

# NUTRI-THEMA®

## PHOSPHOLIPIDS & OMEGA 3

LABORATOIRES LE STUM'S NEWSLETTER N°7

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*Performances on the cellular stage are linked to bioavailability and vectorisation. Phospholipids are awarded as the best protectors against oxidation and the best DHA and EPA carriers to target tissues. They are ahead of ethyl esters, triacylglycerols and free fatty acids. Phospholipids, essential components of cellular membranes, including mitochondrial membranes, are rewarded for acting in a flexible biological capacity.*

*To take on the innovation challenge of the vectorisation of long-chain Polyunsaturated fatty acids (Lc-PUFAs) by the phospholipids path, Laboratoires Le Stum called upon two leading lipid research specialists.*

**VECTOMEGA®** was created through this combined research.

*Prof. Jacques Fanni and Dr. Michel Linder (Institut National Polytechnique de Lorraine (Lorraine National Polytechnical Institute) - Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (National college of Agronomics and Food Industries) co-discoverers of VECTOMEGA® answered some of our questions.*

- **Nutri-Thema:** What were the goals of the research programme launched by the INPL in 2003?
- **Professor Fanni:** It is all summed up in the title: “Development of marine lecithins to meet the needs of food, nutraceutical and cosmetic industries.”  
The project was a follow-up to the programme developed in 2001 in collaboration with the Office National Interprofessionnel des Produits de la Mer et de l'Aquaculture (OFIMER) (National Interprofessional Office for Sea Products and Aquaculture), the theme of which was: “Development of by-products and accessory catches” This study gave rise to the publishing of five articles in international reviews reviewed by reading committees five oral presentations, three public notices and a patent (the one which resulted in **VECTOMEGA®**). Its goal was to “gently” retrieve the lipid and peptide hydrolysis fraction (without organic solvent and at a moderate temperature) from by-products of salmon.
- **N-T:** The transformation process is strongly differentiated from existing methods. Can you give us a quick survey of the production methods used for fish oils containing  $\omega$ 3 Lc-PUFAs currently on the market?
- **Doctor Linder:** The survey is very quick! Marine oils are generally extracted from small cold water pelagics containing high proportions of EPA and DHA, such as the sardine, anchovy, capelin, herring and menhaden, or even by-product or waste resulting from netting operations generated by transformation industries (smoking-canning).  
Standard technologies and patents relating to extraction deal with “cooking and pressing” steps, using a thermal treatment of the raw material at temperatures close to boiling and causing coagulation of the proteins. The raw material is then introduced into presses to separate the proteinaceous aqueous phase containing the oil, from the solid matter.  
The oil can be extracted from the fish flesh using less drastic processes, after adding water, cold pressing and centrifugation.  
The process (patent) uses new biotechnological tools. Very high yields of extraction were obtained from the deconstruction of proteinaceous tissues by the action of specific enzymes, carried out in less than an hour in an inert atmosphere at a temperature lower than 60°C. The full amount of oil can be extracted using the above technique, while keeping its natural proportions

and composition.

