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## **Novel concepts on functional foods and nutrigenomics in healthy aging and chronic diseases: a review of fermented papaya preparation research progress**

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### **Abstract**

Functional foods are an emerging research field corresponding with genetical, epidemiological and clinical studies integrated with the food industry in accordance with the consumer demands. Consequently, the features of the functional foods are being discussed by various researchers and related institutions, and a common view has been pointed out about the availability and the nature of the components of functional foods. Recently, the outcomes of functional foods are being assessed by the help of all the available scientific tools. Genomic medicine is one of the most promising areas of research to reveal the benefits of functional foods and the bioactive ingredients. Nutrigenomics aims at studying the genetic and epigenetic interactions with a nutrient or the functional component in order to lead to a phenotype change and therefore to the cell metabolism, differentiation or even apoptosis. Papaya and its fermentation product are specific products derived from the technologically advanced and controlled environmental-friendly bio-fermentation process.

It has been well known for a long time that the natural anti-oxidant properties of papaya, mainly depending on vitamins A and C in addition to certain amino acids, were consistent both in the fruit and derived from the papain enzyme which is no longer present in the fermented product. In this article, functional foods in genomic medicine are discussed in review of the fermented papaya preparation research progress. Clinical evidences about fermented papaya as a functional food are reported as supported by various research protocols and experimental models. The benefits of fermented papaya preparation are also discussed in nutrigenomic basis and it is reported to have an important antioxidant and transcriptomic potential which deserves further investigation. As a conclusion, fermented papaya preparation represents a Functional Food highly compliant with the novel features of the new nutrigenomic-driven action plan strategy aimed to reduce the incidences of diseases and successful integration within specific pharmacological treatments.

**Keywords:** functional foods, fermented papaya preparation, nutrigenomics

**Functional Food: a recent history dating back in time:** “Functional Foods” represent an emerging opportunity and will certainly play a consistent and important role in future. Contrary to 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 entitled “Japan is exploring limits between food and medicine” [1]. Clearly the success of “Functional Foods” depends on the food industry capacity developing new, effective products which on one side meet any consumer request and on the other must have positive effects on health. Additionally, they must be supported and validated by scientific research and therefore contain far more than simple positive properties, as recently underlined in a meeting organised by a non-profit non-governmental international association [2].

**Definition and demanded features:** The adoption of such a new philosophy in the last few years has led to constant changes in the Functional Food definition which was defined in 1997 by an authoritative scientific European panel as follows: “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” [3]. 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.

From the recommendations of this 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 furthermore 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” [4].

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 interferences [5].

An ancient Chinese proverb specifies that “medicine and food are isogenic”.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 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” (Food for Specified Health Use, FOSHU), stressing and recognising their nutritional value, after undergoing a consistent bio-fermentation process. Given the ever increasing interest in this field, the novel more active definition of functional food given by Dr. Martirosyan comes at no surprise [6]. According to his group, Functional Food Center, a functional food is “a natural or processed food that contains known or unknown biologically-active compounds which, when in defined quantitative and qualitative amounts, provides a documented health benefit and, as a

result, become an important source in the prevention, management and treatment of chronic diseases of the modern age”.

**Synergies, markers and development strategy up to nutrigenomics:** A biochemistry and molecular biology specific development together with biotechnological methods were enhanced to support the hypothesis that some nutrients could modulate the body functions by playing a role in its general good health condition, as well as in the reduction of risk of infection depending on the person’s 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 genome project conclusion [7], the post-genomic era started, which should mainly be correlated with Functional Foods, profiting from sophisticated technologies such as the DNA chip technology and some others, which led to nutrigenomics [8]. 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 as to lead to a phenotype change and therefore to the cell metabolism, differentiation or apoptosis [9]. 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. More recent papers suggest that cells are able to adapt themselves when exposed to excessive quantities of nutrients [10]. 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 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;

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 soya itself [11].

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 answers at 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, 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.

**Fermented Papaya Preparation history: an example of the rational and evidence-based biotechnological study:** That being stated, it is far more interesting to 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 known for a long time that the natural anti-oxidant properties of the papaya, mainly depending on vitamins (A and C) and amino acids, were consistent both in the fruit and derived from the papain enzyme (Arginine among all). Papain plays a digestive activity, but such an activity is no longer present in the FPP. A long fermentation, by means of yeasts, is the unique demanded 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, which is 30 times bigger. In the final fermented product and not in the fresh fruit, many new class of oligosaccharides are present at a different polymerisation as well a monomers similar to the basic structure of  $\beta$  1-3 D-glucan. Such oligosaccharides, mainly oligosaccharides exhibiting a low molecular weight,

exhibit a wide spectrum immune-modulating activity.

