

Polyphenoloxidase (PPO)

Subjects: Biochemistry & Molecular Biology

Contributor: Sergio Nogales

Fresh-cut produce are quite popular among consumers due to their eating ease, high quality and functional content. However, some of the processing steps taking place during minimal processing (such as cutting, peeling, draining, etc.) might speed up decay, e.g., microbial growth, dehydration or browning. When it comes to the latter, polyphenol oxidase (PPO) plays an important role, being the center of many works focused on the understanding of its reaction mechanism and the application of conservative techniques. The aim of this review study was to compare recent research about the effect of PPO on minimally processed fruits and vegetables, trying to understand the way it acts, the measurement of its activity and current treatments, such as modified atmosphere packaging, washing treatments or edible coatings, among others. In conclusion, the combination of conservation techniques (that is, hurdle technology) is vital to guarantee global quality in minimally processed fruits and vegetables, including synergistic effects which will allow the use of mild treatment conditions to decrease PPO activity. However, further research is required to clearly understand PPO inhibition in trendy techniques such as irradiation.

Keywords: minimally processed ; fruits ; vegetables ; antioxidants ; edible coatings ; essential oils ; modified atmosphere packaging

1. Introduction

Minimally processed fruits and vegetables are highly demanded by consumers ^[1], growing their sales all over the world, as in the case of Europe ^[2]. This might be due to an increasing concern about health and lack of time to cook properly. Furthermore, minimally processed fruits and vegetables provide high functional and ready-to-eat food which are convenient products nowadays. Thus, there are a considerable amount of vegetables and fruits undergoing this technology, such as lettuce, cabbage, eggplant, apples, pears, peaches, plums, nectarines, etc. On account of the fact that fresh-cut produces come from whole vegetables and fruits, it is expected that these products have similar functional properties. Consequently, they contain high levels of fiber, minerals or antioxidants such as vitamins C and E, carotenoids, glucosinolates, polyphenols, etc. ^{[3][4][5][6]}. This fact has supposed an interesting research field for scientists, and its relevance has continuously increased. The trend in research about fresh-cut or minimally processed fruits and vegetables is shown in **Figure 1**.

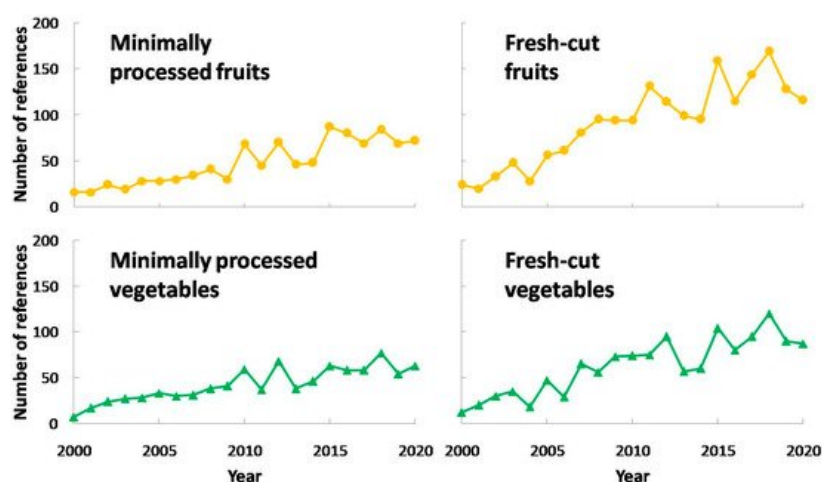


Figure 1. Research works related to minimally processed and fresh-cut fruits (yellow) and vegetables (green) ^[7].

As observed in this figure, there was a steady increase in research works about fresh-cut or minimally processed fruits and vegetables, mainly starting in 2000 with a few works and surpassing 150 studies per year in the past five years. In addition, there were more research works about fresh-cut or minimally processed fruits compared to vegetables. In conclusion, these figures point out the increasing interest in minimally processing so far.

