





Review

# Thermal, High Pressure, and Ultrasound Inactivation of Various Fruit Cultivars' Polyphenol Oxidase: Kinetic Inactivation Models and Estimation of Treatment Energy Requirement

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**Abstract:** Polyphenol oxidase (PPO) catalyses the browning reaction during fruit processing and storage. It is considered a threat to clean labels and minimally processed fruit products. Unwanted changes in fruits' appearance and quality represent a cost to the industry. High pressure and ultrasound, in addition to thermal treatment, are effective in reducing PPO activity and producing high-quality products. PPO from different fruit cultivars behaves differently when submitted to different treatments. A systematic review was conducted, where treatment parameters, PPO inactivation data ( $\geq 80\%$  inactivation), and kinetic inactivation parameters (rate constant ( $k$ ), activation energy ( $E_a$ ),  $D$ -value, and  $z$ -value) by different treatments were collected. Additionally, the estimated energy requirements for the inactivation of PPO ( $\geq 80\%$ ) by different treatments were calculated and compared. Resistance to various treatments varies between fruit cultivars. For the same temperature, the inactivation of PPO by ultrasound combined with heat is more effective than thermal treatment alone, and the high pressure combined thermal process. The majority of the thermal, HPP, and ultrasound inactivation of PPO in fruits followed first-order behaviour. Some fruit cultivars, however, showed biphasic inactivation behaviour. The estimated specific energy requirements calculated based on the mass of processed fruit sample to inactivate  $\geq 80\%$  polyphenol oxidase for the thermal process was 87 to 255 kJ/kg, while for high pressure processing it was 139 to 269 kJ/kg and for ultrasound it was 780 to 10,814 kJ/kg.

**Keywords:** high hydrostatic pressure; sonication; heat; browning; quality; energy

## 1. Introduction

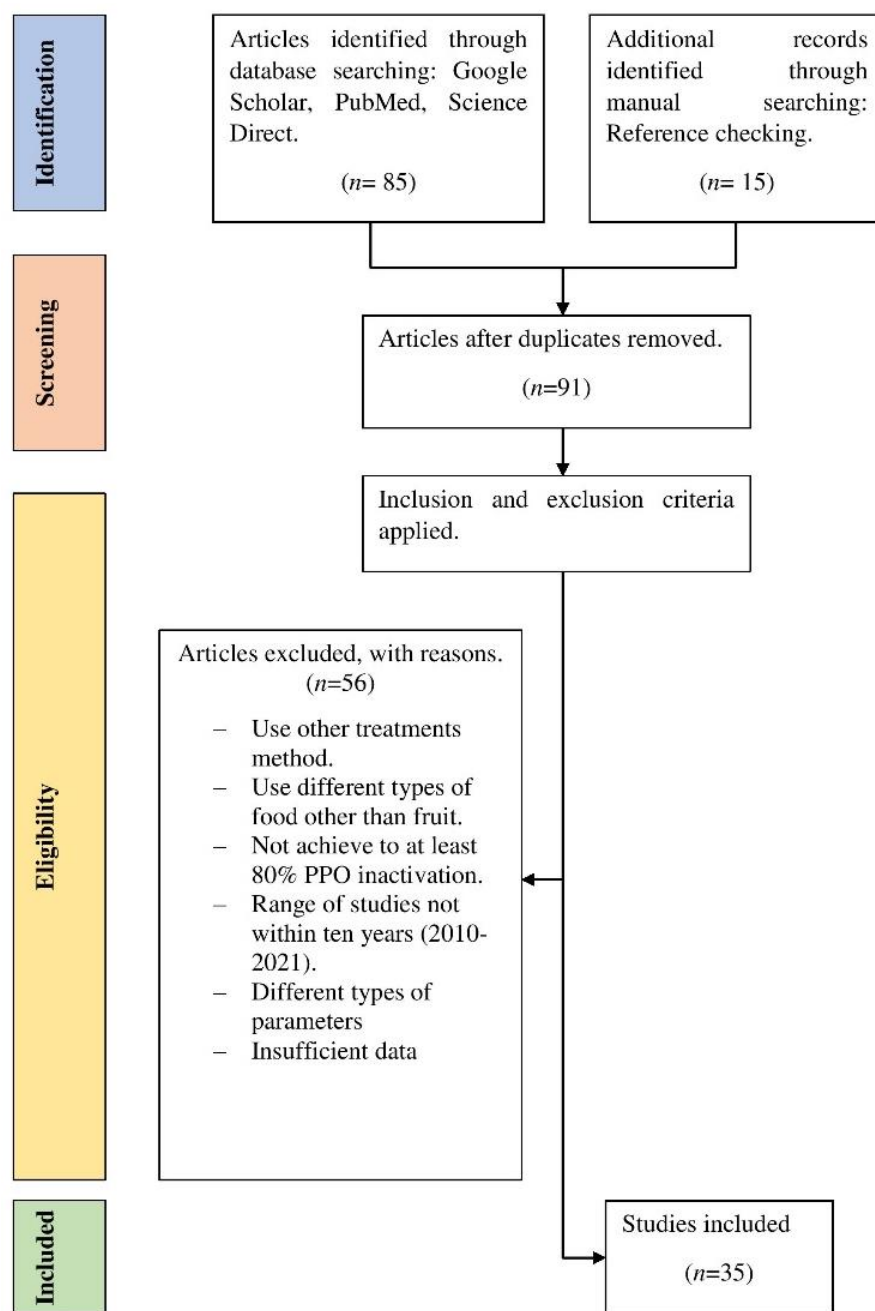
Browning is a common occurrence during the preparation and storage of fruits and vegetables such as apples, potatoes, bananas, and avocado. It occurs when food undergoes chemical reactions that turn it brown. There are two types of browning: enzymatic browning and non-enzymatic browning. Enzymatic browning is believed to cause more than 50% loss during pre-harvest and post-harvest processing of fruits and vegetables [1]. The enzyme that is responsible for food browning is polyphenol oxidase (PPO). Browning occurs when PPO oxidises phenolic substances, resulting in food darkening [2]. PPO activity is a major problem in the production and marketing of food items, particularly fruit juices. When browning occurs, the appearance, and the quality of fruit juices are altered. Many researchers have investigated how to inhibit undesirable browning to increase their

shelf life [3]. However, in certain foods such as coffee, cocoa beans, and tea, browning is needed to produce the desired colour and flavour [4]. This is also the case for dry fruits such as raisins. It is critical in food processing to use physical (thermal and non-thermal processing) or chemical agents (Such as citric or ascorbic acid) to avoid or slow down enzymatic browning.

