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Contemporary food processing techniques as emerging alternatives to enhance bioactive profile of food: A review

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Abstract
The efficacy of non-thermal processing techniques such as on microbial safety of food is already known. From last few years, these technologies are being explored for their extended applications. Present day, popularity of functional foods is on peak due to potential health benefits. Phenolic compounds are the major constituents of these functional foods credited for imparting positive impacts against health related concerns. This effect is generally attributed to the presence of antioxidant properties. The knowledge on the impact of these innovative modifications on bioactive profile of food is still in its infancy. The objective of this review paper is to integrate the recent findings presenting the enhancement in the biofunctional components of food using novel food processing approaches and draw generalized conclusions. The processes discussed here are cold plasma, high pressure processing, and pulsed electric field. Overall, these techniques have proven to be far better than conventional processes like pasteurization in retention of biologically active compounds. Almost all the food groups like fruits and vegetables, dairy, cereals, and meat are taken into consideration.

Keywords: Novel processing, bioactive compounds, antioxidants, vitamins

Introduction
Bioactive compounds are naturally occurring components in food which have been found to have a potential positive impact on human health. They are also referred to as nutraceuticals because of their biological activity and existence in human diet [1]. One of the predominant causes of modern lifestyle diseases such as cancers, Cardiovascular Diseases (CVDs), oxidative stress etc. is the biological toxicity of Reactive Oxygen Species (ROS) [2, 3, 4]. The major health promoting effect of bioactive compounds is attributed to the anti-oxidants present in them. These antioxidants play two significant roles i.e. preserving the food and imparting health benefits to humans. Firstly, antioxidants have the ability to quench singlet oxygen molecules thereby inhibiting the formation of ROS. Secondly, they are potential free radical scavengers and are very effective in inhibiting the chain initiation reaction responsible for oxidation [5]. Thus, beyond their basic nutrition, bioactive compounds imparts promising benefits to human health. They occur naturally in abundance in several foods, especially in fruits and vegetables.

One of the recent research trends is the investigation on retention of bioactive compounds during processing and post-harvest operations. Several researchers have reported that these compounds are heat sensitive and are lost during thermal processing of food. For instance, components like Vit A, Vit B_{12}, carotenoids and polyphenols are sensitive to heat and light. They are generally lost at high temperature processing of food. Novel non-thermal food processing techniques are now-a-days being explored for their impact on nutritional, functional and bioactive profile of food. Techniques such as Cold Plasma, High Pressure Processing (HPP), and Pulsed Electric Field (PEF) have been successfully applied on inactivation of microorganisms at ambient temperatures without thermal degradation of food and thereby maintaining the nutritional value and sensory attributes of the food. In past few years, several scientists have not only reported the retention of bioactive compound using non-thermal techniques but also the increase in the yield of these compounds and enhancement in the bioavailability. The concept of interaction between non-thermal processing variables and phytochemical compounds in food is rather in its infancy and only a scarce information is available [6].

Thus, the objective of this article is to summarize the recent researches and draw some useful conclusions regarding the impact of novel non-thermal food processing techniques (Cold Plasma, High Pressure Processing, and Pulsed Electric Field) on yield and bioavailability of bioactive compounds.

1. Cold Plasma

Plasma, forth state of matter after the three distinct phases of gas, liquid, and solid was first defined by Irving Langmuir in 1920 as whole or partial ionized state of gas [7]. The plasma state is achieved by increasing the energy beyond a certain limit in gas to cause ionization of molecules [8]. It constitutes about 99% matter in the universe. Plasma is merely composed of different species like photons, free electron, reactive items and free radicals in their fundamental state or excited state possessing net neutral charge. This state of matter exists in huge range of temperature and densities. On the basis of energy and temperature, it can be categorized as thermal and non-thermal plasma. The characteristic feature of the thermal plasma is the thermodynamic equilibrium between electrons and heavy species possessing very high temperature profile 5-20 $x10^3$ K. they require high pressure and substantial higher energy. On the other note, non-thermal plasma require less power and low pressure. They do not show local equilibrium and are characterized by electron temperature much above the macroscopic temperature of the air.