However, even though the processing of fresh-cut fruits and vegetables is minimal, some aspects such as cutting and peeling promote a faster deterioration [8], involving physiological, biochemical and microbiological changes [9]. Hence, there are a lot of enzymes that take part in these changes, such as pectin methylesterase, polygalacturonase (both of them affecting texture), lipooxidase (generating off-flavor compounds) and polyphenol oxidase (EC 1.14.18.1 or PPO), among others. The latter plays an important role on visual quality decay causing browning, as it will be discussed in following sections. This deterioration might change consumers' choice as visual appearance is one of the highest rated aspects considered in order to purchase minimally processed fruits and vegetables. As a consequence, PPO has been widely studied (both in whole or minimally processed products) in order to characterize its content or to avoid its effect on quality of fruits or vegetables, (see **Figure 2**).

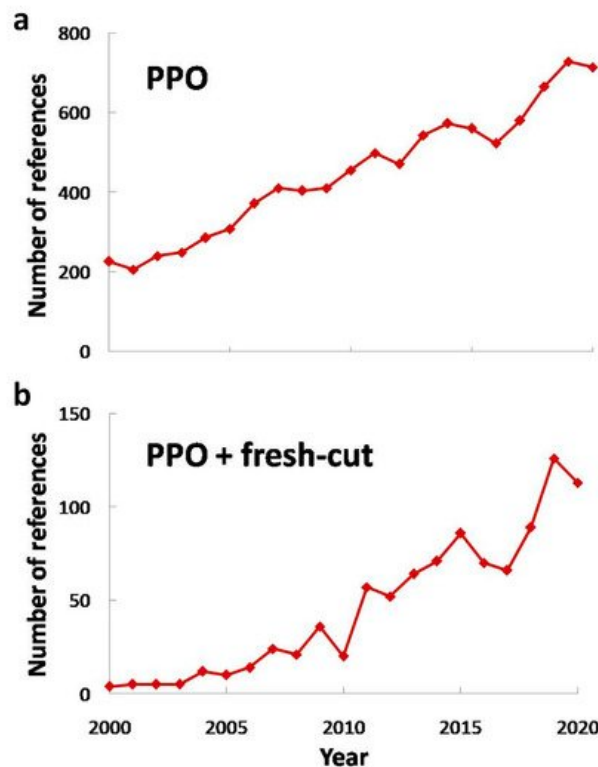


Figure 2. References related to: “PPO” and “PPO + fresh-cut” [7].

This way, PPO has been widely researched, and its interest has increased in the last two decades (**Figure 2 a**). The same trend was observed for research about PPO and fresh-cut produce (**Figure 2 b**), and if **Figure 1** is taken into account, it can be noted that research about fresh-cut produce and its combination with PPO research are almost equivalent, pointing out the need of PPO characterization and the study of its consequences in fresh-cut fruits and vegetables in every research work in this field.

2. PPO Activity Assessment

As explained earlier, there is a special need to assess PPO activity (among other enzymes) in order to assess its effect on browning. Even though there are different ways to assess PPO activity [10], the most common one used in fresh-cut produce was by spectrophotometry. In this case, whole produce and in-vitro assays follow similar procedures. In short, the steps are the ones gathered in **Figure 3** . Additionally, **Table 1** and **Table 2** shows some details about PPO activity extraction and determination for vegetables and fruits, respectively.

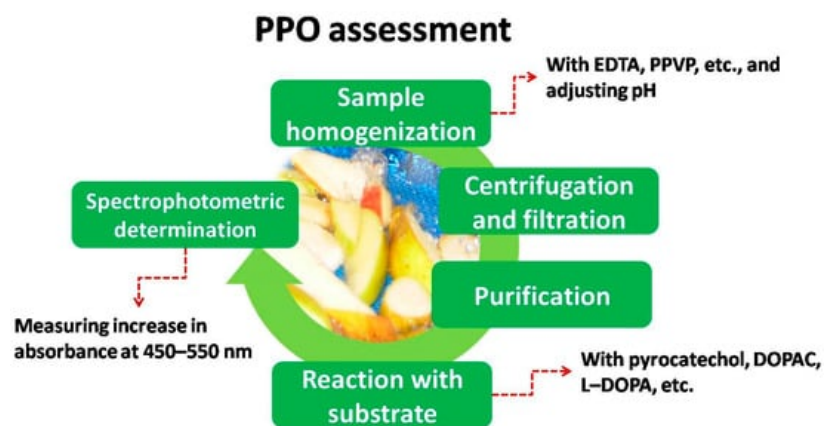


Figure 3. Steps for PPO assessment.