After a series of initial reports written by Japanese scientists a couple of decades ago a group of populations living in the Philippines and eating large amount of papaya on a daily basis, a research institute was set up consecrated to the study of “functional” properties of a series of specific compounds within a fruits- and vegetable-based diet. A leading 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 organic 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 of the Molecular Biology Institute at the Okayama University in Japan, directed by Prof. Mori [12]. Such studies, carried out with sophisticated methods including Electron Spin Resonance, highlighted that such a product consisting of fermented papaya exhibited a powerful anti-oxidizing activity on in vitro cerebral cells [13] as well on the in vivo epilepsy experimental model, where the epileptogenic monoamine neutral release was consistently reduced [14]. Prof. Mori’s group also proved the capacity of fermented papaya to reduce the increase of free radicals concentration as well as superoxide dismutase at the brain level in elderly rats [12] followed by the reduction of experimental ischemia-reperfusion induced cerebral damage . Furthermore highlighted was the consistent in vitro resistant anti-oxidizing product capacities even when tested for one hour at high temperatures (100°C) and acid pH (1,2). What is more, such features were confirmed after a long-term storage. Such potential neuroprotective effects of FPP are currently the issue of a clinical study on Parkinson’s disease patients by a group led by 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 units at the University of Palermo, point toward a significant decrease of plasma oxidative stress parameter in FPP-supplemented patients with varying degrees of dementia.

Then, after thoroughly refining the product and getting its certifications by the governmental body (Table 1), two important studies were carried out with international institutes to further assess the topic of its possible effects on the immune system together with the Kyoto Pasteur Institute [15], 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 the better assessment of its activity mechanisms [16]. Such successful studies, still in progress, lead to a series of extremely interesting *in vitro* and *ex vivo* evidences.

**Table 1.** Fermented Papaya Preparation (100 g) 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	Histidine	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
Folic acid	2 mcg	Methionine	5 mg
Niacin	240 mcg	Valine	13 mg
Calcium	2.5 mg	Glycine	11 mg
Potassium	16.9 mg	Proline	8 mg
Magnesium	4.6 mcg	Glutamic acid	37 mg
Copper	14 mcg	Serine	11 mg
Zinc	75 mcg	Threonine	8 mg
		Aspartic acid	27 mg
		Tryptophan	2 mg

The group from the Pasteur Institute in Kyoto, starting from the evidence of a positive effect of FPP on the Natural Killer population on a sarcoma experimental model, proved its capacity on human beings to affect the  $\gamma$ -interferon production. Such data was further proved by studies supporting the positive activity of FPP on the macrophage function on rats [17] and human beings too. 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 [18] when undergoing a severe effect such as ischemia/reperfusion in the clinical practice, the unique epiphenomenon present during myocardial stroke. Such data have been recently confirmed and gained further insights from Aruoma et al. [19] who have showed the ability of FPP to modulate oxidative DNA damage due to H<sub>2</sub>O<sub>2</sub> in rat pheochromocytoma (PC12) cells and protection of brain oxidative damage in hypertensive rats.

The same Mori's group lead also to important scientific results proving the connection of the immune-modulating activity of FPP to its anti-oxidising features [20, 21]. 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- $\gamma$  upward. FPP [22] would then exhibit a nutrigenomic effect able to change the messenger RNA expression both of inducible nitric acid and of TNF- $\alpha$  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- $\kappa$ B 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)- $\beta$ -D-glucan concentration, which represents the most representing portion of some peculiar yeasts, used in the FPP bio-fermentation process [23].

