CHAPTER

Stability of plant extracts

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5.1 Introduction

In recent years, change in eating habits and increased demand of healthy food have revolutionized the food market. Researchers are now emphasizing more on the functional properties of food along with their nutritional characteristics and esthetic attributes. The successful results of dietary interventions in treating chronic diseases, lifestyle diseases, and degenerative disorders have enforced food industries to focus more on improvising product development to meet the consumers' demand of nutritional and healthy food (Lorenzo et al., 2019). Numerous epidemiological studies revealing the potential of plant foods in reducing the risk of above-mentioned disease conditions are encouraging consumers to take fresh fruits and vegetables or their minimally processed products in their diets. Moreover, scientific developments have changed the concept of traditionally cooked food and explored new strategies of product preparation in combination with various plant parts or their extracts for providing a functional and quality product. The plant extracts constitute various polysaccharides, proteins, fatty acid esters, vitamin, minerals, metal ions, and polyphenolic compounds, accounting to their nutritional and functional properties (Mir, Shah, Ganai, Ahmad, & Gani, 2019). Incorporation of these phytochemicals in food or pharmaceutical formulations increases the exposure of plant extracts to various processing conditions or extraction processes, drastically affecting the stability of plant extracts through oxidation, condensation, polymerization, hydrolysis, or other degradation mechanisms (Thakur, Ghodasra, Patel, & Dabhi, 2011).

Among many factors influencing the plant extract stability, thermal processing is reported as the main cause of structural modifications and alterations in their bioactivity (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012). Heat processing at domestic level (frying, boiling, baking, etc.) or industrial level (blanching, sterilization, roasting, drying, etc.) helps in extending the shelf life of food, bring significant changes in sensory attributes and contributing to their availability (Barba, Sant'Ana, Orlien, & Koubaa, 2018; Putnik et al., 2017). However, thermal treatment also induces the formation of free radicals and other compounds, causing oxidation of bioactives, nutrient loss, quality degradation, or increased toxicity (Boekel et al., 2010). Considering the adverse effects of thermal processing on foods, the nonthermal technologies are gaining more importance at commercial level (Gabrić et al., 2018). Application of high-pressure processing, sonication, irradiation, pulse electric field, electrical voltage, and cold plasma are some of the strategies used alone or in combination for processing (Barba et al., 2017, 2018). Investigations analyzing the effect of

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nonthermal processing on plant extracts have demonstrated significant variations in correlation between treatment conditions and food matrices (Fernández-Jalao, Sánchez-Moreno, & Ancos, 2017; He et al., 2016; Rodríguez-Roque et al., 2015). Furthermore, mechanical pretreatment and processing revealed similar trend with respect to retention and degradation kinetics of polyphenols (Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2011; Radziejewska-Kubzdela & Olejnik, 2016; Makris & Rossiter, 2001). Many studies also conducted to evaluate the effect of storage, pH, light, and other environmental conditions on phytochemicals and other nutrients suggesting the optimized processing conditions to develop quality, and safe product with improved shelf life (Ali et al., 2018; Chaaban, Ioannou, Paris, Charbonnel, & Ghoul, 2017; Nagar et al., 2021). Researchers have also highlighted the contribution of individual phytochemical and other components of plant matrix (fibers, peptides, sugars, and resins) to the stability and bioaccessibility of the plant extracts (Chen et al., 2014; Zhu, 2018).

Different mechanisms inducing modifications in bioactive compounds have been explained and discussed, giving the scientific basis for the selection of various processing factors. These mechanisms include triggering of stress signal, alterations in enzyme activity, auto-oxidation of compounds, free radicals generation and the associated scavenging reactions, polymerization and condensation reactions, stimulation of polyphenol synthesis under different stress conditions, and the presence of other functional groups (Barba et al., 2017; Bayliak, Burdyliuk, & Lushchak, 2016; He et al., 2016; Ioannou et al., 2012; Ismaiel, El-Ayouty, & Piercey-Normore, 2016; Kotsiou & Tasioula-Margari, 2016; Oms-Oliu, Odriozola-Serrano, Soliva-Fortuny, Elez-Martínez, & Martín-Belloso, 2012; Wang, Chen, & Wang, 2009a,b). However, the existing knowledge on above reaction mechanisms is not consistent throughout the various plant products. Therefore it is imperative to develop statistical models to analyze and accurately predict the stability of bioactive compounds after processing or storage. Numerous scientific reports are available on the factors influencing the functional compounds during and after processing or storage, however, no study comprehensively elucidate the effect of all the thermal, nonthermal and storage factors, and presence of other plant components. This chapter describes all the possible parameters affecting the stability of phytochemicals along with their proposed mechanism for better understanding of the response of functional compounds and further process optimization for retaining their activity and stability.

5.2 Stability of plant extracts

The stability of plant extracts in terms of their amount, activity, bioaccessibility, and bioavailability is known to be a factor of the extraction conditions of temperature, pressure, sonication, radiation, cold plasma, electric field, pH, presence of oxygen, and light (Chaaban et al., 2017; Mehta, Sharma, Bansal, Sangwan, & Yadav, 2019; Nagar et al., 2021; Park & Lee, 2021; Rodríguez-Roque et al., 2015; Xia, Wang, Xu, Mei, & Li, 2017). Further incorporation of plant extracts in various food formulations and their processing, and storage also affect the phytochemical potential of the bioactive compounds. Processing parameters such as temperature, light, pressure, pH, and storage conditions of temperature, relative humidity, time, presence of oxygen and light, have been widely studied for their effects on the functional compounds. Besides, numerous researches on phytochemical potential of multicomponent food matrix have revealed the synergistic and antagonistic

interaction of different polyphenolic compounds affecting their overall bioactivity. The bioaccessibility and bioavailability of these compounds in the body during digestion is also an area of interest for scientists nowadays where in vitro studies have been performed to predict the health benefits imparted by some bioactive. These studies further explain the effect of processing parameters on the polyphenolic compounds when these are present along with other dietary components (Lorenzo et al., 2019). The following sections explain the various factors viz. thermal, nonthermal and storage conditions on the plant extract stability.

5.2.1 Effect of processing

Subjecting the plant or food matrices to different stages of processing starting from the extraction of bioactives to incorporation into food and further processing of food using heat or mechanical energy significantly affects the stability of plant active compounds. Along with the type of processing factor, intensity of the treatment has also shown to effect the stability to varying degrees (Galaz et al., 2017; Li, Akram, Al-Zuhair, Elnajjar, & Munir, 2020; Mehta et al., 2019; Teixeira-Guedes, Oppolzer, Barros, & Pereira-Wilson, 2019). Therefore it is imperative to understand the effect of these thermal and nonthermal processing on bioactivity of plant components before selecting the suitable technologies for achieving maximum yield of phenolic compounds without degrading their activity.

5.2.1.1 Thermal processing

Heat processing is one of the most commonly used technologies having significance in food preservation (blanching, pasteurization, and sterilization), and making the food more esthetic for its consumption (boiling, steaming, roasting, and frying). The latter is generally practiced as domestic food processing operations and is very important in deciding the bioaccessibility of polyphenols. There are various factors of type of food matrix, intensity of heat treatment, type of phenolic compound and other related processing effects which are being investigated for their role in predicting nutritional value of food (Tables 5.1 and 5.2). The studies revealed a nonuniform trend in the polyphenolic yield and their antioxidant activities, where some cooking methods facilitate the release of bioactives and others lead to degradation of those compounds (Martini, Conte, Cattivelli, & Tagliazucchi, 2021). A comparative study on steaming, frying and boiling revealed a significant increase in the phenol content of cauliflower with a slow rate of degradation during storage after steaming. These findings were explained by deleterious effects of boiling and frying, where, softening of the food matrix facilitates lixiviation of polyphenols to the solvent after boiling, and reactive species formation and high temperature of frying degrade the active compounds (Girgin & El, 2015).

Besides, the same unit operation does also imply a dual effect on phenolics release and their stability during processing, suggesting an increase in the antioxidant activity to certain level followed by a significant decline of the same (Park & Lee, 2021). The increase in bioaccessibility of compounds in the commodities like cereals was suggested due to softening and disruption of lignocellulosic structure after domestic heat treatment of boiling and steaming. Also, some authors have discussed the formation of more soluble phenolic compounds (Lima et al., 2017). Another important aspect is difference in heat stability within phenolic groups when subjected to same processing conditions. In a study on processing of blackberry jam, the results revealed variation in processing

Table 5.1 Effect of extraction methods on plant extract stability.

