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A MINI REVIEW ON APPLICATION OF NON-THERMAL TECHNIQUES FOR PROTECTION OF FRUIT JUICES

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ABSTRACT

Abstract: Globally, fruit juices are widely consumed due to their nutritional and health benefits. With increased consumer knowledge about health and safety demand for nutritious fresh like juices is also increased. Fruit juices are more susceptible to spoilage mostly by spoilage microorganisms or by intrinsic enzymatic reactions that adversely affect the sensory attributes of juices. Conventional thermal pasteurization is effective to control spoilage, but it also affects heat sensitive functional compounds and the nutritional value of juices. Therefore, to meet increased consumer demand and requirements, it is necessary to process a variety of fruits for juice preparation with improved preservation techniques to control spoilage. Non-thermal preservation like high pressure processing, pulse electric field, ultraviolet radiations and cold press, etc., are recognized as best alternatives to thermal pasteurization as they have substantial potential to completely inactivate spoilage microbes by causing cell disruption either during processing or after juice has packaged. One of the promising features of employing non-thermal preservation methods is that they do not affect nutritional and organoleptic characteristics of processed juice. Also, most of the methods are relatively less expensive and efficient cause maximum microbial load reduction and improve shelf life.

Keywords: Non-thermal Pasteurization; High Pressure Processing; Pulse Electric Field; Cold Plasma; Irradiation; Fruit Juice Preservation

INTRODUCTION

Recent trends of academia and the food industry have revealed the great potential of using innovative non thermal techniques for food preservation **(Prokhasko *et al.*, 2018; Anggono *et al.*, 2022; Rebezov *et al.*, 2022).** Among food groups, highly perishable foods like milk and fruit juices are more susceptible to microbial and enzymatic spoilage (**Chughtai *et al.*, 2021; Sarkar *et al.*, 2021; Meinert *et al.*, 2023**). Thermal pasteurization is usually performed to improve shelf life of juices through the destruction of spoilage agents, but it also has an adverse effect on its functional, nutri-tional and sensory attributes (**Bhattacharjee *et al.*, 2019**).

Therefore, research has shown great interest in exploring alternate non thermal techniques in juice preservation that cause maximum enzymatic and microbial load reduction along with preserva-tion of sensory attributes while using minimal heat. Non-thermal preservation techniques are gaining importance as consumer preferences and requirements for quality foods having 'fresh-like' attributes are increasing. These techniques, including Irradiation, Cold Plasma, Pulsed Electric Field, Ultrasound Waves, and High-Pressure Processing can preserve foods in a better way and re-place the traditional preservation techniques (**Pina-Pérez *et al.*, 2016; Osintseva *et al.*, 2017; Tretyak *et al.*, 2017**). One of the most important concerns of modern food processing industries is to ensure food safety by preserving food through the inactivation of microorganisms and reduction or inhibition of enzymatic activities (**Petruzzi *et al.*, 2017**).

Spoilage can be defined as a chemical process causing food or food products to be unappealing or objectionable for human consumption due to variations in sensory attributes. Such foods might be considered safe to consume as they are not harmful to our health due to the absence of pathogens or toxins, however, they may be rejected or dis-carded owing to the changes in sensory characteristics i.e., appearance, taste, flavor, aroma, or texture (**Sahu and Bala, 2017**). Spoil-age of fruits and their products can happen at any step of the food chain and may arise due to insect damage, phys-ical damage, enzymatic reactions, or microbial activity (**Petruzzi *et al.*, 2017**).

Enzymatic spoilage: Enzymes are chemical compounds, proteinaceous in nature, and exhibit structural variability (**Siddiqui, 2017**). Enzymatic browning is one of the major food spoiling mechanisms occurring in fresh fruits. There-fore, it is mandatory to control enzymatic activities during fruit juice processing operations. The enzymatic activi-ties of polyphenol oxidase and peroxidase were found to be stopped due to the disruptions in the α-helical structure of enzymes. Polyphenol oxidase (PPO) catalyzes the oxidation and hydroxylation of phenolic compounds and re-sults in the browning of fruits. Figure 1 is showing enzymatic reactions that are catalyzed by PPO and peroxidase. Enzymes also cause undesirable changes in color and sensory attributes of fruits and vegetables. They interact with atmospheric oxygen and deteriorate the natural color and produce off-flavors in the fruits (**Ağçam *et al.*, 2018**). Peroxidase enzymes, members of the oxidoreductases group, cause enzymatic browning and undesirable changes in the color and flavor of fruit juices. They use hydrogen peroxide and facilitate the oxidation of phenolic compounds which are naturally present in the fruits. These enzymes have been found to deteriorate the sensory aspects of fruit juices quicker than other enzymes. Polyphenol oxidases, after oxidation form unstable o-quinones which interact with proteins and other molecules and impart a brown, black, or red color to the fruit juices. They have also been reported to alter the flavor of food commodities (**Murtaza *et al.*, 2019**). Thus, in order to maintain the fresh sensory and nutritional value of processed juic-es, a high level of enzymatic inactivation is required (**Marszałek *et al.*, 2016; Murtaza *et al.*, 2019; Benito-Román *et al.*, 2020**).

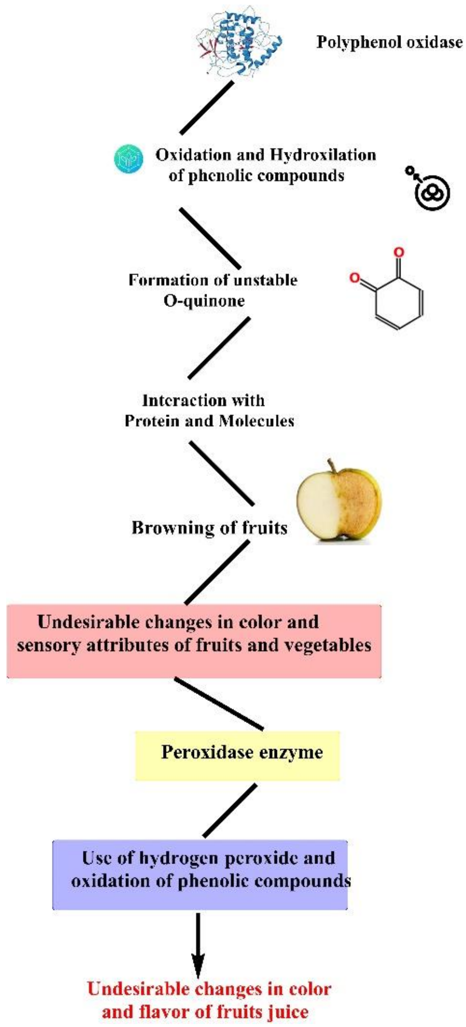

Figure 1 Enzymatic effect on fruits and their juices

Bacterial spoilage

Chemical reactions occurring in foods and resulting in various sensory changes are facilitated by a wide range of microbes, including bacteria, yeasts, molds, and others. Owning to the nature of microbes, some are usually pre-sent in spoiled foods, whereas others exist in a specific type of food. Numerous microbial species could be present in a single spoiled food or there may be a particular species responsible for deteriorating the sensory attributes of foods especially liquid foods. Microbes deteriorate the sensory characteristics of fruit products by producing off-odors, affecting the appearance and development of off-flavors (**Petruzzi *et al.*, 2017**).