Cold plasma is a recent novel non-thermal approach with wide applications at the interface of life sciences. Being capable of working at the moderate temperatures, cold plasma has opened up many opportunities for application in food industry particularly in the field of disinfection and sterilization of heat sensitive materials [9]. The effect of cold plasma on microbial decontamination in food is more in comparison to the conventional sterilization methods like UV treatments, chemical solutions and heat [10]. More so, conventional food preservation methods are time-consuming and causes degradation of sensitive nutrients or adds to the toxicity. Use of cold plasma alters the food properties to desirable characteristics, enhances the nutritional composition with a significant effect on the microbial population. Successful microbial decontamination is dependent on moisture content of food. The effect of cold plasma is higher in moist foods as compared to the dry organisms [11]. The microbial decontamination mechanism of cold plasma is based on the interaction of reactive species and deoxyribonucleic acid (DNA) causing severe damage in the chromosomes. Cold plasma has been successfully used in the microbial destruction of food extending the shelf life in past few years. More recently, the impact of cold plasma on bioactive components beyond their ability to preserve the food has been evaluated.

Impact of cold plasma on bioactive properties of food

Bioactive components present in the food like carotenoids, phenolics, flavonoids, vitamin C, vitamin E etc. are either direct free radical scavengers or act as cofactors of antioxidant enzymes. Apart from microbial safety of food, cold plasma processing of food causes an increment in the bioactive compounds and antioxidant activity. The positive effect of plasma is might be due to the fact that UV radiations formed during plasma treatment are responsible for generation of phenolic compounds extracted from upper epidermis cells of leaves [12]. Major research on the use of cold plasma for enhancement of phenolics and other functional compounds has been done on fruits. Rodriguez *et al*. [13] studied the effect of indirect cold plasma treatment on cashew apple juice and noted an increment in vitamin C, flavonoids and polyphenols content. Operational and configuration conditions illustrated significant effect on these compounds as enhancement of bioactive compounds was observed upto a certain limit beyond which overexposure to plasma degraded the bioactive components. This upsurge in the vitamin C content is attributed to the activation of dehydroascorbate reductase enzyme which facilitates the reduction of dehydroascorbic acid (oxidized state of ascorbic acid) through ascorbate glutathione cycle to bring back into vitamin C. [14] When treating the food with nitrogen plasma, there is generation of several reactive nitrogen species and nitric oxide being one of them is responsible for the activation of dehydroascorbate reeducates [15, 16].

In some fruits, a part of bioactive compounds like flavonoids and phenolics is found in bound form linked in the cell membranes. The exposure to cold plasma gives sufficient energy to release compounds in free state and thus raising the overall concentration of phenolic compounds [13]. However, energy requirement for release of different compounds vary. The flavonoids require lesser energy in comparison to polyphenols for conversion into free state from bound form [17]. In some study, 62% of the phenolics were found in the bound form in rise [18] particularly binding to the cell wall material. The treatment of low temperature of plasma disrupted the cells and in bound polyphenols were released causing and elevation in the total phenolic content of rise [19]. Cold plasma was also found to be a potential processing technique in modifying the wheat flour functional properties to desirable characteristics. The exposure to non-thermal plasma caused a significant effect on the free fatty acids and phospholipids in wheat flour [20]. Both of these compound are highly prone to oxidative damage. Reduction in the whole fatty acid profile was observed on treatment of cold plasma at different voltage and time. However, significant progression in oxidation was evidenced by increments in PV and n-hexanol concentrations at all the levels of cold plasma treatment. At higher levels of treatment, the lipids and the proteins of wheat flour g*et al*tered to the extent that they cause a significant impact on the functional properties of flour affecting the starch and pasting properties [20].

To elucidate the effect of cold plasma treatment, the seeds of sweet basil (after sprouting has occurred and seedlings were clearly visible) were treated with cold plasma. The concentration of eugenol was found higher in treated samples in comparison to the untreated commercial sample analyzed through mass-spectrometry [21]. This increase in the bioactive compound was also evident from the upsurged antioxidant activity of treated sweet basil extracts.

Corona discharge system cold plasma treatment to milk caused significant biochemical changes to the proteins, volatile compounds and free fatty acids profile. It was found that total aldehyde content appraisingly increased along with volatile compounds such as octanol, 2-hexanol, 2-octenal, and nonanal while lipid oxidation remained unaffected [22]. However, cold plasma treated dairy products (milk, cheese and cheddar) revealed significant reductions in sensory quality including odor, flavor and acceptability [23, 24].

