groups, respectively. The increase in DHA/EPA ratio reflects higher β-oxidation on elongation EPA products to produce DHA. The average liver weight after one week was 0.40 g with increasing to 8.00, 8.47 and 8.20 g in the 1% DHA, 2% DHA and 3% DHA group after 8 weeks, respectively.

4.1.2.2 Omega-6 group

It has been reported that GLA reduces body fat content but not body mass and facilitates fatty acid *β*-oxidation in the liver (78). In the present study, the average liver weight after the first week was 0.40 g; whereas, the relative weights of liver to total body weights were 4.4%, 4.6% and 4.1% for 1% GLA, 3% GLA and 5% GLA group after the fourth week, respectively; with no significant difference at the end point.

Concerning liver fatty acids profile (Table 4, p 73), as affected by different Levels of DHA during 8 weeks of age; the data revealed that, LA acts as precursors to LCPsn-6, had higher levels in liver (11.6%- 13.8%) than that obtained in brain of omega-3 and omega-6 groups at birth and after 4 and 8 weeks. In respect to omega-3 and omega-6 groups, the

results showed that, 1%DHA and 1%GLA groups have the same LA level (11.6%) after 8 weeks, but 3% and 5%GLA groups showed lower levels (12.7% and 11.6%, respectively) compared to those obtained in 2% and 3%DHA groups (14.4% and 16.2%, in the same order). The reduction of LA level in 3% and 5%GLA groups is due to the following, LA competes with α-LA for ∆6- desaturase, and thereby evenly inhibits formation of EPA (0.25% and 0.34%, respectively) than those obtained in 2% and 3%DHA groups (1.39% and 2.19%, in the same order), this result is supported by Chapkin (15); Calder and Field (16). With references to liver omega-6 groups, the result showed that, as dietary GLA increased up to 3%, LA and AA increased, then decreased in 5%GLA group, but with higher AA levels than in 1%GLA group. Wherefore, 3%GLA groups showed the highest LA and AA levels (12.7% and 24.1%, respectively) and followed by 5%GLA group (11.6% and 21.2% in the same order), and 1%GLA group (11.6% and 15.6%, respectively). The higher formation of AA in 3%GLA and 5%GLA groups accompany with lower DHA levels after 8 weeks mean that, DGLA compete with omega-3 fatty acids for the ∆5- desaturase to produce AA, and thereby inhibition/ or production of lower DHA. On the other hand, the lower formation of AA in 5%GLA group compared to that in 3%GLA group may be attributed to the presence of GLA, DGLA, prostaglandin E1 and 15-hydroxy-eicosatrienoic acid or to the metabolic at ∆5- desaturase step mostly appeared to be of low enzymatic activity in the synthesis of LCPs n-6 fatty acids (79). Moreover, AA/LA and DGLA/AA ratios (provides a biochemical indexes of product-substrate relationship in the LCPs n-6 pathway and ∆5-6 desaturase activity) has emphasized the same results of AA in omega-6 groups, in which 3%GLA group showed the highest AA/LA and DGLA/AA ratios (1.89% and 14.9%, in the same order) and 1%GLA group (1.34% and 10.4%, respectively) after 8 weeks. The results of EPA/AA support the foregoing findings, in which 3%GLA group

the lowest EPA/AA ratio and followed by 5% and 1%GLA groups at the end of experiment.

DHA/EPA ratio reflects the biochemical synthesis of DHA by β-oxidation, in which 3%GLA group showed the highest ratio (21.7) and followed by 5%GLA group (12.7), and 1%GLA (10.7) after 8 weeks. This result showed that, 3%GLA group possesses the highest capacity of converting the LCPn-3 especially DHA from its dietary precursors. The higher ratio of n-3/n-6 in 1%GLA group (0.37) after 8 weeks reflects the higher enzymatic activity, which reserves a normal brain behavior through n-3 status (59). However, high dietary levels of GLA (3% and 5%) resulted in higher levels of AA and lower EPA and DHA levels after 8 weeks. Generally, omega-6 groups illustrated similar profile of liver fatty acids, composed of 41.3%- 44.2% SFA, 15.3%- 20% MUFA, 39.5%- 45.7% PUFA of total fatty acids and 9.05%- 11.9% oleic acid, which represents 19.8%- 30.1% of MUFA.

4.1.3 Impact of feeding on plasma fatty acids composition

4.1.3.1 Omega-3 group

Effect of supplement of different levels of DHA on the content of plasma fatty acids, along 8 weeks and presented in tables 5 (p 74). It's well known that, after the biosynthesis of the fatty acids and the formation of lipoproteins in liver cells, the latter transports the fatty acids through plasma and uptake by most cells (70 and 71). Wherefore, the profile of plasma fatty acids showed that, LA and α-LA, which acts as precursors for LCPs n-6 and n-3 were found in higher levels (21.1%- 14.3% for the former and 4.41%-1.56% for the latter) than those obtained in liver and brain of omega-3 and omega-6 group, it can be seen that, as dietary DHA increased up to 3%, LA levels increased and AA levels decreased, but with higher LA(21.1%) and lower AA (4.37%) in DHA group means that,

increasing omega-3 in the diet may reduce AA levels (15-17 and 38-39), in which α-LA and C20:4n3 compete with LA and DGLA on ∆6- and 5-desaturase, and thereby production of higher EPA (2.09%). The EPA competes with the lower level of AA for accessing to the cyclooxygenase and lipoxygenase to produce EPA's eicosanoid, and thereby formation of the lowest DHA (4.85%). Contrarily, 1%DHA group showed the lowest LA and EPA levels and the highest α-LA, AA and DHA. This can be explained on the basis that, the reduction of LA level is due to the competition of α-LA for ∆6- desaturase, and thereby reduces the formation of EPA (1.04%) and increases AA (8.93%) and α-LA (4.41%). Furthermore, the higher level of DHA was to be accompanying with decreasing EPA. However, 2%DHA group behaves between 1% and 3% DHA groups. The results of AA can be emphasized by the results of AA/LA and AA/DGLA ratios, in which 3%DHA group had the lowest levels (0.21% and 5.14%, respectively), followed by 2%DHA group (0.41% and 9.03%, in the same order) and 1%DHA group (0.62% and 16.54%, respectively) after 8 weeks.

The results of DHA can be explained by DHA/EPA ratios, which reflect the biosynthesis of DHA from EPA in which 3%DHA group had the lowest DHA level (2.32%) and followed by 2%DHA (3.52) and 1%DHA group (6.68) after 8 weeks. This result showed that, 1%DHA group possessed the highest capacity of converting the LCPs n-3 especially DHA from its dietary precursors.

The results of EPA/AA ratios supported the foregoing results, in which 3%DHA group had the highest ratio (0.48) and followed by 2%DHA (0.22) and 1%DHA group (0.12) after 8 weeks. The higher ratio of n-3/n-6 in 1%DHA group (6.57) after 8 weeks reflects the higher enzymatic activity. However, plasma omega-3 groups presented similar profile of fatty acids, composed of 39.4%- 41.4% SFA, 20.2%- 23.2% MUFA, 37.2%-38.3%

PUFA of total fatty acids after 8 weeks. Palmitic acid was detected (24.7%-26.8%) as a major component (62.7%-66.5%) of SFA.

4.1.3.2 Omega-6 group

Tables 6 present the effect of different levels of GLA on plasma fatty acids, along with eight weeks. As mentioned elsewhere, lipoproteins transport fatty acids, in particular, LCPs by plasma to most cells (70 and 71). Wherefore, LA and α-LA, which consider as precursors for LCPs n-6 and n-3 were found to be in higher levels (ranged between 19.3% to 20.2% for LA and 1.18% to 1.41% for α-LA) than those obtained in liver and brain of omega-6 and control group.

In respect to plasma LA, as dietary GLA increased LA level in significantly decreased, but with significantly increase in 5%GLA group than that of 3%GLA group, after 8 weeks. This may be attributed to the effect of intestinal up take of GLA (1.41%-1.3% in 3% and 5%GLA groups, respectively) after 8 weeks. The results revealed that, there were insignificant differences in DGLA level between all omega-6 groups after 8 weeks. Plasma AA, showed that 3% and 5% GLA groups had significantly higher levels of AA (13.4%- 13.5%) than that obtained in 1%GLA group (12.2%), but with significantly lower levels than obtained in control group(24.2%) after 8 weeks. The highest level of AA in control group accompany with the lowest EPA level (0.48%) and the highest DHA level (4.5%), after 8 weeks reflect the competitive effect of DGLA with C20:4n3 for ∆5- desaturase, and thereby, the formation of AA. The significant higher AA levels accompany with the highest EPA and the lowest DHA in 3%GLA and 5%GLA groups compared to that obtained in 1%GLA after 4 weeks, means that at higher dietary levels of GLA, DGLA compete with C20:4n3 for the ∆5- desaturase to produce higher AA. The highest EPA and the lowest DHA, reflect the slower formation of DHA. On the other hand, the

reduction formation of plasma AA in 1%GLA group than that in other omega-6 groups, reflects the combination effect of EPA (1.18%), DHA (3.05%), which lead to suppress the formation of AA and the inflammatory mediator Leukotrienes in the plasma, because EPA blocks ∆5- desaturase activity, the terminal enzymatic step in AA synthesis (77). These findings were also reflected in the calculated AA/LA in which 1%GLA group had the lowest ratio (0.6). Plasma DHA was found at significant higher concentration in 1%GLA group than those of 3% and 5%GLA groups, which could be supported by the highest DHA/EPA ratio (2.58) in 1%GLA group after 8 weeks. The highest DHA level (3.05%), DHA/EPA (2.58) and n-3/n-6 (0.21) in 1%GLA group, and the lowest AA level (12.2%) and by AA/LA ratio (0.60) reflect the competition between the metabolic pathways between w-3 and w-6 fatty acids (80-83). However, palmitic acid was found as a major component on of SFA in all groups, with significant reverse relationship between PUFA n-3 and PUFA n-6 at all experimental periods.

4.1.3 Impact of feeding on brain weight and fatty acids

4.1.3.1 Omega-3 group

The average brain weight of the 1st week was 0.75 g, increased to 1.42 g in 1% DHA and 1.65 for both of 2% and 3% DHA omega 3 groups with brain to body weights ratios were 0.0079, 0.0086 and 0.0084 at the end of the experiment.

In respect to brain fatty acids composition, as affected by different levels of DHA during 8 weeks of age (Tables 7, p 76).α-LA levels (C18:3n3) showed significant higher levels in all groups (0.41%- 0.52%) after 8 weeks, compared to that obtained at birth (0.15%). The comparatively higher levels of α-LA after 8 weeks can be attributed to the exogenous supply of α-LA to the diets. On the other hand, the insignificant

difference between α-LA level at birth and after 4 weeks (0.06%) means that, the amount of α-LA almost completely used in the metabolic pathway.

The intermediate C18:4n3 and EPA (C 20:5n3) of DHA metabolite were detected in the profile of brain fatty acids. Although C18:4n3 was not detected at birth it showed significantly higher levels in all groups, with comparatively higher levels in omega-3 groups after 4 and 8 weeks. The higher levels of C18:4n3 was found to be 2.33, 2.42 and 2.21 fold in 1%, 2% and 3% DHA groups, respectively more than that obtained in control group after 8 weeks. This can be attributed to the conversion of the supplemented from α-LA to C18:4n3.

In regard to EPA, the results showed significantly lower level at birth (0.24%) compared to those obtained after 4 and 8 weeks in all groups (0.44%-0.49% and 0.45%- 1.78% respectively). After 8 weeks, it could be noted that, 3% DHA group has the highest EPA (1.78%) followed by 2% DHA groups (1.07%) and 1% DHA group (0.45%). However, control group and 1%, 2% and 3%DHA showed higher levels of EPA by about 4.46, 1.88, 4.46 and 7.42, respectively fold more than that obtained at birth (0.24%). Increasing C18:4n3 and EPA in all groups compared to that obtained at birth refers to the metabolism of α-LA, and this is agreement with Sastry (74), Lands (75) and Salem (76).

Brain DHA level was found to be 6.05% at birth, then significantly increased to 6.55% in control group and to 13.9- 14.4% in all groups after 8 weeks, but with no significant differences between omega-3 groups. This results are consistent with the observations of Pawlosky at al.(84), who reported that, a human who consume a high omega-3 have a low rate of conversion of DPA n-3 to DHA.

As expected, depending on inhibition of ∆5- and ∆6- desaturase activity as DHA level increased in diet, it was found that AA (20:4n6) significantly decreased after 4 and 8 weeks and by about 18.6%, 24.9%

and 30.2% after 8 weeks in 1%, 2% and 3% DHA groups, respectively compared to that obtained at birth (12.9%). However, rats in all groups showed similar profile of fatty acids composition, which contains 44.6%-54% SFA, 23.4%- 26.7% MUFA and 20.1%- 29.9% PUFA.

The higher ratio of n-3/n-6 in all groups, after 8 weeks compared to those obtained after 4 weeks, with comparatively higher levels in omega-3 groups reflect the enzymatic activity, which reserve a normal brain behavior through n-3 status (59).

AA/ LA and AA/ DGLA ratios illustrate the changes in AA status in all groups. Control group showed higher levels of AA/ LA and AA/ DGLA after 8 weeks by about 26.9% and 593.8%, respectively than that obtained after 4 weeks. This higher ratios can be attributed to the higher level of EPA (1.07%), which consider as competitive inhibitor that may decreases the formation of eicosanoids through inhibiting oxygenation of AA by cyclooxygenase (COX) and lipoxygenase (LOX) pathways (15-17 and 38-39). On the other hand, in omega-3groups, AA/LA was decreased in 1% and 2% DHA groups by about 13.8% and 0.33%, respectively, but it was increased in 3% DHA group by about 6.88% after 8 weeks than that obtained after 4 weeks. Furthermore, AA/ DGLA was decreased by about 35.5, 58.7 and 61.5% in 1%, 2% and 3% DHA groups, respectively after 8 weeks compared to that obtained after 4 weeks. This means that, addition of EPA inhibits the activity of Δ5- desaturase and thereby, decreases the formation of AA from DGLA (77 and 87).

The results of DHA/ EPA ratio, which reflects the changes in DHA status, showed that, after 8 weeks DHA/ EPA was increased by about 14.7% in 1% DHA group, but decreased by about 50.4% and 72.5% in 2% DHA and 3%DHA, respectively compared to those obtained after 4 weeks. The decrease in DHA/ EPA ratios shows lower β-oxidation to produce DHA and/ or modulation to eicosanoids synthesis from EPA by cyclooxygenase

and lipoxygenase. On the other hand, the increase in DHA/ EPA reflects higher β-oxidation to produce DHA.

4.1.3.2 Omega-6 group

The average brain weight after the 1st week was 0.75 g and after 28 days, the relative weights of brain to total body weights were 1.69%, 1.48% and 1.55% for the 1% GLA, 3% GLA and 5% GLA group, respectively. Hence, the effect of different levels of GLA on brain fatty acids composition, along 8 weeks of age (Tables 8, p 77) showed marked effects of supplementation on special fatty acids in brain. LA levels were significantly decreased to about 0.92%- 1.42% and to 0.76%- 0.92% after 4 and 8 weeks, respectively in all groups, but with comparatively lower levels in omega-6 groups (0.76%- 0.92%). In respect to brain DGLA, 5% GLA group showed the significantly highest DGLA level (1.25%) after 8 weeks. Control group illustrated significant reduction in DGLA (80.5%), after 8 weeks compared to that obtained after 4 weeks, but omega-6 groups showed significantly marked increase (24.2%, 27.4% and 43.2% in 1%, 3% and 5% GLA groups, respectively) after 8 weeks compared to those obtained after 4 weeks. However, high dietary level of GLA (5%) resulted in a slight significantly higher level of AA (11.2%) and lower DHA level (12.9%) after 8 weeks.

AA/ LA and AA/ DGLA ratios reflect the changes in AA status in all groups. In control group AA/LA and AA/ DGLA ratios were increased by about 1.26 and 6.94, respectively fold more than that obtained after 4 weeks. This can be attributed to the higher levels of EPA (1.07%) after 8 weeks. On the other hand, while AA/LA ratio was decreasing by about 8.8% in 1%GLA, it increases by about 87.3% and 72.9% in 3%GLA and 5%GLA compared to that obtained after 4 weeks, respectively. Furthermore, AA/ DGLA was decreased by about 32.4, 29.1 and 45.7% in

1%, 3% and 5%GLA groups, respectively compared to that obtained after 4 weeks. This can be attributed to the effect of EPA in metabolic pathway to the formation of AA from DGLA (77, and 85-87).

The brain DHA level was found to be 6.05% at birth, which significantly increased up to 8 weeks. Brain DHA level in 1%GLA group was found to be 2.13, 1.07 and 1.08 fold more than that obtained in control group, 3%GLA and 5%GLA groups, respectively after 8 weeks. DHA/ EPA ratio reflects the changes in DHA status in all groups. After 8 weeks, DHA/ EPA was increased by about 93.2% in 1% GLA group, but it decreased by about 44.2%, 49.4% and 35.7% in control group, 3% GLA and 5%GLA, respectively compared to those obtained after 4 weeks.

In omega-6 group, rats showed similar profile of fatty acids composition, which contains 44.1%- 54% SFA, 23.5%- 26.3% MUFA and 20.1%- 29.9% PUFA of total fatty acids; and 18%-20.1% as oleic acid which represents 70%-76.5% of total MUFA. The higher ratio of n-3/n-6 in all groups, after 8 weeks compared to those obtained after 4 weeks, observe a normal brain behavior through n-3 status (59).

4.2 Effect of feeding on phospholipids fatty acids composition

4.2.1 Liver phospholipids fatty acids profile

In omega-3 group, they were a fall of AA and increase the level of DHA in liver PL, whereas the level of ω-3 in cell very important But when cell deficient in ω-3 fatty acid has a decrease in DHA and increase levels of the end product of ω-6 metabolism, DPA within the sub-cellular organelles and mitochondria seem to be more sensitive to a low dietary supply as evidenced by the relative abundance of DHA and the changes compositions of these organelles in response to dietary deprivation (77, 88, and 89). The evidence indicates that in early life α-LA precursors are

not sufficiently converted to DHA to allow the biochemical and function of normal cycle (90). Summed of ω-3 FA after 4 week (Table 9, p 78) increased gradually until 8 weeks. On the other hand, in omega-6 group, there was a fall in the level of DHA in liver PL during the fourth week (Table 10, p 79), whereas the decrease was similar in both 3%GLA and 5%GLA groups (about 17-18%). At the end of the experiment, all groups showed decreases of about 1.36, 1.22 and 1.20 folds than the level at the fourth week in the 1% GLA, 3% GLA and 5% GLA groups, respectively. Moreover, rats liver PL had higher levels of AA at 28 day, after that the levels were decreased in all groups till the end. It was found that the 5% GLA group had the highest percentage of AA and DGLA at the end of the experiment. AA was increased by about 25% and 11% than both of the other groups and it had 2.69 and 2.35 folds more than group 1% GLA and 3% GLA of DGLA, respectively.

4.2.2 Plasma phospholipids fatty acids profile

The fatty acids profiles of plasma phospholipids followed closely the degree of supplementation in omega-3 group and illustrate the competitive interactions between fatty acid (ω-3 compared with ω-6) metabolic pathways (77, 82 and 91). For example, in all groups supplement with a constant amount of EPA and AA, fatty acid concentration of AA decreased linearly with increasing amounts of DHA supplements. In our results, DHA recorded more fold decreases incorporation from plasma into the rat brain as well as a reduction in recycling via deacylation/reacylation reactions. The overall rate process of DHA uptake from the circulation into the brain/retina appears slow and may be rate-limiting for DHA repletion. In both of Figures 5 and 6, changes of the plasma phospholipids were shown during the experiment especially after 28 days and 56 days of feeding.

All the omega 6 groups showed similar and significant group mean reduction in AA concentrations in plasma phospholipids with supplementation. After 8 weeks, the reduction in the 5%GLA group was about 25% and 44% in both of the 1% GLA and 3% GLA groups, respectively coming with the first four weeks. The multiple factors that likely account for an actual reduction in AA concentrations include competition by EPA and DGLA for esterification into cellular phospholipids and the attenuating effect of n-3 fatty acids on Δ5-desaturase, which affects the conversion of DGLA to AA (82, 83, 92, and 93). Addition of EPA and DHA to formula increased the proportion of LCPs n-3 in plasma phospholipids but did not prevent n-6 depletion (82, 88).

4.2.3 Brain phospholipids fatty acids profile

Rat brain phospholipids contained only a small amount of α-LA and these results are in agreement with the literature (77, 88). Recycling (deacylation and reacyclation) of PUFAs (AA and DHA) in brain phospholipids is a going and active process, but unlike α-LA they are much susceptible to *β*-oxidation and thus are conserved in the phospholipids by the recycling. Brain phospholipids AA decrease and DHA increase with decrease in 18:4n3 and EPA. At the end of experiment, the proportion of DHA increased in 1%DHA and 2%DHA group (~79% and ~74%, receptively) with similar decrease of EPA (Table 11, p 80). On the other hand, in the omega-6 group, there was a parallel in the n-3 and n-6 fatty acids in PL compared with the brain FA. Between the 4 and 8 weeks, there was found a fall in the level of AA about 26-29% in all groups, related to reverse relationship with DGLA. The proportion of DHA was decreased in 1% GLA group and 3% GLA (~ 13% and ~9%, respectively) at the 28 day and (19% and ~20%, receptively) at the end of experiment comparing to the level at the fourth week (Table 12, p 81). These data are in agree with

that of Wainwright (94) who noted that feeding with oil containing high level of GLA decreased the level of DHA in brain PL by competition between the n-6 fatty acid GLA and the n-3 fatty acid DHA. Thus, a long-sanding question in essential fatty acid research has been whether LCPs are accumulated in tissues *via* metabolism of 18-carbon precursors normally abundant in the diet or rather are incorporated directly from preformed dietary LCPs. Many previous studies had demonstrated that various pathway, either metabolic or involving preformed LCP intermediated, are operative for various tissues. What has been total lacking in any approach that could assess the quantitative contributions of the various known pathways for LCP accumulation? One key aspect of this problem is the source of brain DHA because maintenance of its level is critical for optimal neutral function. The author chose to hold LA and α-LA of the diets constant and change the levels of DHA and GLA at two experiments. Rates with omega 3 diets were present were significantly related after four weeks. Control rat showed decreased level of DHA during 4 weeks. Brain DHA was positively correlated with plasma DHA ($r = 0.82$, $P< 0.05$) and Brain AA was positively correlated with plasma AA ($r = 0.89$, $P< 0.05$). Brain DHA had not related with the increase with DHA in diets. We suggested to plasma DHA may be used as a marker of brain DHA status. On the other side, rates with omega 6 diets were present various metabolic fatty acids pathway, liver and plasma fatty acids were significantly related after four weeks, which liver cells are the main site for the biosynthesis of LPCs from 18 carbon precursors, and for the formation of lipoproteins that transport fatty acids in the plasma and uptake by most other cells. Liver DHA was positively correlated with plasma DHA ($r = 0.81$, $P< 0.05$) and liver AA was positively correlated with plasma AA ($r = 0.86$, $P< 0.05$). Plasma and liver AA were not correlated with brain AA. However, DHA in liver and plasma DHA were significantly associated with DHA in

brain (r = 0.81 and 0.92, P< 0.05) and kidney (r = 0.98 and P< 0.05), respectively.

4.3. Effect of feeding on tocopherol levels

It is important to detect antioxidant status, which is very important when consumed LCPs in infant fed formulas. Free radical especially superoxide ($O_2^{\bullet}$) and other reactive species such as H_2O_2, are continuously produced *in vivo*. Superoxide in particular is produced by leakage from the electron transport chains within the mitochondria system. Risk of oxidative damage for premature infant is very important because they are born with immature antioxidant status.