Major spoilage Bacteria of Fruit juices

In the past few decades, gram-positive nonpathogenic spore-forming bacteria such as *Alicyclobacillus* spp. have been found to cause spoilage in commercially processed fruit juices. Various bacterial species have been re-ported to produce mousy, malty, buttery, or acidic off-flavors which render the food product unacceptable by con-sumers (**Cai *et al.*, 2015**).

Many spoilage microorganisms particularly, *A. acidoterrestris* bacterial species have been found to produce off-flavors in fruits and vegetable products. The spores of *A. acidoterrestris* have ability to withstand commercial pasteurization temperatures and germinate even in low pH environments. They produce volatile and undesirable odorous compounds, known as guaiacol, in various fruit juices. This sour type of spoilage is identified as having a medicinal or antiseptic off flavor without the production of gas or other gaseous compounds (**Pornpukdeewattana *et al.*, 2020**).

Other spoilage agents of juices are mostly yeasts that are classified into four main groups: (a) *Saccharomyces* spp.: these species spoil wine and alcoholic beverages by producing off-flavors and gassiness. (b) *Zygosaccharomyces*: these species spoil salad dressings, dried fruits and vegetables, jams, and soy sauce. These species exhibit slow growth, produce off-odor, off-flavor compounds, and carbon dioxide gas causing the food containers to burst. (c) *Candida* species: these species are involved in the spoilage of fruits and dairy products. They deteriorate the appearance and taste of fresh and processed fruits and vegetables. (d) *Dekkera/Brettanomyces*: these species cause damage to fermented foods, such as alcoholic drinks and certain dairy products by producing volatile phenolics and these compounds spoil the flavor of fruit juices (**Campos *et al.*, 2015; Abdel-Aziz *et al.*, 2016**). The genus *Zygosaccharomyes* comprises six species. Among these, *Z. bailii*, *bisporus*, and *rouxii* are associated with the spoilage of fruits and beverages. These species cause fermentative spoilage in fruit juices, fruit concentrates, honey, and confectionery products. The growth of Saccharomyces pombe renders malic acid in fruit juices unavailable. This increases the pH and ultimately affects the taste and stability of the fruit juice (**Petruzzi *et al.*, 2017; Howell, 2016**). Table 1 is showing effect of microorganisms on quality of juice.

Table 1 Effect of Microorganisms on quality of juice

Microbes	Organisms	Effects on Juice Quality	Spoilage Type	pH Range	References
Acetobacter spp.	*Acetobacter aceti*	Increased acidity due to acetic acid production Vinegar-like aroma and flavor	Sour taste	3.5 - 4.5	(**Gomes *et al.*, 2018**)
Lactic Acid Bacteria	*Lactobacillus spp., Leuconostoc spp.*	Increased acidity due to lactic acid production Cloudiness and off-putting odor	Sour taste	3.2 - 4.0	(**Da Silva, 2017**)
Alicyclobacillus spp.	*Alicyclobacillus acidoterrestris*	Off-flavors, often described as medicinal or phenolic Browning of juice due to enzymatic reactions	Undesirable aroma	3.0 - 4.0	(**Pornpukdeewattana *et al.*, 2020**)
Pectinolytic Bacteria	*Erwinia spp., Pectobacterium spp.*	Pectin degradation leading to juice clarification Changes in color and texture	Loss of turbidity	3.0 - 4.5	(**Shrestha et al., 2021**)
Yeasts and Molds	*Saccharomyces cerevisiae, Aspergillus spp., Candida spp., Zygosaccharomyces, Dekkera/Brettanomyces, Zygosaccharomyces bailii, Pichia bisporus, Candida rouxii*	Fermentation leading to alcohol and CO_2 production Changes in aroma, taste, and color	Fizziness	2.5 - 5.0	(**Zilelidou and Nisiotou, 2021**)

Non thermal techniques for Fruit juice preservation

High Pressure Processing

One of the novel nonthermal processing techniques widely employed for the preservation of liquid foods is high pressure processing that involves high pressure treatment (100-600 Mpa) at room temperature resulted the inactivation of spoilage agents (**Lai *et al.*, 2021**).

Principle of HPP

It works on principle of degradation of the microbial cell membrane, its protein and vegetative cells that eventually lead to cell death (**Lado and Yousef, 2002**). HPP not only inactivates spoilage agents like microorganisms and enzymes but also preserves the organoleptic and nutritional properties of processed food.

Uses

HPP is used as an alternative to conventional thermal treatments to preserve food, especially liquid foods like juices and milk. Numerous databases confirmed high pressure processing attain the same level of preservation as achieved with conventional thermal preservation methods like pasteurization (**Huang *et al*., 2018**). The Food and drug administration (FDA) has also listed HPP as one of the most effective alternatives to thermal pasteurization (**FDA, 2010**). One additional benefit of employing HPP is that it preserves not only the nutritional and organoleptic characteristics of fruit juices but also protects the functional components of juices. Therefore, employed for almost all types of foods particularly for heat sensitive fruit juices to preserve their functional ingredients while maintaining taste (**Wang *et al*., 2016**).

Application of HPP

The high-pressure range varies from 100 Mpa to 1000 Mpa for ultra high-pressure treatment for inactivation of spoilage microorganisms. Pressure has an important functional role when applied at 300 Mpa showed no significant reduction but when it increased to 400 Mpa or above then it inactivates almost all microorganisms (18). Temperature also plays a critical role in high pressure processing particularly in terms of preservation for microbial inactivation. Mostly temperature maintained at room temperature or somehow above and below that give efficient results in HPP.

Processing of apple juice

In a recent research temperature effect in HPP is evaluated by processing apple juice at low temperature 5 ⁰C and room temperature 20 ⁰C. Results confirmed that a substantial reduction of inoculated spoilage organisms (E. coli, Salmonella and *Listeria monocytogenes*) was observed with room temperature HPP treatment (**Cheng *et al*., 2018**).
Current and future trends of the food industry revealed that pasteurization of fruit juices by non-thermal techniques like high pressure processing is now becoming more popular due to its better preservation potential of functional and microbiological characteristics, satisfying both consumer and processor demands (**Huang et al., 2017**).

Processing of Shiikuwasha juice

In a research study, Shiikuwasha (*Citrus depressa*) juice was processed at high pressure 600 Mpa for 150 seconds at room temperature to improve microbiological shelf life of the juice. Evaluated results for 28 days storage period confirmed substantial microbial load reduction of Psychrotrophs and *E. coli* O157:H7 (5.15 log reduction) in HPP processed citrus juice. Also, HP processed juice compared with HTST pasteurized juice that confirmed Shiikuwasha has better shelf stability and microbial safety status than HTST processed **(Lai *et al*., 2021)**.
Apple juice preservation by HPP was achieved best at high pressure (>400 MPa) for 110 seconds that effectively inactivates all spoilage microorganisms by cell disruption (**Petrus *et al*., 2020**). Whereas in some cases recovery of spore former fungi was observed in refrigerated storage of preserved apple juice. A recent research database evaluated HP (600 MPa) treatment and reported substantial reduction (5.3 log reduction) of fungal spp. In apple juice along with modifications in the concentration (aw) and pH of juice (**Buerman *et al*., 2020**).

Processing of Acai juice

In another recent database, Acai juice (pH 4, Brix 2.9) was preserved by high pressure processing to inactivate its common spoilage microorganisms i.e., *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. The study confirmed that high pressure (400 Mpa) treatment for 3 minutes at 5⁰C efficiently inactivates all spoilage microorganisms and improved juice shelf life. HPP cause cell injury and lethality of bacterial cells in such a manner that even no recovery is observed in long refrigeration storage (42 days) period of juice (24).

