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Evolving concepts in nutrition: from functional foods to nutrigenomics: the paradigmatic example of fermented papaya preparation

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Functional Food: recent research with historical beginnings

"Functional Foods" represent an emerging opportunity and if put on a timeline they will certainly play a consistent and important role in the future. Such a new perspective entirely depends on the growing attention paid by nutritionists to the development of new innovating solutions aimed at acting upon organic systems as well as on more general topics related to good consumer health . Different from the past, 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 supplemented by an accurate study on nutrients, genomics and single nutritional requirement diagnostics. Already in 1993, the leading journal Nature published a report "Japan is exploring limits between food and medicine" (Swinbanks 1993). Clearly the success of "Functional Foods" depends on the food industry capacity, too, of developing new effective products which on the one hand meet any consumer request and on the other hand must have positive effects on health, supported and validated by scientific research and therefore far beyond simple positive properties, as recently underlined in a meeting, organised by a non-profit non-governmental international association.

Definition and needed features

Such a new philosophy in the last few years has led to constant changes in 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, 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) be 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 has been changed;

5) a food which one or more component availability has been changed;

6) a combination of the previous features.

It was then underlined how, besides its nutritional properties or physiological effects, it was necessary to offer a consistent administration safety profile. 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". And therefore it is further stressed that "… while Functional Foods are not universal, therefore a nutritional-specific approach would be no longer enough. But mainly and universally a basic specific scientific approach only applies".

It is important to underline a new concept within nutrition on the role played by "Functional Foods science", which is the only one to be followed to get to useful clinical inferences (Roberfroid, 2002).

An ancient Chinese proverb specifies 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, under the patronage of the Ministry of Education, Science and Culture (MESC), aiming at exploring the interface between nutrition and science. Scientists for some 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" (Food for Specified Health Use, FOSHU), 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 generally recalling generic data in literature but not followed by specific validations of the product itself.

Synergies, markers and development strategy leading to nutrigenomics

A biochemistry and molecular biology specific development, together with biotechnological methods, were enhanced so as to support the hypothesis that some nutrients could modulate the body functions playing a role in its general good health conditions as well as in the reduction of contagion risks depending on life style. Such assessments had to be in line with consistent marker identification, both directly connected (functional factors) to the process to be modified as well as indirectly liable (indicators). Suitable marker selection mainly supported the development of genomics. In fact from the human genoma project conclusion (Venter JC 2001), the post-genomic era started, which should mainly be correlated with Functional Foods, profiting from sophisticated technologies such as the DNA tip technology and some others, which lead to nutrigenomics (DellaPenna, 1999). Such a word was only recently introduced and represents a leap forward in comparison with observation 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 so as to lead to a phenotype change and therefore to the cell metabolism, differentiation or apoptosis (Fafournoux 2000). Furthermore to stress the scientific research importance and mainly, as far as natural products are concerned, the simple fact that research is effectively carried out on the nutrient which apparently is "functionally" effective, it is necessary to define the minimum effective quantity leading to the above-mentioned changes. There are in fact many pre-clinical studies which use a bioactive nutritional component at concentrations which cannot practically be administered. More recently papers suggest that cells are able to adapt themselves when exposed to excessive quantities of nutrients. As previously stated, it would be highly confused , if there were no scientific application, to enforce any approach to a natural product:

1) which is only nutrient-specific;

2) and even more, if generally referring to properties simply derived from literature, but with no specific validation or bioavailability study.

What is more, a series a 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 of them, 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 (Crouse 1999).

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

Fermented Papaya Preparation history: an example of the rational and evidence-based biotechnological study.

That being said, it is far more interesting to further and briefly analyse the study and development process, still in progress of fermented papaya preparation (FPP) 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.

It has been well-know for a long time that the anti-oxidant natural papaya properties, mainly depending on vitamins (A & C) and amino acids were consistent both in the fruit and derived from the papain enzyme (Arginine among others). Papain plays a digestive role , but such an activity is no longer present in the FPP. A long fermentation, by means of yeasts, is the unique process, supporting the preservation of papaya anti-oxidant properties while offering important new immune-modulating features. Fermentation deeply modifies, within the product, the ratio between complex carbohydrates and proteins, which in lyophilised papaya accounts for about 10:1, increased up to 10:0.03 in the case of FPP, that is 30 times higher . In the final fermented product and not in the fresh fruit, many new classes of oligosaccharides are present at a different polymerisation as well a monomers similar to the basic structure of ß 1-3 D-glucan. Such oligosaccharides, mainly oligosaccharides exhibiting a low molecular weight, exhibit a wide spectrum of immune-modulating activity.