Thermal or non-thermal processing can be used to inactivate polyphenol oxidase. The PPO enzyme in fruit (juice) is inactivated during thermal processing by heating the juice to a temperature of 70 °C to 90 °C. Thermal treatment of fruits and vegetables is a classic method for destroying bacteria and inactivating enzymes. For a long time, thermal food preservation methods have dominated the food processing sector [5]. Thermal treatment, on the other hand, can result in negative changes in food attributes such as loss of colour, texture, and flavour, as well as nutrient quality degradation [6,7]. Heat treatment of fruits can cause heat-sensitive components including vitamins to be destroyed [1]. Industry and researchers have developed non-thermal food preservation techniques in response to consumer demand for safe and nutritious juices. Some of the technologies include high pressure processing (HPP), high pressure carbon dioxide (HPCD), ultrasound, and irradiation [8–11]. The latter can be used alone or in combination with heat to enhance bacterial and enzyme inactivation. The adverse effects of thermal treatment on a variety of fruits have been mitigated using these strategies [6]. Food will be less impacted by the moderate heat/no heat employed in these processes, allowing for the retention of nutritional, physical, taste, and flavour properties identical to those of fresh foods [12–15]. These non-thermal processing methods have the potential to help with product development in order to address the rising demand for high-value, complex, and diverse food items. The approach for thermal treatment, high pressure processing, and ultrasonic technologies for the inactivation of PPO enzymes are discussed in this study.

The purpose of this review is to observe the resistance of polyphenol oxidase (PPO) enzymes in different fruits and fruit cultivars to thermal treatment, high pressure processing, and ultrasound in terms of inactivation and estimation of energy requirements. The review will include discussions on fruits cultivars that achieved at least 80% PPO inactivation after the specified treatments using the kinetic PPO inactivation models. In addition, the estimation of energy requirements for different processing technologies is thoroughly discussed. In this review, at least 80% inactivation of PPO was regarded to be reasonable to control or slow down the enzymatic browning in fruit products, based on the studies of Sulaiman et al [16,17] that showed no regeneration of PPO activity during storage after thermal, HPP, PEF and ultrasound processing, when <20% residual activity (or >80% enzyme inactivation) was obtained with these processes.

This review summarises the data on the inactivation of PPO in various fruits using different treatments. The systematic review was performed to search different published literature to identify all peer-reviewed fields that investigated the inactivation of PPO in different fruits using thermal or non-thermal treatment. The search for papers on the inactivation of PPO and kinetic inactivation covered papers published over a 10-year period, from 2010 until 2020. The search on papers from previous studies was conducted using the following keywords: 'inactivation of PPO', 'thermal treatment inactivation', 'non-thermal treatment', 'enzyme PPO', and 'kinetics inactivation'. A process flow diagram to summarise the systematic review on PPO inactivation is shown in Figure 1.



**Figure 1.** Study selection process flow diagram for the systematic review of PPO inactivation.

The quality of research is classified according to these six primary factors, which are defined as a priori conditions essential for answering research questions (evaluation of the PPO enzymes inactivation from different fruit cultivars). Study quality was grouped into two categories: satisfactory quality and unsatisfactory quality. Satisfactory quality publications factors are shown in Figure 2.

Papers that do not meet at least four quality factors were considered unsatisfactory publications.

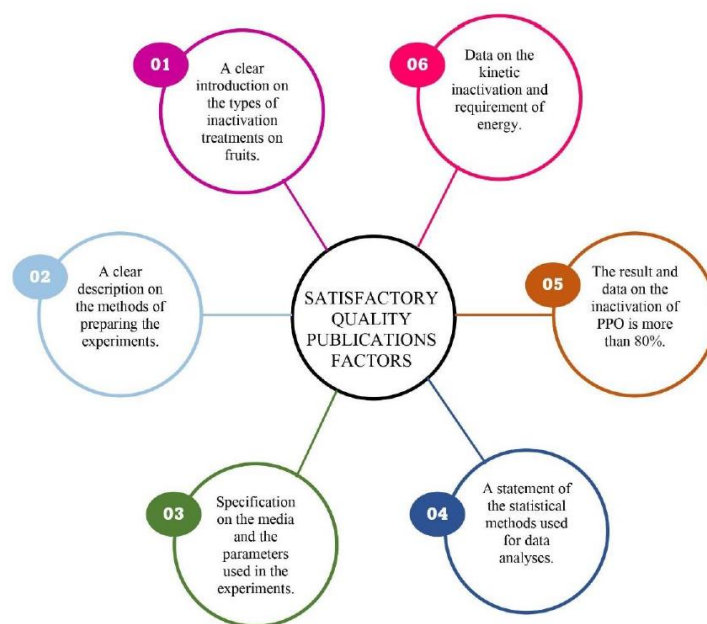


Figure 2. Satisfactory quality factors of publications.

## 2. Enzymatic Browning: Polyphenol Oxidase

Enzymatic browning is a chemical process that discolours fruits or fruit products (juice, puree, etc.) into a brown colour. The enzymes polyphenol oxidase (PPO), 1,2 benzenediol oxygen oxidoreductase, phenolase, monophenol oxidase, diphenol oxidase (DPO), and tyrosinase catalyse browning [18]. Browning is caused by the presence of oxygen in the air in contact with sliced fruit (oxidation reaction). Figure 3 indicates that quick browning occurs in the presence of oxygen due to the enzymatic oxidation of phenols to orthoquinones, which subsequently polymerise in a sequence of processes, resulting in the development of brown or black pigments (melanins) on the food's surface [19].

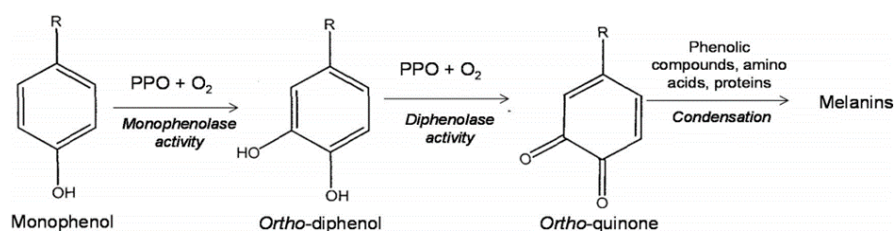


Figure 3. Browning reaction of PPO enzyme taken from [20].