White Grape juice Processing

Chang et al. conducted research aimed to assess HPP as an alternate to TP for white grape juice as it has limited shelf life due to microbial and enzymatic deteriorative reactions. High pressure (600 MPa) applied for 3 minutes resulted in the same microbial load reduction as thermal pasteurization however, other functional, quality and sensory attributes of juice score higher in HPP than TP. Additionally, study results also confirmed that HPP has the potential to limit (50 %) deteriorative activities of polyphenol oxidase and peroxidase that are responsible for quality deterioration and spoilage of grape juice (**Chang *et al*., 2017**).

Processing of mango juice

Mango juice is one of the most widely consumed juice due to its appreciable flavor and antioxidant potential. Thermal pasteurization of juice can cause losses of its sensory and antioxidant profile therefore novel non thermal techniques adopted to preserve its value. In a research database, high isostatic pressure (**Guerrero-Beltrán *et al*., 2005**), a form of HPP (600 MPa, 300 sec, 25 ⁰C) is adopted as an alternative to thermal pasteurization resulted increased shelf life of juice through complete inactivation of spoilage microorganisms while preserving nutritional and sensory attributes of the product (**Moreira *et al*., 2017**).

Processing of other juices

Furthermore, high pressure (550 MPa) processing of carrot juice was performed for 6 minutes resulted into complete inactivation of yeast and mold. Results confirmed that HPP not only improves the shelf storage period of juice by microbial inactivation but also resulted in better sensory attributes than conventional TP (**Zhang *et al*., 2016**). Similarly, sugarcane high pressure (600 MPa for 6 minutes) pasteurization was evaluated in comparison to thermal pasteurization revealed that HP processing resulted in significant microbial load reduction (4.6 log) potential (**Huang *et al*., 2015**). In a research study, when grapefruit juice processed at high pressure (250 MPa) and high temperature (60 °C) for 3 minutes it resulted in significant microbial (aerobic plate count, yeast and mold) and enzymatic (Polyphenol oxidase, Pectin methyl esterase) reduction (**Lado and Yousef, 2002**).

Pulse electric field

Pulse electric field (PEF) has been the most investigated and studied technology of interest by researchers. PEF treatment is thought to be an affordable, environmentally benign method of deactivating microbes and enhancing mass transfer in food items (**Arshad *et al*., 2021**). A unique non-thermal preservation technique holds the promise of producing foods with superior nutritional value, sensory appeal, and shelf life. PEF technology is said to be better than conventional heat treatment for food since it prevents or significantly lessens the negative alterations to food's sensory and physical qualities (**Z. H. Zhang *et al*., 2019**).

Benefits

This non-thermal technique has been used for microbial inactivation in a wide range of fruit as well as vegetable-based drinks, also this technique has no negative impact on the organoleptic properties of these drink. This non-thermal technology keeps the nutritional properties of juice preserved just like fresh juices. This technique has been the center of interest for scientists as the efficiency of PEF is similar to that of thermal treatments without impacting physicochemical or sensory properties of treated product. If PEF is applied continuously for a specific time period, then it will rupture microbial cell-membranes and will ultimately these cells will be destroyed (**Soliva-Fortuny et al., 2009**).

Limitations

Despite PEF has been known for its wide range of pros in producing best quality products especially fresh juices, along with a significant reduction in microbial load, but this technique cannot be adopted widely in food industry due to its high cost than other conventional treatments such as thermal treatments (**Sampedro *et al*., 2013**).

Utilizing PEF Technology

The functioning of PEF technique basically depends on electroporation that leads to the de-polymerization of microorganism's cell membranes' macromolecules (**Arshad *et al*., 2021**).
This de-polymerization of macromolecules results in the inactivation of microorganisms thus reducing overall microbial load in juices without imparting any negative impact on organoleptic or nutritional properties of juices. Researchers have reported that pulse electric field collapse the cell-membranes of microorganisms, alter the overall morphology as well as structure of cell, impacts the functioning of enzymes that are responsible for cell metabolism, destroy the genetic material present in the cell and alter the gene expression of microbial cell (**Huang *et al*., 2014**).
PEF treatment inactivates the microorganisms by multiple aspects and these aspects can be categorized into different groups such as methods of processing, microbial load or nature of microbes and nature of product. The basic parameters involved in different methods of processing are time of treatment, temperature, and width, EFS, shape and pulse polarity. All these parameters play a role in inactivating microorganisms by PEF. Research has evidenced that microbial inactivation is directly related with the treatment time and EFS, as by increasing the treatment time and EFS the inactivation of microorganisms has been observed at a greater level. Electro-permeability, on the other hand, has been found reversible and irreversible depending on the intensity of PEF treatment (30).
Microorganisms cannot be inactivated completely if the electro-permeability of microbial cell membranes is reversible, which is basically due to the suboptimal conditions of PEF treatment (**Demirci and Ngadi, 2012**). Along with all these aspects, the product related factors also play a significant role in inactivating microbial cells. These factors include pH of product, ionic strength and electrical

conductivity of product, while microbes related factors include their growth stage, species, size and shape of cell and concentration of microorganisms (**Huang *et al*., 2012).** In all these microbes related parameters, the most critical one is their specie, as it imparts a great impact on PEF efficiency for microbial cells inactivation. Pulse electric field has been found effective against all the vegetative cells of bacteria, yeast and moulds, while it is inefficient against bacterial spores. PEF treatment has best efficiency against yeasts (**Huang *et al*., 2014**).

Processing of Grape juice

Research study has reported that PEF treatment of 35kV/cm was applied with a frequency of 303Hz, pulse width for almost 1 μs on grape juice and it resulted in a reduction of around 2.5-3.95 logs cell number. The microbes present initially in grapes juice were *Lactobacillus plantarum, S. cerevisiae, Gluconobacter oxydans, Lactobacillus hilgardii* and *Kloeckera apiculata* (**Marsellés-Fontanet *et al*., 2009**).

In another study, the total count of microorganisms in grape juice was found to be reduced by the application of around 20 pulses with 80kV/cm, keeping the temperature 50 °C and no anti-microbial agent was introduced. This treatment resulted in the reduction of almost 3.9 logs of *Zygosaccharomyces bailii* ascospores, *Neosartorya fischeri* ascospores, and *Byssochlamys fulva* conidiospores (**Wu *et al*., 2005**). Likewise, Figure 2 is showing how PEF inactivate microbes and enzyme to extend the shelf life of juices.

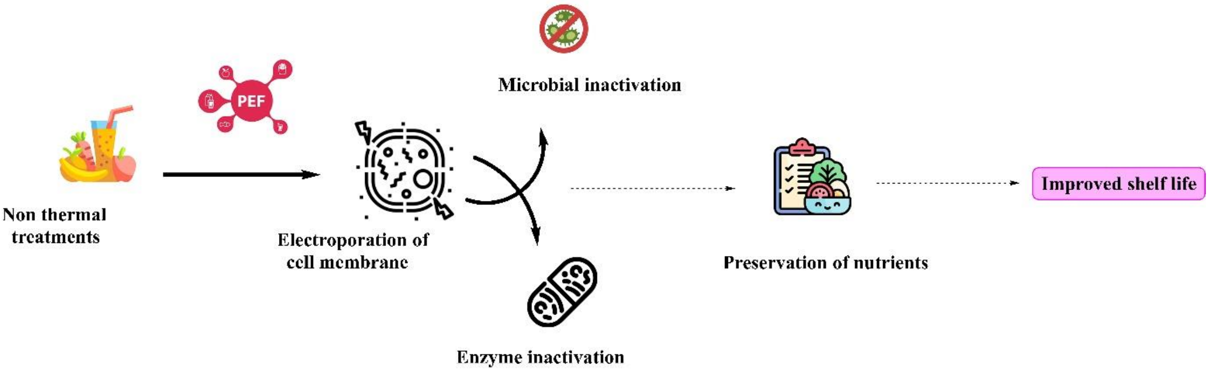

Figure 2 Mechanism of Pulse Electric Field (PEF) to increase shelf life of juice

Processing of APPLE juice

Research on estimating the efficiency of PEF on apple juice revealed that by applying a slight field strength of just 50kV/cm on apple juice with 16 pulses resulted in a strong pasteurizing effect and reduced the cell count up to 4 to 6 logs of yeast, aerobic plate counts and moulds (**Saldana *et al*., 2011**).