4.3.1 Impact of feeding on level of liver tocopherols

Liver is the major storage organ of α-tocopherol (95). Table 13 showed that, liver α-tocopherol was decreased by about 10.7-36.6% and 3-6% and in the omega-3 and omega-6 groups, receptively after 4 weeks compared with the level after 2 weeks. Furthermore, liver α- tocopherol in the 1% DHA group showed 1.58 fold more than the liver in the 3% DHA group after 8 weeks of ages. However it was be found that, 5% GLA group had lesser liver α- tocopherol (40.8%) compared with that obtained with 1%GLA after 8 weeks of age. Our results are in agreement with those of other studies which have shown that diets induced high n-3 FA had oils lower α- tocopherol content in the rats liver (96) or mice(97).

4.3.2 Impact of feeding on level of plasma tocopherols

The α-tocopherol level in plasma rapidly raised to about 0.52–11.1 mg/L in omega-3 group after 4 weeks, while in omega-6 group was increased to about 4.1- 8.82 mg/L at the same period (Table 13, p 81).

Continuous decrease in plasma α- tocopherol was noted after 6 weeks in both groups and with some differences after 8 weeks. This can be due to interaction of omega-3 or omega-6 oils and tocopherol at the gut level, or to enhanced post absorptive utilization of tocopherol compared to rats fed low level and high level of omega oils.

4.3.3 Impact of feeding on level of brain tocopherols

Concentration of α-tocopherol in rat's brain was found to be 12.22 µg/ total brain at birth. Table 13 showed that, α-tocopherol significantly decrease after 4 weeks and up to 8 weeks in all omega-3 and omega-6 groups. The consumption of α-tocopherol was lead to %- loss about 29-40% and 31-38% in the omega-3 and omega-6 groups, respectively after 4 weeks and loss about 87%- 89% in each after 8 weeks. It was found that, the change in brain α–tocopherol being much smaller than that in plasma and other tissues, including liver (98-100). Furthermore, the high content of PUFA and, more specifically, DHA in rat brain fed omega-3 diet may have enhanced the consumption of antioxidants. Gamma-tocopherol was found as very small amount in all tissues and plasma. Summary of this section, all omega-3 and omega-6 groups, there was trend for α- tocopherol to decrease with time, especially between 4 week and 8 weeks of feeding with significance difference between the low and high level of ω-3 as well as low ω-6 and high concentration.

4.4 Free Radical Activity in brain and liver extracted

Pervious studies on radical scavenging activity (RSA) have used different solvents to dissolve the free radicals in different crude oils (91), but were not detected before in brain and liver oil extraction. Hence, the results here were difficult to be compared because the reactions were done under different conditions. Characteristics of the antioxidant-free

radical reactions in the present study were evaluated using total lipids by spectrophotometric method with the stable DPPH radical. This method has been reported as a simple method for evaluation of the radical scavenging activity of a given substance, absorbance at 515 nm decreases as a result of a color change from violet to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H. So, more rapidly of the absorbance decreases, the more potent the antioxidant activity of the compounds was in terms of hydrogen donating ability (101).

Total DPPH radical scavenging capacities of fractions were measured and compared in the two groups with different levels of omega-3 and omega-6. The liver and brain fractions from rat which fed 3% DHA had a much weaker radical scavenging activity compared to 2% DHA and 1% DHA fed rats firstly at all period of age studied. After 4 weeks of the experiment and at a reaction time of 1 min, the liver and brain extract from rat 1% DHA and 1% GLA exhibited the strongest activity, and followed by the another omega-3 and omega-6 groups as shown in Figure 8a and 8b. On the same per weight basis at 4 weeks and after 1 h of incubation, 26.4% of DPPH radicals were quenched by brain extract from rats fed the 1% DHA, while brain extract from the 2% DHA and 3% DHA were able to quench 33.6% and 53.5%, respectively. Free radicals and radical-mediated oxidation play roles in many aging-related health problems including cancers and heart diseases. To better understand the beneficial effects of natural antioxidant in oils, which used in processing infant formula or food and differ in their reactions with free radicals, depending on biological actions, more experiments should be done. In our present study, positive correlation was found between RSA and the levels of tocopherols in brain and liver extracted.

Part II Oxidative stability of formula

5.5 Oxidation stability during oven test

In this part of work, the objectives were to determine the oxidative stability of the oils and their formulas for 12 days at 60℃, whereas the times of storage was roughly comparable more to 6 months at room temperature (20℃), when the general concept of 2 times the oxidation reaction rate increment with 10℃ increment (Q_{10} = 2) was applied. On the other hand, oil stability is determined under accelerated conditions (60℃ or more) for several reasons (i) ambient conditions demand an excessively long period, (ii) ensure that the starting lipid does not contain high levels of oxidation products (e.g. transition metals hexanal, free fatty acids and lipid hydroperoxides) and (iii) at high temperatures can contribute to decomposition of hydroperoxides, decomposition or volatility of antioxidants, and decrease of oxygen solubility rapidly.

5.5.1 Hydroperoxide formation

5.5.1.1 Peroxide and p-anisidine values

Peroxide value is used to quantify the hydroperoixdes formed; however these are intermediate products in the formation of carbonyl and hydroxyl-compound (102). The oils were compared at three different of LCPs (1% GLA containing 1% DHA, 3% GLA and 3% DHA) and oils extracted from manufactured formulas containing the same fatty acids composition. The changes of PV for oils and extracted oils from formulas are illustrated in (Figure 9, p 88). PV remained with small changed at a very low level (7.1 mmol/kg oil) over 192h in EO 1% GLA (Figure 9), whereas the peroxides accumulated in EO 3% DHA to a high level after eight days of storage and then decreased, as a result of hydroperoixdes

decomposition (102). With respect to control oils (Figure 9), PV sharply increase up to 96h and then decreased throughout the rest of the storage period, whereas PV was gradual increased by increasing content of LCPs; suggested more oxidative problems with these group contained 3%DHA compared to the others. To better elucidate degradation of the fat, a *p*-anisidine assay was used to determine the aldehyde content of all oils (Figure 10, p 89). Like peroxides, *p*-anisidine values were increased. Thereafter, *p*-anisidine values were decreased, but to a lesser extent than PV. Therefore, *p*-anisidine values, like peroxide values, proved also unreliable as indicators of the degree of rancidity for fats exposed to extreme oxidative challenge (103).

5.5.2 Ultraviolet absorptivity

LCPs oxidation can be analyzed by the absorptive increase in the UV spectrum (104). During the storage time, lipids that have dienes and polienes showed a changed in the double bonds positions, due to isomeration and conjugation in the molecule (105). Dienes and trienes formation on the oxidation initial steps and they show intense absorption at 233 and 270 nm, respectively (Figure 12, 13, 14 and 15, p 91-94). Absorptive at 233 nm increased gradually with the increase in time, due to the formation of conjugated dienes (CD) (Figure 12) during 288h. The CD from both oils contained 3% DHA and neutral lipid were increased sharply and peaked after 144 h. The sharp increase in the CD might be accounted for by the formation of more and more hydroperoxides as primary products of oxidation according to structure of non-polar lipid, whereas it is the major component of lipid. On the other hand, control with α-tocopherol was more stable than the all samples. After 192 h of storage, the CD values were decreased, possibly due to the breakdown of unstable hydroperoxides. On the another side, CD of EO containing 1% GLA as

well as netural lipid had low change during the storage, from 0 to 288 h, indicating a good oxidative stability. Meanwhile, the changes in conjugated tienes (CT) were less marked than the changes in CD during the storage period. So, increases absorptivity at 270 nm after 6 days of heating being as a result of rancid off-flavor compounds formation (aldehydes and ketones). Parallel was observed between trends for changes in CD, PV and AV.

The polar fractions extracted from formulas containing 1% emulsifier had better OS compared to polar bulk oils fractions at the same induction periods (Figure 14 and 15). It is seem to be due to the refining, bleaching and deodorization process in which the phospholipids were almost completely removed. The incomplete removal of phospholipid, promote the initial breakdown of the primary antioxidants. Thus, any subsequent storage, due to the lower level of antioxidant, will show a decreased stability and a pro-oxidant effect of the PL (106). By comparison, phospholipids gained of emulsifiers may be acting by quenching the free radicals arising from PUFAs oxidation and thus improving the OS (104). Therefore, it could be said that polar fractions added in bulk oils or oils used in food industry were mainly responsible for their stabilities (105). Ultraviolet scans between 220 and 320 nm after 12 days storage at 60 were shown in Figure 11.

5.5.2 Hydroperoxide decomposition

The primary oxidation products for unsaturated fatty acids are the hydroperoxides, highly reactive compounds that decompose rapidly, yielding a complex mixture of non-volatile and volatile compounds such as hydrocarbons, aldehydes and ketones. Aldehydes are practically important in stability of infant foods including DHA, which related to flavor alteration and quality product (107).We were investigated the decomposition of

hydroperoxide as propanal, pentanal, hexanal and nonanal of oils prepared from the same amount of LA, α-LA, AA and EPA, but only different was the amount of DHA and/or GLA with 150 ppm of α-tocopherol. Calibration curves were drawn for the four aldehydes. The curves were followed linear relationship with highly significant correlation coefficients (R^2 better than 0.9 and equal 0.99 for a large number of compounds) (Figure 3b, p 84, Table 3b, p 26).

5.5.2.1 Oxidative stability of bulk oils

Pentanal and hexanal were originated from n-6 fatty acids (LA and AA), while the propanal and nonanal were originated from n-3 and n-9 fatty acid, respectively. In the control oil, the initial rate of hydroperoxides decomposition increased sharply after storage period of 96 h. The low propanal, hexanal, pentanal and nonanal values in 1% GLA oil containing 1% DHA agreed with the corresponding PV obtained for this oil (Figure 9). With respect to the high content of n-3 fatty acids in 3%DHA oil was higher propanal (33%), lower hexanal (49%) and pentanal (53%), after 192 h than those obtained from 3% GLA oil (Figure 16, p 95-102). The lower hexanal content without any off-flavor may be due to the addition of coriander oil. However, these differences in relative stability may be due to not only to fatty acids composition but also to interaction of α-tocopherols as antioxidant (108).

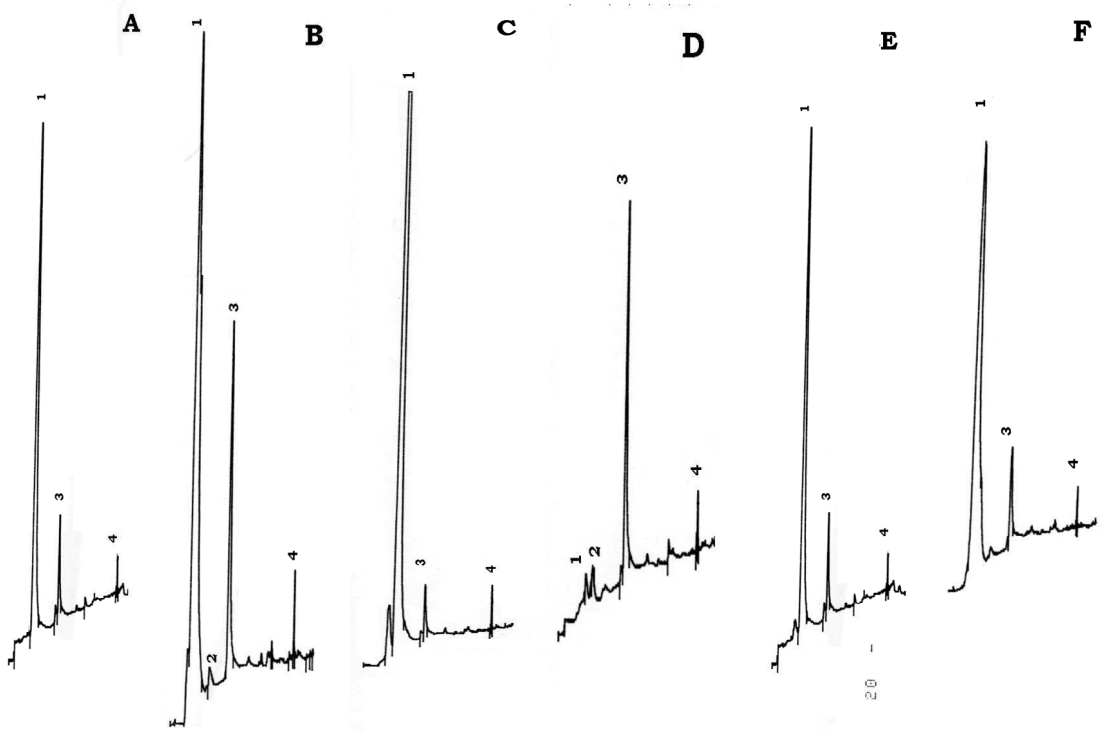
5.5.2.2 Stability of extracted oils (EO)

Oil-in-water emulsions were prepared with oil 10 wt% of the same LCPs containing DHA and/or GLA described above and 1% soy lecithin as emulsifier in phosphate buffer at pH 3.8. The oxidative stability (OS) of these extracted oils were significantly higher during the first 96 h than that of the corresponding oils (Figure 16); and this may be resulted from many

factors (i) scavenging free radicals by amino acids of protein (109); (ii) lecithin may be increasing the contact between the antioxidant and the oxidizing fat (103) and/or; (iii) effecting of PC on the decomposition of hydroperoxides by catalyze a non-radical pathway (104). Propanal analysis was found to be the highest oil after 192 h in 3% DHA oil, while the oppositely effect was observed in pentanal formation (Figure 16). On the basis of hexanal formation, hexanal did not increase until 96 h of storage period, and after 192 h the level was decreased. Decrease of hexanal could be as a result of finished fatty acids oxidation, although this assertion can not be categorical, because the differences could also be ascribed to the manufacturing process

5.5.2.3 Oxidative stability of oil fractions

Bulks oils were sequentially fractionated with chloroform to recover NL; followed by acetone to recover GL then methanol to recover PL. Numerous studies have been focused on the oxidative stability of emulsion containing lecithin. The OS of NL, GL and PL neither is so far nor reported in the published literature. Hence, we may have for the first time definitively established the OS of oil fractions. The importance of studying oil stability fractions is reflected in the utilization of each fraction in the industry. As expected in oils, NL (constituted mainly of triacylglycerols) was the major fraction followed by GL and PL, respectively (Table 14, p 81).



Propanal and hexanal by GC-HS: chromatography of glycolipids of oils (A) 1%GLA, (B) 3%GLA, (C) 3%DHA and extracted oils (D) EO 1%GLA, (E) EO 3%GLAand (F) 3%DHA after 96h at 60 °C. Peaks numbered correspond to 1, propanal; 2, pentanal; 3, hexanal; and 4, nonanal.

The ratio of saturated to polyunsaturated fatty acids (S/P) in the oil fractions is summarized in Table 14. The fractions had a rather similar S/P pattern wherein the ratio increased with the increase of the polarity of the oil fraction. It was worthy to mention that the S/P ratio recorded the highest level in the polar fractions (GL and PL).This may be explanation; the major propanal content in polar fractions. On the basis of hexanal formation, in the control oil, the rate of hexanal formation increased sharply after 96 h (Figure 16), but a different order of stability was observed after 96 to 192 h of storage period, detected at the higher level in polar lipids than the neutral lipid fraction with increasing in the 3% GLA (Figure 16).Extended storage, without daylight, results in high concentrations of propanal and hexanal in polar fractions.

5.5.2.4 Thiobarbituric acid reactive substances (TBARS) Assay

A portion of the lipid hydroperoxides that form in the early stages of lipid oxidation are broken down to form the low molecular weight volatile compound (secondary products) that impart rancidity. Various low molecular weight aldehydes, alkanals and nonvolatile precursors of these substances react with 2-thiobarbituric acid (TBA), resulting in chromogens termed 2-thiobarbituric acid-reactive substances (TBARS) that can be determined spectrometric at 532 nm. TBARS values of oils and extracted oils (EO) as well as the control are shown in Figure 17. During the test; statistical evaluations ($p< 0.05$) showed that PUFAs significantly affected the TBARS values. After the first 2 days of incubation, the lowest values were observed for oil containing the lowest level of PUFAs. As the accelerated storage period was extend up to 72 h, TBARS values of 3% DHA oil increased gradually (Figure17, p 103).

TBARS of this oil was considerably higher than those of the EO 3% DHA over the entire storage period. As explained earlier, removal of antioxidant during process is responsible for the compromised stability of the modified product. Meanwhile, TBARS values of the EO 1% DHA, as such, remained nearly closed with control during the entire storage period, indicated its good stability under oven conditions at 60 ºC. The general increase in TBARS values of oils during storage time may be due to the breakdown of lipid hydroperoxides and the production of secondary products (110). Correlations were existed between peroxide value (PV) and *p*-anisidine value (AV) ($P <0.05$) as well as 2-thiobarbituric acid-reactive substances (TBARS) and headspace volatiles (propanal) content ($P<0.001$) for most oils and extracted oils. Besides, it was found that a negative correlation was demonstrated between the TBRAS formation and total phenolic contents.

For each of the time periods studies, we measured the ratio of EPA and DHA to palmitic acid. These values were used as markers of the lipid oxidation progress. We would expect to decreases this ratio with an increase in oxidation of EPA and DHA.

5.5.3 Effect of α-tocopherol in bulk oils and extracted oils (EO)

Lipid oxidation is the most critical parameter affecting in the shelf life of the newer food products, which LCPs lipids have been incorporated. The many different fatty acids hydroperoxide positional and geometrical isomers that are formed during the oxidation of the ω-3 and ω-6 PUFAs up on storage rise to a complex mixture of secondary oxidation products. Therefore, we was investigated the effect of the conditions of (O/W emulsion), pH 3.8, 150 ppm α-tocopherol and temperatures 50℃ on the oxidative stability in ω-3 and ω-6 enriched formulas and compared with the same composition of bulk oils in a storage period (12 days) at 60℃. Hydoperoxide formation and decomposition as well as antioxidant activity were quantified for oils and finished products in storage. Quality testing of several indicators of degradation in new oils enriched in omegas is essential to enhancing quality, stability, and nutritional value of these foods especially infant formulas.

The tocopherols are the most important natural antioxidants in fats and oils. To understand the mechanism of α-tocopherol as an antioxidant compound, it is well known that lipid oxidation started by the abstraction of a hydrogen atom from an allylic or *bis*- allylic position of an USFA (RH) to generate an alkyl radical , which combines with molecular oxygen at diffusion- controlled rate to produce a lipid peroxyl radical ($ROO^{\bullet}$). The $ROO^{\bullet}$, having longer lifetimes (ca. 7s) than $R^{\bullet}$ (ca. 10^{-8} s), propagate the oxidation reaction by selectively abstracting hydrogens from RH to form

lipid hydroperoxides (ROOH) and another $R^{\bullet}$ Unsaturated fatty acids have different susceptibilities to hydrogen abstraction, depending on the dissociation energies of labile C-H bonds within the molecules. C-H bonds adjacent to a double bond or at a tertiary carbon atom are weaker and easier to break. For the FA series $-CH_2-(CH=CH-CH_2)n-CH_2-$ (n = 1-3), the relative oxidation rates increase in the order 1: 40: 80, respectively. It was established that the fatty acids oxidizability is much lower for MUSF substrates (*e.g*. oleic acid) than mainly PUFAs. Thus the oxidize ability of FA mixture is mainly depended on the number of allylic methlenes therein. Thus, the α-tocopherol affected not only the overall formation of volatile secondary oxidation products, but also the composition of this group of oxidation products (109). In our study, the control oil without antioxidant oxidised very rapidly and all the treatments related to hydroperoxide formation and decomposition. The inhibition of hydroperoxide decomposition by α-tocopherol between the control and sample containing α-tocopherol increased with storage time (Table 15, p 82). With 150 ppm of α-tocopherol, hexanal and propanal increased after 96 h. The inhibition was increased after 144h with decreased in α-tocopherol amount; no hexanal detected after this time. Mixture oil with 1% GLA in the presence of 150 ppm had a good scavenging peroxyl radicals activity compared with others but all oil mixtures had a very low total polyphenols. However; polar lipids are found in high levels in EO, strong RSA of these components can be expected as well as synergistic activity with α-tocopherol. On the other hand, addition of lecithin containing phosphatidylcholine and phosphatidyelethanolamine apparentely all facilitate hydrogen or electron donation to tocopherols by the amine group. Hence, PL could be extending the effectiveness of tocopherols by delaying the irreversible oxidation to tocopheryl quinone, thereby delaying the oxidation. As detected, we conclusion that these oils in stable up to 144 hour at 60ºC. Additional

studies are necessary to show the antioxidant activity between different levels of tocopherols and PL, metal ions and poly phenols plus the effect of LCPs n-3 on the oxidative and flavour stability of triglycerides remains however an open and important question.

5 CONCLUSIONS

Each of the two experiment design contributes some evidence that is relevant to possible causal linkages between tissues and plasma concentrations of DHA and eicosanoid precussour. The relationship between dietary fat and structural fats are very complex. The diet-induced shifts in the fatty acid composition were greatest in liver, followed by brain and then plasma. Plasma DHA can be used as proxies for tissues DHA. Accretion liver of LCPs n-6 is more rapid than the LCPs n-3 series, rendering the latter more dependent upon dietary supplementation than the former, while brain accretion is reversible, depending on the metabolic pathway.

It would be expected that human infant who received vegetable oil-based formulas which the essential fatty acids supplied by corn oil, sunflower or safflower oil would exhibit a marked decline in brain DHA in the first months of life, similar to that observed for the n-3 Def rats in the present study.

It could be recommended that higher levels of DHA not contribute to increase of DHA in brain, liver or plasma. Whereas addition 1% DHA to formula had significant effect in an important time period during four weeks, wherein this period is encompasses the majority of rat brain.

In the present study, adding a source of EPA+ DHA to the diet concomitant with different amounts of GLA reduced mean AA concentration in plasma and increased concentration of EPA. However, DGLA increased when the ratio of EPA+DHA to GLA was 1: 0.5, 1: 1.5 or 1: 2.5. That is may be important to use for infant fed formula with a family history of inflammatory or AD diseases.

Reduction of plasma AA in the second stage of our experiment between 4 weeks and 8 weeks, is very important factor for patients with CD or inflammatory condition, such as rheumatoid arthritis, are needed for the development of additional treatment strategies of these patients.

Direct evaluation of antioxidants from tissues and plasma contributed information to the antioxidant status of an individual. This maybe useful for evaluating the risk for degenerative diseases and radical scavenging activity could be a good marker for lipid peroxidation.

The OS of NL, GL and PL is neither so far nor reported in the published literature. Hence, we may have for the first time definitively established the OS of oil fractions. The importance of studying oil stability fractions is reflected in the utilization of each fraction in the industry especially infant rich PUFAs formulas processing.

The results of the present investigation indicated that level of LCPs, initial PV and the level of polar lipids were significantly affect in the RSA of the oils and EO. Beside, α-tocopherol was affected by the overall formation of volatile secondary oxidation products.

In the light of these results, further studies will be required to:

1. Clarify the effects of long terms administration of DHA oil on lipid metabolism and peroxidation.
2. Molecular and enzymatic studies for consumption a mixtures of DHA, EPA plus GLA in some infant or adult inflammatory history diseases.
3. Antioxidant activity between different levels of tocopherols and PL, metal ions and polyphenols plus the effect of LCPs n-3 on the oxidative and flavour stability of triglycerides remains an open and important question.

