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Inhibition of Enzymatic Browning and Oxidation in Pineapple Fruit by Different Thermal and Non-Thermal Techniques

Rana Fahad Ahmed[1*], Safoora Murad[2], Laiba Shafiq[1], Shahrooz Basharat[1], Khurram Saeed[3], Rabia Abid[4], Zareena Ashfaq[4], Waqar ul Hassan[3], Alishba Mazhar[5]

[1]National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan
[2]Department of Chemistry, University of Agriculture Faisalabad, Pakistan
[3]Department of Human Nutrition and Dietetics, Kohat University of Science and Technology, Kohat, Pakistan
[4]Department of Food Engineering, University of Agriculture Faisalabad, Pakistan
[5]Department of Human Nutrition and Dietetics, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

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Correspondence to: Rana Fahad Ahmed, National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan.
Email: ranafahadahmad7@gmail.com

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ABSTRACT

Pineapple (*Ananas comosus* L.) is a renowned tropical and subtropical fruit growing all around the world, having large nutritional and commercial values. Pineapple is greatly consumed in both ways, fresh and processed as dried pineapple, jam, jelly, juice, and dehydrated sweets. Pineapple has huge demand in food industry as it is rich in minerals, vitamins, antioxidants, and micronutrients, possessing many health benefits, such as improving digestion, lowering cholesterol, decreasing inflammation, ageing, and fighting cancer. A frequent issue that drastically influence the quality parameters of number of fruits and vegetables, is enzymatic browning. Enzymatic browning is the generation of dark pigment in fruit that doesn't only change the color but also changes the texture and lowers the nutritional values and flavor of the product. To avoid the fall in quality standards of pineapple, proper inhibition of enzymatic browning is a need of hour. Based on the recent research, this review summarizes the thermal and non-thermal techniques in prevention of browning. The treatments effectively target the enzymes that catalysis the oxidation of phenolic like polyphenol oxidase (PPO) and pectin methylesterase (PME) that increase the availability of phenolics to PPO, ultimately causing the pigmentation (browning) in pineapple. The efficiency of treatments enhances by combining both thermal and non-thermal techniques, leading to greater suppression of enzymatic browning with hybrid treatments. Though, the gap between researchers, government officials, and industry stakeholders still needed to be addressed to cross the barriers about infrastructure, cost, and consumer acceptance.

INTRODUCTION

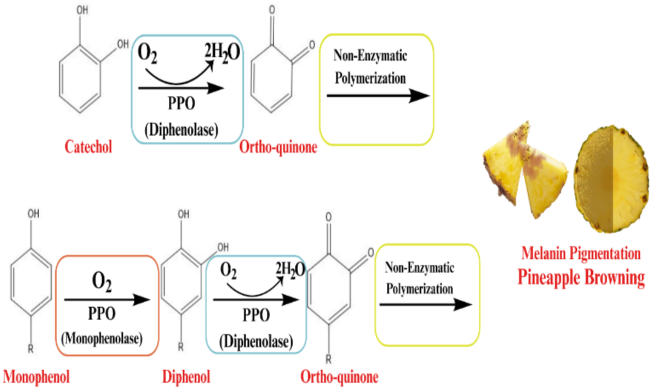
A renowned tropical and subtropical fruit with great nutritional and commercial value, the pineapple (*Ananas comosus* L.) is growing all around the globe (Guillén et al., 2022). One of the most significant commercial fruits, used in both ways, fresh and processed, is the pineapple (*Ananas cosmosus*) (Chakraborty et al., 2015). The market offers a variety of processed pineapple products, such as pulp, dried pineapple, tinned slices, pickles, jam, jelly, pasteurised juice, and dehydrated sweets. Pineapple juice distinguishes among these manufactured food products because of its mouthwatering flavour and pleasant aroma (Pipliya et al., 2022). Almost 80 percent of the pineapple sold is processed; 48 percent is sold as concentrated juice, 30 percent as canned fruit. Because of its good taste and scent, pineapple juice is a common good in marketplaces throughout Southeast Asia (Costa et al., 2013). Originated by European explorers who perceived the fruit as resembling a sizable pinecone, the English term "pineapple" derived from those explorers. High rates of water loss, respiration, senescence, and ethylene generation cause pineapple, an easily spoilt fruit with a limited life to be stored, after harvest, to quickly ripening and deterioration. With the intent of extending shelf life, thermal processing techniques are implemented in inactivating rotting bacteria and polyphenol oxidase (PPO), which catalyses the browning activities in the freshly extracted pineapple juice Shaik & Chakraborty, 2022). Appropriate preservatives such benzoates, sorbates, ascorbic acid, citric acid, and others added to a defined measure as advised by the FSSAI can help processed juice keep safe up to the duration of 6–7 months (Rattanathanalerk et al., 2005). Pineapple possesses unique sweet and savory taste, and more importantly pineapples are rich in minerals and antioxidants, and major source of vitamins and micronutrients, (Chadar et al., 2021) which pleases clients due to its health advantages. Because they encourage bowel movements, enhance intestinal health, and cleanse the kidneys, pineapples are commonly included in diets to treat or prevent constipation (Wang et al., 2024) Pineapple is much demanded in beverage industry and exhibits many health benefits, such as its ability to lower cholesterol, improve digestion, promote healing, and lessen inflammation, ageing, and fights cancer (Pipliya et al., 2022). Containing these health benefits, pineapple also includes phenols and bromelain, the main proteolytic enzyme complex that is characteristic of pineapple (Hale, Greer, Trinh, & James, 2005). In addition to its anti-inflammatory and tumour growth-controlling effects, bromelain has pharmacological and therapeutic qualities. Global production of pineapple is approximately 28 million tonnes annually, with Philippines, China, Brazil, Indonesia, and Costa Rica having largest productions worldwide. After citrus and bananas, pineapple is the third most top-selling fruit right now (Puglisi, 2023). The juice market is anticipated to grow at a rate of 4.75% year, with pineapple juice alone generating US$6818.7 million in revenue worldwide in 2021 (Abraham et al., 2021). Around the world, there are many cultivars and variations. Cayenne and Queen are two important varietal groups. With an annual yield of over 1,530,000 tonnes, Comte de Paris is the most common pineapple cultivar in southern China, making up over 90% of the country's total pineapple yield (Hong et al., 2013). In developing nations, PHL for all crops is anticipated to be between 20 and 40 percent. The United Nations Food and Agriculture Organisation estimates that 1.04 million hectares of pineapple land will yield 28.64 million tonnes (MT) of pineapple worldwide in 2021 (Choubey, 2021). In the world, smooth cayenne makes up almost 70% of pineapple production. The top producers for 2021 are the Philippines (2860.20 metric tonnes), Indonesia (2886.42 metric tonnes), and Costa Rica (2938.33 metric tonnes). Vietnam accounted for 38,554 hectares of the 437,571 hectares of pineapple cultivation in Asia as of 2020. More than 50–60% of solid waste is produced by the pineapple processing industry. Instead of microbial deterioration, another significant matter is the breakdown of pineapple puree by endogenous enzymes (Chakraborty et al., 2016). Peroxidase (POD) and polyphenol oxidase (PPO) enzymes were observed playing a major role in pineapple juice deterioration, particularly those involved in processes like oxidation and browning (Pipliya et al., 2022). Enzymatic browning primarily participates in processing and preserving the majority of fruits and vegetables while greatly impacting the quality of food, such as colour, flavour, and texture (Dong et al., 2021). Food products may sustain cuts, bruises, and other injuries when they are manufactured, transported, and stored, which eventually causes browning of product. For the manufacture of preserved fruit purees chunks, inactivating the enzyme polyphenoloxidase (PPO) is crucial, to prevent the catalysis of degradation processes following damage to tissue during the cutting process. Moreover, the creation of a dark pigment in fruit is also one of the responses of browning. In addition to changing its colour, browning lowers the nutritional and flavour qualities of the product. Therefore, inactivating enzymes that degrade quality is part of primary aims of fruit and vegetable preservation (Costa et al. 2013). The cloudiness retention in juice can be affected by pectinmethylesterase (PME). PME reduces cloud stability by hydrolysing pectin ester connections in juices. Enzymatic browning, a frequent problem for a number of vegetables and fruits, drastically influence the standards of liquid extracts by altering its colour, flavour, and consistency during processing and storage (Shevkani, 2024) thus, from an industrial standpoint, inactivating spoilage enzymes is critical to ensure the microbial safety of pineapple juice. This review concentrates on the techniques used nowadays to prevent fruit wastage in as a consequence of enzymatic reactions, browning of fruit. The efficacy of enzymatic processes in pineapple was reduced by using different heat and non-thermal techniques, which led to non-enzymatic browning and a lower grade. By concentrating on reaction elements like oxygen, copper ions, substrate, products, or even the enzyme itself, the majority of anti-browning treatments employ physical and chemical techniques to inhibit PPO activity. The most prevalent physical-based preservation methods for the production of beverages, smoothies, purees, nectar, dehydrated, and canned fruits and vegetables are the thermal technologies, classic heating, and cutting-edge thermal processing techniques, such as ohmic and microwave heating.