Processing of Strawberry juice

Studies conducted for strawberry juice evidenced the reduction of *E. coli* by almost 3.8 logs by applying PEF of 18.6kV/cm for around 150 μs (**Gurtler *et al*., 2011**). A slight increase in the intensity of PEF treatment was also studied and resulted in the higher level of microbial inactivation. Scientists have suggested that the inactivation of microbes can be increased if these treatments are coupled with mild thermal treatments (**Geveke *et al*., 2015**). The efficiency of PEF against microbial load has been investigated by multiple researchers on different juices including apple juice (**Saldona *et al*., 2011**), grape juice (**Huan *et al*., 2014**), tomato juice (**Kim *et al*., 2017**), and orange juice (**Timmermans *et al*., Roobab *et al*., 2018)** etc., along with processing conditions are shown in table 1.

Irradiation

Irradiation refers to the use of ionizing radiations and these include the applications of electromagnetic rays including high-energy gamma rays, accelerated electrons, and X-rays. These rays are administered according to the Codex General Standard for Irradiated Foods (**Commission, 2003**). Irradiation helps increase the shelf life via the inactivation of microorganisms in foods and food products. Gamma X-rays and UV rays, among the other types of electromagnetic rays, are generally used by food processors for preserving fruit juices (**Roboob *et al*., 2018**).

Limitations

Generally, the radiation dose below 1 kGy is considered sufficient to inhibit the growth of microorganisms however, to kill or remove microbes completely from foods, a radiation dose of 1-10 kGy is required (**Mesquita *et al*., 2020**). Gamma X-rays are particularly used by the fruit juice processors to limit the growth the microorganisms, such as bacteria, yeast, and mold. When used at 5 kGy dose, gamma rays resulted in complete elimination of bacterial and yeast and mold count (**Kalaiselvan *et al*., 2018**).

Application of UV rays

Another important aspect of irradiation is the application of UV rays. UV rays, in the electromagnetic spectrum, lie in the range of 200-800 nm and have a lethal effect on microbial growth (**Roboob *et al*., 2018**). Bacteria included in Alicyclobacillus genus cause serious damage to the quality of fruit juices due to their spore forming ability. UV-C rays have been reported to be very effective in decreasing and limiting the spores of these species in orange juices (**do Prado *et al*., 2019**).

The mechanism of inactivating growth of microorganisms begins as the DNA absorbs electromagnetic rays followed by the formation of cross-linked nitrogenous bases which causes a mutation in the microbial DNA. This mechanism impairs the normal functioning of DNA and reproductive capacities of the microorganism. Application of UV rays at an energy concentration of 400 J/m2 is sufficient to control microbial growth. Exposure time, nature of microorganism, irradiation concentration, and dimensions of food are important factors of irradiation. The application of gamma radiations causes radiolysis and results in the formation of a series of reactive compounds, such as oxygen species, radicals, and ionized water. These reactive compounds facilitate the formation of secondary products i.e. hydrogen peroxide (**Mesquita *et al*., 2020**). These cell-damaging reactive compounds generate a chain of reactions causing oxidative damage to the biological molecules. The outcomes of these propagating reactions are impairment of physiological functions which inhibit the growth of microorganisms and development of off-flavors (**Roboob *et al*., 2018**).

The application of UV irradiation has been found to be effective for enzyme inactivation in different fruit juices, including apple, pear, and grape juices (**Mesquita *et al*., 2020**). The influence of UV-Vis irradiation on quality attributes and enzymatic activities of nectarine juices was observed by (Aguilar). Irradiation was applied for 2 hours using a power lamp of 460-W, emitting rays in the range of 250-740 nm. Results showed that irradiation was effective in inactivating enzymes such as polyphenol oxidase (PPO) and peroxidase by up to 60%. The impact of UV irradiation on microbial and enzymatic inactivation in apple juice treated with UV rays by using a UV device for 40 minutes was investigated (**Akgün *et al*., 2017**). The UV device is comprised of four LEDs with different peak and coupled emissions. Results showed a significant decline in antimicrobial and enzymatic activity. Polyphenol oxidase activity was reduced to 65.62% when a combination of 280-365 nm and 280-405 nm were used. The efficiency of enzymatic inactivation in fruit juices using UV irradiation is dependent on the juice composition and its matrix (**Wang *et al*., 2020**).

Uses

Gamma X-rays and UV rays were widely used to inactivate microorganisms such as E. coli and S. cerevisiae in apple juice at 60 W/m2 and 254 nm. Results showed a significant reduction count in spoilage yeasts (**Gabriel, 2012**) and a 4- to 5- log reduction. The sensitivity of microbes also affects these results. For instance, a similar log reduction level was observed for A. acidoterrestris at 13.44 W/m2 (**Gabriel, 2012; Tremarin et al., 2017**). The application of UV at different radiation concentrations to limit the growth of E. coli in apple juice was studied. Results showed a 2- to 5-log reduction in viability of these pathogenic microbes (**Usaga *et al*., 2015; Yin *et al*., 2015**). In another study, the effect of UV-C radiation to inactivate the spores of A. acidophilus and the growth of yeast and molds were studied (**Tremarin *et al*., 2017**). Results showed a 5-log reduction at the maximum treatment dose, which was 13.44 W/m2. Watermelon juice irradiated by UV-C showed a log reduction of 2.6, 1.47, and 0.99 at a concentration of 2.7 and 37.5 J/mL in Total Plate Count of coliforms and yeast and molds without affecting the physicochemical properties (pH, TSS, lycopene, phenolic content) of juice (**Feng *et al*., 2013**). The impact of UV irradiation was studied for the pasteurization of orange juice. Results showed a log reduction of 2.8- and 0.34- in the aerobic plate count and yeast and mold count respectively (**Pala and Toklucu, 2013**). The application of centrifugal UV to pasteurize grapefruit was studied at the following conditions: 4.8 to 24 mJ/cm2 and 450-750 rpm. Results showed a log reduction of 5.1- in E. coli and 6.0- in S. cerevisiae species (**Geveke and Torres, 2012**).

Ultrasound

During the last decade, ultrasonication has emerged to the greater extent as innovative and eminent technology that can be utilized as a perpetual alternative to thermal techniques (**Dolas *et al*., 2019**). Ultrasounds are sound waves or pressure waves having a frequency greater than the threshold for human hearing (≥ 16 kHz) or about 20 kHz.