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Table 1: Composition of the two experimental groups and deficient group diets

Ingredient (g/ 100 g diet)	Omega-3 group			Omega-6 group			Deficient group
	1% DHA	2% DHA	3% DHA	1% GLA	3% GLA	5% GLA	
Skim milk powder	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Carbohydrate	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Sucrose	10	10	10	10	10	10	10
Malto-dextrin	15	15	15	15	15	15	15
Cornstarch	15	15	15	15	15	15	15
Dextrose	20	20	20	20	20	20	20
Fat	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hydrogenated coconut oil	7.12	7.1	7	7.12	6.95	6.87	8.1
Mixed oils	2.88	2.91	3.00	2.87	3.05	3.23	1.9
Additives:	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Cellulose	5	5	5	5	5	5	5
Salts	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamins	1	1	1	1	1	1	1
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Cystine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
THBQ*	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Fatty acids composition							
SFA	69.8	70.9	71.2	69.8	68.7	66.3	75.5
MFA	10.2	9.8	9.2	10.2	9.5	8.9	14.2
C18:2n-6	15.24	15.2	15.53	15.19	15.25	15.51	15.96
C18:3n-3	3.01	2.98	3.11	2.98	3.01	3.04	3.2
C18:3n-6	0.099	0.098	0.105	0.99	3.01	4.98	nd
C20:4n-6	0.58	0.55	0.53	0.58	0.59	0.57	nd
C22:5n-3	0.89	0.89	0.85	0.89	0.87	0.88	nd
C22:6n-3	0.99	2.01	3.1	0.99	1.01	1.11	nd
n-6	15.92	15.85	16.17	16.76	18.85	21.06	15.96
n-3	4.89	5.88	7.06	4.86	4.89	5.03	3.2
n-6/n-3	3.26	2.70	2.29	3.45	3.85	4.19	4.99
18:2/18:3	5.06	5.10	4.99	5.10	5.07	5.10	4.99

* THBQ, t-butylhydroquinone; SFA, total saturated fatty acids; MFA, total monosaturated fatty acids; nd, not detected

Table 3: Liver fatty acids composition (%) of different levels of DHA during eight weeks*.

% DHA		Control		1% DHA		2% DHA		3% DHA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.57± 0.00	0.25 ± 0.00	0.28 ± 0.00	0.40 ± 0.01	0.64 ± 0.02	0.63 ± 0.05	0.69 ± 0.05	0.46 ± 0.01	0.43 ± 0.05
C16:0	21.7 ± 0.21	25.2 ± 0.21	25.6 ± 0.11	20.4 ± 0.25	22.4 ± 0.74	21.2 ± 0.45	23.3 ± 0.55	21.3 ± 0.35	23.6 ± 0.23
C16:1n-7	1.06 ± 0.00	0.23 ± 0.00	0.28 ± 0.02	1.86 ± 0.12	3.91 ± 0.09	1.83 ± 0.17	3.31 ± 0.60	1.40 ± 0.14	1.31 ± 0.07
C18:0	19.9 ± 0.17	11.3 ± 0.05	12.5 ± 0.09	16.5 ± 0.36	16.1 ± 0.26	15.6 ± 0.35	15.4 ± 0.27	16.0 ± 0.45	15.6 ± 0.23
C18:1n-9	10.8 ± 0.08	19.5 ± 0.10	19.2 ± 0.12	12.0 ± 0.25	10.9 ± 0.14	13.1 ± 0.27	8.24 ± 0.11	10.2 ± 0.15	9.21 ± 0.12
C18:1n-7	2.02 ± 0.04	3.20 ± 0.05	3.10 ± 0.03	2.01 ± 0.04	4.59 ± 0.07	2.52 ± 0.09	3.89 ± 0.02	2.01 ± 0.07	1.31 ± 0.04
C18:2n-6	11.9 ± 0.12	12.3 ± 0.16	13.8 ± 0.13	13.3 ± 0.06	11.6 ± 0.11	15.3 ± 0.21	14.4 ± 0.45	14.0 ± 0.23	16.2 ± 0.14
C18:3n-6	0.61 ± 0.02	0.06 ± 0.00	0.04 ± 0.00	0.43 ± 0.00	0.21 ± 0.00	0.25 ± 0.00	0.22 ± 0.05	0.19 ± 0.02	0.18 ± 0.00
C20:0	0.16 ± 0.00	nd	nd	0.44 ± 0.01	0.19 ± 0.01	0.92 ± 0.05	0.12 ± 0.00	0.59 ± 0.01	0.33 ± 0.00
C18.3n-3	0.12 ± 0.00	0.45 ± 0.04	0.52 ± 0.01	nd	0.05 ± 0.00	nd	0.04 ± 0.00	nd	0.08 ± 0.00
C18:4n-3	nd	nd	nd	0.23 ± 0.02	0.29 ± 0.00	0.35 ± 0.00	0.41 ± 0.03	0.86 ± 0.04	0.46 ± 0.01
C20:3n-6	0.91 ± 0.05	0.06 ± 0.00	0.04 ± 0.00	0.84 ± 0.03	1.50 ± 0.03	1.21 ± 0.14	1.58 ± 0.05	1.28 ± 0.07	1.46 ± 0.04
C22:0	0.31 ± 0.00	nd	nd	nd	0.19 ± 0.00	0.73 ± 0.05	nd	0.24 ± 0.00	0.04 ± 0.00
C20:4n-6	19.8 ± 0.23	14.1 ± 0.12	16.9 ± 0.07	17.6 ± 0.15	10.5 ± 0.20	12.1 ± 0.39	9.88 ± 0.38	11.3 ± 0.24	9.72 ± 0.11
C22:1n-9	nd	nd	nd	0.13 ± 0.00	nd	0.10 ± 0.00	nd	0.29 ± 0.00	0.27 ± 0.00
C20:5n-3	0.31 ± 0.02	0.49 ± 0.02	0.45 ± 0.03	1.5 ± 0.05	0.76 ± 0.01	1.75 ± 0.16	1.39 ± 0.09	5.52 ± 0.04	2.19 ± 0.02
C24:0	2.33 ± 0.02	3.68 ± 0.01	0.24 ± 0.00	0.66 ± 0.00	0.64 ± 0.06	0.39 ± 0.02	1.13 ± 0.04	0.53 ± 0.00	0.65 ± 0.01
C24:1	nd	2.45 ± 0.01	0.12 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.15 ± 0.00	0.09 ± 0.00	0.42 ± 0.01	0.32 ± 0.00
C22:5n-3	1.49 ± 0.09	1.63 ± 0.02	0.75 ± 0.02	1.77 ± 0.12	1.39 ± 0.16	1.86 ± 0.09	1.60 ± 0.10	2.97 ± 0.12	1.82 ± 0.14
C22:6n-3	5.86 ± 0.11	4.93 ± 0.04	6.03 ± 0.05	9.41 ± 0.28	13.9 ± 0.52	9.91 ± 0.89	14.3 ± 0.33	9.98 ± 0.24	14.8 ± 0.19
∑ SFA§	42.7 ± 0.35	40.43 ± 0.35	38.6 ± 0.28	38.4 ± 0.41	40.2 ± 0.67	39.5 ± 0.55	40.6 ± 0.64	39.1 ± 0.48	40.7 ± 0.41
∑ MUFA#	14.2 ± 0.12	25.38 ± 0.15	22.7 ± 0.12	16.2 ± 0.21	19.6 ± 0.12	19.6 ± 0.32	15.5 ± 0.23	14.3 ± 0.28	12.4 ± 0.14
∑ PUFA$	43.1 ± 0.42	34.02 ± 0.35	38.6 ± 0.29	45.1 ± 0.33	40.5 ± 0.49	42.7 ± 0.95	43.8 ± 0.45	46.1 ± 0.35	46.9 ± 0.25
n-3/n-6	0.29	0.28	0.25	0.4	0.69	0.48	0.68	0.72	0.7
AA/LA	1.66	1.15	1.23	1.32	0.91	0.79	0.69	0.81	0.6
AA/DGLA	21.79	235	423.25	20.95	7	10	6.25	8.83	6.66
EPA/AA	0.12	0.03	0.03	0.09	0.07	0.14	0.14	0.49	0.23
DHA/EPA	2.52	10.06	13.4	6.27	18.29	5.66	10.29	1.81	6.76
PI**	164.70	122.63	138.73	182.47	182.87	169.4	191.97	196.72	203.13

*Each parameter is present as the mean form three rat ± SD; SFA: Total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; LA: linoleic acid; DGLA: dihomogamma-linolenic acid; AA: arachidonic acid; EPA: eicosapentanoic acid and DHA: docosahexanoic acid. nd, not detected

** PI: Peroxidizability index (sum of percentages of individual fatty acids x number of active methylenes).

Table 4: Liver fatty acids composition (%) of different levels of GLA during eight weeks**

% GLA		Control		1% GLA		3% GLA		5% GLA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.57± 0.00	0.25 ± 0.00	0.28 ± 0.00	0.40 ± 0.01	0.64 ± 0.02	0.40 ± 0.05	0.42 ± 0.00	0.49 ± 0.01	0.51 ± 0.05
C16:0	21.7 ± 0.21	25.2 ± 0.21	25.6 ± 0.11	20.4 ± 0.18	22.5 ± 0.23	19.3 ± 0.32	22.0 ± 0.11	19.3 ± 0.21	23.6 ± 0.35
C16:1n-7	1.06 ± 0.00	0.23 ± 0.00	0.28 ± 0.02	1.86 ± 0.00	3.50 ± 0.06	1.35 ± 0.05	1.40 ± 0.02	1.64 ± 0.04	2.11 ± 0.11
C18:0	19.9 ± 0.17	11.3 ± 0.05	12.5 ± 0.09	16.5 ± 0.12	16.1 ± 0.18	17.6 ± 0.29	18.2 ± 0.14	17.2 ± 0.16	18.9 ± 0.21
C18:1n-9	10.8 ± 0.08	19.5 ± 0.10	19.2 ± 0.12	12.0 ± 0.08	11.9± 0.21	9.20 ± 0.18	9.05 ± 0.11	9.82 ± 0.18	10.77 ± 0.14
C18:1n-7	2.02 ± 0.04	3.20 ± 0.05	3.10 ± 0.03	1.97 ± 0.02	4.59 ± 0.04	2.10 ± 0.04	2.10 ± 0.02	1.78 ± 0.12	2.13 ± 0.01
C18:2n-6	11.9 ± 0.12	12.3 ± 0.16	13.8 ± 0.13	13.3 ± 0.09	11.6 ± 0.07	12.3 ± 0.10	12.7 ± 0.16	12.5 ± 0.14	11.6 ± 0.18
C18:3n-6	0.61 ± 0.02	0.06 ± 0.00	0.04 ± 0.00	0.43 ± 0.02	0.21 ± 0.00	0.98 ± 0.04	0.31 ± 0.01	1.10 ± 0.00	0.40 ± 0.01
C20:0	0.16 ± 0.00	nd	nd	0.44 ± 0.01	0.19 ± 0.00	0.17 ± 0.00	0.12 ± 0.00	0.37 ± 0.01	0.18 ± 0.00
C18.3n-3	0.12 ± 0.00	0.45 ± 0.04	0.52 ± 0.01	0.03 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
C18:4n-3	nd	nd	nd	0.23 ± 0.00	0.29 ± 0.04	0.30 ± 0.01	0.23 ± 0.03	0.47 ± 0.01	0.27 ± 0.00
C20:3n-6	0.91 ± 0.05	0.06 ± 0.00	0.04 ± 0.00	0.84 ± 0.03	1.5 ± 0.11	1.10 ± 0.03	1.47 ± 0.08	1.17 ± 0.01	1.42 ± 0.07
C22:0	0.31 ± 0.00	nd	nd	nd	0.19 ± 0.02	nd	0.15 ± 0.00	nd	0.16 ± 0.00
C20:4n-6	19.8 ± 0.23	14.1 ± 0.12	16.9 ± 0.07	18.1 ± 0.23	15.6 ± 0.32	25.9 ± 0.45	24.1 ± 0.32	24.9 ± 0.25	9.72 ± 0.11
C22:1n-9	nd	nd	nd	0.13 ± 0.00	nd	nd	nd	0.07 ± 0.00	0.27 ± 0.00
C20:5n-3	0.31 ± 0.02	0.49 ± 0.02	0.45 ± 0.03	1.50 ± 0.01	0.76 ± 0.04	0.28 ± 0.00	0.25 ± 0.04	0.45 ± 0.01	2.19 ± 0.02
C24:0	2.33 ± 0.02	3.68 ± 0.01	0.24 ± 0.00	0.66 ± 0.01	0.64 ± 0.06	0.90 ± 0.12	0.52 ± 0.11	1.39 ± 0.04	0.85 ± 0.04
C24:1	nd	2.45 ± 0.01	0.12 ± 0.00	nd	nd	0.30 ± 0.01	0.36 ± 0.04	0.03 ± 0.00	0.26 ± 0.02
C22:5n-3	1.49 ± 0.09	1.63 ± 0.02	0.75 ± 0.02	1.77 ± 0.04	1.39 ± 0.04	1.72 ± 0.01	1.14 ± 0.09	1.35 ± 0.04	0.93 ± 0.07
C22:6n-3	5.86 ± 0.11	4.93 ± 0.04	6.03 ± 0.05	9.41 ± 0.13	8.13 ± 0.24	5.96 ± 0.11	5.43 ± 0.17	5.59 ± 0.08	4.33 ± 0.21
SFA	42.7 ± 0.35	40.43 ± 0.35	38.6 ± 0.28	38.4 ± 0.28	40.3 ± 0.41	38.3 ± 0.61	41.4 ± 0.35	38.8 ± 0.37	44.2 ± 0.54
MUFA	14.2 ± 0.12	25.38 ± 0.15	22.7 ± 0.12	16.0 ± 0.07	20.0 ± 0.28	13.0 ± 0.21	12.9 ± 0.15	13.6 ± 0.35	15.3 ± 0.24
PUFA	43.1 ± 0.42	34.02 ± 0.35	38.6 ± 0.29	45.6 ± 0.35	39.5 ± 0.74	48.6 ± 0.58	45.7 ± 0.68	47.6 ± 0.55	40.5 ± 0.68
n-3/n-6	0.29	0.28	0.25	0.4	0.37	0.21	0.18	0.20	0.17
AA/LA	1.66	1.15	1.23	1.36	1.34	2.11	1.89	2.00	1.82
AA/DGLA	21.79	235	423.25	21.6	10.4	23.5	16.4	21.3	14.9
EPA/AA	0.12	0.03	0.03	0.08	0.05	0.01	0.01	0.02	0.02
DHA/EPA	2.52	10.06	13.4	6.27	10.70	21.29	21.72	12.42	12.72
PI	164.70	122.63	138.73	184.6	157.1	181.4	165.6	174.6	143.7

** Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 5: Plasma fatty acids composition (%) of different levels of DHA during eight weeks**

% DHA		Control		1% DHA		2% DHA		3% DHA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	1.41± 0.00	0.43 ± 0.00	0.38 ± 0.00	1.05 ± 0.03	1.36 ± 0.09	1.72 ± 0.02	1.92 ± 0.01	1.88 ± 0.02	1.56 ± 0.02
C16:0	28.7 ± 0.10	30.1 ± 0.14	31.1 ± 0.11	23.8 ± 0.15	26.3 ± 0.19	25.2 ± 0.25	26.8 ± 0.36	23.9 ± 0.16	24.7 ± 0.17
C16:1n-7	1.64 ± 0.00	0.52 ± 0.00	0.58 ± 0.02	0.53 ± 0.01	1.58 ± 0.02	1.74 ± 0.08	1.77 ± 0.02	1.32 ± 0.05	1.84 ± 0.04
C18:0	8.38 ± 0.07	12.5 ± 0.06	12.7 ± 0.09	12.1 ± 0.09	12.4 ± 0.06	8.91 ± 0.14	10.1 ± 0.15	10.3 ± 0.28	11.8 ± 0.19
C18:1n-9	17.5 ± 0.08	14.1 ± 0.11	15.2 ± 0.12	15.4 ± 0.14	16.4 ± 0.17	18.4 ± 0.21	18.8 ± 0.25	19.0 ± 0.06	19.3 ± 0.26
C18:1n-7	1.62 ± 0.04	nd	nd	1.94 ± 0.04	1.91 ± 0.09	1.16 ± 0.05	1.45 ± 0.06	1.69 ± 0.07	1.78 ± 0.09
C18:2n-6	21.1 ± 0.11	16.4 ± 0.16	9.70 ± 0.13	14.5 ± 0.28	14.3 ± 0.19	18.0 ± 0.24	17.1 ± 0.19	21.9 ± 0.29	21.1 ± 0.19
C18:3n-6	1.23 ± 0.02	0.11 ± 0.00	0.06 ± 0.00	0.20 ± 0.00	0.66 ± 0.00	0.39 ± 0.04	0.76 ± 0.04	0.27 ± 0.01	0.88 ± 0.03
C20:0	0.42 ± 0.00	nd	nd	0.32 ± 0.00	0.43 ± 0.03	0.34 ± 0.01	0.47 ± 0.01	0.35 ± 0.08	0.42 ± 0.02
C18.3n-3	1.26 ± 0.00	0.64 ± 0.04	0.54 ± 0.01	6.05 ± 0.11	4.41 ± 0.09	2.67 ± 0.09	2.64 ± 0.06	2.78 ± 0.07	1.56 ± 0.01
C18:4n-3	nd	0.28 ± 0.00	nd	1.69 ± 0.09	1.02 ± 0.11	1.56 ± 0.06	1.36 ± 0.05	1.59 ± 0.03	1.14 ± 0.04
C20:3n-6	0.73 ± 0.05	0.03 ± 0.00	0.02 ± 0.00	0.54 ± 0.00	0.54 ± 0.04	0.98 ± 0.04	0.78 ± 0.03	0.59 ± 0.02	0.85 ± 0.02
C22:0	0.63 ± 0.01	nd	nd	0.36 ± 0.00	0.31 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.27 ± 0.01	0.35 ± 0.01
C20:4n-6	8.89 ± 0.09	18.1 ± 0.12	24.2 ± 0.07	14.3 ± 0.35	8.93 ± 0.28	10.7 ± 0.35	7.04 ± 0.38	7.16 ± 0.29	4.37 ± 0.19
C22:1n-9	nd	nd	nd	0.38 ± 0.01	0.30 ± 0.02	0.63 ± 0.05	0.45 ± 0.04	0.21 ± 0.02	0.22 ± 0.01
C20:5n-3	3.08 ± 0.02	2.5 ± 0.02	0.48 ± 0.03	1.63 ± 0.04	1.04 ± 0.01	2.15 ± 0.07	1.57 ± 0.15	2.58 ± 0.05	2.09 ± 0.08
C24:0	0.24 ± 0.02	0.18 ± 0.01	0.21 ± 0.00	0.53 ± 0.01	0.63 ± 0.08	0.64 ± 0.01	0.67 ± 0.03	0.66 ± 0.08	0.59 ± 0.07
C24:1	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:5n-3	0.61 ± 0.09	0.60 ± 0.02	0.15 ± 0.02	0.85 ± 0.02	0.47 ± 0.01	0.79 ± 0.03	0.42 ± 0.01	0.71 ± 0.04	0.45 ± 0.08
C22:6n-3	2.02 ± 0.11	3.50 ± 0.04	4.50 ± 0.05	3.26 ± 0.18	6.95 ± 0.48	3.58 ± 0.36	5.53 ± 0.29	2.77 ± 0.13	4.85 ± 0.19
SFA	39.5 ± 0.18	43.2 ± 0.18	44.4 ± 0.18	38.2 ± 0.18	41.4 ± 0.28	37.2 ± 0.35	40.3 ± 0.45	37.4 ± 0.35	39.4 ± 0.28
MUFA	23.8 ± 0.11	14.6 ± 0.12	15.8 ± 0.09	18.3 ± 0.13	20.2 ± 0.21	21.9 ± 0.28	22.5 ± 0.28	22.2 ± 0.18	23.1 ± 0.29
PUFA	36.3 ± 0.25	42.1 ± 0.28	39.8 ± 0.23	43.9 ± 0.58	38.3 ± 0.94	40.9 ± 0.95	37.2 ± 0.65	40.4 ± 0.55	37.3 ± 0.59
n-3/n-6	0.14	0.22	0.17	0.46	0.57	0.36	0.45	0.35	0.37
AA/LA	0.42	1.11	2.49	0.99	0.62	0.60	0.41	0.33	0.21
AA/DGLA	12.18	603.33	1210.0	26.56	16.54	10.99	9.03	12.14	5.14
EPA/AA	0.05	0.14	0.02	0.11	0.12	0.20	0.22	0.36	0.48
DHA/EPA	4.70	1.40	9.38	2.00	6.68	1.67	3.52	1.07	2.32
PI	86.10	138.40	148.39	133.62	130.48	122.23	115.80	106.64	104.34

** Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 6: Plasma fatty acids composition (%) of different levels of GLA during eight weeks**

% GLA		Control		1% GLA		3% GLA		5% GLA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	1.41± 0.00	0.43 ± 0.00	0.38 ± 0.00	1.05 ± 0.03	1.26 ± 0.09	1.22 ± 0.02	1.28 ± 0.01	1.18 ± 0.02	1.24 ± 0.02
C16:0	28.7 ± 0.10	30.1 ± 0.14	31.1 ± 0.11	24.5 ± 0.18	25.6 ± 0.24	24.1 ± 0.23	26.7 ± 0.29	23.2 ± 0.23	27.0 ± 0.35
C16:1n-7	1.64 ± 0.00	0.52 ± 0.00	0.58 ± 0.02	1.53 ± 0.01	2.95 ± 0.01	1.01 ± 0.01	1.90 ± 0.07	1.91 ± 0.04	2.69 ± 0.02
C18:0	8.38 ± 0.07	12.5 ± 0.06	12.7 ± 0.09	10.1 ± 0.11	11.0 ± 0.14	11.2 ± 0.14	11.5 ± 0.24	11.7 ± 0.18	11.5 ± 0.17
C18:1n-9	17.5 ± 0.08	14.1 ± 0.11	15.2 ± 0.12	15.6 ± 0.12	14.4 ± 0.21	13.2 ± 0.08	14.4 ± 0.29	13.0 ± 0.12	15.0 ± 0.21
C18:1n-7	1.62 ± 0.04	nd	nd	1.94 ± 0.04	2.91 ± 0.05	1.85 ± 0.04	2.55 ± 0.05	1.76 ± 0.01	2.35 ± 0.04
C18:2n-6	21.1 ± 0.11	16.4 ± 0.16	9.70 ± 0.13	14.3 ± 0.21	20.2 ± 0.21	15.5 ± 0.09	19.3 ± 0.32	16.5 ± 0.18	19.5 ± 0.32
C18:3n-6	1.23 ± 0.02	0.11 ± 0.00	0.06 ± 0.00	0.25 ± 0.00	0.23 ± 0.00	1.62 ± 0.04	1.40 ± 0.04	1.70 ± 0.04	1.30 ± 0.07
C20:0	0.42 ± 0.00	nd	nd	0.32 ± 0.01	0.43 ± 0.01	0.32 ± 0.00	0.14 ± 0.00	0.33 ± 0.00	0.20 ± 0.00
C18.3n-3	1.26 ± 0.00	0.64 ± 0.04	0.54 ± 0.01	6.05 ± 0.17	1.41 ± 0.04	6.51 ± 0.21	1.18 ± 0.11	6.91 ± 0.15	1.19 ± 0.02
C18:4n-3	nd	0.28 ± 0.00	nd	1.89 ± 0.03	1.02 ± 0.02	0.43 ± 0.02	0.99 ± 0.08	0.90 ± 0.01	0.81 ± 0.01
C20:3n-6	0.73 ± 0.05	0.03 ± 0.00	0.02 ± 0.00	0.54 ± 0.02	0.84 ± 0.01	0.46 ± 0.04	1.01 ± 0.01	0.37 ± 0.04	0.89 ± 0.03
C22:0	0.63 ± 0.01	nd	nd	0.16 ± 0.00	0.11 ± 0.00	0.43 ± 0.00	0.10 ± 0.00	0.17 ± 0.00	0.21 ± 0.00
C20:4n-6	8.89 ± 0.09	18.1 ± 0.12	24.2 ± 0.07	14.3 ± 0.07	12.2 ± 0.21	13.6 ± 0.21	13.4 ± 0.35	13.8 ± 0.21	13.5 ± 0.17
C22:1n-9	nd	nd	nd	0.08 ± 0.00	0.03 ± 0.00	0.20 ± 0.00	0.14 ± 0.00	0.24 ± 0.00	0.12 ± 0.00
C20:5n-3	3.08 ± 0.02	2.5 ± 0.02	0.48 ± 0.03	1.63 ± 0.02	1.18 ± 0.02	5.28 ± 0.11	2.14 ± 0.07	3.07 ± 0.02	2.01 ± 0.09
C24:0	0.24 ± 0.02	0.18 ± 0.01	0.21 ± 0.00	1.01 ± 0.02	0.63 ± 0.01	0.85 ± 0.04	0.60 ± 0.02	0.85 ± 0.11	0.78 ± 0.04
C24:1	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:5n-3	0.61 ± 0.09	0.60 ± 0.02	0.15 ± 0.02	0.55 ± 0.04	0.47 ± 0.00	0.43 ± 0.01	0.25 ± 0.01	0.61 ± 0.04	0.35 ± 0.00
C22:6n-3	2.02 ± 0.11	3.50 ± 0.04	4.50 ± 0.05	4.06 ± 0.10	3.05 ± 0.04	1.64 ± 0.42	1.04 ± 0.01	1.82 ± 0.05	1.01 ± 0.08
SFA	39.5 ± 0.18	43.2 ± 0.18	44.4 ± 0.18	37.2 ± 0.16	39.0 ± 0.40	38.1 ± 0.12	40.4 ± 0.52	37.4 ± 0.16	40.9 ± 0.54
MUFA	23.8 ± 0.11	14.6 ± 0.12	15.8 ± 0.09	19.2 ± 0.48	20.3 ± 0.27	16.2 ± 0.51	18.9 ± 0.39	16.9 ± 0.19	18.75 ± 0.23
PUFA	36.3 ± 0.25	42.1 ± 0.28	39.8 ± 0.23	43.5 ± 0.18	40.6 ± 0.54	45.5 ± 0.23	40.7 ± 0.94	45.6 ± 0.37	40.4 ± 0.74
n-3/n-6	0.14	0.22	0.17	0.48	0.21	0.46	0.16	0.41	0.15
AA/LA	0.42	1.11	2.49	1.00	0.60	0.88	0.70	0.83	0.70
AA/DGLA	12.18	603.33	1210.0	26.39	14.52	29.70	13.28	37.19	15.21
EPA/AA	0.05	0.14	0.02	0.11	0.10	0.39	0.16	0.22	0.15
DHA/EPA	4.70	1.40	9.38	2.49	2.58	0.31	0.49	0.59	0.40
PI	86.10	138.40	148.39	138.5	112.8	136.8	107.2	130.2	104.7

** Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 7: Brain fatty acids composition (%) of different levels of DHA during eight weeks**.