ENZYMATIC BROWNING MECHANISM IN PINEAPPLE

Enzymatic browning in pineapple is a complex physiological process that significantly impacts fruit quality, particularly during post-harvest storage and handling. This browning, often observed as internal browning (IB) or blackheart, is primarily triggered by the oxidation of phenolic compounds (Sidhu et al., 2023). When pineapple tissue is damaged by cutting, chilling, or senescence, cellular compartmentalization is disrupted, allowing polyphenol oxidase (PPO) to access its phenolic substrates in the presence of oxygen. PPO catalyzes the oxidation of these phenolics to quinones, which subsequently polymerize to form brown pigments such as melanins, leading to visible discoloration in the fruit. The severity of browning correlates with both the accumulation of phenolic compounds and the activity of PPO, making these factors central to the browning mechanism (Tilley et al., 2023). The activity of PPO in pineapple is closely linked to the degree of browning. Studies have shown that varieties of pineapple susceptible to internal browning exhibit significantly higher PPO activity and a greater number of PPO isoforms compared to resistant varieties. For example, after exposure to chilling stress, susceptible varieties displayed up to six PPO isoforms and heightened overall PPO activity, while resistant varieties like MD2 exhibited minimal PPO presence. The regulation of PPO expression can also be influenced by plant hormones such as gibberellins; increased gibberellin levels upregulate PPO gene expression and enhance browning, whereas low temperature and abscisic acid downregulate PPO and reduce browning symptoms (Zhang et al., 2016).

Figure 1

Demonstrates the Mechanism Involved in Enzymatic Browning of Pineapple by Polyphenol Oxidase (PPO).



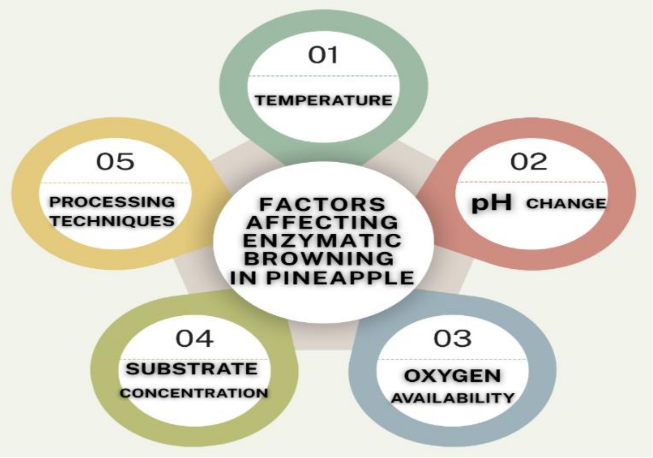
zPectin methylesterase (PME) also plays an indirect but important role in enzymatic browning. PME modifies the fruit's cell wall by demethylating pectin, which can alter tissue structure and increase the accessibility of phenolic substrates to PPO after cellular disruption. While PME does not directly catalyze browning reactions, its activity can exacerbate the process by making phenolic compounds more available for oxidation by PPO. Thus, the interplay between phenolic biosynthesis, PPO activity, and PME-mediated cell wall modification collectively determines the extent of enzymatic browning in pineapple. Understanding these mechanisms offers potential strategies for mitigating browning, such as manipulating hormone levels or targeting specific transcription factors to control phenolic accumulation and enzyme activity (Li et al., 2023).

Factors Affecting Enzymatic Browning

Enzymatic browning is a chemical process that occurs in many fruits and vegetables when they are cut or damaged, leading to the formation of brown pigments. This process is primarily catalyzed by polyphenol oxidase (PPO), a copper-containing metalloprotein that oxidizes phenolic compounds to quinones in the presence of oxygen (Murata, 2022). These quinones then undergo non-enzymatic polymerization to form melanins, which are pigments of high molecular mass and dark color. Several factors influence the rate and extent of enzymatic browning, with temperature and pH being particularly significant. Temperature plays a crucial role in enzymatic browning. The reaction generally accelerates with rising temperature up to an optimal point (Nath et al., 2022). For many fruits and vegetables, enzymatic browning is most pronounced at moderate temperatures, typically between 30°C and 70°C. Beyond this range, especially above 60°C, the enzymes begin to denature, resulting in a sharp decline in browning activity. This temperature sensitivity is utilized in food processing, where heating (blanching) is commonly employed to inactivate browning enzymes and preserve the color and quality of produce (Rasane et al., 2024). Research has shown that PPO, along with related enzymes like peroxidase (POD) and phenylalanine ammonia-lyase (PAL), are easily deactivated at high temperatures above 60°C. pH is another critical factor affecting enzymatic browning. The optimum pH for PPO activity varies depending on the specific enzyme and substrate, but generally ranges between 4 and 7. At this near-neutral pH range, the enzyme maintains its optimal structure and function. When the pH drops below 4.0, the enzyme becomes less active or is completely inactivated, drastically reducing browning. This is why acidic substances like citric acid or ascorbic acid are often used in the food industry to control browning by lowering the pH of fruit and vegetable tissues (Moon et al., 2020). Oxygen availability directly affects the browning reaction, as PPO requires molecular oxygen to convert phenols to orthoquinones. Higher oxygen concentration accelerates the browning reaction, while limiting oxygen exposure can significantly slow the process (Wang et al., 2024a).This principle is applied in various food preservation techniques such as vacuum packaging or submerging produce in water. The substrate concentration and type also influence enzymatic browning. Common substrates include phenols, catechols, caffeic acid, chlorogenic acid, and catechins. The rate of browning depends on both the availability of these substrates and the substrate specificity of the enzymes present. For example, PPOs can oxidize mono, di, and polyphenols with varying efficiency (Tilley et al., 2023). Processing methods significantly impact enzymatic browning. Cutting, peeling, or any mechanical damage destroys cell membranes, exposing cellular components to air and initiating the browning reaction. Delayed processing of minimally processed fruits and vegetables extends exposure time to browning factors, increasing discoloration. Understanding these factors is crucial for developing effective strategies to prevent or control enzymatic browning in food processing and preservation, helping to maintain the visual appeal, flavor, and nutritional value of fruits and vegetables (Weaver, 1974).

Figure 2

Shows the Factors that Affect the Pigmentation (Browning) in Pineapple.



THERMAL TECHNIQUES

Thermal techniques for controlling enzymatic browning in pineapple involve carefully managed heat application to inactivate polyphenol oxidase (PPO) enzymes while minimizing quality loss. Recent studies highlight that drying at lower temperatures (e.g., 50°C) better preserves color compared to higher temperatures (70°C), which can exacerbate browning (Marcel et al., 2014). Thermal processing, such as pasteurization, effectively reduces PPO activity but requires precise temperature control to balance enzyme inactivation and sensory quality (Silva & Sulaiman, 2022). We have discussed the different thermal techniques which are frequently used in the following writing.