The jet plasma application on lamb's lettuce using argon caused reduction in flavonoid compounds (diosmetin and leteolin) while chlorogenic acids and caffeic acids were comparatively stable [6]. It implies that phenolic acids showed slow decrease in contrast to flavonoids. On contrary, phenolic

acids decreased but strong increment was observed in diosmetin for *Valerianella locusta*.

Antioxidants are the most potential defense material against free radicals. The ability of the antioxidants is attributed to scavenging properties of singlet molecular oxygen and peroxyl radicals. Cold plasma treatment to fruits and other commodities lead to increase in radical scavenging activity. Several plant species are tolerant to UV radiations and when these radiations formed during the generation of cold plasma comes in contact with food, synthesis of phenolic compounds and flavonoids occurs [25]. Since cold plasma works with fractions of air, the electrons/protons transfer generates a phenoxyl radical which upon tautomerization cause damage to the aromatic ring resulting in the formation of small phenolic acids. Chemical changes in food with treatment of cold plasma are shown in table 1.

The application of cold plasma in microbial decontamination and surface modification has already been accepted. It has also proven to be a potential candidate in preserving food at moderate temperature, causing lower impact on food-matrix. The unique property of cold plasma to enhance the bio functional profile of food has opened many doors in the field of functional foods and nutraceuticals. However, no significant effect have been studied on the toxicity of treating cold plasma in food matrices.

Table 1: Chemical changes in food with treatment of cold plasma

Food product	Cold plasma treatment	Chemical changes	References
Kiwi fruit	Atmospheric double barrier discharge plasma treatment for 10-20 minutes	Better color retention Comparatively lower pigment loss Increased dry mass	[26]
Cashew apple juice	Nitrogen plasma treatment @ 10-50 ml/min for 5-15 minutes under vacuum	Increase in vitamin C, flavonoids and total phenols. Increase in antioxidant activity	[13]
Sweet basil	Plasma jet: 30 seconds treatment once a week for 1 month	Increase in eugenol content Increment in antioxidant activity	[21]
Basmati rice	Air plasma treatment at 30-40 W for 5-10 minutes	Increase in total phenol content Increase in antioxidant activity Reduction in characterized flavor	[19]
Choke berry juice	Cold atmospheric plasma jet at 0.75 dm^3/min for 3-5 minutes	Improved stability of hydroxycinnamic acids Lower stability of flavonols and anthocyanins	[27]

2. High-pressure processing

High-pressure processing (HPP) is a minimal processing technique which exhibits extensive industrial applications in retaining food quality attributes such as color, flavor, and nutritional characteristics. This technique is unique due to its minimal thermal effects on non-covalent bonds which lead to less nutritional losses. In HPP food materials are subjected to isostatic pressures ranging from 40MPa (5kpsi) to 1000MPa (145kpsi) for a period of 1–20min [28]. As compared to thermal processing at atmospheric pressure, HPP has a great advantage of instantaneously transmitting uniform pressure throughout the food surface, irrespective of the size and shape of the product [29]. When pressure is applied to foods, many food-borne microorganisms are inhibited/ destroyed. On the other hand, bioactive compounds are less affected as a result of which foods can hence be pasteurized to preserve/ retain the freshness and healthier characteristics [28]. Bioactive compounds remain stable under high pressure compared to heat treatment as because high pressure affects only non-covalent bonds (hydrogen, ionic, and hydrophobic bonds). This causes macro-molecules such as protein chains to unfold under high pressure but has little effect on chemical constituents associated with essential food components [29]. HPP is accompanied by increase in temperature of food materials which is due to compression heating (a thermodynamic outcome of high pressure processing). Many food materials are considered as incompressible (at atmospheric and low pressures), but they do get compressed significantly under very high pressures [30].

HPP is commonly used in combination with moderate to high temperatures whose effects on food components are governed by activation volume (difference in sensitivity of substances to pressure) and activation energy (difference in sensitivity of substances to temperature). These two properties define the possibility of retention or risk of destruction of food quality attributes. As a direct consequence of the stability/ instability of the bioactive compounds during HPP antioxidant activity and bioavailability are affected.