S. No.	Extraction method	Food matrix	Processing conditions			Impact on extract stability			References
			Temperature	Pressure	Other parameters	TP	AOX	Others	
1.	Ultrasound assisted solvent extraction	Black chokeberry	20°C, 40°C, 60°C, 80°C	–	Time- 4 h, initial solid−solvent ratio- 1:10, 1:20, 1:40; continuous mode sonication at 30.8 kHz frequency; 100 W power	Threefold increase at 60°C than at 20°C.	Increased with temperature	–	d'Alessandro et al. (2012)
2.	Subcritical water extraction	Date pits	120°C − 180°C	35 bar	Extraction time- 10 −30 min; impeller speed 500 rpm; solid content- 2 −10% w/w	First increased up to 150°C, and then decreased	Decreased with increase in temperature	TEY increased up to 150°C, dietary fibers increased with temperature	Li et al. (2020)
3.	Pressurized hot water extraction	Thyme	50°C − 200°C	1500 psi	Contact time- 5 − 30 min	Twofold increase in TP with temperature increment from 50°C to 200°C; high temperatures with long extraction times produced small number of polyphenols (methylflavones and flavonols)	1. fivefold increase in AOX with temperature increment from 50°C to 200°C	>twofold increase in TEY with increase in temperature from 100°C to 200°C	Vergara-Salinas et al. (2012)
4.	Hydro-alcoholic extraction	Oregano	22°C, 40°C, 60°C	–	Solvent:Ethanol−water; ethanol conc.%-0%, 60%, 80%, 96%, solid-to-liquid ratio- 1:20, 1:40 g/mL; particle size of plant material- <315 μm, 315−600 μm, 600−800 μm, 800−100 μm, >1000 μm, and not ground	TP increased with increase in temperature, maximum yield (49.80 mg GAE/gdw) obtained at 40°C	–	Increase in solvent selectivity with increase in temperature up to 40°C	Oreopoulou, et al. (2020)

5.	Accelerated solvent extraction (ASE)	Olive fruit	60°C, 80°C, 100°C	100 atm	Extraction cycles- 2/run; static time between runs- 10 min; rinse volume- 20%; solvents- acetone, EtOH, water	High phenolic (gallic acid, quercetin, luteolin, rutin) yield obtained at 60°C	–	Water at 100°C showed maximum TEY and % recovery of 130 mg/g and 13%	Ahmad, Ahmad, Aljamea, Abuthayn, and Aqeel (2020)
6.	Subcritical water extraction	Onion skin	170°C −230°C	30 bar	Mixing at 400 rpm; extraction time-30 min	Decrease in TP after 165°C	Nearly 38% loss in AOX at 230°C	Highest TF yield observed at 230°C; quercetin yield was high at 170°C than at 230°C	Munir, Kheirkhah, Baroutian, Quek, and Young (2018)
7.	Hot pressurized liquid extraction	Grape Pomace	90°C, 120°C, and 150°C	10 MPa	Solvent: water-glycerol mixtures (15%, 32.5%, and 50%) solvent volume-50 mL, extraction cycle-1, 250 s nitrogen purge time, and static extraction time- 5 min	∼34 times increase in TP at 150°C	–	∼13 times and 18 times more gallic acid and flavanol yield at high temperature	Huamán-Castilla, Mariotti-Celis, Martínez-Cifuentes, and Pérez-Correa (2020)
8.	Microwave-assisted extraction	*Hibiscus sabdariffa*	50°C−150°C	–	Solvent- 15%−75% EtOH; 1500 W microwave power; extraction time- 5−20 min	Highest TP yield at 164°C	–	Highest TFC achieved at 158°C (14.43 mg QE per g), high TEY of ∼47% obtained at 150°C	Pimentel-Moral, et al. (2018)

AOX, *Antioxidant activity;* TAC, *total anthocyanin content;* TF, *total flavonoid;* TEY, *total extraction yield;* TP, *total phenols content.*

Table 5.2 Effect of temperature on polyphenols during domestic and industrial processing of food.

S. No.	Food matrix	Processing technique	Processing conditions			Impact on plant extract			References
			Temperature	Pressure	Other parameters	TP	AOX	Others	
1.	Omija fruit	Roasting	120°C, 150°C, 180°C	–	Roasting time- 5, 10, 15 min.	Nearly twofold increase in TP at 180°C	–	5 to 15-fold increase in TF at 180°C; lignan content increased up to 150°C and decreased at 180°C, ester content decreased with increase in temperature	Park and Lee (2021)
2.	Pomegranate peel	Drum drying	Drying conditions- 422 s at 100°C, 400 s at 110°C, 257 s at 120°C	–	–	No significant effect on TP	An insignificant decrease of 7.5% observed at 100°C	Least change in color at 120°C for 257 s	Galaz et al. (2017)
3.	Mulberry leaves	Air drying	Air-drying conditions- 45 h at 40°C; 7 h at 60°C, 4.3 h at 70°C, 2.5 h at 80°C, 1.7 h at 110°C	–	–	TP increased up to 60°C and then decreased (three times) at >60°C	AOX increased up to 60°C and then decreased at >60°C	kaempferol; quercetin; chlorogenic acid; rutin; astragalin; isoquercitrin showed degradation after 60°C	Katsube, Tsurunaga, Sugiyama, Furuno, and Yamasaki (2009)
4.	Apple Pomace	Blanching and air drying	Blanching in stainless steel steam-jacketed kettle at 95°C to achieve a stable temperature of 86°C for 4 min; dehydration temperatures- 50°C, 60°C, 70°C, and 80°C until 0.05 kg H_2O/kg dry matter achieved	–	–	1.25-fold increase in TP after blanching; significant loss in TP at 70°C	–	Twofold increase in TF after blanching; significant loss in TF at 70°C; decreased browning in blanched samples	Heras-Ramírez et al. (2012)
5.	Plum extract	Thermal treatment	Anthocyanin solutions submerged in thermostatic bath at temperatures 25°C−110°C	–	Time: 10 min	Heating for 5 min at 70°C −90°C reduced TP between 4%−23%, and decreased the content to 43%−72% at >90°C	61% AOX loss occurred after 20 min. of heating at 110°C	Maximum reduction of 71% and 91% in TFC and TAC was recorded at 110°C, after 20 min of holding	Turturică et al., 2016

6.	Onions	Frying Boiling Microwaving Sautéing	Frying. onions were fried for 2 min, submerged in 350 mL of 100% soybean oil heated to 150°C Boiling. onions were boiled for 5 min in 100 mL of distilled water containing 1 or 3% NaCl Microwaving. at high heat for l min in a microwave oven SautéIng. Chopped onions were cooked on a stovetop for 3 min in soybean oil	–	–	–	–	Frying. 32.8% loss in TF Boiling. 13.7% loss in TF Microwaving. 4.4% loss in TF SautéIng. 20.6% loss in TF	Lee et al. (2008)
7.	Kale	Steaming, boiling, frying	Steaming (over boiling water, 5 min−60 min); Boiling (5−60 min); Frying (140°C, 5 min−20 min)	–	–	Steaming caused a TP decrease of 5%−30%; stir frying showed 35%−41% decrease in TP	Steaming showed an initial increase of AOX, followed by 15% decrease for >20 min. cooking time, frying decrease the AOX significantly	Total carotenes, β-carotene and lutein content increased after boiling, 54%−42% decrease in lutein observed after steaming, stir frying caused a 10%−23% decrease in β-carotene	Giambanelli, Verkerk, D'Antuono, & Oliviero, 2016)
8.	Pepper, squash, green beans	Boiling, steaming, microwaving	–	–	Boiling (5 min); Steaming (over boiling water, 7.5 min, atmospheric pressure); Microwaving (1000 W, 1 min for squash, 1.5 min for pepper & beans)	Increase in TP of pepper & green beans, decrease in TP of squash	Increase in AOX of pepper & green beans, no change in AOX of squash	–	Turkmen, Sari, and Velioglu (2005)

(*Continued*)

Table 5.2 Effect of temperature on polyphenols during domestic and industrial processing of food. *Continued*

S. No.	Food matrix	Processing technique	Processing conditions			Impact on plant extract			References
			Temperature	Pressure	Other parameters	TP	AOX	Others	
9.	Cauliflower	Boiling, steaming	Steaming (over boiling water, 10 min); Boiling (10 min)	–	–	Increase in TP by 14.83% after steaming, decrease in TP by 1.8% after boiling	AOX increased by 47% after steaming, and decrease 8% after boiling	–	Girgin and El (2015)
10.	Eggplant	Baking, boiling, frying, grilling	Boiling (20 min at 100°C); grilling (cooking at 120°C for 10 min on a grilling plate); baking (30 min at 180°C in electric oven); frying (170°C for 10 min)	–	–	34.5% decrease after grilling; increased 42%, 67% & >300% after boiling, baking and frying, respectively	Frying increased (~3.5 times) the AOX; grilling induced 20% decrease; baking and boiling had no effect on activity	74.2% of increase in hydroxycinnamic acids after frying; 27%, 51%, and 60% decrease in total hydroxycinnamic acids was observed after boiling, grilling, and baking, respectively	Martini et al. (2021)
11.	Kidney bean, pinto bean, black bean, soybean	Boiling and pressure cooking	Soaking (12 h at room temp.), followed by boiling (100°C, for 50 min) or pressure cooking (115°C, for 20 min)	–	–	175%, 80%, 72%, 56% increase in TP of kidney, pinto, and borlotti beans, respectively, after boiling	109%, 74%, 42%, 64% increase in AOX of kidney, pinto, and borlotti beans, respectively, after boiling	TEY increased for all beans after boiling and pressure cooking (94%, 50%, 30%, 5% increase after boiling; 130%, 20%, 5%, 10% increase after pressure cooking of kidney, pinto, black and soy beans, respectively)	Teixeira-Guedes et al. (2019)
12.	Wild rocket leaves	Hot water pretreatment and steaming	Hot water pretreatment (90°C for 5 min)	–	Steaming (10 min)	Pretreatment with hot water yielded highest TP (45.4 mg/100 g); steaming of leaves reduced TP by 20%	Pretreatment with hot water gave highest AOX (5.8 μmol Trolox/1 g)	Steaming and pretreatment with hot water reduced the glucosinolate content by 21%, and 37%, respectively	Radziejewska-Kubzdela, Olejnik, and Biegańska-Marecik (2019)

