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# APPROACHES FOR DELIVERY OF HEAT SENSITIVE NUTRIENTS THROUGH FOOD SYSTEMS FOR SELECTION OF APPROPRIATE PROCESSING TECHNIQUES: A REVIEW

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## Abstract

Food contains many heat sensitive nutrients which include vitamins, minerals, and nutrients having functional properties such as pigments, antioxidant, Bioactive compounds. Many processes during manufacturing of food cause detrimental effects on these nutrients. Retention of these nutrients in food products requires innovative approaches for process design because of their sensitivity to a variety of physical and chemical factors, which causes either loss of biological functionality, chemical degradation and premature or incomplete release.

This article reviews effect of Different Processes on Heat Sensitive Nutrients and approach for selecting appropriate processing technology. Proposed target application of nutrient is first analyzed using scientific principles, including materials science, physical chemistry and biophysics. The scientific understanding is used to develop a range of potential solution strategies from which the most feasible is selected for further development. Based on technological considerations, such as cost, ease of manufacturing, adaptability, one of these various possible solutions is finally implemented in the actual food product.

The major advantage of Retro-design approach is that it does not focus from the outset on a specific technology. Application of Sensitive Nutrient is placed at the centre and from there systematically works back to find a feasible technology to introduce or retain sensitive nutrient in the food product.

A wide selection of delivery systems is available for the use in food systems. Ultimately, one would like to relate the characteristics of the delivery systems to the functional attributes of the final product, such as sensory, physico-chemical and biological nutritional impact. Studies have shown that use of Novel Thermal as well as Non-thermal processing techniques such as Pulsed X-ray Processing, Oscillating Magnetic Fields, Low-Temperature Plasma, Ozone processing, Dense-Phase Carbon Dioxide Processing of Fluid Foods, Ultra-sound Processing of Food, High Voltage Arc for better retention of Sensitive nutrients.

***Key words**: Nutrient delivery system, Food system, Heat sensitive nutrients, Retro-design approach, Processing impact, Non-thermal processing techniques.*

## 1. Introduction

### 1.1 Effect of processing on nutrients

#### 1.1.1 Nutrients directly affected by heat treatments

Food contains many heat sensitive nutrients which include vitamins, minerals, and nutrients having functional properties. Vitamins are organic components in food that are needed in very small amounts for growth and for maintaining good health. The vitamins include vitamin D, E, A and K (fat-soluble vitamins), and folate, vitamin $B_{12}$, biotin, vitamin $B_6$, niacin, thiamin, riboflavin, pantothenic acid, and vitamin C (water-soluble vitamins). Vitamins are required in the diet in only tiny amounts, in contrast to the energy components of the diet. Many processes during manufacturing of food cause detrimental effects on these nutrients. Maximum destruction during heat processes is of vitamin and minerals. Vitamins are unstable in foods. Processing and cooking conditions cause vitamin loss. The losses vary widely according to processing method and type of food. Vitamin degradation depends on specific parameters during the culinary process, e.g., temperature, oxygen, light, moisture, pH, and obviously length of exposure. Vitamin A is stable under an inert atmosphere; however, it rapidly loses its activity when heated in the presence of oxygen, especially at higher temperatures.

Carotenoids are extremely susceptible to degradation. Their highly unsaturated structure makes them sensitive to heat, oxygen, and light [1 and [2]. Vitamin D is

susceptible to the alkaline pH range, light, and heat [3 and 4]. However, fat content is probably the crucial factor affecting retention during culinary treatment. A high-fat content usually results in a high vitamin D loss due to dripping off, while low-fat content might probably disrupt thermal isolation and vitamin D is more easy accessible to other aggressors (e.g., light). Retention of vitamin D varied in the range of 60–90% during culinary treatment of meat and fish [5].

In case of vitamin E most common heat treatments, such as broiling or roasting, cause a high loss of the nutrient. The vitamin E content in food treated in vegetable oil increases or remains stable because vegetable oils are a good source of the fat-soluble vitamin. Vitamin E is unstable in the presence of reducing agents: oxygen, light, and peroxides (occurring as a result of unsaturated fat auto-oxidation). Retention of vitamin E is in the range of 44–95% during culinary treatment of various types of meat, and 60 - 93% in the case of legumes.

Vitamin K shows stability during culinary treatment combined with sufficient human intake of this nutrient world-wide. Cooking losses of L-ascorbic acid depend on the degree of heating, leaching into the cooking medium, surface area exposed to water and oxygen, pH, presence of transition metals, and any other factors that facilitate oxidation [6]. Higher retention values were often observed in vegetables prepared by steaming (up to 99%), microwave steaming and stir-frying with oil, followed by stir-frying with water, and finally by boiling which caused the most extensive damage, with losses of up to 75%. Losses of AA are minimal when vegetables are cooked without any water, while maximum losses are associated with cooking in a large amount of water and oxygen present [5].

Thiamine is highly unstable at alkaline pH. Stability depends on the extent of heating and on the food matrix properties. Thermal degradation occurs even under slightly acid conditions. Thiamine is highly unstable at alkaline pH. Stability depends on the extent of heating and on the food matrix properties. Thermal degradation occurs even under slightly acid conditions. Thiamine is more sensitive to heat than is riboflavin [6].

Riboflavin is stable with respect to both oxidation and heat, but sensitive to light [7]. Riboflavin is very resistant to dry heat, acid solutions, and air (oxygen), but very sensitive to light, especially at high temperatures and pH values. During cooking riboflavin leaches into water. Among the heat treatments and vegetables mentioned, the highest losses (up to 66%) were observed in cabbage. Folate losses during cooking and preparation are the result of a combination of thermal degradation and leaching of the vitamin into the cooking water [6].

Folate is sensitive to sunlight, air, and light and being heated in acid solutions. Folate is lost in food during cooking because it breaks down under heat and leaches into the cooking water. The presence of reducing agents (AA) in the food can increase folate retention during thermal processing. Folates of animal origin appeared to be stable during boiling and frying.

Pantothenic acid is the most stable vitamin during thermal processing with pH levels of 5 - 7. Large losses can occur through leaching into cooking water during preparation of vegetables. Niacin is the most stable water-soluble vitamin. Processing and cooking procedures do not deactivate niacin. Leaching is usually the primary pathway of its loss during food preparation [6].

Retention of niacin is in the range of 45 - 90% within the various culinary treatments of meat and legumes. Thermal degradation of vitamin B6 increases as pH rises. Vitamin B6 is resistant to heat, acid, and alkaline, but sensitive to light in neutral and alkaline solutions. Pyridoxal and pyridoxamine are more heat, oxygen, and light labile than pyridoxine (the primary vitamer in plants).

Vitamin $B_{12}$ is generally considered to be stable under most food processing operations, but like all water-soluble vitamins, it is subject to large losses through leaching into the cooking water. Vitamin $B_{12}$ is normally stable during pasteurization of milk, but up to 20% can be lost during sterilization [8].

Biotin is stable when heated in the presence of light and in neutral or even in strong acid solutions, but it is labile in alkaline solutions. In general, biotin retention is relatively high during heat treatment (80% in meat, 85–90% in milk pasteurization, 85–95% in legumes, 70% in preservation of fruits and vegetables) [5].

### 1.1.2 Nutrients became unavailable during heat processing

Minerals are the inorganic elements, as calcium, iron, magnesium, potassium, or sodium, that are essential to the functioning of the human body and are obtained from foods. Minerals also get affected by heat treatment during processing. The study of processing impact on the fate of minerals is modelled after the analysis of vitamin or macronutrient loss during processing. Since ash values (determined before and after processing) do not drastically change, minerals seemed not to be affected during food processing. The late Robert Harris has summarised the maximum losses of minerals during cooking to be not larger, on average, than 3%. However, the classical nutritional evaluation of food processing by global estimation of mass losses might be misleading in the assessment of processing impact on bioavailability [9].

It might be possible that minerals were not lost during processing, but combined with co-nutrients or non-food components. They may become unavailable for

digestion due to these interactions. Moreover, the opposite effect may also occur: increased bioavailability because of destruction of binding ligands (e.g. phytates). Various review articles have focused on the mass losses in unit operations, mostly concerned with the losses of Ca, Zn and Fe [9].

Hazell and Johnson [10], proposed that the reaction products of depolymerisation processes under the high temperature and shear might change the chemical form of iron and make it more soluble which increases its availability. There were no established changes in the bioavailability of zinc and iron [11].

Since the inorganic and organic forms of metal ion complexes can have different absorbability, knowledge of the chemical form is pre-eminent for the understanding of the physiological impact of the mineral. For example, it was shown that peptides liberated by the digestion of meat enhance the bioavailability of the soluble iron in the intestinal lumen [12]. Results of interaction of minerals with unit processes are summarised below [13].

**Table1. Effect of heat treatments on minerals**

| Processing | Possible causes of losses or gains |
|---|---|
| **Boiling/Cooking** | Leaching oxidative losses phytate retention |
| **Blanching** | HCl extractability of Zn and Ca increased |
| **Pasteurisation, steaming** | Few losses |
| **Canning** | Complex destruction |
| **Baking** | Phytate hydrolysis increase absorption |
| **Frying** | Iodine losses |
| **Drying** | Denaturation of binding proteins, Maillard reaction |
| **Fermentation** | Phytate content reduction, hydrolysis |
| **Extrusion** | Phytase deactivation effects controversial |
| **Home preparation** | Too much water, no use of cooking water (pasta 20%, veg. 15%) reduces mineral bioavailibity |

### 1.1.3 Nutrients having functional properties

This comprises wide range of bioactive compounds such as pigments, antioxidant, and immune boosting naturally occurring bioactive compounds. Volden [14] reported that blanching, boiling and steaming resulted in losses of 59%, 41% and 29% respectively in anthocyanin content of red cabbage. Curcumin loss from heat processing of turmeric was 27–53%, with maximum loss in pressure cooking for 10 min. Curcumin loss from turmeric was similar even in the presence of red gram. In the presence of tamarind, the loss of Curcumin from turmeric was 12–30%. Capsaicin losses from red pepper ranged from 18% to 36%, with maximum loss observed in pressure cooking. Presence of either red gram or tamarind or both did not influence the loss of capsaicin. Piperine losses from black pepper ranged from 16% to 34%, with maximum loss observed in pressure cooking [15].

## 2. Nutrient Delivery Systems - Basics

The creation of novel functionality of heat sensitive nutrients in complex food materials is of increasing importance for the food scientists. Application of active ingredients in food products often requires innovative approaches because of their sensitivity to a variety of physical and chemical factors, which causes either the loss of biological functionality, chemical degradation or a premature or incomplete release. The situation is challenging not only because of the high sensitivity to temperature, but also because of the complexity of many food products and the conditions prevalent in many food matrices. In addition, product safety, appearance, storage conditions, ease of preparation by the consumer, freshness and sensory properties of the food product are not to be compromised by the incorporation of the heat sensitive nutrients.

Job Ubbink and Jessica Kruger [16] proposed target application first analyzed using scientific principles, including materials science, physical chemistry and biophysics. The scientific understanding is used to develop a range of potential solution strategies from which the most promising may be selected for further development. Based on technological considerations, such as cost, ease of manufacturing, adaptability, one of these various possible solutions may finally be implemented in the actual food product. The major advantage of this approach is that it does not focus from the outset on a specific technology to solve an issue, which might be poorly understood, and for which the selected technology may ultimately be ineffective.

In a retro-design approach to the delivery of heat sensitive nutrients, the food application is placed at the centre and from there one systematically works back to find a feasible technology to introduce the sensitive nutrient in the food product. The principle of retro-design, as developed in organic synthesis, allows the systematic evaluation of all steps and routes starting from the final product down to the raw materials. In organic chemistry, such a retro-design allows the evaluation of all possible reaction pathways and intermediates leading to the desired product and facilitates the choice of the favoured synthesis route based on a rational compromise between reaction yields, number of reaction steps, and availability of starting materials. In addition, the approach has proved useful as it allows the defini-

tion of chemical transformations which do not yet exist but whose development may then be attempted [17].