Effect of HPP on Stability of Bioactive Compounds

Vitamin C or L-Ascorbic Acid

A number of researches have been published on vitamin C stability under pressure in controlled environment and during subsequent storage. Most studies have shown that L-ascorbic acid is resistant to pressure and survives pressure treatments below 50°C. However, there is significant loss during high-pressure and temperature combination treatments in presence of oxygen, since this degradation is a rapid oxygen-dependent reaction that continues until oxygen is fully used up, followed by anaerobic degradation [31]. Pressure stability is dependent on concentrations of both ascorbic acid and oxygen. Among food products, ascorbic acid stability has been extensively studied in fruit and vegetable products. In strawberry puree and strawberry coulis, pressure treatment (400–600MPa/ 20°C /15–30min) resulted in ~10–12% decrease in vitamin C levels [32, 33]. Fruits such as oranges, apples, apple purees, mixed citrus juices, carrots, tomatoes, and frozen raspberries showed no significant difference after HPP treatment (400–800MPa/ 25–44°C/ 6min) compared to unprocessed samples [34, 35]. Higher degradation of ascorbic acid was observed in case of vegetable-based products compared to fruit-based products. In green peas, pressure treatment between 400 and 700MPa/ 33–40°C/ 5–10min showed only 50–80% degradation of L-ascorbic acid [36]. Alfalfa sprouts pickled in citric acid showed 77% loss of L-ascorbic acid after pressure treatment at 500MPa/ room temperature/ 10min [37]. Cowpeas germinated for 4–6 days showed 10–69% decrease after HPP treatment (300–500MPa/ room temperature/ 15min) [38]. Higher retention of ascorbic acid with increase in pressure was correlated to reduction in peroxidase activity [36]. Stability of vitamin C during storage in HPP food products is related to enzyme activity and availability of oxidants that catalyze oxidation of ascorbic acid. To summarize, L-ascorbic acid or vitamin C is stable under high-pressure treatment carried out at mild temperatures (<60°C). Vitamin C is found to be unstable at high-pressure, high-temperature combination treatments.

Vitamins A, E, and K

The effect of high-pressure treatment on fat-soluble vitamins is less identified compared to water-soluble vitamins. Pressure stability of retinol and vitamin A has been researched in model systems and some food products. Vitamin A (retinol) stability has been more commonly studied in its provitamin form (β-carotene). In model systems, high-pressure treatment has been shown to degrade vitamin A. High pressure treatment at higher temperatures (600MPa/ 40, 60, and 75°C/ 5min) resulted in a decrease in retinol levels by 45% [39]. When the treatment time was increased to 40min, the retinol level was found to decrease by 70% [40]. In food products, vitamin A has been considerably more stable under pressure compared to in model systems. Longer time and higher temperatures combined with pressure treatment increases vitamin A content in food products. This increase in vitamin A levels could be explained by an increase in extractability after HPP. Sanchez-Moreno *et al*. [41] studied that vitamin A level was increased by 39% for treatment of 400MPa/40°C/1min. This increase in vitamin A levels could be explained by an increase in extractability after HPP. Other fat-soluble vitamins (E and K) are also found to be less stable in model systems compared to food products. Human milk that was high-pressure treated (400–600MPa/ 22–27°C/ 5min) also retained delta-, gamma-, and alpha-tocopherols post-processing [42]. Vitamins A and E in food products were relatively stable to high-pressure processing at room temperature.

Carotenoids

Carotenoids, pigments commonly found in fruits and vegetables, are found to be somewhat pressure stable in food matrices. Various studies have also tested the stability of carotenoids in model systems. During HPP treatment, isomerization of all- *trans* -lycopene to 13-cis- isomers has been observed in hexane and tributyrin solutions after HPP [43, 44]. HPP treatment has also been shown to increase extraction of carotenoids from their plant matrix [45, 34]. In 2008, Hsu, Tan, and Chi studied the effects of HPP on tomato juice's lycopene and carotenoid content [46]. It was observed that there was a 56–62% increase in lycopene and total carotenoid levels of tomato juice processed at 300MPa at 4 and 25°C for 10min. This increase in lycopene concentration could explain the increased redness in HPP-processed tomato juice compared to untreated juice. In watermelon juice, lycopene level was unchanged after moderate-pressure treatment (300–600MPa/ room temperature/ 5min) and increased only after higher-pressure treatment between 600 and 900MPaat room temperature for 20–50 min [47]. Applied pressure is another factor influencing extraction yield of carotenoids after HPP. Pressure holding time and temperature have a much smaller influence on carotenoid yield. Orange juice carotenoids have been found to be relatively stable to combination treatments of pressure/ temperature/ time. Carotenoids are stable at low temperatures as well mostly below 10°C.