After a series of initial reports by Japanese scientists on a series of populations living in the Philippines and eating large amount of papaya on a daily basis, over 20 years ago, a research institute was set up committed to the study of "functional" properties of a series of specific compounds within a fruit - and vegetable-based diet. Special attention was paid to Carica Papaya Linn, which was collected in the Philippines and was further processed in Japan with other exotic fruits through a long fermentation process according to organic methods.

Basic research: a compulsory process to follow the development of biotechnologies

From extraction of the final product, a series of experimental scientific activities and studies were carried out by the Neuro-science Department, Molecular Biology Institute at the Okayama University in Japan, directed by Prof. Mori (Santiago 1991). Such studies, carried out with sophisticated methods, among which Electron Spin Resonance, highlighted that such a product consisting of fermented papaya exhibited a powerful anti-oxidizing activity on *in vitro* cerebral cells (Santiago 1993) as well as on the *in vivo* epilepsy experimental model, where the epileptogenic monoamine neutral release was consistently reduced (Santiago 1993). 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. The consistent *in vitro* resistant anti-oxidizing product capacities was furthermore highlighted even when tested for one hour at high temperatures

(100°C) and acid pH (1,2). What is more, such features were confirmed after long-term storage. Such potential neutoprotective effects of FPP are at the moment the issue of a clinical study on Parkinson's disease patients by the group of Dr. Nordera in northern Italy. which is showing some preliminary promising results especially in rigidity symptoms. Interestingly, some still uncontrolled data from Prof. Barbagallo, chief of Geriatrics unit at the University of Palermo pointing towards a significant decrease of plasma oxidative stress parameters in FPP-supplemented patients with varying degree of dementia.

Then, after thoroughly refining the product and getting certification by the governmental body (table 1), two important studies were carried out with international institutes so as to further assess the topic such as its possible effects on the immune system together with the Kyoto Pasteur Institute (Kishida 1994) as well as its effects on the oxidizing stress in co-operation with the Molecular Biology Department at UC at Berkley directed by Prof. Packer, a widely recognised authority on the subject, leading to a better assessment of its activity mechanisms. Such successful studies, still in progress, lead to a series of extremely interesting *in vitro* and ex vivo evidence . The group from the Pasteur Institute in Kyoto, starting from the evidence of positive effects of FPP on the Natural Killer population of a sarcoma experimental model proved its capacity to affect the γ-interferon production on human beings. Such data was further proved by studies supporting the positive activity of FPP on the macrophage function on rats (Marcocci 1996) and human beings as well . In the same time period, the working group co-ordinated by Prof. Mori proved the consistent protection effect by FPP on oxidizing stress on isolated rat hearts (Haramaki 1995) when undergoing a severe effect such as ischemia/reperfusion in the clinical practice, the unique epiphenomenon present during a myocardial stroke. Such data have recently been confirmed and have gained further insights from Aruoma et al. (2006) who has shown the ability of FPP to modulate oxidative DNA damage due to H_2O_2 in rat pheochromocytoma (PC12) cells and protection of brain oxidative damage in hypertensive rats.

The same Mori group also led to important scientific results proving the connection of the immune-modulating activity of FPP to its anti-oxidising features. In fact, on a rat macrophage line, important experimental evidence was put forward on how FPP can adjust the nitric acid production induced by interferon-γ upward. FPP (Kobuchi 1997) would then exhibit a nutrigenomic effect able to change the messenger RNA expression both of inducible nitric acid and of TNF-a and of interleukin 1β.

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-κB binding to DNA as a clear explanation of the transcriptional increase of inducible nitric acid 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)-β-D-glucan concentrations, which represents the most representative portion of some peculiar yeasts, used in the FPP bio-fermentation process.