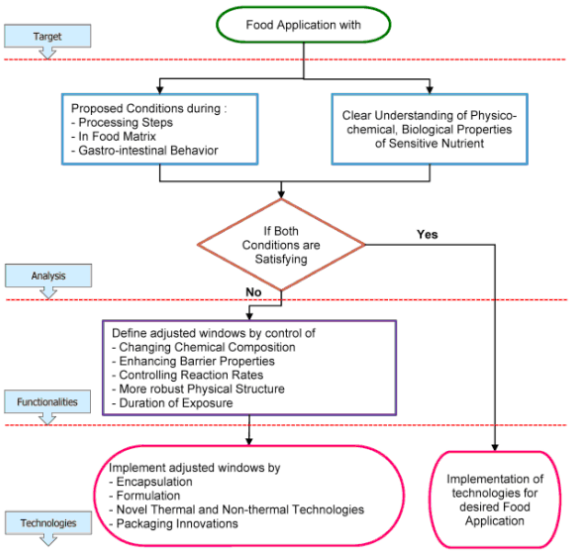


**Figure 1. Flow diagram for retro-design approach application for food application**

In the food field, application of this approach is very useful, in particular in the development of complex food products containing active ingredients. In adopting a retro-design approach towards the delivery of sensitive nutrients in foods, one starts by defining precisely the functionality and performance of the active ingredient desired in the final application (Figure 1). This target sets the required functionality and performance of the active ingredient. This could be the presence of a defined quantity of vitamins or minerals (heat sensitive nutrients) in a food product or the maintenance of a bioactive form during the shelf life and the digestion of a food product. Subsequently, the physical, chemical and biological properties of these nutrients and the conditions prevailing in the food matrix are analyzed (Figure 1). Such properties of these nutrients include the physical properties, including phase behaviour and molecular mobility, chemical reactivity and conditions under which the physiological and sensory characteristics are maintained and they effectively determine the application window of the sensitive nutrients. If the target can be satisfied is then determined by the conditions prevailing in the food matrix, during manufacturing, storage and consumption. For delivery of heat sensitive nutrients through food product, we have only a very limited flexibility in adapting the conditions in the food matrix to match the window of application of these ingredients. Consequently, often conditions in the food matrix have to be accepted which are detrimental to the stability and functionality of the sensitive ingredient. In such situations, the sensitive ingredient and the product may be said to be incompatible and a successful product development satisfying the original requirements on the functionality and performance of the active ingredient turns out to be impossible. However, having clearly identified both the conditions required for maintenance of the performance of the sensitive ingredient and the limits to the conditions set by the food matrix, one may define the functionality needed to resolve the causes of the incompatibility of sensitive ingredient and food matrix. The functionality is defined solely based on the analysis of the interaction of sensitive ingredients, its stability and food matrix and it does not yet relate to a specific technology. In fact, a functionality defined in this way may not even have an existing associated technology.

The major advantage of introducing the concept functionality is that it postpones the selection of a technology to a later stage in order to allow the optimal matching of the different factors indicated in Figure-1. It thereby enables a clear definition of the requirements a novel technology should fulfil in satisfying the specific application. Based on the defined functionalities, one is in a position to select or develop an appropriate technology.

### 2.1 Case Study: Delivery of Probiotic Cultures through Food Systems

Job Ubbink [16] has studied application of Retro-design approach for stability of probiotic cultures in Food Matrix (Figure 2).

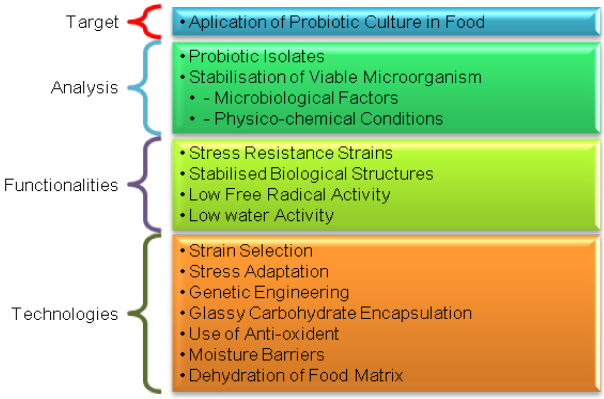


**Figure 2. Retro-analysis of the delivery of probiotics in food matrices**

Probiotics are used as living microbial food additives that beneficially affect the host organism by improving its intestinal microbial balance [18]. Most of the probiotic bacteria, in particular bifidobacteria, are sensitive microorganisms with low survival to stresses occurring during the production, storage and consumption of food products. Large fraction of the microorganisms will lose their viability already before the moment of consumption of the food product. These losses are commonly larger than 1 log during the shelf life of the product, but may be much higher. A number of technologies and strategies are being developed to support the survival of probiotics in food products during

processing and storage, but the range of application of such technologies and strategies is usually restricted to a limited variation in conditions. One of the solutions for probiotic stability is to use probiotic isolates and extracts instead of Functionalities, viable microorganisms. However, studies have shown that probiotic extracts and isolates cannot completely replace live probiotics. Therefore, effective technologies are required to stabilize microorganisms in the food matrix to be able to develop probiotic food products. Strategies for the stabilization of probiotic cultures in such dehydrated states develops based on general observations on the response of the cultures to varying environmental conditions. Hence, application of Probiotic microorganisms is done in dehydrated food products, with a water activity typically between 0.3 and 0.5 having a shelf life of 1 - 2 years at room temperature. Under these conditions, probiotic microorganisms lose their viability in few months. In order to increase probiotic stability in dehydrated products, the cultures store in a very dry state (dehydrated regime). The probiotic viability rapidly increases with decreasing water activity. This can be achieved by drying the product matrix to the required water content. This is costly solution as product weight loses from 1% up to 10% during dehydration. In addition, rendering a product extremely dry may alter numerous product characteristics like texture, palatability, and solubility. Another strategy to protect probiotic microorganisms against the effects of moisture is by encapsulation of the dry biomass in materials, which form a barrier towards water. However this has got limitations because hydrophobic food materials like lipids have appreciable rates of moisture migration. Protection against moisture strongly enhances probiotic viability during storage; may be due to moisture dependence reactions, including oxidation, or to the effects of water on the conformations of biological macromolecules. The impact of oxidation reactions on probiotic stability may be limited by encapsulation of the microorganisms in a material with high oxygen barrier properties, such amorphous glassy carbohydrates which are having multiple associated functionalities. [19]. Glassy carbohydrates are thought to play an important role in the stabilization of fragile biological structures such as lipid membranes, proteins and nucleic acids by forming a physical stable matrix interacting with the biological structure via hydrogen bonding [20]. Microbiology is used as Stabilization strategies complementary to previously discussed physicochemical strategies. Strain selection and stress adaptation, are become an important strategy to adjust probiotic viability [21]. Stress adaptation consists in the application of sub-lethal or gradually increasing doses of stress in order to stimulate an adaptive cellular response that enables the microorganism to resist a similar, but more intense stress at a later stage [22]. Genetic tools, including bioinformatics of entire genome sequences, were being used to increase the understanding of molecular mechanisms of microbial adaptation and protection [23].

Thus, structured analysis based on the 'retro-design' concept is useful as it offers a rational way to disentangle the various physico-chemical and biological factors determining probiotic stability. The final processing strategy will therefore not be optimal regarding a single process step or ingredient but rather the best compromise in terms of the target product and the requested product specifications.

In this review, we have focused upon the types of delivery systems used for sensitive products, Novel thermal and Non-thermal processing techniques with their impact on sensitive nutrients. Finally heat sensitive nutrients with their food application have been described. Bio-fortification of nutrients is relatively recent concept that needs to be studied extensively before implementation on Food Sector.

### 2.2 Types of Nutrient Delivery Systems

A wide selection of delivery systems is available for the use in food systems. Ultimately, one would like to relate the characteristics of the delivery systems to the functional attributes of the final product, such as sensory, physico-chemical and biological nutritional impact [17].

#### 2.2.1 Powder particles

Spray-drying (micro) encapsulation has been used in the food industry to provide, protection against degradation/oxidation, and to convert liquids to powders. Microencapsulation is defined as a process in which tiny particles or droplets of the active ingredient(s) are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix, to give small capsules with many useful properties. Microencapsulation can also provide a physical barrier between different active ingredients in the solid product. microencapsulation is done by spray drying, freeze drying, fluid bed coating and extrusion. Solid microcapsules represent the large majorities of delivery systems used in food. Particle size ranges from 1 to 2 µm. Such colloidal systems are much less susceptible to creaming or sedimentation in the final fortified liquid product. In order to avoid creaming sedimentation in the latter systems, the aqueous continuous phase has to be viscous or gellified or density matching components have to be added.

#### 2.2.2 Oil in water emulsion

Oil in water emulsion such as milk, yogurt drinks, dressings, sauces or mayonnaise, are ubiquitous in food. Their oil droplets can easily be used for the delivery of

lipophilic active ingredients. For example Vitamin E or its derivatives are frequently added to the oil phase of o/w emulsion products for fortification reasons or in order to stabilize unsaturated oils against oxidation [24 and 25].

Since vitamin E acetate is chemically more stable than vitamin E itself, it is especially used in food technology for fortification reasons. For many nutrients, however, a classical emulsion delivery system does not offer the desired properties in terms of solubilization (e.g. preventing crystallisation), protection against chemical degradation or inducing the desired nutritional activity. For example, classical emulsion systems do not protect unsaturated triglycerides, essential oils, vitamins A and D efficiently against degradation.

Therefore other ideas are needed to deliver such ingredients without losing their nutritional effect during shelf-life of the product. One way to achieve this is by controlling the composition and structure of the oil droplet interface, i.e., by building around the oil droplets multilayer of surfactants.

- **Multilayer emulsion system**

McClements and co-workers [26] showed that when stabilizing oil droplets first with an anionic surfactant, such as a phospholipid, and then adding a positively charged polymer, such as chitosan to the emulsion, the droplets are coated with a surfactant-polymermembrane, which gives the globules a positive charge. It was observed that this kind of emulsion protects more efficiently Ω 3 fatty acids and essential oils (citral and limonene) from oxidation that ordinary emulsions stabilized by a single surfactant or amphiphilic layer. The observed effect against oxidation of the oil droplets in this multilayer emulsion system was attributed to the net positively charged interface. A positive charge around oil droplets hinders the contact with transition metals, like iron or zinc, and as a consequence, prevents them to act as a pro-oxidant of the oil droplets. It is also suitable for water–oil droplet interface, reducing oxidation of sensitive oil droplets, like poly-unsaturated fatty acids (PUFA), essential oils such as citral and limonene [27].

-**Double Emulsion**

Double emulsions, also often denoted as 'multiple emulsions', are "emulsions of an emulsion", e.g. a water-in-oil emulsion dispersed in an aqueous phase (water-in-oil-in-water, W/O/W). Such emulsions are interesting as delivery systems, since, in principle, the water droplets inside the oil droplets can be used to deliver (unstable) hydrophilic active ingredients separating them from the outer aqueous phase of the food product. Therefore, most studied applications of double emulsions are related to the control of the release of hydrophilic substances from the inner to the outer aqueous phase [28]. Benichou [29] studied the double emulsion stabilization potential of WPI (Whey Protein Isolate)/ polysaccharide(e.g. xanthan gum) complexes in comparison to each of the biopolymers alone. A synergistic positive effect with regard to the double emulsion stability was demonstrated, which was associated to modified surface properties induced by the adsorption of the complexes. The authors also showed that these double emulsions can be used for entrapping hydrophilic vitamins, such as vitamin B1, into the inner aqueous phase. By means of Differential Pulse Polarography it was possible to follow the real-time release of the entrapped vitamins from the core of the W/O/W double emulsion droplets to the outer aqueous phase.

- **Nanoemulsions**

Nanoemulsions, often also called miniemulsions, are emulsions consisting of droplets which are significantly (by a factor of 10 or so) smaller than the droplets present in ordinary emulsions. The very small droplet size of nanoemulsions (20 - 200 nm) makes them resistant to physical destabilization via gravitational separation, flocculation and/or coalescence. Nanoemulsions are resistance due to creaming because of Brownian movements but they are particularly prone to growth in particle size over a time by a process known as Ostwald ripening [30]. Yuang [31] investigated oil-in-water nanoemulsions of β-carotene produced by high pressure homogenization. While the physical stability of the nanoemulsions, which were stabilized by polysorbate emulsifiers, was quite acceptable, significant chemical degradation of the delivered β-carotene occurred during storage.

-**Solid lipid nanoparticles carriers**

Solid lipid nanoparticles carriers (SLNs) have some similarities with nanoemulsion systems. The diameter of such lipid particles can be also quite small, i.e. in the range between 50 nm and 1 µm. SLNs consist of a solid or semi-solid lipidic core containing lipophilic active ingredients. Active ingredients can be solubilised homogeneously either in the core of the SLNs or in the outside part. This physical property allows a better control of both the physical (against recrystallisation) and chemical (against degradation) stability of the delivered nutrients. The preparation of SLNs is achieved by heating the lipidic core components above their melting point, and then using common emulsion or microemulsion technology, i.e., homogenisation or mixing of the melted lipidic phase with a cold aqueous solution generating re-crystallised lipidic particles. The main difficulty associated with SLNs production is to control the lipid

polymorphism. Triglycerides, for example, can be present in three different crystalline structures α (spherical), β′ (needle shaped), and β (needle shaped) [32]. Carlotti [33] found that between 50 and 70% of retinol remains undegraded when 'encapsulated' in SLNs made of Cetyl palmitate (30% of vitamin A was degraded), Glyceryl behenate (49% degradation) and palmitic acid (34% degradation), while 8% retinol remains when delivered in standard oil-in-water emulsions.

But during heat treatment active element may be exposed to high temperature during the preparation of the lipid carrier material leading to chemical degradation. Finally, saturated lipids are needed to obtain these kinds of delivery system. Such lipids are not the preferred ones in terms of nutrition and health.