Most fruits and vegetables that are exposed to air after being chopped, sliced, or pulped, will be affected by enzymatic browning due to mechanical damage of plant tissues and cells. Enzymatic browning can occur during frozen or cold food transit and storage. PPO produces quinones that bind to plant proteins, reducing protein digestion and nutritional value for herbivores [21]. The PPO enzyme is structurally composed of two copper ions in its active region, which is surrounded by six histidines and one cysteine residue [1]. Copper ions in the enzyme are important factors in the oxidation–reduction process by cyclically transitioning the active site between met-, oxy-, and deoxy- forms during the catalysis reaction [22]. PPO has two copper atoms in its activity site, and the enzyme catalyses two distinct processes in the presence of molecular oxygen: the hydroxylation of monophenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) [23]. This is followed by non-enzymatic polymerisation of the quinones, which produces the pigment melanins, resulting in dark colours [24,25].

### 3. Thermal and Innovative Technologies for PPO Inactivation

In general, different fruits/cultivars required different processing conditions for >80% PPO inactivation. Additionally, for the same fruit/cultivar, the processing time can vary depending on the technology used for PPO inactivation. For example, apple puree cv. Royal gala PPO inactivation of 80% occurred after a 3 min TS treatment at 460 W/cm<sup>2</sup> and 72 °C, while 50 min were required for HPP-71 °C treatment 600 MPa. The combination of HPP and ultrasound with thermal processing offers an advantage in terms of PPO inactivation and has been discussed in the literature [9,26–31]. Different forms of the same fruit (e.g., juice, puree, concentrate) can also affect PPO inactivation for similar treatments, but more studies are needed in this area.

#### 3.1. Thermal Treatment

Thermal processing is a traditional food preservation method that employs heat to preserve and process food products. The inactivation of polyphenol oxidase (PPO) by heat treatment is the most effective and standard method to control enzymatic browning. Heat is used in this treatment to destroy microorganisms and inactivate enzymes. However, the thermal treatment causes undesirable changes in food qualities, such as loss of colour, flavour, texture, and nutrients [26]. Blanching is a common thermal treatment used to inactivate enzymes in fruits and vegetables. Blanching is a water-intensive technology and is deemed as uneconomical technology [32]. It involves rapid heating of fruits and vegetables to a predetermined temperature and holding it for a set period, usually 1 to less than 10 min [29]. The most common method of blanching is hot water blanching, during which the products are immersed in hot water (70 to 100 °C) for several minutes [33,34].

Figure 4 shows studies on the thermal inactivation of PPO enzymes that have been carried out by various authors [28,35–39]. The effect of temperature on PPO stability is commonly investigated by incubating the enzyme at a higher temperature. Processes from 70 °C to 80 °C for 5–25 min were seen to provide more than 80% of PPO inactivation. However, studies by Sreedevi [35] on sugarcane and Sulaiman [28] on strawberry PPOs show that at a lower temperature of 60 °C, 80% of PPO inactivation can also be achieved. This proves that different fruits can react at different temperatures and times to achieve similar PPO inactivation. Different technologies used to produce juice and puree, different fruit cultivars, and different methods used to determine PPO activity could explain the differences in the inactivation after processing.

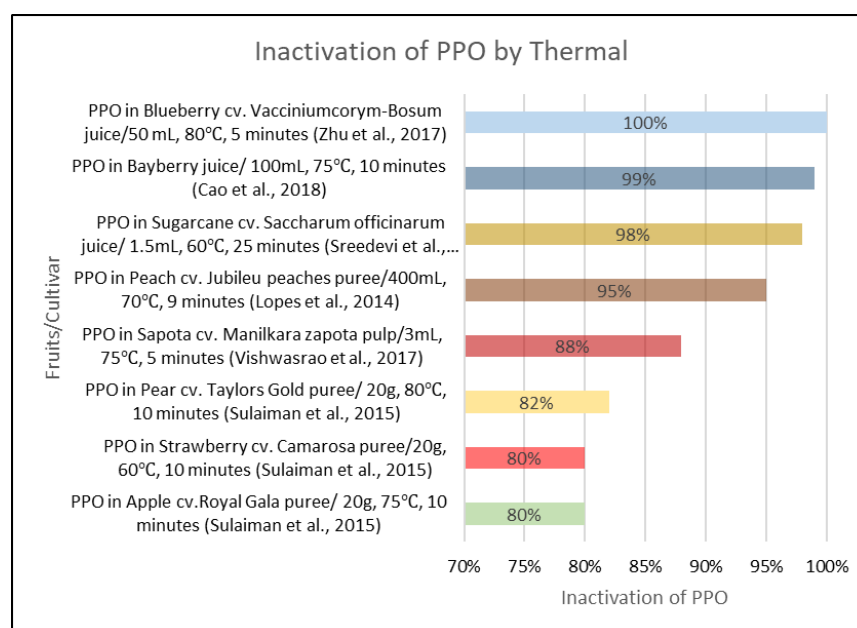


Figure 4. Inactivation of PPO ( $\geq 80\%$ ) by thermal treatment [28,35–39].

### 3.2. High Pressure Processing

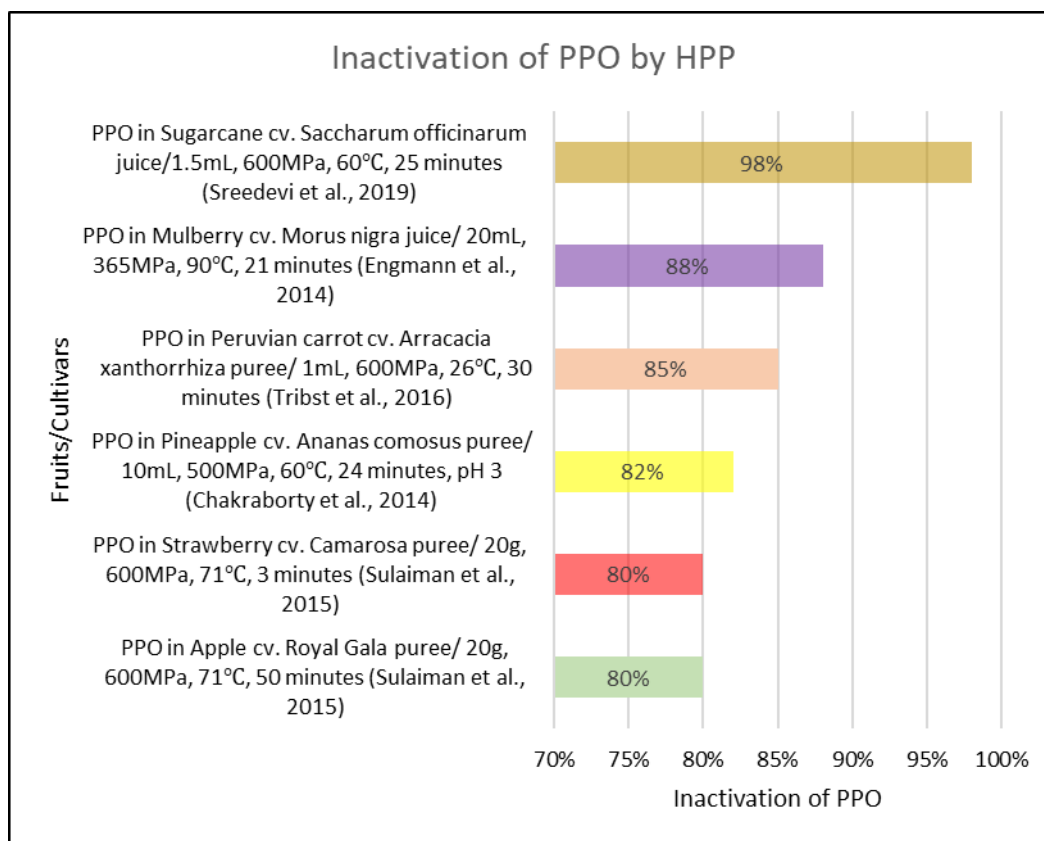
High pressure processing (HPP) has been considered as an alternative technology to preserve food without heat or chemical preservatives [40]. HPP is a non-thermal process that is very effective in assuring the safety and quality of minimally processed food products. In contrast to thermal treatment, this technology can inactivate foodborne microorganisms and enzymes while maintaining food quality [41]. For high pressure processing, raising treatment pressures will generally result in faster microbial inactivation in shorter times. However, the increased pressure can cause protein denaturation and other potentially harmful changes in food quality, which might affect the appearance and texture of the food [40].