Flavonoids

Isoflavones, flavonols, and flavonones exhibit diverse effects upon processing under high pressure. This conversion is attributed to adiabatic heating that promotes conversion of malonyl- to β-glucoside isoflavones [8]. Use of higher temperatures (>30 °C) in combination with pressure can cause detrimental effects on the flavonols [49]. The isoflavone profile changes can be attributed to pressure-induced changes in enzyme activity, disruption of bean cell walls, and modification in isoflavone-protein interactions. Anthocyanins in pressure-treated fruits and vegetables are unstable during successive storage conditions. Various mechanisms have been implicated in anthocyanin instability during storage of pressurized food products. The probable reasons are the incomplete inactivation of enzymes in fruits that catalyze anthocyanin degradation reactions; specificity of β-glucosidase responsible for the selective degradation of anthocyanins; influence of ascorbic acid on the stability of anthocyanins during storage; and condensation reactions between anthocyanins and flavanols or organic acids through covalent association resulting in the formation of pyran ring compounds [50]. Most common enzymes found to be involved are polyphenol oxidase (PPO), peroxidase (POD), and β-glucosidase. Pressure sterilization at 600MPa/ 110°C/ 3min led to 80–90% retention of total anthocyanins in strawberry paste [51]. Ascorbic acid, apart from being an antioxidant, accelerates the degradation of anthocyanins. Table 2 represents the effect of HPP on selected bioactive compounds.

Table 2: The effect of HPP on selected bioactive compounds

Bioactive compounds	Raw material	HPP Conditions	Findings	Reference
Tocopherol	Beef	0.1, 200 & 800 MPa/ 60ºC/ 20min	No change was observed	[52]
Ascorbic acid	Tomato puree Carrot puree	400-600 MPa/ 20°C/ 15min	90 % retained	[53]
α carotene and β carotene	Carrots (Whole)	400-600 MPa/ 25°C/ 2min	100% retention	[54]
α carotene and β carotene	Broccoli (Whole)	400-600 MPa/ 25°C/ 2min	95- 83% retention	[54[
Lycopene	Tomato puree	400 MPa/ 25°C/ 15min	49% increased content	[55]
Total phenol content Flavonol content	Onion Onion	100 MPa/ 50°C/ 5min 400 MPa/ 5°C/ 5min	12% increased content	[49]
Total phenols	Strawberry and blackberry	400-600 MPa/ 20°C/ 15min	9.8% increased content in strawberry and 5% increased content in blackberry at 600 MPa	[53]
Total Phenolics	Litchi fruit	200-500 MPa/ 30°C/ 2.5- 30min	No significant difference was observed	[56]
Ascorbic acid	Orange juice	500MPa/ 35°C/ 5min	Discoloration of juice was observed during storage at 0, 5, 10, and 15 °C	[57]
Ascorbic acid	Orange juice	600MPa/ 40°C/ 4min	Positive correlation between color changes and loss in ascorbic acid	[58]
Carotenoid	Mediterranean vegetable soup (gazpacho)	150–350MPa/ 60°C/ 15min	Total carotenoid loss of up to 46% observed over a storage period of 40days at 4°C	[59]

Anthocyanin	Raspberry	200 & 800 MPa/ 22°C/ 15 min	Highest retention of the pigment when processed under mentioned condition and in refrigerated storage conditions	[60]

3. Pulsed Electric Field

Pulsed Electric Field (PEF) is one of the promising non-thermal technology used in the food industry for the preservation of foods and enhancing their quality [61]. PEF is a novel emerging processing technology or an alternative to conventional heat treatments which preserve the sensory and nutritional characteristics of foods [62]. PEF is basically used for increasing permeability of cellular membrane with low energy cost [63].

PEF involves the application of short/high voltage electric pulses for short duration (nanoseconds to milliseconds) to a food product which is placed in treatment chamber/ liquid material passed through the chamber and the chamber is confined in between electrodes [64].

Principle

The basic principle of PEF-assisted extraction is electroporation which disrupts the cell membrane of the food substance. Cell membranes act like a capacitor with low dielectric constant, having natural trans-membrane potential due to the presence of free charges of opposite polarities across the membrane. When the external electric field is applied, the transmembrane potential is increased because of the accumulation of charges across the membrane.