### 2.2.3 Molecular Complex

Another strategy to deliver active ingredients in aqueous foods is by physically complexing them with other molecules, hoping that in this way a better solubilisation and/or an increase in the chemical stability of the complex bioactive can be achieved. In this context a molecular complex is referring to the physical association between a host and a guest (active ingredient) molecule.

- **Cyclodextrins**

Cyclodextrins are cyclic (or taurus shape) oligosaccharides having a hydrophilic outer surface insuring good dissolution of the complex in an aqueous environment. Cyclodextrins (α-6, β-7, γ-8) contain a lipophilic cavity (0.5-0.8 nm) enabling to host relatively small lipophilic or amphiphilic constituents, such as fatty acids, vegetable and essential oils, nucleic acids, vitamins and hormones [34 and 35].

- **Molecular association with biopolymer**

Active molecules form physical complexes with a variety of other naturally occurring food components. Such systems are the base for designing 'natural' delivery systems. Amylose present in starch, which adopt a helical structure generating a cavity of about 0.5 nm in diameter. Small molecules like aromas solubilize in this cavity and can bbe used in Food Delivery systems. Proteins and peptides are amphiphilic molecules.

They are also relatively soluble in water and can bind lipophilic or amphiphilic active ingredients. Semo [36] used casein micelles to solubilize vitamin $D_2$. Sodium caseinate, $CaCl_2$ and $K_2HPO_4$ were used to encapsulate the vitamin and reconstitute the casein micelle solution and study their protective properties. It was found that the casein micelle can provide a partial protection against UV-light induced degradation compared to the serum media of the casein micelle dispersion, which was used as a control.

### 2.2.4 Self-assembly delivery structures

Tanford [37] define the dimensionless surfactant packing parameter for a semi quantitative description of the relation between surfactant molecular shape and self-assembly phase formation. Packing parameter is ration of the molecular volume of the hydrophobic moiety, the molecular length of the hydrocarbon chain and is the effective (or hydrated) cross-sectional area of the polar head-group. Depending on packing factor, different self-assembly structures are formed. If Packing parameter is less than 1, structures like normal micelles, hexagonal (Hi) or cubic phases are formed. If Packing parameter is close to 1, a lamellar liquid crystalline (Lα) phase is formed, which when dispersed into water gives rise to vesicles or liposomes formation. If packing parameter is more than 1, reversed self-assembly structures, such as reversed micelles, reversed hexagonal or reversed cubic structures are formed.

Self-assembly structures, such as micelles, microemulsions, and liquid crystalline phases, are formed by the spontaneous association of surfactants in aqueous (or oil) phases. These consist of polar lipids such as monoglyceraldehydes and phospholipids. These are thermodynamically stable and are formed spontaneously and as compared to nanoemulsion , microemulsion requires large amount of surfactant [38]. For example-polysorbate used to prepared β-carotene, lycopene, lutein or phytosterols.

- **Liposomes**

Liposomes, often also denoted as vesicles, are formed when the surfactant molecules have a Packing parameter close to 1. Contrary to microemulsions their formation is often not completely spontaneous. When mixed with water the surfactant spontaneously forms a lamellar phase, which then needs to be dispersed to form vesicles. Liposomes can contain (i) one bilayer forming unilamellar vesicles (ULV), (ii) several concentric bilayers forming multi lamellar vesicles or (iii) non concentric bilayers forming multi vesicular vesicles (MVV). The size of these structures can be rather small (in the range of 20 nm) or rather large (exceeding 1 μm) [39].

### 2.2.5 Dispersed reversed surfactant systems

Reversed phases are made out of surfactants that have a Packing parameter more than 1. They are formed by lipophilic surfactants such as unsaturated monoglycerides or phospholipids. Reverse microemulsion droplets can solubilize non-esterified phytosterol molecules in

much larger amounts than in ordinary oil droplets can do. Another interesting application of reversed micro-emulsion droplets deals with the controlled release of aromas [39]. Table 2 summarizes the various types of systems which can be used for the delivery of sensitive ingredients in aqueous liquid products.

**Table 2. Delivery systems for sensitive nutrients**

| Delivery systems | Particle Size | Salient Feature | Limitations |
|---|---|---|---|
| **1. Powder particles** | | | |
| – Glass encapsulation<br>– Core-shell capsule<br>– Matrix capsule | 10 µm – 1 mm | Good encapsulation for solid products | Limited application for delivery of sensitive nutrient in liquid foods |
| **2. Oil in Water Emulsion** | | | |
| – Ordinary emulsion<br>– Multilayered emulsion<br>– Double emulsion<br>– Nano-emulsions<br>– Solid lipidnanoparticles carriers (SLNS) | 100 nm – 10 µm<br><br>50 nm – 1 µm | - Hosts lipophilic molecules<br>- Better chemical protection of sensitive oil achieved when multi-layered emulsion or SLNS used<br>-controlled released with SLNS | - Physical stability<br>- Polymorphism stability and encapsulation for SLNS difficult to control |
| **3. Molecular Complex** | | | |
| – Cyclod3rins<br>– Molecular associated with biopolymers (Amylose, Proteins, Protein aggregates) | 10nm-600nm | - Solubilisation of small lipophilic molecules<br>- Protection of sensitive molecules | Limited Loading Capacity of Sensitive Nutrient with Molecular Complexes |
| **4. Self-assembly delivery systems** | | | |
| Oil in Water Micro-emulsion | 5nm - 100 nm | -Solubilisation of lipophilic molecules<br>-Solubilisation of crystallizing molecules<br>-Increased in bioavailability<br>-Transparent appearance(water) | -Large amount of surfactant needed<br>-often off taste<br>-Used surfactant often not well accepted |
| Liposomes, Vesicles | 20 nm - 100 µm | - Solubilisation of hydrophilic and lipophilic molecules<br>- Sustained released of nutrients | - Higher cost (ingredients and process)<br>- Poor loading efficiency and capacity |
| **5. Self - assembly Structures** | | | |
| – Cubosomes<br>– Hoxosomes<br>– Dispersed reversed<br>- Microencapsulations<br>– Micellosomes | 100 nm – 1 µm | -Solubilised amphiphilic and lipophilic mol.<br>-Controlled released<br>-Solubilisation of crystallizing molecules | Large amounts of surfactant may be needed |

## 2.3 Use of Novel Thermal and Non-Thermal Technologies for Heat Sensitive Materials

Traditional thermal treatments are a cornerstone of the food industry providing required safety profiles and extensions of shelf-life. However, such treatments may lead to losses of desired organoleptic properties and damage to temperature labile nutrients and vitamins. Consequently, the food industry has long sought alternative or synergistic approaches to provide the treatment objectives. Novel thermal and non-thermal technologies have been designed to meet the required food product safety or shelf-life demands while minimizing the effects on its nutritional and quality attributes. The potential of novel thermal electromagnetic technologies such as Ohmic and microwave heating, are promising alternatives to conventional methods of heat processing. Such technologies are regarded as a volumetric form of heating, in which thermal energy is generated directly inside the food, does not rely on limiting heat transfer coefficients and the requirement of high wall temperatures. Non-thermal processing is often used to designate technologies that are effective at ambient or sub-lethal temperatures. Some of these technologies are enlisted below with their mechanism of microbial inactivation and Food Applications. Very limited number of studies has been done on effect of these technologies on Heat Sensitive Nutrients in Food.

### 2.3.1 Microwave and Radio-Frequency processing

Microwave and radio frequency heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material [40 and 41]. Microwave food processing uses different frequencies, allocated by Federal Communications Commission (FCC), such as for United States, 2450 (Domestic Use) and 915 MHz (Industrial Purpose), similarly 5800 and 24125 MHz is used for Heating Purpose. Radio frequency is used at three different frequencies, 13.56 MHz, 27.12 MHz and 40.68 MHz. This is used in Food pasteurization, baking and other processes in the food industry [42 and 43]. Heating principle of Microwave is similarly as that of Radio Frequency involves two mechanisms - Dielectric and Ionic. Water present in the food is the primary component responsible for dielectric heating. Due to dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the very high frequencies. Food Material to be heated is placed between two metal plates which form an electrical capacitor. The material becomes "lossy" dielectric and absorbs energy from radio frequency generator which is connected across the two plates known as electrodes. Such oscillations of the water molecules produce heat. The second mechanism of heating with microwaves and radio frequency is through the oscillatory migration of ions in the food that generates heat

under the influence of the oscillating electric field [44 and 45]. Salient features are Rapid and Uniform heating, Selective heating, Instant control, energy efficient.

Two mechanisms for inactivation of microorganisms by microwaves have been suggested. The first states that microbial inactivation by heat through mechanisms comparable to other biophysical processes induced by heat, such as denaturation of enzymes, proteins, nucleic acids, or other vital components, as well as disruption of membranes [46]. A second mechanism for inactivation by microwaves involves non-thermal effects due to selective heating, electroporation, cell membrane rupture, and magnetic field coupling [47].

### 2.3.2 Ohmic & Inductive heating

Ohmic heating (also referred as Joule heating, electrical resistance heating, direct electrical resistance heating, electroheating, and electroconductive heating) is defined as a process wherein electric currents are passed through foods with the primary purpose of heating them. The heating occurs in the form of internal energy generation within the material. Salient feature of Ohmic heating is presence of electrodes contacting the food (unlike to microwave heating), frequency, and waveform [48]. Ohmic heating is a thermal process in which heat is internally generated by the passage of alternating electrical current through a body such as food system that serves as an electrical resistance. During Ohmic heating, AC voltage is applied to the electrodes at both ends of product body. The rate of heating is directly proportional to electric field strength, electrical conductivity & the type of food being heated. The electrical field strength can be controlled by adjusting the electrode gap or applied voltage, while the electrical conductivities of foods vary greatly, but can be adjusted by the addition of electrolytes. Ohmic Heating is having salient features: Rapid heating independent of Heat transfer coefficients, without hot surfaces for heat transfer, heat sensitive food components are not damaged by localized over-heating, suitable for viscous liquids, energy efficient, suitable for continuous processing [49].

The principal mechanisms of microbial inactivation are thermal and mild electroporation occurs during Ohmic heating. Electroporation is due to its low frequency (50 - 60 Hz), which allows cell walls to build up charges and form pores. This is in contrast to high-frequency methods such as radio or microwave frequency heating, where the electric field is essentially reversed before sufficient charge buildup occurs at the cell walls. Ohmic Heating is used for blanching, evaporation, dehydration, fermentation, extraction, sterilization, pasteurization and heating of foods to serving temperature [49].

### 2.3.3 High Pressure processing

High-pressure processing (HPP) is a method of food processing where food is subjected to elevated pressures (up to 87,000 pounds per square inch or approximately 600 MPa), with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities. The technology is also referred as High Hydrostatic Pressure Processing (HHP) and Ultra High Pressure Processing (UHP). During pressure treatment, the application of pressure is governed by Le Chatelier's principle. Due to iso-static pressure treatment, pressure is transmitted in a uniform and quasi-instantaneous manner throughout the whole sample, thus making the process independent of volume and geometry of the product. It has been generally accepted that the iso-static principle is assumed to be true for high-pressure food processing applications except heterogenous large solid samples. Once the desired pressure is reached, it is maintained for an extended period of time without any further energy input. Due to microscopic ordering at constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Fruits containing Air-spaces such as Marshmallows or strawberries containing large air packets are deformed during pressure treatment due to differences between the compressibility of air and the rest of the food constutuents.

Fluid foods are processed in batch or semi-continuous mode, consists of Pressure vessel, Top and bottom end closures, Yoke (structure for restraining end closures), High pressure pump and intensifier for generating target pressures, Process control and instrumentation, A handling system for loading and removing the product. In the batch mode, liquid product is pre-packaged, preconditioned and pressure treated. Semi-continuous pressure equipment employs two or more pressure vessels with free-floating pistons arranged to compress the liquid foods [50].

In Pressure-assisted thermal processing (PATP), the food is subjected to a combination of elevated pressures and moderate heat for short duration. One of the unique advantages of PATP is its ability to provide a rapid and uniform increase in the temperature of treated food samples. High pressures at ambient or chilled temperatures have been employed for processing a number of liquid and semi-solid foods such as fruit juices, purees, smoothies, jellies, guacamole, etc. Several studies evaluated the beneficial effects of pressure treatment over conventional treatment on preserving quality attributes of foods. Small molecules such as vitamins, and flavor compounds remain unaffected by high pressure, while the structure of the large molecules such as proteins, enzymes, polysaccharides, and nucleic acid may be altered [50].

