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## Functional Foods as catalysts of the nutrition evolution into nutrigenomics: a scholastic example of a fermented papaya preparation (Immun-Âge)

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### Functional Food: a recent history dating back in time

“Functional Foods” represent an emerging opportunity and the development of innovative solutions aimed at acting on organic systems as well as on more general topics relating to consumer health. This is different to previous situations, when mainly retrospective epidemiological studies or empirical experiences were carried out on single nutrients. Such a new and growing interest by the scientific community follows research deeply oriented to clinics and supplemented by an accurate study on nutrients, genomics and single nutritional requirement diagnostics. Already in 1993, Nature published a report that Japan is exploring roles between food and medicine. Clearly the success of “Functional Foods” depends also on the food industry’s capacity to develop new effective products which on the one side meet consumers’ needs and on the other must have positive effects on health, supported and validated by scientific research and therefore far beyond a simplistic claim of positive properties, as recently outlined in a meeting, organised by a non profit, non governmental international association.

### Definition and demanded features

This approach has lead, in the past few years, to constant changes in the Functional Food definition

which an authoritative scientific European panel defined as follows, in 1999 “ A nutrient can only be easily considered functional if it was satisfactorily proved that it can positively change one or more target functions, besides nutritional effects, as to consistently improve health, and well-being while reducing any affection risk. A Functional Food should ideally be a nutrient and should not change its efficacy when entering into a diet; it should not be either a pill or a capsule”. It was then agreed that, from a practical view point, a Functional Food should comply with the following features:

- 1) a natural food;
- 2) a food which was simply supplemented by a component;
- 3) a food which was no longer holding a component;
- 4) a food which the nature of one of more components had been changed;
- 5) a food which one or more component availability had been changed;
- 6) a combination of the previous features.

It was then outlined how, besides its nutritional properties or physiological effects, it is mandatory to offer a consistent safety profile of its administration. Such a condition is nothing but a prerequisite to further develop any Functional Food. From the recommendations of such a European commission, it is possible to come to the conclusion that “The design and development of a Functional Food is a key factor, besides a scientific challenge, which should be mainly based on consistent scientific knowledge in terms of target functions and

their possible modulations by nutritional components". It is furthermore stressed that "Functional Foods are not universal, therefore a nutritional-specific approach would be no longer suitable, but rather a basic scientific approach would only apply".

It is important to outline a new concept within nutrition, on the role played by Functional Foods science, which must be followed to get useful clinical interventions (**tab. 1**).

An ancient Chinese proverb says that "medicine and food are isogenic" and it is not by chance that in 1984, in Japan, a unique national study group was set up, sponsored by the Ministry of Education, Science and Culture (MESC), aiming at exploring the interface between nutrition and science. Scientists in time studied and defined a series of foods and nutrients which were officially listed in the category "foods to be specifically administered for health-care", stressing and recognising their nutritional value, after undergoing a consistent bio-fermentation process. Such a classification is still a legally-binding tool against media communication of wrongly defined natural products, misleading or simply recalling data from the literature but not followed by specific validations of the product itself.

## Synergies, markers and development strategy of nutrigenomics

A biochemistry and molecular biology specific development together with biotechnological methods were conducted to support the hypothesis that some nutrients could modulate body functions, playing a role in general good health as well as in the reduction of disease risk. depending on life style. Such assessments had to be in line with consistent identification markers, both directly connected (functional factors) to the process to be modified, as well as indirectly associated (indicators). In fact from the human genome project conclusion, a post-genomic study has started, which should mainly be correlated with Functional Foods, availing itself of sophisticated technologies such as DNA tip technology and others, which lead to nutrigenomics. Such a word was only recently introduced and represents a leap forward in comparison with observational studies which were mainly based on research in the bioactive nutritional component field. Nutrigenomics mainly aims at studying genetic and epigenetic interactions with a nutrient as to lead to a phenotype change and therefore to the cell metabolism, differentiation or apoptosis. Furthermore, to stress the scientific research importance, the simple fact that research is effectively carried out on the nutrient which apparently is "functionally" effective, requires that to the minimum effective quantity leading to the above-mentioned changes is defined. There are in fact many pre-clinical studies which use a bioactive nutritional component at concentrations which can not be practically

administered. More recent papers suggest that cells are able to adapt themselves when exposed to excessive quantities of nutrients. As previously stated, it would be highly incoherent, if not with no scientific application, to enforce any approach to a natural product:

1) which is only nutrient-specific;  
2) and moreso, if generally referring to properties simply retrieved from literature, but with no specific validation or bioavailability study.