For the inactivation of enzymes, the pressure is strongly dependent on the type of endogenous enzymes—some enzymes can deactivate at room temperature by a few hundred MPa [29,35,42,43], while others can withstand 1000 MPa [44,45]. As some food enzymes are highly stable, pressure must be combined with other factors such as temperature to inactivate them. During the process, hydraulic fluid (typically water) is poured into a chamber and pressurised with a pump. This pressure is then transferred to the food isostatically through the packaging, where the pressure at all sides is at the same magnitude (Isostatic principle). This procedure can increase the product's shelf life without the addition of preservatives while maintaining the product's quality (e.g., nutrients).

When using a high pressure treatment for food processing, it is crucial to understand the interactions between the processing parameters (pressure, time, and temperature) to figure out the best conditions to achieve desired levels of microbial inactivation while maintaining a high degree of nutritional quality, good flavour, and texture [46]. Increased treatment pressures for high pressure processing will generally result in higher microbial inactivation. However, some food enzymes are extremely stable, so the pressure must be paired with other factors such as temperature to inactivate them. Several studies [29,47] demonstrated that by utilising higher pressure (600 MPa) and a moderate temperature, fruits juice and puree could achieve PPO inactivation of more than 80%. For example, data by Engmann [48] show that Mulberry can achieve 88% of inactivation with pressure 365 MPa and a combination of thermal treatment at 90 °C. Applying pressure without heat treatment in fruits puree and juice is insufficient to totally inactivate the oxidative enzymes. Temperature assistance is required to achieve significant inactivation of these enzymes, which may improve the shelf stability of the products. The efficiency and effectiveness of HPP can be determined by various factors such as the type of food, processing time, initial pH, and the presence of certain additives in the food. The effect of high pressure processing (HPP) on PPO inactivation of various fruits is shown in Figure 5 [9,29,35,43,48].

### 3.3. Ultrasound Processing

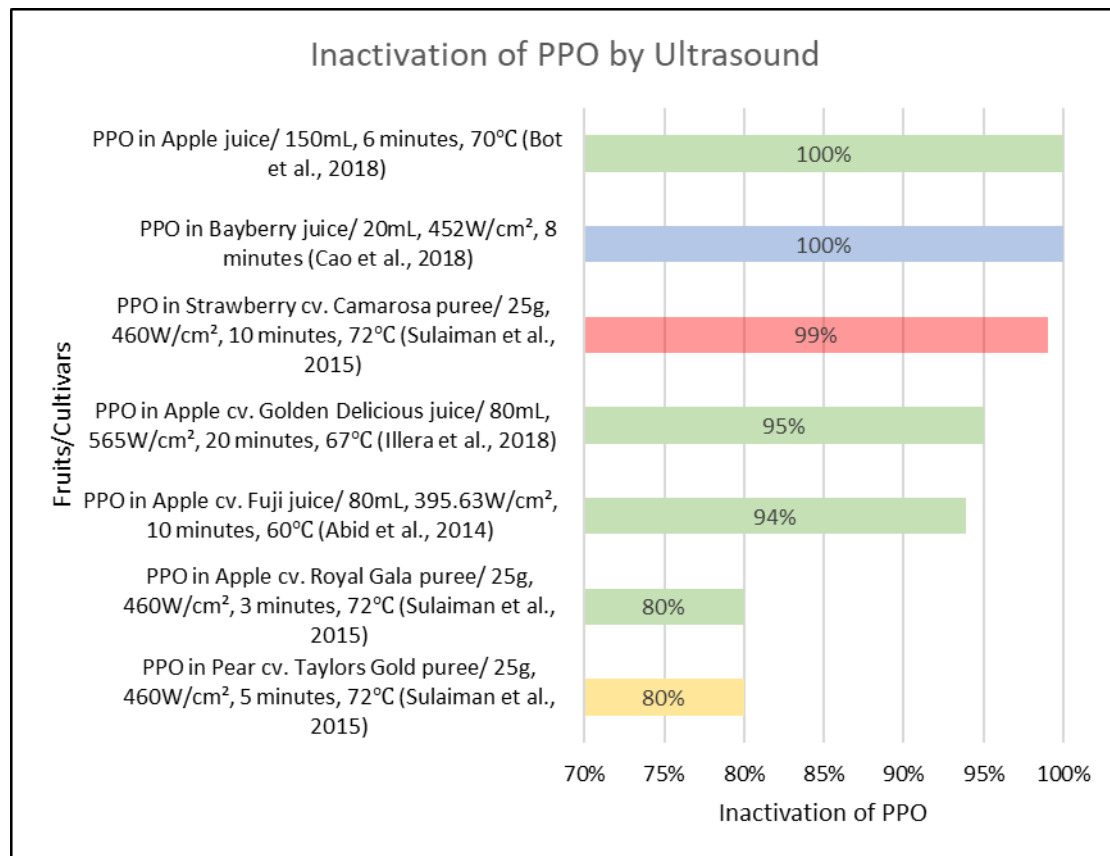
Food products that are free of additives or preservatives have become a consumer demand for high quality and fresh products, especially juice products. This demand has led researchers or producers to employ ultrasonic technology in the production of fruit juice. Ultrasonic or ultrasound is a promising technology that can be used to replace traditional thermal treatment processes. It has the potential to improve the quality and safety of juice by converting electrical energy into mechanical energy through piezoelectric materials [47]. When sound waves pass through the fluid, continuous compression and relaxation will occur, and then the negative pressure overcomes the tensile force, the transmission of sound waves to the relaxed position causes the formation of micron-sized bubbles and spaces [49]. Ultrasound is also transmitted at frequencies higher than the audible frequency of 20 kHz [50]. The frequency of ultrasonic equipment is usually 20 kHz to 10 MHz [51].