Three major factors are being considered for effective PEF processing- 1) Electric field strength 2) Treatment temperature and 3) Energy delivery. The electric field, pulse shape, width and frequency, total treatment time, electrode configuration and temperature are also considered for PEF treatment. PEF treatment is restricted to only those food products that can tolerate the high electric field and do not undergo any change in their size and shape and liquid material do not form any bubble. Along with these factors, food product also should have low electrical conductivity [61].

PEF treatments

Pulsed Electric Field (PEF) treatment can be categorized into 2 categories depending on the pulse treatment.

a. Mild Treatment

In this treatment low electric field in the range of 100-300V/cm is applied to the food product. Under the effect of this mild treatment, the cell membrane of the food product is punctured and losses its semi-permeability temporarily/ permanently which is used for recovery of high-value components from different matrices. Thus this treatment is a promising technique for drying, freezing and extraction processes. In this method potential permeability of tissue structure increases [65] due to the formation of hydrophilic pores in the membrane and forced opening of protein channels [64].

b. High- Intensity treatment

This treatment constitutes an alternative to traditional thermal technologies. In this treatment, electric impulses in the range of 30-50 KV/cm are applied to food products to inactivate pathogenic micro-organisms and related enzymes [65]. This treatment retains the sensory, nutritional and other health-promoting attributes of different processed foods. It is applied to ensure irreversible effects leading eventually to the death of microbes. Several thousand volts per 20-1000µs is required for effective inactivation of microorganisms [62]. Thus this treatment is used for the preservation of food products and inactivation of microbes and enzymes [66].

Advantages and Applications of PEF in Food industry

PEF treatment exhibits a lower treatment temperature, shorter processing time and potential continuous flow in comparison to traditional thermal processing technologies. PEF exhibits high-intensity electric pulses for short duration which induce irreversible changes in cell membrane due to rupture or breakdown of tissues of the cell membrane and enhance membrane permeability [61].

PEF treatment causes less degradation of nutritional and sensory attributes of foods as compared to the conventional processing methods [67]. PEF is effective in the destruction of microorganisms such as vegetative bacterial cells, yeasts, and molds [62]. Beside these PEF have a lethal effect on microbial spores. Thus PEF is considered as pasteurization method for partial destruction of microorganisms [68].

PEF-assisted processing is used to increase mass transfer, improve extraction yield, decrease energy cost and provide purified compounds [65]. PEF treatment is used to increase the rate of extraction of bioactive components as well as other components like colorants (anthocyanins, carotenoids, etc.), sugar, etc. and reduce the loss of thermosensitive bioactive components because electroporation of cell membrane occurs in an electric field treated substances which enhances the permeability to the transfer of ions and macromolecules [69].

PEF treatment is done to accelerate some mechanical processes like freezing or drying [70]. This treatment can also be useful for a synergistic effect on bacterial inactivation and this effect can be controlled by rapid cooling after treatment is given to food product [71].

PEF-assisted processing is widely used in food processing industries for enhancement of juice extraction, pasteurization, and sterilization of liquid milk, dairy products, liquid eggs, fruit juices, wine, beer and also used for osmotic dehydration [62].

Impact of PEF treatment on bioactive components of food

Phenolic compounds

Phenolic compounds are ubiquitous components of higher plants and are secondary metabolites of plants generally involved in defense against ultraviolet radiation or aggression by pathogens. Balasa *et al*. investigated the effect of low-intensity PEF (0.5 KV/cm and 50 pulses) on wine grapes which increased the total phenol content up to 13-28%. Total phenols were increased because low electric field induced stress in grape cells which increased the production of phenols as a secondary metabolite [72]. Anthocyanins are plant phenolic compounds that are responsible for the natural coloring of plant foods. Tedjo *et al*., studied that PEF induced cell permeability when wine grapes were treated with 3 kV/cm and 50 pulses which increased the total anthocyanin content in wine grapes due to more cell permeabilization [73]. Odriozola *et al*., studied the effect of PEF treatment on strawberry juice and concluded that high electric field strength treatment increased the retention of anthocyanin in strawberry juice and increased the concentration of ellagic and coumaric acid [74]. It has also been reported that high PEF treatment increased the concentration of phenolic acid and flavonol (quercitin) in tomato juice [75].