Table 1. Different conditions for PPO activity determination in some fresh-cut vegetables.

Produce	Reference	PPO Extraction	Spectrophotometric Determination		
			Substrate Solution	pH	Wavelength (nm)
Artichoke	[11]	PVP and acetate buffer (pH 5.6)	Chlorogenic acid	6.0	410
Artichoke	[12]	Benzamidine, ascorbic acid, PMSF, triton and phosphate buffer (pH 7.0)	MBTH	4.5	400
Carrot	[13]	PVPP and phosphate buffer (pH 6.5)	Catechol	6.5	420
Carrot	[14]	Phosphate buffer (pH 7.0)	Catechol	7.0	410
Eggplant	[15]	Citrate phosphate buffer (pH 7.0)	DOPAC, MBTH, methanol	7.0	466
Eggplant	[16]	PVP, PVPP, triton, ascorbic acid and phosphate buffer (pH 6.8)	4-methylcatechol	6.8	420
Lettuce	[17]	PVPP and phosphate buffer (pH 6.5)	Catechol	6.5	400
Lettuce	[18]	PVPP and phosphate buffer (pH 7.0)	Catechol	7.0	420
Lettuce	[19]	Acetone, MBTH and citrate phosphate buffer (pH 7.5)	DOPAC	7.0	505
Lettuce	[20]	Phosphate buffer (pH 7.0)	MBTH	7.0	467
Lotus root	[21]	PVPP and phosphate buffer (pH 7.0)	Catechol	7.0	410
Mushroom	[22]	PVPP and NaCl (pH 6.5)	Catechol	7.0	400
Mushroom	[23]	PVPP, triton and phosphate buffer (pH 6.8)	Catechol	6.8	420
Potato	[24]	PVPP and phosphate buffer (pH 6.0)	4-methylcatechol	6.0	410
Potato	[25]	Phosphate buffer (pH 6.5)	Catechol	6.5	410
Potato	[26]	PBS (pH 6.8)	Catechol	5.5	405
Potato	[27]	PVP, triton and phosphate buffer (pH 6.8)	Catechol	7.0	475
Red beet	[28]	NaCl, PVP and phosphate-citrate buffer (pH 6.5)	Pyrocatechol	6.5	420
Red beet	[29]	NaCl and phosphate buffer (pH 6.0)	Pyrocatechol	7.0	420
Sweet peppers	[30]	Acetone and citrate phosphate buffer (pH 7.5)	DOPAC	7.0	505

DOPAC = 3,4-dihydroxyphenyl acetic acid; EDTA = Ethylenediaminetetraacetic acid; MBTH = 3-methyl-2-benzothiazolinone hydrazine; PBS = Phosphate buffered saline; PMSF = Phenylmethylsulphonyl fluoride; PVP = Polyvinylpyrrolidone; PVPP = Polyvinyl polypyrrolidone.

Table 2. Different conditions for PPO activity determination in some fresh-cut fruits.