**Figure 5.** Inactivation of PPO ( $\geq 80\%$ ) by HPP [9,29,35,43,48].

The applications of ultrasound in the food industry include low-energy and high-energy ultrasound. Low energy intensities of ultrasound, less than  $1 \text{ W}\cdot\text{cm}^{-2}$ , and frequency greater than 100 kHz [47] can be used to monitor food composition and physico-chemical properties of food during processing and storage, for quality control purposes. High-energy ultrasound is also called power ultrasound because the intensity is higher than  $1 \text{ W}\cdot\text{cm}^{-2}$ , and the frequency range is 20 to 100 kHz [47]. Ultrasound can be applied to a product using two methods: submergence in an ultrasonic bath [52] or direct treatment to the product using a probe sonicator [53,54].

Figure 6 summarises PPO inactivation by ultrasound in fruit juice [28,39,55]. Ultrasound occurs at frequencies ranging from 20 kHz to 24 kHz, inactivating PPO by more than 80%. Ultrasound combined with heat, also known as thermosonication (TS), is another emerging preservation method. TS treatment on apple juice of different cultivars shows an inactivation PPO of more than 80% at various temperatures (ranging between  $60^\circ\text{C}$  and  $70^\circ\text{C}$ ) and time (6 to 20 min) [30,55,56]. A comparison of apple juice shows that different parameters used such as sound intensity, time, and temperature would affect the inactivation of PPO in the fruits. The higher the intensity (or amplitude) of ultrasound applied is, the more enzyme is inactivated, because high intensity releases high energy. The wide range of data on PPO inactivation in fruits shows that enzyme inactivation is influenced by various parameters, including source, sub-type, environment, and physicochemical conditions such as pH and temperature.



**Figure 6.** Inactivation of PPO ( $\geq 80\%$ ) by Ultrasound [28,30,39,55,56].

#### 4. Kinetic Modelling of PPO Inactivation

##### 4.1. Kinetic Inactivation Model

All data and results were tabulated into different treatment methods (thermal treatment, ultrasound, high pressure processing) of PPO inactivation. The selection of data on inactivation of PPO is more than 80% of inactivation. Standard models used to demonstrate the kinetic inactivation of PPO in different fruits or cultivars by different processing processes are shown in Table 1. A typical model for fitting or predicting PPO enzyme inactivation is the first-order kinetics, as illustrated in this review [28,36,39,55,57–60]. Enzyme inactivation is a complex process involving several events. However, most of the time the inactivation shows apparent first-order inactivation where the intermediate reaction occurs instantaneously and unobserved. Using linear regression analysis, the activation energy can be determined from the slope of the  $\ln(k)$  against a  $1/T$  plot. Other models, such as biphasic models showing the occurrence of different stabilities of isoenzymes and the Weibull distribution that is usually used for microbial inactivation with scale (b) and shape (n) factors as important parameters, were used to model PPO inactivation in some fruits.



**Table 1.** Models for inactivation of PPO.

Models	Equations
First-order kinetic model	$\ln\left(\frac{A}{A_0}\right) = -k t \quad \log\left(\frac{A}{A_0}\right) = -\frac{t}{D}$ <p>where A is the enzyme activity of sample at different treatment times (t), A<sub>0</sub> is the initial enzyme activity of the raw unprocessed sample, A/A<sub>0</sub> is the residual activity of the enzyme, k is the first-order inactivation rate constant at the operating conditions (min<sup>-1</sup>), and t is the treatment time (min).</p> <p>The decimal reduction time (D: the time required to reduce enzyme activity by 90%) can be calculated from the inactivation rate constant (D = 2.303/k). The negative reciprocal slope of the regression line of log D as a function of T was used to calculate z, which is defined as the temperature increase required to reduce the D value by 90%.</p> $\log\left(\frac{D_1}{D_2}\right) = \frac{(T_2 - T_1)}{z}$ <p>Furthermore, the temperature dependence of the PPO inactivation can be described using the linearized version of the Arrhenius equation as follows:</p> $\ln\left(\frac{k_1}{k_2}\right) = \left(\frac{E_a}{RT}\right)\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$ <p>where temperatures T<sub>2</sub> and T<sub>1</sub> correspond to decimal reduction times D<sub>2</sub> and D<sub>1</sub> or constants k<sub>2</sub> and k<sub>1</sub>, respectively. E<sub>a</sub> is the activation energy (kJ/mol), and R is the universal gas constant (8.31 J/(mol·K)). Using linear regression analysis, the activation energy was calculated from the slope of the ln (k) versus 1/T plot.</p>
Biphasic model	$A = A_s \exp(-k_s t) + A_L \exp(-k_L t)$ <p>For non-linear data, the biphasic model can be used, where A<sub>S</sub> and A<sub>L</sub> are activities of the stable and the labile fractions, respectively, and k<sub>S</sub> and k<sub>L</sub> are the inactivation rate constants of stable and labile fractions, respectively.</p>
Weibull model	$\log\left(\frac{A}{A_0}\right) = -bt^n$ <p>Weibull model is also suitable for non-linear, where b and n are scale and shape factors, respectively [61].</p>

#### 4.2. Thermal Inactivation Kinetics of PPO

In terms of kinetic modelling, first-order kinetics adequately described the semi-logarithmic relationship between residual PPO activity and thermal treatment in different fruits, ranging from 60 to 85 °C. Table 2 shows that, according to the Arrhenius equation, the rate constants increased with temperature. Higher k was obtained for strawberry puree PPO, confirming that the resistance of the fruit enzyme is low. The D-values of PPO are crucial for a better understanding of enzyme stability. When PPO in different fruits was treated either in puree or in juice form, the D-values of PPO decreased as the temperature increased. When comparing the D-value of PPO in different fruits such as sapota (75 °C, 8 min), bayberry juice (75 °C, 6 min), pear puree (75 °C, 121 min), and apple puree (75 °C, 22 min), it could be concluded that the PPO in bayberry juice is more thermosensitive [28,36,39]. The minimum energy required for a chemical reaction is known as activation energy, and it varies depending on the reaction. The susceptibility to temperature changes is indicated by the activation energy (E<sub>a</sub>). High E<sub>a</sub> indicates greater changes in k as a function of temperature [28]. These differences in PPO inactivation kinetic parameters could be due to the different fruit sources or the difference between the form of the fruits, such as puree or juice.