Types of ultrasonication

It is divided into three types depending upon the frequency range: power ultrasound also known as high power ultrasound utilizes the sound waves ranges from 16 to 100 kHz; high-frequency ultrasound (0.1–1 MHz) and diagnostic ultrasound with wider frequency ranges from 1 to 10 MHz (**Zhang *et al*., 2019**).

Mechanism of ultrasonication

When the sound waves propagated through the liquid medium, they produce gas bubbles, and their bursting (cavitation) results in a rise in temperature and pressure. These bubbles have greater surface area during the expansion cycle that enhances the diffusion rate of gas thus causing bubbles to expand. Afterward, prompt condensation occurred at a point when ultrasound energy is not enough to maintain the vapor phase in the bubble. Finally, resulting in the intense collisions of condensed molecules, generating shock waves raising the temperature and pressure of the liquid medium up to 5500 °C and 50 MPa respectively (**Jan et al., 2017**).

The microbial destruction caused by ultrasonic waves is mainly due to the pressure changes that occur during these implosions (collapse or falling or air bubbles), thus hastening the mass transfer, disruption of the particles, and destruction of the microbial cell membrane (**Kentish and Feng, 2014; Shen *et al*., 2017**). Though the temperature rise is also responsible for the bactericidal effect but it is very confined and does not affect the large surface area.

Application

Succinctly, numerous ultrasound systems with varying ranges of frequency and energy density are employed with widespread application in food preservation (destruction of microorganisms and enzymes), food processing (defoaming, degassing, emulsification and filtration), and also assist in the extraction of active ingredients or bioactive from various food products (**Aliasghari Aghdam *et al*., 2015; Kumari *et al*., 2017**).

Ultrasonic is a novel technique that has discovered its widespread application in the food industry especially the fruit juice and beverage industry due to its multidimensional positive effects on fruit juice processing and preservation. It is a simple, economical, and reliable method that exerts a minimal negative effect on the environment. Ultrasonication is a profoundly utilized technique for the preservation of fruit juices while keeping the nutritional quality and sensory attributes intact. It has proved to have strong antimicrobial potential against a wide continuum of microorganisms and thus has been perceived as an imminent technology that meets the FDA requirement of safety for fruits and vegetables. Fruits have a short shelf life because they are highly susceptible to microbial and enzymatic spoilage that may result in the loss of color and nutritional quality attributes. Subsequently, sonication is an impending technology that can be employed to preserve fruit juices without affecting nutritional quality. Sonication has been investigated for its utilization on a wide range of fruit juices including apple, melon, pomegranate, blueberry, and orange juice (**Dolas *et al*., 2019**).

Pomegranate fruit is highly nutritious and rich in bioactive compounds. Thermal processing of pomegranate juice for its preservation and microbial destruction considerably affects its nutritional profile or reduces its functional ingredients. Thus, the application of sonication at frequency of 20 kHz at 100% amplitude level for 15 min has been reported to significantly reduce the microbial count especially *E. coli* and *S. cerevisiae* in pomegranate juice thus extending its shelf life. Moreover, the results showed that the lower amplitude levels were not found effective in reducing microbial count (**Alighourchi *et al*., 2014**).

Similarly, an ultrasonication probe was employed by **Pala et al. (2015)** (надо вставить не год, а номер ссылки), to study the effect of ultrasonication on the microbial inactivation and physicochemical properties of pomegranate juice. The application of 20 kHz for 30 min at an amplitude level of 100% was found to be effectual in the inactivation of *E. coli* ATCC 25922 to 5 log and *S. cerevisiae* ATCC 2366 to 1.36 log with no significant change in the phenolic count, pH, and soluble solids. However, the anthocyanin count showed 92% and 89% retention at amplitude levels of 75 and 100% respectively. Thus, ultrasound technology was found potent in improving the quality and safety of pomegranate juice.

In another research, the effect of continuous ultrasound treatment (US) by intensifying the amplitude and flow rate on reduction of microbial count in blueberry juice was investigated. It was concluded that the continuous application of US resulted in a substantial reduction of total count, aerobic plate count, yeast, and mold count. Moreover, continuous US treatment exerts no negative effect on the colour and the anthocyanin content of juice thus preserving the quality and safety of juice. So, it can be considered as an alternative to thermal treatment (**Mohideen *et al*., 2015**).

Moreover, juice blend (apple+strawberry+Lemon) was treated with ultrasound at 376 W/10 min/35 °C and its effect on the safety and quality of juice was evaluated. The results showed a significant reduction in the microbial count during the 10 days storage at 4°C (**Feng *et al*., 2020**). **Oliveira et al. (2018)** observed the effect of ultrasound, ozone, and their combined treatment on the quality and microbiological safety of acai juice. Assay with ultrasound treatment at an energy density of 700 J·mL−1 and no ozonation showed maximum potential in a significant reduction in mesophilic bacteria, molds and yeasts count (**Oliveira *et al*., 2018**).

Comparison of thermal and non-thermal technologies

Ultrasonication (non-thermal) and thermal heat treatment both were employed as preservation technologies that significantly reduced the microbial count. But the thermal treatment was reported to have adverse effect on the nutritional characteristics of food. A comparative study was conducted by **Farhadi Chitgar et al. (2017)**, to evaluate the effect of sonication and heat treatment on the quality and safety attributes of barberry juice that is rich in anthocyanin and antioxidants. Results showed that sonication was reported to enhance the total phenolic count and antioxidant profile of barberry juice and showed very less effect on the colour and anthocyanin content in comparison to thermal treatment. Moreover, both the treatments resulted in the reduction of microbial count below the detection limit. The greater efficiency of sonication treatment to exert the lethal effect on microorganisms was accomplished by intensifying the amplitude and increasing the time of sonication (**Farhadi Chitgar *et al*., 2017**). Likewise, **Saikia et al. (2016)** also reported the positive effect of ultrasound treatment on the total phenolic count and antioxidant activity of juices from five fruits (carambola, pineapple, litchi, black jamun and watermelon) in comparison to thermal pasteurization (**Saikia et al., 2016**).

Furthermore, the high-power ultrasound in combination with slight heating (thermosonication) showed its potential in significantly reducing the count of selected mold and yeast in apple, blueberry, cranberry juice. Application of ultrasound of 20 kHz, at 60°C for the period of 3, 6 and 9 min resulted in the complete reduction of yeast and mold that was not visible at lower temperatures (20 or 40 °C). It was concluded that temperature is an important factor that significantly influences the inactivation of yeasts and molds in fruit juices (**Jambrak *et al*., 2018**). Guerrouj et al. (2016 надо вставить не год, а номер ссылки), also reported the positive impact of thermosonication in reducing the microbial account along with the positive impact on the nutritional quality of orange juice. As it also resulted in the general augmentation of bioactive compounds such as flavonoids, total phenolics, carotenoids, vitamin C and anthocyanin in orange juice. Conclusively, ultrasonication in combination with mild heat allowed the inactivation of microorganisms in various fruit juices and can be adopted on a piolet scale (juice processing industries) for the production of safe, nutritious and high-quality fruit juices with extended shelf life (**Guerrouj *et al*., 2016**).

Pulsed Light

Pulsed light treatment is an emerging non-thermal food processing and preservation technique that can be employed in the replacement of conventional thermal pasteurization techniques. It is composed of white light with a wide spectrum of wavelength (200-1100nm) including ultraviolet (UV) ranges from 200 to 400 nm, the visible light spectrum from 400 to 700 nm, and near-infrared spectra from 700-1100 nm (**Bhavya and Umesh Hebbar, 2017**). It involves the discharge of high energy and high voltage electric pulses upto 70 kV/cm for every short interval of time (picosecond or femtosecond). Pulsed light is generated by various sources including ytterbium ions doped silica fibers and flash lamps filled with inert gas (xenon or krypton) (**Santamera et al., 2020**).