AOX, *antioxidant activity;* TAC, *total anthocyanin content;* TEY, *total extraction yield;* TF, *total flavonoid;* TP, *total phenols content.*

effect on different bioactive compounds where bioaccessibility of total phenolics increased with a significant decrease in the bioaccessibility of flavonoids. The decrease in activity was ascribed to either degradation of the polyphenolic (anthocyanin) compound caused by oxidation and cleavage of intramolecular bonds after exposure to heat or formation of complex with reduce activity (procyanidins) in association with other polyphenolics. On the contrary, release of bound phenolic compounds have an additive effect on the accessibility (Tomas et al., 2017).

Application of temperature during the extraction of plant extracts increases the extraction efficiency; however, after a certain range of temperature, the adverse effect of temperature on the yield of bioactive compounds is more prominent. The effect of different extraction methods (ultrasound assisted, hydroalcoholic, solvent, subcritical-water extraction etc.) and food processing techniques (roasting, drying, blanching, etc.) involving heat on the functional bioactive of plant extracts has been summarized in Table 5.1. In the process of extraction the temperature generally facilitate the extraction of polyphenols. However, after certain maxima the effect of temperature negatively affect the extraction yield and bioactivity of the compounds (d'Alessandro, Kriaa, Nikov, & Dimitrov, 2012; Li et al., 2020). The temperature aided high polyphenol yield is observed to be due to improved solvation power of the solvent, high solubility of polyphenolics at high temperatures along with increased diffusivities of the extracted compounds and improved mass transfer between the food matrix and the solvent. Furthermore, temperature-induced disruption of plant cell membrane and hydrolysis of ester or ether bonds between different complex bioactive compounds leads to the release of bound phytochemicals and therefore enhances the extraction yield (Gonzales et al., 2015; Prasad, Yang, Yi, Zhao, & Jiang, 2009). However, at very high temperature the content of some active compounds tend to decrease which is attributed to their thermal sensitivity and probable structural changes (Oreopoulou, Goussias, Tsimogiannis, & Oreopoulou, 2020; Park & Lee, 2021). Various studies on polyphenol extraction suggested the synergistic and antagonistic interaction between temperature and other extraction parameters such as type of food matrix, particle size, pretreatment of food, type of solvent, solvent concentration, solid and solvent ratio, time of extraction, pressure, presence of enzyme, microwave energy, ultrasound energy, etc. (Li et al., 2017; Nishad, Saha, & Kaur, 2019; Oreopoulou, et al., 2020; Vergara-Salinas, Pérez-Jiménez, Torres, Agosin, & Pérez-Correa, 2012). Few studies have also depicted an increase in total antioxidant activity whereas the concentration of individual polyphenolic compound decreased after extraction. Nishad et al. (2019) in their study on *Citrus sinensis* peel revealed similar findings where extraction resulted increase in antioxidant activity and a decrease in naringin and other phenolics attributed to the conversion of galloylated form of polyphenolic compounds to agalloylated form having higher reducing activities.

Moreover, heat processing of food has some effects on the polyphenolics yield. Treatment like pasteurization, blanching, drying, roasting, steam heating etc. plays an important role in signifying the phytochemical potential of the foods (Fuleki & Ricardo-Da-Silva, 2003; Galaz et al., 2017; Heras-Ramírez et al., 2012; Park & Lee, 2021; Zhang, Chen, Li, Pei, & Liang, 2010; Zhang, Cardon, Cabrera, & Laursen, 2010). Most of these studies depicted a significant loss in total phenolic content, flavonoids and antioxidant activity. Where the degree of loss is a factor of process method used for for example, effect of hot air drying on flavonoids is more intense than the freeze drying (Zainol, Abdul-Hamid, Bakar, & Dek, 2009). However, some studies showed positive impact of temperature on prevention of polyphenols by inactivating different hydrolytic and oxidative enzymes (Dewanto et al., 2002; Galaz et al., 2017). The effect of thermal treatment on

water-soluble plant pigments having antioxidant potential is more prominent. Study on monomeric anthocyanins revealed highest degradation rate of anthocyanins to anthocyanidins after thermal processing attributed to oxidation and disruption of covalent bonds (Turturică, Stănciuc, Bahrim, & Râpeanu, 2016). On the contrary the water insoluble plant pigments like carotenes are more stable to heat treatment. A study on thermal processing of tomatoes depicted stability of lycopene at high temperatures of 130°C, beyond which the pigment start degrading but to a lower extent (Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010).

Furthermore, individual polyphenolic compounds behave differently at similar thermal processing conditions. A study on buckwheat groats revealed higher thermal stability of rutin than other flavonoids (vitexin, isovitexin, homoorientin and orientin) (Zielinski, Michalska, Amigo-Benavent, del Castillo, & Piskula, 2009). Similarly, pasteurization of strawberry juices at 90°C for 60 s did not affect the quercetin and kaempferol contents but significantly reduced the naringin, quercetin, naringenin content in processed grapefruit juices (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2011; Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2008). These findings are attributed to the structural differences in the polyphenolic compounds (Buchner, Krumbein, Rhon, & Kroh, 2006). The variation in thermal stability, however, could also be due to structural difference of food matrices where it act as barrier to the heat applied. More promising and uniform results for bioactive stability are reported in case of novel technologies viz. high-intensity pulsed electric fields, microwave, infra-red heating, high-pressure processing (Odriozola-Serrano et al., 2008; Srinivas, King, Monrad, Howard, & Zhang, 2011; Xi & Shouqin, 2007). The degradation of bioactives is not only a function of temperature but also depends on the pH, oxygen, and other phytochemicals (Buchner et al., 2006; Murakami, Yamaguchi, Takamura, & Matoba, 2004).

5.2.1.2 Nonthermal processing

Preparation and processing of foods include primary and secondary unit operations where mechanical processes of cutting, peeling, trimming, chopping, pressing, and filtration significantly affect the functional properties of food (Pap et al., 2012; Pérez-Gregorio et al., 2011; Renard et al., 2011). Further, with increasing concern of consumers for wholesome and safe food, various nonthermal processing have occupied significant place in food industries. Pressure, pulse electric field, electrical voltage, ultrasonication, microwave, ultraviolet radiation, cold plasma are few important components of these new processing and preservation technologies. Numerous studies are based on these technologies where researchers have focused on analyzing the short- and long- term effects on physicochemical and functional properties of different food components (Table 5.3).

5.2.1.2.1 Mechanical processing

Different stages of preparation of raw material viz. peeling, trimming, chopping, slicing, to further processing of food matrix through crushing, pressing, and filtration involve the use of mechanical energy. This processing has significant effect on phytochemicals with respect to their extraction yield, bioactivity, and bioavailability (Nicoli, Anese, & Parpinel, 1999). The major loss of polyphenolics was observed in initial stages of processing or preprocessing steps. The reduction is primarily attributed to the removal of parts during peeling and trimming of the fruits or vegetables, having high content of bioactives. Slicing also revealed a negative effect on the polyphenols (Makris & Rossiter, 2001). However, the cutting has shown contrary results in many studies where the step was responsible for a significant increase of flavonol content (Pérez-Gregorio et al., 2011; Tudela,

Table 5.3 Effect of nonthermal processing on plant extract stability.