Produce	Reference	PPO Extraction	Spectrophotometric Determination		
			Substrate Solution	pH	Wavelength (nm)
Apple	[31]	PVPP and phosphate buffer (pH 7.0)	Cathecol	5.8	420
Apple	[32]	PVPP and phosphate buffer (pH 5.0)	4-methylcatechol	5.0	494
Apple	[33]	Triton and phosphate buffer (pH 7.2)	Chlorogenic acid	5.2	420
Apple	[34]	PVPP, triton and Phosphate buffer (pH 7.0)	Citrate-phosphate	5.0	420
Apple	[35]	Potassium phosphate buffer (pH 7.0)	Sodium acetate	5.5	405
Banana	[36]	PVPP, triton and phosphate buffer (pH 6.5)	Catechol	6.5	420
Carambola	[37]	PVPP, KCl and phosphate buffer (pH 6.8)	Catechol	7.2	410
Carambola	[38]	PVPP, KCl and phosphate buffer (pH 6.8)	Catechol	7.2	410
Coconut water	[39]	Phosphate buffer (pH 5.5)	Pyrocatechol	5.5	470
Jicama	[40]	PVPP and phosphate buffer (pH 7.0)	Catechol	7.0	420
Papaya	[41]	Sodium phosphate buffer (pH 6.5)	Catechol	6.5	420
Peach	[42]	PMSF, PVPP, triton and phosphate buffer (pH 6.8)	L-Dopa	6.8	475
Peach	[43]	EDTA, MgCl ₂ , PMSF, PVPP, triton and phosphate buffer (pH 7.0)	Resorcinol	7.0	500
Peach	[44]	EDTA, PVPP and phosphate buffer (pH 7.0)	--	7.0	420
Pear	[45]	Dihydrogen phosphate buffer (pH 6.8)	Catechol	6.8	398
Pear	[46]	Triton and phosphate buffer (pH 7.2)	Chlorogenic acid	4.2	420
Strawberry	[47]	PVPP, triton and phosphate buffer (pH 6.5)	Catechol	6.5	420

EDTA = Ethylenediaminetetraacetic acid; PMSF = Phenylmethylsulphonyl fluoride; PVPP = Polyvinyl polypyrrolidone.4. Current studies focused on PPO activity.

Thus, homogenization is required, mixing up the sample with some components such as EDTA, PMSF or PVPP and adjusting pH (generally between 6 and 8) with buffer [10]. After centrifugation and filtration, the spectrophotometric reaction takes place adding the extract to a reactive environment with suitable substrates and adjusting pH. Molecular structures of some of the most used substrates are shown in **Figure 4**.

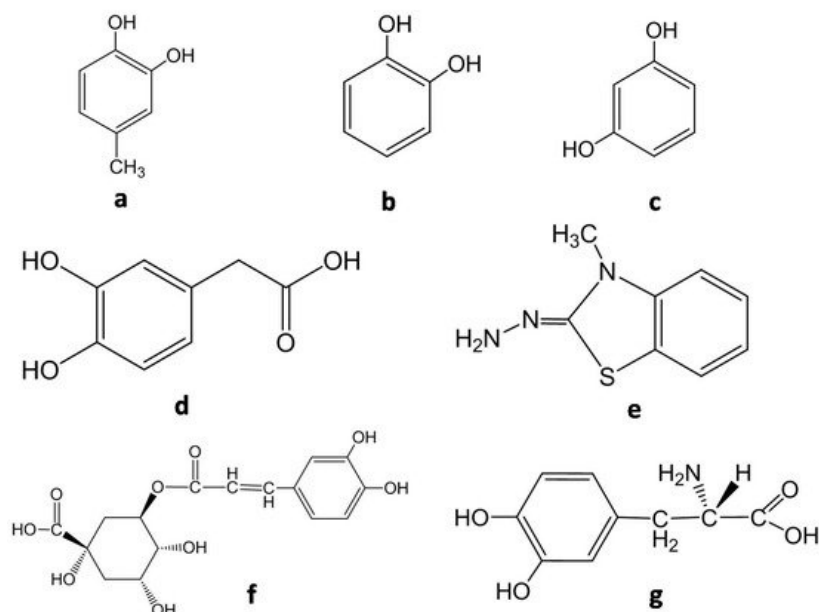


Figure 4. Molecular structures of some common substrates used in PPO activity determination for fresh-cut produce: (a) 4-methylcatechol; (b) pyrocatechol (catechol); (c) resorcinol; (d) DOPAC; (e) MBTH; (f) chlorogenic acid; (g) L-DOPA.

Finally, the increase in absorbance is measured at the beginning of the reaction (usually for 3 min), using wavelengths from 450 to 550. **Figure 3** and **Figure 4** show some of the most common extracts used in spectrophotometric determination of PPO activity applied to different fresh-cut vegetables and fruits, respectively.