- **N-T:** What about enriched oils?
- **Doctor Linder:** To enrich oils with specific molecules, for example, to bring the DHA level up to 30%, maybe even more (starting from 10% for salmon), other techniques are required. Lipid fractions can be separated by cryoconcentration or “winterisation”, according to their fusion point and can be concentrated by repeated processes. Concentration using a urea complex is often implemented at the same time as the pre-treatment of oils rich in PUFAs. Urea is a molecule, the particularity of which is to make complex associations with certain saturated acylglycerols, which can be eliminated following crystallization. However, the concentration process requires large quantities of solvents, various chemical products (soda, hexane and methanol) and generates a huge amount of waste which needs to be reprocessed. Extraction by supercritical fluid is very neat, applicable on an industrial scale; however, because of its **high cost** it can only be imagined for products with a high added value. Fatty acids can be fractioned and concentrated using preparative chromatography separating them by their chain lengths. These techniques are used to obtain highly purified concentrates of PUFA. Lipases of fungal, bacterial, pancreatic or gastric origin catalyse a large number of specific reactions relating to the position of the fatty acids to the glycerol. The first step is to specifically hydrolyse the saturated fatty acids of the glycerol and to eliminate them. The second step is to **re-esterify the PUFAs** in the form of acylglycerols with the help of other lipases, using organic **solvents**.
- **N-T:** Do these oils all have the same bioavailability?
- **Professor Fanni:** The bioavailability essentially depends on the oil’s composition in fatty acids and especially their regiodistribution on the glycerol molecule. One must remember that marine oil Lc-PUFAs are generally located at the central position (sn-2) on triacylglycerol. This is the best position for maximum bioavailability. Certain fish oils considered to be “naturally enriched in PUFAs” have undergone treatments which modify the arrangement of the fatty acids and their concentration. This is the case with the total hydrolysis of triacylglycerols re-esterified as ethyl-esters, whose bioavailability is highly questionable. There are also the “fish oil in the form of free fatty acids” formulas, very easy to obtain, but whose bioavailability is merely a reflection of a marketing drive. There are also a number of marine oils on the nutraceutical and cosmetic market, presenting high concentrations in Lc-PUFAs (microseaweed, sardine and anchovy oil mixture with “optimised” EPA/DHA ratio, Krill oil, etc.).
- **N-T:** Incidentally, what are we to think of Krill oil?
- **Professor Fanni:** Its value is indisputably very high. The main criticism that can be made about it is linked to its high cholesterol content (this is a characteristic of all seafood) **eliminated by organic solvents**. It should be observed that the presence of endogenous lipases rapidly deteriorates the lipid compounds before extraction. Moreover, one could object to the fact that Krill is a natural resource, taken from the arctic seas and that it might be a good idea to treat it with care.
- **N-T:** What types of fatty acids are contained in an “Omega-3 enriched” product?
- **Doctor Linder:** If this is an “Omega-3 enriched” fish oil, the treatments mentioned above are generally translated by a fairly heterogeneous increase in the concentration of unsaturated fatty

acids with a low bioavailability. In general, 30 to 50% of DHA and EPA can be reached with this enrichment. Where fish oils are concerned, laboratories always talk of the proven or supposed effect of certain fatty acids, to enrich their formula to **above what is reasonable**. There are formulas with high concentrations of Omega-3 (90%), and high levels of EPA (70%), called “mood regulators”, and others with a very high concentration of Omega-3 (85%) and a 70% DHA level, for cardiovascular problems, and there is even triDHA!

The success of “Omega-3s” in the media, brought to light by the Bang and Dyeberg team in the 1970s, has now brought about **disorderly marketing drives** in numerous food sectors, and we are now seeing an increase in the number of cheeses, ham, milk drinks, eggs and butter, enriched with Omega-3.

These can contain deodorized fish oil, fractions of enriched oil or precursors of long chains with uncertain metabolic evolution.

- **N-T:** If we refer to advertising for the general public, DHA is generally thought of as a poor parent of the  $\omega$ 3 family. However, it plays a major role in many systems. Why is there so little information? How can it be situated in the Cretan diet?
- **Doctor Linder:** It is more a problem of confusion generated by the media and picked up again by the medical profession, than a lack of information. For most people, the word “Omega-3” means nothing. When you think of information available, we haven't seen the end of it.

In the Cretan diet, Long-chain Omega-3s intervene without the inhabitants' knowing, because these populations do not eat much meat, relatively little fish and lots of fruit, vegetables and wild herbs. So, where do the Omega-3s in their diet come from?

It comes from snails which they eat a lot of. These are full of polyunsaturates through the intermediary of purslane, a plant that contains an awful lot.

Of course, their food rich in vegetable oil also takes part in the beneficial quality of this diet.

- **N-T:** Let us continue to talk about DHA. Did you imagine there would be such a large bioavailability of DHA, when you were perfecting the future **VECTOMEGA®**?
- **Professor Fanni:** No. Information on the bioavailability of polyunsaturates by the intermediary of phospholipids was still only sketchy. Recent work shows that DHA fed to rats in the form of liposomes (phospholipid vesicles) is better assimilated than in the form of neutral oil. It should be remembered that these molecules easily cross the enterocyte membrane. Moreover, it should be observed that DHA is naturally in *sn-2* position and that its concentration in phospholipids can reach 30 to 50% depending on the nature of the amphiphilic molecule carrier.
- **N-T:** Here is a table of the phospholipid composition of **VECTOMEGA®** and their actions in brief.