S. No.	Treatment	Food matrix	Processing conditions			Impact on functional quality and stability of plant extract			References
			Pressure	Time	Others	TP	AOX	Others	
1.	HPH	Apple, grape and orange juice	250 MPa	10 min	–	Apple juice. 28.5% decrease in TP Grape juice. 14.6% increase in TP Orange juice. 29% increase in TP	Apple juice. 39% reduction in AOX Grape juice. 16% increase in AOX Orange juice. 29% increase in AOX	29% reduction of phenolic bioaccessibility in apple, and preservation in grape and orange juice	He et al. (2016)
2.	HPP	Onion	400 MPa	5 min	Temperature: 25°C; compression rate: 500 MPa/min	TP increased by 6%	9.6% increase in DPPH value	Bioaccessibility of total flavonols was preserved (~17.63%)	Fernández-Jalao et al. (2017)
3.	HVED, PEF and US	Exotic fruit juice with *Stevia rebaudiana*	–	–	Energy level. 32 kJ/kg, 256 kJ/kg; *PEF*. 50 to 400 pulses; electric field strength – 25 kV/cm; temperature- 35°C *HVED*. 50 to 400 discharge number, initial voltage peak amplitude- 40 kV *US*. 400 W, 24 kHz frequency; 100% amplitude, cycle- 1.	TP after high energy HVED and PEF were increased (2%–4%)	ORAC values depicted an increase (16%–22%) in AOX after PEF and US	Increase in bioaccessibility of TP (34.2%) after HVED at low energy level, further decrease to 16.7% at 256 kJ/kg; in case of PEF and US increase in TP bioaccessibility at both energy levels; Total carotenoids increased (18%) after low energy PEF treatment, while high energy HVED and USN caused 28%–45% decrease in total carotenoids	Buniowska, et al. (2017)

(*Continued*)

Table 5.3 Effect of nonthermal processing on plant extract stability. ***Continued***

S. No.	Treatment	Food matrix	Processing conditions			Impact on functional quality and stability of plant extract			References
			Pressure	Time	Others	TP	AOX	Others	
4.	HIPEF, HHP	Water-(WB), Milk-(MB) and soymilk-fruit (SB) juice beverage	HHP. 400 MPa	5 min	HIPEF. 35 kV/cm, 1800 μs	10% and 20% decrease in WB after HPP and HIPEF; whereas HPP and HIPEF of MB and SB reported ~20% increase	Around 12% and 16% decrease of AOX in WB; 30% and 27% increase in MB; 18% and 10% decrease in SB was observed after HIPEF and HPP, respectively	HIPEF reduced the vitamin C in the range of 8%−15% in all beverages; HPP revealed a decrease of 10.5% in vitamin C in SB	Rodríguez-Roque et al. (2015)
5.	HHP	Germinated brown rice	100, 300, and 500 MPa	10 min	Temperature-18°C	–	At 500 MPa, AOX increased by 12.72% as depicted by DPPH value	The *in vitro* bioaccessibility of calcium and copper was increased by 12.59%−52.17% and 2.87%−23.06%, respectively, after HHP	Xia et al. (2017)
6.	Thermal treatment, US, UV-C	Mango juice	–	–	*Thermal treatment*:90°C for 30 and 60 s *US*: 15, 30 and 60 min at 25°C, 40 kHz frequency *UV-C light treatment:* 15 min, 30 min and 60 min at 25°C	Significant improvement in extraction of quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin, and tannic acid after US and UV-C	Nearly 3% increase in AOX in non-thermally treated samples	Thermally and non-thermally treated samples depicted extended shelf life of 4−5 weeks at 4°C	Santhirasegaram et al. (2015)

7.	UV, US, cold plasma treatment, thermal processing	Tomato based beverage	–	–	*UV processing*: 10 min and 15 min in UV cabinet at 254 nm *Cold plasma treatment:* 260 V, 60 kV at 50 Hz for 10 min and 15 min *Ultrasonication:* 240 V, 37 kHz for 10 and 15 min *Thermal processing:* 80°C for 2 min	Significant increase in TP and individual phenolic compound after non-thermal treatment, highest increment was observed after cold plasma treatment	–	1 log reduction in bacterial, yeast and mold count; no effect on glucose and fructose content	Mehta et al. (2019)
8.	UV radiation	Starfruit juice	UV radiation dose of 2.158 J/m^2	30 and 60 min	Temperature: 25°C ± 1°C.	3.1% and 6.2% increase in TP at 30 min and 60 min, respectively	Percentage increase of 1.9 and 3.4 in AOX at 30 min and 60 min of UV exposure time, respectively	% increase in flavonoids was 17.4 at 30 min and 60 min; ascorbic acid was decreased by 10% and 20% after 30 min and 60 min, respectively	Bhat, Ameran, Voon, Karim, and Tze (2011)
9.	Pressing	Apple juice	20 kg apple/ pressing	–	Temperature 4°C, 11°C, 18 and 25°C	Increase in TP content after thermal pressing	–	Increase of the proanthocyanidins (> 50%)	Renard et al. (2011)
10.	Ultrafiltration	Blackcurrant juice	1 bar to 2.75 bars	–	recirculation flow rate of 220 L/h at feed temperatures of 25°C and 45°C	–	–	50% and 46% decrease in anthocyanin and flavonol content after filtration	Pap et al. (2012)
11.	HHP	Orange juice	600 MPa	4 min	–	~6% reduction in TP after treatment	25% loss in AOX as measured by FRAP assay	Preservation of bioaccessibility of TP	Mennah-Govela and Bornhorst (2017)

(*Continued*)

Table 5.3 Effect of nonthermal processing on plant extract stability. ***Continued***

S. No.	Treatment	Food matrix	Processing conditions			Impact on functional quality and stability of plant extract			References
			Pressure	Time	Others	TP	AOX	Others	
12.	Soaking	Coleslaw mix	–	5 min	soaking in 5 g/L ascorbic acid and 5 g/L citric acid solution	–	–	A reduction of 20% in glucosinolate, 26% in glucoiberin, and 14% in sinigrin levels after pretreatment	Radziejewska-Kubzdela and Olejnik (2016)
13.	Chopping	Asparagus	–	–	Chopped into small pieces (0.5-cm length), kept at room temperature, under open air, for 60 min	–	–	18.5% decrease of rutin content	Makris and Rossiter (2001)
14.	HVED	Cocoa	–	15, 30, and 45 min	40 and 80 Hz	–	–	11% and 70% loss in epicatechin gallate and catechin concentration, respectively	Barišić et al. (2020)
15.	UV-light	Elderberry fruit	–	Pulsed UV duration: 5, 10, 20, 30 s	3 energy dosages:4500, 6000, 11,000 J/m^2/pulse	Highest increase in TP ~50% was found with 11,000 J/m2/pulse for 10 s	–	–	Ramesh, Valérie, and Mark (2012)

AOX, *antioxidant activity;* HHP, *high hydrostatic pressure;* HIPEF, *high-intensity pulsed electric fields;* HPH, *high pressure homogenization;* HPP, *high pressure processing;* HVED, *high voltage electrical discharges;* MWH, *microwave heating;* PEF, *pulsed electric fields;* TAC, *total anthocyanin content;* TEY, *total extraction yield;* TF, *total flavonoid;* TP, *total phenols content;* US, *ultrasonication;* UV, *ultraviolet radiation.*

Cantos, Espin, Tomás-Barberán, & Gil, 2002). The results were supported by the fact that wounding induces activation of plant defense system which further activate enzymes like phenylalanine ammonia-lyase, enhancing the biosynthesis of polyphenols (Tudela et al., 2002).

Further studies on pressing and filtration have a positive effect on flavonoid content in association with temperature, solvent, and enzyme; however, the single step processing yielded a significant decrease in the values (Oszmianski, Wojdylo, & Kolniak, 2009; Renard et al., 2011; Van Der Sluis, Dekker, Skrede, & Jongen, 2004). The reduction was associated with the compounds left behind in the pomace or residues. Application of other factors (temperature, solvent, and enzyme) lead to cell lysis, disruption of bonds, breakdown of complex food structure, solubilization of active compounds, and their release into the solvent, thereby improving the yield of phytochemicals with simultaneously reducing their loss in pomace or residues (Korus, Słupski, Gębczyński, & Banaś, 2014; Pap et al., 2012; Van Der Sluis et al., 2004).

5.2.1.2.2 Novel processing technologies

Application of hydrostatic pressure, pulse electric field, ultrasonication, pressure-induced homogenization, ultraviolet radiation, irradiation, and cold plasma are extensively used in food industries. The commercialization of these technologies for food processing and preservation has been attracting researchers to investigate their interaction with different food components responsible for bringing nutritional and sensory changes in the products. Largely, these processing methods have reported to impart positive effect on phytochemical bioavailability and bioaccessibilty (Table 5.3).

The pressure treatment in the form of high hydrostatic pressure (HPP) processing or high pressure homogenization (HPH) reduces the negative effects of processing on phytochemicals, and even increase their content (Lorenzo et al., 2019; Rodríguez-Roque et al., 2015). However, depending upon the food matrix the treatment may reduce the bioaccessibility (He et al., 2016). This variation in results was explained by the difference in composition of the food. The increase in after treatment phenolic content is attributed to structural changes in tissues, facilitating the release of bioactives. High pressure cause alterations in physical structure, induces chemical reactions, and decrease the volume under the effect of molecular volume change. The compression in volume affects the integrity of cell structure and bring modifications in its permeability, thus facilitating the release of bioactives from the cell and further improve the solubilization of the compounds (Patterson, 2014). Further this can also affect the in-vivo accessibility and availability of the compound, though no correlation has yet been found between the structure disruption and bioavailability of phytochemicals (Barba, Terefe, Buckow, Knorr, & Orlien, 2015). Conversely, the reduction in bioactive content could be due to release of polyphenol oxidase along with other cytoplasmic compounds which are known to trigger oxidation reactions, epimerization, and finally the degradation (He et al., 2016).