3. Current Studies Focused on PPO Activity

According to many researchers, as included in **Figure 5**, the main factors affecting PPO activity are temperature, pH, cell integrity, pre-harvest conditions and microbial activity, which will be explained in the following sections.

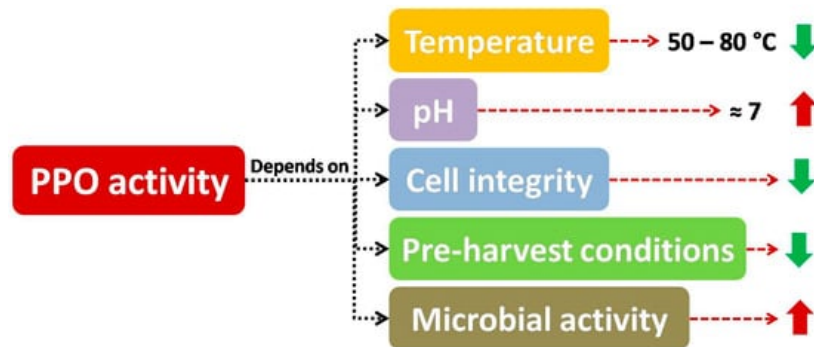


Figure 5. Influence of main factors on PPO activity, indicating its positive (green down arrow) or negative effect (red up arrow) and some specific conditions.

Apart from the activity of endogenous quality degrading enzymes, spoilage microorganism reaction considerably shortens the shelf life of fruit and vegetable products, both whole and fresh-cut produce ^{[48][49]}. Furthermore, microbial growth can be a matter of concern, with many studies considering microbial safety in minimally processed samples ^[50], as in the case of cantaloupe pieces ^[51].

As included in some studies, the effect of enzymes and microbial growth on shelf life of minimally processed fruits and vegetables could be synergistic, as microorganisms can worsen cell integrity of tissues, provoking cell lysis, which is highly related to the increase in enzyme activity, as enzymes and substrates are easily put in contact, accelerating decay in fruits and vegetables (both whole and fresh-cut samples) ^[52]. On the other hand, microbial growth and enzymatic reactions can present a common starting point (i.e., cell lysis) to put some components included in cell content (such as sugar or phenols for microbial counts or PPO activity, respectively), and consequently these two adverse effects can act in parallel. As a consequence, if some techniques promote cell reinforcement are used (such as the application of calcium treatments), a combined decrease in microbial and PPO activity can be observed, as outlined below.

This way, the effect of microbiological activity on some visual defects has been investigated in several papers that considered the relationship between psychrotrophic counts and visual decay ^[51]. As some authors have pointed out, the use of protective treatments seemed to be related to a direct decrease in browning, enzyme activity (such as PPO) and microbial growth ^{[50][53]}, as in the case of edible coatings applied to fresh-cut fruits and vegetables ^[54]. Specifically, the reduction of microbial population may result in PPO activity inhibition, on account of the fact that pectinolytic microorganisms could break down cell walls resulting in stress-related exposure of enzymes and substrates, which also could lead to enzymatic browning. Hence, some barriers such as modified atmosphere packaging or washing treatments might contribute (besides their direct effects on browning inhibition) to reduce microbial population and therefore reduce PPO activity, improving visual quality ^{[55][56]}, as explained in many papers included in this review, although the decrease in microbial growth is not thoroughly covered in this work.

4. Current Treatments to Avoid or Reduce PPO Activity in Fresh-Cut Produce

In order to avoid the negative effects of PPO activity, or at least to reduce them, many treatments have been used in fresh-cut or minimally processed produces. Some of them can be exclusively used to reduce PPO activity, whereas others can show other beneficial effects on fresh-cut produce, such as antimicrobial effect, inhibition on other enzymes, etc. On the other hand, some of these treatments can be combined, such as the use of washing with antioxidants or essential oils, the use of edible coatings containing antioxidants or the combined use of washing and modified atmosphere packaging, presenting additive or synergistic effects in many cases. In any case, some of the treatments that have drawn attention to

researchers are included in **Figure 6** . It should be pointed out that the search for mild treatments (by combining techniques or using the hurdle technology) seems to be one interesting and resourceful option for researchers, in order to avoid other undesirable effects on fresh-cut fruits and vegetables [57].