% DHA		Control		1% DHA		2% DHA		3% DHA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.87 ± 0.00	1.40 ± 0.00	1.60 ± 0.01	0.24 ± 0.00	0.41 ± 0.02	0.21 ± 0.01	0.34 ± 0.02	0.52 ± 0.03	0.4 ± 0.01
C16:0	25.3 ± 0.11	26.8 ± 0.12	26.4 ± 0.14	21.2 ± 0.15	21.6 ± 0.12	21.8 ± 0.11	22.4 ± 0.16	23.1 ± 0.15	22.1± 0.15
C16:1n-7	1.46 ± 0.01	1.15 ± 0.01	1.24 ± 0.03	0.47 ± 0.01	0.47 ± 0.03	0.45 ± 0.00	0.41 ± 0.01	0.6 ± 0.05	0.4 ± 0.02
C18:0	18.8 ± 0.12	21.7 ± 0.05	22.1 ± 0.08	20.2 ± 0.31	21.5 ± 0.25	21.7 ± 0.14	22.1 ± 0.18	21.7 ± 0.12	20.9 ± 0.15
C18:1n-9	19.5 ± 0.04	18.0 ± 0.07	18.4 ± 0.04	19.5 ± 0.25	19.1 ± 0.08	18.4 ± 0.23	18.0 ± 0.35	18.7 ± 0.28	18.2 ± 0.23
C18:1n-7	2.82 ± 0.01	4.98 ± 0.03	3.50 ± 0.01	4.26 ± 0.03	4.01 ± 0.01	4.4 ± 0.1	4.01 ± 0.01	4.3 ± 0.15	4.01 ± 0.02
C18:2n-6	8.51 ± 0.02	1.42 ± 0.00	1.53 ± 0.02	0.94 ± 0.01	0.97 ± 0.05	0.93 ± 0.02	0.8 ± 0.02	1.12 ± 0.05	0.95 ± 0.02
C18:3n-6	0.15 ± 0.00	1.04 ± 0.00	0.11 ± 0.00	0.07 ± 0.00	nd	0.05 ± 0.00	0.1 ± 0.01	0.85 ± 0.04	0.11 ± 0.01
C20:0	0.15 ± 0.00	0.12 ± 0.00	0.30 ± 0.00	0.07 ± 0.00	nd	0.03 ± 0.00	nd	0.16 ± 0.00	nd
C18.3n-3	0.15 ± 0.00	0.06 ± 0.00	0.41 ± 0.00	0.49 ± 0.02	0.52 ± 0.00	0.5 ± 0.01	0.41 ± 0.02	0.46 ± 0.01	0.44 ± 0.01
C18:4n-3	nd	0.75 ± 0.01	0.78 ± 0.01	1.61 ± 0.03	1.82 ± 0.01	1.36 ± 0.02	1.89 ± 0.05	1.74 ± 0.21	1.72 ± 0.04
C20:3n-6	1.01 ± 0.01	1.28 ± 0.04	0.25 ± 0.00	0.69 ± 0.01	0.91 ± 0.06	0.53 ± 0.00	1.1 ± 0.02	0.65 ± 0.02	1.53 ± 0.05
C22:0	0.14 ± 0.00	0.42 ± 0.00	0.45 ± 0.01	0.47 ± 0.02	0.52 ± 0.01	0.55 ± 0.00	0.5 ± 0.01	0.48 ± 0.02	0.69 ± 0.02
C20:4n-6	12.9 ± 0.15	9.74 ± 0.08	13.2 ± 0.13	11.8 ± 0.15	10.5 ± 0.23	11.3 ± 0.14	9.69 ± 0.24	9.93 ± 0.25	9.01 ± 0.21
C22:1n-9	nd	0.12 ± 0.00	0.05 ± 0.00	0.4 ± 0.01	0.19 ± 0.01	0.31 ± 0.00	0.29 ± 0.01	0.35 ± 0.01	0.71 ± 0.02
C20:5n-3	0.24 ± 0.00	0.49 ± 0.00	1.07 ± 0.03	0.49 ± 0.01	0.45 ± 0.00	0.49 ± 0.03	1.07 ± 0.04	0.44 ± 0.01	1.78 ± 0.06
C24:0	1.68 ± 0.01	3.56 ± 0.02	1.45 ± 0.03	2.82 ± 0.02	1.85 ± 0.04	2.42 ± 0.01	1.55 ± 0.03	1.65 ± 0.02	1.71 ± 0.03
C24:1	nd	1.46 ± 0.01	0.35 ± 0.00	0.59 ± 0.00	0.96 ± 0.01	0.96 ± 0.01	0.63 ± 0.01	0.24 ± 0.01	0.6 ± 0.01
C22:5n-3	0.23 ± 0.00	0.25 ± 0.00	0.23 ± 0.00	0.32 ± 0.02	0.25 ± 0.00	0.3 ± 0.00	0.25 ± 0.02	0.43 ± 0.01	0.65 ± 0.02
C22:6n-3	6.05 ± 0.05	5.10 ± 0.07	6.55 ± 0.03	13.2 ± 0.11	13.9 ± 0.08	13.3 ± 0.11	14.4 ± 0.41	12.6 ± 0.53	14.0 ± 0.24
∑ SFA[§]	45.3 ± 0.22	54.0 ± 0.18	52.3 ± 0.21	45.0 ± 0.23	45.9 ± 0.19	46.7 ± 0.16	46.9 ± 0.19	47.3 ± 0.11	45.8 ± 0.12
∑ MUFA[#]	24.0 ± 0.04	25.7 ± 0.09	23.5 ± 0.03	25.2 ± 0.18	24.7 ± 0.23	24.5 ± 0.23	23.4 ± 0.34	24.2 ± 0.12	23.9 ± 0.15
∑ PUFA[$]	30.7 ± 0.19	20.1 ± 0.10	24.1 ± 0.21	29.6 ± 0.14	29.3 ± 0.28	28.8 ± 0.12	29.7 ± 0.48	28.4 ± 0.38	29.9 ± 0.31
n-3/n-6	0.36	0.49	0.60	1.19	1.37	1.25	1.54	1.27	1.58
AA/LA	1.52	6.86	8.63	12.55	10.82	12.15	12.11	8.87	9.48
AA/DGLA	12.77	7.61	52.80	17.10	11.54	21.32	8.81	15.28	5.89
EPA/AA	0.13	0.05	0.08	0.04	0.04	0.04	0.11	0.04	0.20
DHA/EPA	3.60	10.41	6.12	26.94	30.89	27.14	13.46	28.64	7.87
PI[*]	123.19	94.02	119.78	168.17	169.13	165.48	174.04	159.66	173.89

** Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 8: Brain fatty acids composition (%) of different levels of GLA during eight weeks**

% GLA		Control		1% GLA		3% GLA		5% GLA	
Weeks	**At birth**	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.87 ± 0.00	1.40 ± 0.00	1.60 ± 0.01	0.24 ± 0.00	0.41 ± 0.02	0.32 ± 0.01	0.13 ± 0.02	0.30 ± 0.03	0.13 ± 0.01
C16:0	25.3 ± 0.11	26.8 ± 0.12	26.4 ± 0.14	21.2 ± 0.32	20.3 ± 0.24	21.1 ± 0.23	21.2 ± 0.18	21.4 ± 0.12	20.1 ± 0.32
C16:1n-7	1.46 ± 0.01	1.15 ± 0.01	1.24 ± 0.03	0.47 ± 0.01	0.47 ± 0.01	0.61 ± 0.01	0.45 ± 0.05	0.46 ± 0.01	0.38 ± 0.01
C18:0	18.8 ± 0.12	21.7 ± 0.05	22.1 ± 0.08	20.2 ± 0.12	21.3 ± 0.21	21.4 ± 0.29	21.7 ± 0.24	19.9 ± 0.14	21.9 ± 0.21
C18:1n-9	19.5 ± 0.04	18.0 ± 0.07	18.4 ± 0.04	19.5 ± 0.11	20.1 ± 0.14	18.9 ± 0.24	19.4 ± 0.32	19.2 ± 0.12	18.5 ± 0.25
C18:1n-7	2.82 ± 0.01	4.98 ± 0.03	3.50 ± 0.01	4.33 ± 0.09	4.58 ± 0.09	4.29 ± 0.11	4.58 ± 0.17	4.32 ± 0.09	4.08 ± 0.17
C18:2n-6	8.51 ± 0.02	1.42 ± 0.00	1.53 ± 0.02	0.94 ± 0.02	0.92 ± 0.05	1.35 ± 0.02	0.79 ± 0.04	1.37 ± 0.03	0.76 ± 0.04
C18:3n-6	0.15 ± 0.00	1.04 ± 0.00	0.11 ± 0.00	0.13 ± 0.00	0.20 ± 0.00	0.24 ± 0.00	0.02 ± 0.00	0.48 ± 0.00	0.02 ± 0.00
C20:0	0.15 ± 0.00	0.12 ± 0.00	0.30 ± 0.00	nd	nd	0.02 ± 0.00	nd	0.02 ± 0.00	nd
C18.3n-3	0.15 ± 0.00	0.06 ± 0.00	0.41 ± 0.00	0.49 ± 0.01	0.52 ± 0.00	0.39 ± 0.00	0.45 ± 0.02	0.56 ± 0.01	0.54 ± 0.01
C18:4n-3	nd	0.75 ± 0.01	0.78 ± 0.01	1.61 ± 0.04	2.00 ± 0.02	1.41 ± 0.07	1.98 ± 0.06	1.73 ± 0.09	1.78 ± 0.11
C20:3n-6	1.01 ± 0.01	1.28 ± 0.04	0.25 ± 0.00	0.69 ± 0.01	0.91 ± 0.01	0.53 ± 0.01	0.73 ± 0.04	0.71 ± 0.04	1.25 ± 0.07
C22:0	0.14 ± 0.00	0.42 ± 0.00	0.45 ± 0.01	0.47 ± 0.00	0.52 ± 0.00	0.39 ± 0.00	0.39 ± 0.00	0.52 ± 0.00	0.38 ± 0.00
C20:4n-6	12.9 ± 0.15	9.74 ± 0.08	13.2 ± 0.13	11.8 ± 0.14	10.5 ± 0.21	11.1 ± 0.23	10.9 ± 0.21	11.7 ± 0.11	11.2 ± 0.25
C22:1n-9	nd	0.12 ± 0.00	0.05 ± 0.00	nd	nd	0.16 ± 0.00	0.28 ± 0.00	0.21 ± 0.00	0.29 ± 0.00
C20:5n-3	0.24 ± 0.00	0.49 ± 0.00	1.07 ± 0.03	0.31 ± 0.02	0.30 ± 0.00	0.28 ± 0.00	0.56 ± 0.04	0.63 ± 0.02	1.03 ± 0.06
C24:0	1.68 ± 0.01	3.56 ± 0.02	1.45 ± 0.03	2.82 ± 0.12	1.65 ± 0.08	3.35 ± 0.01	2.43 ± 0.02	3.34 ± 0.08	2.88 ± 0.07
C24:1	nd	1.46 ± 0.01	0.35 ± 0.00	0.59 ± 0.01	0.96 ± 0.04	0.66 ± 0.07	0.61 ± 0.01	0.73 ± 0.01	1.64 ± 0.03
C22:5n-3	0.23 ± 0.00	0.25 ± 0.00	0.23 ± 0.00	0.32 ± 0.01	0.25 ± 0.01	0.30 ± 0.01	0.21 ± 0.00	0.18 ± 0.00	0.25 ± 0.00
C22:6n-3	6.05 ± 0.05	5.10 ± 0.07	6.55 ± 0.03	13.4 ± 0.21	13.9 ± 0.23	12.9 ± 0.28	13.1 ± 0.21	12.3 ± 0.18	12.9 ± 0.23
∑ SFA§	45.3 ± 0.22	54.0 ± 0.18	52.3 ± 0.21	45.0 ± 0.45	29.6 ± 0.51	46.8 ± 0.28	45.7 ± 0.58	45.5 ± 0.45	46.4 ± 0.62
∑ MUFA#	24.0 ± 0.04	25.7 ± 0.09	23.5 ± 0.03	25.1 ± 0.25	17.0 ± 0.24	24.6 ± 0.52	25.3 ± 0.32	24.9 ± 0.29	24.8 ± 0.35
∑ PUFA$	30.7 ± 0.19	20.1 ± 0.10	24.1 ± 0.21	29.9 ± 0.17	11.6 ± 0.23	28.6 ± 0.29	28.7 ± 0.24	29.6 ± 0.18	29.7 ± 0.32
n-3/n-6	0.36	0.49	0.6	1.21	1.36	1.16	1.31	1.08	1.25
AA/LA	1.52	6.86	8.63	12.5	11.4	8.25	13.8	8.5	14.7
AA/DGLA	12.77	7.61	52.8	17.0	11.5	21.0	14.9	16.4	8.9
EPA/AA	0.13	0.05	0.08	0.04	0.029	0.03	0.05	0.05	0.09
DHA/EPA	3.60	10.41	6.12	27.43	46.5	46.32	23.34	19.52	12.55
PI*	123.19	94.02	119.78	170	169.8	161.7	164.5	162.3	167.9

** Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 9: Fatty acids compositions (%) of liver phospholipids after 4 and 8 weeks*

% DHA	1% DHA		2 % DHA		3% DHA	
Weeks	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	1.51 ± 0.02	1.53 ± 0.12	1.22 ± 0.10	1.26 ± 0.02	0.58 ± 0.03	0.68 ± 0.02
C16:0	34.0 ± 0.23	34.0 ± 0.49	33.3 ± 0.32	33.1 ± 0.35	32.2 ± 0.39	32.1 ± 0.65
C16:1n-7	1.52 ± 0.11	1.79 ± 0.12	1.63 ± 0.11	1.65 ± 0.14	0.88 ± 0.08	0.97 ± 0.08
C18:0	24.1 ± 0.24	25.6 ± 0.35	24.6 ± 0.32	25.7 ± 0.28	25.2 ± 0.31	25.7 ± 0.24
C18:1n-9	13.2 ± 0.15	13.3 ± 0.12	13.7 ± 0.15	12.7 ± 0.18	11.3 ± 0.12	11.1 ± 0.21
C18:1n-7	2.75 ± 0.12	2.93 ± 0.09	2.90 ± 0.05	3.94 ± 0.06	1.68 ± 0.08	1.72 ± 0.09
C18:2n-6	5.96 ± 0.54	5.13 ± 0.32	6.90 ± 0.35	6.94 ± 0.27	11.2 ± 0.35	12.3 ± 0.32
C18:3n-6	0.96 ± 0.15	0.60 ± 0.11	0.58 ± 0.02	0.51 ± 0.00	0.26 ± 0.00	0.21 ± 0.11
C20:0	0.18 ± 0.00	0.16 ± 0.00	nd	nd	nd	nd
C18.3n-3	1.89 ± 0.12	0.62 ± 0.08	1.90 ± 0.03	0.92 ± 0.03	0.82 ± 0.11	0.45 ± 0.12
C18:4n-3	0.49 ± 0.12	0.33 ± 0.00	0.42 ± 0.02	0.26 ± 0.02	1.11 ± 0.01	0.80 ± 0.13
C20:1n-9	0.62 ± 0.11	0.55 ± 0.00	0.43 ± 0.03	0.46 ± 0.01	1.10 ± 0.09	0.96 ± 0.21
C22:0	0.58 ± 0.02	0.65 ± 0.01	0.88 ± 0.12	0.72 ± 0.09	0.50 ± 0.02	0.40 ± 0.04
C20:4n-6	7.02 ± 0.28	4.27 ± 0.24	6.10 ± 0.15	2.97 ± 0.21	5.81 ± 0.56	2.94 ± 0.19
C22:1n-9	0.35 ± 0.02	0.10 ± 0.00	0.50 ± 0.03	0.55 ± 0.01	0.12 ± 0.00	0.40 ± 0.03
C20:5n-3	0.71 ± 0.04	0.50 ± 0.02	0.73 ± 0.11	0.15 ± 0.00	1.76 ± 0.05	1.02 ± 0.04
C24:0	1.23 ± 0.13	1.13 ± 0.01	1.03 ± 0.03	1.14 ± 0.03	0.66 ± 0.03	0.68 ± 0.08
C22:5n-	0.22 ± 0.00	0.18 ± 0.00	0.21 ± 0.01	0.06 ± 0.00	1.67 ± 0.04	0.39 ± 0.03
C22:6n-3	2.62 ± 0.14	6.54 ± 0.31	2.69 ± 0.21	6.95 ± 0.32	2.89 ± 0.21	6.98 ± 0.54
SFA	61.6 ± 0.45	63.1 ± 0.65	61.0 ± 0.65	61.9 ± 0.63	59.1 ± 0.69	59.6 ± 0.75
MUFA	17.8 ± 0.32	18.1 ± 0.21	18.7 ± 0.21	18.9 ± 0.32	13.9 ± 0.25	14.2 ± 0.27
PUFA	20.5 ± 0.65	18.7 ± 0.60	19.9 ± 0.52	19.2 ± 0.71	26.6 ± 0.87	26.1 ± 0.75
n-3/n-6	0.41	0.77	0.42	0.77	0.45	0.59
AA/LA	1.18	0.83	0.88	0.43	0.52	0.24
AA/DGLA	11.32	7.76	14.19	6.46	5.28	3.06
EPA/AA	0.10	0.12	0.12	0.05	0.30	0.35
DHA/EPA	3.69	13.08	3.68	46.33	1.64	6.84
PI	69.93	83.92	66.43	80.97	87.29	95.15

* Each parameter given is the mean from three rat's ± SD. For abbreviations see table 3.

Table 10: Fatty acids composition (%) of liver phospholipids after 4 and 8 weeks*

% GLA	1% GLA		3% GLA		5% GLA	
Weeks	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	1.56 ± 0.08	1.62 ± 0.04	0.49 ± 0.01	0.82 ± 0.03	0.57 ± 0.01	0.64 ± 0.03
C16:0	22.4 ± 0.17	26.4 ± 0.21	26.8 ± 0.29	28.0 ± 0.32	24.4 ± 0.38	27.7 ± 0.39
C16:1n-7	1.93 ± 0.11	1.66 ± 0.03	1.25 ± 0.03	2.08 ± 0.08	1.23 ± 0.04	0.99 ± 0.04
C18:0	24.0 ± 0.32	25.6 ± 0.21	23.0 ± 0.17	25.2 ± 0.28	24.9 ± 0.28	25.8 ± 0.38
C18:1n-9+n-7	15.1 ± 0.27	16.9 ± 0.24	11.2 ± 0.21	13.3 ± 0.15	12.3 ± 0.21	13.2 ± 0.28
C18:2n-6	6.15 ± 0.12	5.05 ± 0.11	7.92 ± 0.15	6.59 ± 0.21	8.74 ± 0.15	7.28 ± 0.19
C18:3n-6	0.99 ± 0.05	0.64 ± 0.07	0.58 ± 0.02	0.27 ± 0.00	0.85 ± 0.11	0.19 ± 0.00
C20:0	0.68 ± 0.01	0.16 ± 0.00	0.26 ± 0.00	0.75 ± 0.01	0.16 ± 0.00	0.19 ± 0.00
C18.3n-3	1.10 ± 0.06	0.65 ± 0.05	0.96 ± 0.04	0.99 ± 0.05	0.82 ± 0.08	0.82 ± 0.05
C18:4n-3	0.51 ± 0.01	0.35 ± 0.03	0.30 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.42 ± 0.04
C20:3n-6	0.65 ± 0.02	0.58 ± 0.01	0.76 ± 0.06	0.65 ± 0.01	0.89 ± 0.03	1.54 ± 0.07
C22:0	1.48 ± 0.06	1.41 ± 0.07	0.55 ± 0.01	1.59 ± 0.09	0.39 ± 0.01	0.81 ± 0.06
C20:4n-6	12.1 ± 0.34	10.5 ± 0.11	16.8 ± 0.19	12.4 ± 0.32	16.9 ± 0.21	13.9 ± 0.18
C22:1n-9	0.37 ± 0.00	0.10 ± 0.00	nd	nd	nd	nd
C20:5n-3	0.74 ± 0.07	0.53 ± 0.02	0.99 ± 0.08	0.62 ± 0.01	0.88 ± 0.08	0.19 ± 0.00
C24:0	0.66 ± 0.03	0.93 ± 0.07	0.74 ± 0.04	0.63 ± 0.03	0.68 ± 0.04	0.68 ± 0.07
C22:5n-3	0.98 ± 0.03	0.23 ± 0.00	0.82 ± 0.05	0.59 ± 0.02	0.63 ± 0.02	0.62 ± 0.01
C22:6n-3	8.51 ± 0.11	6.25 ± 0.21	6.53 ± 0.09	5.35 ± 0.15	6.02 ± 0.21	4.99 ± 0.11
SFA	50.9 ± 0.55	56.2 ± 0.51	51.9 ± 0.46	57.0 ± 0.68	51.1 ± 0.67	55.9 ± 0.45
MUFA	17.4 ± 0.37	18.7 ± 0.26	12.4 ± 0.23	15.3 ± 0.23	13.5 ± 0.25	14.2 ± 0.32
PUFA	11.8 ± 0.27	8.01 ± 0.32	9.61 ± 0.25	7.85 ± 0.24	8.65 ± 0.32	7.03 ± 0.22
n-3/n-6	0.60	0.48	0.37	0.39	0.32	0.31
AA/LA	1.97	2.08	2.12	1.88	1.93	1.91
AA/DGLA	18.62	18.10	22.11	19.08	18.99	9.03
EPA/AA	0.06	0.05	0.06	0.05	0.05	0.01
DHA/EPA	11.50	11.79	6.60	8.63	6.84	26.26
PI	140.91	107.22	144.33	111.65	140.21	114.79

* Each parameter given is the mean from three rat's ± SD. For abbreviations see Table 3.