Other processing treatments also revealed on par results with pressure treatment where the stability of plant extracts were manifested by the polyphenol content and antioxidant activity (Fig. 5.1) (Buniowska, Carbonell-Capella, Frigola, & Esteve, 2017; Rodríguez-Roque et al., 2015). Treatment of food using pulse electric field (PEF) revealed a significant interaction of food matrix and individual phenolic compound and their effect on the yield and bioaccessibility of phytochemicals (Rodríguez-Roque et al., 2015).

The optimization of PEF process involves tuning of several factors of electric field intensity, properties of pulse, treatment time, electrode configuration, and temperature. In addition the

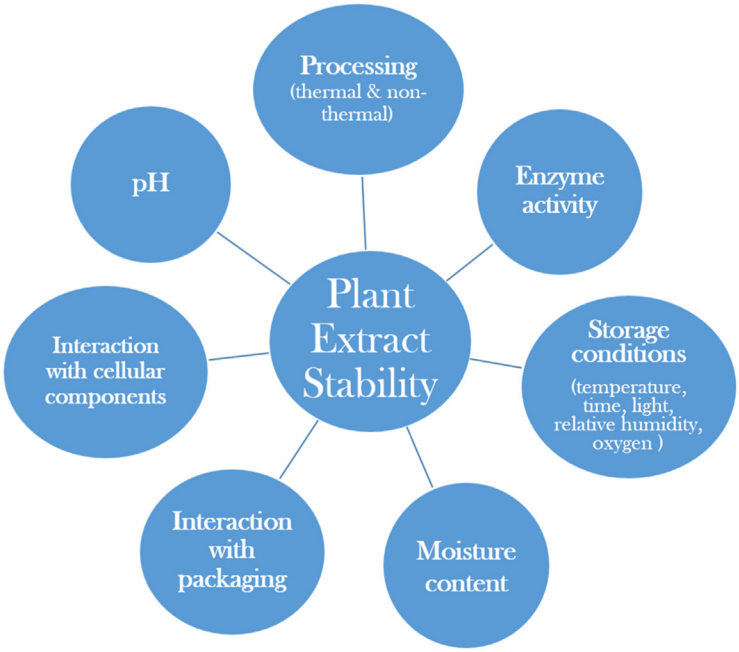

FIGURE 5.1

Factors affecting stability of plant extracts.

properties of food including type of matrix, pH, the ionic strength, and conductivity also become crucial during the PEF treatment of food with the objective of maximizing the phytochemical yield (Knorr et al., 2011; Li & Farid, 2016). The increase in phenolics content and their activity is suggested to be an effect of electroporation. This phenomenon causes perforation and compression of the cell membrane and leakage of intracellular content, breaking of bonds and complex compounds, thereby increasing their solubility and extractability (Barba et al., 2017).

Another important novel technology is based on sound waves ultrasonication. The positive effects of this treatment on bioactive release is associated with acoustic cavitation. Sonication generates microbubbles in the system which continuously grow in the medium, and collapse after certain time, giving rise to shock waves. This is these waves which generate high temperatures and pressures, resulting in the cavitation mechanism (Cravotto & Binello, 2016). The cavitation in food matrix is responsible for producing shear forces and thereby disrupt the cell structure, breaks down the polysaccharides or other polymers, and hence helps in releasing bound phytochemicals. Also disruption in cell structure leads to an increase in the contact between solvent and solute (bioactive compounds), enhancing penetration of solvent and improving extractability of phytochemicals. Ultrasonication under controlled parameters are widely reported to increase the phenolics yield and antioxidant activity. However, the treatment has also depicted negative effects on plant phenolics when the matrix was subjected to extreme conditions of sonication energy, this is attributed to the degradation of the compounds due to their scavenging actions on newly formed free radicals

(Nishad et al., 2019). Sonication as pretreatment is also reported to be very efficient in increasing the bioaccessibility of the compounds (Fonteles et al., 2016).

Ultraviolet radiation (UV) is also another important processing technology, which is widely investigated for its effects on the levels of phytochemicals and on the capability of plants to produce them at different levels. Various conditions of radiation exposure, exposure and storage temperatures, wounding of the plant matrix, sensitivity of the compounds, and effect of treatment on other constituents which are responsible for production or accumulation of phytochemicals, may increase the concentration of bioactive compounds. Enhanced antioxidant activity of a plant after treatment is mainly attributed to two mechanism: (1) increase in enzyme activity of phenylalanine ammonia-lyase and peroxidase, (2) increase in extractability of compounds from the cellular matrix. Increase in activity of these enzymes trigger the polyphenolic synthesis and hence add to their content. On the contrary, inactivation of some enzymes like polyphenol oxidase tends to retain the antioxidant power of the system (Alothman et al., 2009; Oms-Oliu et al., 2012). Also, disruption in chemical bonds of polyphenols under the effect of treatment facilitates the release of more soluble phenols of low molecular weight, enhancing the phenolic yield and antioxidant activity. Numerous studies have also reported formation of free radicals after radiation treatment which further results in a decrease of phytochemical content (Sajilata & Singhal, 2006).

Similarly, other novel technologies like high-voltage electrical discharges (HVED) showed promising results in increasing the yield of phenolics and their bioavailability (Buniowska et al., 2017). These findings highlight the importance of nonthermal processing techniques to stabilize the plant extracts for their further utilization in functionalization.

5.2.2 Effect of pH

pH is another very important factor widely investigated for its effects on extraction yield and stability of phytochemicals (Table 5.4). The plant matrix, their extracts, and foods are generally exposed to a wide pH range during their processing and storage, affecting their functionality. Therefore it becomes imperative to know about the antioxidant systems and their sensitivity toward acidity or alkalinity for the optimization of technological and processing conditions. Many researchers have confirmed a significant interaction between the type of phenolic compound and the pH. Many polyphenolic compounds like anthocyanin are more stable at acidic pH and showed a decrease in bioaccessibility at high pH, on the contrary phenolic compounds like procyanidin B, kaempferol, chlorophyll revealed opposite trend with respect to their stability (Ismaiel et al., 2016; Nagar et al., 2021; Roy & Urooj, 2013). There are many other factors associated with the effect of pH where pretreatment, heat processing, exposure to high pressure, presence of other food components or polysaccharides have depicted the change in trend of availability, extraction and overall stability of phytochemicals (Nagar et al., 2021; Roy & Urooj, 2013). In a study on blue green algae, the spirulina was found to be stable at wider pH range but showed a decreased growth rate at pH above 10 and depicted a significant color change with the inhibition of chlorophyll and carotenoids synthesis. This degradation in growth rate was explained by correlation of photosynthetic activity and pH, high pH inhibit the photosynthesis due to inaccessibility of carbon dioxide. The bicarbonate if available as an only source of carbon then it cause a rise in pH to 8 and eventually limiting the content of free CO_2. This deficiency further brings cells under stress and induce formation of free radicals or ROS or oxidative stress. The variation in the content of phenolics at high pH is further

Table 5.4 Effect of pH on plant extract stability.

S. no.	pH range	Other processing conditions	Food matrix	Impact on plant extract			References
				TP	AOX	Others	
1.	3.0, 5.0, 7.0 and 9.0	Storage: at 4°C in closed containers (0.5-l flasks) in the dark for up to 400 days	Grape marc extract	No significant change in TP for all pH	Loss of AOX at pH 7 and 9 over the first 100 days	Clouding of extracts was observed in all the pH solutions during stoarge	Amendola, De Faveri, and Spigno (2010)
2.	4.0, 7.0 and 9.0	Preincubation at various pH values for 24 h	Methanolic antioxidant extract of leaves of: Pomegranate (PM), sweet potato (SPL), carrot (CL), kilkeerae (KL), shepu (SH), beet greens (BL)	–	Highest AOX activity of PM, CL and KL at pH 4, for SPL and SH at pH 9, for BL at pH 7	–	Roy and Urooj (2013)
3.	3.0, 4.0, 5.0, 6.0 and 7.0	At different temperatures (4°C, 25°C, and 100°C) for 24 h	Tea	Tea polyphenols were more stable at low pH; Catechins degraded (21%) at pH 7 after 24 h	–	~50% decrease in clarity of the extracts at high pH of 7.0°C and 100°C	Zeng, Ma, Li, & Luo, (2017)
4.	3.0, 5.0, 7.0 and 8.0	–	Sweet potato leaf	TPC was higher in neutral and weak acid solvent; optimum pH- 5.0–7.0	AOX were higher (2.71 mg TE/mL) at pH 7	–	Sun, Mu, and Xi (2017)
5.	7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0	Incubation: at 31°C with continuous cooling white fluorescent lights (60 μmol/photons m^2 s)	Blue green algae	Highest TP (12.1 mg GAE/g DW) observed at pH 9.5	Highest AOX at pH 9.0 was reported with a percent increase of 567%	Optimum pH for growth was 9.0; highest chlorophyll a (10.6 mg/g DW), carotenoids (2.4 mg/g DW) at alkaline pH of 8.5	Ismaiel et al. (2016)
6.	6.0 and 7.8	Plant extracts were mixed with 50 mM KPi, pH 6.0 or 7.8, at the ratio 1:20 (v/v) and incubated for 30 min in	*Rosa canina* L., *Rhodiola rosea* L., *Hypericum perforatum* L.,	-	AOX at pH 6.0 was 1.4-fold higher for *R. rosea* and *G. lutea* extracts and 1.2-fold higher for	–	Bayliak et al. (2016)

