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Extraction and characterization of polyphenol oxidase from pulp of apple fruit

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Abstract
Polyphenol Oxidase is present in majority of plant tissues and it is involved in adverse browning of plant products. Crude polyphenol oxidase (PPO) from the pulp of apple fruit was extacted by homogenization with potassium phosphate buffer followed by precipitation with 1.5 volumes of cold acetone. The concentration of enzyme in apple pulp was determined spectrophotometrically. The activity of enzyme in apple pulp was found to be2.65 µmol /$min^{-1}cm^{-3}$. Some properties like optimum pH and optimum temperature of the isolated PPO were also studied. The optimum pH for apple pulp PPO activity was found to be 7 and the optimum temperature of PPO activity was 20 °C.

Keywords: Polyphenol oxidase, enzyme activity, optimum pH, optimum temperature

1. Introduction
Polyphenol oxidase also known as tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, or catecholase which is widely found in nature is typically present in the majority of plant tissues (Whitaker 1994, 1996; Fraignier *et al*., 1995; Haruta *et al*., 1999) [27, 15, 18]. Polyphenol oxidases are a widespread group of enzymes found in plants, fungi, bacteria, and animals. In plants, these enzymes are usually found in the chloroplasts, although they can be released from this compartment during ripening or senescence.
The enzymes contain copper at their active site. This metal ion enables them to oxidize the phenolic group of an aromatic compound to a reaction group known as a quinone. Quinones are reactive oxygen molecules bound to a carbon atom by two double bonds. Polyphenol oxidase catalyzes two reactions; the first, a hydroxylation of monophenols to diphenols, which is relatively slow and results in colourless products. The second, the oxidation of diphenols to quinines which is rapid and gives coloured products (Queiroz *et al*., 2008) [24].
This enzyme is almost found in all living organisms including plants, animals and microorganisms. In plant, it is involved in defense mechanism. When a plant gets a bruise or cut, certain phenolic compounds are oxidized in the presence of oxygen to form a polymeric structure which prevents microbial contamination (Whitaker, 1994) [27]. The catalytic action of polyphenol oxidase is connected to undesirable browning and off-flavor generation in stored and processed foods. On the other hand, PPO has been also shown to have important applications such as the use in the synthesis of valuable added products like the substituted catechol, L-DOPA for the treatment of Parkinson's disease (Pialis and Saville, 1998) [23]. A number of other catechols have found applications as fine chemicals or as starting materials for pharmaceutical drug synthesis (Halder *et al*., 1998) [17]. PPOs also play an important role as efficient reagents for cleaning polyphenols-containing wastewater (Freire *et al*., 2002) [16]. In the view of the increasing commercial applications of PPO in various fields and the development of more effective preservation conditions and methods in order to prevent the enzymatic browning, the properties of PPO from its various sources need to be studied.
Polyphenol oxidase (PPO) causes oxidative browning in many food products (Chi *et al*., 2014) [13]. Enzymatic browning is a significant problem in a number of fruits and vegetables resulting in discoloration of fruits and vegetables. This occurs as a result of conversion of phenolic compounds to *o*-quinones which subsequently polymerize to be a brown or dark pigment. Polyphenol oxidase has received much attention from researchers in the field of plant physiology and food science because of its involvement in adverse browning of plant products.

Plants and fruits account for a substantial fraction of the world's agricultural output (Ali *et al*., 2015; Ashraf *et al*., 2015; Asif, 2015a, b, c, d, e, f, g, h, i, 2016; Hussain *et al*., 2016; Mensah and Golomeke, 2015) [1, 2, 3-11, 19, 21] and some (such as the apple and the pomegranate) have acquired extensive cultural and symbolic meanings. Wounds inflicted during the preparation of fresh-cut on fruits and tubers promote many physical and physiological changes that hasten loss of product quality. Foremost among these, are the removal of the protective epidermal layer and/or exposure of internal cells. These changes not only facilitate water loss, but also provide an easy entry for microbial pathogens and chemical contaminants. When fruits or tubers are peeled or cut, enzymes contained in the plant cells are released. One of these enzymes is polyphenoloxidase which is involved in enzymatic browning which occurs readily at warm temperatures when the pH is between 5.0 and 7.0 (Cisneros, 1995) [14].

Enzymatic browning affects nutritional properties, flavor and texture of foods and feeds during storage or processing and is therefore detrimental to food quality. Browning and discoloration causes substantial losses in a wide range of fresh and processed fruits and tubers. Traditionally, browning in foods has been controlled by using sulfating agents; such food additives have been used in a wide range of fresh, frozen and processed food products.

The objective of our study was to extract and characterize polyphenol oxidase from pulp of apple.

2. Material and Methods

2.1 Source of fruits

Fresh and matured fruit of apple were purchased from local market.

2.2 Reagents

0.2M potassium phosphate buffer, cold acetone, 20mM catechol, 0.2M sodium acetate buffer.

2.3 Preparation of crude PPO extract: The enzyme was extracted by homogenizing20 gm of sliced fruit with 250 ml cold potassium phosphate buffer (0.2 M, pH 7).The homogenate was filtered through cheese cloth and centrifuged at 5000 g for10 minutes. The enzyme was precipitated from the supernatant by adding 1.5volumes of cold acetone (-5 °C) with gentle stirring for 60 minutes. The mixture was centrifuged at 10000g for 15 minutes and the precipitate was dissolved in 50 ml of potassium phosphate buffer. This crude enzyme extract was used for the enzyme characterization.

