Chapter 10
Effect of Nonthermal Treatments on the Properties of Natural Food Additives

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10.1 Introduction

Consumers demand fresh and minimally processed foods with natural ingredients that enhance health or prevent disease. This trend raises industries' and researchers' interest in developing processing techniques that result in higher quality foods free of chemical additives. Thermal treatment, commonly used to increase the shelf life of foods through the inactivation of microorganisms and enzymes, has detrimental effects on processed foods'nutritional and sensory attributes, including the loss of antioxidant activity, phenolics, and discoloration. Nonthermal technologies have been highly recommended in the food industry as an alternative to conventional processes to prevent quality losses in food products. High-pressure processing (HHP), ultrasound (Us), pulsed light (PL), UV-light, cold plasma (CP), pulsed electric field (PEF), and radio frequency (RF) are some nonthermal techniques of the emerging research that can improve, maintain or change properties of compounds related to natural additives in food manufacturing.

A suitable nonthermal technology may promote several modifications in natural food additives (NFAs), improving sensory and texture properties, digestibility, and antimicrobial and antioxidant activities. An increase efficiency when extracting intracellular compounds such as phenolics, pigments, starches, and proteins has been the main effect reported in using nonthermal technologies in foods [1–6]. In contrast, structural changes such as depolarization and crosslink are reported mainly in macromolecules (e.g., starch and protein) [7–9]. Furthermore, technologies such

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as CP and PEF can act as abiotic stressors, inducing reactions and the formation of bioactive compounds [4, 5, 10, 11].

In food processing, aiming at improving natural compounds, nonthermal technologies have advantages over traditional processes due to the possibility of reducing the use of solvents, energy efficiency, and shorter processing time, in addition to the quality of the final product [1]. Knowing the impact of these emerging technologies on changing the food compounds allows the development of strategies to improve food properties, reduce the number and amount of additives in a given product, or improve the quality of natural compounds that can be used as food additives. The following sections will present the impact of nonthermal techniques benefiting NFAs and discuss the mechanisms associated with them.

10.2 High-Pressure Processing

High-pressure processing (HPP) – also known as high isostatic pressure (HIP) or high hydrostatic pressure (HHP) – is a nonthermal treatment using pressures up to 1000 MPa into a product in controlled time and temperature conditions [12]. The observed HPP effects converges to increase surface hydrophobicity, change the structure of the non-covalent bonds, and cause molecules denaturation and aggregation (e.g., proteins) [13]. HPP can also improve protein functionality and digestibility of cereals and legumes [14] while reducing microorganisms for juice preservation [15].

High-pressure homogenization (HPH) – also called dynamic high-pressure (DHP) – imposes high-pressure conditions by pumping liquid food through a tiny gap in a valve, which results in a high velocity that causes high shear stresses. Consequently, it causes changes in food rheological properties [8]. HPH combines the effectiveness of high-frequency vibration, high-velocity impact, quick pressure drop, cavitation, and intense shear stress in a short time [16]. Typical HPH pressures are moderate and usually up to 100 MPa [17], while HPP can reach ten times more. HPH was also recently applied to food products aiming at microbial inactivation and changes in the protein's techno-functional properties [12].

High pressures favor extracting bioactive compounds from plants (e.g., carotenoids, chlorophylls, sterols, fibers, and phenolics) [8] by decreasing solvent consumption, increasing extraction yields, and shortening the extraction time. Studies demonstrated that HPP could be applied to foods and increase their antioxidant capacity by maintaining/improving the concentration of anthocyanins [18, 19], ascorbic acid/vitamin C [20], tocopherols/vitamin E [21], which are natural antioxidants in foods. Likewise, the carotenoids (lutein, α-carotene, and β-carotene), which can be used as colorings and antioxidants, were evaluated after HPP treatment (600 MPa) and no differences between the untreated and treated pumpkin purées was observed [22]. Table 10.1 shows more examples of high-pressure technology applied to foods aiming their role as natural additives.

Table 10.1 Examples of high-pressure processing (HPP) applied to foods aiming their role as natural additives

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Apple juice	Ascorbic acid, quercetin, gallic acid, procyanidin B2, and catechin	Antioxidant	300–600 MPa 5–15 min	HPP treatment caused the degradation of ascorbic acid, quercetin, gallic acid, procyanidin B2, and catechin after storage	[15]
Persimmon fruit	Gallic acid and catechin	Antioxidant	100 MPa 10 min	Low intensity of HPP significantly increased the extractability of phenolics	[23]
Nectarine purée	Criptoxantin, β-carotene, zeaxanthin and lutein, and gallic acid	Coloring and antioxidant	450–600 MPa 5–10 min	HPP at 600 MPa/10 min showed the highest phenolics content. Zeaxanthin + lutein and criptoxantin was significantly highest in purées treated at the lowest pressure intensity and shortest holding time	[19]
Açaí juice	Anthocyanins, tocopherols, and gallic acid	Antioxidant	400–600 MPa 5 min 20 °C	HPP was effective for the preservation of anthocyanins and phenolics. Tocopherols activity were not affected	[21]
Turmeric	Gallic acid, curcumin, ferulic acid, vanillin, and vanillic acid	Antioxidant	100–550 MPa 15 min	HHP at 400 MPa for 20 min was the optimal extraction condition for the highest antioxidant activity	[24]
White tea	Catechin and caffeine	Antioxidant	300–500 MPa 120–600 s	The maximum total phenolic content (1949.2 mg/L) and total antioxidant activity (91.9%) were achieved at 300 MPa for 600 s	[25]
Coconut water	Ascorbic acid and gallic acid	Antioxidant	500 MPa 5 min	HPP treatment substantially delayed losses of ascorbic acid, phenols, and antioxidant capacity	[20]

