CHARACTERIZATION OF ENZYMES OF RIPEN BANANA (*Musa sp*.) FRUIT PEEL AND PULP

M. Sc. THESIS

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APPROVAL SHEET

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DEDICATION

This thesis work is dedicated to my mother Etagegn Fetene and my father MulukenTiru for their encouragement and support in all my life

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this M.Sc Thesis is my own work. I have followed all ethical and technical principles of scholarship in conducting studies, data collection, data analysis, and compilation of this thesis. Any scholar matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

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ACRONYMS/ABBREVIATIONS

CATA	Catalase activity
CATspa	Catalases specific activity
DPPH	2,2-diphenyl-1-picrylhydrazyl
PO	Peroxidase assay
PG	Polygalacturonase
PME	Pectin methylesterase
PODA	Peroxidase activity
PODspa	Peroxidase specific activity
PPOA	Polyphenol oxidase activity
PPOspa	Poly phenol oxidase specific activity
HPSA	Hydrogen peroxide scavenging activity

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ABSTRACT

The increasing interest for natural sources of bioactive compounds and the popularity of the concept of functional foods, food products enriched with fruit peels are being developed .The present study was planned to characterize enzyme extracts from ripen banana fruit peel and pulp with respect to enzymatic and antioxidant activities. The study was conducted to investigate characteristics of enzyme extracts from banana fruit peel and pulp. The enzyme extraction involved homogenization of the sample, filtrate in cold acetone and then the filtrate was homogenized in 0.1M sodium acetate buffer, pH 7. The protein content was determined using Lowry assayusing bovine serum albumin (BSA) as a standard protein. Then enzymatic activity was determined using assay mixture and absorbance unit. The antioxidant activities of the enzyme extract was conducted using DPPH and hydrogen peroxide free radical scavenging activities. The relationships among enzyme assay parameters were determined using correlation coefficient and cluster methods. The results of enzymatic assay of ripen banana peel and pulp showed significantly higher, total soluble protein (12.67mg/ml), peroxidase activity (PODA, 81.60u/ml), peroxidase specific activity (PODspa, 6.44u/mg), catalase activity (CATAu/ml) and catalase specific activity (CATspa, 6.83u/mg), in pulp than in peel extract .in Contrast, significantly higher polyphenol oxidase activity (PPOA, 92.40u/ml) and polyphenol specific activity (PPOspa, 9.61u/mg) were observed in the banana peel extract than in the pulp extract. In both banana fruit pulp and peel peroxidase was most active at PH 6. Peroxidase of both fruit peel and pulp has presented a wide range of temperature for activity (0 to 30^0C). The antioxidant activity of the enzyme extracts indicated that significantly higher DPPH radical scavenging activity (83.12±0.12) was observed for peel extract. However, significantly higher hydrogen peroxide scavenging activity (HPSA) for banana fruit pulp extracts. The Pearson correlation coefficient of enzyme activity parameters and free radical scavenging activities demonstrated that the DPPH free radical scavenging activity was found to be significantlyand positively correlated to PPOA and PPOspa. In contrast, hydrogen peroxide scavenging activity (HPSA) was significantly and positively correlated toCATA, CATspa, PODA, PODspa and protein content. The dendrogram for clustering of enzyme activity parameters and free radical scavenging activities grouped the parameters into two clusters. The first cluster includes PODA, HPSA, CATA, CATspa, PODspa, and protein content while the second cluster contained PPOA, DPPH &PPOspr.It can be concluded from the result of the present study that enzymatic extractslike POD and PPO will have significant role as antioxidants.

Keywords: *Catalase, DPPH, Hydrogen peroxide scavenging activity, Peroxidase, Polyphenoloxidase, Protein content.*

1. INTRODUCTION

By-products of fruit processing industry considered as fruit wastes consist mainly of core, seeds, pomace and peels, contain large amounts of water and are in a wet and easily fermentable form. If not processed further, these agrowastes produce odor, soil pollution, harborage for insects and can give rise to serious environmental pollution (Shalini and Gupta, 2010). Scientists were able to develop high value products from these by-products such as cosmetics, medicines and the recovery seems to be economically attractive (Ashoush and Gadallah, 2011). The idea of utilizing fruit by-products mainly the peels which in some fruits represent almost 30% of the total weight, has slowly gained popularity especially when researchers found that peels possessed better biological activities than other parts of the fruit. With increasing interest for natural sources of bioactive compounds and the popularity of the concept of functional foods, food products enriched with fruit peels are being developeddeveloped. However the potential application of fruit peels in food supplementation depends strongly on their chemical composition (Babiker*et al.,* 2001; Altunkaya*et al.,* 2013).