Hot Air Treatment on Harvested Pineapples

According to the results mentioned in previous studies, the browning of fruit can be greatly reduced by heat-based treatments. Interior browning is considerably decreased by treatment with heated air at 38°C for a duration of three days prior to storage at 25°C by activating heat shock proteins that shield the object from getting damaged by cold (Zhang et al., 2017). The heat treatment causes a heat shock reaction in the fruit, which helps lessen interior browning and chilling sensitivity. Polyphenol oxidase (PPO) and peroxidase (POD) activities, which rise during cold storage and cause internal browning, are decreased as a result of the therapy, altering the activity of the enzymes involved in the browning response (Sui et al., 2023). And hot air therapy mitigates oxidative stress by lowering the fruit's levels of superoxide anion (O2) and malondialdehyde (MDA). Based on the previous studies, hot air therapy has a major impact on the pathways of alpha-linolenic acid metabolism, phenylpropanoid biosynthesis, MAPK signal pathway, and protein processing in the endoplasmic reticulum (Bae et al., 2020). The discovery of IB-related genes in these pathways illustrates the benefits of hot air technology that it reduces internal browning in winter-harvested pineapple by inhibiting the expression of lipoxygenase (LOX), dioxygenase (DOX), respiratory burst oxidase homologue (RBOH), phenylalanine/tyrosine ammonia-lyase (PAL/TAL), cinnamate-4-hydroxylase (C4H), p-coumarate: coenzyme A ligase (4CL), and polyphenol oxidase (PPO), while up-regulating the expression of heat shock factors (HSF), heat shock proteins (HSPs), phospholipase A2 (PLA2), and ascorbate peroxidase (APX) (Song et al., 2022).

Hot Water Bath Treatment

Thermal processing is commonly utilized in commercial fruit beverages for the deactivation of enzymes and microorganisms, hence reducing organoleptic modifications that impact product shelf life and quality (Polak et al., 2024). Enzyme inactivation is mostly required to prevent quality changes associated with cloud loss, while mild temperature allows for slight modifications both in terms of its sensory and nutritional parameters. Pineapple juice has a significant residual pectin methylesterase (PME) activity. It is a product of diluting the concentrate juice and pasteurizing it before shipping it to Europe. Accordingly, the current study offers a thorough kinetic analysis of how pasteurization affects PME inactivation in pineapple juice (Cautela et al., 2018). The fresh juice was subjected to temperatures between 70 and 95 °C for varying durations until PME was completely denatured. The results of thermal stabilization treatments demonstrated that, within the temperature range under study, the behavior of the inactivation of an enzyme is a first-order kinetic process (Kabir & Ju, 2023). Additionally, the reaction rate constants for denaturing PME were found; specifically, the z value (z), decimal reduction time (Dref), and activation energy (Ea) were 36 ± 3 °C, 16 s (at reference temperature of 106 °C), and 78.2 ± 4.5 kJ/mol, respectively. So, to maintain the particle suspension of sterilized pineapple juice, a methodology of 106 °C with a retention period of 1 minute averagely likely to be used, according to the thermal resistance data here given in this study to inactivate PME (Cautela et al., 2018). The technique of thermally inactivating polyphenol oxidase (PPO) in pineapple puree has been thoroughly investigated in order to prevent browning and maintaining the fruit's quality standards. In the thermal inactivation method, the enzyme is denatured and rendered inactive by heating the pineapple puree to a particular temperature. The ideal temperature range for the thermal inactivation of PPO in pineapple puree is 40°C to 90°C, with an activation energy of 82.8 kJ/mol, surpassing the value recorded for rice at 23.3 kJ/mol (Zawawi et al., 2022). The heat inactivation of polyphenol oxidase (PPO) in pineapple puree adheres to first-order kinetics, with the rate constant (k) escalating as temperature rises. The kinetic parameters, such as the rate constant (k), half-life (t1/2), and inactivation rate constant (k-inact), exhibit dependence on temperature (Chutintrasri & Noomhorm, 2006).

Low Temperature Treatment

Pineapple plants of cultivar ‘Trad-Srithong’ exhibit demonstrates crassulacean acid metabolism (CAM), as the levels of organic acids in crown and stem leaves are greater at 0600 than at 1200. The fruit, which is very prone to internal browning (IB) at temperatures below 15 °C, exhibited IB after being stored for 10 days at 8 °C or 13 °C, but not at temperature of 20 °C. The time of harvest (0600 or 1200) had no impact on IB development (Dolhaji et al., 2019). During storage, the titratable acidity (TA) rose, resulting in a higher TA and lower pH in juice than in core tissue. IB symptoms were more pronounced in the flesh close to the core, and the level of ascorbic acid declined towards the end of storage (Youryon et al., 2011). Another study examined internal browning (IB) and associated biochemical changes in the pineapple cultivars Mauritius and Kew during a 3-week cold storage period at 10°C and 85% RH. Mauritius showed quicker IB onset respiration, ripening and acid accumulation compared to Kew. The tissues of both cultivars that underwent browning exhibited elevated levels of PPO and peroxidase activity, as well as increased electrolyte leakage. In both cultivars, early harvesting at the 100% green stage led to a reduction in IB severity (Weerahewa & Adikaram, 2005). studied the effects of storage temperatures of 6, 10, and 25 °C on the quality and internal browning (IB) of pineapple cv. ‘Comte de Paris’. Fruit kept at 6 °C retained greater amounts of glucose, fructose, vitamin C, and TA while reducing IB and better preserving quality compared to higher temperatures. Increased temperatures sped up the loss of sucrose, the decline of protein levels, the activity of PPO, and the development of IBs, indicating that 6 °C is ideal for storage. The effect of packaging materials on internal browning (IB) in pineapple cv. ‘Mauritius’ during cold storage (at 15°C and 85–90% RH) and subsequent ambient storage was examined by (Hussain et al., 1998) according to which, after 10 days of cold storage and 4 days of ambient storage, the incidence of IB in fruits packed with dried straw was significantly lower (16%) compared to those using shredded paper (75%) and the control group (78%). Fruits packed with straw exhibited superior quality, characterized by minimal browning, acceptable organoleptic properties, and total soluble solids ranging from 11% to 12% (Table. 1).

Table 1

Repressing Effect of Thermal Techniques on Enzymatic Browning of Pineapple

Product Type	Technique	Processing Parameters	Reduction of Enzymes / Internal Browning (%)	References
Pineapple	Hot air treatment	Treated: 3 days at 38 °C, 80–90% RH + 5 days at 25 °C, 80–90% RH (total 8 days) Untreated: 8 days at 25 °C	20 PPO 45 PPO, 80 IB 0 PPO, 0 IB	(Song et al., 2022)
Pineapple juice	Thermal inactivation of pectin methylesterase	90 °C, 100 sec 106 °C, 1 min	90.4 PME 100 PME	(Cautela et al., 2018)
Pineapple juice	Thermal inactivation of polyphenoloxidase	40–60 °C, 5–30 min 85 °C, 5 min 90 °C, 5 min	~60 PPO 93 PPO 98.8 PPO	(Chutintrasri & Noomhorm, 2006)
Pineapple	Chilling temperature	20 °C, 20 days	100 IB	(Youryon et al., 2011)

Pros and Cons of Thermal Treatments

Thermal techniques effectively combat enzymatic browning in pineapple by inactivating polyphenol oxidase (PPO) while balancing quality retention. Recent studies demonstrate that pasteurization at 85°C with dual thermal treatments maintains vitamin C stability and microbial safety in pineapple juice during 12-month storage, though lower temperatures (75°C) initially preserve more nutrients but degrade faster (Chadar¨¦ et al., 2021). Optimized drying at 60°C with parallel airflow minimizes color loss (ΔE) in slices by reducing PPO activity and preserving lightness (L-value) (Marcel et al., 2014). These methods also enhance shelf life by preventing microbial growth, particularly in low-pH environments (Chadar¨¦ et al., 2021). While emerging technologies like ohmic heating integrate thermal principles for energy efficiency, traditional thermal processing remains vital for large-scale applications due to its reliability in enzyme inactivation and quality control (Gavahian & Chu, 2022). Thermal techniques are effective in deactivating polyphenol oxidase (PPO), but also present some drawbacks. High processing temperatures (e.g., above 60°C) can induce non-enzymatic browning through Maillard reactions and caramelization, leading to undesirable color changes and hydroxymethylfurfural (HMF) formation in pineapple juice. Prolonged heating also degrades heat-sensitive nutrients like carotenoids and vitamin C, reducing yellowness (b-value) and lightness (L-value) in dried slices. Additionally, thermal drying may cause textural issues, such as lignification and poor rehydration, compromising product quality (Guangsen et al., 2022). These limitations underscore the need for precise temperature control or hybrid approaches to mitigate quality losses in thermally processed pineapple products.