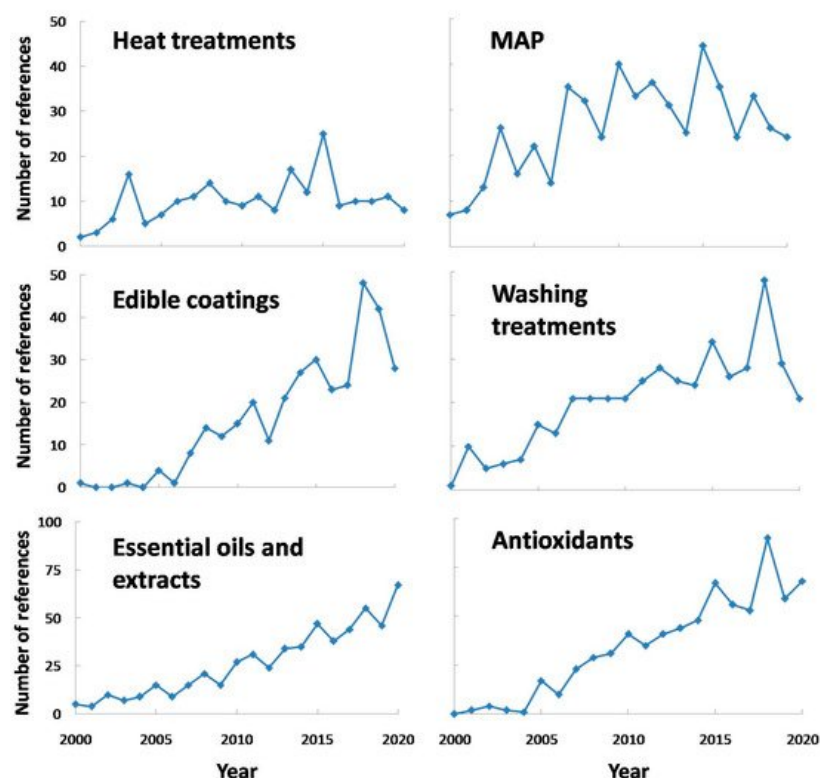


Figure 6. Main trends for treatments in order to keep quality of fresh-cut produce. Search words: Heat treatments, modified atmosphere packaging (MAP), edible coatings, washing treatments, essential oils and extracts and antioxidants. Source: Scopus [7].

On the other hand, N -acetylcysteine is specifically used as a dietary supplement, being used in order to avoid cut browning. For instance, it is worth mentioning the optimization of a washing treatment including N -acetylcysteine in minimally processed apples. Thus, the use of 100 mM isoascorbic acid, 5 mM Caascorbate, 5 mM Capropionate and 5 mM N -acetylcysteine washing treatment on different cultivar apples was the optimum combination used in order to keep visual quality [58].

This product could be used included in washing treatments, as the literature showed promising results when it comes to PPO control. As some authors have appointed, the use of salicylic acid (SA) in washing treatments have showed good results concerning PPO inhibition in fresh-cut chestnut, and its effect was higher with concentration (up to 10 mM or at least 0.3 g/L, depending on the case). However, its inhibition properties are not clear yet, needing further studies [59][60].

Ultrasound (US) might cause enzyme inactivation by cell lysis due to vibration energy, which produces cavitation bubbles and temporarily generates spots of high pressure and temperature when imploded. Thus, its use might be suitable especially when combined with antioxidant treatments (normally included in washing treatments), just as in the case of fresh-cut apples. Specifically, PPO activity was reduced by combining US (40 kHz) and ascorbic acid (1%), pointing out the fact that the individual application of these treatments was not effective at inactivating enzyme activity [32]. Other recent study was focused on the suitability of this technique combined with ascorbic acid and citric acid for fresh-cut potatoes, showing around 40 % decrease in PPO activity in treated samples, compared to control samples [26].

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