Table 11: Fatty acids compositions (%) of brain phospholipids after 4 and 8 weeks*

% DHA	1% DHA		2% DHA		3% DHA	
Weeks	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.93 ± 0.02	1.04 ± 0.06	0.62 ± 0.03	0.64 ± 0.05	0.67 ± 0.04	0.62 ± 0.09
C16:0	37.5 ± 0.25	37.6 ± 0.32	37.0 ± 0.28	37.2 ± 0.35	33.7 ± 0.39	33.8 ± 0.21
C16:1n-7	1.20 ± 0.02	1.44 ± 0.05	1.09 ± 0.06	1.26 ± 0.02	0.82 ± 0.02	1.37 ± 0.05
C18:0	17.3 ± 0.15	17.6 ± 0.21	17.2 ± 0.18	17.3 ± 0.16	18.3 ± 0.21	18.6 ± 0.24
C18:1n-9	19.5 ± 0.18	19.8 ± 0.23	20.4 ± 0.31	20.6 ± 0.24	20.4 ± 0.25	20.9 ± 0.21
C18:1n-7	5.47 ± 0.06	5.77 ± 0.11	4.72 ± 0.08	4.83 ± 0.09	5.60 ± 0.14	4.78 ± 0.11
C18:2n-6	0.76 ± 0.02	0.72 ± 0.05	0.95 ± 0.05	1.56 ± 0.02	0.95 ± 0.07	2.16 ± 0.09
C18:3n-6	0.44 ± 0.01	0.57 ± 0.02	0.30 ± 0.00	0.68 ± 0.03	0.28 ± 0.02	0.79 ± 0.07
C20:0	nd	nd	0.36 ± 0.00	0.41 ± 0.08	0.43 ± 0.04	0.41 ± 0.04
C18.3n-3	1.36 ± 0.06	0.84 ± 0.09	1.74 ± 0.02	0.31 ± 0.00	1.88 ± 0.06	0.91 ± 0.02
C18:4n-3	2.07 ± 0.08	1.53 ± 0.07	1.72 ± 0.03	1.42 ± 0.05	2.67 ± 0.04	2.13 ± 0.11
C20:3n-6	0.20 ± 0.00	0.24 ± 0.00	0.33 ± 0.05	0.55 ± 0.02	0.36 ± 0.01	0.54 ± 0.03
C22:0	0.41 ± 0.04	0.18 ± 0.05	0.41 ± 0.02	0.26 ± 0.01	0.60 ± 0.01	0.28 ± 0.04
C20:4n-6	6.28 ± 0.19	3.92 ± 0.21	5.90 ± 0.09	3.79 ± 0.11	5.82 ± 0.18	3.70 ± 0.10
C22:1n-9	0.48 ± 0.01	0.24 ± 0.02	0.13 ± 0.00	0.43 ± 0.03	0.08 ± 0.00	0.06 ± 0.00
C20:5n-3	0.40 ± 0.02	0.22 ± 0.01	0.56 ± 0.02	0.22 ± 0.00	0.58 ± 0.00	0.28 ± 0.00
C24:0	1.40 ± 0.04	1.41 ± 0.02	1.68 ± 0.11	1.72 ± 0.12	1.61 ± 0.08	1.56 ± 0.09
C22:5n-	0.15 ± 0.02	0.10 ± 0.00	0.32 ± 0.00	0.22 ± 0.03	0.21 ± 0.00	0.12 ± 0.00
C22:6n-3	3.78 ± 0.14	6.76 ± 0.28	3.98 ± 0.19	6.91 ± 0.21	4.78 ± 0.32	6.81 ± 0.21
SFA	57.5 ± 0.37	57.8 ± 0.48	57.3 ± 0.41	57.5 ± 0.42	55.3 ± 0.58	55.3 ± 0.41
MUFA	26.7 ± 0.18	27.3 ± 0.35	26.4 ± 0.34	26.8 ± 0.28	26.9 ± 0.38	27.1 ± 0.28
PUFA	15.4 ± 0.30	14.9 ± 0.41	15.8 ± 0.28	15.7 ± 0.25	17.5 ± 0.45	17.4 ± 0.32
n-3/n-6	1.01	1.73	1.11	1.38	1.37	1.43
AA/LA	8.26	5.44	6.21	2.43	6.13	1.71
AA/DGLA	31.40	16.33	17.88	6.89	16.17	6.85
EPA/AA	0.06	0.06	0.09	0.06	0.10	0.08
DHA/EPA	9.45	30.73	7.11	31.41	8.24	24.32
PI	72.37	82.50	73.95	84.07	83.60	87.52

* Each parameter given is the mean from three rat's ± SD. For abbreviations see Table 3.

Table 12: Fatty acids composition (%) of Brain phospholipids after 4 and 8 weeks*

% GLA	1% GLA		3% GLA		5% GLA	
Weeks	**4**	**8**	**4**	**8**	**4**	**8**
C14:0	0.95 ± 0.02	1.07 ± 0.05	0.71 ± 0.02	0.98 ± 0.08	0.45 ± 0.02	0.54 ± 0.02
C16:0	30.4 ± 0.23	32.5 ± 0.32	32.5 ± 0.21	32.9 ± 0.35	30.9 ± 0.32	32.0 ± 0.38
C16:1n-7	1.47 ± 0.02	1.23 ± 0.04	1.28 ± 0.04	1.15 ± 0.09	0.59 ± 0.00	0.53 ± 0.02
C18:0	18.8 ± 0.25	19.1 ± 0.19	18.2 ± 0.21	17.5 ± 0.11	20.1 ± 0.09	22.5 ± 0.21
C18:1n-9+n-7	25.2 ± 0.32	25.3 ± 0.21	24.0 ± 0.30	24.0 ± 0.24	24.1 ± 0.12	24.1 ± 0.25
C18:2n-6	0.78 ± 0.05	0.73 ± 0.01	2.83 ± 0.11	3.31 ± 0.13	2.55 ± 0.06	0.95 ± 0.01
C18:3n-6	0.46 ± 0.01	0.59 ± 0.03	0.58 ± 0.07	0.91 ± 0.07	0.48 ± 0.02	0.99 ± 0.01
C20:0	0.40 ± 0.02	0.62 ± 0.01	0.44 ± 0.05	0.44 ± 0.01	0.57 ± 0.03	0.63 ± 0.02
C18.3n-3	0.87 ± 0.08	1.39 ± 0.05	1.23 ± 0.04	2.27 ± 0.12	0.59 ± 0.03	0.89 ± 0.01
C18:4n-3	1.57 ± 0.04	2.11 ± 0.11	1.18 ± 0.03	1.31 ± 0.10	1.70 ± 0.01	2.04 ± 0.03
C20:3n-6	0.21 ± 0.00	0.25 ± 0.00	0.22 ± 0.00	0.87 ± 0.02	0.48 ± 0.01	0.36 ± 0.01
C22:0	0.83 ± 0.05	0.95 ± 0.02	0.50 ± 0.01	0.85 ± 0.01	0.57 ± 0.04	0.79 ± 0.01
C20:4n-6	7.98 ± 0.15	5.64 ± 0.11	8.23 ± 0.21	5.98 ± 0.09	8.45 ± 0.11	6.25 ± 0.11
C22:1n-9	0.49 ± 0.02	0.27 ± 0.01	0.12 ± 0.00	0.23 ± 0.00	0.50 ± 0.02	0.33 ± 0.00
C20:5n-3	0.41 ± 0.00	0.43 ± 0.02	0.23 ± 0.00	0.47 ± 0.01	0.60 ± 0.01	0.79 ± 0.01
C24:0	1.41 ± 0.11	1.44 ± 0.03	1.32 ± 0.01	1.59 ± 0.18	1.57 ± 0.11	1.61 ± 0.03
C22:5n-3	0.10 ± 0.00	0.16 ± 0.00	0.11 ± 0.00	0.14 ± 0.00	0.19 ± 0.00	0.13 ± 0.00
C22:6n-3	7.62 ± 0.15	6.18 ± 0.11	6.36 ± 0.21	5.12 ± 0.14	5.57 ± 0.24	4.61 ± 0.08
SFA	52.8 ± 0.48	55.7 ± 0.54	53.6 ± 0.47	54.2 ± 0.49	54.2 ± 0.44	58.1 ± 0.60
MUFA	27.2 ± 0.33	26.8 ± 0.25	25.4 ± 0.33	25.4 ± 0.32	25.1 ± 0.13	24.9 ± 0.26
PUFA	20.0 ± 0.48	17.5 ± 0.35	20.9 ± 0.54	20.4 ± 0.61	20.6 ± 0.41	17.0 ± 0.25
n-3/n-6	1.12	1.42	0.77	0.84	0.72	0.99
AA/LA	10.2	7.73	2.91	1.81	3.31	6.58
AA/DGLA	38.00	22.56	37.41	6.87	17.60	17.36
EPA/AA	0.05	0.08	0.03	0.08	0.07	0.13
DHA/EPA	18.59	14.37	27.65	10.89	9.28	5.84
PI	106.76	89.84	98.09	85.82	96.18	81.61

* Each parameter given is the mean from three rat's ± SD. For abbreviations see Table 3.

Table 13: Concentration of alpha tocopherols at all time points in tissues and plasma in omega 3 group and omega 6 groups.

Groups	Tissues	Weeks / Formulas	2	4	6	8
Omega-3 group	Brain µg /total brain	1 DHA	78.62	48.57	18.57	9.88
		2 DHA	69.21	41.46	13.58	8.18
		3 DHA	55.34	39.04	8.44	6.11
	Liver µg/total liver	1 DHA	89.97	80.29	77.9	24.68
		2 DHA	77.26	65.74	57.26	18.99
		3 DHA	65.67	41.61	19.57	15.53
	Plasma mg/ L Plasma	1 DHA	5.34	11.1	4.30	5.66
		2 DHA	7.48	8.52	5.70	5.74
		3 DHA	8.90	8.62	4.78	3.00
Omega-6 group	Brain µg/total brain	1 gamma	72.51	47.57	18.97	7.88
		3 gamma	65.91	45.46	16.24	6.94
		5 gamma	53.12	32.54	16.82	6.84
	Liver µg/total liver	1 gamma	80.29	77.9	24.68	21.13
		3 gamma	77.15	68.41	21.13	16.6
		5 gamma	62.52	58.77	18.04	12.5
	Plasma mg/L plasma	1 gamma	3.10	5.34	4.30	5.66
		3 gamma	1.98	8.82	6.58	5.68
		5 gamma	1.76	4.08	2.50	2.48

Table 14: Percent of fatty acids classification of oils and extracted oils (EO)

	Oils			EO		
Oils component / Oils fractions	1%GLA	3%GLA	3%DHA	1%GLA	3%GLA	3%DHA
NL	90.1	91.0	93.2	81.1	80.2	79.9
GL	6.9	6.7	4.9	8.1	8.6	9.5
PL	2.9	1.5	1.8	10.2	11.0	10.3
S/ P*	0.652	0.523	0.498	0.751	0.526	0.485
Total phenolic**	4	5	10	5	7	11

* S/ P ratio saturated to unsaturated fatty acids in phospholipids.
**Total phenolic excess as caffeic acid (ppm).

Table 15: Inhibition of formation of propanal and hexanal by tocopherol in bulk oils and extracted oils (EO) and their fractions (Percent Mean Inhibition SD)[a]

	Propanal		**Hexanal**	
sample	**day 4**	**day 6**	**day 4**	**day 6**
control oil	0.0	0.0	0.0	0.0
1% GLA	33.5	41.7	62.5	66.7
3% GLA	3.1	18.8	41.7	33.3
3% DHA	-2.6	6.1	45.8	66.7
EO control	0.0	0,0	0.0	ND
EO 1%GLA	22.7	79,9	44.4	ND
EO 3% GLA	-0.3	75,8	22.2	ND
EO 3% DHA	-30.9	63,8	33.3	ND
NL				
control oil	0.0	0.0	0.0	0.0
1% GLA	37.2	50.8	91.1	92.7
3% GLA	-1.0	31.8	73.2	78.2
3% DHA	12.6	20.5	ND	ND
EO control	0.0	0.0	0.0	0.0
EO 1% GLA	6.2	60.1	16.3	98.1
EO 3% GLA	-10.4	54.3	-44.9	96.3
EO 3% DHA	-71.3	25.9	-4.1	96.3
Polar lipid	**day 4**	**day 8**	**day 4**	**day 8**
control oil	0.0	0.0	0.0	0.0
1% GLA	59.8	75,4	38.7	90.1
3% GLA	45.9	74,5	-79.9	76.7
3% DHA	33.5	51,7	96.2	93.5
EO control	0,0	0,0	0,0	0,0
EO 1%GLA	47,8	70.8	40.7	77.0
EO 3% GLA	43,3	60.4	-75.2	41.0
EO 3% DHA	0,1	51.9	97.4	90.1

[a] % inhibition = (C-S)/S*100; C=hexanal or propanal formation in control and S = hexanal or propanal formation in sample. Negative represent prooxidant activity; SD standard deviations; the initial inhibition of hexanal formation was not calculated because hexanal formation did not increase until after 4 days of oxidation.

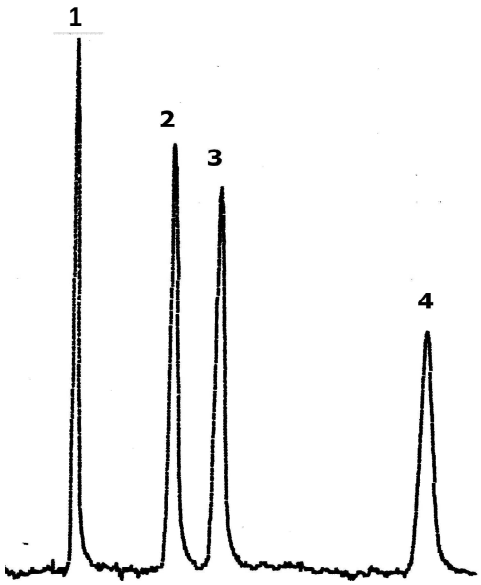


Figure 3a: Tocopherols Standard, 1: α-Tocopherol, 2: β-Tocopherol, 3: ɣ-Tocopherol, 4: Δ-Tocopherol detection with NP-HPLC at 295 nm and used isooctane: acetylacetate 96:4 (v/v) as a mobile phase.

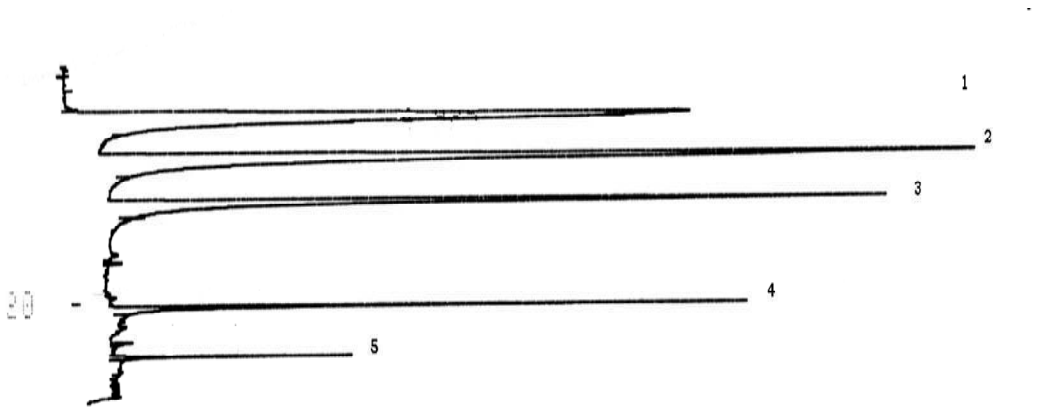


Figure 3b: Chosen volatile aldehyde standards by GC-HS.Peaks numbered correspond to **1**, propanal; **2**, penatanl; **3**, hexanal; **4**, octanal and **5**, nonanal

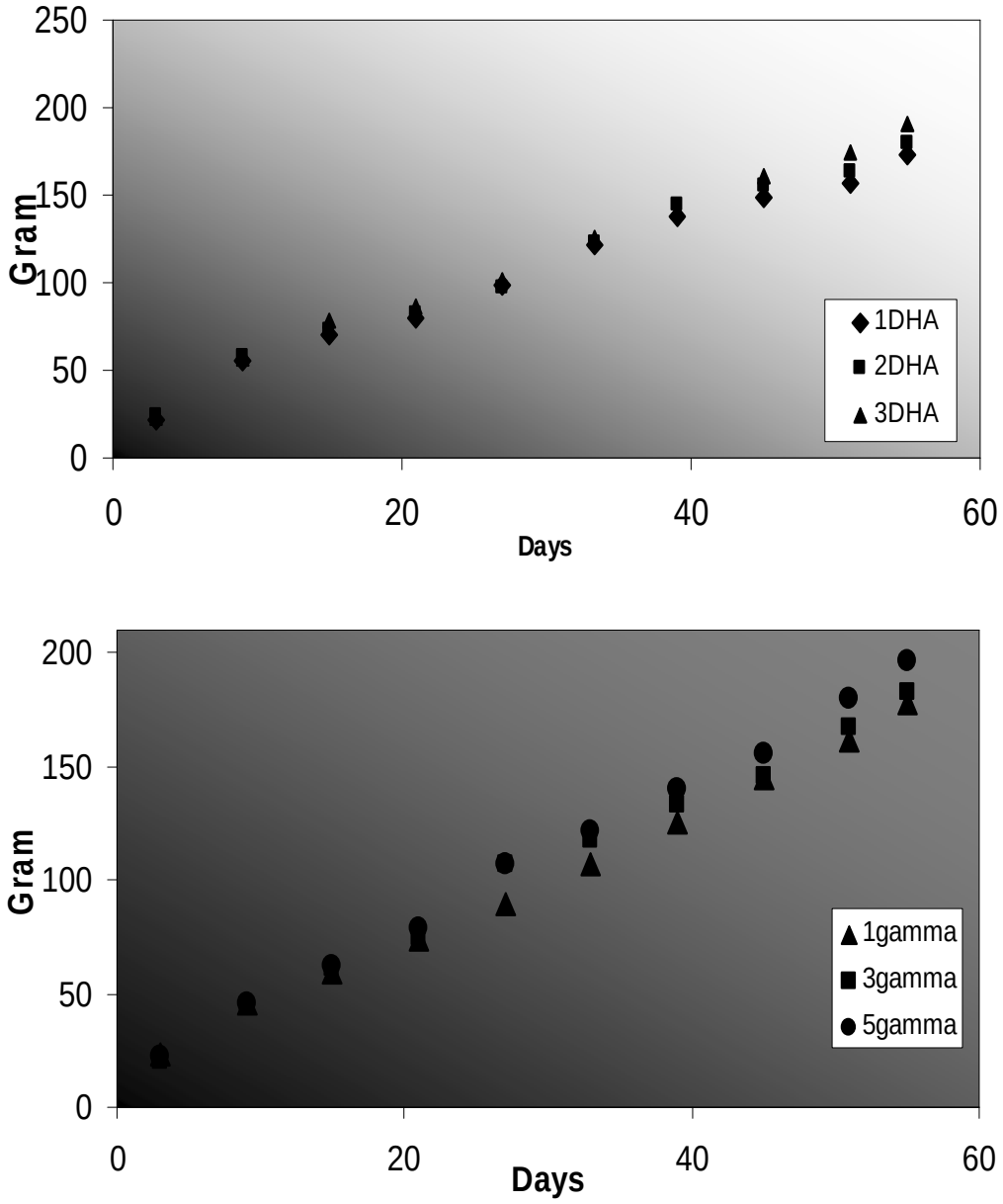


Figure 4: Changes in rat weight gains during 56 days.

Figure 5: Changes in the fatty acids in plasma phospholipids after 4 weeks

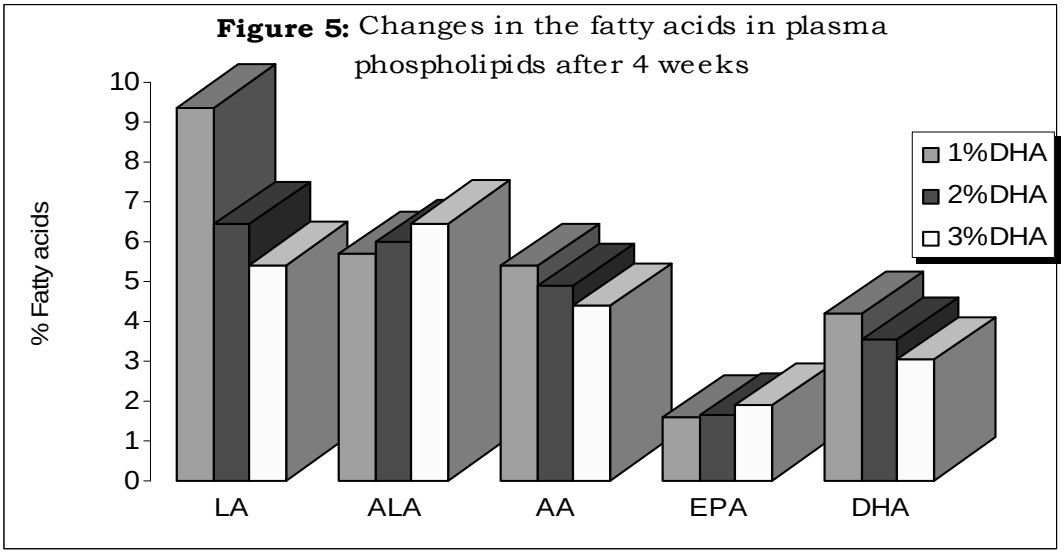


Figure 6: Changes in the fatty acids in plasma phospholipids after 8 weeks

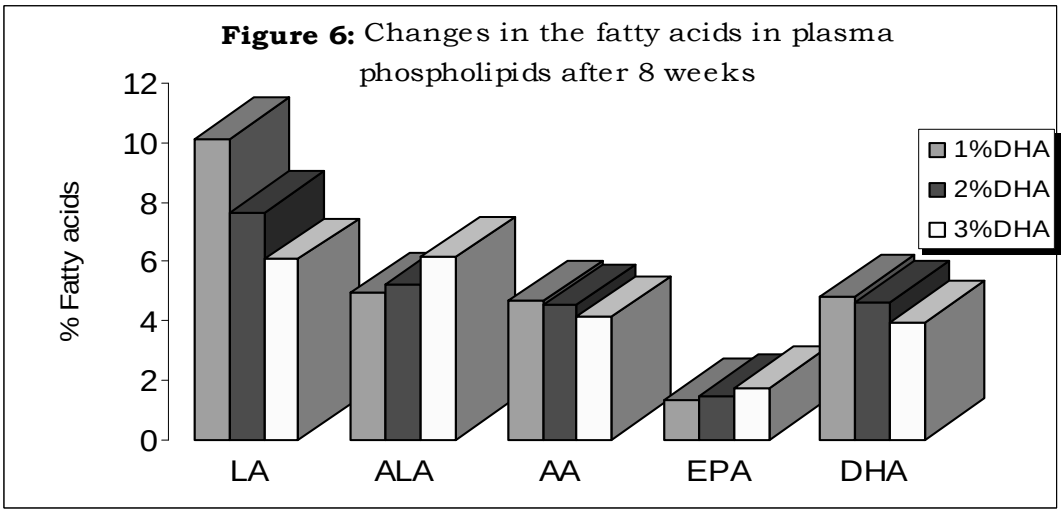
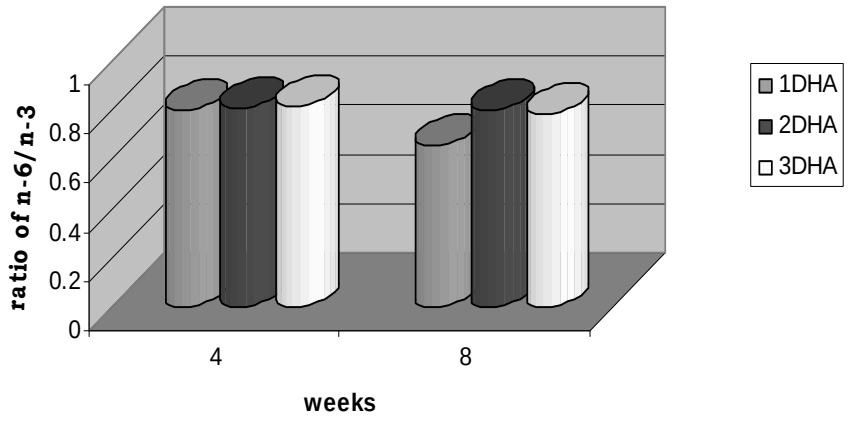