Limitations

Pulsed light treatment must not exceed 12 J/cm2 for the duration of 2 milliseconds for the treatment of food to control surface microorganisms has been approved by the Food and Drug Administration (FDA). The efficiency of pulsed light treatment on microbial destruction has been significantly influenced by the wavelength of the light spectrum, number of pulses generated (amount of energy), and fluence level applied on the sample. The exposure of the sample to pulsed light treatment with higher intensity and pulse number resulted in microbial inactivation to a greater extent (**Shankar *et al*., 2014**).

Utilization

It is most commonly reported as an appropriate technique for surface decontamination or sterilization of raw and fresh-cut fruits and vegetables or meat cuts, processing equipment, and food packaging materials. Pulsed light has also found its application for the inactivation of foodborne pathogens in liquid foods including fruit juices, milk and infant foods (**Bhavya *et al*., 2017**).

Preetha et al. (2021) investigated the effect of continuous flow pulsed light treatment (flow rate of 100 ml/sec) on the inactivation of E. coli in fruit juices including coconut water, pineapple and orange juice. The maximum reduction of E. coli in fruit juices was reported, at the dose of 95.2 J/cm2 as it resulted in the damage to cell integrity examined by scanning electron microscopy (SEM). Moreover, pulsed light treatment was found more effective for clear juices such as coconut water as compared to cloudy juices (**Preetha *et al*., 2021**).

Currently, another research was conducted to investigate the effect of pulsed light treatment on the microbiological safety and phytochemical composition of pineapple juice in comparison to thermally pasteurized juice. Results showed that pulsed light treatment was fount effective in the reduction of the microbial count to acceptable level (5 log reduction) along with preserving the colour and antioxidant profile of pineapple juice. Moreover, thermal pasteurization has also completely inactivated the microorganism but exerted adverse effects on antioxidant activity, colour and ascorbic acid content. Thus, pulsed light can be adopted as an alternative to other thermal techniques of juice preservation (**Vollmer *et al*., 2020**).

Moreover, pulsed light treatment alone or in combination with ultrasound treatment showed great potential in ensuring the microbiological safety and sensory characteristics of the product. **Ferrario et al. (2016)** investigated the effect of continuous pulsed light treatment used alone or combined with ultrasound treatment on the quality and microbial safety of apple juice. The combined treatment improved microbial reduction rate, delayed browning and showed wide consumer acceptance comparison to when applied alone (**Ferrario *et al*., 2016**).

Previously, a similar study was conducted to evaluate the effect of combined treatment (pulsed light and thermosonication) on the inactivation of E. coli and results showed its reduction to 6 log cfu/ml in apple juice (**Muñoz *et al*., 2012**).

Therefore, pulsed light treatment can be used alone or in combination with other non-thermal techniques and is considered an efficacious alternative to thermal pasteurization techniques.

Cold Plasma

Plasma is defined as the fourth state of matter and it is entirely different from the other three (solid, liquid, gas) states of matter (**Bourke *et al*., 2018**). The basic difference among the states of matter is energy due to which the states can be changed. Energy breaks and shapes the structures between molecules and atoms and during this process free electrons and ions are generated.

Plasma is categorized as an ionized gas comprising uncharged molecules, free electrons, and a mixture of oppositely charged ions. This matrix of different molecules interacts with one another and gas molecules and transfer of energies take place. This results in the formation of highly reactive compounds (radicals, hydrogen peroxide, ozone, and UV radiation) which interact with the food surface (**Carrillo *et al*., 2017**).

Uses

Cold plasma technology is being used extensively at various stages of food production (**Ozen and Singh, 2020**). There are various ways to ionize the gas and generate plasma, including the use of heat, electricity, and the application of lasers. Thus, the composition and nature of plasma is distinct and depends on the type of carrier gas (which could be air, nitrogen, argon, or helium), plasma generator (which could be a microwave, plasma jet, or dielectric), and external conditions under which it is generated (pressure, humidity, and temperature) (**Bourke *et al*., 2018**). Generally, cold plasma is produced at or around room temperature and is not dependent on heat to destroy the pathogens, so it poses no threat to the quality of food products (**Ozen and Singh, 2020**). The Cold Plasma technique uses a carrier gas, such as air, oxygen, or nitrogen to which electrical energy is provided via electrodes. The CP generation system comprises a carrier gas, power supply, and electrodes (**Misra *et al*., 2016**).

Application

Depending on the food product or stage of application, the cold plasma technique can be direct, indirect, or in-package. Direct cold plasma finds its applications for bulk food products and is administered by a conveyor belt system (85). Indirect cold plasma finds its application through plasma-activated water and is applied in the form of sprays. It can be applied for the preservation of fresh produce or disinfection of various food equipment where liquid chemicals are commonly used (**Schnabel *et al*., 2014; Shen *et al*., 2016**).

In-package cold plasma is used for packaged foods and provides a decontamination effect by generating reactive species for a longer period of time and prevents recontamination. Cold plasma has been found to inactivate microorganisms and inhibit their growth with minimum impact on the quality characteristics of fruit juices (**Ozen and Singh, 2020**).

Among others, the most observed mechanism is the interaction of cell membrane components with free radicals, excited molecules, or charged particles. These reactive species are generated by the breakdown of O3, peroxides, or hydroxide radicals and play a significant role in the inactivation of microorganisms. Oxides of nitrogen, NO and NO2, have been found to play an important role in limiting microbial growth by disrupting the chemical components like proteins and lipid. A study revealed that argon plasma, when used as a carrier gas, generated OH, N2, and O radicals. These reactive species significantly limited the growth of Bacillus subtilits species. OH radicals produced in water, when Helium-oxygen is used as a carrier gas, had a significant role in the inactivation of S. aureus species (**Edelblute *et al*., 2015**). Reactive oxygen species, particularly O3 and NO2 were found to be effective for bacterial inactivation when used with atmospheric plasma. An ionic compound peroxynitrite ion produced by the reaction of plasma-generated radicals has been reported to play an important role in bacterial inactivation (**Misra *et al*., 2014**).

Recent studies have shown the effectiveness of UV photons in limiting the growth of microorganisms by CP technology (**Guo *et al*., 2015**). These photons disrupt the genetic material of microorganisms and prevent DNA replication. There are various ways by which plasma damages the microbial DNA, such as moderation of nucleo-bases, formation of thymine dimers, and oxidation by reactive species (84). The application of cold plasma is independent of surface irregularities of foods as it can flow around the edges and ensure effective treatment. However, the texture of the product surface can affect the efficacy of cold plasma treatment (**Mandal *et al*., 2018**). It is a quick and contact-free pathogen removing technique from the food surfaces. The penetrating capacity of cold plasma and reactive species generated by it is limited. CP has been applied for the surface decontamination of various food products, such as tomatoes (**Schnabel *et al*., 2015; Kim and Min, 2017**). The nature of microorganisms affects the efficiency of decontamination with cold plasma technology. Microorganisms inhabiting the surface of foods are easily removed as compared to the ones that reside inside the seeds or form spores (**Butscher *et al*., 2016**). Due to its potential to inhibit microbial growth, cold plasma can prolong the shelf life of fruit juices by delaying food spoilage processes. Microbial inactivation by atmospheric cold plasma has been studied for various fruit juices, such as apple juice (**Liao *et al*., 2017; Xiang *et al*., 2018**) orange juice (**Xu *et al*., 2017**), white grape juice (**Pankaj *et al*., 2017**), and blueberry juice (**Hou *et al*., 2019**). The detailed effects of cold plasma application on microbial inactivation in different fruit juices along with processing conditions are shown in Table 2.