(continued)

Table 10.1 (continued)

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Pomegranate juice	Anthocyanin and rutin	Antioxidant	400–600 MPa 3–10 min 20 °C	For anthocyanins and antioxidant activity, the maximal retention of 95.69% and 95.89% was achieved at 600 MPa/3 min	[18]
Jussara juice	Anthocyanin	Antioxidant	200–500 MPa 5–10 min	While 200 MPa/5 min retained anthocyanins, 82% were lost at the 500 MPa/10 min	[26]
Carambola purée	β-carotene, gallic acid, and rutin	Coloring and antioxidant	200–800 MPa 5–15 min 25 °C	The color change was caused by the β-carotene release. The phenolics and the antioxidant activity increased with the increase of pressure	[27]
Fruit juice mixture sweetened with *Stevia rebaudiana*	Ascorbic acid, anthocyanin, and gallic acid	Antioxidant	300–500 MPa 5–15 min	HPP conducted at 300 MPa/14 min led to a beverage with the greatest presence of antioxidant compounds	[28]
Citrus beverages	Hesperidin, anthocyanins (cyanidin 3-O- glucoside), and ascorbic acid	Antioxidant	450–600 MPa 180 s	Phenolic compounds were little affected by the HPP. Ascorbic acid showed significant degradation after processing under any condition	[29]
Soybean protein isolate	Protein	Foaming properties	100–300 MPa	Foaming increased after HPP treatment	[30]
Peanut protein isolate	Protein	Water- and oil-holding capacities	50–200 MPa 5 min	HPP can be used to modify the properties of peanut protein isolate at the appropriate pressure within a short time	[31]

(continued)

Table 10.1 (continued)

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Sweet potato protein	Protein	Water-holding and gelation properties	250–550 MPa pH 3–9	The hardness, springiness, chewiness, and water-holding capacity of gels treated at moderate pressure (250 and 400 MPa) were improved, leading to a compact and uniform three-dimensional gel network	[32]
Sweet potato protein	Protein	Gelation properties	400 MPa 25 °C 30 min	Textural properties of gels were improved by sulfur-containing amino acids and HHP	[13]
Fababean protein	Protein	Emulsifying and foaming properties	103–207 MPa 32–45 °C 6 cycles	Improvement in foaming capacity and decreased emulsifying capacity by HPP	[33]
Potato protein isolate	Protein	Gelation properties	300–500 MPa	300–500 MPa allows the formation of physical gels only at pH 3, and when the system crosses 30 °C by adiabatic heating during pressurization	[34]
Pork batters with gum	Protein-gum	Water-holding and gelation	400 MPa 15 min	Water-holding capacity and gel strength increased with the increase in pressure	[35]

10.3 Ultrasound

Ultrasound (Us) technique uses low-frequency and high-intensity soundwaves, ranging from 20 to 100 kHz [36]. As consequence, it leads to the cavitation phenomenon, forming gas bubbles within the liquid phase and causing local microexplosions and volume increase [37]. The Us provides high shear forces in the extractive agent, accelerates the mass transfer of bioactive compounds [38], and improves solubility due to cellular structure's high stress and deformation [37]. The increased temperature, turbulence, and cavitation caused by the Us treatment also increase extraction efficiency [8] and reduce extraction time and protein aggregates [39]. Additionally, the cavitation bubbles result in micro-jetting and particle breakdown, improve solvent permeation into the food matrix, and enhance protein functionality [36, 39].

High-intensity ultrasound is a quick and cost-effective technology to modify proteins' structural and functional properties [40] while recovering valuable bioactive compounds, such as natural additives from plants (e.g., carotenoids, chlorophylls, and phenolics) [41]. Table 10.2 displays examples of Us technology applied to foods aiming their role as natural additives.

10.4 Cold Plasma

Plasma is described as ionized gas containing reactive species (e.g., in air it forms oxygen reactive species, ROS: atomic oxygen (O), superoxide anion (O_2^-), ozone (O_3), singlet oxygen ($^1O_2$), and hydroxyl radical (OH•), and reactive nitrogen species, RNS: atomic nitrogen (N), nitric oxide (NO•), and nitric dioxide (NO_2•)), ultraviolet radiation (UV), free radicals, electrons, and charged particles [6, 8]. Usually, the plasma is generated by applying a high electrical potential difference between two electrodes that causes gas ionization due to free electrons colliding with the gas molecules. The plasma is classified as thermal and nonthermal. There is a local thermal equilibrium in thermal plasma, and all the species are at the same temperature. Conversely, in the nonthermal or cold plasma (CP), there is no local thermal equilibrium, characterized by an electron temperature much above that of the ions and neutral molecules [60].