What is more, a series of far-sighted companies and food industries are consistently sponsoring independent validation studies on natural products, even when not imposed by the regulation in force;

3) taking into account the negative effect of the variable efficacy of the nutrient according to the different formulation (lyophilised products, dehydration processes at low or high temperature, extracts, etc.) or associations. Isoflavons and soy proteins stand out among all, where the role of each single component is not clear yet, as well as the effects of any possible association or the best formulation of soy itself.

As for new generation studies, however, it is too early yet and still many are the interactions to be assessed between nutrients and host and between nutrients themselves, and possibly many mechanisms will play an important role all together. Biological modifications in the presence of a Functional Food would briefly be anti-oxidant (followed by a series of possible genic sequences mediated by an increased transcriptional rate by: cytochrome P450s, glutathione-S-transferase, NAD(p)H: kinone-reductase, UDP-glucuronosyltransferase, microsomal hydrolysis, aphta-toxin B1-aldehyde reductase, dihydrodiol-dehydrogenase, aldehyde-dehydrogenase, glutathione-reductase, etc.), supporting the detoxigenic enzymes, carcinogen build-up and metabolism block, hormonal homeostasis change, delaying the cell division or inducing apoptosis.

Immun-Âge history: it is an example of the rational and evidence-based biotechnological study.

That being stated, it is far more interesting to further and briefly analyse the study and development process, still in progress of Immun-Âge, a specific product derived from the technologically advanced and controlled bio-fermentation process of *Carica Papaya* Linn, in the absence of genetic manipulation, within a Japanese research institute carried out in compliance with every quality control and environmental-friendly validated standards. After a series of initial reports, a couple of decades ago, by Japanese scientists on a series of populations living in the Philippines and eating a lot of papaya fruit, 15 years ago, a research institute was set up devoted to the study of "functional" properties of a series of specific compounds within such a diet. The most attention was paid to *Carica Papaya* Linn, which collected in the Philippines, was further processed in Japan, with

other exotic fruits through a long fermentation process according to natural methods.

## **Basic research: a compulsory process to follow in the development of biotechnologies.**

From the extraction of the final product, a series of experimental scientific activities and studies were carried out by the Neuroscience Department, Molecular Biology Institute at the Okayama University in Japan, directed by Prof. Mori. Such studies, carried out with sophisticated methods among which was Electron Spin Resonance, highlighted that such a product consisting of fermented Papaya exhibited a powerful anti-oxidizing activity on *in vitro* cerebral cells as well on the *in vivo* epilepsy experimental model, where the epileptogenic monoamine neutral release was consistently reduced. Prof. Mori's group also proved the capacity of fermented papaya to reduce the increase of free radical concentration as well as superoxide dismutase at the brain level in elderly rats followed by the reduction of experimental ischemia/reperfusion-induced cerebral damage. It was furthermore highlighted to be consistent *in vitro* anti-oxidizing product capacities even when tested for one hour at high temperatures (100°C) and acidic pH<sup>(1,2)</sup>. What is more, such features were confirmed after a long-term storage.