Clinical evidence supported by research: a needed evolution from the empirical

Supports offered by scientific evidence and a series of works on human beings represented a foundation to plan a series of clinical studies. In 1995 in fact a oncological- haematologic Russian study group (Korkina 1995) proved, on young subjects undergoing radiotherapy against severe mielo- and lympho-leukaemia, how the administration of FPP, as proved in the previous experimental studies of Prof. Mori, managed to significantly reduce clinical side effects (encephalopathy score: anorexia, nausea, vomiting, convulsions, dizziness) and bio-humoral effects (change of the redox state due to erythrocyte gluthatione depletion and leukocyte SOD increase, deficit of the monocyte bactericidal activity). During the same time period a group of Italian, French and Japanese scientists co-ordinated a series of studies on the alcoholic liver disease which proved how FPP allows the reduction of alcoholic oxidative stress (reduction of plasma and erythrocyte level of malonyldialdehyde as well as of plasma lipoperoxides) both during the initial phases of withdrawal, when it is possible to observe a persistence of the microsomial system activation leading to ethanol oxidation (with a consequent maintenance of the pro-oxidative state) and during chronic alcoholic abuse. More precisely, taking into account the low clinical practice compliance in the case of withdrawal, it was proved how the administration of FPP 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 a specific membrane). Such a consistent increase of the malonylaldehyde concentration in the erythrocytes in the case of chronic alcoholics leads to, through lipoperoxidising effects, a lipid asymmetry destabilisation (Marotta et al. 2001). Part of these data have recently been confirmed in a small group of generally healthy elderly individuals (Marotta et al, 2006). In a different setting of chronic liver disease unrelated to alcohol, i.e. HCV-related, the same research group has then shown that A significant improvement of redox status was obtained by both alpha-tocopherol 900 IU/day or 9 g/day of a FPP regimens. However, only FPP significantly decreased 8-OHdG and the improvement of cytokine balance with FPP was significantly better than with vitamin E treatment. A few years later, a similar group of patients was further studied (Marotta 2010) and it was found that patients with liver cirrhosis showed a significantly time-dependent upregulated TNF-α production from ex-vivo LPS-stimulated monocyte, this effect being more pronounced in more advanced stages of the disease together with a higher serum level of thioredoxin (Trx). Again, FPP showed a normalization of Trx anda partial but significant downregulation of TNF-α mRNA.

2. The previously mentioned haematological data also proved to be interesting for an authoritative (CORRECT) Israeli group led by Prof. Rachmilewitz (2002, Amer 2008) which has shown that *in vitro* treatment of blood cells from beta-thalassemic patients with FPP increased the glutathione content of red blood cells, platelets and polymorphonuclear leukocytes, and reduced their reactive oxygen species, membrane lipid peroxidation and externalization of phosphatidylserine. These effects result in (a) reduced thalassemic RBC sensitivity to hemolysis and phagocytosis by macrophages; (b) improved PMN ability to generate oxidative burst - an intracellular mechanism of bacteriolysis, and (c) reduced platelet tendency to undergo activation, as reflected by fewer platelets carrying external phosphatidylserine. Oral administration of FPP to beta-thalassemic mice (50 mg/mouse/day for 3 months) and to patients (3 g x 3 times/day for 3 months), reduced all the above mentioned parameters of oxidative stress (Fibach 2010). Quite recently, this group has studied the effect of FPP on two groups of beta-thal patients: beta-thal, major and intermediate, (in Israel) and E-beta-thal (in Singapore). The results indicated that in both groups FPP treatment increased the content of reduced glutathione in red blood cells, and decreased their reactive oxygen species generation, membrane lipid peroxidation, and externalization of phosphatidylserine, indicating amelioration of their oxidative status. Further corroborative hints come from a concomitant case report of a beneficial administration of FPP to a patient with paroxymal nocturnal haemoglobinuria (Ghoti 2010).

3. a significant recovery of the latent malabsorption of vitamin B12 due to the interference of alcohol-induced oxidising effects on the gastric mucus at the binding site level between the intrinsic factor and cyanocobalamin (Marotta 2000).

Such evidence on the efficacy of FPP on oxidising stress induced by alcohol on the gastric mucus was also based on the associated evidence of the significant protective effect (macro and microscopic and biochemical as well) on healthy subjects, after being administered a test-dose of ethanol (40 ml 80% ethanol) (Marotta 1999).

According to the previous results on the antigenotoxic effect and on the DNA *in vitro* protection by FPP from the group of Prof. Mori and more recently of Prof. Packer's group (Rimbach 2000) who highlighted the iron chelating effect, a new clinical trial was carried out on the gastric pre-cancerous lesions. A group of Italian and Japanese scientists proved in fact in a controlled and randomised study carried out for a six month period on patients suffering from chronic atrophic gastritis without the presence of Helycobacter pylori that both a multivitamin anti-oxidant mixture and high dosage vitamin E and FPP led to the reduction of a series of mucosal markers related to oxidative stress. However, FPP only managed to significantly reduce the two markers used as an expression of a pre-mutagenic biochemical changes, that is ornithine decarboxylase and 8-oxoguanine. This is one of the most frequently used biochemical markers relating to the DNA oxidative damage, since being a mutated base, it can lead to severe replication errors and anaplastic transformation) (Marotta 2004).