Banana is the second largest produced fruit after citrus, contributing about 16% of the worldtotal production (Mohapatra*et al.,* 2010). Banana peel is, hygienic and easy-to-remove packaging, making handling easy and convenient. The absence of seeds and availability throughout the year also contribute to its acceptance (Lichterberg, 1999). Usually, only ripe pulp is consumed due to its high sugar content and sensory aspects. However, the green fruit is also consumed in cooking dishes typical of some regions due to its high starch content. In addition, the incorporation of green fruit flour in some products, like biscuits, bread rich in fibers and edible films, is increasing. The consumption of green bananas (peel and pulp) is beneficial to human health due to the high content of resistant starch, which acts in the body as food fiber (Rodríguezambriz*et al*., 2008; Oliveira *et al.,* 2015). Moreover, banana flour can be an important source of polyphenols, compounds considered as natural antioxidants (Vergara-Valencia *et al.,* 2007).

In addition to the importance of carbohydrates, attention has been directed to the study of the antioxidant activity present in the most diverse fruits, including banana. The presence of antioxidant components such as phenolic compounds in the human diet is associated with

protective effects in the prevention of some chronic-degenerative diseases related to oxidative stress, i.e., preventing the harmful action of free radicals on proteins, DNA and lipids (Isabelle *et al.,* 2010). In addition, antioxidant compounds have proven action on synergistic effects and protective properties against various degenerative diseases, including cancer, stroke, cardiovascular diseases, Parkinson's and Alzheimer's disease (Abdel-Hameed, 2009).

Various studies have demonstrated the occurrence of phenolic compounds and antioxidant action in banana (Fatemeh*et al.,* 2012; Shian*et al*., 2012; Rebello*et al*., 2014). Shian*et al.* (2012) reported for 'Berangan' (AA), 'Mas' (AA) and 'Raja' (ABB) banana cultivars in Malaysia radical removal percentage varying from 3.2 to 63.1% in mature pulp, making imperative the evaluation of the pulp and peel at different ripening stages.

Banana is one of the most important crops of the tropical plants. It belongs to the family *Musaceae*and the genus *Musa* .The plant consists of long, overlapping leaf stalks and bears a stem which is 1.22 to 6.10 m high. It has life span of about 15 years (Hassan *et al.,* 2018). The fruits grow in clusters, each separate banana of the cluster being about 1 inch in diameter. Banana fruit requires about two and a half to four months after shooting before the fruit becomes ready for harvesting ora total of about eight to twelve months after planting. Banana contains high fibre content, and is capable of lowering cholesterol level and helps to relieve constipation and prevention of colon cancer. Its high potassium content is found to be useful in the prevention of raising blood pressure and muscle cramp. Various parts of the plant such as the leaves, roots, fruit stalks, bracts and fruits have been used for medicinal and domestic purposes. Its sap is used as a remedy for diarrhoea, dysentery, hysteria and epilepsy. A cold infusion of the root is used to treat venereal diseases and anaemia. Banana is said to prevent anaemia by stimulating the production of haemoglobin in the blood (Rolfes*et al.,* 2009).

Damages from free radicals at the cellular level cause cell inflammation, increasing the risk of damage from sunlight radiation, and related development of skin cancer. Antioxidants work by preventing onset of disorder at the cellular level. The development of natural antioxidants is still continuing to be studied because it is difficult to maintain the antioxidant activity of material and its stability (Wanasundara and Shahidi, 2005; Mandal *et al.,* 2009). Waste of yellow banana peels is rich in flavonoids and another phenolic in addition to carbohydrates,

cellulose, minerals such as potassium and sodium. Flavonoid and phenolic are bioactive compounds that useful as antioxidant (Azmi, 2010).

