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ω-3 Fatty Acids, the Importance and Yeast Expression

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Omega fatty acids are very important for human healthiness. Dietary á-linolenic(trienoic fatty acid) sources are varying from plant sources to fishes. 18:3 is an essential fatty acid and it is converted by the human body into eicosapentaenic acid (EPA, 20:5n-3) and docosahexaenic acid (DHA, 22:6n-3) which are very important in fighting heart disease. α-linolenic reduces human serum LDL- cholesterol, lowering LipoproteinA, suppresses the production of interleukin-1 (IL-1), tumor necrosis factor and leukotriene B4 (LTB4) oxygen free radicals (OFRs), reduce mammary cancer risk, as well as modify emotional reactivity and learning abilityand performance. Omega-3 fatty acids are precursors for industry valuable substances such ascyclopentenones, ketols, or uses as an alternative fuel for the small diesel engine.Yeast (*Saccharomyces cerevisiae*) can absorb and integrateseveralvarieties of fatty acids from growth medium. Exogenously feeding oleic acid to yeast transformed with Δ-12 desaturase successfully lead tolinoleate invention. Expressed yeast with oilseeds FAD3 and fedwith 18:2produced α- linolenate. Yeast as expression ideal may affordoutstandingdesigns for understanding the transcriptional and post-transcriptional toolselaborated in the regulation of plant fatty acid desaturases, which offer the implementconstructing engineered plants contains nutritional and industrial valuable fatty acid contents.

Key words: ω- linolenic, Helth, Industry, Yeast expression

One of the most important biochemical factories is the plant cell. In it, many biochemical compounds are manufactured including the fatty acids. Fatty acid biosynthesis in higher plant cells occurs in the plastids and it is formed initially from acetyl-CoA and malonyl-CoA, which are the precursors of acetyl-and malonyl-ACPS[1]. The chloroplast membranes of all higher plants contain very high proportions of trienoic fatty acids. These lipid structures are important in photosynthesis. Trienoic fatty acid content 16:3 + 18:3 are important to ensure the correct biogenesis and maintenance of chloroplasts during growth of plants at low temperatures[2]. Dietary á- linolenic sources are fish oil, walnuts, soybeans, spinach, canola (rapeseed), linseed, perilla, and dragonhead. Linseed (*Linum usitatissimum*) has a very high content of linolenic acid in the triacylglycerol (TAG), normally more than 45% of the total fatty acid content[3,4,5,6,7].

ω-3 fatty acids, the importance

The trienoic fatty acid (alpha- linolenic, octadeca-9, 12, 15-trienoic acid, 18:3n-3), is an essential fatty acid and it is converted by the human body into two acids eicosapentaenic acid (EPA, 20:5n-3) and docosahexaenic acid (DHA, 22:6n-3) which are very important in fighting heart disease. Flaxseed oil-containing human diet resulted in significant increases in alpha- linolenic concentration in the plasma phospholipids, cholesterol ester, triglyceride fractions (an 8-fold increase), and neutrophil phospholipids was increased by 50%, also the EPA concentration increased by 2.5-fold in plasma lipid fractions and

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neutrophil phospholipids[9,8,10]. Linolenic acid was found to significantly reduce human serum LDL-cholesterol as a result of diet on flaxseed, also diet on flaxseed results in lowering LipoproteinA (Lpa) which is a strong predictor of cardiovascular disease[11]. Perilla oil (58% 18:3) is found to have a more potent serum cholesterol- lowering ability than safflower oil (77% 18:2)[12]. ω-3 fatty acid from flaxseed suppresses the production of interleukin-1 (IL-1), tumor necrosis factor and leukotriene B4 (LTB4) as well as oxygen free radicals (OFRs) and would prevent the development of hypercholesterolemic atherosclerosis in rabbits, dietary flaxseed supplementation could, therefore, prevent hypercholesterolamia-related heart attack and strokes[13]. Dietary á- linolenic acid significantly enriches 18:3n-3, 20:5n-3, 22:5n-3 in plasma, red blood cells, aorta, platelet neutral, and reduces plasma TAG in pigs as well as significantly reducing serum cholesterol and TAG levels in rats[14,15]. Feeding of flaxseed, which is rich in alpha-18:3 and secoisolariciresionol diglycoside (SDG), the latter is the precursor of mammalian lignans, can affect the rat mammary gland structures that may potentially reduce mammary cancer risk[16]. Safflower oil and perilla oil, rich in n-6 and n-3 respectively, alter the membrane fatty acid composition of rat livers and suppress the development of liver cell carcinoma[17]. Dietary flaxseed affects ω-3 fatty acids metabolism and helped to restore the health of sick dogs as a possible implications in companion animal nutrition, also in rat dietary18:3 from perilla oil caused characteristic changes in the activity of hepatic enzymes in fatty acid and glucose metabolism[18,19]. Exogenous PUFA from Perilla oil at 2% of rat diet was sufficient to suppress lipogenic enzyme gene expression, also eicosapentaenoic acid increased within only 1 h of rat stomach intubations[20]. Semi-purified diets containing either perilla oil (high in 18:3n-3) or safflower oil (high in 18:2n-6) were fed to SAMR1 mice, the n-6/n-3 ratio of its brain phospholipids were affected and this may modify emotional reactivity and learning ability, the biochemical characteristics membrane surfaces of brain microsomes were affected significantly in a dietary oil-dependent manner, the relatively large changes in n-3 and n-6 PUFA in brain membranes caused by dietary manipulation do not cause significant alterations in most of membrane-bound enzyme activities. However a small but significant change in Na+, K+-ATPase activity that may affect the altered learning behavior was found. In addition dietary oil-rich induced morphological changes in synapses in the hippocampus of rats, led to differential learning performance when a brightness discrimination learning task was carried out. Perilla oil-fed lines found to have more correct responses through the learning sessions compared with those fed with safflower oil, moreover the decrease in the discrimination- learning ability induced by á-linolenate deficiency is a relatively reversible process, both the DHA content in the brain and the learning performance were restored by supplementing alpha-linolenate[21,22,23,24,25].