### 2.3.4 Irradiation of food

Irradiation is a food-processing treatment endorsed by a variety of professional and governmental organizations. In reviewing the scientific literature on the process, the US Food and Drug Administration (CFR, 2000) the US General Accounting Office (GAO, 2000) the American Dietetic Association (ADA, 2000) and the UN World Health Organization (WHO, 1999) have endorsed the technology as safe and effective. Recent Studies shows that no detectable furan was produced in most fresh and fresh-cut fruits and vegetables following a dose of 5 kilogray (10 kGy), a very high dose in the context of food processing [51]. Irradiation Dose ranges are characterized as low (less than 3 kGy), medium (more than 3 and less than 10 kGy), or high (more than 10 kGy). The irradiation treatment is based on the energy absorbed by Food Material. There are currently three methods of delivering doses of ionizing radiation for food processing. Gamma radiations are produced by radioactive isotope sources such as cobalt-60 (Co-60) and Cacsium-137(Cs-137). These are highly penetrative rays. Gamma ray sources produce radioactivity constantly and so need heavy containment and shielding for safety. Dosing is controlled by exposure time. Electron beams (e-beams) are streams of high energy electrons (beta particles) produced by an electron gun (can be turned on and off. Electron beams have limited penetration (3-4 cm) into food, and do not require such high levels of containment and shielding to protect workers. X-rays are used to process foods is more recent and coming as separate processing technology [52].

Irradiation is a non-thermal process that can be applied to juices, beverages, and other fluid foods. Successful use of this technology is contingent on developing irradiation protocols that achieve the desired antimicrobial and food-safety goals while preserving (or improving) the sensory and nutritional value of the product [53]. Reyes and Cisneros-Zevallos [54] showed Irradiation effects on anthocyanin pigments depend upon the nature of anthocyanin for example; diglycosides are relatively stable towards irradiation dose compared to monoglycosides. Effect of irradiation (1 - 3.1 kGy) on mango shows minor effect of irradiation dose on the total phenolic content while there was a significant increase in flavonols after an 18 day storage period for the irradiated fruits (at 3.1 kGy). Study conducted by Lopez-Rubira [55] demonstrated insignificant changes in anthocyanins and antioxidant activity of pomegranate arils after exposure to UV-C (0.56 - 13.62 kJ/m$^2$).

### 2.3.5 Pulsed X-ray processing

Electrons have a limited penetration depth of about 5 cm in food, while X-rays have significantly higher penetration depths (60 - 400 cm) depending upon the energy used. Pulsed X-rays are generated using radionuclide sources that utilizes a solid state-opening switch to generate electron beam X-ray pulses of high intensity.

The radionuclides Co-60 and Cs-137 are produced by neutron bombardment of Co-59 and Cs-136 as a fission fragment of a nuclear power reactor operation. They emit γ-radiation of discrete energy. These radionuclide sources require permanent massive concrete shielding to protect workers and the environment from their permanent radiation.

Second approach is electrically driven radiation sources that switch off when the radiation is no longer needed are easier to incorporate into existing food processing plant. Linear Induction Electron Acceleration (LIEA) generates broad spectrum ionizing radiation by targeting the accelerated electron beam to collide with a heavy metal converter plate. This plate converts the electron beam in X-rays with a broad-band photon-energy spectrum. Then, by filtering the energy spectrum of the radiation, high-energy, highly penetrating radiation is produced, resulting in smaller variations in dose uniformity of food packages and higher quality. LIEA can deliver dose rates many orders of magnitude higher than possible with Co-60 sources. Consequently, ultra-short, high-intensity radiation treatments can be applied, resulting in higher local radical concentrations and favoring radical-radical recombination reactions. This reduces the diffusion of radical species, which are thought to be responsible for undesirable effects of irradiation on food quality. Salient Features of this technology are (1) flexibility of controlling the direction of the electrically produced radiation; (2) the flexibility of shaping the geometry of the radiation field to accommodate different package sizes; and (3) its high reproducibility and versatility. The kinetic energy limit for X-ray irradiation is 5MeV [56].

X-ray treatment reduces or eliminates Salmonella serovars in poultry, mold growth on strawberries and sprout development in potatoes. Salmonella serovars have been found to be the most radiation sensitive of all pathogenic organisms on foods. As a method of food preservation, X-ray treatment has low energy requirements.

Microbial inactivation by all types of ionizing radiation is thought to happen through 2 main mechanisms: direct interaction of the radiation with cell components and indirect action from radiolytic products, such as the water radicals. The primary target of ionizing radiation appears to be chromosomal DNA, although effects on the cytoplasmic membrane may also play a role. Changes to chromosomal DNA and/or cytoplasmic membrane can cause microbial inactivation or growth inhibition. Many studies have shown that ions, excited atoms and molecules generated during irradiation have no toxic effect on humans [57].

### 2.3.6 Ultra-Violet pasteurization

Ultraviolet processing involves the use of radiation from the ultraviolet region of the electromagnetic spectrum for purposes of disinfection. Typically, the wavelength for UV processing ranges from 100 to 400 nm. This range is further subdivided into UVA (315 to 400 nm) ; UVB (280 to 315 nm) ; UVC (200 to 280 nm) UVC is called the germicidal range since it effectively inactivates bacteria and viruses, and the vacuum UV range. (100 to 200 nm) that can be absorbed by almost all substances and thus can be transmitted only in a vacuum. The germicidal properties of ultraviolet irradiation are due to the DNA absorption of the UV light, causing crosslinking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand [58]. Due to the mutated base, formation of the hydrogen bonds to the purine bases on the opposite strand is impaired. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death. The amount of crosslinking is proportional to the amount of UV exposure. The level of mutations that can be reversed depends on the UV repair system present in the target microorganism. Once the threshold of crosslinking has been exceeded, the number of crosslinks is beyond repair, and cell death occurs [59]. This mechanism of inactivation results in a sigmoidal curve of microbial population reduction. To achieve microbial inactivation, the UV radiant exposure must be at least 400 J/m$^2$ in all parts of the product. Critical factors include the transmissivity of the product, the geometric configuration of the reactor, the power, wavelength and physical arrangement of the UV source(s), the product flow profile, and the radiation path length. UV may be used in combination with other alternative processing technologies, including various powerful oxidizing agents such as ozone and hydrogen peroxide, among others. Applications include disinfection of water supplies and food contact surfaces. Recently, interest has increased in using UV to reduce microbial counts in juices [60].

UV light has broad antimicrobial action, providing effective inactivation of viruses, vegetative bacteria, bacterial spores, yeasts, conidia and parasites. UV-light treatment of liquid foods is performed by use of continuous UV sources. Continuous UV treatments are performed by mercury vapour-lamps that continuously emit UV photons and are called continuous-wave UV (CW UV) lamps in both monochromatic and polychromatic modes [61]. UV-C illumination of grapes induces stilbene synthesis, especially that of trans-resveratrol, which will yield a phytochemical-enriched grape juice [60].

### 2.3.7 Pulsed Light processing

Pulsed light is a technique to decontaminate surfaces by killing microorganisms using short time pulses of an intense broad spectrum, rich in UV-C light (portion of the electromagnetic spectrum corresponding to the band between 200 and 280 nm). Material to be sterilized is exposed to xenon flash lamp that multiply the power many fold. Power is magnified by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths or thousandths of a second). The emitted light flash has a high peak power and consists of wavelengths from 200 to 1100 nm. The technique used to produce flashes originates, besides high peak power, a greater relative production of light with shorter bactericidal wavelengths [62].

The germicidal effect of UV light on bacteria is primarily due to the formation of pyrimidine dimers, mainly thymine dimers. The dimer inhibits the formation of new DNA chains in the process of cell replication, thus resulting in the inactivation (inability to replicate, called clonogenic death) of affected microorganisms by UV rays. On bacterial spores, UV-C treatment results mainly in the formation of the "spore photoproduct" 5-thyminyl-5,6-dihydrothymine, and in single-strand breaks, double-strand breaks and cyclobutane pyrimidine dimers. As per photothermal effect, UV light with fluence (measured in Joule/meter$^2$ and is the energy received from the lamp by the sample per unit area during the treatment) exceeding 0.5 J/cm$^2$, the disinfection is achieved through a rupture of bacteria during their momentous overheating caused by absorption of all UV light from a flash lamp [62]. Advantages of Pulsed Lights Processing are rapid disinfection, lack of residual compounds, absence of any extraneous chemical, environment friendly. Areas need improvements are Sample Heating by lamp, shadowing effect of microorganism, limited application in caser of irregular and opaque surfaces [62]. UV-C illumination decreases ascorbic acid content of juices at a similar level to that caused by thermal treatments [63]. Also for orange juice, a treatment of 299 mJ/cm2 destroyed about 50% riboflavin and β-carotene, 17% vitamin C, 11% vitamin A, and did not affect folic acid or vitamin E. In apple juice, the reduction is reported to be from 5.4 to 4.0 mg/100 ml of juice. Besides the effect on micronutrients, the potential effect of UV light on antioxidant capacity and related compounds is also important to assess due to the beneficial effects of phytochemicals [64].

### 2.3.8 Oscillating Magnetic Fields for food preservation

Static Magnetic Field (SMF) and oscillating Magnetic Fields (OMF) are used for their potential as microbial inactivation techniques. For SMF, the magnetic field intensity is constant with time, while an OMF is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. The magnetic fields may be homoge-

neous (uniform magnetic field intensity) or heterogeneous (magnetic field intensity is inversely proportional to distance from coil) [65]. OMF is used in the form of pulses reverses the charge for each pulse, and the intensity of each pulse decreases with time to about 10% of the initial intensity [66]. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperatures in the range of 0 to 50°C for a total exposure time ranging from 25 to 100 milli-seconds. Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material [67]. Magnetic field treatments are carried out at atmospheric pressure and at moderate temperatures. The temperature of the food increases 2-5 $^0$C [65].

Studies have proposed two theories to explain the inactivation mechanisms for micro-organism and pathogenic cells placed in SMF or OMF. The first theory states that OMF loose the bonds between ions and proteins. Many proteins vital to the cell metabolism contain ions such as enzymes, hormones, pre-cursors which get damaged by OMF. A second theory considers the effect of SMF and OMF on calcium ions bound in calcium-binding proteins, such as calmodulin. Changing magnetic field to calmodulin causes cyclotron resonance resulting in loosening of the bond between the calcium ion and the calmodulin. This ultimately causes metabolic disorder followed by cell death [66].

### 2.3.9 Use of Pulse Electric Field

PEF utilizes high intensity electric field pulses to inactivate microorganisms mainly in liquid foods at relatively low or moderate temperatures (less than 60$^0$C), whilst preserving the fresh flavour, colour and integrity of heat sensitive components. A typical PEF food processing unit comprises of a high voltage pulse generator, a treatment chamber, a fluid handling system and control and monitoring devices. Depending on the particular PEF systems used, typical PEF treatment parameters include pulsed field intensity of 15 - 50 kV.cm$^{-1}$, pulse width of 1 - 5 microseconds, and pulse frequency of 200 - 400 Hz (pulses/s). Key parameters influencing microbial inactivation in PEF are pulse width, pulse shape, adequate design of the treatment chamber and polarity. Square-wave pulses are considered to be superior to exponentially decaying pulses as the former gives the treatment in a sustained and constant intensity for the total duration of the pulse. Bipolar pulses are reported to be generally more effective for microbial inactivation than monopolar pulses.

The total phenolic content of a blend of orange, kiwi, pineapple juice and soymilk was not affected by PEF treatments conducted at 35 kV/cm, 4 microseconds bipolar pulses at a frequency of 200 Hz for a total treatment time of 800 microseconds and 1400 microseconds. No effect of PEF was detected on phenolic compounds. However, significant reductions were observed for vitamin C concentration which was reflected in a decrease in the antioxidant activity of the product. Under the tested processing conditions the PEF treatment caused a reduction in the vitamin C and antioxidant capacity which decline over time compared to conventional thermal treatment [68 and 69].

The microbial inactivation principle is Electroporation theory. PEF treatment at electric field intensity greater than a critical threshold of trans-membrane potential of 1 V across the target cells causes irreversible pore formation and destruction of the semi-permeable barrier of the cell membrane. More recent studies also show that hypothesis of microbial inactivation due to membrane permeabilisation caused by PEF. It is generally reported that yeast cells are more sensitive to PEF treatment than bacterial cells, and that Gram-negative are more sensitive than Gram-positive bacterial cells [70].

### 2.3.10 Low-Temperature Plasma for Food processing

A neutral gas is converted to plasma by the application of energy in several forms including; thermal, electric or magnetic fields and radio or microwave frequencies, resulting in an increase in the kinetic energy of the electrons of constituent gas atoms. This causes a cascade of collisions in the gas resulting in the formation of plasma products of electrons, ions, radicals and radiation of varying wavelengths including that in the UV ranges. The effectiveness of plasma to inactivate microorganisms on inert surfaces will depend greatly on the equipment design and operating conditions like gas type, flow rate and pressure. The methods of generating discharges of low-temperature plasmas by using electric fields of either DC, AC, pulsed DC, radiofrequency, microwave, dielectric barrier, or electron and laser beams. Electric fields are the most commonly used method of generating plasmas for technological applications.