Figure 7: Ratio of n-6/n-3 during 4 and 8 weeks in brain



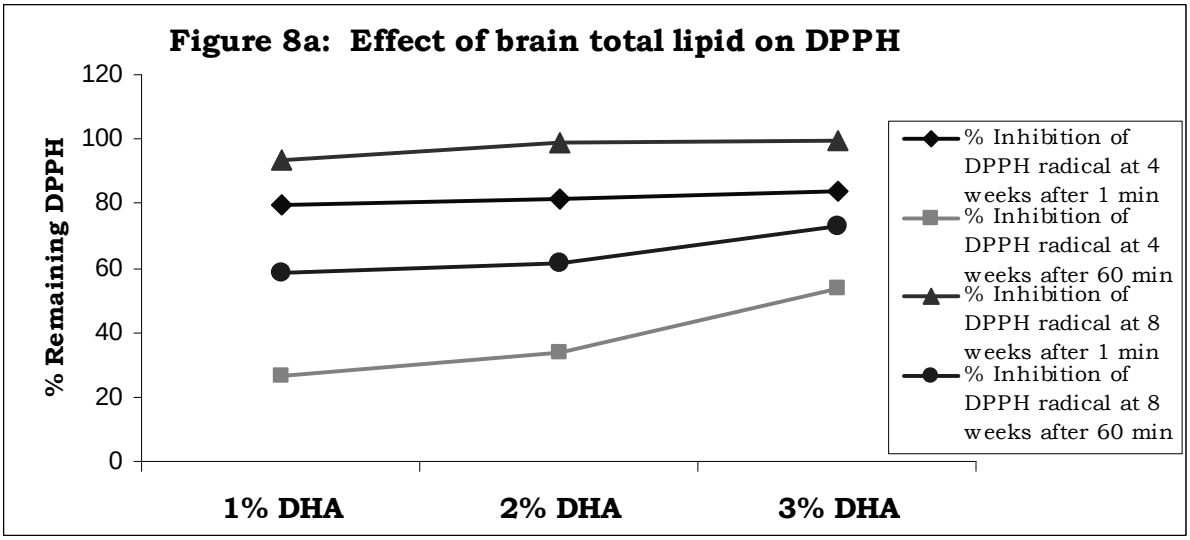


Figure 8a: Effect of brain total lipid on DPPH
% Remaining DPPH
120
100
80
60
40
20
0
1% DHA
2% DHA
3% DHA
% Inhibition of DPPH radical at 4 weeks after 1 min
% Inhibition of DPPH radical at 4 weeks after 60 min
% Inhibition of DPPH radical at 8 weeks after 1 min
% Inhibition of DPPH radical at 8 weeks after 60 min

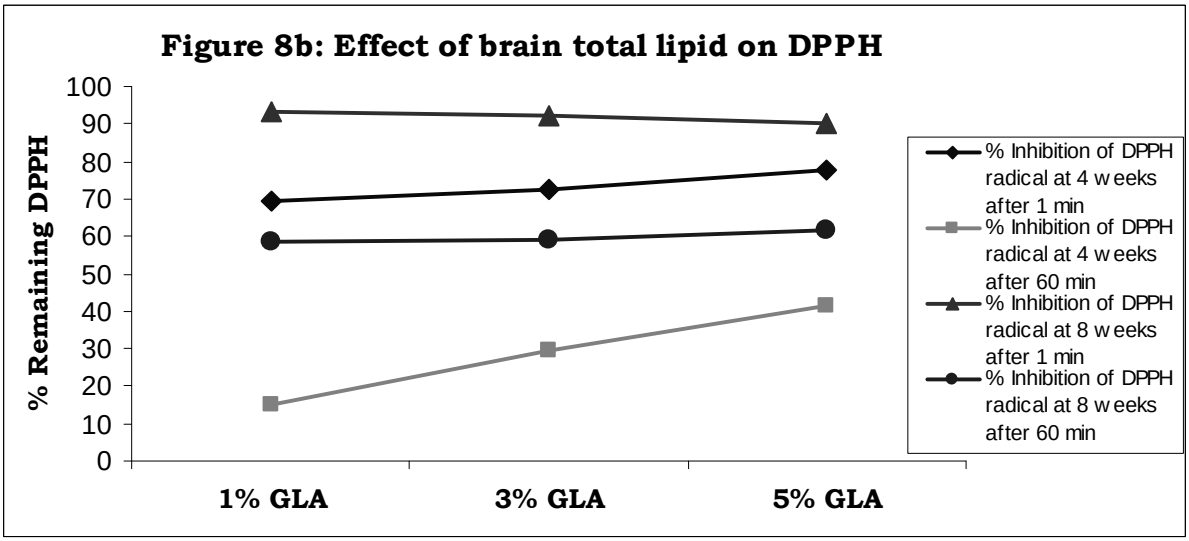


Figure 8b: Effect of brain total lipid on DPPH
% Remaining DPPH
100
90
80
70
60
50
40
30
20
10
0
1% GLA
3% GLA
5% GLA
% Inhibition of DPPH radical at 4 weeks after 1 min
% Inhibition of DPPH radical at 4 weeks after 60 min
% Inhibition of DPPH radical at 8 weeks after 1 min
% Inhibition of DPPH radical at 8 weeks after 60 min

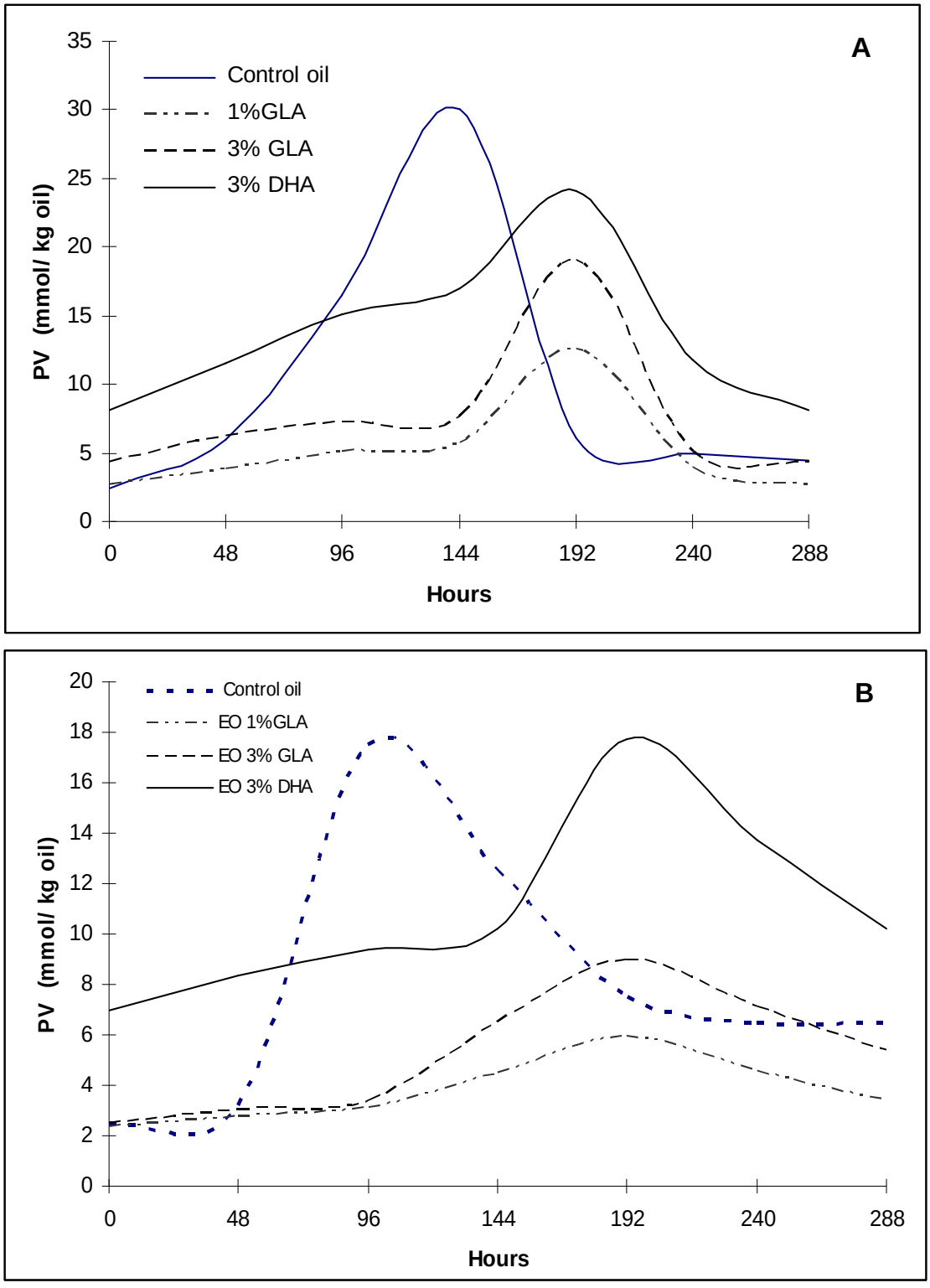


Figure 9: Changes in peroxide levels of bulk (**A**) and extracted (**B**) oils during storage test

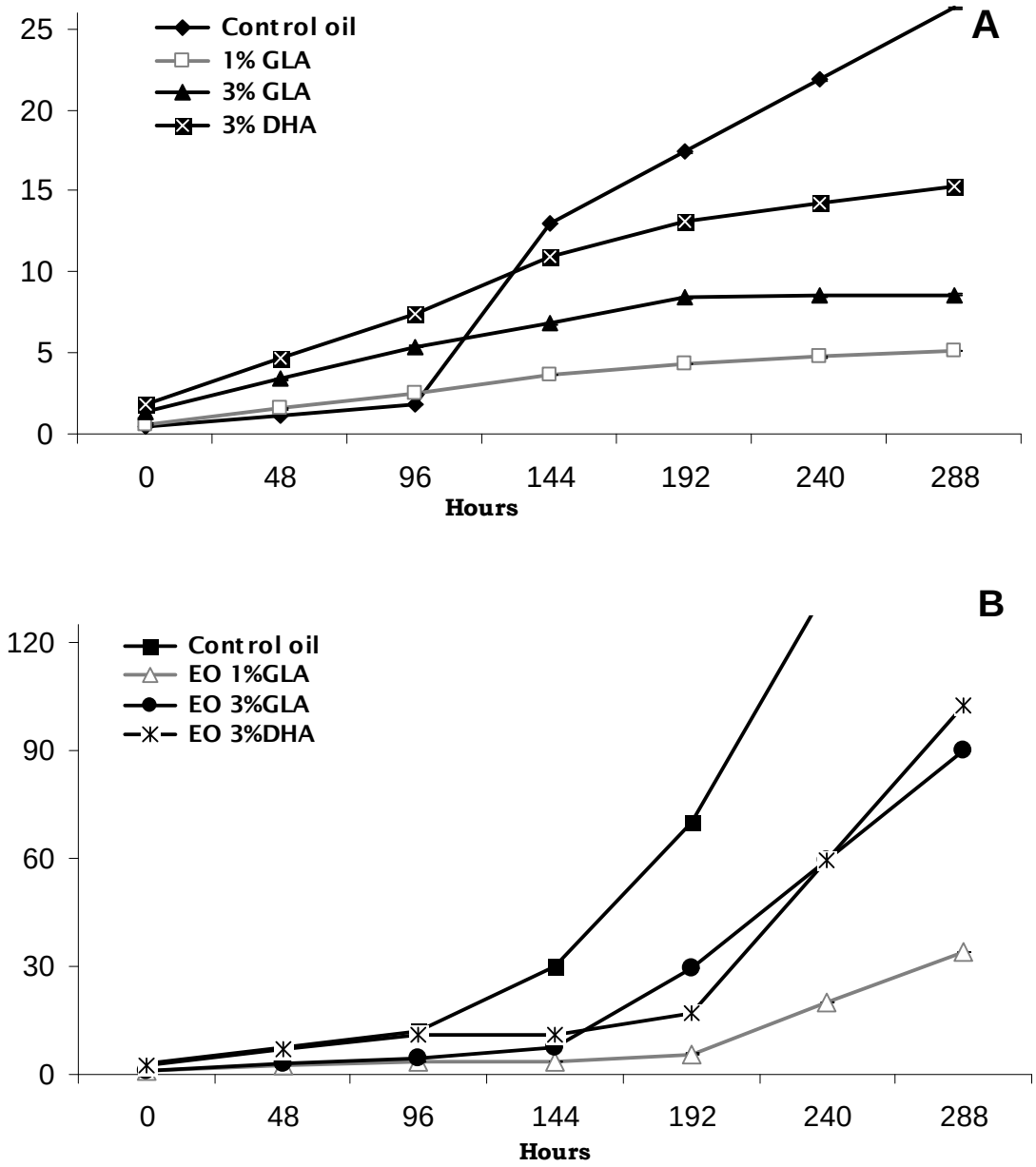
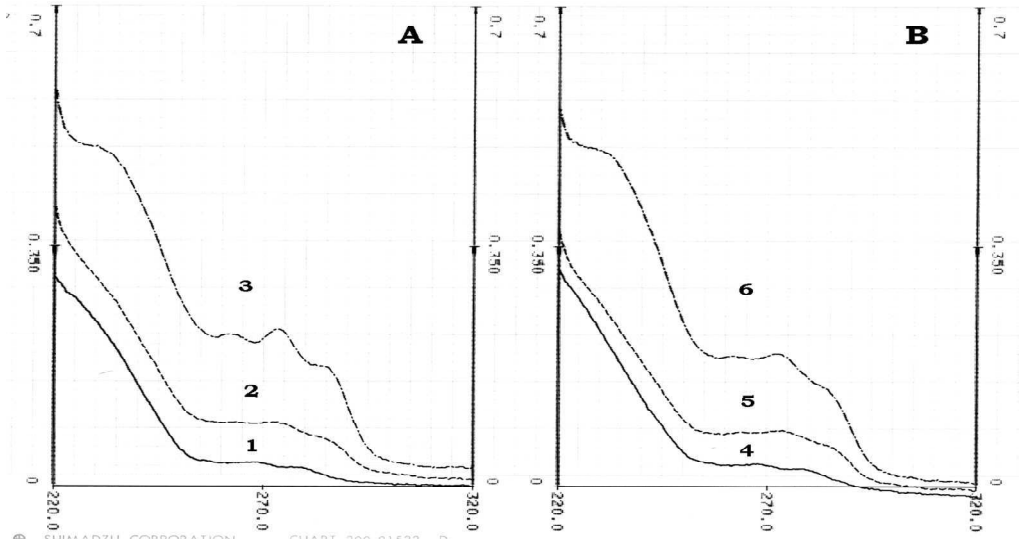
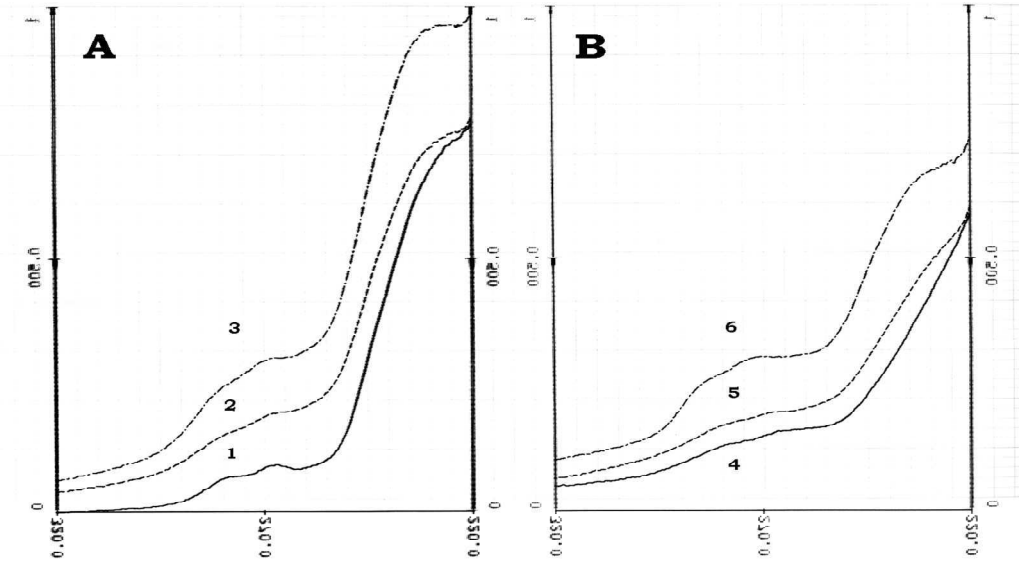


Figure 10: Changes in *p*-anisidine values of bulk (**A**) and extracted (**B**) oils during storage test

(I) Neutral lipids



(II) Glycolipids



(III) Phosopholipids

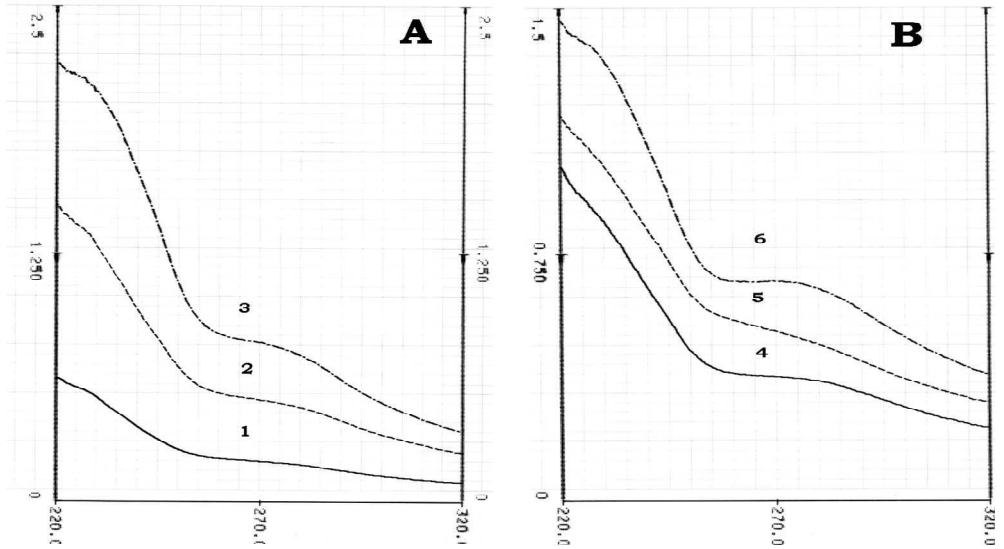


Figure 11: Ultraviolet scans between 220 and 320 nm after 12 days storage at 60 °C, **(A)**, Oils: **1**, 1%GLA; **2**, 3%GLA; **3**, 3%DHA; **(B)**, EO: **4**, EO 1%GLA; **5**, EO 3%GLA; **6**, EO 3%DHA.

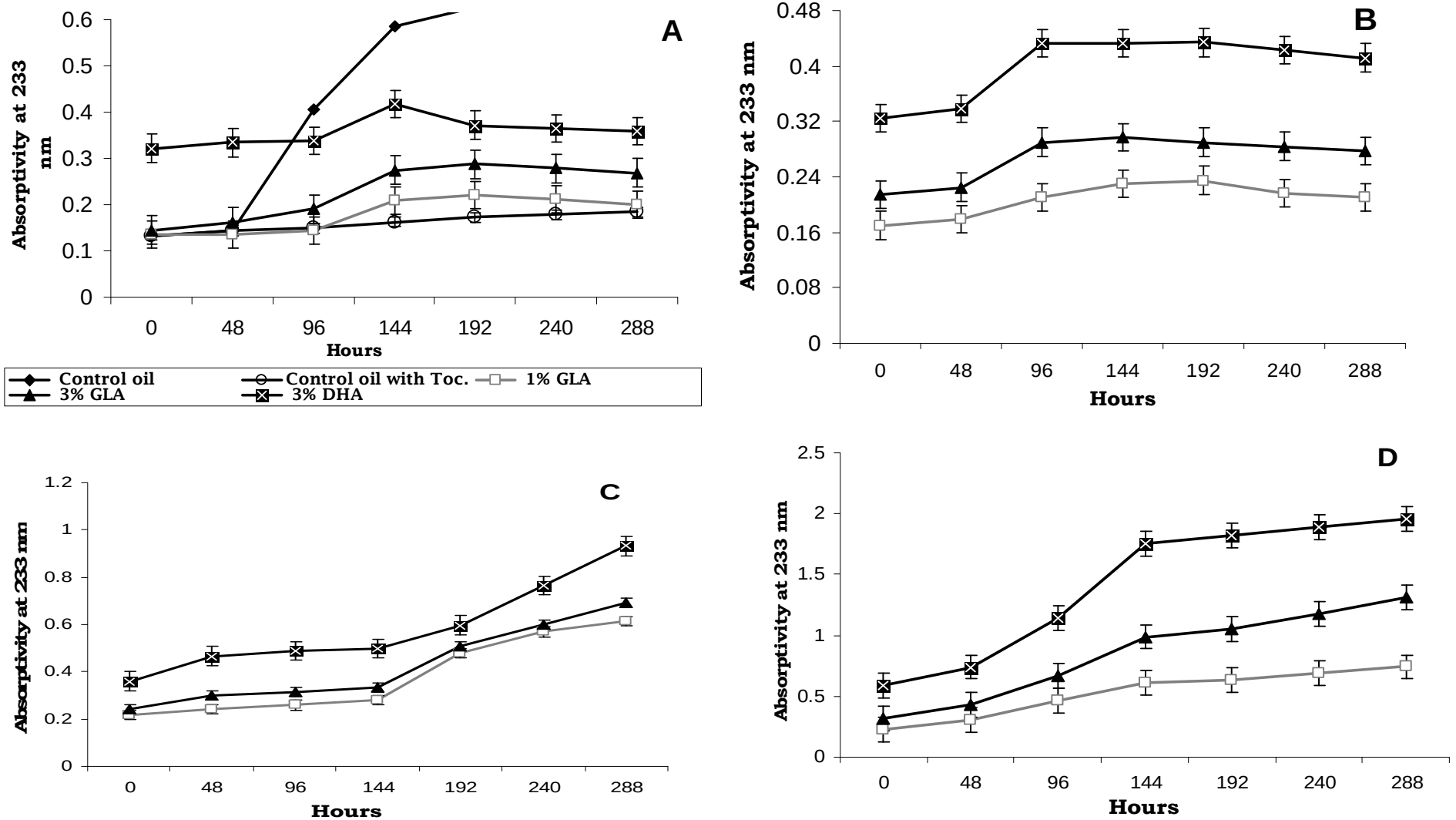


Figure 12: Absorpitivity at 233 nm of oils (A), Neutral lipids (B), Glycolipids (C) and phospholipds (D) during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