At the same time as the first clinical trials by the Kyoto Pasteur group on the immuno-modulating FPP effects and related reports (increase of the CD8+ and QOL score), on the positive beneficial effect which HIV-affected patients could benefit from (Mimaya 1998), a series of studies were started by Prof. M. Weksler of Cornell University in the USA (2002) 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 enlarged further, it was proved that FPP administration for 3 weeks before the anti-flue vaccination in 10 hospitalised elderly patients consistently improved their specific antibody response in comparison to a control group which was only administered the vaccine. What is more, Prof. Montagnier's group (2003) carried out a study on the administration of FPP to poor immunological-responder HIV-positive patients and data from the open preliminary research proved how such a compound, when associated with the anti-retroviral treatment, could significantly improve the CD4+ concentration as well as hemoglobineamia, weight increase and cenaesthesia. Such immune-modulating effects of FPP are now under consideration in a clinical research project aimed at ascertaining its potential properties in reducing the upper respiratory tract infections in the overall population and, principally , in elderly subjects (Marotta 2010)

Taking into account the overall previously mentioned data, one can also suggest that either the antioxidant effect of FPP and its beneficial microrrheological and macrophage activity-enhancing properties must have played a role in the successful study of the Comprehensive Wound Center, Department of Surgery at Ohio State University Medical Center, USA. Indeed, Drs Collard and Roy studied (2010) the effects of FPP on wound healing in adult obese diabetic (db/db) mice and found that FPP supplementation improved respiratory-burst function as well as inducible NO production together with a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved proangiogenic response. Interestingly, the authors also noted that FPP blunted the gain in blood glucose and this somehow parallels the intriguing clinical findings of the Italian researcher Danese (2006) who, by administering 3 grams of FPP daily, during lunch, for two months to 25 patients affected by type-2 diabetes mellitus under treatment with glybenclamide and to 25 controls, noticed a significant decrease in plasma sugar levels in both groups. This data needs further confirmation in a larger study but it may open new avenues to an integrated medical approach.

It goes without saying that it is extremely important to promote a diet rich in organically-grown vegetables, which if correctly enforced, offers the availability of micro-nutrients and anti-oxidants which are sufficient to comply with the body requirement in the case of normal health conditions and in the absence of important psychological and physical burdens. What simply depended on common sense, was underlined a long ago by an authoritative international non-profit institute which stressed how a healthy diet should not be replaced by a non-controlled diet rich in supplements or food-like compounds such 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 cannot be counterbalanced by general data from the literature. Legislation and standards are still open about fortified foods supplemented by specific nutrients which deserve a discussion of each one . As previously underlined by Prof. Packer during an international congress (2003), we are facing a consistent evolution of anti-oxidants, implying the study of 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 genomic adjustment.

In particular, a polymorphism-profile designed placebo-controlled study (Marotta 2006) carried out in 54 elderly patients without major diseases has shown that only the GSTM1 (-) subgroup was the one that, under FPP treatment, decreased lymphocyte 8-OHdG. Such preliminary data show that FPP is an advisable nutraceutical for improving antioxidant defences even without any overt antioxidant-deficiency state while helping explain some inconsistent results of prior interventional studies. A further study (Marotta 2007) showed that in a similar group of patients, there may occur a proinflammatory profile acting also as a downregulating factor for inducible Hsp70, particularly if Interleukin-6 promoter -174 G/C-negative while FPP supplementation at the dosage of 9g/day sublingually (a preferable route) proved to normalize such phenomena. The understanding of the complex intracellular/epigenomic mechanisms of FPP still needs further investigations and posttranscriptional/translation protein modifications that also occur need to be unfolded as Prof. Migliore from Pisa University in addressing her research studies stated. Nonetheless, a recent small pilot study showing FPP-induced upregulation of gene expression of leukocyte GPx, SOD, catalase and hOGG1 (Marotta 2010) seems to suggest that a transcriptomic modification of key redox and DNA repair genes may offer further insights when attempting to interrelate "nutragenomics" to clinical phenomena.

FPP certainly represents a Functional Food, highly compliant with the novel features of the new nutrigenomic-driven action plan strategy aimed at disease risk reduction and successful integration within specific pharmacological treatments.

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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
Calcium	2.5 mg	Glycine	11mg
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