Type of Phospholipid (PL)	Vectomega® PL %	Action in brief
Phosphatidylcholine (PC) or Marine Lecithin	68.00%	Plays a part in growth, and cellular differentiation and regeneration. Assists in the introduction of DHA into the heart muscle. Protects the mitochondrion from oxidative damage, decreasing the impact of auditory and visual aging. Reduces fatty liver

Phosphatidylethanolamine (PE)	11.00%	Combined with PC helps in the building of the myelin sheath and the astrocyte development of synapses. It is strongly concentrated around the medullar motoneurons.
Phosphatidylserine (PS)	9.00%	The most widespread of all membrane PLs, protects from ischaemic attacks or Alzheimer's disease of the brain functions, particularly in the hippocampus.
Phosphatidylinositol (PI)	5.00%	Has a major role in cellular identification, a precursor of intracellular signal molecules. Acts on the regulation of cellular calcium. Has shown its effectiveness on OCDs, panic attacks, depression, manic depression and Alzheimer's disease.
Sphingomyelin (Sph)	5.00%	Its pro-apoptotic activity decreases the risks of coronary heart disease and cancer of the bowel (colon). Decreases parietal cell toxicity of bile salts. Sphingolipids are mostly present in the myelin sheathes

- **N-T:** The proteinaceous hydrolyses and amino acids highly present in **VECTOMEGA®** are created during the fabrication process. What are their physiological properties and their actions along with fatty acids?
- **Professor Fanni:** We know that proteins and peptides take part in transporting polyunsaturates, by associating themselves with phospholipids. For the consumer, the first most obvious effect is the masking of smells after ingestion. There is no reflux. The presence of peptides gives increased phospholipid peptide complex solubility. We also know from literature on the subject, that peptides stemming from fish proteins have serotoninergetic properties, however for the moment no formal proof has been provided. We suspect as much, but that is all.
- **N-T:** Lc-PUFAs are particularly vulnerable to oxidation, what is the situation with those transported and vectorised by phospholipids?
- **Doctor Linder:** PUFAs attached to phospholipids are reputed to be less sensitive to oxidation. Work on the resistance of DHA to oxidation shows that this stays intact when it transported by a phospholipid, while under oxidative stress, its concentration plummets to more than 90% in two weeks when it is in the triacylglycerol form.
- **N-T:** Must we add large volumes of antioxidants to the matter of **VECTOMEGA®**?

- **Doctor Linder:** No. The high concentration in phospholipids and the presence of caretenoid compounds in fish oil strengthen its natural resistance to oxidation.
- **N-T:** What are the tissues likely to benefit most from the  $\omega$ 3 Lc-PUFA content?
- **Professor Fanni:** Since phospholipids are universal cellular components in the human, we are able to say that all tissues will benefit from the  $\omega$ 3 Lc-PUFA content of **VECTOMEGA<sup>®</sup>**, however the tissues with a greater demand for it, following phospholipid metabolisation: enterocytes, hepatocytes, cardiac muscle and arterial cells, mammary tissue and finally all the neurotissues (brain, spinal cord, nerves including the visual and auditory system). Their intervention in the metabolic regulation of inflammatory states should not be forgotten either.
- **N-T:** What features help us to realize the importance of this biotechnological discovery and its clinical applications?
- **Professor Fanni and Doctor Linder:** This is undeniably the observation of a neurone culture boosted by DHA. It has reinforced our thoughts that by directly providing Lc-PUFAs to the brain using a **phospholipid, the best carrier there is**, this could only be beneficial. Of course, it is obvious that the special sn-2 position of DHA on the glycerol is capital. So it would be a good idea to reflect upon the interest that this observation raises with regard to the bioavailability of Lc-PUFAs. It is not unreasonable to think that the phospholipid associated with a proteinaceous complex and new integrated molecules could have a particularly interesting effect. To be continued.!....

### **Bibliography :**

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