CP technology has a great diversity of applications in various industry sectors. Specifically, agency regulators have not yet approved the CP application in food [6]. However, a wide range of studies demonstrates the application of CP for nutritional improvements. For example, for a natural food addictive present in food products, CP can be used to alter the physicochemical properties of starches and proteins, the bioactive content and properties, modulate aromas, and change the pigment's color. In addition, CP effectively inactivates microorganisms [61] and enzymes [62], enhances antioxidant activity [63], and degrades mycotoxin [64], pesticides [65], and allergenic [66]. Table 10.3 displays examples of CP technology applied to foods aiming their role as natural additives.

The design aspects of each CP generating system and operational parametric setup lead to different CP properties and, consequently, different food product properties after the treatment. Among others, the most impacting characteristics of a CP system are the source (piece of equipment design), feed gas, electrode material, and operating humidity, frequency, and voltage [6]. Therefore, plasma induces numerous reactions, and the synergistic contributions of them make plasma chemistry rather complex. Besides, multiple reaction pathways are plausible, including activating complex metabolic pathways in fruits and vegetables [10, 67], in special when CP interacts with foods matrix, that are complex and multicomponent systems. The interactions of reactive plasma particles with each food component lead to specific changes in chemical composition, generation of new products, and altering the component characteristics [68].

Table 10.2 Examples of ultrasound (Us) technology applied to foods aiming their role as natural food additives

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Maqui berries	Anthocyanin and gallic acid	Antioxidant	10–70 °C 30–70% amplitude	The optimal extraction time was 15 min for gallic acid, while 5 min for anthocyanins	[42]
Raspberry seed oil	Tocopherol	Antioxidant	250 W 0–70 °C 40 kHz 10–40 min	30 min and 50 °C were the best conditions to extract tocopherol (15.1 mg/g sample)	[43]
Lemon balm and peppermint leaves	Gallic acid, carotenoids, and chlorophylls	Antioxidant	35 kHz 140 W 5–30 min 28.2–56.4 °C	A significant increase in all studied bioactive compounds was found during 5–20 min extraction. The maximum of total chlorophylls and carotenoids were determined during 20 min of ultrasonic extraction	[41]
Green propolis	Gallic acid	Antioxidant	40 kHz 20 min 25 °C	Extracts were suitable to produce natural ingredients with antioxidant capacity aiming for food use	[44]
Red beet	Betalain	Coloring and antioxidant	165 W 0–100% 30 °C	Us resulted in higher betalains content at low temperature using less extraction time	[45]
Mung bean coat	Gallic acid, catechin, coumaric acid, vitexin, and isovitexin	Antioxidant	70 °C 46 min	Compared with conventional methods (maceration and Soxhlet), optimized US was much more efficient for extracting antioxidant ingredients	[46]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Blueberry pomace	Anthocyanin, gallic acid, and catechin	Antioxidant	64 W 35 kHz	Us under slightly basic pH conditions positively affected total phenolic content and antioxidant activity compared to acidic pH, but lowered the anthocyanin content	[47]
Green tea	Gallic acid, catechins, caffeine, epicatechin gallate, ellagic acid, and astragalin	Antioxidant	360 W 25–85 °C 0–35 min	The combined treatment of tannase and ultrasound markedly increased the antioxidant activity of the green tea extract	[48]
Olive and fig leaves	Gallic acid, catechin, and carotenoids	Antioxidant and antimicrobial	375 W 10 min	Results showed that Us extracted more carotenoids than conventional extraction while impacting on higher flavonoids (olive leaves) and total phenolics (fig leaves). Extracts presented the highest bacterial growth inhibition and showed the highest anti-inflammatory activity	[49]
Thyme and sage	Chlorophylls, carotenoids (β-carotene, lutein, zeaxanthin)	Coloring and antioxidant	60 °C 10.3 MPa 3 cycles/10 min	The extracted pigments were determined in the range of 73.8–127.6 mg/100 g	[2]
Soybean protein isolate	Protein	Emulsifying properties	200–600 W	Us pretreatment results in hydrolysates with improved emulsifying capability	[50]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Flaxseed gum	β-carotene	Coloring and antioxidant	500 W 22 kHz 50% amplitude 10–30 min	Us showed highest β-carotene extraction when compared to microwave or alkaline extractions	[51]
Cashew-apple coproduct	Gallic acid and quercetin	Antioxidant	150 W 25 kHz 30 °C	The optimized process provided a great yield of gallic acid (750 mg/100 g) and quercetin (479 mg/100 g)	[52]
Sweet potato and wild carrot	β-carotene	Antioxidant	Ni	The Us approach is the preferred method for extracting β-carotene from carrots, sweet potato, and marketed formulations	[53]
Kiwi peel	Catechin and quercetin-3-O-glucoside	Antioxidant	5–500 W 20 kHz 1–45 min	The sonication at 94.4 W for 14.8 min, using 68.4% ethanol, resulted in a maximum of 1.5 mg of flavonoids per g of extract	[54]
Soybean protein isolate	Protein	Gelation and water-holding properties	20 kHz 150–450 W	Under 300 W, the gel hardness reached a maximum of 998.9 g, with a water-binding capacity of 87%	[55]
Pea protein concentrate	Protein	Emulsifying properties	412.5–712.5 W 336–582 s	Emulsions were greatly improved	[56]
Pea protein isolate	Protein	Foaming properties	20 kHz 30–90% 30 min	Foaming ability increased from 145.6 to 200% and foaming stability from 58 to 73.3%	[40]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Soybean and rice protein isolates and pea protein concentrate	Protein	Oil- and water-holding properties	20 kHz 562.5–712.5 W 120–600 s	Properties are improved as the dispersibility of protein materials increases (712.5 W, 600 s)	[57]
Tamarind seed protein isolate	Protein	Emulsifying, foaming, oil-and water-holding properties	100–200 W 15–30 min	The functional properties were the highest when both time and intensity of treatment were high	[58]
Shell eggs	Protein	Foaming properties	200–450 W 2–5 min 24 °C	Us caused stability of foam and maintained both foaming properties/ whipping capacity due to avoiding changes in the pH during storage	[59]