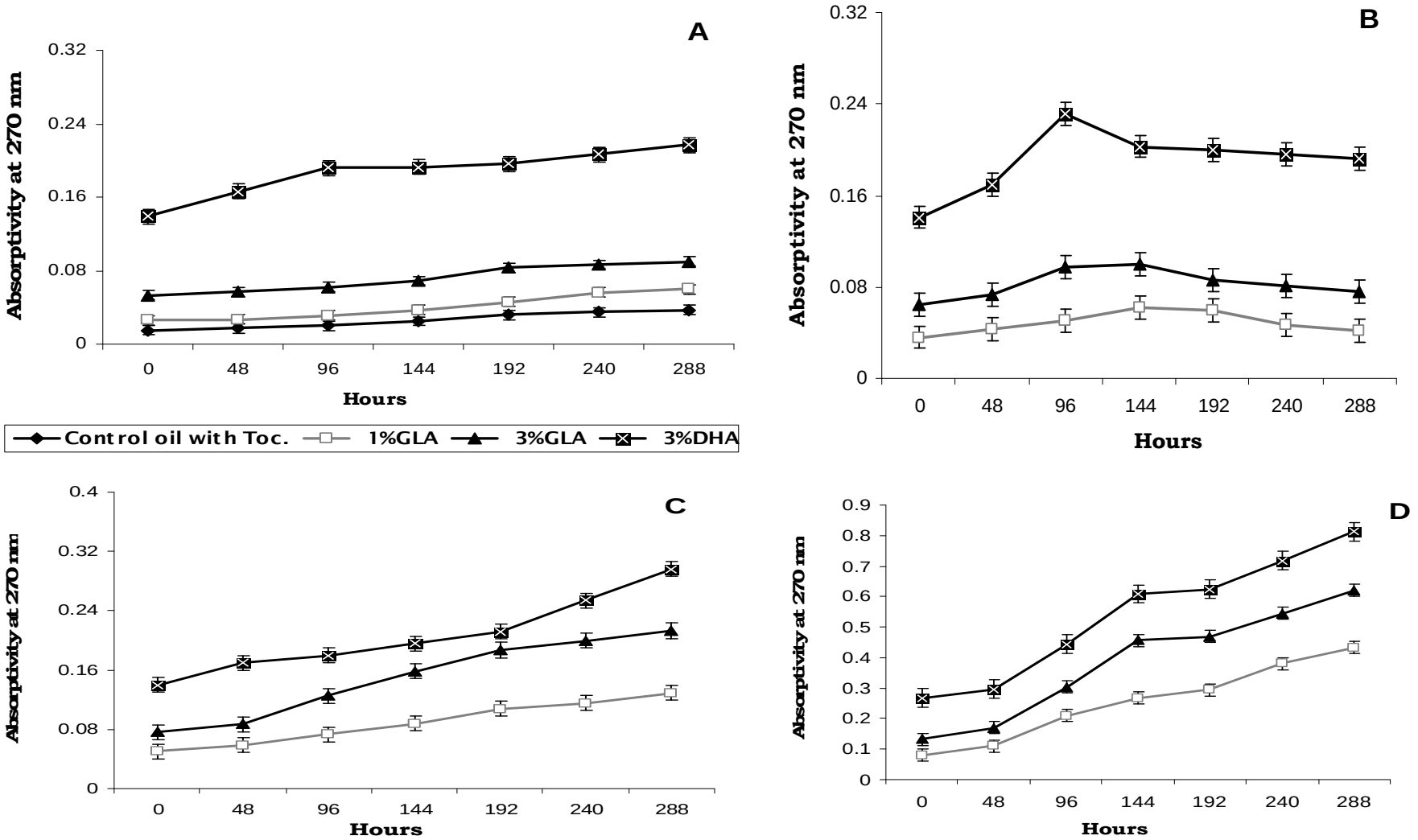


Figure 13: Absorptivity at 270 nm of oils (A), Neutral lipids (B), Glycolipids (C) and phospholipds (D) during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

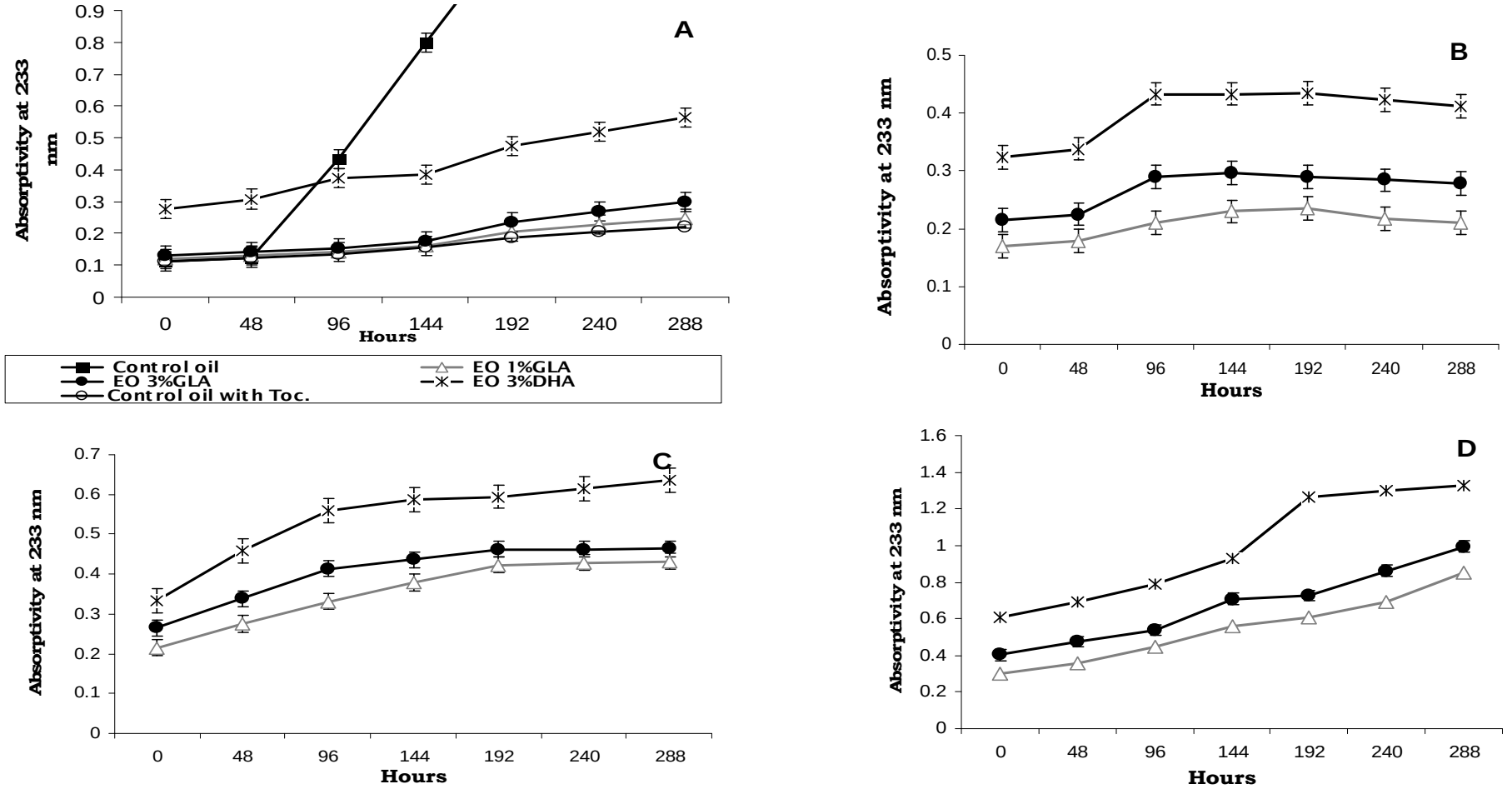


Figure 14: Absorpitivity at 233 nm of extracted oils (EO); (A), Neutral lipids (B), Glycolipids (C) and phospholipds (D) during storage period at 60 °C. Error bars shows the variations of the three determinations in terms of standard deviation.

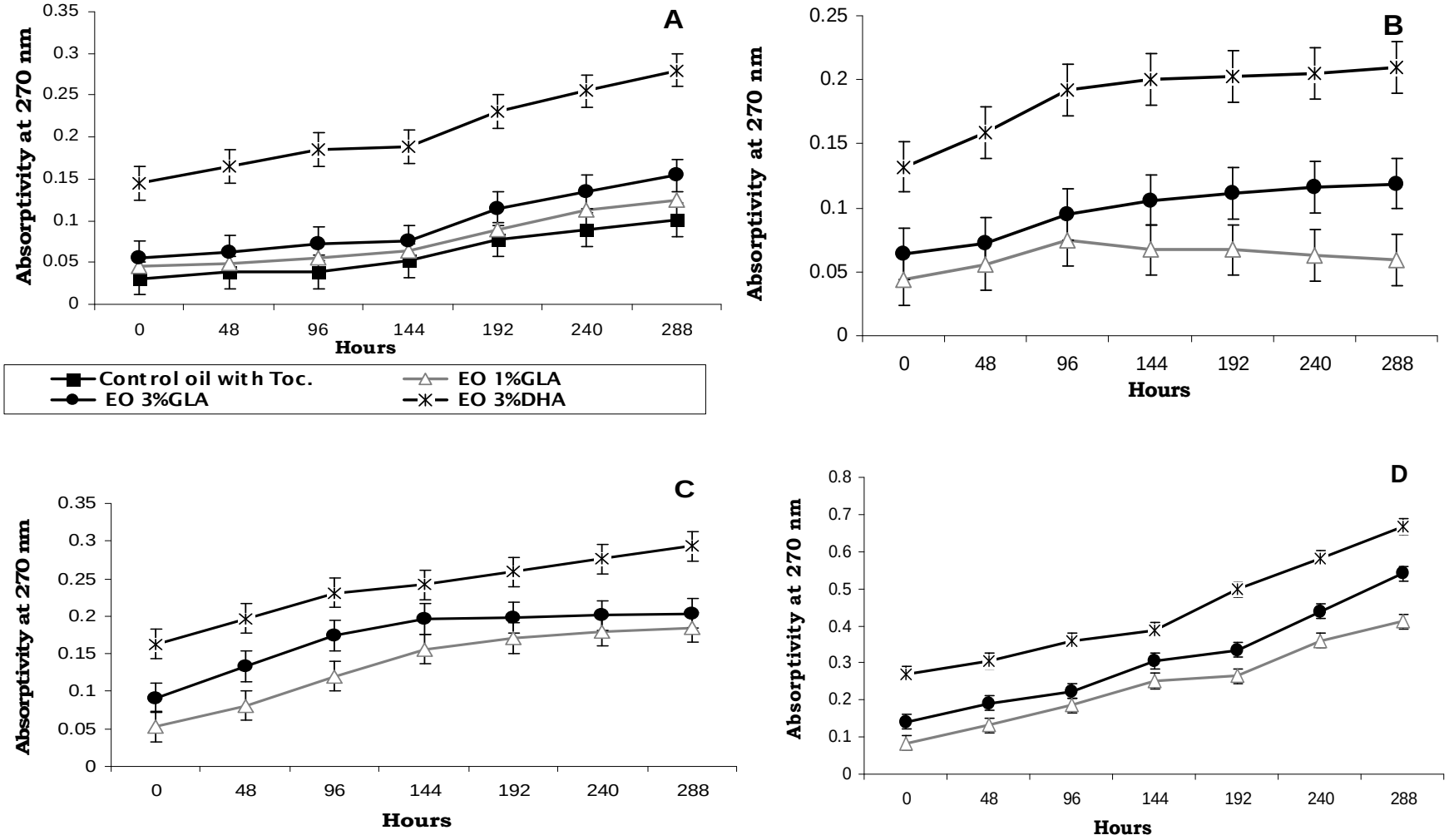


Figure 15: Absorpitivity at 270 nm of extracted oils (EO); (A), Neutral lipids (B), Glycolipids (C) and phospholipds (D) during storage period at 60 ºC. EO, extracted oil from formulas .Error bars shows the variations of the three determinations in terms of standard deviation.

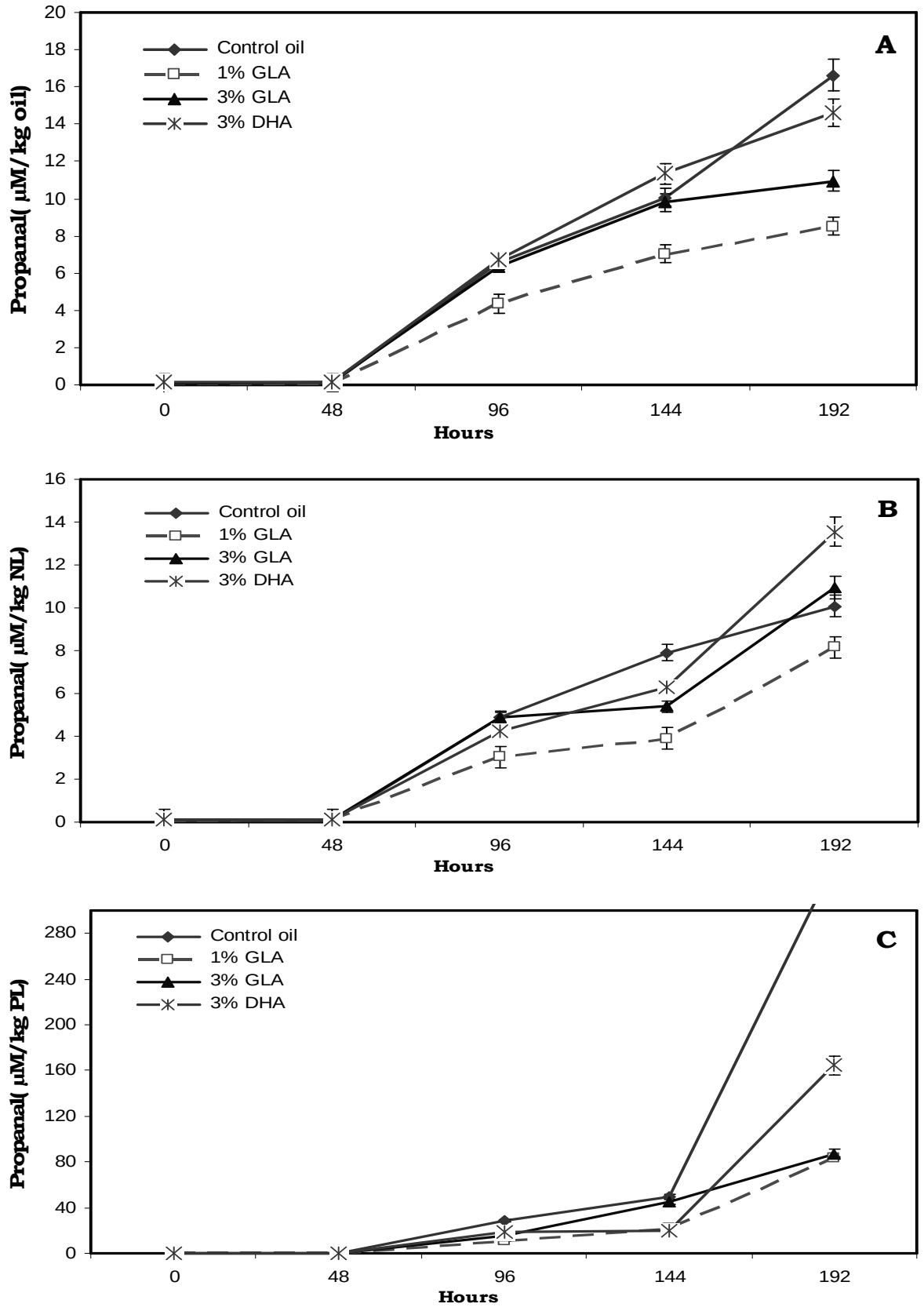


Figure 16a: Propanal content in **(A)**, Oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 ºC. Error bars show the variations of the three determinations in terms of standard deviation.

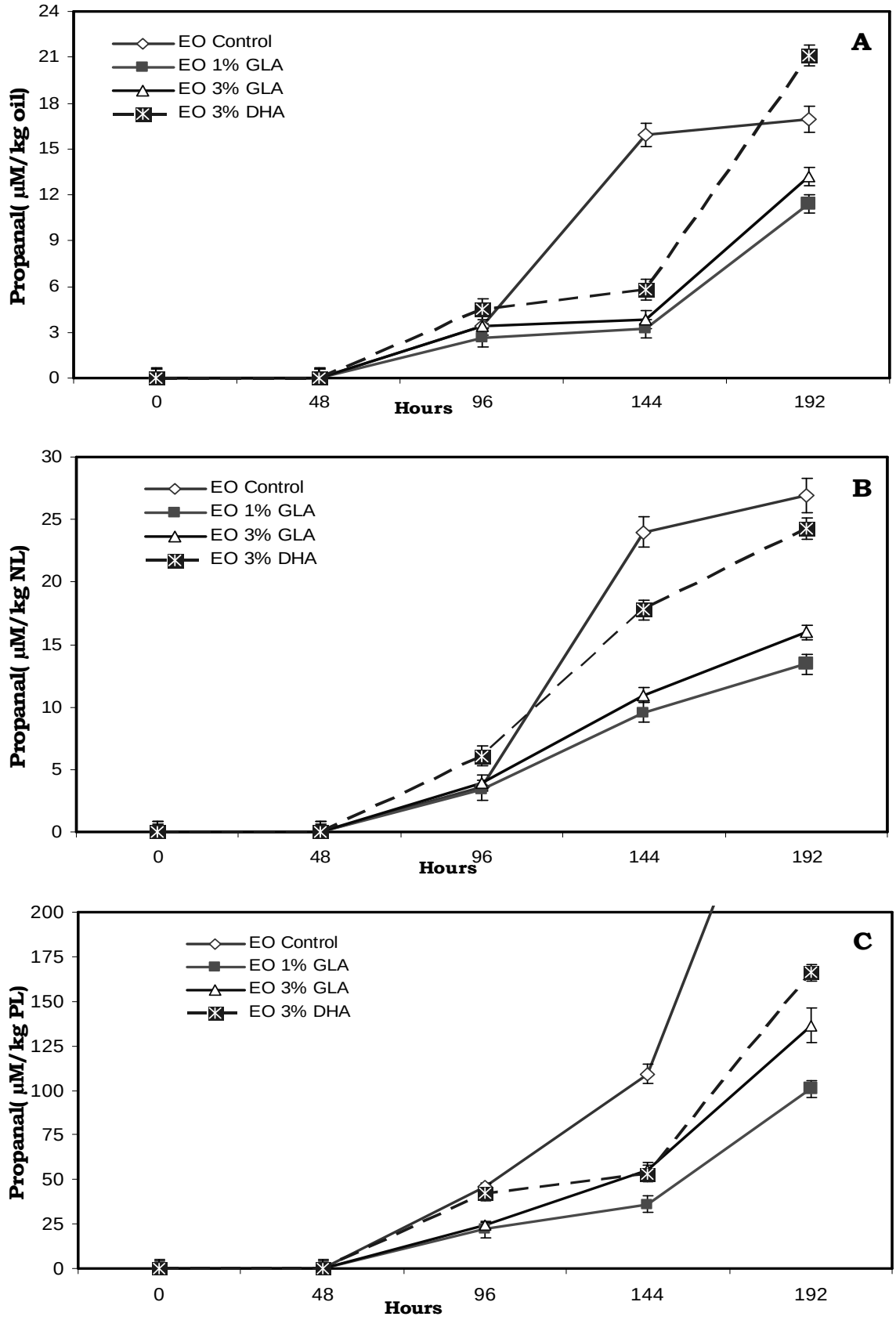


Figure 16b: Propanal content in **(A)**, Extracted oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

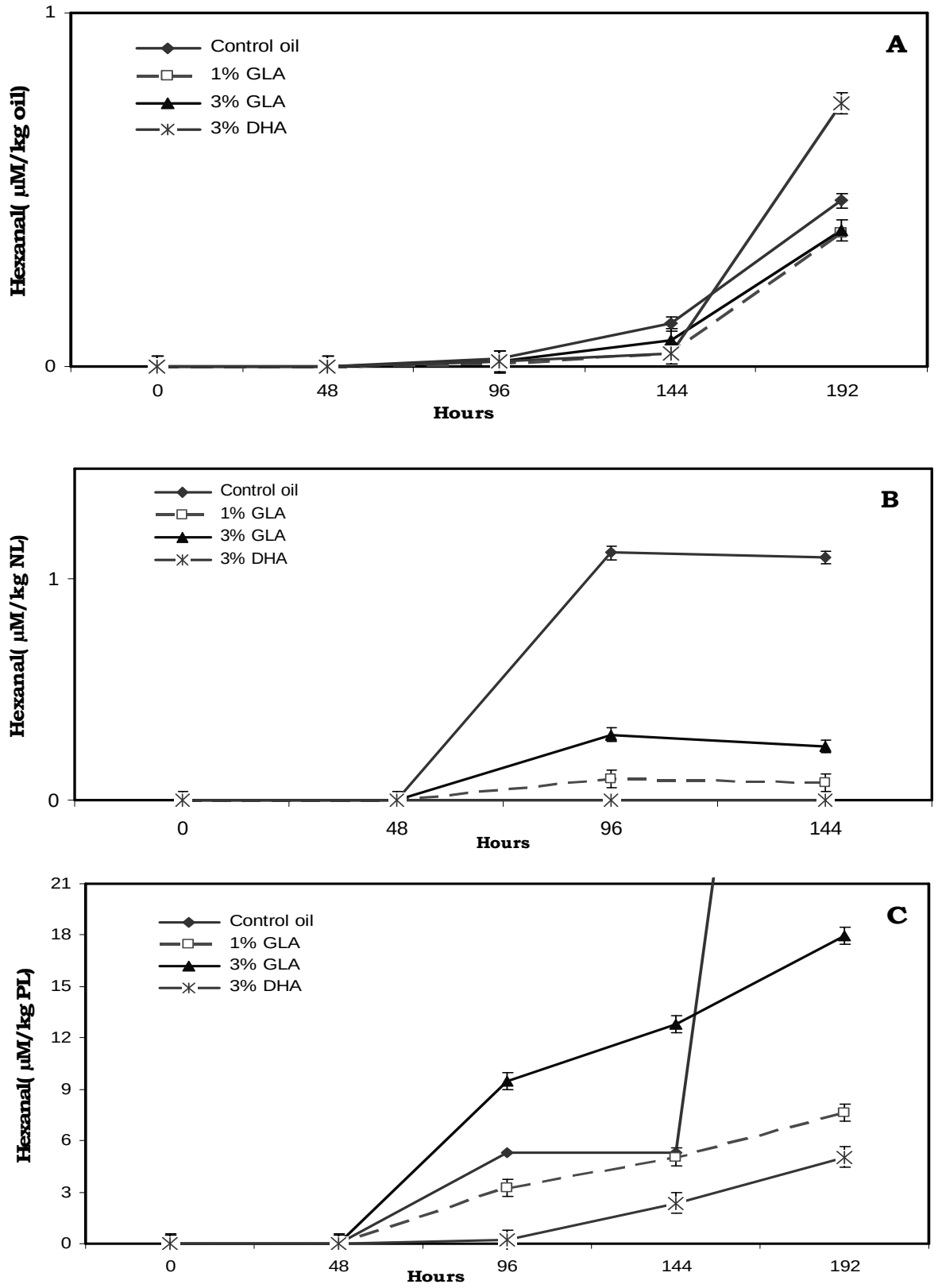


Figure 16c: Hexanal content in **(A)**, Oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 ºC. Error bars show the variations of the three determinations in terms of standard deviation.