NON-THERMAL TECHNIQUES

In food processing and preservation, non-thermal technologies are becoming more and more popular as alternatives to traditional methods (Misra et al., 2017). By inactivating enzymes and microbes, it improves food safety and shelf life. They are also sustainable systems since they enable the manufacture of high-quality products with less of an adverse effect on the environment in terms of reduced emissions, water consumption, and energy efficiency (Meng et al., 2018). Recent studies highlight those non-thermal methods like high-pressure CO_2 processing better preserve color and phenolic content while maintaining lower enzymatic activity compared to traditional thermal treatments (Chacha et al., 2021). Some of the non-thermal techniques are discussed below:

Dielectric Barrier Discharge Plasma Technology

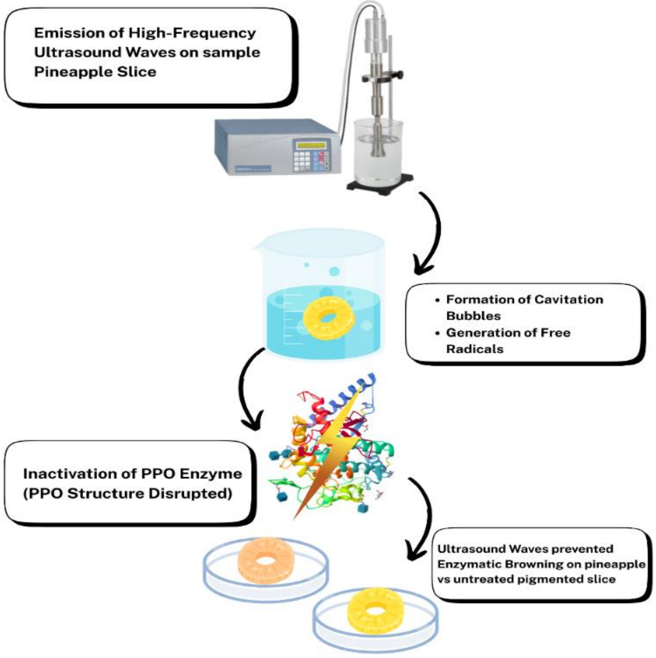
Dielectric barrier discharge plasma is the most popular technique for enzyme inactivation due to its low operating cost, eco-friendliness, absence of harmful by-products, low temperature, and simple construction (Kogelschatz & processing, 2003). DBD plasma has demonstrated promising results in inactivating PPO and POD in fruit products like mushrooms, strawberries, fresh-cut apples, orange juice, apple juice and tender coconut water (S. Pankaj et al., 2013). Three distinct voltages, 25, 35, and 45 kV, were used to examine the inactivation kinetics of PPO and POD in pineapple juice for a maximum of 10 minutes of plasma treatment. It was discovered that the Weibull model best described the kinetics of enzyme inactivation. In comparison to POD, which had scale factors of 40.74, 19.76, and 7.28 minutes at the same voltages, PPO had scale factors of 15.95, 10.87, and 5.73 minutes at 25, 35, and 45 kV, respectively. This indicates that POD exhibited greater resilience than PPO against deactivation by DBD plasma (Pipliya et al., 2022). Comparable investigations were conducted on the spoilage enzymes in deteriorated coconut water that are inactivated by atmospheric cold plasma technology. Dielectric barrier discharge (DBD) plasma treatments in ambient air, with voltage levels ranging from 18 to 28 kV, were employed to examine the kinetics of peroxidase (POD) and polyphenol oxidase (PPO) inactivation (Dong et al., 2021). In comparison to PPO, which exhibited half maximum activity values of 0.67, 1.18, and 1.35 minutes at equivalent voltages, POD required a longer duration to attain half maximal activity, necessitating 0.84, 1.67, and 2.53 minutes at 18 kV, 23 kV, and 28 kV, respectively. Assuming that POD exhibits a stronger resistance to cold plasma than PPO, its deactivation by cold plasma in soft coconut water serves as an essential quality indicator (Chutia et al., 2019). An investigation was conducted to explain the kinetics and processes of polyphenol oxidase (PPO) inactivation in plant-derived meals, wherein 2 mL of PPO solution was subjected to dielectric barrier discharge (DBD) plasma for different durations. PPO activity decreased by 70.2%, 85.7%, and 94.2% after three minutes of exposure to DBD plasma at 19.4, 26.4, and 32.6 W, respectively. Both the logistic and Weibull models accurately correspond to the PPO inactivation curves. Moreover, exposure to DBD plasma significantly diminished the intrinsic fluorescence intensity of PPO, indicating a disruption of its tertiary structure (Dong et al., 2021). Air dielectric barrier discharge plasma treatments of 30, 40, and 50 kV for a duration of up to 5 minutes were employed to investigate the kinetics of tomato peroxidase inactivation as a model enzyme. Enzyme activity was diminished by both voltage and treatment duration, with the impact of treatment duration being more significant. Model parameters were determined by fitting experimental data to various kinetic models, including logistic, Weibull, and first-order models (S. K. Pankaj et al., 2013).

Multi-Frequency Power Ultrasound

Ultrasonography is a novel non-thermal physical processing method having a broad variety of implementations in the food business and related fields (Jin et al., 2015). One of the variables affecting the quantity and severity of cavitation in liquids is the ultrasonic frequency (Mason, 2002). The yield of ultrasonic cavitation was known to be boosted by multiple frequency sonication (Feng et al., 2002). Pineapples are often dried by natural or traditional hot-air drying, however these processes take a long time, degrade the quality, and use more energy (Chauhan, 2019). Power ultrasonography has recently become a popular nonthermal pretreatment before drying, and because of its superiority over conventional techniques, it has become a key subject of experimental research (Llavata et al., 2020). The application of ultrasound pretreatment prior to pineapple drying by (Fernandes et al., 2008) demonstrated that osmosonication pretreatment can cut drying times by up to 31% when evaluated against pineapple samples with no treatment, this was demonstrated in a different investigation, the formation of micropores in the slices' interior structure (Fernandes et al., 2009). According to a major research by (Xu, Sylvain Tiliwa, et al., 2022), ultrasound pre-treatments reduced drying times of fruit slices and altered the microstructure as well. The effects of mono-frequency ultrasound (MFU) at 20 kHz, dual-frequency ultrasound (DFU) at 20/40 kHz, and tri frequency ultrasound (TFU) at 20/40/60 kHz on the mass transfer, drying kinetics, and qualitative traits of infrared-dried pineapple slices were investigated. The drying duration was shortened by 19.01–28.8% for the US, 15.33–24.41% for the US-OD, and 38.88–42.76% for the US-ET as compared to the control group and ultrasound treatment (Xu et al., 2022). Comparing tri-frequency ultrasound to MFU, the biggest decreases were obtained demonstrating higher product quality and time savings of 6.36-11.20%. Compared to pretreatment sample groups, US-OD slices showed better preservation of nutrients and bioactive chemicals, higher hardness values, and decreased browning and rehydration capabilities (Osae et al., 2019). Thus, when combined TFU and osmotic dehydration may increase ultrasonic effectiveness, and shorten drying times, and yield high-quality products (Figure 3).

Figure 3

Represents the Steps Involved in Prevention of Enzymatic Browning by Ultrasound Waves Technology.