		the presence of 10 mM H_2O_2	and *Gentiana lutea*		*R. canina* and *H. perforatum* extracts		
7.	2.5 and 6.5	*HPP:* samples were treated for 10 min at 600 MPa (initial temperature 25°C) *Pasteurization*: 90°C for 30 s	Strawberries	Decrease in polyphenols at high pH except procyanidin B, kaempferol-3-O-glucuronide and kaempferol-3-O-malonyl-glucoside which showed opposite trend	–	Decrease in bioaccessibility of anthocyanins by 50% at neutral and high pH	Nagar et al. (2021)
8.	4.0 and 9.0	Storage: (a) in dark under refrigeration (5°C) (b) in dark at room temperature (25°C)	Drumstick leaves, mint leaves and carrot tuber	–	AOX of mint leaves and carrot tuber extracts was higher at pH 9 than at pH 4, for drumstick leaves it was unchanged in both alkaline and acid pH	–	Arabshahi-D, Devi, and Urooj (2007)
9.	3.3, 6.3 and 8.3	Extraction: 50% ethanol with a solid/liquid ratio of 1/15; 40°C for 40 min	Blueberry pomace	TPC significantly increased (9%) with increase in pH above 6.3	AOX increased by 9% at pH 8.3	TAC significantly decreased (5.5%) above pH 6.3	Bamba et al. (2018)
10.	4.0 to 9.0	–	Lettuce extract (LE) with quercetin (QC), green tea extract (GTE) or grape seed extract (GSE)	–	14%–40% increase in AOX at pH 9.0 in LE with different phenolics	–	Altunkaya, Gökmen, and Skibsted (2016)

AOX, *antioxidant activity;* HPP, *high pressure processing;* TEY, *total extraction yield;* TP, *total phenols content;* TF, *total flavonoid;* TAC, *total anthocyanin content.*

explained by the generation of oxidative stress. The increase in the concentration corresponds to plant reaction toward the oxidation for alleviation of free radicals. The further increase in pH demonstrated a reduction of phenolics, attributing to inability of cells to function under very high pH conditions (10.5−11.0). Impairment in cell functioning results in cessation of phenolics production, and growth rate, depicting low biomass, and pigment production. The hydrogen peroxide model have been successfully used to evaluate the prooxidant/oxidant properties of the plant extracts at varying pH range (Bayliak et al., 2016). Thus the pH showed effects on auto-oxidation, non-enzymatic antioxidants (phycocyanin and phenolics), and antioxidant enzymes activities, which are responsible for the oxidative stability of plant extracts (Chu, Lim, Radhakrishnan, & Lim, 2010; Ismaiel et al., 2016).

5.2.3 Effect of storage

Storage of food products is an integral part of food supply chain and has been widely studied for its impact on nutritional and functional profiles of foods. This is a major concern for food industries to control and monitor the quality loss during storage. Storage conditions of time, temperature, relative humidity, presence of oxygen and light are the major factors deciding the degree of loss in foods. Mostly researchers have investigated combinations of different factors for their effect on fresh produce and processed products (Table 5.5). Significant variability in the responses of plants was observed on the basis of their processing state i.e. fresh or processed, where fresh commodities or juices revealed no effect on the polyphenolics, on the contrary processing of matrices induced the degradation of active compounds (Ali et al., 2018; Bazinet, Araya-Farias, Doyen, Trudel, & Têtu, 2010; Radziejewska-Kubzdela & Olejnik, 2016; Santhirasegaram, Razali, George, & Somasundram, 2015). However, the effect of processing is not consistent and did depict a different trend in other products (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009; Zafrilla, Ferreres, & Tomás-Barberán, 2001). This discrepancy could be explained by the fact that the parameters of storage have synergistic or antagonistic effects on bioactive stability and shelf life (Ioannou et al., 2012). Photostability of plant and their extracts is another important deciding factor of its antioxidant activity and stability. Presence of light act as stress signal and expedite the process of phenol degradation by keeping up the mechanism of their synthesis active even at low temperature (Tudela et al., 2002; Wang et al., 2009a,b). Light is known to effect phytochemicals during different stages of plant growth, its processing, and storage. Further, the efficiency of light in affecting the plant extracts is mainly a factor of wavelength of light, duration of exposure, pH of matrix, physicochemical properties, concentration of the compound, and the structure (Ioannou et al., 2012). Exposure to light with low wavelength revealed photo-induced molecular rearrangement when compared with high wavelength light which triggered photooxidation (Tommasini et al., 2004). Polyphenolic compounds can either increase or degrade in presence of light depending on the processing state of the food. In fresh foods, light induce a stress signal and triggers the mechanism of synthesis of active compounds (Pérez-Gregorio et al., 2011). Conversely, in processed food products photo degradation of the functional compounds was observed by many researchers. Among different polyphenolic compounds some are more prone to get affected by light such as anthocyanins, chlorophylls, carotenoids etc. (Boon, McClements, Weiss, & Decker, 2010; Lee, Ahn, & Choe, 2014; Março, Poppi, Scarmino, & Tauler, 2011). On the contrary some studies yielded insignificant or no effect on anthocyanins after exposure to light (Dyrby,

Table 5.5 Effect of storage conditions on plant extract stability.

S. no.	Food matrix	Storage conditions				Impact on plant extract			References
		Temperature	Duration	Light/gaseous atmosphere	Other conditions	TP	AOX	Others	
1.	*Piper betle* extracts	5°C and 25°C	6 months	With and without light	–	TP extract stored at 5°C with and without exposure to light showed >99% retention; storage at 25°C lead to retention of 97% (dark) and 93% (light)	High AOX stability was observed at 5°C in dark which retained 99.98% activity after 180 days; extracts at 25°C with light were least stable (90% of activity)	Complete loss of isoeugenol at 25°C with or without the presence of light after 30 days of storage	Ali et al. (2018)
2.	Sweet potato leaf	55°C, 65°C, 80°C, and 100°C	0, 10, 30, 60, and 90 min	Light treatments: direct sunlight from 10:00 am to 3:00 pm	–	TPC of samples after thermal treatment was higher than 91% and after light treatment for 5 h was 98%	AOX retention at 80°C and 100°C, decreased significantlyafter 90 min, and remained at 62.14% and 61.86%, respectively; light treatment for 5 h retained the AOX up to 92.5%	–	Sun et al. (2017)
3.	Rutin, naringin, mesquitol, eriodictyol, luteolin, luteolin-7-O-glucoside	25°C	15 days	Model solutions exposed to 0% light (darkness) and 100% light (equivalent to exposure at 16.5 klux) 2 models: nitrogen bubbling of 2 min (O_2 conc. of 15%); without bubbling (O_2 conc. of 85%)	–	–	Antioxidant activity of rutin and mesquitol increased in the presence of O_2 and light	Decrease in flavonoid content as a function of light intensity, O_2 conc. and structure of compound	Chaaban et al. (2017)

(*Continued*)

Table 5.5 Effect of storage conditions on plant extract stability. ***Continued***

S. no.	Food matrix	Storage conditions				Impact on plant extract			References
		Temperature	Duration	Light/gaseous atmosphere	Other conditions	TP	AOX	Others	
4.	Coleslaw mix	4°C	12 days	Packaged under modified atmosphere consisting of 5/10/85, 20/25/55, 50/30/20, 70/30/0% of O_2/CO_2/N_2, as well as air	Pretreatment-soaking in an ascorbic acid (5 g/L) and citric acid (5 g/L) solution	–	–	Total glucosinolates was highest with 38% increase in the samples packaged under the modified atmospheres with 5/10/85%O_2/CO_2/N_2	Radziejewska-Kubzdela and Olejnik (2016)
5.	Garlic clove	20°C ± 2°C	12 weeks	1000 lx light/dark (12 h/12 h) cycle	45% RH	TP reached maximum values (839.96 μg/g DW) after 6 weeks	AOX reached maximum values at 8 weeks (26% incraese), then decreased significantly	Levels of 18 organosulfur compounds increased from 2 to 6 weeks, reached maximum level 41.36 ± 2.34% at 8 weeks, then decreased significantly	Fei, Tong, Wei, and De Yang (2015)
6.	Mango juice	4°C ± 1°C	5 weeks	–	–	–	–	Aerobic plate counts increased from 2.74 to 8.32 log CFU/mL and yeast and mold counts increased from 2.42 to 6.10 log CFU/mL after 5 weeks storage	Santhirasegaram et al. (2015)
7.	*Anemopsis californica*	50°C, 25°C, 4°C, and −20°C	180 days (stability was measured every 30 days)	Under ambient light and dark conditions (using amber bottle)	–	79% of total phenols was conserved at the end of storage, at −20°C in dark	Best conditions for AOX stability were 4°C (95%) and −20°C (98%) under dark	Retention of 73% of total flavonoid after 180 days at −20°C in dark	Del-Toro-Sánchez et al. (2015)
8.	*Hypericum perforatum*	–	6 months	Storage conditions: 1. 25°C with uncontrolled humidity with daylight 2. 25°C with uncontrolled humidity without daylight, dark	–	–	–	Chlorogenic acid was the most stable, decay of phenolics was lowest at −20°C and highest at 40° C and 75% RH; dark condition decreases breakdown within 4 months	Koyu and Haznedaroglu (2015)