2.4 Enzyme activity assay: Polyphenol oxidase activity was determined according to the method of Ying and Zhang, 2008 by measuring the increase in absorbance at 420nm with spectrophotometer. The sample cuvette contained 2.0ml of catechol, 0.9ml of 0.2M sodium acetate buffer pH 4.0 and 0.1ml of enzyme solution. Reference cuvette (blank) contained 2.0ml of the same substrate solution and 1.0ml of 0.2M sodium acetate buffer. Each sample was assayed in triplicates. One unit of PPO activity is defined as the amount of enzyme that causes an increase in absorbance of 0.001/minute.

2.5 Determination of optimum temperature: The activity of PPO was measured at different temperatures ranged from 10 to 30 °C.

2.6 Determination of optimum pH: The rate of catechol oxidation by PPO was estimated in the pH range of 5.8-7.9 using potassium phosphate buffer (0.2 M).

3. Results and Discussion

3.1 Optimum pH of polyphenol oxidase activity: PPO activity was measured in the pH range 5.8 7.9 using potassium phosphate buffer (0.2 M) with 20 mM catechol as the substrate. Optimum pH was found to be 7.0 for apple PPO (Fig.1). As shown in the Figure apple PPO showed good activity in the neutral and alkaline pH while the activity sharply declined in the acidic pH.In general, most plant polyphenol oxidases show a maximum activity at neutral pH (Benjamin and Montomery, 1973; Siddiq *et al*., 1992; Unal, 2007; Yue-Ming, 1999) [12, 25, 26, 29]. The optimum pH of PPO activity may vary depending on some factors such as the enzyme source,maturity of the fruit, extraction method, temperature, substrate and type and concentration of the buffer (Whitaker, 1994; Ziyan and Pekyardimic, 2004) [27, 30].

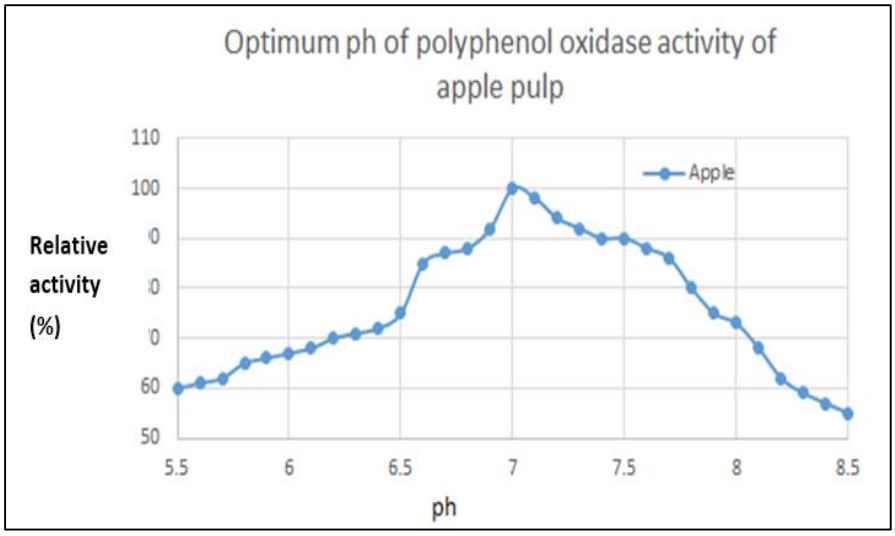
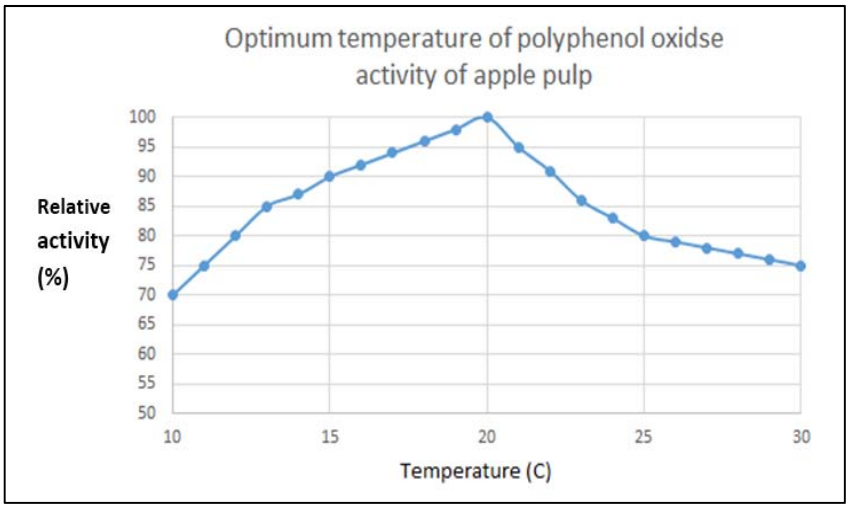


Fig 1

3.2 Optimum temperature of polyphenol oxidase activity
PPO activity was assayed at temperature range of 10-30 °C. The activity was found to be remarkably affected by temperature (Fig.2).The enzyme isolated from apple showed maximum activity at 20 °C. Above these temperature values, the activity was rapidly decreased. Oktay *et al*. (1995) [22] and Ziyan and Pekyardimic (2004) [30] had reported optimum temperatures of 18 and 20 °C for apple and pear PPOs, respectively. Optimum temperature of polyphenol oxidase may change depending on the type of the used substrate.



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