Continuous Pressure Change Technology (PCT)

The freshly developed non-thermal technique is called pressure change technology (PCT), depends on a physical procedure. The juices or other liquid foods are compressed to an adequate level, in the environment provided of an inert gas (Vollmer et al., 2022). The gas is absorbed into liquid extracts of fruits and enters microbial and plant cells when pressures reach 50 MPa for a short time. An immediate decline in pressure to room temperature causes the inert gas to expand and destroy the organized structures, including microbial or plant cells. The PCT's working depends on dynamic relaxation phase. In comparison to fresh and pasteurized (90 °C) juices, short-term consequences of a single and a double PCT treatment on the most significant quality parameters were investigated and evaluated (Vollmer, Santarelli, et al., 2020). The outcomes of the research showed that trained panellists' assessments of descriptive sensory profiles were not greatly influenced by thermal pasteurization or non-thermal PCT. Similarly, Aschoff *et al.,* (2016) conducted non-thermal pasteurization of orange juices applying a consistent (PCT) apparatus. The pressurization of orange juice and nitrogen were employed in the PCT treatment at a moderate level (P = 25 or 50 MPa) in a tubular continuous reactor (Aschoff et al., 2016). The remaining activity of pectin methylesterase was 26–27%, whereas peroxidase was completely inactivated. Meanwhile, overall the measure of aerobic plate lowered by at least of 3.4 log10 cfu/mL(Vollmer, Santarelli, et al., 2020).

Pulsed Light Technology

An efficient germicidal technique, pulsed light (PL) uses short, strong bursts of visible (VIS), ultraviolet (UV), and infrared (IR) light with wavelengths ranging from 100 to 1100 nm (Oms-Oliu, Martín-Belloso, et al., 2010). Three voltage settings (1.8, 2.1, and 2.4 kV) and pulse counts (47, 94, and 187) were used in a laboratory-scale PL batch system to examine the impact of PL on important pineapple juice quality parameters. A 5-log decrease in the numbers of aerobic mesophiles, yeasts, and molds was observed in treatments that used 2.4 kV with either 94 or 187 pulses (757/1479 J·cm^{-2}) (Vollmer, Chakraborty, et al., 2020a). All juices treated with PL maintained their bromelain activity, however peroxidase exhibited greater resistance to PL than polyphenol oxidase. Treatment dosage is the primary determinant of the PL's effectiveness in reducing PPO activity. (Manzocco et al., 2013) demonstrated that pulsed light dosages exceeding 8.75 J cm^{-2} were sufficient to achieve complete enzyme deactivation in this context. Pulsed light treatment significantly inactivated the enzyme at low PPO concentrations (4 to 10 U) because of protein structural alterations such as unfolding/aggregation events and fragmentation. In addition, the aftereffects of PL treatment on the nutritional and physical qualities of fresh-cut mangoes were examined by (Charles et al., 2013). He used PL treatments which amounted to 8 J cm^{-2}, and the activity of the enzymes Phenylalanine ammonia-lyase (PAL) and Polyphenol oxidase (PPO) was evaluated. Three days later, the treatments maintained PAL activity while increasing PPO activity. (Pellicer et al., 2018) examined the PPO activity in mushroom by using spectrophotometric and fluorometric PL methods for deactivation of enzyme. The work was concluded that PPO enzyme activity was reduced following the Weibull model, which was strongly linked to unfolding of protein. Nonetheless, the product type also affects how much PPO is inactivated. Due to the thermal degradation brought on by the treatments, (Oms-Oliu, Aguiló-Aguayo, et al., 2010) showed that the texture of sliced mushrooms was significantly impacted by the placement of high-pulsed light fluencies (12 and 28 J cm^{-2}). Polyphenol oxidase activity significantly increased with the use of the maximal pulsed light dosage, improving enzymatic browning. A significant decrease in phenolic compounds, vitamin C, and antioxidant capability was observed at 28 J cm^{-2}. The results suggest that pulsed light at 4.8 J cm^{-2} dosages may extend the lifespan of fresh-cut mushrooms without appreciably altering their texture or antioxidant capacity (Vollmer, Chakraborty, et al., 2020a).

Ozone Treatment

Ozone (O3) treatment is regarded as a highly effective sanitizer in maintaining the post-harvest standards of vegetables and fruits due to its potent oxidizing properties (Tzortzakis & Chrysargyris, 2017). The efficacy of ozone (O3) in deactivating Polyphenoloxidase correlates with ozone concentration and exposure duration. Concerning this issue, (Jaramillo Sánchez et al., 2018) shown the working of PPO in peach beverage reduced over time in a non-linear manner, with reductions of 94% and 97% observed after 12 minutes at O3 concentrations of 0.11 and 0.20 mg/min/mL, respectively, in a bubble column at a temperature of 20°C. The combined application of O3 treatment (1.2 g/h for 10 minutes) and 0.5% lactic acid led to a 60% decrease in PPO activity (Garud et al., 2018). It is essential to meticulously assess the processing settings to avert possible losses of antioxidants (Alothman et al., 2010). Ozone treatment enhanced enzyme activities in papaya fruit throughout the storage period, indicating improved physiological processes (Ong et al., 2014b). Ozone treatment postponed ripening of fruit by inhibiting the production of ethylene and modulating degradation of cell wall, thereby preserving fruit firmness and nutrient composition (Ong et al., 2014b).

High-Intensity Ultrasound Technology

Ultrasound is a promising food preservation technique that exposes food to high-intensity, low-frequency waves with a power density of 10 to 1000 W/cm^2 and a frequency range of 20 to 100 kHz. Longer exposure and higher intensity (376 W/cm^2 and 10 min) decreased polyphenol oxidase (PPO) activity by 20% when compared to fresh pineapple juice, according to a study on the effects of ultrasound processing on the physicochemical properties of pineapple juice (Costa et al., 2013). He investigated the combined impact of ultrasonic and ultraviolet (UV) treatments to extend the shelf life of pineapple juice in a different study (Anjaly et al., 2022). The results of that study showed that while the qualitative parameters were maintained, the combined treatment had no discernible impact on the physicochemical properties. A product that was microbiologically safe and had an organoleptic quality similar to fresh juice was produced by the ideal combination of UV dosage of 1.577 J/cm^2 and ultrasound treatment for 22.95 minutes. In support of earlier research, the inactivation of PPO and POD in bayberry juice with ultrasonic treatment was examined in (Aslam et al., 2023; Cao et al., 2018). For PPO and POD, they found that inactivation rates reached 90.72% and 73.18%, respectively, at the greatest ultrasonic intensity and exposure duration. With an energy density of 1610 W cm^{-2} pulsed at 5 seconds on and 5 seconds off for 7 minutes 30 seconds, the improved ultrasound procedure inactivated PPO by 76.42% and POD by 64.57% (Table. 2).

Table 2

Reduction in Internal Browning of Pineapple Fruit by Non-Thermal Techniques.

Product Type	Technique	Processing Parameters (Temperature, Voltage, Time, Pressure, Pasteurization, UV)	Reduction of Enzymes (PPO, POD, PME, Bromelain, IB %)	References
Pineapple juice	Dielectric Barrier Discharge (DBD) plasma	10 min, 25 kV 10 min, 35 kV 10 min, 45 kV	61.3 POD, 52 PPO 48.9 POD, 40.33 PPO 30.9 POD, 23.9 PPO	Pipliya et al., 2022
Pineapple slices	Multi-frequency power ultrasound	MFU (20 kHz) DFU (20/40 kHz) TFU (20/40/60 kHz) 35°Brix sucrose (US-OD) , 75% ethanol (US-ET)	9.67–38 PPO, 27.2–78 POD 41.94–70.96 PPO, 82.98–95.21 POD 38.71–64.52 PPO, 98.32–99.16 POD 21.23–32.21 BRM, 64.42–75.27 BRM	Xu, Tiliwa, et al., 2022