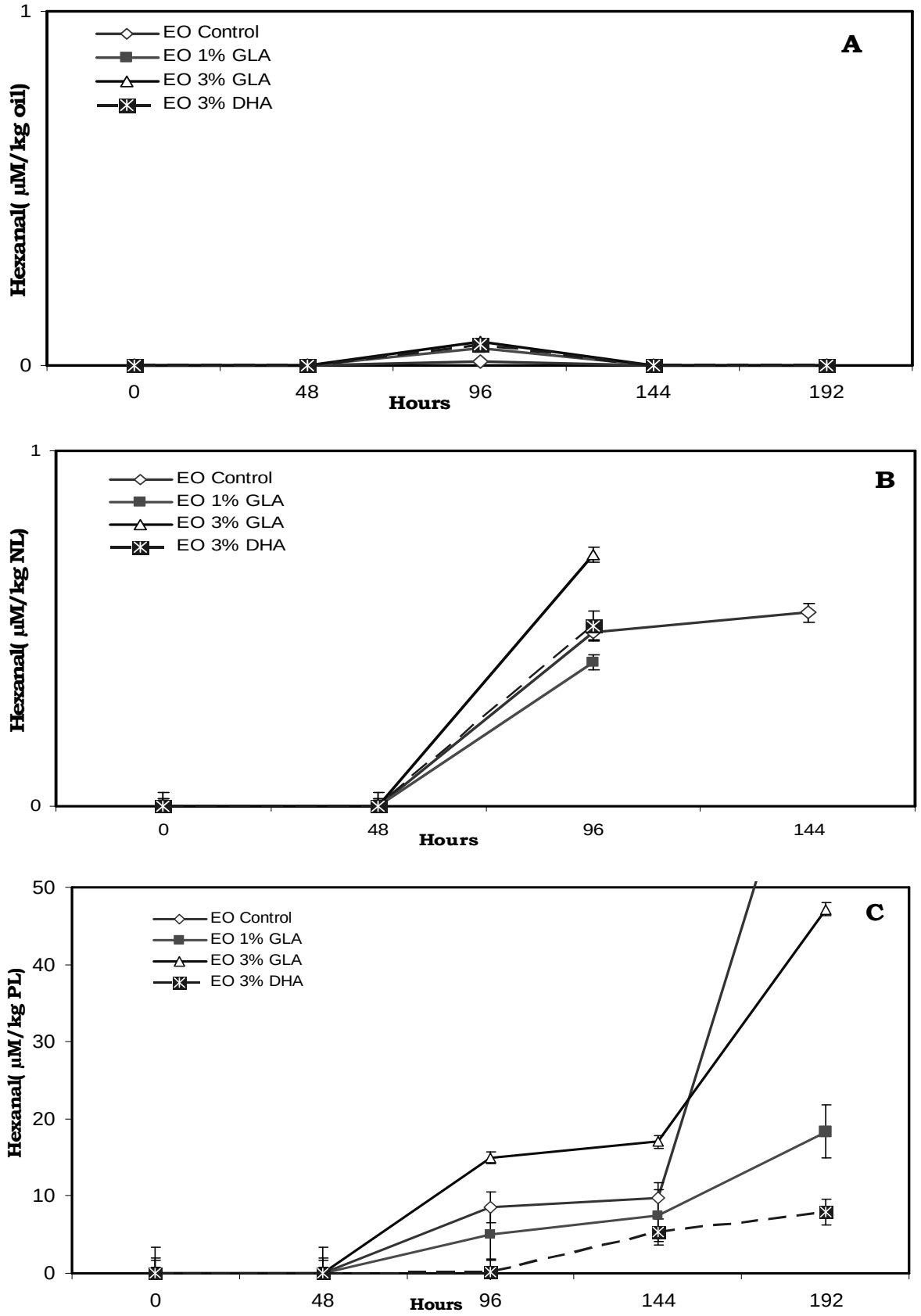


Figure 16d: Hexanal content in **(A)**, Extracted oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

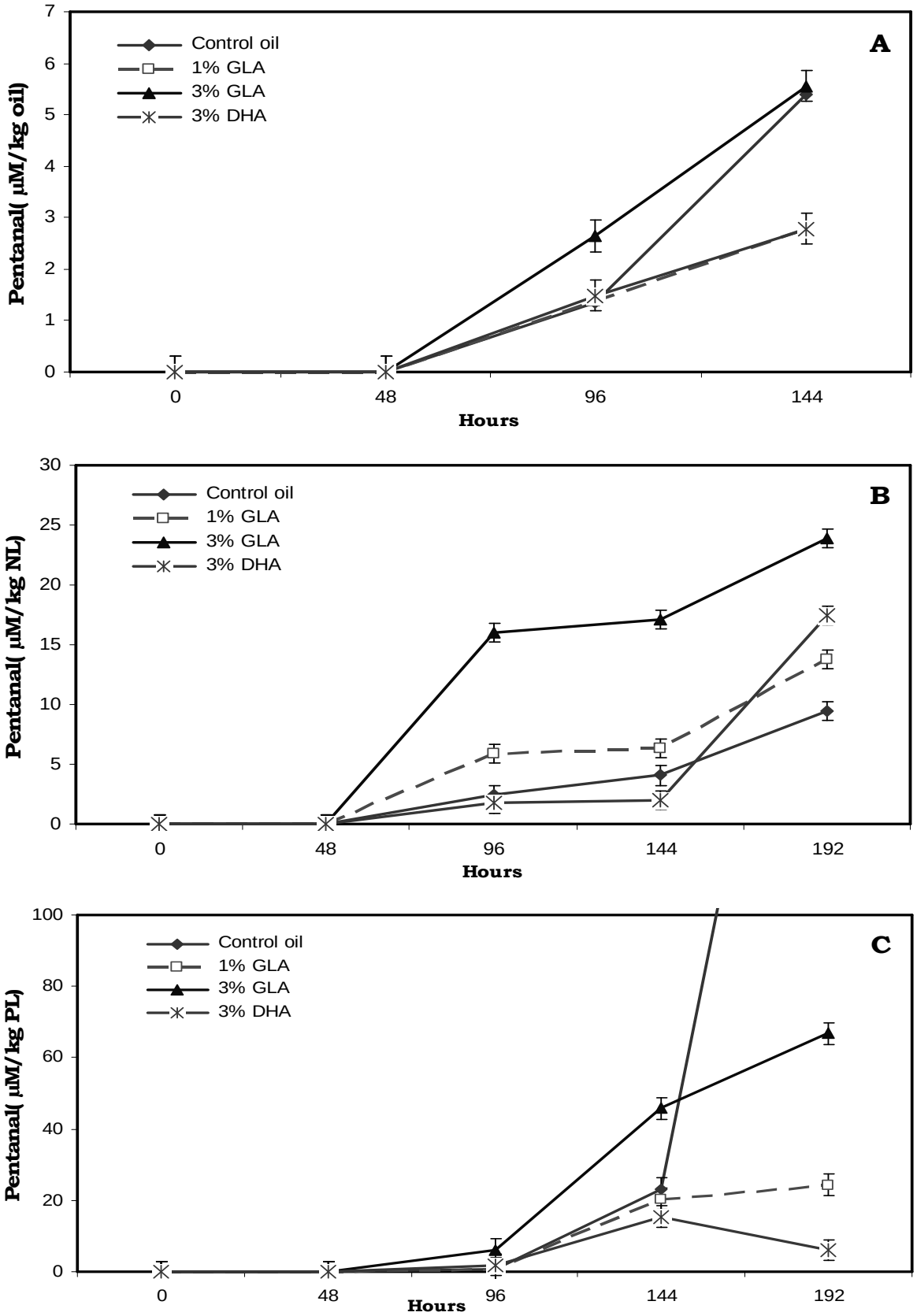


Figure 16e: Pentanal content in **(A)**, Oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 ºC. Error bars show the variations of the three determinations in terms of standard deviation.

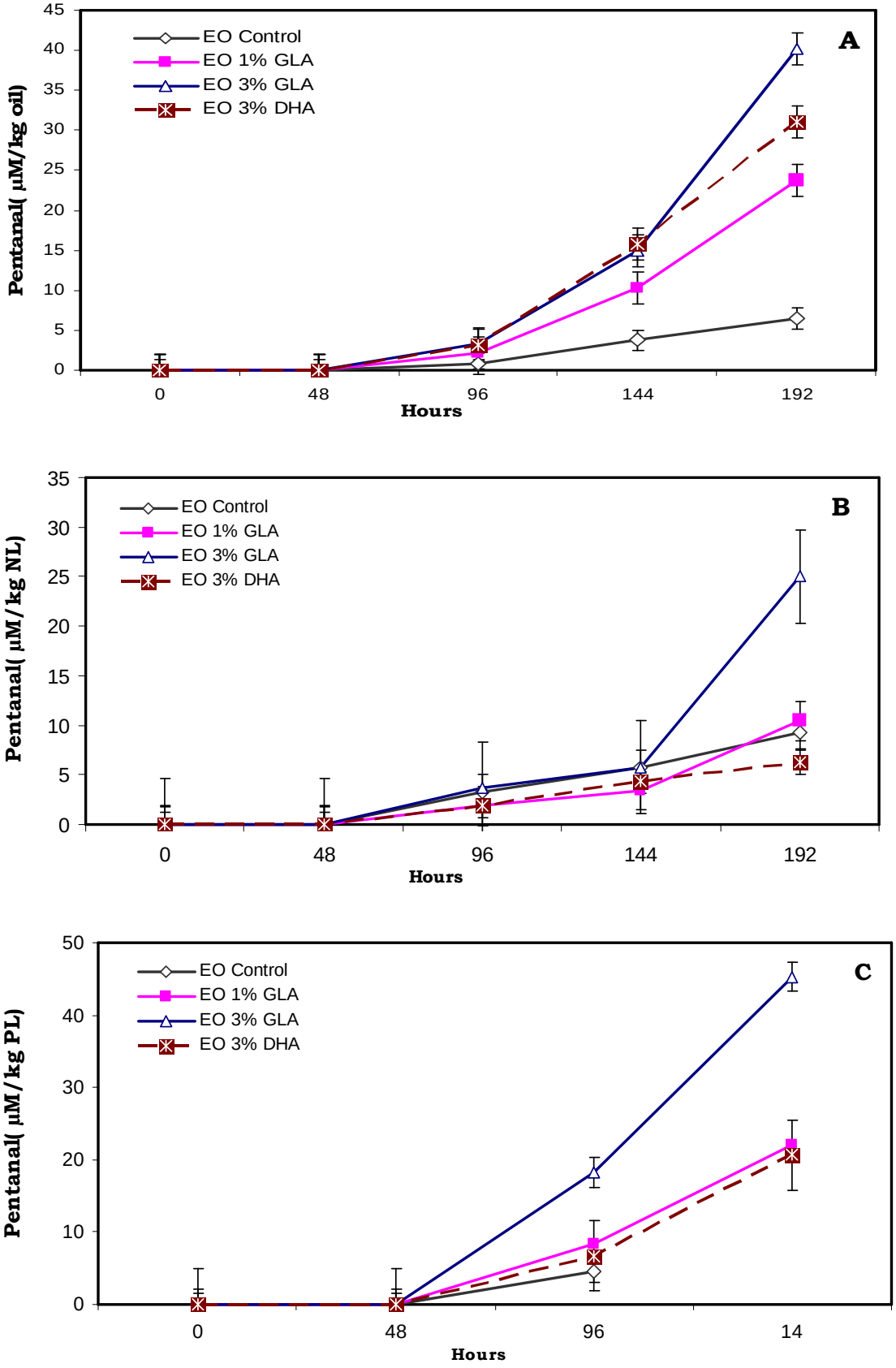


Figure 16f: Hexanal content in **(A)**, Extracted oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

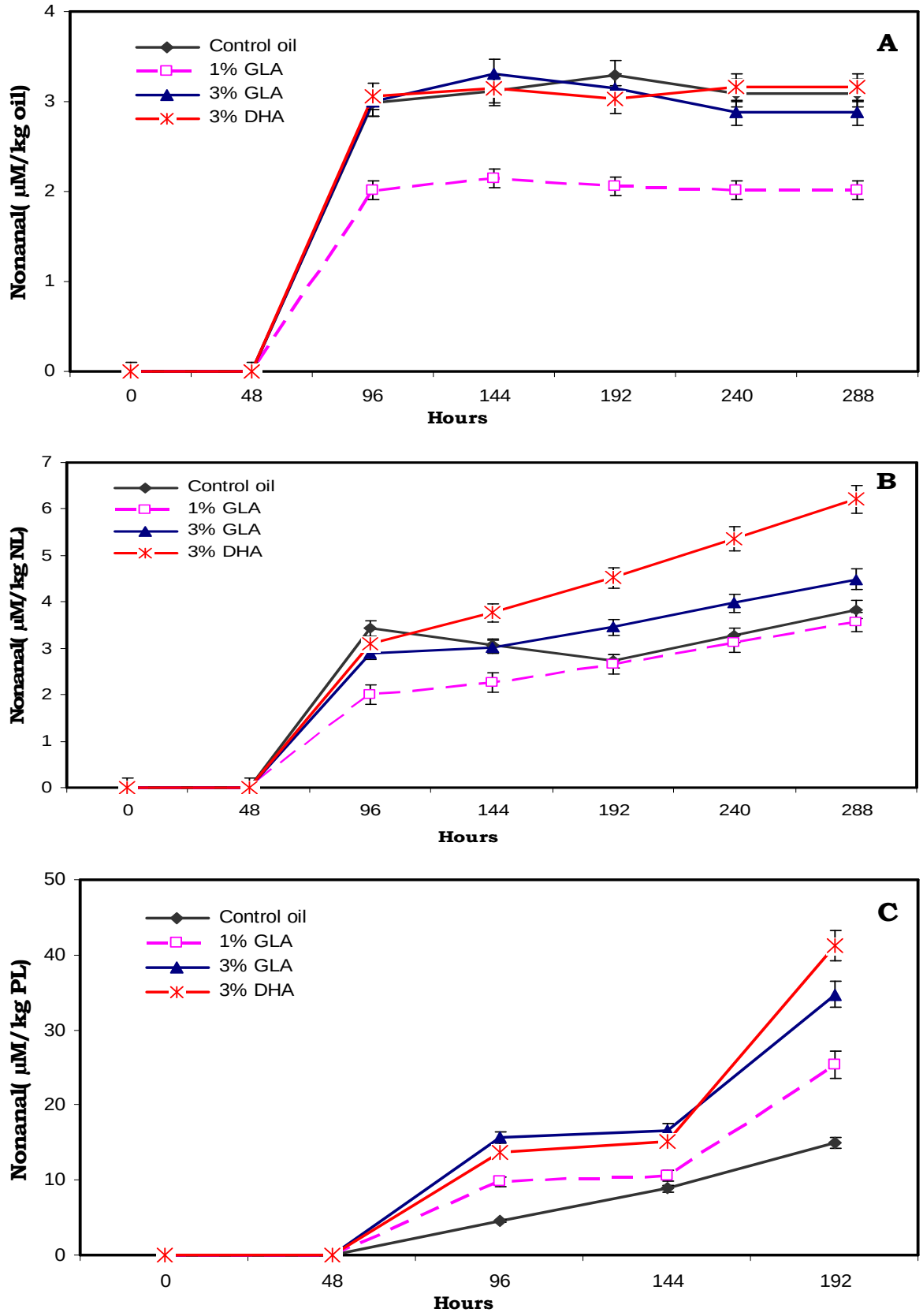


Figure 16g: Pentanal content in **(A)**, Oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 °C. Error bars show the variations of the three determinations in terms of standard deviation.

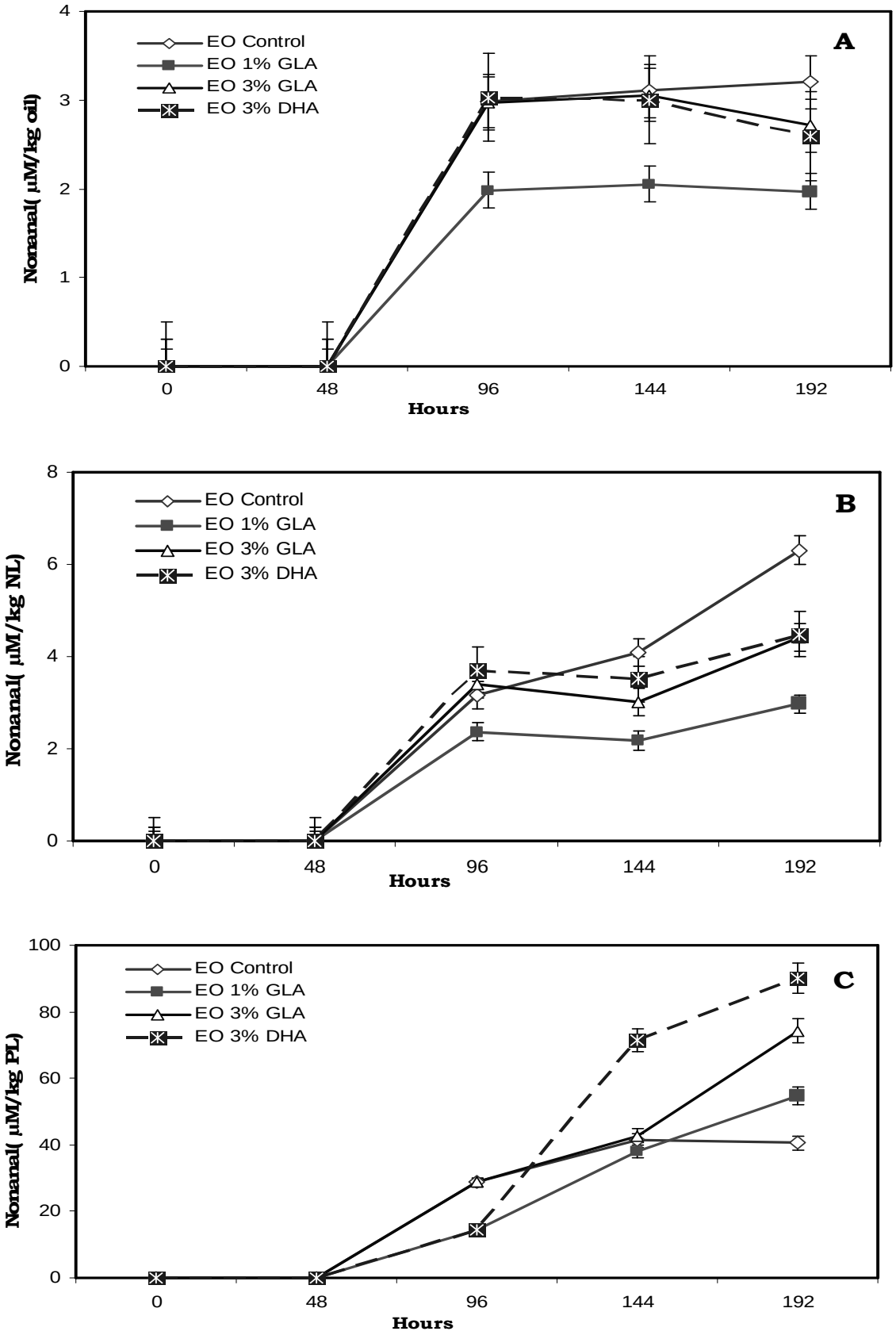


Figure 16h: Nonanal content in **(A)**, Extracted oils; **(B)**, Neutral lipids and polar lipids **(C)** during storage period at 60 ºC. Error bars show the variations of the three determinations in terms of standard deviation.

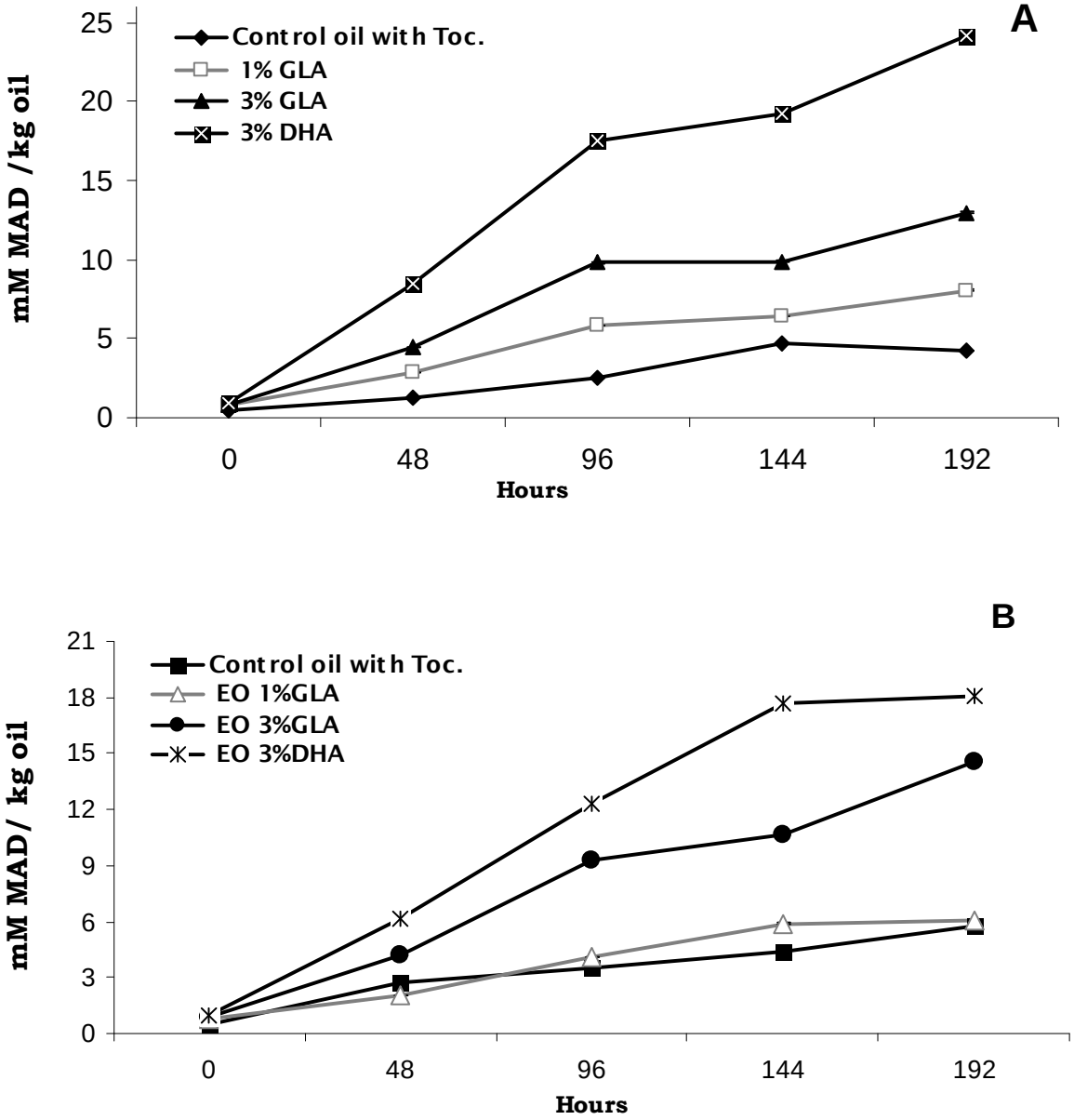


Figure 17: Malondialdehyde contents in (A) bulk and (B) extracted oils during storage period.

Curriculum Vitae

Rokaia Ramadan Abdelsalam, she was born in Minia, Egypt, on the 1 th of July, 1974. She is married since 2000. She graduated (B.SC.) in Agricultural Science from the University of Minia, Egypt, in June 1995. She received the M.SC. degree (May, 2001) in Agricultural Science (Food Science from the Faculty of Agricultural, University of Minia, Egypt. From December 1995 to June 2001, she worked as a Demonstrator and taught Food Science and Technology at the Faculty of Agricultural, University of Minia, Egypt. From June 2001 till now she is an Assistant Lecturer at the Food Science and technology Department, Faculty of Agricultural, University of Minia, Egypt. In March 2002 she started her Ph.D at Food Science and Technology Institute, Technical University of Berlin, Germany.

LIST OF PUPLICATION RELATED TO THE STUDY

1. Abdelsalam, R. and Mörsel T. 2007. Docosahexanoic acid does not raise LC-PUFA n-3 in rat brain, liver and plasma. (Submitted for publication).

2. Abdelsalam, R. and Mörsel T. 2007. Effect of long time feeding with different levels of gamma-linolenic acid with a constant amount of docosahexanoic acid and eicosapentanoic acid on the developing and lipids profile of rat. (under puplications).

3. Abdelsalam, R. and Mörsel T. 2007. A Rapid Method for Determination of Vitamin e and Radical Scavening Activity on Plasma and Tissues. (submitted for publication).