Then, after thoroughly refining the product and getting its certifications by the governmental body (**table 2**), two important studies were carried out with international institutes to further assess the product, such as its possible effects on the immune system, together with the Kyoto Pasteur Institute, as well as its effects on the oxidizing stress in co-operation with the Molecular Biology Department at the UCLA in Berkley directed by Prof. Packer, a widely recognised authority on the subject, leading to a more detailed assessment of its activity mechanism. Such successful studies, still in progress, led to a series of extremely interesting *in vitro* and *ex vivo* evidence data. The group from the Pasteur Institute in Kyoto, starting from the evidence of a positive effects of Immun-Âge on the Natural Killer population on an experimental sarcoma model, proved its capacity on human beings to affect the interferon-g production. Such data was further proven by studies supporting the positive activity of Immun-Âge on the macrophage function isolated from rats and human beings. In the same time period, the working group co-ordinated by Prof. Mori showed the consistent protecting effect by Immun-Âge on oxidizing stress on isolated rat hearts. These data may have potential clinical implications when considering that severe injury such as ischemia/reperfusion is a unique epiphenomenon present during myocardial infarction and in stroke. Such data were then confirmed in oxidative damage of other tissues such as kidney, liver and brain.

The same group also shed some light on the causal connection of the immune-modulating activity of Immun-Âge with its anti-oxidising features. In fact on a rat macrophage line, important experimental evidence was put forward on how Immun-Âge can upregulate nitric acid production induced by interferon-g. Immun-Âge would then exhibit a nutrigenomic effect able to affect the messenger RNA expression both of inducible nitric acid and of TNF-a and of interleukin 1 $\beta$ .

Such an activity was further assessed when two different fractions were arbitrarily separated, according to their different molecular weight (cut off: MW 3.000), both confirming the previous results as well as the new important evidence of their action on the NF-kB binding to DNA as a clear explanation of the transcriptional increase of inducible nitric oxide gene. The two different fractions however proved a series of differences in terms of macrophage stimulation and anti-oxidising scavenging activity. It is therefore possible to prove, for example, that a different immune-modulating activity could depend on the different (1-3)- $\beta$ -D-glucan concentration, which represents the most representing portion of some peculiar yeasts, used in the Immun-Âge bio-fermentation process.

Clinical evidence supported by research: a long-awaited evolution from empirism

Support offered by scientific evidence and a series of works on human beings represented the foundations to plan a series of clinical studies. In 1995 a oncology-haematological Russian study group proved, on young subjects undergoing radiotherapy against severe myelo- and lympho-leukaemia, how the administration of this specific fermented papaya preparation, as proved in the previous experimental studies by Prof. Mori, managed to significantly reduce clinical side-effects (encephalopathy score: anorexia, nausea, vomiting, convulsions, dizziness) and bio-humoral abnormalities (change of the redox status due to the erythrocyte glutathione depletion and leukocyte SOD increase, deficit of the mononuclear cell activity). During the same time period a group of Italian, French and Japanese scientists co-ordinated a series of studies on the alcoholic liver diseases which proved how Immun-Âge allows reduction of alcoholic oxidative stress (reduction of plasma and erythrocyte level of malonyldialdehyde as well as of plasma lipoperoxides) during the initial phases of withdrawal, when it is possible to mark a persistence of the microsomal system activation leading to the ethanol oxidation, with a consequent continuance of the pro-oxidative state and during chronic alcoholic abuse. More precisely, taking into account the low compliance in the case of withdrawal, it was proved how the administration of Immun-Âge to alcoholics led to the following effects:

1. a significant improvement of haemorheology (reduction of the whole blood viscosity, recovery of the erythrocyte deformability and increase of blood filtration capacity through specific membrane). Such a consistent

increase of the malonylaldehyde concentration in the erythrocytes in the case of chronic alcoholics leads, through lipoperoxidising effects, to a lipid asymmetry destabilisation. Such data proved also to be interesting for an Israeli working group on thalassaemia which got preliminary in vitro data about the protective effects of Immun-Âge on erythrocytes collected from subjects suffering from b-thalassaemia

2. a significant recovery of the latent malabsorption of vitamin B12 due to the interference of alcohol-induced oxidising effects on the gastric mucosa as the binding site level between intrinsic factor and cyanocobalamin.

Such evidence on the efficacy of Immun-Âge on oxidising stress inducted by alcohol on the gastric mucosa was also based on the concomitant evidence of the significant protective effect (macro- and micro-scopic and biochemical as well) on healthy subjects, after being administered a test-dose of ethanol (40 ml 80% ethanol).