The cold plasma technique has been found to control the PPO activity and enzymatic browning in fruits (**Surowsky *et al*., 2014; Han *et al*., 2019**). The impact of cold plasma on the PPO activity in fresh-cut apples was investigated. Results showed that the application of plasma technology was effective in reducing the PPO activity by about 62%. Pectin methylesterase (PME) is an enzyme present in the cell wall that de-esterifies pectin and produces pectic acids (**Bußler *et al*., 2017**). Recent studies have addressed the sensory attributes of plasma-treated fruit juices and the effects of cold plasma on the appearance and color of fruit juices. A significant effect of cold plasma treatment on the appearance of fruit juices has been observed depending on the treatment application and juice type (**Ozen and Singh, 2020**). An increased treatment time of cold plasma results in color degradation of fruit juices (**Pankaj *et al*., 2018**). The effect of gas flow on the color profile of pomegranate juice was observed by (**Kovačević *et al*., 2016**). Results showed that the color of cloudy pomegranate juice changed significantly and increasing gas flow raised the total anthocyanin content in the juice. Overall, color comparisons of different juices (apple juice, orange juice, white grape juice, and pomegranate juice) showed no significant variation upon treatment with atmospheric cold plasma (**Almeida et al., 2015; Kovačević et al., 2016; Pankaj et al., 2017; Liao et al., 2018**). The quality of processed fruit juices depends mainly on pH and acidity. Changes in these attributes could result in an undesirable effect on consumer preference. The acidity in fruit juices is affected by the solubility of OH radicals produced during the cold plasma generation process (**Pankaj *et al*., 2018**) and the change in pH could be due to the production of nitric acid and related nitrogen oxides. Various studies described that the application of cold plasma affects the pH and acidity of apple, orange, and white grapefruit juices (**Xu et al., 2017**; **Pankaj *et al*., 2017; Liao et al., 2018)**

CONCLUSION

As reported research databases have confirmed that non-thermal techniques have a promising and effective avenue in fruit juice preservation by meeting the increasing demand for nutritious and fresh-like products while ensuring safety and extending shelf life. They have been proven to the effective in microbial inactivation to the acceptable limit without disturbing the nutritional profile, functional ingredients, and sensory attributes of the fruit juices. Therefore, they can be used alone or in combination with other non-thermal techniques to obtain more nutritious, safer products to address food safety and security issue. Traditional thermal techniques were effective in controlling spoilage but often compromises the nutritional quality and sensory attributes of juices due to its impact on heat-sensitive compounds. Non-thermal methods such as high-pressure processing, pulse electric field, ultraviolet radiation, and cold pressing offer viable alternatives by effectively inactivating spoilage microorganisms without compromising nutritional or sensory characteristics. Moreover, these techniques are relatively cost-effective, efficient, and have potential of achieving significant microbial load reduction, thereby enhancing the overall quality and shelf life of fruit juices. Embracing non-thermal preservation methods signifies a pre-emptive approach to meeting consumer expectations for safe, nutritious, and high-quality fruit juice products in an increasingly health-conscious market.

Table 2 Application of non-thermal techniques and their potential for microbial load reduction at specific conditions

Sr #	**Juice**	**Technique**	**Experimental Conditions**	**Target microorganism**	**Microbial Reduction**	**Improved Period of storage**	**Reference**
1	Shiikuwasha (Citrus depressa) juice	HPP	600 MPa, 150 sec, 25 ºC	*E. coli O157:H7*	5.15-log	28 days	(**Lai *et al*., 2021**)
				E. coli O157:H7	5 log		
			>400 MPa, 110 sec	*L. monocytogens*		28 days	
2	Apple Juice	HPP	600 MPA 90 sec	*Fungal spp*. Mostly *Penicillium spp.* *Aspergillus niger*, *Candida parapsilosis*	5.3 log		(**Buerman *et al*., 2020; Petrus *et al*., 2020**)
3	Açaí juice	HPP	400 Mpa, 180 sec, 5 ºC 600 Mpa , 60 sec	*E. coli* *L.monocytogenes* *Salmonella spp.*	5 log - 6 log reduction	42 days	(**Gouvea *et al*., 2020**)
4	White Grape juice	HPP	600 MPa, 180 sec	Aerobic bacteria Yeast/Mold	2 log CFU/mL	20 days	(**Chang *et al*., 2017**)
				Yeast / mold count	<1.0 log CFU/mL −1		
5	Mango Juice	HPP	600 MPa, 300 sec, 25 ºC	Total plate count Aerobic mesophiles Salmonella spp.	5 log	30 days	(**Moreira *et al*., 2017**)
				Yeast and mold	1 log CFU/mL		
6	Carrot Juice	HPP	550 MPa, 360 sec	TPC	4.3 log	20 days	(**Zhang *et al*., 2016**)
				Yeast	Complete inactivation		
7	Grapefruit juice	HPP	250 MPa, 180 sec, 60 ºC	Total plate count	2.5 log	-	(**Aadil *et al*., 2017**)
				Yeast and Mold	1.1 log		
8	Sugar cane juice	HPP	600 MPa, 360 sec, 20 ºC	Aerobic plate count Coliforms	4.6 log	28 days	(**Huang *et al*., 2015**)
				Yeast and mold	<1.0 CFU/mL		
9	Grapefruit juice	PEF	1 kHz 600µs 25 kV cm^{-1} 40 °C	Total plate count Yeast and Mold	1.72 log 1.66 log	-	(**Aadil *et al*., 2015**)
10	Grape juice	PEF	120 Hz 34 µs - 275 µs 9-27 kVcm^{-1} 40°C	*Staphyllococcus aureus* *Staphyllococcus cerevisiae*	3.36 log	-	(**Huang *et al*., 2014**)
				E.coli	2.27 log		
11	Strawberry juice	PEF	150µs 18.6 kVcm^{-1} 45°C,50°C,55°C	*Escherichia coli*	5 log	90 days	(**Geveke *et al*., 2015**)
12	Tomato juice	PEF	80 kVcm^{-1} 50 °C	Total plate count	4.4 log	28 days	(**Nguyen and Mittal, 2007**)
13	Blueberry juice	PEF	36 kV/cm, 100 µs	Total plate count	50 %	-	(**Barba *et al*., 2017**)
14	Orange juice	PEF ,	30kVcm^{-1} 240µs 480µs	Total plate count Yeast and mold	5 log	60 days	(**Sampedro *et al*., 2013**)
15	Apple juice	PEF	40 kj per Kg 7.86µs 55°C	*Escherichia coli*	6 log	-	(**Saldana *et al*., 2011**)
16	Watermelon juice	PEF	188Hz 35 kVcm^{-1} 1727 µs 40 °C	*E. coli* *L.monocytogenes*	3.6 log 3.5 log	91 days	(***Bhattacharjee et al*., 2019**)
17	Sour cherry juice	Irradiation (gamma rays)	Gamma rays 6 kGy , 4ºC for 2 months	Bacteria, yeast and mold	Complete reduction in microbial at ≥3 kGy	-	(**Arjeh *et al*., 2015**)