				3. 25°C, 65% relative humidity 4. 40°C, 75% RH 5. −20°C 6. 4°C					
9.	Seeds from four *Brassica oleracea* varieties: Broccoli, kale, Penca cabbage, and red cabbage	–	Harvesting of sprouts: green sprouts (GS) after 7, 9, 12 and 15 days of germination and white sprouts (WS) after 5, 6, 7, 9 and 12 days	Photoperiod regimes: for GS production a cycle of 16 h of light and 8 h of darkness; for WS under dark	Sprouting: 12 h in darkness, at room temperature, light agitation; 25°C temperature, and different photoperiod regimes	TP of red cabbage and Penca cabbage WS decreased about 10% along all the experiment (from day 5 to day 12). WS of kale and broccoli showed an increase in TP from day 5 to 7, followed by a decrease (day 9 to day 12 of 20% and 10%, respectively)	Red and Penca cabbage sprouts produced under light cycles showed highest AOX (57.11 μg/mL) than kale	Seeds revealed TFC maxima after different germination days; Broccoli GS showed after 12 days of germination (24.0 mg QE/g), red cabbage and Penca cabbage after 7 days (41.2 mg and 24.7 mg QE/g) and Galega kale after 9 days of germination (25.4 mg QE/g)	Vale, Cidade, Pinto, and Oliveira (2014)
10.	Hazelnut	In-shell hazelnuts storage-ambient temperatures (10°C−26°C) Shelled hazelnuts: 4°C	1 year	Shelled hazelnuts: with or without modified atmosphere (1% O_2, 99% N_2) for	In-shell hazelnuts: 60%−80% RH Shelled hazelnuts: 55% RH	TP decreased after 8th month for shelled and in-shell hazelnuts, then showed no change, with a slight increase (13%) in refrigerated kernels	Highest AOX (6.29 TE mmol/kg) in refrigerated kernels after 12 months	Refrigerated storage reduced the lipid oxidation, with best result in modified atmosphere (0.057 O_2 mmol/kg) after 12 months	Ghirardello et al. (2013)
11.	Pomegranate peel		0, 1, 5, 10, 30, 60, 90, and 180 days	2 packaging methods (no light and exposure to light)	pH values (3.5, 5.0, and 7.0)	67% retention of TP at low pH (3.5) in dark packaging	AOX was retained to 58% at low pH (3.5) in dark packaging	Extracts stored at high pH were more opaque and looked darker and chromaticity deeper in color than that at the low pH	Qu, Breksa, Pan, Ma, and Mchugh (2012)

(*Continued*)

Table 5.5 Effect of storage conditions on plant extract stability. *Continued*

S. no.	Food matrix	Storage conditions				Impact on plant extract			References
		Temperature	Duration	Light/gaseous atmosphere	Other conditions	TP	AOX	Others	
12.	Fresh cut onions	–	0, 1, 3, 8 and 16 days	Treatment: white visible light at a 45-cm distance (fluorescent tube-lamp at 14 W 230–240 V, 50–60 Hz)	Packaging 1: under vacuum and refrigerated storage at 1–2°C in the absence of light in PA/PE 20/70 (90-lm thickness) bags Packaging 2 and 3: in closed cups of PS or PET cups (12.5X 9 cm, 4 cm depth) stored under refrigeration (1°C–2°C) in the absence of light Packaging 4: closed cups of PS stored under refrigeration in the presence of visible light	–	–	A 12%–30% reduction in TAC after 16 days in different packaging; increase of total flavonols by 28% under PS packaging, increase of total flavonols by 58% and total anthocyanins by 39% in presence of light	Pérez-Gregorio et al. (2011)
13.	Litchi	–	–	Continuous flow (30 mL/min) of humidified air (control) and 100% O_2	Dipping treatment: dipped for 3 min in 0.1% Sportak fungicide solution and air-dried for 2 h at 28°C	After 4 and 6 days of storage, TP in pure oxygen–exposed fruits was maximum	Exposure to oxygen reduces the reduction rate of AOX	Exposure to pure oxygen enhanced the activity of superoxide dismutase by 20%, and catalase by 40%, compared with that of 0 days	Duan et al. (2011)

14.	Sprouted seeds (wheat, radish and lentils)	27°C	–	Light wavelengths: 385, 445, 510, 595, 638, 669, and 731 nm; photosynthetic photon flux density of about 100 μmol/m^2 s and a 12 h photoperiod were maintained during treatment	Sprouting: for 24 h in germination plates at 18°C; under light conditions	Accumulation of TP was greater in red light radiated lentil, radish, and wheat seeds	After 3 days germination, the AOX was increased by about 12% in wheat seeds in green light (510 nm), whereas decreased by 50% in radish	Positive effect of green light on vitamin C was observed in all treated seeds, whereas red radiation led to a significant decrease of vitamin C	Samuolienė et al. (2011)
15.	Enriched tea drink	4°C and 25°C	6 months	-	-	-	-	No effect on catechin was observed at 4°C however at 25°C reached to 0 after 30 days	Bazinet et al. (2010)
16.	Ginger	-	-	The plants were grown under four level of glasshouse shade (0%, 20%, 40% and 60% shade) corresponding to 790, 630, 460 and 310 μmol/m^2s of photosynthetically active radiation (PAR)	Harvesting- 16 weeks	790 μmol/m^2s was best for maximum TP production	AOX were higher in the leaves under 310 μmol/m^2s	Higher TF (5.95 mg/g DW and 8.45 mg/g DW) under 310 μmol m^2s of light intensity	Ghasemzadeh, Jaafar, Rahmat, Wahab, and Halim (2010)
17.	Mulberry fruits	40°C, 50°C and 70°C	0, 1, 2, 3, 6, and 10 h	Fruit extracts placed under normal fluorescent lights (220 V, 50 Hz and 0.37 A) at about 18 in. distance at room temperature for 10 h	–	–	After thermal and light treatment for 10 h, the AOX decreased significantly	Around 25% loss in TAC after 10 h at 70°C; 18% decrease in TAC after light treatment for 10 h at room temperature	Aramwit, Bang and Srichana (2010)

(*Continued*)

Table 5.5 Effect of storage conditions on plant extract stability. *Continued*

S. no.	Food matrix	Storage conditions				Impact on plant extract			References
		Temperature	Duration	Light/gaseous atmosphere	Other conditions	TP	AOX	Others	
18.	Raspberries	24°C during the day (0700–1900) and 16°C at night (1900–0700)	1, 2, 3, and 4 days	RH at 75% and under three different light intensities (fluorescent lamps for 12 h/day (0700–1900)); Light treatment: photosynthetically active radiation (PAR) of 56 ± 0.5 μmol/m^2s (H), 31 ± 0.2 μmol/m^2s (L), and in the dark (D)	Harvesting at five different stages based on surface red color: (1) 0%–5% red, (2) 20% red, (3) 50% red, (4) 80% red, (5) 100% red	Fruits of greener stages showed highest TP; fruit exposed to higher light intensities had higher TP especially during the first 2 days of storage	Fruit harvested at greener stages (5% and 20%) consistently yielded higher AOX	TAC increased with fruit maturity and during storage	Wang et al. (2009a, 2009b)
19.	Blueberries	–	–	Light treatment: UV-C lamp, 254 nm, time- 1, 5, 10, and 15 min equal to the dosages of 0.43, 2.15, 4.30, and 6.45 kJ/m^2	–	2.15 and 4.30 kJ/m^2 yielded highest TP (~60%)	4.30 kJ/m^2 yielded highest AOX (44%)	54% increase in anthocyanin at 4.30 kJ/m^2	Wang et al. (2009a, 2009b)
20.	Longan fruit	28°C	6 days	Atmosphere of 5%, 21% (control) or 60% O_2 (balance N_2) at 28°C and 90%–95% relative humidity	–	Highest TP (12 mg/g FW) was observed in fruits stored at 5% O_2 after 4 days of storage	Fruit exposed to 5% O_2 exhibited highest AOX after 4 and 6 days of storage	Exposure of 5 or 60% O_2 resulted in a higher level of total soluble solids and lower ascorbic acid	Cheng et al. (2009)

AOX, *antioxidant activity;* TAC, *total anthocyanin content;* TEY, *total extraction yield;* TF, *total flavonoid;* TP, *total phenols content.*

Westergaard, & Stapelfeldt, 2001; López-Rubira, Conesa, Allende, & Artés, 2005). Additionally, position of hydroxyl group in benzene ring decides the stability of compound against light, where glycosylation in position 3 gives more stability (Zhang, Cardon, et al., 2010; Zhang, Chen, et al., 2010).