When rats were fed diets containing perilla seed oil rich in alpha- linolenic acid, liver peroxisome proliferation was not affected and heart mitochondrial cytochrome C oxidase activity also was not affected. Feeding on perilla oil was found to be beneficial for suppression of carcinogensis, allergic hyperreactivity, thrombotic tendency, apoplexy, hypertension, and aging in animals which provides evidence that n-3-enriched oils are safe under conditions applicable to human nutrition and n-3 acid should be increased to levels higher than current dietary levels for prevention of chronic diseases found in the industrialized countries considering that the rats were fed at levels several-fold higher than the maximal human intake[26,27]. Dietary supplementation with perilla seed oil in selected patients with asthma suppresses the generation of leukotriene (LTC4) and its association with clinical features such as respiratory function and lipometabolism[28]. Compared with corn oil-rich supplementation, Perilla seed oil-rich supplementation (n-3 fatty acids) is useful for the treatment of asthma in terms of suppression of leukotriene B1 (LTB1) and LTC4 generation by leucocytes, and improvement of pulmonary functions[29].

ω-3 fatty acids, food and industry

Due to the human health and nutrition importance of 18:3, researchers tend to reproduce 18:3-rich foods for human diets. By feeding hens on diets rich in n-3 fatty acids, egg yolk lipids composition was significantly affected. Total and percentage yolk lipids became lower, in some cases yolk lipids had more C16:0 and less C18:0 and C18:1, and significant decrease in the ratio of n-6 to n-3 fatty acids in platelet phospholipids. The á-linolenic acid content was significantly increased in the produced egg yolk[30,31,32,33,34]. Another example is a new flax variety for 18:3-rich edible oil is reproduced[35,36]. In a study on the effect of various sources of unsaturated fatty acids on beef dietetic quality and growing bulls, 2% supplement of unground full-fat flaxseeds was more effective in modifying the composition of fatty acids in longissimus dorsi muscle lipids, 18:1 is decreased while docosahexaenoic acid was increased in additions to increasing 18:3 levels in the produced meat[37].

In addition to its nutritional and health importance, 18:3 is a precursor for industry valuable substances such as fatty acids hydroperoxids, alpha-linolenic acid is commercially produced by hydrolyzing flax seed oil or perilla oil using polyethylene-immobilized lipase powder[38,39]. The cyclopentenones is naturally formed from cyclization of allene oxide fatty acids precursors such as linoleic hydroperoxide (HPOD), alpha-linolenic hydroperoxide (HPOTa), and gamma-linolenic hydroperoxide (HPOTg) in the presence of flaxseed allene oxide synthase, also 18:3 is a precursor of other important compounds known as ketols in the presence of flaxseed hydroperoxide dehydrase preparations[40,41]. Also it is possible to use n-3 rich-perilla oil as an alternative fuel for the small diesel engine[42].

Yeast expression with ω-3 desaturases

Yeast (*Saccharomyces cerevisiae*) works as a very useful host for heterologous desaturase expression[43]. It has a very simple fatty acid profile and only one major fatty acyl desaturase (Δ-9 desaturase). Yeast cell contains eukaryotic endoplasmic reticulum, cytochrome b_5, and cytochrome b_5 reductase. In addition, yeast can take up and incorporate many kinds of fatty acids from the growth medium, also it has a low level of â-oxidation and carbon sources needed for the accumulation of the fed substrate and any fatty acid products[44,45,46,47]. For instance exogenously feeding oleic acid to yeast transformed with Δ-12 desaturase successfully resulted in the production of linoleate[45,48]. Yeast espression with *FAD3* of the oilseeds produces α- linolenate with 18:2 feeding[43]. However, in terms of its substrate specificity, *FAD3* showed the ability to desaturate exogenously fed 16 to 22 carbon substrates. In addition it showed ù-3 regioselectivity[49,50]. The uptake of these unsaturated fatty acids such as 18:2 and 18:3 from the growth media led to a severe reduction in the amount of endogenously synthesized monounsaturated fatty acids (16:1 and 18:1), which was likely due to repression of endogenous yeast desaturase activity[43,51]. Co-expression of yeast with *Brassica napus FAD2* and *FAD3* genes using a fatty acid-inducible peroxisomal gene promoter resulted in the induction of a new metabolic pathway converting oleic acid (18:1) into linolenic acid (18:3)[43]. Also the cultivation of yeast cells in the presence of triacylglycerols and exogenously supplied lipase promotes extensive incorporation of triglyceride fatty acids into yeast cells[52].

CONCLUSION

In general, omega-3 fatty acids have nutritional as well as industrial importance. expression of plant desaturases in yeast has offered a rapid method to verify the enzyme activities as well as the characteristics of their relationships with the substrates and the products, in addition to study their behaviors under different physiological conditions such as the effect of temperature on the accumulation of different fatty acids into storage lipids[45,49,53,54,55,56,57]. The yeast expression model may provide excellent ideas for understanding the transcriptional and post-transcriptional mechanisms involved in the regulation of plant fatty acid desaturases[43,54], which in turn could provide the tool producing engineered plants containing nutritionally and industrially useful fatty acid compositions[52,54,58,59,60,61].

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