Low-temperature plasmas are differentiated into atmospheric or low pressure (in the order of 10 Pa. In the atmospheric plasma, many collisions between the particles occur due to the density of the gas. This leads to rapid exchanges of energy between the electrons and heavier particles (ions, radicals and molecules) reaching a steady state and resulting in temperatures of the order of 10,000's degrees Celsius. The chemical composition of low-temperature plasmas of nitrogen, oxygen and carbon dioxide gas mixtures are dominated by ions free radicals and highly reactive intermediate species. Also if water vapour is present, highly reactive species including $H_2O$, H, OH are formed and also cluster ions containing $H_2O$. The generation of UV radiation oc-

curs in the ranges 10 - 290 nm, and those wavelengths above 200 nm, at a fluence of several $mWs.cm^{-2}$, are responsible for microcidal effects. Plasma inactivates both vegetative cells and bacterial endospores. Three basic mechanisms have been attributed to the inactivation of microbial spores in plasma environments. These include destruction of DNA by UV irradiation, volatilization of compounds from the spore surface by UV photons and erosion or so called 'etching' of the spore surface by adsorption of reactive species like free radicals. Synergistic effects between these possible mechanisms of inactivation can be expected, depending on the operational conditions and the design of the plasma generator [70].

The effect is limited to the surface layers of food products, but since plasmas are able to 'flow' over the treated surface into fissures and depressions. The entire surface can be treated, in contrast to other surface decontamination processes such as UV light. A number of researchers have investigated the potential for cold plasma treatment as a method of non-thermal decontamination of ready to cook food, nuts, fresh fruits and vegetables, cooked meats, raw chicken pieces and also for packaging [71].

### 2.3.11 Chlorine Dioxide processing

Chlorine dioxide ($ClO_2$) is one of the few compounds that exists almost entirely as monomeric free radicals. $ClO_2$ cannot be compressed and stored under the pressure because it is explosive, therefore the shipping of $ClO_2$ gas is impossible, and it has to be generated on-site. Easy-to-use commercial systems exist on the market for on-site $ClO_2$ generation. They generally consist of two sachets each one containing a precursor for $ClO_2$ generation, which takes place upon mixing two components.

Cell membrane has been identified as the primary target of $ClO_2$ on microbial cells. Studies show chlorine dioxide directly affects microbial cells by inhibiting protein synthesis, loss of permeability control. The effect of $ClO_2$ was related to non-specific oxidative damage of the outer membrane leading to the destruction of the trans-membrane ionic gradient. Some studies also show inhibition of division and associated metabolic damage or damage to genetic material. Use of chlorine dioxide for microbial deactivation on Lettuce, Cabbage, Green bell, Baby Apple pepper, Apples, Mungbean sprout, Blueberry, Melon, cucumber have shown satisfactory results. The main advantage of $ClO_2$ gas over aqueous sanitizers is that gas has more penetrability; it could therefore reach microorganisms protected from aqueous disinfectants by surface irregularities or biofilms [72].

### 2.3.12 Ozone processing

Ozone ($O_3$) results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electric discharge. The product is a bluish gas with a pungent odor and strong oxidizing properties Ozone inactivates microorganisms through oxidization, and residual ozone spontaneously decomposes to non-toxic products (i.e. oxygen), making it an environmentally friendly antimicrobial agent for use in the food industry. The strong biocidal characteristics of ozone are due to a combination of its high oxidizing potential and its ability to diffuse through biological membranes. Ozone is generated at Industrial scale on demand, in situ through various methods. Electrical (Corona) Discharge Method includes, adequately dried air or $O_2$ (free from particulate matter and dried to a dew point of at least - 60 $^0$C) is passed between two high-voltage electrodes separated by a dielectric material, which is usually glass. The ozone/gas mixture discharged from the ozonator normally contains 1 - 3% ozone when using dry air, and 3 - 6% ozone when using high purity oxygen as the feed gas. In Electrochemical (Cold Plasma) Method, an electrical current is applied between an anode and a cathode in an electrolytic solution containing water and a solution of highly electronegative anions. A mixture of oxygen and ozone is produced at the anode used for microbial reduction. In Radiochemical Ozone Generation (RCOG), high-energy irradiation of oxygen is happened to produce ozone. In Ultraviolet Method, ozone is formed when oxygen is exposed to UV light of 140 - 190 nm wavelengths. This splits the oxygen molecules into oxygen atoms, which then combine with other oxygen molecules to form ozone. Some of Extrinsic Factors such as flow rate, ozone concentration, temperature and intrinsic factors such as pH and Organic Matter affect the ozone efficacy. Ozone is very unstable both in the gaseous phase and in solution, decomposing into hydroxyl, hydroperoxy and superoxide radicals. The reactivity of ozone is attributed to the oxidizing power of these free radicals. Microorganisms are inactivated by disruption of the cell envelope or disintegration leading to cell lysis. The bacterial membrane seems to be the first site of the attack with proteins and unsaturated lipids in the cell membrane being the primary targets. Additionally, ozone causes alteration in membrane permeability leading to leakage of cell contents and eventually causing lysis. Ozone shows favorable microbial reduction (gram negative, gram positive bacteria and spores) in milk, gelatin, albumin, casein, meat products, apple juice, whey, water, orange juice [73].

Kiwi fruit is a rich source of vitamin C and contains more ascorbic acid than citrus fruits. Barboni [74] compared the effect of ozone rich storage and air storage over a period of 7 months on the vitamin C content of kiwi fruit. Gaseous ozone concentration was 4 mg/h in

the chamber at a temperature of 0 $^0$C and a humidity of 90 - 95%. The authors did not observe any significant change in ascorbic acid content of kiwi fruit over a 7 month storage period at an ozone concentration of 4 mg/h in the chamber (2 m$^3$) and a storage temperature of 0 $^0$C. Reports on the effect of ozone on other bioactive compounds of exotic fruits are limited [74].

Ozone treatments were also reported to have minor effects on anthocyanin contents in strawberries [75] and blackberries [76].

### 2.3.13 Dense-Phase Carbon Dioxide processing of fluid foods

Carbon dioxide is used because of its safety, low cost, and high purity. Dense-phase carbon dioxide (DPCD) treatment has attracted great interest in the non-thermal treatment of liquid foods or liquid model solutions. DPCD has been shown to inactivate microorganisms as well as conventional heat pasteurization without the loss of nutrients or quality changes that may occur due to thermal effects. The temperature increase induced by the pressure build-up is negligible. The treatment times can range from about 3 to 9 min for continuous, or from 120 to 140 min for semi-continuous or batch DPCD processes.

A typical batch system has a $CO_2$ gas cylinder, a pressure regulator, a vessel, a water bath or heater, and a $CO_2$ release valve. The sample is placed into the vessel and the temperature is set to the desired value. Then $CO_2$ is introduced into the vessel until the sample is saturated at the desired pressure and temperature. The sample is left in the vessel for a period of time and then the $CO_2$ outlet valve is opened to release the gas. Some systems contain an agitator to decrease the time to saturate of the sample with $CO_2$. A continuous high-pressure $CO_2$ system has been developed to process 1 L/h of liquid at 40.0 MPa. The sample liquid was stored in two 5 liter high-density polyethylene containers, both connected to the pump. $CO_2$ passed through an in-line 0.5 µm filter and a cooling system, then pumped to four mixing points. The pressurized $CO_2$ was mixed with the liquid and the mixture went to a temperature-controlled holding tube. Several valves along the holding tube allowed for sampling at different residence times. The treated liquid depressurized through a capillary tube inside a thermostatic bath. The liquid was degassed in two containers.

The bacteriostatic action of pressurized $CO_2$ compromises different steps such as solubilization of pressurized $CO_2$ in the external liquid phase, cell membrane modification, intracellular pH decrease, key enzyme inactivation/cellular metabolism inhibition due to internal pH lowering, direct inhibitory effect of molecular $CO_2$ and $HCO_3$ on metabolism, disordering of the intracellular electrolyte balance, extraction of vital constituents from cells and cell membranes, physical disruption of cell membrane. Most of these steps occur consecutively and simultaneously in a complex and interrelated manner. Another mechanism shows that carbon dioxide is having very high affinity for plasma consists in great part of lipid components. Due to the increased membrane permeability caused by the reaction of $CO_2$ with water, which lowers the extracellular pH, pressurized $CO_2$ may easily penetrate through the bacterial cell membrane and accumulate in the cytoplasmic interior of bacterial cells then structurally and functionally disrupt the cell membrane due to a loss of the order in the lipid chain If too much dissolved $CO_2$ enters the cytoplasm, the cells may be unable to expel all the resulting protons and internal pH starts to decrease. If the internal pH is lowered too much, cell viability will be impaired leading to inhibition of cell metabolism or denature certain proteins and enzymes essential for metabolic and regulatory processes, such as glycolysis, amino acid and peptide transport. Finally internal damage of the metabolic processes induces microbial inactivation. Studies on Microbial Inactivation in Liquid Foods such as Whole skim milk, fruit juices like orange, peach, carrot, mandarin, watermelon, pear, apple, grapefruit a well as many harmful enzymes by Dense-Phase shows 5 to 7 log reduction of Pathogenic bacteria and yeast [77].

Chen J, investigated the effects of DPCD treatment of 8, 15, 22, 30 and 35 MPa for 5, 15, 30, 45, 60 min at 35°C, 45°C, 55°C, 65°C on vitamin C in Hami melon juice during storage at 4 $^0$C for 4 weeks. The authors found that vitamin C concentration decreased following DPCD processing, but percentage loss was lower than of the untreated samples. DPCD also appear to prevent losses of other potential bioactive compounds such as β-carotene. The study conducted by Chen J showed better retention of β-carotene in DPCD (55 $^0$C, 60 minutes, and 35 MPa) treated melon juice compared to conventional HTST pasteurization. Significant losses (57.87%) in β-carotene content was observed in heat pasteurized samples. It should be noted that exact mechanism for β- carotene stability is difficult to establish [78].

Many examples of the applications of the DPCD to juices demonstrated the protective nature of the process to antioxidants, phytochemicals, organoleptic attributes such as taste, color, and appearance. The relatively low process temperatures, the lack of oxygen in the environment, and for some nutrients, the lower pH, protect the vitamins such as vitamin C. Since the process can be made continuous, its control is easy [78].

However this technology is facing some challenges such as lack of the commercially successful DPCD operation, higher cost of the operation, stringent environmental regulations regarding the release of $CO_2$ into the atmosphere, both total capture and recycling of

the gas needs to be designed into new systems, or a carbon-neutral source of $CO_2$ needs to be used, limited data to satisfy the regulatory requirements [77].

### 2.3.14 Ultra-sound processing of food

Power ultrasound employed in food processing uses the lower-frequency ranges of 20 - 100 kHz with a sound intensity of between 10 and 1,000 W/cm$^2$. The vibrational energy is provided by ultrasonic transducers that convert electrical energy to sound energy of which there are two types in common usage, piezoelectric transducer and magnetostrictive transducers. The driving force for the processing effects of sonication is acoustic cavitation. The cavitation bubbles are generated by the ultrasound wave as it passes through the liquid. Like any sound wave, it is transmitted as a series of compression and rarefaction cycles affecting the molecules of the liquid. When the negative pressure of the rarefaction cycle exceeds the attractive forces between the molecules of the liquid, a void is formed. This void or cavity in the structure takes in a small amount of vapor from the solution so that on compression it does not totally collapse, but instead continues to grow in size in successive cycles to form an acoustic cavitation bubble. There are many thousands of such bubbles in a liquid, some of which are relatively stable but others expand further to an unstable size and undergo violent collapse to generate temperatures of about 5,000 K and pressures of the order of 50 MPa. The cavitation bubbles formed in this way are divided into two types: Stable cavition and Transient cavitation [79].

For the sterilization of liquid foods, higher acoustic energies are employed and the approaches can be classified as sonication alone, manosonication (pressure and ultrasound), themosonication (heat and ultrasound), or monothermosonication (heat, pressure, and ultrasound). The mechanisms through which ultrasound affects microbial inactivation are induced by acoustic cavitation which results in the weaking or disruption of bacterial cells through various processes. Bacteria cell wall damage, due to mechanical effects induced by pressure gradients generated during the collapse of cavitation bubbles within or near the bacteria. Second process is shear forces induced by micro-streaming which occurs in the bacterial cell itself. Third approach suggest, chemical attack due to formation of free radicals during cavitation which attack the cell wall structure leading to disintegration. In addition there will be the formation of a small amount of hydrogen peroxide via sonication, which itself is a bactericide. One of the major bactericidal effects of ultrasound is attributed to intracellular cavitation; that is, micromechanical shocks that disrupt cellular structural and functional components up to the point of cell lysis. Use of ultra-sound processing for desired microbial reduction is studies with various food products such as orange juice, apple cider, mango juice, guava juice, tomato juice, whole milk, skim milk. In terms of dairy products, especially milk, ultrasound providing considerable benefit at lower-temperature pasteurization [80].

Rawson investigated the effect of thermosonication on the bioactive compounds of freshly squeezed watermelon juice. They observed a higher retention of ascorbic acid and lycopene at low amplitude level and temperature. They also observed a slight increase in lycopene at low amplitude level [81].

### 2.3.15 High Voltage Arc discharge

High voltage arc discharge is a method to pasteurize liquid foods by applying rapid discharge voltages through an electrode gap below the surface of aqueous suspensions of microorganisms. When rapid high voltages are discharged through liquids, a multitude of physical effects (intense waves) and chemical compounds (electrolysis) are generated, referred to as electrohydraulic shock, which inactivate the microorganisms [82]. Enzymes are also inactivated by high voltage arc discharges.