Ni Not informed

The phenolic compounds, responsible for the natural antioxidant activity, antimicrobial activity, flavor, and color in fruits and vegetables, can be altered by CP treatment by different mechanisms. The UV radiation and reactive species active cell defense mechanisms, acting as an abiotic stressor and inducing the biosynthesis of the phenolic [63], cause structural tissue damage, enhancing the extractability of bioactive compounds from the vacuoles [69, 70]. CP promotes the tannins depolymerization by breaking covalent bonds and forming smaller phenolic molecules (e.g., tannins to gallic acid) [71]. Also, the chemical transformations in the volatile compound profile are obtained [72, 73]. However, degradation of the compounds may occur depending on the treatment conditions, such as treatment time and feed gas [74, 75].

In response to the abiotic stress caused by CP, there is a higher consumption of sugars as a source of energy for the biosynthesis of phenolic compounds [63]. In contrast, the increase in sugar content is related to the depolymerization of starch, sucrose, and oligosaccharides, forming glucose, fructose, and other small-chain sugars [76]. The depolarization can also form molecules of small-chain of starch and oligosaccharide [9, 77].

CP also results in alterations of starch's chemical, physical, and mechanical properties. Crosslinking induced by CP occurs due to the cleavage at the extremity of two polymeric starch chains (C–OH) and forming of a new C–O–C linkage [78], resulting in a decrease in the viscosity and retrogradation, which increases the stability of the paste at high temperatures and on cooling [79].

Table 10.3 Examples of cold plasma (CP) technology applied to foods aiming their role as natural additives

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Grape pomace	Anthocyanins, quercetin, gallic acid, protocatechuic acid, and stilbenes	Coloring and antioxidant	DBD 60 kV 60 Hz He 5–15 min	CP pretreatment disrupted the epidermal cell structures, increased the grape peels hydrophilicity, accelerated grape drying, and increased the yield of phenolic extracts (10.9–22.8%) and antioxidant capacity (16.7–34.7%)	[75]
Camu-camu pulp	Terpenoids and sesquiterpenoids	Flavor and aroma	Glow discharge 80 kV 50 kHz Air 0.3 bar 10–30 min 10–30 mL/min	Chemical transformations in the volatile compound profile varied with the operating conditions. The main change in aroma profile was in the woody, pine, and spicy notes, and in the flavor profile was in the woody, camphoraceous, and citrus notes	[73]
Apple cubes and apple juice	Sucrose	Sweetness	DBD: 20 kV, 50–900 Hz, air, 15 min Glow discharge: 80 kV, 50 kHz, synthetic air, 0.3 bar, 10–30 min, 10–30 mL/min	Glow discharge decreases sucrose content and increases glucose, fructose, and malic acid, increasing sweetness power up to 27%. DBD reduced the sucrose, glucose, and fructose content and increased malic acid content, reducing the sweetness power up to 44%	[76]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Pomegranate juice	Tannins, anthocyanins, procyanidins, phenolic acids, and flavonol glycosides	Antioxidant	Plasma jet 25 kHz Ar 3–7 min 0.75–1.25 L/min	Pasteurization and CP increased total phenolic content by 29.55% and 33.03%, respectively. Ellagitannins depolymerization by CP increased ellagic acid content three times	[71]
Apple juice	Ascorbic acid, polyphenols, and pectin	Antioxidant	Plasma jet (spark and glow) 7.9–10.9 kV 20–65 kHz 1–5 min	Spark discharge at 10.5 kV for 5 min almost completely inactivated the polyphenol oxidase. As a result, juice color was lighter, increased antioxidant capacity (up to 17%), and polyphenols content (up to 69%), and the juice was stable during storage	[69]
Fresh-cut pitaya	Glucose and fructose gallic acid, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, and p-coumaric acid	Sweetness, antioxidant, antimicrobial	DBD 60 kV Air 5 min	Treatment inhibited up to 2 log cfu growth of total aerobic bacteria, increased up to 27% phenolic total, and increased up to 21% antioxidant activity. The sugar consumption was triggered (after 48 h, glucose decreased 21.6% (control) and 27.4% (CP), fructose decreased by 20.1% (control) and 26.7% (CP)), increased energy supply and ROS signal, activating phenylpropanoid metabolism	[63]