According to the previous results on the antigenotoxic effect and on the DNA in vitro protection by Immun-Âge from the group of Prof. Mori and more recently of Prof. Packer who highlighted its iron chelating effect, a new clinical trial was carried out on gastric precancerous lesions. A group of Italian and Japanese scientists proved in a controlled and randomised study carried out for a six month period on patients suffering from atrophic chronic gastritis without the presence of *Helicobacter pylori* that both a generic anti-oxidant mixture, high dosage vitamin E and Immun-Âge reduced of a series of mucosa markers relating to oxidative stress. However, Immun-Âge alone managed to significantly reduce the two markers used as an expression of a precancerous biochemical change, that is ornithine decarboxylase and mainly 8-oxoguanine which is one of the most frequently used biochemical markers relating to DNA oxidative damage.

At the time of the first clinical trials by the Kyoto Pasteur group on the immuno-modulating Immun-Âge effects and relating observation reports (increase of the CD8+ and QOL score), on the positive beneficial effect which HIV-affected patients could benefit from, a series of studies were started by Prof. M. Weksler of the Cornell University in the USA and Prof. L. Montagnier, former director of the virology laboratory of the Pasteur Institute in Paris and present chairman of the World AIDS Research and Prevention Foundation. In a preliminary study, which is going to be further enlarged, it was proved that the Immun-Âge administration for 3 weeks before anti-flu vaccination in 10 hospitalised elderly patients consistently improved their specific antibody response in comparison with a control group which was only administered the vaccine. What is more, Prof. Montagnier's group carried out a study on the administration of Immun-Âge to HIV-positive patients and data from the open preliminary research proves how such a product, when associated to an anti-retroviral treatment, can significantly improve the CD4+

concentration as well as hemoglobineamia, weight increase and cenaesthesia.

Finally, at present in Great Britain studies are being carried out on Immun-Âge on experimental Parkinson models while in a highly qualified French laboratory a series of studies are being enforced on the possible fraction separations inside the product to better assess its specific features.

It goes without saying that it is highly important to move to a diet rich in vegetable foods which, if correctly enforced, offers the availability of micro-nutrients and anti-oxidants which are more than enough to comply with the body requirement in the case of normal health conditions and in the absence of important psychical and physical burdens. What simply depended on common sense, was underlined long ago by an authoritative international no-profit institute which stressed how a healthy diet should not be replaced by a non-controlled diet rich in supplements or food-like compounds as vitamins, extracts or lyophilised products, mainly when the variability of such products in each single batch is uncontrolled or even worse, when no certified titration was carried out. However the absence of specific and referenced studies on each single nutraceutical attempt can not be counterbalanced by general data collected from literature. However legislation and standards are still open about fortified foods supplemented by specific nutrients which deserve a discussion on its own. As previously underlined by Prof. Packer during an international congress we are in front of a consistent evolution of anti-oxidants implying the study on how some of them from a simple scavenger function are instead able to interact in a complex way with the redox balance and immune-modulating network through a genic adjustment.

Immun-Âge certainly represents a Functional Food highly complying with the new features of the new nutrigenomic product category

## References

- 1) Nature 1993; 364:180.
- 2) "Functional Foods - Scientific and Global Perspectives", Intl. Life Science Institute symposium, Paris, France 2001.
- 3) European Commission's Concerted Action on Functional Food Science in Europe- FUFOS, 1999.
- 4) EU Novel Food Regulation, European Commission 1997.
- 5) Roberfroid MB et al. Br J Nutrition 2002; 88 :133-138.
- 6) Food for Specified Health Use, FOSHU.
- 7) Ventre JC et al. Science 2001.
- 8) DellaPenna D. Science 1999.
- 9) Milner JS. Moving beyond observational studies, in Functional foods and Health: a US perspective. Br J

Nutrition 2002.

10) Jackson AA, Adv Exp Med Biol 2000 e Fafournoux P et al. Biochem J 2000.

11) Kneale C et al. Survey on health claims, University of Sydney, Nutrition Res. Found.,1997.