Palaniappan and Sastry presented an extensive literature review on the effect of electrohydraulic shock on the inactivation of microorganisms. They reported that bacterial inactivation was not due to heating, but mainly to irreversible loss of membrane function as a semipermeable barrier between the bacterial cell and the environment and to the formation of toxic compounds (oxygen radicals and other oxidizing compounds). In their review, it was concluded that chemical action is a complex effect and depends not only on the voltage applied but also on the type of microorganism, initial concentration of cells, volume of the medium used, distribution of chemical radicals, and electrode material [83].

Inactivation is attributed to oxidation reactions mediated by free radicals and atomic oxygen. There is no significant temperature increase during treatment by arc discharge [84]. Gilliland and Speck found electrohydraulic treatment to be effective in inactivating at least 95% of the vegetative cells of *E. coli, Enterococcus faecalis, Micrococcus radiodurans, Bacillus subtilis* and its spores. High voltage electrical impulses were discharged at a rate of 1 V/s [85].

## 2.4 Sensitive Nutrients with their food systems

Thus delivery of heat sensitive nutrients through food systems posed a challenge for food scientist to satisfy often conflicting requirements with respect to the incorporation of sensitive active ingredients in food products of increasing complexity have provoked a

growing interest in the development and application of delivery systems for foodstuffs. In many cases, however, it is difficult to apply established encapsulation technologies without modification to an application of interest, principally because the physical and chemical behaviour of the sensitive ingredients and the functionality of the delivery system are poorly understood. So systematic evaluation of various aspects of food systems should be done at the time of developing food systems delivering heat sensitive nutrients. Some examples of such food systems are described below with appropriate explanation.

According to FAO/WHO (2004), the recommended intake for vitamin A is 375 µg Retinol Equivalent (RE) per Day (for Infants) upto 600 µg RE per Day (for Adults). Iron requirements are 4.2 mg per Day (for children) upto 9.1 mg per day for adult males and 7.5 – 19.6 mg per day for adult females at 15% bioavailability. Calcium requirements are 500 - 700 mg per Day (for children), 1000 mg per day for adult males and 1000 – 1300 mg per day for adult females. Vitamin C requirements are 30 - 35 mg/Day (for children), 45 mg/day for adult males and females. The recommended daily intake (RDI) for Iodine for adult is 100–150 µg/day for Adults and 90 -120 µg/day for children [86].

#### 2.4.1 Vitamin A, Iron and Iodine:

Rutkowski and Diosady [87], developed a triple fortified which contained Vitamin A (250 IU per gram of salt), Iron (1000 ppm) and Iodine (50 ppm). Vitamin A is used in the form of vitamin-A palmitate, iron in the form of ferric NaEDTA and iodine in the form of potassium iodate. Here stability of vitamin A is maintained by using Shellac as a hydrophobic coating agent. They also found good stability of vitamin A, Iron and iodine during processing and storage. Vitamin A is also used in the form of retinyl acetate at 257.85 µg per 100 g of cookies and shows minimum losses during baking i.e. 8.69 - 11.11%. It fulfils our 45 % RDA of vitamin A [88].

#### 2.4.2 Calcium

Food products find wide range of calcium fortified food products in market. Meat sausage fortified with calcium lactate and calcium glutamate at concentration of 27 - 32 mg per 100 g of product [89]. Apple slices are also impregnated with calcium upto 140 - 250 mg per 100 gm [90]. Often vitamin C in combination with calcium fortified in food to enhance the bioavailability of calcium. Calcium enriched food products contains wheat flour tortillas (48 g per 100 g) [91], mango yogurt (50 mg per 100 g) [92], soy milk (24.96 - 28.8 mg per 100 g) [93] which provides 20 to 40 % RDA for calcium (1000 mg per day).

#### 2.4.3 Zinc

Zinc, in the form of zinc sulphate and zinc oxide is used to fortified parboiled rice at concentration 13.2 - 44.1mg per 100 g [94]. Zinc oxide (30 ppm) in combination with NaFeEDTA (40 ppm) used in whole wheat flour [95].

#### 2.4.4 Iodine

Potassium iodide is used to enriched salt in combination of iron (ferrous fumarate) at concentration up to 50 mg per kg. It is also used during manufactured of meat burger and meat balls with wheat fibre and soy isolate impregnated with KI and $KIO_3$ (43 µg per 100 g) which provides 30 % RDA (150 µg per day) [96].

#### 2.4.5 Vitamin D

RDA for Vitamin D is 5 µg per day for children, 10 - 15 µg per day for adult (FAO WHO 2004). It is used to enrich milk (upto 5000 IU per 100 g), yogurt (5000 IU per 100 g) and ice-cream (5000 IU per 100 g) in the emulsified form of vitamin D in butter oil [97]. In many countries, milk, milk products, margarine, vegetable oil fortified with vitamin D, used as a major dietary source. This vitamin along with vitamin A and calcium used to enrich dairy products. This fat soluble vitamin should not cross the limit of 50000 IU per day through food to avoid toxic effect.

#### 2.4.6 Vitamin E

The RDA for α - tocopherol is 4 - 15 mg per day for adults and children. Vitamin E is used to fortify ground beef pattice (300 ppm)[98]. α – tocopherol succinate which is heat and storage stable form of vitamin E is used in soft drinks and ice-crieams. Microdispersion of vitamin E (alpha-tocopheryl acetate) in milks showed increased molar ratio of plasma tocopherol to cholesterol (2 times) compared with Vitamin E capsules [99].

#### 2.4.7 ω-3 long chain PUFA

These are used to fortify yogurt, cream (1 - 5 mg per 100 g). Also in cheese (30 mg per 100 g), processed cheese (40-60 mg per 100 gm), spreadable fresh cheese (20 mg per 100 g) [100].

#### 2.4.8 Vitamin C

Ascorbic acid is most heat liable vitamin and also water soluble so it is enriched in the combination of iron in various food systems such as dairy products such as cheese, yogurt, chocolate milk which increases the bioavailability of iron in presence of vitamin C. It is also

**Table 3. Sensitive nutrients with their food systems**

| Sr. No. | Chemical form | Added concentration | Food product | References |
|---|---|---|---|---|
| **Calcium** | | | | |
| 1. | - Calcium citrate<br>- Calcium lactate<br>- Calcium gluconate | 27 - 32 mg/100 g of product | Meat Sausage | [89] |
| 2. | - Calcium lactate | 140 - 250 mg/100 g of product | Vacuum impregnated apple slices | [90] |
| 3. | - Calcium carbonate<br>- Calcium citrate<br>- Calcium lactate | 48 mg/100 g of product | Wheat flour tortillas | [91] |
| 4. | - Calcium lactate | 50 mg/100 g of product | Calcium fortified cow milk | [105] |
| 5. | - Calcium lactate pentahydrate | 50 mg/100 g of product | Mango yogurt | [92] |
| 6. | - Calcium glutamate | 24.96 - 28.28 | Soymilk | [93] |
| **Iodine** | | | | |
| 7. | - Dextrin encapsulated<br>- Potassium iodide<br>- Ferrous fumarate | KI - 50 mg /100 g<br>Iodine - 100mg/100g of product | Salt | [106] |
| 8. | - Wheat fibre and soy isolate impregnated KI and Potassium iodate | 43 µg/100 g<br>(30 % RDA) | Meat burgers and meat balls | [96] |
| **Zinc** | | | | |
| 9. | - Zinc sulphate<br>- Zinc oxide | 13.2 - 44.1 mg/ kg | Parboiled rice (polished) | [94] |
| 10. | - Zinc oxide and NaFeEDTA | 30 ppm | Whole wheat flour | [95] |
| **Iron** | | | | |
| 11. | - Ferrous sulphate<br>- Fe-EDTA | 10 - 30 mg/100 g | Bakery products- flour Bread, Cookies, Wheat bread | [107] |
| | - Ferric pyrophosphate<br>- Ferrous fumarate | 12 mg/L | Milk infant formula | |
| | - $FeCl_3$ | 10 mg Fe/100mL | Yogurt | |
| | - Caesin-chilated Iron ferric chloride | 25 - 50 mg Fe/kg | Mozzarella cheese | |
| | - Ferrous Sulphate | 15 mg/L | Milk | |
| **Vitamin E** | | | | |
| 12. | - Vitamin E | 300 ppm (µl of Vit E/g lipid meal) | Ground beef patties | [98] |
| 13. | - α-tocopherol | - | B-lactoglobuline and Hen egg white protein | [108] |
| **Vitamin D** | | | | |
| 14. | - Vit. $D_3$ emulsified in butter oil | 5000 IU/100g of product | Cheese, Yogurt, Ice-cream | [97] |
| **Vitamin A** | | | | |
| 15. | - Retinyl acetate | 257.85 µg/100 g | Cookies | [15] |
| 16. | - Vitamin A, palmitate iron and Iodine | Vit A - 250 IU of vit.A/100 g<br>Iron - 1000 ppm<br>Iodine - 50 ppm | Triple fortified salt | [87] |
| 17. | - Retinol | - | O/W/O emulsion | [109] |
| 18. | - Retinol | - | Glyceryl behenate SLN | |
| 19. | - All trans retinoic acid | - | 2-Hydroxypropyl-β-cyclodextrin complex | |
| 20. | - Retinol | - | B-Lactoglobulin complex | |
| **Folic Acid** | | | | |
| 21. | - Folic acid (low methoxy pectin and ethyl cellulose) | 400 µg/g | Parboiled rice | [104] |
| 22. | - Folic Acid | 0.05 g/100 g of flour | Asian noodle | [110] |
| 23. | - Folate | 131 - 191 µg/100g | Bakery Products, Sourdough, French loves, Potato rolls, Sandwitch. | [103] |
| | | 33 - 229 µg/100g | Cereal products-flours, baking n\mix, bread mix | |
| | | 154 - 308 µg/100g | Instant rice, Parboiled rice yellow rice, precooked rice. | |
| | | 198 - 264 µg/100g | Enriched macaroni products, Spagetti, Pasta. | |
| | | 198 - 264 µg/100g | Noodle | |
| | | 80 - 400 µg/100g | Ready to eat breakfast cereals(corn, oat, wheat) | |
| | | 40 - 120 µg/100g | Cereal bars | |
| **Riboflavin** | | | | |
| 24. | - Riboflavin | - | Soy protein cold set hydrogel | [102] |
| **Vitamin C** | | | | |
| 25. | - Vitamin C | 33 mg/100 g | Ascorbic acid | [101] |
| | - Vitamin A palmitate | 300 IU/100 g | Vit. A palmitate | |
| **Nutrients having functional properties** | | | | |
| 26. | - Ω-3 long chain PUFA | 1 - 5 mg/100g | Cheese, butter | [100] |
| | | 20 mg/100g | Spreadable fresh cheese | |
| | | 30 mg/100g | Cheese, butter | |
| | | 40 - 60 mg/100g | Processed cheese | |
| 27. | - Curcumin | 4.1 mg/mL of lecithin and Tween 80 as the surfactants and ethyl oleate as oil phase | - | [111] |

used to enrich different fruit juices which undergoes minimum heat treatments and retained maximum quantity of vitamin C [101].

### 2.4.9 Vitamin B

Vitamin $B_1$ is sensitive to heat or oxidation. The major challenge in this context is, these are water soluble vitamins and leaching losses are more. They are often delivered through breakfast cereals, juices, milk, and infant formulas. Generally enriched in food products as vitamin B complex. There are new novel approaches for delivery of these vitamins such as soya protein cold set hydrogel is used to entrap vitamin B for incorporation in food products [102].

### 2.4.10 Folic acid

Folic acid is soluble in water and sensitive to heat. The RDA for folic acid is 400 µg per day [86]. Folate is used to fortify different bakery products (Sourdough banquettes, French loves, Buttertop breads, Italian bread, Potato rolls, Enriched Floors (229 µg per 100g), white breads upto 131 - 191 µg per 100g ), cereal products (All purpose flours, wheat flour, bread mix, All-purpose baking mix upto 33-229 µg per 100g), Instant rice (Precooked rice, parboiled rice, yellow rice, instant rice at 154 - 308 µg per 100g), macaroni (198-264 µg per 100g), pasta, noodles (198-264 µg per 100g), ready to eat breakfast cereals (80 - 400 µg per 100g) and cereal bars (40 - 120 µg per 100g) [103]. Another way of delivering folic acid is addition to rice up to 400 µg per 100g of rice and coating with edible polymers ethyl cellulose [104].

Thus different combinations of heat sensitive nutrients and their interaction with each other determine their bioavailability. Appropriate encapsulation, Use of more chemically stable form and novel and non-thermal processing techniques provides desired retention levels of sensitive nutrients in Food matrices during processing, storage and consumption. Following table 3 explains in details some more examples of vitamins, minerals and bio-active compounds with respective food systems.