Other factors of pretreatment or mechanical processing of foods prior to storage also play a significant role in the activity and stability of phytochemicals by stimulating the process of oxidation. Storage under modified atmospheric conditions depicts an increase in phenolic content at elevated CO_2 content during certain period and shows a decline over long duration storage. This finding is supported by the stress-induced accumulation of phenolic compounds under high carbon dioxide and oxygen levels, and mechanical damage. Some researchers have also suggested inactivation of enzymes at high CO_2 level, leading to increased content of bioactives. Whereas, during long period storage high CO_2 and oxygen levels may cause damage in cell membrane and enzymatic degradation of polyphenolics. Higher levels of oxygen during high demand for energy can further augment the oxidation of respiratory substrates and make them incapable of transporting increased electrons. This trigger the formation of free radicals and depletion of phytochemicals in scavenging mechanism (Radziejewska-Kubzdela & Olejnik, 2016; Sørensen, 1990; Xu, Guo, Yuan, Yuan, & Wang, 2006). Storage at higher temperatures has negative impact on polyphenolics and ascorbic acid due to increase in oxidation and hydrolytic reactions, which has more deteriorative effects in presence of light (Del-Toro-Sánchez et al., 2015; Kotsiou & Tasioula-Margari, 2016).

Similar to other factors discussed earlier, the stability of plant extracts during storage does also get effected by individual bioactive compound due to presence of structural differences. The reactivity of the polyphenolic is dependent on the position of functional groups. Rice-Evans, Miller, and Paganga (1996) have reported susceptibility of position 3 and 4 in flavonoids toward dihydroxylation than others. Further, presence of hydroxyl group decreases the stability whereas methyl groups makes more stable compounds (Bkowska-Barczak, 2005).

Anthocyanin, in many studies, revealed instability during storage ascribed to residual activity of enzymes, susceptibility of monomeric anthocyanins toward polymerization, and condensation reactions with other phenolics (Brownmiller, Howard, & Prior, 2008; Ochoa, Kesseler, Vullioud, & Lozano, 1999; Reed, Krueger, & Vestling, 2005). Additionally, pH, temperature, oxygen, light, sugars, metal ions have also investigated as limiting factor of anthocyanin stability (Ioannou et al., 2012).

5.2.4 Miscellaneous factors

A food matrix constitute of many components viz. carbohydrates, protein, fats, vitamins, minerals, and polyphenolic compounds. The interaction of these constituents is very significant for the nutritional and functional values of the food, corresponding to their positive and negative synergies. Different bioactive compounds when present in a solution have also shown synergistic interaction with respect to their antioxidant activity and stability, whereas, a different combination of polyphenolics revealed antagonistic effect (Hidalgo, Sanchez-Moreno, & De Pascual-Teresa, 2010). Plant polyphenols are located within the cell prior to processing and do not interact with other cell wall material, however, on processing or extraction these compounds are released and come in contact with carbohydrates, minerals and metal ions and form complexes (Zhu, 2018). The binding of active compounds to these components can either increase or decrease their activity,

bioaccessibility, and bioavailability. The interactions between polyphenols and polysaccharides could be covalent or non-covalent and their strength is affected by molecular size of compounds and the conformational flexibility, demonstrating a difference in the behavior of the polyphenols (Chirug, Okun, Ramon, & Shpigelman, 2018). A study on dietary fiber interaction suggested a negative effect on bioavailability of polyphenol glucoside, attributing to gelation, increased viscosity, or binding and entrapment of compounds (Bohn et al., 2015). Presence of ascorbic acid in conjunction with phenols has also reported to positively affect the stability of both the compounds, suggesting an antioxidation and cooperation effect between the two (Chen et al., 2014). Moreover, investigations have depicted protective effect of water-soluble antioxidants on lipids as compare to lipid-soluble ones, due to the "polar paradox" (Porter, Levasseur, & Henick, 1977).

The naturally occurring plant enzymes including catalases, polyphenol oxidases, peroxidases, amylases are also extensively studied for their impact on plant bioactives. These enzymes can break the complex structure of plant cells, chemically modifying the secondary metabolites, inducing oxidation, and significantly affecting the antioxidant activity and stability of the plant extracts (Ravimannan & Nisansala, 2017; Sachadyn-Król et al., 2016).

Further, the water present in the raw material is critical in plant extract stability. Presence of moisture allows the redox reaction to produce free radicals, having detrimental effects on primary and secondary plant metabolites. Water also facilitate the enzyme activity and degrade the functional quality of the extracts. High moisture further allows the microbial growth in the products and adversely affect the stability of bioactive components (Gafner & Bergeron, 2005).

5.3 Improving stability of plant extracts

Incorporation of plant extracts in food or other formulations subjects the phytochemicals to various processing conditions, which affect their activit and stability. The loss of active ingredients at different unit operations restrict the use of natural plant extracts in food and pharmaceutical industries. The bioavailability of the extracts is another important concern in utilizing the plant extracts in the food. The bioavailability is affected by the solubility of the active compound, stability during different digestion stages, and absorption of the nutrients in the body. Thus it has attracted researchers to find plausible solutions for utilizing the antioxidant potential and improving the solubility, sustainability, absorption, availability, and stability of bioactive compounds (Rahman et al., 2020).

There are many approaches studied for their effectiveness in improving the stability and shelf life of phytochemicals. Characterization of the plant extract is the first step in the process of stabilization where knowledge of physical, chemical, functional, and biological attributes direct the selection of appropriate technique. Nanotechnology is the widely accepted method in stabilization including the formation of nanocoating on the active component and nanoemulsion preparations (Zorzi, Carvalho, von Poser, & Teixeira, 2015). Besides, encapsulation of bioactives, stabilization using water-soluble chelating agents for example, polyvinylpyrrolidone (PVP), use of suspending agent and nonionic surfactant for sparingly soluble or insoluble or sparingly insoluble plant extracts revealed their effect on protecting phytochemicals (Armendáriz-Barragán et al., 2016; Bosch et al., 2004; Rijo et al., 2014; Thakur et al., 2011; Wolf et al., 2007). Controlled storage condition also play an important role in alleviating the adverse effect of temperature, oxygen, light, moisture,

interactions with other ingredients, cross metal contamination from containers, and microbes (Thakur, Prasad, & Laddha, 2008). Furthermore, in a biological plant system inactivation of enzymes and chemical modification of the phytochemicals are very successful in stabilization of the plant bioactives especially pigments. Pretreatments using high temperatures such as steaming, and hot water blanching, and chemical soaking in acid or alkaline solution are the widely used methods for stabilization (Ngamwonglumlert, Devahastin, & Chiewchan, 2017). Biotechnology has also emerged as a novel method of providing stability to phytochemicals where plants are genetically transformed for robustness (Miller, Fatnon, & Webb, 2004).

Nanocarrier is another strategy to thwart the existing problem of pharmaceutical industries. Encapsulation of plant metabolites into biocompatible and biodegradable nanoparticles facilitates the targeted delivery of the compounds and improves their bioavailability (Bharali et al., 2011). Besides, nanonization has other advantages of improved solubility, reducing recommended doses and side effects, and increasing the absorption of pharmaceutical herbs over their crude counterparts. Nanocarrier in the form of solid lipid nanoparticle, nanostructured lipid carrier, nanoemulsion, nanocapsule, drug conjugates, liposome, transferosome, nanosphere, nanocrystals, nanofiber, metal nanoparticle, nanotube, and biological nanocarrier overcome the limitations of utilizing natural plant extracts (Loredo-Tovias et al., 2017; Luo et al., 2011; Rahman et al., 2020; Tully, Fakhrullin, & Lvov, 2015).

5.4 Conclusion

Plant extract is a concoction of numerous components and holds an important place in human nutrition and dietary interventions. Bioaccessibility, bioavailability, and stability thus become imperative to utilize the potential of these phytochemicals. This chapter comprehensively illustrates the factors of consideration for bioactive stability. There are many intrinsic and extrinsic factors influencing the concentration and antioxidant activity of the compounds. Processing of food using thermal or mechanical energy has revealed their effect on individual polyphenols differently. Similarly, other factors of pH, storage conditions (duration, temperature, relative humidity, gaseous environment, and light), and other food components also demonstrated positive and negative correlation with the phytochemical stability and availability. Besides, characteristic of phenolic compounds, their structure conformations, and molecular size decide their sensitivity toward processing factors. A wide variation has been observed between operating conditions and selected samples, which are used in different studies; therefore it is difficult to give a comparable explanation for the effects of processing and food matrix. Presently, there is no explicit scientific ground that can be used for predicting the plant extract stability after processing or during storage. Therefore development of statistical models to analyze the interaction between different polyphenolic compounds and with other food components and surroundings, and understanding of degradation mechanisms would facilitate the accurate prediction of phytochemical stability. The phytochemicals have wider applications in food industries, pharmaceuticals, and cosmetics, requiring more extensive research for developing advance food processing technologies and efficient delivery system. Nonconventional processing technologies like high pressure processing, pulse electric field, cold plasma, and irradiation along with encapsulation and nanoemulsification should be further exploited for increasing their effectiveness in protecting the potential of plant extracts.

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