**Table 2.** Thermal inactivation kinetic parameters.

Fruit/Cultivar	Temperature (°C)	k (min <sup>-1</sup> )	D-Value (min)	z-Value (°C)	E <sub>a</sub> , (kJ/mol)	Reference
Kalipatti sapota ( <i>Manilkara zapota</i> ) pulp	75	0.288	8	15.2 °C	158	[36]
Bayberry ( <i>Myrica</i> ) juice	65	0.0888	26	13.2 °C	152	[39]
	75	0.370	6			
Pear ( <i>Pyrus communis</i> cv. Taylor's Gold) puree	75	0.019	121	6.0 °C	375	[28]
	80	0.214	11			
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	75	0.103	22	17.0 °C	134	[28]
Strawberries ( <i>Fragaria ananassa</i> cv. Camarosa) puree	60	0.144	16	14.0 °C	147	[28]

#### 4.3. High Pressure Inactivation Kinetics of PPO

The inactivation kinetics of PPO of different fruits processed at 400–600 MPa typically followed first-order behaviour [57–59]. PPO inactivation in lychee, sapodilla, and PPO in pear showed first-order inactivation kinetics [57–59]. However, in terms of kinetic inactivation by HPP in apple cv. Royal Gala puree and strawberry cv. Camarosa puree, Sulaiman [9] reported that these data showed non-linear first-order kinetics, better fitted with biphasic models. The difference in models could be due to the presence of isoenzymes with similar structures but different resistance to pressure and temperature that could explain the biphasic behaviour [62]. High pressure inactivation kinetic parameters for different fruits and cultivars are summarised in Table 3. The differences in E<sub>a</sub> could be attributed to the enzyme extract being processed in a buffer rather than the fruit itself because the medium in which PPO is suspended can change its resistance, as Rapeanu [63] previously reported.

**Table 3.** Kinetic parameters of inactivation PPO by HPP. PPO inactivation with biphasic behaviour (a) and first-order inactivation of PPO (b).

(a)										
Fruit/Cultivar	Parameters	k <sub>s</sub> (min <sup>-1</sup> )	E <sub>a</sub> (s) (kJ/mol)	k <sub>L</sub> (min <sup>-1</sup> )	E <sub>a</sub> (L) (kJ/mol)	D <sub>S</sub> -Value (min)	z <sub>S</sub> -Value (°C)	D <sub>L</sub> -Value (min)	z <sub>L</sub> -Value (°C)	Reference
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	600 MPa 57 °C	0.0121	31	0.0266	54	190	69	86	40	[9]
	600 MPa 71 °C	0.0184		0.0613		124		38		
Strawberry ( <i>Fragaria ananassa</i> cv. Camarosa) puree	600 MPa 57 °C	0.0182	99	0.213	57	127	22	11	38	[9]
	600 MPa 71 °C	0.0805		0.514		29		4.5		
(b)										
Fruit/Cultivar	Parameters	k (min <sup>-1</sup> )	D-Value (min)	z-Value (°C)	E <sub>a</sub> , (kJ/mol)	Reference				
Lychee ( <i>Litchi chinensis</i> Sonn) pulp	400 MPa 50 °C	0.0256	90	65	40	[57]				
Sapodilla ( <i>Manilkara zapota</i> ) Jam	600 MPa 65 °C	0.0136	169	108	18	[58]				
Pear ( <i>Pyrus communis</i> cv. Packham) juice	600 MPa 60 °C	0.0830	28	31	64	[59]				

#### 4.4. Ultrasound Inactivation Kinetics of PPO

Table 4 shows that the rate constants increased with temperature when the data were fitted using the first-order kinetic model for ultrasound-processed data. For the same temperature (72 °C) and ultrasound intensity (460 W/cm<sup>2</sup>), the highest inactivation rate constant was observed in apple puree, followed by strawberry puree and lastly pear puree [28]. PPO inactivation could be affected by different food matrices and enzyme sources.

**Table 4.** Kinetic parameters inactivation of PPO by Ultrasound \*.

Fruit/Cultivar	Parameters	k (min <sup>-1</sup> )	D-Value (min)	z-Value (°C)	E <sub>a</sub> (kJ/mol)	References
Apple ( <i>Malus domestica</i> cv. Golden Delicious) juice	565 W/cm <sup>2</sup> 64 °C	0.036	28	18	123	[55]
	565 W/cm <sup>2</sup> 67 °C	0.056	18			
Pear ( <i>Pyrus communis</i> cv. Taylor's Gold) puree	460 W/cm <sup>2</sup> 1.3 W/g 72 °C	0.356	7	50	40	[28]
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	460 W/cm <sup>2</sup> 1.3 W/g 72 °C	0.540	4	39	52	[28]
Strawberries ( <i>Fragaria ananassa</i> cv. Camarosa) puree	460 W/cm <sup>2</sup> 1.3 W/g 72 °C	0.483	5	80	25	[28]
Apple ( <i>Malus domestica</i> cv. Golden Delicious) juice	60 °C	0.178	13	19	105	[60]

\* Authors of paper [28,60] used an equipment with 24 kHz, while the other study's frequency was 20 kHz.

For D-values of PPO inactivation, Illera [55] reported that D-values decreased with temperature. Temperature-sensitive parameters, such as z-values and E<sub>a</sub>, were evaluated through slopes by plotting the linearized form of the Arrhenius equation. Processes with high activation energy and low z-values are extremely temperature sensitive. The differences in PPO inactivation could be due to a difference in fruits' cultivar or the fruits' form, such as puree and juice. The cavitation of formation and collapsing of microbubbles could affect the inactivation of the enzyme by ultrasound [31]. As the temperature rises, the vapor pressure inside the bubbles rises, potentially creating a cushioning effect and reducing the number of effective collapses [28].

## 5. Energy Requirement Estimation

### 5.1. Energy Calculations

The inactivation of PPO in different fruits can be compared in terms of energy requirements. Equation (1) was used to calculate the energy for the thermal inactivation in fruit juice and puree.

$$Q = mC_p\Delta T \quad (1)$$

where Q is the specific heat energy required to heat the fruits samples (J), m is the mass of the fruits samples (kg), C<sub>p</sub> is the fruits heat capacity (J/kg·°C). ΔT for thermal processing is the temperature difference between the final temperature and initial temperature, which was assumed to be 20 °C.