### 2.5 Recent trends in delivery of heat sensitive nutrients

More and more efforts are being taken by food scientist to incorporate these sensitive nutrients in food by using newer technologies such as membrane extrusion, vacuum impregnation. New vehicles for these nutrients such as β-lactoalbumine and Hen white protein for α-tocopherol, soy protein cold set hydrogel for riboflavin, milk caesinates are also developed for protection against processing conditions [112].

Also biofortification of food plants for vitamins by using metabolic engineering is also encouraging. Minerals fortification is done by manipulating manure dosage during plant growth become more precise and successful [113].

## 3. Conclusions

- Food contains many heat sensitive nutrients such as vitamins, minerals, nutrients having functional properties. Many processes during manufacturing of food cause detrimental effects on these nutrients. Among these nutrients vitamins (Vit. A,D,E, B,C) are most susceptible for heat causes upto 80 % destruction due to processing treatments and minerals get unavailable due to interaction with co-nutrients during the heat treatments such as pasteurisation, sterilisation, ultra high temperature.
- In order to maintained the level of these sensitive nutrients different approaches are applied including process modifications, fortification or encapsulation. The nature of methodology depends exclusively in interest of functionality of particular nutrients in food irrespective of techniques.
- In case of delivery of heat sensitive nutrients through food systems we concentrate on different delivery systems such as 0/w emulsion, nano-emulsions, encapsulation, molecular complexes, and powder particles with their uses in food application.
- Many recent trends are also becoming more and more successful to prevent sensitive nutrients from degradation such as use of: Microwave and Radio Frequency Heating, Ohmic and Inductive Heating, High Pressure Processing, Irradiation of Food, Pulsed X-ray Processing, Pulsed Light processing, Ultra-Violet Pasteurization, Oscillating Magnetic Fields, Pulse Electric Field, Chlorine Dioxide Processing, Low-Temperature Plasma, Ozone processing, Dense-Phase Carbon Dioxide Processing of Fluid Foods, Ultra-sound Processing of Food, High Voltage Arc Discharge and bio-fortification. Thus lots of work is to be done by food scientist to come up with more and more efficient technology.

## 4. References

[1] Clydesdale F.M., Francis F.J. (1976). *Pigments*. In: Fennema, O.R. (Ed.), Principles of Food Science - Food Chemistry. Marcel Dekker, New York, pp. 417 - 430.

[2] Britton G. (1992). *Carotenoids*. In: Hendry G. F. (Ed.), Natural Foods Colorants. G. F. Blackie, New York, pp. 141 – 148.

[3] Bolin H.R. (1982). *Effect of processing of nutrient composition of food: fruits and fruit products*. In: Rechcigl Jr. M. (Ed.), Handbook of Nutritive Value of Processed Food.

Food for Human Use, Vol. I. CRC Press, Boca Raton, FL, pp. 310.

[4] Harris R.S. (1987). *General discussion on the stability of nutrients*. In: Karmas E., Harris R. S. (Eds.), Nutritional Evaluation of Food Processing. Van Nostrand Reinhold, New York, pp. 4.

[5] Emilla L., Janna K., Eva Kovacikova, Martina K., Janka P., Kristina H. (2006). *Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models.* Journal of Food Composition and Analysis, 19, pp. 252 - 276.

[6] Eitenmiller R. R., Laden W. O. (1999). *Vitamin A and b-carotene. Ascorbic acid. Thiamin. Vitamin B-6. Folate*. In: Eitenmiller R. R., Laden W. O. (Eds.), Vitamin Analysis for the Health and Food Science. CRC Press, Boca Raton, FL (pp. 15 - 19, 226 - 228, 275, 375, 411 - 465).

[7] Le Roche. (1976). *Vitamin Compendium*. F. Hoffmann - La Roche, Basle, Switzerland.

[8] Ottaway P. B. (2002). *The stability of vitamins during food processing: vitamin K*. In: Henry C. J. K., Chapman C. (Eds.), The Nutrition Handbook for Food Processors. CRC Press, Boca Raton, FL, pp. 247 - 264.

[9] Karmas E. and Harris R. S. (1988). *Nutritional Evaluation of Food Processing*, Van Nostrand Reinhold, New York.

[10] Hazell T. and Johnson I. T. (1989). *Influence of Food Processing on Iron Availability in Vitro from Extruded Maize-based Snack Foods*. Journal of Science, Food and Agriculture, 46, pp. 365 - 374.

[11] Johnson P. E. (1991). *Effect of Food Processing and Preparation on Mineral Utilization in Nutritional and Toxicological Consequences of Food Processing (Friedman, M., Ed.)*. Advances in Experimental Medicine and Biology, 25, pp. 125 - 128.

[12] Kapsokefalou M. and Miller D. D. (1995). *Iron Speciation in Intestinal Contents of Rats Fed Meals Composed of Meat and Non-meat Sources of Protein and Fat*. Food Chemistry, 52, pp. 47 - 56.

[13] Heribert J. W. Watzke. (1998). *Impact of processing bioavailibility examples of minerals in foods*. Trends in Food Science and Technology, 9, pp. 320 - 327.

[14] Volden J. Borge A. I. G., Bengtsson B. G., Hansen M., Thygesen E. I., Wicklund T. (2008). *Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (Brassica oleracea L. ssp. capitata f. rubra)*. Food Chemistry, 109(3), pp. 595 - 605.

[15] Suresh D., Manjunatha H., Krishnapura S. (2007). *Effect of heat processing of spices on the concentrations of their bioactive principles: Turmeric (Curcuma longa), red pepper (Capsicum annuum) and black pepper (Piper nigrum)*. Journal of Food Composition and Analysis, Volume 20, Issues 3 - 4, pp. 346 - 351.

[16] Job U. and Jessica K. (2006). *Physical approaches for the delivery of active ingredients in foods*. Trends in Food Science and Technology, 17, pp. 244 - 254.

[17] Corey E. J., and Cheng X. M. (1989). *Chapter 1 - The Basis for Retro-synthetic Analysis*. In:The logic of chemical synthesis. New York: John Wiley & Sons. pp: 1 -15.

[18] Salminen S., Ouwehand A., Benno Y., and Lee Y. K. (1999). *Probiotics: How should they be defined?* Trends in Food Science and Technology, 10(3), pp. 107 - 110.

[19] Sigler K., Chaloupka J., Brozmanova J., Stadler N., and Hofer M. (1999). *Oxidative stress in microorganisms - I. Microbial vs. higher cells - Damages and defences in relation to cell aging and death.* Folia Microbiologica, 44(6), pp. 587 - 624.

[20] Crowe J. H., Carpenter J. F., and Crowe L. M. (1998). *The role of vitrification in anhydrobiosis*. Annual review of Physiology, 60, pp. 73 - 103.

[21] Beales N. (2003). *Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. Comprehensive Reviews*. Food Science and Food Safety, (3), pp. 1 - 20.

[22] Crawford D., and Davies K. (1994). *Adaptive response and oxidative stress*. Environmental and Health Perspectives, 10(Suppl. 10), pp. 25 - 28.

[23] De Angelis M., and Gobbetti M. (2004). *Environmental stress responses in Lactobacillus: A review*. Proteomics, 4, pp. 106 - 122.

[24] Byers T., Perry G. (1992). *Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers.* Annual Review Nutrition, (12). pp. 139 -159.

[25] Serfert Y., Drusch S., Schwarz K. (2009). *Chemical stabilisation of oils rich in long-chain polyunsaturated fatty acids during homogenisation, microencapsulation and storage,* Food Chemistry, (113), pp. 1106 - 11012.

[26] Mcclements D. J. , Ogawa S., Decker E. A. (2007) *Production and characterization of O/W emulsions containing cationic droplets stabilized by lecithin-chitosan membranes.* Journal Agriculture Food Chemistry, 51(28), pp. 06 - 12.

[27] Mcclements D. J., Shaw L. A., Decker E. A. (2007). *Spray-dried multilayered emulsions as a delivery method for omega 3 fatty acids into food systems.* Journal of Agriculture Food Chemistry, 55(31), pp. 9 - 12.

[28] Muschiolik G.(2007). *Multiple emulsions for food use*. Current Opinion in Colloid Interface Science, (12) pp. 213 - 20.

[29] Benichou A., Aserin A. A., Garti N. (2007). *W/O/W double emulsions stabilized with WPI-polysaccharide complexes*. Colloids Surface Physicochemistry Engineering Aspects, (294) pp. 20 - 32.

[30] Wooster T. J., Golding M., Sanguansri P. (2008). *Impact of oil type on nanoemulsion formation and Ostwald ripening stability.* Langmuir, (24), pp. 12758 –12765.

[31] Yuan Y., Gao Y., Zhao J., Mao L. (2008). *Characterization and stability evaluation of betacarotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions.* Food Research International. (41). pp. 61 - 68.

[32] Bunjes H., Steiniger F., Richter W. (2007). *Visualizing the structure of triglyceride nanoparticles in different crystal modification*. Langmuir, (23), pp. 4005 - 4011.

[33] Carlotti M. E., Sapini S., Trotta M., Battaglia L., Vione D., Pelizzit E. (2005). *Photostability and stability over time of retynil palmitate in an O/W emulsion in SLN introduced in the emulsion*. Journal of Dispersion Science Technology, 26, pp. 125 - 138.

[34] Buschmann H. J., Schollmeyer E. (2002). *Applications of cyclodextrins in cosmetic products: A review*. Journal of Cosmetic Science, 53, pp. 185 - 191.

[35] Davis M. E., Brewster M. E. (2004). *Cyclodextrin-based pharmaceutics: past, present and future*. National Review Drug Discovery, 3, pp. 1023 - 1035.

[36] Semo E., Kesselman E., Danino D., Livney Y. D. (2006). *Casein micelle as a natural nanocapsular vehicle for nutraceuticals*. Food Hydrocolloids, 21, pp. 936 - 942.

[37] Tanford C. (1980). *The hydrophobic effects: formation of micelles and biological membranes*. New York: John Willey.

[38] Leser M. E. , Sagalowicz L. , Michel M. , Watzke H. J. (2006). *Self-assembly of polar food lipids*. Advance Colloid Interface Science, 123, pp. 125 - 136.

[39] Sagalowicz L., Leser E. M. (2010). *Delivery systems for liquid food products*. Current Opinion in Colloid & Interface Science. (15) pp. 61 - 72.

[40] Metaxas R. (1996). *Foundations of electro heat: a unified approach*. John Wiley & Sons. Chichester, UK.

[41] Roussy G. and Pearce J. (1995). *Foundations and industrial applications of microwaves and radio frequency fields*. New York. Wiley.

[42] Kasevich R. S. (1998). *Understand the potential of radio-frequency energy*. Chem Eng Progress. pp.75 - 81.

[43] Minett P. J. and Witt J. A. (1976). *Radio frequency and microwaves*. Food Processing Industry, 36 - 37.

[44] Buffler C. R. (1993). *Microwave cooking and processing* In: Engineering fundamentals for the food scientist, Van Nostrand Reinhold, New York.

[45] Anantheswaran D. (2000) *Fundamentals of heat and moisture transport for microwaveable food product and process development.* A. K. Datta and R. C. Anantheswaran (eds.). Handbook of Microwave Technology for Food Applications. Marcel Dekker, Inc. New York.

[46] Heddleson R. A. and Doores S. (1994). *Factors affecting microwave heating of foods and microwave induced destruction of foodborne pathogens - A review.* J. Food Protect. 57(11). pp. 1025 - 1037.

[47] Kozempel M. F., Annous B. A., Cook R. D., Scullen O. J. and Whiting R. C. (1998). *Inactivation of microorganisms with microwaves at reduced temperatures.* Journal of Food Protection 61(5). pp. 582 - 585.

[48] USA - FDA Center for Food Safety and Applied Nutrition (2011). *Kinetics of microbial inactivation for alternative food processing technologies: ohmic and inductive heating*. < URL: http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm101246.htm. Accessed on 11 July 2013.

[49] Marcos C. K., Carolina Alves dos Santos, Soares O. M. V. A. A. and Thereza Christina Vessoni Penna (2010). *Ohmic heating - A review. Trends in Food Science & Technology*. 21, pp. 436 - 441.

[50] Rockendra G., and Balasubramaniam M. V. (2012). *Chapter 5: High-Pressure Processing of Fluid Foods*. In: Novel Thermal and Non-Thermal Technologies for Fluid Foods, Elsevier pp. 109 - 133.

[51] Fan X., Sokorai K. J. B. (2008). *Effect of ionizing radiation on furan formation in fresh fruits and vegetables*. Journal of Food Science, 73 (2) pp. C79 - C83.

[52] Food Engineering & Ingredients (2008). *Food irradiation: a technology wasted or simply unwanted*. Vol. 33, Issue 2, pp. 16 - 19.

[53] Brendan A. N., Meixu G. (2012). *Chapter - 7 : Irradiation of Fluid Foods*. In : Novel Thermal and Non-Thermal Technologies for Fluid Foods. pp. 167 - 183.