Carotenoids

Carotenoids are widespread pigments present in plant foods which act as an antioxidant, anti-inflammatory, have anti-tumor promoting properties and have a good effect on the immune system. Recent research suggested that PEF treatment significantly increased the carotenoid content in PEF treated foods. PEF treatment in the range of 0.6-2.6KV/cm with 5-100 pulse of 1Hz frequency enhanced the pigment extraction rate. Carrot juice was treated with 2.6KV/cm at 50 pulses produced a higher amount of β-carotene[76]. High-intensity electric field treatment of tomato juice in the range of 35KV/cm for 1000µS increased the lycopene content by 46.2% than untreated sample [77].

Chlorophyll

PEF treatment increased the green color pigment of the treated sample. Spinach was treated with 60KV/cm electric field which increased the green color of spinach due to the destruction of microorganisms and enzymes that helps in chlorophyll degradation reaction [78].

Vitamins

PEF treatment is effective in achieving higher vitamin content as compared to the treatment with high-temperature processing methods. PEF processing (35KV/cm for 1500µS at 100 Hz) of tomato and strawberry juices induced greater retention of vitamin C than heat processed juices (90°C for 60sec) stored at 4°C [79]. The greater retention of vitamin C in PEF treated products can be explained by the fact that heat processing affects the ascorbic acid in the presence of oxygen while with PEF treatment it is not degraded through the aerobic pathway [80].

While in the case of vitamin A, when orange juice was treated with 30KV/cm for 100µS 7.52% vitamin loss occurred while heat pasteurization induced a loss of 15.62% [81]. The PEF treatment effect both water soluble and fat soluble vitamins in milk. A treatment at 22.6 kV/cm for 400 µs was effective in pasteurization of milk and preserving the overall vitamin content in milk. Thus PEF treatment of milk preserves only vitamin C [82].

Flavor compounds

PEF treatment of fruit juices and other food products preserves the overall flavor components such as ethyl butyrate in orange juice treated with 30KV/cm for 480µs [83]. When citrus juices were treated with PEF there was not any loss of volatile aroma components occurred. Tomato juice treated at 40 kV/cm for 57µs retained more flavor compounds such as trans-2-hexenal, 2-isobutylthiazole, cis-3-hexanol) than thermally processed or unprocessed tomato juice [84].

Table 3: Effect of PEF on bioactive compounds in food.

Product	Component	PEF treatment	Chemical changes	References
Spearmint	Phenolics	99 pulses of 3 kV/cm	PEF pre-treatment produced higher TPC, AC, and AA than heat and microwave pre-treatments.	[63]
Peach by-products	polyphenols	0 to 5 kV/cm	Maximum amount of bioactive compounds and individual phenols (chlorogenic acid, coumaric acid, and neochlorogenic acid) were achieved.	[69]
Tomato	Carotenoid content	30 pulses at 200KV/m	1) Total carotenoids and lycopene concentrations were enhanced by 50% and 53%, respectively. 2) Significant improvement in lipophilic antioxidant capacity was observed. 3) Total soluble solids were increased.	[88]
Orange peels	Polyphenols	3 kV/cm and 10 kV/cm,	1) Polyphenols concentrations in the juice significantly enhanced by the treatment of citrus peels with PEF at high electric field strength.	[89]
Red cherries	Anthocyanin and polyphenols	0.3 and 2.5 kV/cm at 100 Hz frequency for 20 µs.	1) Maximum production of Cyanidin-3-rutinoside, an anthocyanin was achieved. 2) Myricetin polyphenol content was also increased.	[90]

Essential oils

PEF treatment in the range of (0.6-1.3 KV/cm) on plants (soybean, maize, and olive) which accumulated high recovery of plant oils with the release of intracellular pigments such as phytosterols in maize germ and isoflavonoids in soybean [85]. When rapeseed was treated with 5 kV/cm at 120 pulses gave higher oil yield as compared to the control sample and the higher amount of tocopherol, polyphenolics and phytosterols were also measured [86]. Schilling *et al*., (2007) investigated the antioxidant activity of juice obtained from PEF treated apple mash with 3KV/cm at 30 pulses which enhanced the polyphenols and antioxidant activity [87]. Table 3 represents effect of PEF on bioactive composition of food.

Apart from the above applications, PEF treatment before the mechanical operation has its impact on functional food ingredients such as antioxidants, tocopherols, polyphenolics, and phytosterols [74].

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