In some studies, HPP combined with thermal treatment was applied for the inactivation of enzyme PPO, so Equation (1) was also used to estimate the energy requirement during the pre-heating of the sample before HPP treatment. For the sake of the estimation

energy calculation for HPP, the mass of the product treated was taken to be half of the maximum capacity of HPP chamber volume multiply with the density of water (assuming density of water is approximately the density of the sample) which is more industrial relevance considering the cost-effective capacity of HPP. The temperatures used to calculate the  $\Delta T$  was the temperature used before starting the HPP process, where the initial temperature was assumed at 20 °C to a final temperature before pressurisation that is associated to each treatment pressure. The temperature increase per 100 MPa for fruit juice and puree was assumed to be the same as pure water which is ~3 °C [64]. The temperature increase due to heat of compression during pressurisation is 18 °C for a high pressure process at 600 MPa.

In addition to the heat generated during the pressure come-up phase of the HPP cycle, the compression work ( $W_{\text{compression}}$  in J) during pressurisation also needs to be calculated. The following Equation (2) is used to calculate the compression work during the pressurisation phase of the HPP cycle:

$$W_{\text{compression}} = \left[ \int_{P_1}^{P_2} PV\beta dP - \int_{T_1}^{T_2} PV\alpha dT \right] \quad (2)$$

$$\frac{1}{2} \beta V(P_2^2 - P_1^2) - P_2 V \alpha (T_2 - T_1)$$

where  $P_1$  and  $P_2$  are the initial and final pressure (Pa) during pressurisation,  $V$  is the volume of the chamber containing liquid water ( $\text{m}^3$ ),  $\beta$  is the isothermal compressibility of water at the average compression temperature,  $\alpha$  is the volume expansivity or thermal expansion coefficient of water,  $T_2 - T_1$  is the adiabatic temperature increase during pasteurisation.

For the ultrasound process, the energy from the probe was estimated using Equation (3).

$$E = \text{sound intensity} \times A \times t \quad (3)$$

where  $E$  is the sound energy or acoustic energy,  $A$  is the area of the sonotrode,  $t$  is the total ultrasound processing time. All specific energies for the treatments were obtained by dividing the energy per mass of processed samples. In addition, the energy required to preheat the food before thermosonication was not considered as it is of much lower magnitude compared to ultrasound energy. Furthermore, the heat released during TS to the surroundings could possibly be used to preheat the food samples before TS treatments.

The specific energies for the treatments were obtained by dividing the energy per kg of the processed sample, which was different for each process.

## 5.2. Thermal Processing

The estimation of energy requirements for at least 80% inactivation of PPO in different fruits by thermal processing is shown in Table 5. The heat from the thermal process denatures enzymes. The activity of PPO enzymes is rapidly lost at higher temperatures as the protein undergoes irreversible denaturation. In addition, heat increases the kinetic energy of the molecules, causing them to vibrate rapidly and violently that the bonds are disrupted.

The major difference in energy during heating treatment could be due to the difference in temperature changes during the heating in the water bath. When the temperature increases, atoms and molecules move faster and collide, producing thermal energy (also known as heat energy) [65]. The rate of thermal energy is determined by the temperature differences between the fruits—the greater the temperature difference is, the greater the thermal energy is. Different levels of PPO inactivation and thermal energy observed in different cultivars could also be due to the mass and type of fruits ( $C_p$ ).

**Table 5.** Energy estimation for at least 80% PPO inactivation by thermal treatment.

Fruit/Cultivar	Mass (kg)	$\Delta T$ ( $^{\circ}C$ )	T Final ( $^{\circ}C$ )	Cp (kJ/kg.K)	$Q = mc_p \Delta T$ (kJ)	Specific Energy (kJ/kg)	Data Collected from
Kalipatti sapota ( <i>Manilkara zapota</i> ) pulp	0.004	55	75	3.94 [66]	0.87	217	[36]
		65	85		1.02	256	
Peach ( <i>Prunus persica</i> cv. Jubileu) puree	0.414	50	70	1.92 [67]	39.8	96	[37]
		70	90		55.7	134	
Blueberry ( <i>Vacciniumcorym-Bosum</i> ) juice	0.050	40	60	3.64 [67]	7.28	146	[38]
		60	80		10.9	218	
Bayberry ( <i>Myrica</i> ) juice	0.099	45	65	1.94 [67]	8.64	87	[39]
		55	75		10.6	107	
Sugarcane ( <i>Saccharum officinarum</i> ) juice	0.001	40	60	3.64 [68]	0.21	146	[35]
Pear ( <i>Pyrus communis</i> cv. Taylor's Gold) puree	0.020	60	80	2.06 [67]	2.47	124	[28]
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	0.02	55	75	1.88 [69]	2.07	103	[28]
Strawberries ( <i>Fragaria ananassa</i> cv. Camarosa) puree	0.02	40	60	3.60 [70]	2.88	144	[28]

T = temperature;  $\Delta T$  = Final T – Initial T ( $20^{\circ}C$ ).

### 5.3. High Pressure Processing

For HPP, the compression work ( $W$ ) during pressurisation must be calculated in addition to the heat required to warm the fruit juices or puree to the processing temperature. Assuming that the temperature increase per 100 MPa for fruit juice and puree is the same as pure water which is  $\sim 3^{\circ}C$  [64], the temperature increase due to pressurisation is  $18^{\circ}C$  for a high pressure process at 600 MPa. Meanwhile, for compression work, as the temperature used for the high pressure process increases with a constant pressure of 600 MPa, the compression energy decreases. Table 6 show the energy estimation ranging between 139 to 269 kJ/kg. Heat and compression work equally contribute to the overall energy estimation for HPP-thermal processing. The latter explains the fact that HPP-thermal treatment require higher energy consumption compared to heat alone treatment at the same temperature, unless HPP alone is used for the treatment.