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Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Fresh-cut apples	Phenolics (Catechin, Epicatechin, Procyanidin, Caffeoylquinic acid, Quercetin)	Antioxidant	DBD 12.7 kHz 150 W Air 10–30 min	CP decreased the pH and the browning. Phenolic increase up to 20% at 10 min treatment and a progressive decrease was observed with increasing exposure time	[74]
Rice starch	Starch	Thickening and gelling	DBD 13.56 MHz 40–60 W Air 0.15 mbar 5–10 min	Treatment increased in gel hydration properties, syneresis, and final viscosities, decreased amylose content (from 29.3 to 22.8), pH (from 7.42 to 6.94), turbidity (from 30.0 to 18.5), gelatinization temperature, and pasting temperature. The addition of carboxyl and hydroxyl groups, the formation of fissures on granules, and depolymerization were observed	[80]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Potato starch	Starch	Thickening and gelling	Glow plasma 1.1 A 245 V N_2 and He 20 mbar 30–60 min	Polymerization and crosslink on molecular-scale increased starch branched, decreasing the viscosity and retrogradation, increasing the high-temperature paste stability and paste cooling stability. Gelatinization and pasting were facilitated, and the granules' surface etching was observed	[79]
Corn starches	Starch	Thickening and gelling	DBD and RF 13.56 MHz 90 W HMDSO 0.35 m^3/min 10 min	RF treatment formed cavities allowing the active species to modify the internal structure of the granule, increasing the amylose helix order and thermal stability. DBD treatment promoted a thicker coating deposition and HMDSO functional groups inclusion, increased the granular interaction and the decomposition temperature	[89]

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Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Whey protein	protein	Emulsifier, gelling, flavor, and texture	DBD 70 kV Air 1–60 min	ROS and RNS increased yellow color and decreased pH. The treatment within 15 min caused mild oxidation, increased the carbonyl groups and the surface hydrophobicity, reduced free SH groups, improved foaming, and emulsifying capacity. Treatments at 30 and 60 min decreased the foaming and emulsifying capacity drastically and increased the foam stability	[87]
Wheat flour	Protein	Emulsifier, gelling, and texture	DBD 15–20 V 9 kHz Air 1–2 min	The treatment did not change the total aerobic bacterial count, mold count, concentration of non-starch lipids, non-polar, and glycolipids. At 20 V accelerated lipid oxidation, reducing total free fatty acids and phospholipids, and increased molecular protein weight, resulting in the stronger dough	[88]

The etching caused by CP treatment on the starch granule surface indicates surface-structural disorganization, easing water permeation into the granules, decreasing long-range crystallites and short-range orders, gelatinization temperature, and melting enthalpy [79]. The starch granules are oxidized by CP reactive oxygen species, decreasing the pH due to forming chemical groups with acidic characters, such as the carbonyl group [80].

Concerning natural pigments, generally phenolics, the main effect caused by CP treatment is their extraction from the vacuoles by the cell membrane degradation.

Also, the anthocyanin chromophores (responsible for coloring) can undergo oxidative cleavage and conjugated double bonds break by the reactive species, resulting in product color loss [81]. Besides, anthocyanins show different conformations at different pH, and as the CP treatment tends to decrease the medium pH, a color change can occur. Whereas chlorophyll degradation can occur by several routes, resulting in different colors. Colored intermediate compounds formed in the porphyrin ring's pathways remain unchanged. The additional oxidation cleaves these intermediates' porphyrin ring, producing fluorescent catabolites and, subsequently, colorless compounds [82, 83]. Groups can be changed or removed from the chlorophyll molecule periphery. This pathway can remove the phytol, forming green derivatives [83]. The acid environment also can change the chlorophyll color, where two hydrogen ions replace the Mg-atom of the porphyrin ring, converting chlorophylls into pheophytins, an olive-brown pigmentation [84, 85].

The main changes triggered by CP in proteins are ROS and RNS. The reactive species interact with the side chain of amino acid residue and protein polypeptide backbone, resulting in unfolding, crosslinking, fragmentation, and conformational changes [86]. The protein oxidation changes its functionalities, increasing the emulsifying and foaming capacity and foam stability at CP long exposure [87]. The CP in wheat flour promotes polymerization, solubility alteration, and forming of a gluten network, resulting in a stronger dough [88]. Also, CP can inactivate enzymes involved in undesirable reactions, such as peroxidase, polyphenol oxidase, and lipoxygenase, as well as the inactivation of the allergenic protein [6].

10.5 Pulsed Electric Field

The pulsed electric field (PEF) applies high voltage pulses for a very short time (from several nanoseconds to milliseconds) to a food product placed between two electrodes [8]. PEF induces the formation of irreversible or reversible pores in biological cell membranes, known as the electroporation phenomenon (cell electrical breakdown) [90, 91]. This nonthermal technology can improve food products through microbial inactivation due to the dielectric breakdown of the cell membrane and enzyme inactivation. The PEF pretreatment of food products is effective for osmotic dehydration [92], improvement of freezing and thawing processes [93], and reducing drying process time [94]. The main effect of PEF on bioactive compounds relays on the disruption of cells which increases the mass transfer of intracellular compounds, making them more available. As a result, bioactive extraction efficiency increases, shorting extraction time, reducing solvent consumption, and maintaining the quality of these compounds [5]. Table 10.4 displays examples of PEF technology applied to foods aiming their role as NFAs.