[54] Reyes L. F., and Cisneros - Zevallos L. (2007). *Electron-beam ionizing radiation stress effects on mango fruit (Mangifera indica L.) antioxidant constituents before and during postharvest storage*. Journal of Agriculture and Food Chemistry, 55, pp. 6132 - 6139.

[55] Lopez-Rubira V., Conesa A., Allende A., and Artés F. (2005). *Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C.* Postharvest Biology and Technology, 37, pp. 174 - 185.

[56] Codex Alimentarius General Standard on Food Irradiation (2003). 106 - 1983, REV. 1 - 2003.

[57] USA - FDA Center for Food Safety and Applied Nutrition (2011). *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies - Pulsed X - rays*. <URL:http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm105787.htm. Accessed on 12 July 2013.

[58] Miller R., Jeffrey W., Mitchell D. and Elasri M. (1999). *Bacterial responses to ultraviolet light. American Society for Microbiology.* 65(8) pp. 535 - 541.

[59] USA-FDA Center for Food Safety and Applied Nutrition (2011). *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies -- Ultraviolet Light*. <URL:http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm103137.htm . Accessed on 12 July 2013.

[60] Gonzalez - Barrio R., Vidal - Guevara M. L., Tomas - Barberan F. A., Espın J. C. (2009). *Preparation of a resveratrol-enriched grape juice based on ultraviolet C-treated berries.* Innovative Food Sci. Emerg. Technol. (10). pp. 374 - 382.

[61] Gomez-Lopez M. V., Koutchma Tatiana, Linden K. (2012). *Chapter 8 - Ultraviolet and Pulsed Light Processing of Fluid Foods* In : Novel Thermal and Non-Thermal Technologies for Fluid Foods. Elsevier, pp. 185 - 223.

[62] Gomez-Lopez M. V., Ragaert P., Debeverea J., and Devlieghere F. (2007). *Pulsed light for food decontamination: A review*. Trends in Food Science & Technology, (18), pp. 464 - 473.

[63] Tran M. T. T., Farid M. (2004). *Ultraviolet treatment of oran-*

*ge juice*. Innovative Food Sci. Emerg. Technol., 5, pp. 495 - 502.

[64] Walkling-Ribeiro M., Noci F., Cronin D. A., Riener J., Lyng J. G., Morgan D. J. (2008). *Reduction of Staphylococcus aureus and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields*. J. Food Eng., 89, pp. 267 - 273.

[65] USA-FDA Center for Food Safety and Applied Nutrition (2011). *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies - Oscillating Magnetic Fields*. <URL:http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm103131.htm. Accessed on 11 July 2013.

[66] Pothakamury U. R., Barbosa-Cánovas G. V., and Swanson B. G. (1993). *Magnetic-field inactivation of microorganisms and generation of biological changes*. Food Technol., 47(12) pp. 85 - 93.

[67] Barbosa-Cánovas G. V., Gongora-Nieto M. M., and Swanson B. G. (1998). *Nonthermal electrical methods in food preservation*. Food Science International, 4(5) pp. 363 - 370.

[68] Morales-de la Peña M., Salvia-Trujillo L., Rojas-Graü M. A., and Martín-Belloso O. (2010). *Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice-soymilk beverage in chilled storage.* LWT Food Science and Technology, 43, pp. 872 - 881.

[69] Morales-de la Peña M., Salvia-Trujillo L., Rojas-Graü M. A., and Martín-Belloso O. (2010). *Isoflavone profile of a high intensity pulsed electric field or thermally treated fruit juice-soymilk beverage stored under refrigeration*. Innovative Food Science & Emerging Technologies, doi:10.1016/j.ifset.2010.08.005.

[70] Wan J., Coventry J., Swiergon P., Sanguansri P., and Versteeg C. (2009) *Advances in innovative processing technologies for microbial inactivation and enhancement of food safety - pulsed electric field and low-temperature plasma Trends.* Food Science & Technology, (20), pp. 414 - 424.

[71] Food Engineering & Ingredients. (2012). *A tale of two technologies: How innovative processing methods can help keep businesses competitive*. Volume 37, pp. 11 - 14.

[72] Gomez-Lopez M. V., Rajkovic A., Ragaert P., Smigic N., and Devlieghere F. (2009) *Chlorine dioxide for minimally processed produce preservation: A review*. Trends in Food Science & Technology, (20) pp. 17 - 26.

[73] Patil S., and Bourke P. (2012) *Chapter 9 - Ozone Processing of Fluid Foods. Novel Thermal and Non-Thermal Technologies for Fluid Foods.* Elsevier Inc., pp. 225 - 261.

[74] Barboni T., Cannac M., and Chiaramonti N. (2010). *Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in Actinidia deliciosa*. Food Chemistry, 121(4), pp. 946 - 951.

[75] Perez A. G., Sanz C., Rios J. J., Olias R., and Olias J. M. (1999). *Effects of ozone treatment on postharvest strawberry quality*. Journal of Agricultural and Food Chemistry, 47(4), pp. 1652 - 1656.

[76] Barth M. M., Zhou C., Mercier J., and Payne F. A. (1995). *Ozone storage effects on anthocyanin content and fungal growth in blackberries.* Journal of Food Science, 60(6), pp. 1286 - 1288.

[77] Ferrentino G., Balaban O. M. (2012) *Chapter 10 - Dense-Phase Carbon Dioxide Processing of Fluid Foods.* Novel Thermal and Non-Thermal Technologies for Fluid Foods, Elsevier Inc., pp. 263 - 303.

[78] Chen J., Zhang J., Feng Z., Song L., Wu J., and Hu X. (2010). *Changes in microorganism, enzyme, aroma of Hami melon (Cucumis melo L.) juice treated with dense phase carbon dioxide and stored at 4 °C.* Innovative Food Science & Emerging Technologies. Volume 11, Issue 4, pp. 623 - 629.

[79] Tiwari K. B. and Mason J. T. (2012). *Chapter - 6: Ultrasound Processing of Fluid Foods. Novel Thermal and Non-Thermal Technologies for Fluid Foods.* Elsevier Inc., pp. 135 - 165.

[80] Rawson A. Patras B. K. Tiwari F. N., Koutchma T., Brunton N. (2011). *Effect of thermal and non-thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances*. Food Research International, (44), pp. 1875 - 1887.

[81] Rawson A., Tiwari B. K., Patras A., Brunton N., Brennan C., Cullen P. J., O'Donell C. (2011). *Effect of thermosonication on bioactive compounds in water-melon juice.* Food Research International, Volume 44, Issue 5, pp. 1168 - 1173.

[82] Edebo L., Selin I. (1968). *The effect of pressure shock-wave and some electrical quantities in the microbicidal effect of transient electric arcs in aqueous systems*. J. Gen. Microbiol., 50, pp. 253 - 259.

[83] Palaniappan S., Richter E. R. and Sastry S. K. (1990). *Effects of electricity on microorganisms: A review.* J Food Process Preserv., 14, pp. 393 - 414.

[84] Barbosa-Canovas G. V., Gongora-Nieto M. M., Pothakamury U. R. and Swanson B. G. (1999). *Preservation of foods with pulsed electric fields*. Academic Press Ltd. London.

[85] Gilliland S. E., and Speck M. L. (1967). *Inactivation of microorganisms by electrohydraulic shock*. Appl Microbiol., 15(5). pp. 1031 - 1037.

[86] WHO & FAO (2004). *Chapter 2 - Vitamin A. In Vitamin and mineral requirements in human nutrition. Second edition.* World Health Organization and Food and Agriculture Organization of the United Nations, ISBN: 9241546123.

[87] Rutkowski K., Diosady L. L. (2006). *Vitamin A stability in triple fortified salt*. Food Research International, 40, pp. 147 - 152.

[88] Masood S., Muhammad U., Muhammad S., Muhammad T. (2007). *Bioavailability and storage stability of vitamin A fortificant (retinyl acetate) in fortified cookies*. Food Research International, 40, pp. 1212 - 1219.

[89] Caceres E., Garcıa M. L., Selgas M. D. (2005). *Design of a new cooked meat sausage enriched with calcium*. Meat Science, 73, pp. 368 - 377.

[90] Barrera C., Betoret N., Corell P. F. (2009). *Effect of osmotic dehydration on the stabilization of calcium-fortified apple*

*slices (var. Granny Smith): Influence of operating variables on process kinetics and compositional changes.* Journal of Food Engineering, 92, pp. 416 - 424.

[91] Romanchik-Cerpovicz E. J. (2007). *Fortification of All-Purpose Wheat-Flour Tortillas with Calcium Lactate, Calcium Carbonate, or Calcium Citrate Is Acceptable*. Journal of The American Dietetic Association, 107, pp. 506 - 509.

[92] Singh G., Kasiviswanathan M. (2008). *Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt*. LWT, 41, pp. 1145 - 1152.

[93] Rasyid F., and Hansen P. M. T. (1991). *Stabilization of soy milk fortified with calcium gluconate*. Food Hydrocolloids, Vol-A No.5, pp. 415 - 422.

[94] Chanakan P., Benjavan R., Ismail C., Longbin H. (2010). *Zinc fortification of whole rice grain through parboiling process*. Food Chemistry, 120, pp. 858 - 863.

[95] Saeed A., Anjum F. M., Salim M. A., Kalsoom F. (2008). *Effect of fortification on physico-chemical and microbiological stability of whole wheat flour.* Food Chemistry, 110, pp. 113 - 119.

[96] Katarzyna W., Krystyna S. (2008). *The application of wheat fibre and soy isolate impregnated with iodine salts to fortify processed meats.* Meat Science, 80, pp. 1340 - 1344.

[97] Syed A., Reinhold V., Derick R. (2008). *Vitamin D3 fortification and quantification in processed dairy products.* International Dairy Journal, 17, pp. 753 - 759.

[98] Wills M. T., Mireles DeWitt A. C., Sigfusson H. (2007). *Improved antioxidant activity of Vitamin E through solubilisation in ethanol: A model study with ground beef*. Meat Science, 76, pp. 308 - 315.

[99] Hayes K. C., Pronczuk A., and Perlman D. (2001). *Vitamin E in fortified cow milk uniquely enriches human plasma lipoproteins*. The American Journal of Clinical Nutrition, (74) pp. 211 - 218.

[100] Wojciech K., Jenny W. (2007). *Sensory quality of dairy products fortified with fish oil*. International Dairy Journal, 17, pp. 1248 - 1253.

[101] Ilic D. B., and Ashoor S. H. (1987). *Stability of Vitamins A and C in Fortified Yogurt*. Journal of Dairy Science, 71. pp. 1492 - 1498.

[102] Anne M., Gabriel E. R., Muriel S. (2009). *Soy protein cold-set hydrogels as controlled delivery devices for nutraceutical compounds*. Food Hydrocolloids, (23) pp. 1647 - 1653.

[103] Jeanne I. R., Carol M. W., Gerry A. (2000). *Total folate in enriched cereal-grain products in the United States following fortification.* Food Chemistry, 70. pp. 275 - 289.

[104] Ashok K. S., Jayashree A., Janet L. (2003). *Edible coating materials-their properties and use in the fortification of rice with folic acid.* Food Research International, 36. pp. 921 - 928.

[105] Singh G., Arora S., Sharma G. S., Sindhu J. S., Kansal V. K., Sangwan R. B. (2007). *Heat stability and calcium bioavailability of calcium-fortified milk.* LWT, (40). pp.625 - 631.

[106] Diosadya L. L., Albertib J. O., Venkatesh M. M. G. (2002). *Microencapsulation for iodine stability in salt fortified with ferrous fumarate and potassium iodide.* Food Research International, 35, pp.635 - 642.

[107] Martınez N., Camachoa J., Martınez L., Martınez M,. Fitoa P. (2002). *Iron deficiency and iron fortified foods-A review*; Food Research International, 35, pp. 225 - 231.

[108] Somchue W., Sermsri W., Shiowatana J., Siripinyanond A. (2009). *Encapsulation of a-tocopherol in protein-based delivery particles*. Food Research International, 42, 909 - 914.

[109] Loveday M. S. and Singh H. (2008). *Recent advances in technologies for vitamin A protection in foods*. Trends in Food Science & Technology, 19, pp. 657 - 668.

[110] Cheung F. H. R., Hughes G. J., Marriott J. P., Small M. D. (2009). *Investigation of folic acid stability in fortified ins tant Asian noodles by use of capillary electrophoresis.* Food Chemistry, 112, pp. 507 - 514.

[111] Chuan-Chuan L., Hung-Yin L., Hsu-Chih C., Ming-Wen Y., Mei-Hwa L. (2009). *Stability and characterisation of phos pholipid-based curcumin-encapsulated micro-emulsions*. Food Chemistry, 116, pp. 923 - 928.

[112] Livney D. Y. (2010). *Milk proteins as vehicles for bioactive*. Current Opinion in Colloid & Interface Science, (15), pp. 73 - 83.

[113] Johns T., Eyzaguirre B. P. (2006) *Bioforti Wcation, biodi versity and diet: A search for complementary applications against poverty and malnutrition*. Food Policy, (32). pp. 1 - 24.