Pineapple juice	Continuous Pressure Change Technology (PCT)	1 L, 50 MPa, Argon, 3 min, 35 °C Flow 1.3 L/min 90 °C (Thermal pasteurized)	15 BRM, 23 POD 97 BRM, 85 BRM 83.3 BRM	Vollmer, Santarelli, et al., 2020
Pineapple juice	Pulsed Light Treatment	50 mL, 90 ± 1 °C, 5 min 1.8 kV/187 pulses, 1479 $J \cdot cm^{-2}$ 2.1 kV/187 pulses, 1479 $J \cdot cm^{-2}$ 2.4 kV/187 pulses, 1479 $J \cdot cm^{-2}$	81 POD, 61 PPO, 93 BRM 18 POD, 32 PPO, 0 BRM 31 POD, 39 PPO, 0 BRM 41 POD, 50 PPO, 0 BRM	Vollmer, Chakraborty, et al., 2020a
Papaya fruit	Ozone treatment	Ozone: 0, 1.5, 2.5, 3.5, 5 $\mu L \ L^{-1}$ 25 ± 3 °C, 70 ± 5% RH, 10 days & 14 days Untreated: 14 days	Day 10: PPO & POD increased above control Day 14: PPO reduced to control level PP same as ozone-treated	Ong et al., 2014a
Pineapple juice	High-intensity ultrasound	376 W/cm^2, 10 min 54 °C, 10 min	20 PPO 0 PPO	Costa et al., 2013
Pineapple fruit	UV-C Irradiation	Stored at 10 °C, 21 days: 26.4 $kJ \ m^{-2}$, 39.6 $kJ \ m^{-2}$ Stored at 10 °C, 28 days: 26.4 $kJ \ m^{-2}$, 39.6 $kJ \ m^{-2}$	90 IB , 90 IB 45 IB, 45 IB	Sari et al., 2016

UV-C Irradiation

The quality of postharvested pineapple cv. 'Phulae' was investigated in relation to UV-C radiation. Four distinct treatments were applied to the pineapples: UV-C irradiation for 10 minutes (13.2 kJ m^{-2}), UV-C irradiation for 20 minutes (26.4 kJ m^{-2}), UV-C irradiation for 30 minutes (39.6 kJ m^{-2}), and a control group that did not receive any UV-C irradiation. Quality attributes such disease incidence, internal browning, color, total soluble solids (TSS), titratable acidity (TA), vitamin C levels, total phenolic compounds, total flavonoid content, and antioxidant activity were evaluated every seven days for 28 days after irradiation. The results showed that UV-C radiation significantly ($P < 0.05$) decreased the incidence of illness and internal browning in pineapples after 28 days of storage at 10 °C. A decreased incidence of illness was linked to higher UV-C radiation doses. Color, TSS, and TA did not significantly differ between treatments. UV-C irradiation increased the peel's total phenolic component, total flavonoid, and antioxidant capacity significantly ($P < 0.05$); nevertheless, UV-C increased the pulp's vitamin C content (Sari et al., 2016). Similar studies investigated that how 10 °C stored fresh-cut pineapples were affected by UV-C radiation for 60 and 90 seconds. While the UV-C treatment lowered vitamin C and enhanced browning, it also preserved firmness and total soluble solids (TSS) and decreased sugar loss. There were no noticeable variations between exposures of 60 and 90 seconds. UV-C radiation can preserve quality but browns stored food (Pan & Zu, 2012). Another study examined the antioxidant capabilities of fresh-cut pineapple, banana, and guava after UV-C irradiation (0–30 min). Guava and banana had higher total phenol and flavonoid content, but all fruits had lower vitamin C. Long-term treatment boosted banana antioxidant capacity (FRAP and DPPH), but pineapples stayed steady and guavas rose after 30 minutes (Alothman et al., 2009).

Pros and Cons of Non-Thermal Techniques

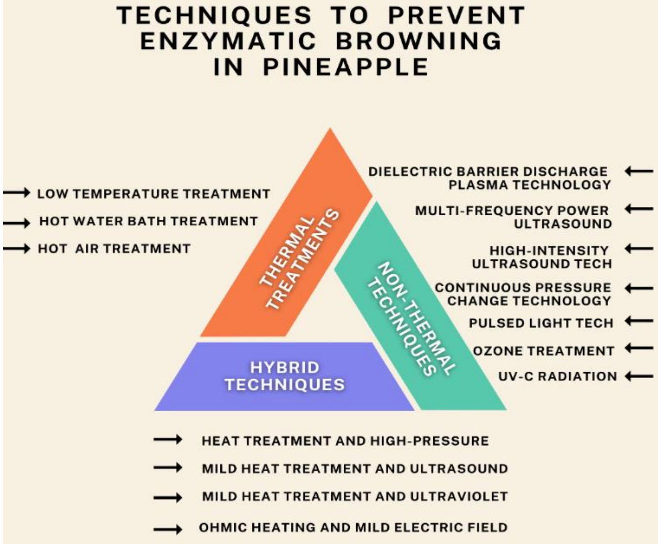
Non-thermal techniques, such as pulsed electric fields (PEF) and high-pressure processing (HP), offer significant advantages in preserving pineapple by minimizing enzymatic browning while maintaining nutritional and sensory qualities. PEF effectively reduces polyphenol oxidase (PPO) activity and internal browning while preserving antioxidant compounds like ascorbic acid during storage, making it ideal for extending shelf life under sub-optimal temperatures (Moura et al., 2024).Similarly, HP treatments using argon or nitrogen gases lower respiration rates and ethylene production, reducing browning and nutrient degradation without compromising tissue firmness or juice leakage (Silva & Sulaiman, 2022). Non-thermal techniques like ultraviolet (UV) and ultrasound offer significant advantages in preserving pineapple by reducing enzymatic browning while maintaining nutritional and sensory quality. UV treatment, particularly at optimized dosages, is effective in microbial reduction while retaining phenolic compounds and bromelain activity in pineapple juice (Anjaly et al., 2022). These methods ensure better retention of phenolic compounds and antioxidant capacity compared to thermal approaches, offering a promising alternative for high-quality preservation of minimally processed pineapples (Moura et al., 2024). Non-thermal techniques for preserving pineapple and preventing enzymatic browning offer advantages in nutrient retention but also have notable limitations. One major drawback is their limited effectiveness on foods with complex shapes or uneven surfaces, as folds and fissures can shield microorganisms from treatments like pulsed light, reducing microbial inactivation efficiency (Franco-Vega et al., 2021). Some microbial strains, such as Listeria monocytogenes, may exhibit resistance to certain non-thermal methods, compromising food safety. Additionally, processes like pulsed electric fields (PEF) can cause undesirable changes in juice quality, including sedimentation, discoloration, and flavor degradation. Equipment costs and operational complexities, such as the need for specialized waveforms and temperature control, also pose challenges for widespread adoption. Furthermore, non-thermal methods often have limited penetration depth, restricting their use mainly to liquids or surface treatments rather than solid or whole fruits. These factors highlight the need for careful optimization and sometimes combination with other preservation methods to ensure efficacy and quality in pineapple products (Chacha et al., 2021) (Leong et al., 2024) .

Combination of Thermal & Non-Thermal Treatment

Due to some of the drawbacks found in thermal and non-thermal treatments, scientists experimented by combining both kind of treatments to obtain best results. The combination of both kind of treatments gives the combined benefits to the novel treatment and make it more favorable for avoiding melanin generation. By applying both methods together, synergistic effects can be achieved, allowing for reduced intensity of each treatment. This not only enhances food safety by more effectively inactivating pathogens, but also helps maintain the food's original taste, texture, and nutritional value (Cano-Lamadrid & Artés-Hernández, 2022). Additionally, the combined use of these technologies can lower processing costs and energy consumption, making food preservation more efficient and sustainable Here are some hybrid techniques, which gave excellent preservation against enzymatic browning (Figure 4).

Figure 4

Illustrates the Techniques Utilized to Prevent Enzymatic Browning in Pineapple.