18	Pitaya juice	Irradiation (UV-C rays)	UV 57 µW/cm^2	*Zygosaccharomyces bailii*	1.8-log		(**Ochoa-Velasco and Beltrán, 2013**)
19	Apple juice	Irradiation (UV-C rays)	UV-C rays 254 nm 90 s	*Bacteria* *E. coli* Spoilage yeast species	0.13-2.4 log 0.20 log	-	(**Gabriel, 2012**)
20	Apple juice	Irradiation (UV-C rays)	Irradiance at 13.44 W/m^2	*A. acidoterrestris*	5 log	-	(**Tremarin *et al*., 2017a, 2017b**)
21	Apple juice	Irradiation (UV rays)	Irradiance at 14 mJ/cm	*E. coli*	5 log	-	(**Usaga *et al*., 2015**)
22	Apple juice	Irradiation (UV rays)	Irradiance at 75 mJ/cm^2	*E. coli*	>2 log	-	(**Yin *et al*., 2015**)
23	Watermelon juice	Irradiation (UV-C rays)	Irradiance at 2.7 and 37.5 J/mL	TPC Yeast and Mold	1.47 log 0.9 log	-	(**M. Feng *et al*., 2013**)
24	Grapefruit juice	Irradiation (Centrifugal UV rays)	Irradiance at 24 mJ/cm^2 at 750 rpm	*E. coli* Spoilage yeast	5.1 log 6.0 log	-	(**Geveke and Torres, 2012**)
25	Orange juice	Irradiation (UV-C rays)	Irradiance at 73.8 mJ/cm^2	Aerobic Plate Count Yeast and Mold	2.8 log 0.34 log	-	(**Pala and Toklucu, 2013**)
26	Chokanan Mango Juice	Irradiation (UV-C rays)	Irradiance at 254 nm up to 60 min with intervals	APC Yeast and Mold	1.22 0.77 log	-	(**Santhirasegaram *et al*., 2015**)
27	Pummelo fruit juice	Irradiation (UV-C rays)	Irradiance at 254 nm	*S. typhimurium*	>5 log	-	(**Shah *et al*., 2014**)
28	Pineapple juice	Irradiation (UV-C rays)	Irradiance at 10.76 mJ/cm^2	TPC Yeast and Mold	1.91 log 1.4 log	-	(**Shamsudin et al., 2014**)
29	Pineapple juice	Irradiation (UV-C rays)	Irradiance at 10.10 mJ/cm^2	*S. typhimurium*	5 log	-	(**Mansor *et al*., 2014**)
30	White grape and apple juice	Irradiation (UV-C rays)	Irradiance at 0.38 mW/cm^2 and 15 min	*A. acidoterrestris spores*	5.5 log 2 log	-	(**Baysal *et al*., 2013**)
31	Blended Carrot and orange juice	Irradiation (UV-C rays)	Irradiance at 10.6 kJ/m^2	*E. coli*, Yeasts *P. fluorescens*	2.6 6.0 log	-	(**Carrillo *et al*., 2017**)
32	Blended Lemon and melon juice	Irradiation (UV-C rays)	Irradiance at 2.46 J/mL for 71 s	*E. coli*	>6 log	-	(**Kaya *et al*., 2015**)
33	Pomegranate Juice	Ultrasound	20 kHz/100%/15 min/25 ± 1 °C	*E.coli* *S. cerevisiae*	3.47 log 1.86 log	-	(**Alighourchi *et al*., 2014**)
34	Pomegranate Juice	Ultrasound	20 kHz/100%/500 W/30 min	*E. coli* *S. cerevisiae*	5 log 1.36log	-	(**Pala *et al*., 2015**)
35	Blueberry juice	Ultrasound	20kHz/93.5 mL/min/100A/25 °C ± 0.5.	APC TCC Y&M	1.36 log cycles	-	(**Mohideen *et al*., 2015**)
36	Juice Blend (Strawberry + Apple + Lemon)	Ultrasound	US at 376 W/10 min/35 °C	Total Aerobic Bacteria Y&M TCC	> 2 log$_{10}$ 1.3 log$_{10}$ (MPN/100 mL) was less than 3	10 days at 4 °C	(**X. Feng *et al*., 2020**)
37	Açai Juice	Ultrasound	19 kHz/5 min/700 J·mL^{-1}/500W/32 ± 1.2 °C	Mesophilic Bacteria Y & M	Significant	-	(**Oliveira *et al*., 2018**)
38	Barberry Juice	Ultrasound	20 kHz/70%, 100%/15 min/200 W	APC Y & M	-	4C for 42 days	(Farhadi Chitgar et al., 2017)
39	Apple Blueberry Cranberry juice	Ultrasonication+ Thermosonication	20 kHz/120 μm/600 W 60 °C	*A. ochraceus* *Rhodotorula* sp. *S. cerevisiae* *Penicillium expansum*	5 log	-	(**Jambrak *et al*., 2018**)
40	Orange Juice	Thermosonication	24 kHz/105 µm/ 33.31 W ml^{-1}/46 °C	TPC Y&M	1.6 log 0.9 log	10 days at 5 °C.	(**Guerrouj *et al*., 2016**)
41	Pineapple Juice	Pulsed Light Treatment	2.4 kV Number of pulses-94 or 187 pulses Dose-757/ 1479 J·cm^{-2}	Aerobic Mesophilic count Y & M	5-log	-	(**Vollmer *et al*., 2020**)
42	Orange Juice Pineapple juice	Pulsed Light Continuous Treatment System	Flow rate-100 ml/s Dose-95.2 J/cm^2	*E.coli*	4 log 4.5 log	-	(**Preetha *et al*., 2021**)

	Coconut Water				5.33 log		
43	Apple Juice	Pulsed Light Continuous Treatment Pulsed Light+ Ultrasound Treatment	Max Fluence-0.73 J/cm^2, 155 mL/min Pulse rate-3 pulses/s Pulse width-360 μ	*E.coli Salmonella Enteritidis S. cerevisiae* Indigenous flora	1.8–4.2 log 3.7–6.3 log	-	(**Ferrario and Guerrero, 2016**)
44	Apple Juice	Pulse Light Pulsed light+ Thermosonication	PL Fluenc-4 J/cm^2/ 1.20 μs/3 Hz 5.1 J/cm^2/ 1.52 μs/ 3 Hz	*E.coli*	4.9 log 6 log	-	(**Muñoz *et al.*, 2012**)
45	Orange juice	Cold Plasma Cold Plasma	HVACP Direct Treatment for 30 s HVACP Direct treatment for 120 s	Bacteria	>5 2.9	-	(**Xu *et al.*, 2017**)
46	Apple juice	Cold Plasma	Treatment: 3 mL juice for 30 s	*Escherichia coli* O157:H7	4.3	-	(**Liao *et al.*, 2018**)
47		Cold Plasma	Treatment: 3 mL juice for 140 s	*Zygosaccharomyces rouxii*	5	-	(**Xiang *et al.*, 2018**)
48		Cold Plasma	Treatment: 11 mL juice for 120s	*Escherichia coli*	4	-	(**Dasan and Boyaci, 2018**)
49	White grape juice	Cold Plasma	Treatment: Packaged sample for 4 min	*Saccharomyces cerevisiae*	7.4	-	(**Pankaj *et al.*, 2017**)
50	Blueberry juice	Cold Plasma	Treated for 6 min	*Bacillus sp.*	7.2	-	(**Hou *et al.*, 2019**)

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