**Table 6.** Energy estimation for at least 80% PPO inactivation by HPP.

Fruit/Cultivar	Conditions	* Mass (kg)	$\Delta T$ <sup>1</sup> (°C)	C <sub>p</sub> (kJ/kg·K)	Q = mc <sub>p</sub> ΔT (kJ)	$\alpha$ <sup>2</sup> (10 <sup>-4</sup> , K <sup>-1</sup> )	$\beta$ <sup>2</sup> (10 <sup>-10</sup> pa <sup>-1</sup> )	Compression Work, W (kJ)	Total Energy, Q + W (kJ)	Specific Energy (kJ/kg)	Data Collected from
Sugarcane juice ( <i>Saccharum officinarum</i> ) juice	600 MPa 25 min 60 °C	1	22	3.64 [68]	80	5.2307	4.4496	149	229	229	[35]
Pineapple ( <i>Ananas comosus</i> L.) puree	500 MPa 24 min 60 °C	1	25	3.68 [69]	92	5.2307	4.4496	103	195	195	[29]
Strawberry ( <i>Fragaria ananassa</i> cv. Senga Sengana) puree	500 MPa 15 min 50 °C	0.75	15	3.60 [70]	41	4.5759	4.4173	79	120	159	[42]
Mulberry ( <i>Morus nigra</i> ) juice	250 MPa 10 min 85 °C	3.25	58	1.94 [67]	366	6.6886	4.6748	86	452	139	[48]
	365 MPa 17.5 min 90 °C		59		372			6.9623		4.7429	
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	600 MPa 70 min 57 °C	1	19	1.88 [69]	36	5.0401	4.4362	149	185	185	[9]
	600 MPa 50 min 71 °C		33		62			5.8960		4.5246	
Strawberry ( <i>Fragaria ananassa</i> cv. Camarosa) puree	600 MPa 15 min 57 °C	1	19	3.60 [70]	68	5.0401	4.4362	149	217	217	[9]
	600 MPa 3 min 71 °C		33		119			5.8960		4.5246	

\* The mass of the product treated is taken to be half of the maximum capacity of HPP chamber volume multiplied with density of water (assuming density of water is approximately the density of the sample); <sup>1</sup> ΔT (Final temperature before HPP—initial temperature assumed at 20 °C); <sup>2</sup> α and β retrieved from *Journal of Chemical and Engineering Data* [71].

#### 5.4. Ultrasound

For the inactivation of PPO by ultrasound, the energy from the probe during processing was produced by the production of sound waves during the treatments on fruit juices and puree. Table 7 summarises the energy requirement in this process for different fruits or cultivars, which is sound energy. A study by Cao [48] shows that the higher the intensity or amplitude of ultrasound applied, the greater the energy released. Inactivation studies of PPO by ultrasound processing were performed in different fruits and cultivars, which showed different sound energy (E) values required to inactivate the enzyme. For example, PPO in bayberry juice is completely inactivated at 452 W/cm<sup>2</sup>/480 s or E = 288 kJ [39], PPO in strawberry puree achieved 99% of inactivation at 460 W/cm<sup>2</sup>/600 s or E = 19.5 kJ [32], PPO in apple juice cv Golden Delicious is 95% inactivated at 565 W/cm<sup>2</sup>/1200 s or E = 900 kJ, while apple juice cv. Fuji is 94% inactivated at 396 W/cm<sup>2</sup>/600 s or E = 315 kJ [30,55]. As a result, the amount of E required to inactivate PPO in different fruits or cultivars varies greatly. Furthermore, applying ultrasound to fresh fruits and vegetables can affect the firmness, depending on the intensity [72]. However, other than the amount of sample, the sound energy (E) value also will be affected by the amount of water, fat, sugars, and other substances present in the whole fruit if processed as juices or purees. Most of the energy used in ultrasound was used to create sound waves for the process and dependent on the sound intensity, area of sonotrode, and processing time, as described by Equation (3) in Section 5.1. The specific energy value was found to be a large number due to the small volume of sample used for the ultrasound.

**Table 7.** Energy estimation for at least 80% of PPO inactivation by ultrasound.

Fruit/Cultivar	Conditions	Mass (kg)	Sound Intensity (W/cm <sup>2</sup> )	Area (cm <sup>2</sup> )	E = Sound Intensity × A × t, (kJ)	Specific Energy (kJ/kg)	Data Collected from
Apple ( <i>Malus domestica</i> cv. Golden Delicious) juice	20 min 67 °C	0.083	565	1.327	900	10843	[55]
Apple ( <i>Malus domestica</i> cv. Fuji) Juice	10 min 60 °C	0.083	396	1.327	315	3795	[30]
Bayberry ( <i>Myrica</i> ) Juice	8 min 60 °C	0.198	271	1.327	173	874	[39]
	8 min 70 °C		452	1.327	288	1455	
Pear ( <i>Pyrus communis</i> cv. Taylor's Gold) Puree	10 min 72 °C	0.025	460	0.07068	19.5	780	[28]
Apple ( <i>Malus domestica</i> cv. Royal Gala) puree	10 min 72 °C	0.025	460	0.07068	19.5	780	[28]
Strawberry ( <i>Fragaria ananassa</i> cv. Camarosa) puree	10 min 72 °C	0.025	460	0.07068	19.5	780	[28]

For studies [30,39,55] the frequency of ultrasound applied by authors was 20 kHz, while the other studies were 24 kHz.

## 6. Final Remarks and Challenges

The inactivation of PPO enzymes in fruits and the energy requirements for various treatments were evaluated. PPO in different fruit juices was effectively inactivated by thermal, HPP, and ultrasound treatment, with residual enzyme activity decreasing with increasing temperature, pressure, or ultrasound intensity and treatment time. PPO in different fruits showed first-order kinetics in many studies of kinetic inactivation. However, kinetic inactivation by HPP exhibited non-linear kinetics in some fruits, which fit better with first-order biphasic models. The activation energy data, D-values, and z-values summarised from this review can be used to better understand inactivation kinetics and to develop an industrial process that can produce stable products with longer shelf life. The estimation of energy calculated in this study based on Equations (1) and (3) can be used to compare the energy consumption and requirements for different types of food processing technologies. Estimation of energy is important, especially for food industries to develop solutions towards sustainable food production and more efficient energy use. Most food processing operations involve adding heat energy to ensure microbiological safety, improve quality, and extend product shelf life. Furthermore, most alternative processes (HPP and ultrasound) use electricity, so energy calculation allows the estimation of energy efficiency and comparison of novel food processing technologies. Researchers could explore more in this area, as it is significant to determine the cost effectiveness of fruit processing.

Some challenges and limitations during this study should be noted. It was difficult to find temperature data that combined thermal and non-thermal treatments in previous studies. Additionally, for thermal treatment, the calculation is based on the heat absorbed by the sample rather than the energy provided by the equipment based on the power supply, and this could provide an underestimation of energy supplied as heat losses were not included. Aside from that, few studies have used PPO enzyme inactivation as a baseline comparison to estimate the energy requirements for fruit processing using thermal and non-thermal technologies, particularly HPP and ultrasound. More research on energy estimation is needed, particularly in the food industry, to develop solutions for sustainable food production and more efficient energy use.

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