Ohmic Heating and Mild Electric Field (MEF)

Hot water treatment (HWT) and ohmic heating have been examined for their impact on enzyme activity and qualitative characteristics in pineapple juice. Both HWT and OH treatments effectively inactivated PPO, with OH demonstrating superior efficacy at identical temperature and duration (Rattanathanalerk et al., 2005). Ohmic heating employing a mild electric field (MEF) efficiently deactivates PPO in pineapple juice, with the deactivation rate dependent upon the voltage gradient (VG), temperature, and duration of holding time. The inactivation of PPO in pineapple juice exhibits an exponential correlation with the voltage gradient (VG) ranging from 16 to 36 V/cm, whereby greater VG leads to increased inactivation. Ohmic heating using MEF has been evaluated against typical heating methods and has demonstrated higher effectiveness in inactivating PPO and other rotting enzymes in pineapple juice. Ohmic heating with MEF can also influence many enzymatic activities, including cell wall disintegrating enzymes, ethylene production enzymes, and protective-associated proteins (H. A. Makroo et al., 2022). The quality characteristics of pineapple juice, including colour, pH, and total phenolic content (TPC), are influenced by ohmic heating with MEF. The TPC concentration diminishes by 18% over hot water treatment, but observations are made for losses of just 7–10% with ohmic heating at 16–24 V/cm. The application of MEFs in ohmic heating also boosted the antioxidant capacity of pineapple juice. It arises from the improved extraction of antioxidant molecules and the inhibition of pro-oxidant enzymes such as PPO. The remaining PPO activity diminished after hot water treatment and ohmic heating at 16 V/cm, recorded at 50.06% and 56.67%, respectively, despite comparable time-temperature profiles in both treatments (H. Makroo et al., 2022). The somewhat elevated residual PPO activity in the ohmic heating sample may result from enhanced enzyme-substrate interactions caused by modifications in the enzyme, facilitating optimal substrate utilization(Gavahian & Chu, 2022).

Heat Treatment and High-pressure

Pectin methyl esterase (PME) is a principal enzyme that deteriorates the quality of pineapple puree, particularly affecting its viscosity and pectin precipitation. The inactivation of pectin methyl esterase (PME) in pineapple puree was examined by a combination of high-pressure and thermal treatments, ranging from 0.1 to 600 MPa and 30 to 70 °C for durations of 1 to 40 minutes. As the temperature and pressure increased, PME gradually became inactive. At 600 MPa and 70°C, the most notable inactivation a 66% decrease in PME activity was noted. To clarify the PME inactivation kinetics under various combination treatments, mathematical models were created. These models can predict optimal PME inactivation settings and mitigate negative effects on pineapple puree quality. The most significant enhancement in phenylalanine ammonia-lyase (PAL) activity and total phenolic content (TPC) in pineapple peel samples occurred at optimal circumstances for PME inactivation, specifically at 225 MPa and 7.6 minutes (Chakraborty et al., 2019).

Combined high-pressure and temperature treatments have been researched for their effects on the enzymatic activity in pineapple. The study used pressures between 0.1-600 MPa and temperatures between 30-70°C for 0-20 minutes (Roobab et al., 2022). The results demonstrated that high-pressure processing (HPP) had a substantial effect on the color and bioactive components in pineapple puree in these settings. The combined effects of high pressure and temperature on the enzyme activity in pineapple suggest that the activity of polyphenol oxidase (PPO) and peroxidase reduced dramatically with increasing pressure and temperature. The maximum inactivation of PPO and peroxidase was reported at 600 MPa and 70°C, with a reduction of 96% and 92%, respectively. This treatment greatly lowered the activity of bromelain, which is an essential enzyme in pineapple (Chakraborty et al., 2015). In the another study, the effects of pressure, temperature, duration, and post-pressurization storage on pineapple polyphenol oxidase and peroxidase activity were examined independently or in combination. The studies used the following scales for the independent variables and were conducted in accordance with a 27-basic treatments design: 12.40 and 60 degrees Celsius; pressure: 0.100.300, 500, and 600 MPa; duration: 15.30 and 45 minutes: Storage options include 24 hours at 5 or no storage following pressure treatment, prior to tile activity determination. Tris buffer (pH=6.5) was blended with the fruit's core to produce an enzymatic extract consisting of both peroxidase and polyphenol oxidase. After filtering, the extract to be compressed was separated into tiny parts and placed in thermosealed polythene bags. Just before the pressurization, the samples were defrosted after being frozen and stored in a freezer at -18° (Kuang et al., 2023). By calculating the absorbance that came from the remaining enzyme activity on catechol, the substrate, polyphenol oxidase activity was identified using a spectrometric approach. In similar way, Peroxidase can be identified using hydrogen peroxide as the substrate and phenylenediamine as the acceptor. Modified second-degree polynomial models were used for polyphenol oxidase and peroxidase activities (Thatoi et al., 2023). Due to aleatorization limitations, a mixed model was altered by applying fixed effects to polynomial coefficients and aleatory effects to the factor day in which the experiment was carried out. Controlled and maintained temperature for three treatments on each experiment day contributes to aleatory impact on treatment day (Rosenthal et al., 2002). Polyphenol oxidase statistical study revealed that storage factor had a considerable negative impact on enzyme activity, indicating that stored samples had reduced the tile activity. The independent variables did not seem to interact with one another. There was an insignificant negative linear influence on operational time, referring to decreased linear activity over time. Temperature or time did not linearly or quadratically much impact the activity of polyphenol oxidase. At 30.8 C of temperature and 274 IVlPa of pressure, the maximum polyphenol oxidase activity was found. Temperature variations had more effect on polyphenol oxidase activity than pressure fluctuations (Cheng et al., 2021). Another study revealed that the storage term or time of the enzymatic extract post pressurization had no major effect on the peroxidase case's enzymatic activity. However, the way temperature and pressure interacted still had a big effect on peroxidase activity (Liang et al., 2024). In other words, when the pressure went up, the enzyme's activity stayed almost the same at low temperatures. But when the temperature went up to high levels, the peroxidase activity dropped by a lot. In the case of peroxidase, the quadratic effect only caused bending when it came to temperature. In the pressure vessel, the maximal peroxidase activity was confirmed at a tile ambient temperature of 36.8~ without any operating pressure (0 MPa) being applied. The least degree of activity for both enzymes came from the highest degree of quantitative factors. The highest decrease in enzymatic activities was 60.08% for peroxidase and 33.17% for polyphenol oxidase when contrasted with first values acquired for the untreated enzymatic extract (Amoghin et al., 2024).

Mild Heat Treatment and Ultraviolet

It has been demonstrated that the enzymatic activity in pineapple juice is `enhanced when mild heat treatments and ultraviolet (UV) light treatments are combined. Bromelain, oxidoreductases (POD and PPO), and PME enzymes that can degrade the juice have been discovered to be effectively inhibited by this combination. It has been observed that pineapple juice's bromelain activity is decreased by UV and mild heat treatments. The combination of UV at 5.61 mJ·cm^{-2} and mild heat at 55°C for 10 minutes maintained 61.57 ± 0.21% of bromelain activity(Chew et al., 2014). Pineapple juice browning is largely caused by the actions of oxidoreductases, including peroxidase (POD) and polyphenol oxidase (PPO), discovering that these enzyme activities are decreased by both UV and moderate heat treatments. For example, POD and PPO were inactivated by about 81% and 61%, respectively, by heat pasteurization, whereas these activities were lessened by pulsed light (PL) treatments (Vollmer, Chakraborty, et al., 2020b). According to the study, mild heat treatment considerably decreased PME activity. When pineapple juice was exposed to mild heat at 55°C for 10 minutes and UV light at 5.61 mJ·cm^{-2}, the activity decreased by 60.53%. According to the study, TPC depletion increased with increasing holding duration and UV dosage. UV at 7.55 mJ·cm^{-2} and mild heat at 55°C for 20 minutes combined to reduce PME activity by 69.42±0.33% (Table 3) (Sew et al., 2014).

Table 3

The Inhibitory Effect of Combined Thermal & Non-Thermal Techniques on the Enzymatic Activity in Pineapple.