12) Crouse JR et al. Arch Intern Med 1999.

13) Santiago LA et al. Free Rad Biol Med 1991; 11:379-383

14) Santiago LA et al. Med Sci Res 1992 ; 21 :139-141.

15) Santiago LA et al. Oxygen Radicals 1992 ; 405-408.

16) Santiago LA et al. Neurochem Res 1993; 18:717-717.

17) Mori A et al. NeuroReport 1993; 4:1031-1034.

18) Osato JA et al. Med Sci Res 1992; 20:27-28.

19) Okuda H et al. Clinical Rep. 1993; 27:4249-4258.

20) Kishida et al. Neurosciences 1994; 20:149-152

21) Kishida et al. J Interferon res 1994; 14:179.

22) Shinohara M et al. Canad J Physiol Pharmacol 1994; 72:1

23) Osato JA et al. Phys Chem Biol Med 1995; 2:87-95.

24) Osato JA et al. Nutrition 1995; 11:568-572.

25) Haramaki N, Packer L et al. Biochem Mol Biol Int 1995; 36:1263-1269.

26) Marcocci L et al. Biochem Mol Biol Int 1996; 38:535-541.

27) Kobuchi H et al. Biochem Mol Biol Int 1997; 43:141-152.

28) Rimbach G et al. Life Sci 2000; 67:679-694.

29) Korkina LG et al Nutrition 1995; 11:555-558.

30) Marotta F et al. HepatoGastroenterol. 1997; 44:1360-1366.

31) Marotta F et al.. Hepato-Gastroenterology 2001; 48: 511-17.

32) Prof. Rachmilewitz, ORI Report, UNESCO, Paris, France, 2002.

33) Marotta F, Hepato-Gastroenterology 2000; 47: 1189-94.

34) Marotta F et al. Digestion 1999; 60: 538-43.

35) deCastro-Bernas G et al. Med Sci Res 1993; 21:107-108.

36) Rimbach G et al. Anticancer Res 2000; 20:2907-2914.

37) Marotta F et al. Annals of N Y Acad Sci, 2004.

38) Mimaya J. Life Living Guidance-Ministry of Health, Japan, 1998.

39) Weksler M. Personal communication, The Press Club, Paris 2002.

40) Chenal H, Montagnier L. Personal communication at From Genomics to Nature, University of Tor Vergata, Rome, 2003.

41) "Antioxidant: Scientific Basis, Regulatory Aspects and Industry Perspectives", Antioxidant Task Force-Intl. Life Science Institute symposium, Bruxelles, 1996.

42) Ghosh S et al. Commercial Validity of Claims for Biological Activity and Regulatory Issues, Clinical Science; 2003 104:547-556.

43) Howe PCR What makes a Functional Food

functional? -substantiating health claims. Asia-Pacific J Clin Nutrition 2000; 9:108-112.

44) International Symposium on Free Radicals and Health: Molecular Intervention and Protection of Lifestyle-Related Disease, 23-25 Ottobre 2003, Sakata, Japan.