The PEF treatment efficacy depends, among other factors, on the electric field intensity, temperature, treatment time, pulse wave, and physical properties of food, such as electrical conductivity, size, and shape of cells. For example, foods with more current-conducting compounds within their cells (e.g., ions of dissociated

Table 10.4 Examples of pulsed electric field (PEF) technology applied to foods aiming their role as natural food additives

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Carrot	Phenolics	Antioxidant and pigment	0.8–3.5 kV/cm 5–30 pulses	At 5 pulses of 3.5 kV/cm and 30 pulses of 0.8 kV/cm was the highest increase in phenolics (about 40%) after storage for 24 h at 4 °C; color and hardness were maintained. Weight loss reached 9.3% at 3.5 kV/cm (the control was 1%). A higher increase in media conductivity was observed after treatment at 2 and 3.5 kV/cm. The cell viability was reduced up to 73% for carrots treated	[97]
Spinach	Chlorophyll carotenoids	Antioxidant and pigment	3.3–26.7 kV/cm 1 kHz 20 μs	PEF treatment increased the chlorophyll a, b, carotenoids, and antioxidant activity up to 26.3, 21.0, 41.5, 14%, respectively, compared to the untreated. In addition, promoted the crosslink reaction with other chlorophyll molecules and affected carotenoids' unsaturated bond, changing conformation from cis to trans	[101]
Tomato peel	Lycopene	Antioxidant and pigment	1–5 kV/cm 10 Hz 20 μs 10–833 pulse number	PEF before the solvent extraction process enhanced the extraction rate (27–37%), lycopene yields (12–18%), and antioxidant power (18%). PEF induced size reduction and separation between the plant cells due to pore formation and leakage of intracellular matter	[110]

(continued)

Table 10.4 (continued)

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Freshwater mussel	Protein	Nutrient	10–35 kV/cm 2 μs 2–12 pulse number 40–3000 Hz	The protein extraction yield was 77.08% at 20 kV/cm, 8 pulse number, and 2 h enzymolysis time. Compared with other extraction methods (NaCl, alkali, and enzyme method), the PEF increased the speed and yield extraction from the mussel	[103]
Canola seeds	Protein	Emulsifier, foaming, and water-holding	10–35 kV 100–1000 Hz 1–10 μs 60–210 s (residence time)	PEF pretreatment increased the functional properties of canola protein: solubility (up to 46%), water-holding capacity (up to 68%), emulsibility (up to 13%), emulsion stability (up to 21%), oil-holding capacity (up to 74%), foamability (up to 40%), and foam stability (up to 51%). PEF changed the secondary structure, increased free sulfhydryl groups, and surface hydrophobicity, and formed protein aggregates with low molecular mass	[107]
Macroalgae (*Ulva Ohnoi*)	Protein and starch	Nutrient	1 kV/cm 30 Hz 50 μs	PEF treatment increased the conductivity sample (indicating that treatment affected membrane permeability), and the extraction of starch, protein, and ash (60, 15, and 68%, respectively) compared to the control (52, 3, and 47%, respectively)	[104]

(continued)

Table 10.4 (continued)

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Esterified Potato starch	Starch	Emulsifier and digestibility	1.25–5 kV/cm 1000 Hz 40 μs 60 min (residence time)	The slowly digestible starch fractions increased from 6.6% (control) to 17.5% (PEF-treated). As the electric intensity was increased, more deformations, protrusions, and pits were observed on the starch granules' surface. The sample treated with higher electric intensity resulted in a stable emulsion	[109]
Waxy rice starch	Starch	Thickening and digestibility	3–50 kV/cm 1 kHz 40 μs	PEF treatment decreased the gelatinization temperature (up to 17%), enthalpy (up to 45%), crystallinity (up to 23%), and slowly digestible starch level (up to 23%), and increased rapidly digestible starch (up to 55%). The changes were more pronounced as the intensity of the electric field was increased	[108]

salts and charged molecules of proteins) are more susceptible to electroporation [95].

The PEF treatment increased the content of phenolic compounds and antioxidant activity of fruits and vegetables and improved the extraction of natural pigments (e.g., carotenoids and anthocyanins) [96, 97]. However, depending on the PEF operating parameters, it may decrease the bioactive compounds content [11, 95]. Also, different PEF parameters can modulate the chemical composition of bioactive extracts [98]. The co-pigmentation and pigments formation may be favored by PEF pretreatment, as observed for winemaking before the macerating fermentation step, in which the polyphenols extraction increased 48%, and the wine color attributes increased 56% [99].

The PEF voltage can cause dissociation of water and other molecules, producing free radicals and hydrogen peroxide [100]. Thus, PEF treatment can act as an abiotic stressor for the biosynthesis of secondary metabolites, increasing the phenolic content in the food products [97]. Also, the pigments chlorophyll are affected by free radicals, mainly the chemical bonds between the pyrrole ring and central magnesium ions of the molecule that can form the chlorophyll aggregated structures and increase the stability [101].