Product Type	Technique	Processing Parameters Temperature, Voltage, Time, Pressure, Pasteurization, UV	Reduction of Enzymes Bromelain, PPO, POD, PME (%)	References
Pineapple juice	Influence of mild electric field (MEF during ohmic heating	80 ± 2 ◦ C, 16 - 36 V/cm, 1 min	50.06 PPO 56.67 PPO	(H. Makroo et al., 2022)
Pineapple Puree	Combined high-pressure and temperature treatments	200 MPa, 30 °C, 40 mints 600 MPa, 70 °C, 40 mints	15 PME 66 PME	(Chakraborty et al., 2019)
Pineapple Puree	High-pressure and thermal treatments	200 MPa, 70 °C, 20 min 600 MPa, 70 °C, 20 min	63 POD, 64 PPO 96 POD, 92 PPO	(Chakraborty et al., 2015)
Pineapple juice	Combination of ultraviolet and mild heat treatment	55 ◦ C, 5.61 mJ·cm^{-2}, 10 min 55 ◦ C, 7.55 mJ·cm^{-2}, 20 min	60.5 PME, 61.57 ±0.21 Bromalin 69.42 ± 0.33 PME	(Sew et al., 2014)
Pineapple	Effect of pressure, temperature and time treatment	12.40, 60°C 0, 100., 300, 500, 600 Mpa 15, 30 ,45 min.:	33.17 PPO 60.08 POD	(Rosenthal et al., 2002)

Mild Heat Treatment and Ultrasound

Over a 60-day period at normal temperature, the nutritional value, physicochemical properties, and microbiological attributes of pineapple juice were evaluated. The accumulative consequences of ultrasound treatment, thermal pasteurization (80˚C for 15 minutes), and mild heat pasteurization (65˚C for 15 minutes) was evaluated (Chiozzi et al., 2022). The degree of pigmentation was significantly reduced as a consequence of the ultrasonic treatment. Ultrasound and ultrasound combined with moderate heat pasteurization (UMP) effectively maintained the total phenolic content of pineapple juice over the 60-day storage period, in contrast to the untreated juice sample and thermal treatment stored at ambient temperature. The proliferation of germs in pineapple juice was effectively inhibited by both mild heat pasteurization combined with ultrasonic treatment (UMP) and thermal pasteurization (TP), according to the order of increasing efficacy. This research suggests that pineapple juice can be effectively inactivated by pectin methylesterase and microorganisms through the use of ultrasound and mild thermal pasteurization, while still maintaining a fairly significant phenolic content (Lagnika et al., 2017). Another study compared thermosonication (TS) to standard pasteurization for fresh pineapple juice. To achieve effective microbial inactivation and maintain high bromelain activity (63.24 U/100 mL) and total phenolic content (177.3 µg GAE/100 mL), the temperature and amplitude were adjusted to 62.33 °C and 35.32% for 2 minutes. TS-treated juice had higher ascorbic acid and protein contents than untreated juice after 28 days at 4 °C . This suggested that TS-treated juice could be a juice business alternative (Mala et al., 2021). A study compared standard pasteurization (95 °C for 5 minutes) to ultrasonic (US) treatments for pear juice processing. Ultrasound at 65 °C (10 min, 70% amplitude) was found to be more effective in preserving bioactive compounds (7.44% loss vs. 16.39% via pasteurization), while ensuring safety and prolonging shelf life to 21 days at 4 US pasteurization improved nutrient retention at low temperatures (Saeeduddin et al., 2017). (Aadil et al., 2020) examined apple-grape juice blends' antioxidant indices and polyphenolic profile after blanching, high-temperature-short-time treatment, ultrasonication, and thermo-ultrasound. Ultrasonication (10 min) increased chlorogenic acid (422.12 mg/L) and resveratrol (33.08 mg/L) while keeping quality better than heat.

CHALLENGES AND FUTURE TRENDS

Recent research highlights several sustainable and green alternatives for preventing browning in pineapple, focusing on natural extracts, edible coatings, and innovative packaging. Plant-based antioxidants such as ascorbic acid, isoascorbic acid, and N-acetylcysteine have shown strong anti-browning effects, especially when combined with modified atmosphere packaging (MAP), which reduces oxygen exposure and maintains fruit quality during storage (AYÓN-REYNA et al., 2019). Edible coatings made from natural polymers like sodium alginate, often enriched with isoascorbic acid, further enhance browning inhibition by forming a barrier against oxygen and moisture loss (Liao et al., 2023). Additionally, the use of natural extracts derived from fruit byproducts, such as pineapple shell or mango waste, not only controls browning but also supports circular economy practices by utilizing agricultural residues. These eco-friendly strategies offer effective, scalable, and consumer-preferred alternatives to synthetic preservatives for maintaining the freshness and visual appeal of pineapple (Hamdan et al., 2022). But the prevention of enzymatic browning in pineapple is still a significant difficulty because of scalability and technological limitations. According to recent research, natural antioxidants such as ascorbic acid and pineapple shell extract (PSE) exhibit varying levels of effectiveness based on storage conditions and application techniques. They frequently need to be used at specific quantities to prevent flavor or texture changes (Hamdan et al., 2022). However, for cutting polyphenol oxidase (PPO) and peroxidase (POD) activity while maintaining ascorbic acid levels, scalability is restricted by the requirement for specialized tools and energy-intensive procedures. By identifying transcription factors such as AcbHLH144, which control phenolic biosynthesis linked to internal browning, genetic research has opened the door to the possibility of creating resistant cultivars through genome editing (Li et al., 2023). However, practical acceptance is slowed by administrative hurdles and consumer skepticism about genetically modified crops. Though it is difficult to maintain airtight conditions, modified atmosphere packaging (MAP) substantially reduces oxygen exposure, especially for small-scale producers without infrastructure (Wang et al., 2024b). Effectiveness and scalability gaps may be closed by hybrid strategies that combine natural antioxidants with affordable technologies or optimized storage protocols; however, cooperative efforts between researchers, government officials, and industry stakeholders are crucial to addressing barriers related to infrastructure, cost, and consumer acceptance (Moon et al., 2020 ; Moura et al., 2024)).

CONCLUSION

Pineapple (Ananas comosus L.) is a well-known fruit that holds significant nutritional and commercial importance. It features a distinctive sweet and savory flavor and is particularly notable for its richness in minerals and antioxidants, serving as a major source of vitamins and micronutrients. An important challenge encountered by consumers is enzymatic browning of pineapple which leads to degradation of nutrients and spoil the texture of fruit as well. Peroxidase (POD) and polyphenol oxidase (PPO) enzymes were found to significantly contribute to the deterioration of pineapple fruit, especially in processes such as the oxidation of phenolics which ultimately caused enzymatic browning. A variety of treatments are employed to address this issue of food wastage, which can be classified into thermal, non-thermal, and combined methods. In thermal techniques, the application of high temperatures to PPO and PME enzymes resulted in a notable reduction in internal browning. Also, subjecting pineapple to low temperatures for several days achieved a complete 100% reduction in browning. However, during thermal treatments involving high temperatures, non-enzymatic browning can occur, resulting in altered texture and the degradation of nutrients in pineapple. Meanwhile, non-thermal methods such as dielectric barrier discharge plasma (DBD), multi-frequency power ultrasound, pulsed light, ozone, and UV-C irradiation treatment are utilized to inhibit enzymatic browning in pineapple, demonstrating a reduction of up to 90% in internal browning. These non-thermal techniques are good in inhibition of pigmentation but have some limitations as well including, high costs, shallow penetration, requiring careful optimization of equipment. To come over these limitations, combination of thermal and non-thermal techniques not only enhances food safety by more effectively inactivating pathogens, but also helps maintain the food's original taste, texture, and nutritional value. However, addressing the prevention of enzymatic browning in pineapple remains a considerable challenge due to issues related to scalability and technological constraints. Hybrid strategies that integrate natural antioxidants with cost-effective technologies or enhanced storage protocols could bridge effectiveness and scalability gaps along with beneficial policies for making products cost-effective and consumer accepted.

Abbreviations

PPO	Polyphenols Oxidase
POD	Peroxidase
PME	Pectin Methylesterases
BRM	Bromelain.

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