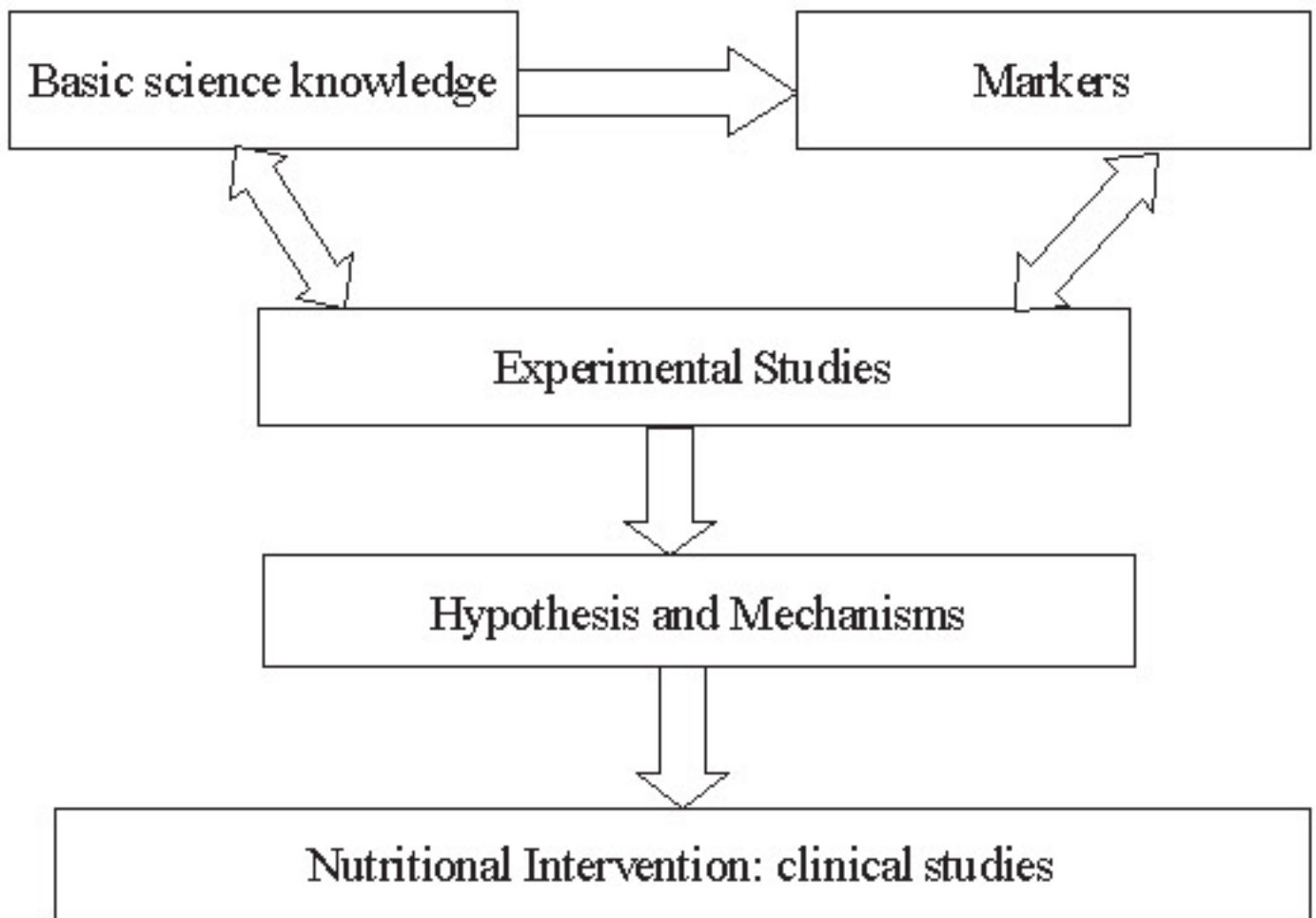
**Table 2**

**Fermented Papaya Preparation (100 gr)**

FPP/100g Composition. (Japan Food Res.Lab, Tokyo)

Carbohydrates	90.7 g	Arginine	16 mg
Moist	8.9 g	Lysine	6 mg
Proteins	0.3 g	Hystidine	5 mg
Fats	absent	Phenylalanine	11 mg
Ashes	0.1 g	Tyrosine	9 mg
Fibres	Absent	Leucine	18 mg
Vitamin B6	17 mcg	Isoleucine	9 mg
Pholic acid	2 mcg	Methionine	5 mg
Niacin	240 mcg	Valine	13 mg
Iron	0.29 mg	Alanine	12 mg
Calcium	2.5 mg	Glycine	11 mg
Potassium	16.9 mg	Proline	8 mg
Magnesium	4.6 mg	Gluthamic acid	37 mg
Copper	14 mcg	Serine	11 mg
Zinc	75 mcg	Treonine	8 mg
		Aspartic acid	27 mg
		Triptophane	2 mg

**Table 1.** A correct strategy to discover and develop Functional Foods



A diagram of the correct synergy between the discovery and development of Functional Foods.