PEF treatment can influence biomacromolecules' physicochemical and functional properties [102]. In addition to improving the extraction of proteins [103] and starch [104] from the cell tissue, treating proteins with PEF can induce structural and functional changes. The ionization of various chemical groups or breaking of electrostatic interactions alters the secondary and tertiary structure of proteins, consecutively in the loss of α-helix and β-sheet, resulting in modifications such as unfolding, crosslinking, and aggregation of proteins [105–107]. Also, PEF alters starch properties by disintegrating amylopectin linkages and damaging starch granules, allowing water molecules to ingress into the crystalline region, decreasing crystallinity, gelatinization temperatures, and increasing water holding capacity [108]. These damages facilitate enzymes attack to the granules, increasing the digestibility. On the other hand, starch acetylation can increase the content of slowly digestible starch fractions [109].

10.6 Pulsed Light/UV-Light

Ultraviolet (UV) and pulsed light (PL) treatments are used as alternatives to chemical and thermal processing to inactivate microorganisms on surfaces, liquid foods, beverages, ingredients, and packaging, producing foods with better quality, extended shelf-life, and often with enhanced health benefits [111–113]. However, both technologies are based on irradiation, so the products can suffer photoreactions, depending on the food's optical properties, such as absorption, transmission, reflection, and the light spectrum emissions and irradiation doses. Once foodstuffs are exposed for too long and high doses of light energy, secondary products can be produced and cause matter changes, such as discoloration, off-flavors, loss of vitamins, and other essential nutrients [114–116].

The efficacy of both treatments is generally related to the absorption of UV-C light by microbial nucleic acids, causing photochemical changes, but for PL treatment, photothermal and photophysical changes on microorganisms are also related [111]. Therefore, UV-light technology is often found as monochromatic light in the UV-C spectrum ($\lambda = 254$ nm). UV-C devices work with low power; thus, long times are needed to be effective against microorganisms, which can cause the degradation of some other compounds, such as carotenoids, chlorophylls, flavonoids, and lipids [114]. In contrast, PL is found as a polychromatic light that includes ultraviolet (200–400 nm), visible light (380–780 nm), and infrared radiation (700–1100 nm) [111]. A capacitor stores high-intensity power energy and is released in short-intense pulses no longer than 2 ms. US Food and Drug Administration (FDA) approved PL to treat food surfaces with fluence levels not higher than 12 J cm^{-2} [117]. Together with other technologies and even mild temperatures, these technologies can enhance results further.

The literature is scarce on NFAs as ingredients in foodstuffs treated by light. However, natural compounds extracted from raw foods can be added to improve nutritional or functional properties of other products, Table 10.5. Plants exposed to

Table 10.5 Examples of UV and Pulse Light (PL) technology applied to foods aiming their role as natural food additives

Food	Natural food additive present	Additive role	Processing conditions	Results	Reference
Ergosterol incorporated into purple sweet potato pastes	Ergosterol transformed into Vitamin D2	Supplementation - Phosphorus and Calcium absorption	UVC-irradiation	Pastes with ergosterol concentrations lower than 0.65 mg/g could be well printed. Reducing the internal filling ratio was noted as an effective means to improve the conversion. When such a ratio was 70%, more formation of vitamin D2 at minimal use of raw materials could be realized	[120]
Mangoes	Vitamins C, B1, B3, B5, and B6	Participants in human metabolism	PL (3.6–10.8 J/cm^2)	Mangoes were pretreated by PL and then convective dried. Highest concentrations of vitamin C, B1, B3, and B5 were found in dried mangoes subjected to fluences PL treatment, while vitamin B6 decreased by 40 to 50% in the pretreated mangoes	[115]
Strawberry	Ascorbic acid (Vit C) and anthocyanin	Antioxidants	PL (4, 8, 12, and 16 J/cm^2)	Vitamin C and total anthocyanin contents of the samples treated at low energy doses were maintained, whereas those of slices treated at the highest energy dose decreased between 20 and 30%	[121]
Mushrooms	Gallic acid (GA), caffeic acid, chlorogenic acid, and quercetin contents	Antioxidants	PL (68.8 mJ/cm^2)	Short PL treatment (3 pulses each side) enhanced GA, caffeic acid, and quercetin contents of shiitake mushrooms	[129]

(continued)

Table 10.5 (continued)

Food	Natural food additive present	Additive role	Processing conditions	Results	Reference
White 'Gijnlim' asparagus	Anthocyanins	Antioxidant and colorant	After harvest, spears were exposed to weak white, red and blue light (30 μmol/m^2s) for 3 h and to UV-C (254 nm, 1 kJ/m^2) for 8 min	White-light triggered anthocyanin synthesis via an associated phenylalanine ammonia-lyase increase. Red light and UV-C irradiation tendentiously resulted in an anthocyanin inhibition or even degradation, coinciding with changes in phenylalanine ammonia-lyase activity	[116]
Sausage coated by curcumin-hydrogels	Curcumin	Photosensitizer, antioxidant, antimicrobial, and colorant	UV-A lamps (320–400 nm; 32 ± 0.2 W/m^2 for 5 and 15 min)	Curcumin can generate reactive oxygen species (ROS) in solution after excitation by a light causing microbial inactivation	[119]
Corn and potato	Starch	Thickener	UVB-irradiation	The potato amylose was more susceptible to changes upon UV-B irradiation, whereas corn ones. Similarly, functional properties were not significantly influenced by UV-B treatment	[130]
Cheese	Proteins	Nutrition	PL (1.3, 3.1, 7.5, 15 J/cm^2)	PL induced the formation of aggregates of small protein particles with lipids and carbohydrates, which reduced protein solubility. The formation of melanoidins and carbonyls confirmed protein photoreaction	[125]
Whey Protein Isolate (WPI) solution	Proteins	Nutrition/ Supplementation	PL (4–16 J/cm^2)	PL treatments increased the concentration of total and free sulfhydryl groups and protein carbonyls. In addition, PL treatments induced dissociation and partial unfolding of WPI, improving solubility and foaming ability	[127]