### **Clinical evidences supported by research: a most demanded evolution from empirics:**

Supports offered by scientific evidences and a series of works on human beings represented the foundations to plan a series of clinical studies. In 1995 in fact, an oncological-haematological Russian study group [24] proved on young subjects undergoing radiotherapy against severe mielo- and lympho-leukaemia how the administration of FPP, as proved in the previous experimental studies by Prof. Mori, managed to significantly reduce clinical side effect (encephalopathy score: anorexia, nausea, vomiting, convulsions, dizziness) and bio-humoral effects (change of the redox state due to the erythrocyte glutathione 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 to reduce the 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 microsomal system activation leading to the ethanol oxidation (with a consequent maintenance of the pro-oxidative state) and during the chronic alcoholic abuse [25]. 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 specific membrane). Such a consistent increase of the malondialdehyde concentration in the erythrocytes in the case of chronic alcoholics leads to, through lipoperoxidising effects, a lipid asymmetry destabilisation [26]. Part of these data has been recently confirmed in a small group of generally healthy elderly individuals [27]. In a different setting of chronic liver disease unrelated to alcohol, i.e. HCV-related, the same research group has then showed 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. Few years later, a similar cohort of patients was further studied [28] and it was found that patients with liver cirrhosis showed a significantly time-dependent upregulated TNF- $\alpha$  production from *ex vivo* LPS-stimulated monocyte and this effect was more pronounced in more advanced stages of the disease together with higher serum level of thioredoxin (Trx). Again, FPP showed to reach a normalization of Trx and partial but significant downregulation of TNF- $\alpha$  mRNA [29].

2. The previously mentioned haematological data proved also to be interesting for an authoritative Israeli group led by Prof. Rachmilewitz [30, 31] which has showed 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 [32]. Quite recently, this group has studied the effect of FPP on two groups of beta-thalassemic patients: beta-thalassaemia major and intermedia, (in Israel) and E-beta-thalassaemia (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 paroxysmal nocturnal haemoglobinuria [33].

3. A significant recovery of the latent malabsorption of vitamin B12 due to the interference of alcohol-induced oxidising effects on the gastric mucosa at the binding site level between intrinsic factor and cyanocobalamin [34].

Such evidences on the efficacy of FPP on oxidising stress inducted by alcohol on the gastric mucosa was also based on the concomitant 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) [35].

According to the previous results on the antigenotoxic effect and on the DNA in vitro protection by FPP from the group of Prof. Mori [36] and more recently of Prof. Packer's group [37] 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 *Helicobacter pylori* that both a multivitamin antioxidant mixture and high dosage vitamin E and FPP lead to the reduction of a series of mucosa markers related to oxidative stress. However, FPP only managed to significantly reduce the two markers used as an expression of 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, as being a mutated base; it can lead to severe replication errors and anaplastic transformation [38].

At the same time of the first clinical trials by the Kyoto Pasteur group on the immunomodulating FPP effects and related reports (increase of the CD8+ and QOL score), on the positive beneficial effect which HIV-affected patients could benefit from [39], a series of studies were started by Prof. M. Weksler of the Cornell University in the USA [40] 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 will be further enlarged, it was proved that FPP 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 [41] 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 to the anti-retroviral treatment, could

significantly improve the CD4+ concentration as well as hemoglobinemia, weight increase and anesthesia. Such immune-modulating effects of FPP are now under consideration in a clinical research project aimed to ascertain its potential properties in reducing the upper respiratory tract infections in overall population and, namely, in elderly subjects [42].

Taking into account the overall previously mentioned data, one can also suggest that either the antioxidant effect of FPP and its beneficial microcirculatory and macrophage activity-enhancing properties must have played a role in the successful study of the Comprehensive Wound Center, Department of Surgery from Ohio State University Medical Center, USA. Indeed, Drs Collard and Roy studied [43] 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. The same research group has very recently provided first evidence that compromised respiratory burst performance of diabetic polymorphonuclear cells may be corrected by a nutritional supplement FPP via a Sp-1 dependent pathway [44]. 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 [45] 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 integrated medical approach.

The importance of promoting 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 psychical and physical burdens is commonly understood. 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 [46]. However, the absence of specific and referenced studies on each single nutraceutical attempt can not be counterbalanced by general data from literature [47, 48].

Legislations and standards are still open about fortified foods supplemented by specific nutrients which deserve a discussion on their own. As previously underlined by Prof. Packer during an international congress [49], 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 genomic adjustment.

In particular, a polymorphism-profile designed placebo-controlled study [50] carried out in 54 elderly patients without major invalidating diseases have shown that only the GSTM1 (-) subgroup was the one that, under FPP treatment, increased 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 [51] showed that in a similar cohort of patients, there may occur a proinflammatory profile playing 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 also occur and need to be unfolded as Prof. Migliore from Pisa University is addressing her research studies [30]. Nonetheless, a very recent small pilot study showing FPP-induced upregulation of gene expression of leukocyte GPx, SOD, catalase and hOGG1 [52] 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.

**Conclusion:**

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

**Competing interests:** No competing interests.

**Authors' Contributions:** Authors contributed equally to this work.

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