Egg white	Proteins	Functional properties	PL (1.75–31.5 J/cm^2)	PL caused browning, protein aggregation by disulfide exchange, and protein backbone cleavage. These structural modifications cause an increase in immunoreactivity and a decrease in gelling temperature. Also, foams treated by PL showed higher stability due to the jamming of protein aggregates and fragments in the fluid interstices between bubbles	[128]
Milk	β-Lactoglobulin and α-lactalbumin	Nutrition/ Supplementation	PL (4 cm from the lamp); 2.2 J/cm^2 per pulse (1, 3, 5, 7, and 10 pulses)	PL treatments showed no conformational changes in milk proteins despite aggregation by disulfide bonds, and the products had no oxidation appearance	[126]
Beeswax	Esters, hydrocarbons, fatty acids, and alcohols.	Glazing or coating for fruits and candies	UV light exposure (4.5 mW/cm^2 for 50 h)	The lifetime of beeswax at 23 °C was shortened by about 60%. FTIR showed the extinction of ester groups accompanied by an increase in free fatty acids content was observed	[131]
Fish	Lipids and proteins	Nutrition	Systematic review comparing UV-treatments with low doses (0.05–0.16 J/cm^2) and high (0.30–0.79 or about 0.30 J/cm^2).	Lipid and protein oxidation increased by 6% and 7% for low UV-light doses and 13% and 20% for high doses	[123]

light with different wavelengths have shown synthesized pigments, such as anthocyanins, associated with enzymes changes [116], which is often related to the visible blue light [118]. However, the exposition of these compounds to UV-C light causes their degradation [116]. Curcumin is natural pigment extracted from plants that have been discussed in the literature because of its photosensitizer property, which generates reactive oxygen species (ROS) after excitation, causing microbial inactivation [119].

Vitamins are crucial for human metabolism, and many foods can be fortified with these additives, which can be natural or synthetic. The literature reports UV-C light transforming ergosterol into vitamin D2 [120], and to break chemical bonds between vitamin B3 and nucleotides, and vitamin B5 and coenzyme A, turning into more bioavailable vitamins [115]. Nevertheless, light radiation also degrades vitamins C and B6 [115, 121].

Light control is essential in the flavor stability of vegetable oils and other unsaturated fats. The literature reports many cases of light-induced oxidation, as rapeseed, corn, soybean, and coconut oils and milk fat subjected to light in the wavelength ranging from 350 to 750 nm [122]. Fishes are rich in unsaturated fatty acids such as Omega-3 polyunsaturated fatty acids (PUFAs), a natural health additive used in supplements. Lipid oxidation increased by 6% when a sample was submitted to low UV-light doses and 13% for high doses [123]. A study on the addition of essential oils to foods aiming to improve the lethality of a UV-light treatment showed a synergistic effect on inactivating biofilms of *S.* Typhimurium [124].

Proteins are functional molecules that, after exposure to PL, aggregate with lipids and carbohydrates, reducing their solubility [125]. In milk proteins, it was reported that the only conformational modification was the aggregation of disulfide bonds [126]. However, when whey protein isolated (WPI) was exposed to PL treatment, its solubility and foaming ability were improved due to the dissociation and partial unfolding of WPI [127]. In addition, egg white proteins treated by PL showed structural changes, resulting in different functional properties, such as increased immunoreactivity, decreased gelling temperature, and higher foam stability [128].

UV-light and PL treatment have shown the potential to inactivate bacteria in clear and transparent liquids. However, their efficiency is compromised as turbidity increases, and for solid foods; low absorption and shadowing effects are challenging to be solved for the application of PL in the food industry. On the other hand, photodegradation products are restricted to the product's surface, often having low impact on the sensory and nutritional properties of the products.

10.7 Conclusions

The technologies reported herein are attractive due to their capacity to produce and preserve natural additives in foods, superior to the food quality when conventional thermal processes are applied for inactivating pathogenic and spoilage microorganisms and enzymes. In addition, those nonthermal methods cause structural

changes in cell membranes and structural modifications in some (macro)molecules, favoring extraction, digestibility, and desirable functional/nutritional properties.

Some of these emerging nonthermal technologies are on a small scale (laboratory or pilot level) and need to be scaled up before industrial use. On the other hand, the development of industrial equipment after scientific development has been intense and fast, as is the case of high-pressure systems. It is crucial to consider governmental regulations in each country and the safety aspects of each pair of technology-food product. Furthermore, costs, cultural changes, and consumer awareness are challenges in implementing a nonthermal process to obtain and modify natural compounds. Based on the state-of-the-art, these emerging nonthermal methods will keep evolving and reaching the food industry since they require fewer chemical additives, favoring natural additives usage.

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