The banana peels waste is normally disposed on lands which contribute to the existing environmental problems. The problems can however be overcome by utilizing its high-added value compounds, including the dietary fibre fraction that has a great potential in the preparation of functional foods. Dietary fibre has shown beneficial effects in the prevention of several diseases, such as cardiovascular diseases, constipation, irritable colon, colon cancer, and diabetes (Rodriguez *et al*., 2006). The fruit fibre has a better quality than other fibre sources due to its high total and soluble fibre content, water and oil holding capacities, and colonic fermentability, as well as a lower phytic acid and caloric value content. The high dietary fibre content of banana peel (about 50 g/100g) is indicative of a good source of dietary fibre (Figuerola*et al,* 2005). Management and processing of the waste from different industries are the major problem in different parts of the world (Anupama*et al*. 2013). The harmful impact of molecules that are generated from various different process industries pollutes air and water by its dangerous and mutagenic properties (Busca*et al*. 2008). Major organic pollutants include phenol and its derivatives like chlorophenol, cresols, orthophenols, nitro phenols, etc., produced by various industrial activities, such as coal mining, petroleum refining, resin, plastic and pharmaceutical production, wood preservation, metal coating and textile dyeing. Production and degradation of pesticides also release huge quantities of phenols and its derivatives to the environment (Varsha et al. 2011). The aim of the present study was to characterize enzyme extracts from ripen banana fruit peel and pulp with respect to enzymatic and antioxidant activities.

General Objective

To investigate enzymatic and antioxidant activities of ripen banana fruit.

Specific Objectives:

- To extract crude enzymes from banana fruit pulp and peel.
- To assess enzyme activities of the crude extracts from banana fruit pulp and peel.
- To determine optimum pH and temperature for enzymatic activities;
- To evaluate free radical scavenging activities of the crude enzymes from banana fruit peel and pulp.

2. LITERATURE REVIEW

2.1. Plant Description

Banana belongs to the genus Musa of the family *Musaceae*. The genus *Musa* contains four sections, *Eumusa*, *Rhodochlamys*, *Australimusa*, and *Callimusa*. *Rhodochlamys*and*Callimusa*are of ornamental interest only. *Australimusa*species are utilized across a large area of the Pacific as a cooked vegetable. However, *Eumusa*is the largest and most widespread geographically and contains the all the major edible species of banana. Most edible bananas are delivered from two members of the sect of *Eumusa*: *Musa acuminate* and *Musa balbisiana* (Salunkhe and Kadam, 1998). Most of the edible bananas are either derived solely from the *Musa acuminate* or are hybrid between two wild diploid species, *Musa acuminate Colla*and*Musa balbisianaColla;* which contributed to A and B genomes respectively (Hailu*et al.,* 2013). There are 300 -500 cultivars which are naturally occurring banana hybrids which can be grouped according to their genome constitutions, as AA, AAA, AB, AAB, ABB and ABBB. Tetraploid AAAA cultivars have only been produced through breeding program. Nevertheless triploid and tetraploid B cultivars have not been identified yet (Hirimburagama and Gamage, 2015). Three most common species of banana which are mostly grown in the world are *Musa cavendishi*, *Musa paradisiaca*and*Musa sapientum* (Mohapatra*et al.,* 2010). It is one of top most fruit in the world in production and as well as in consumption. Bananas are climacteric fruits which are artificially ripened regularly. Ripening process of banana can be divided into three distinct phases namely the pre-climacteric or 'green life' stage, the climacteric and ripening stage and finally eat-ripe and senescence stage (Gowen, 1995). During ripening process banana undergoes different physiological, biochemical, and organoleptic changes that lead to a soft and edible ripe fruit (Gowen, 1995).

2.2. Biochemical changes during ripening of Banana

Fruit ripening is a genetically programmed, highly coordinated process of organ transformation from unripe to ripe stage, to yield an attractive edible fruit with an optimum blend of colour, taste, aroma and texture. A set of biochemical and physical changes occur in banana during ripening which makes it an edible fruit. These changes involves several

biochemical and physical pathways like degradation of starch to sugar, change in the peel and pulp colour, cell wall changes, change in the concentration of volatiles and acids (Gowen, 1995).

2.3. Tissue softening

Softening is a very important aspect of the ripening syndrome. Loss of turgor, degradation of starch and enzyme catalyzed changes to wall structure and composition are the mechanisms which lead fruit softening. According to Finney *et al* (1967) textural change in banana fruit during ripening is predominantly due to the changes in chemical structure of starch grains. Many researchers have shown that starch content in the pulp of banana decreases drastically during the short period of ripening and then starch is no longer detected Agravante*et al.*, (1990). However Kojima (1996) suggests that banana pulp softening process is due to the associated process whereby the contents of pectic and hemicellulosic polysaccharides and starch decrease during ripening. According to Ali (2004) 50% firmness loss was occurred in 3 days during ripening in Mas banana (*Musa acuminata*, AA group).

2.4. Carbohydrates

During ripening process starch is converted in to simple sugars through enzymatic browning process (Yang and Hoffman, 1984). Starch forms about 20 to 25% of the fresh weight of the pulp of unripe bananas. Sugars are present in the green fruit only about 1 to 2% in the fresh pulp which rises up to 15 to 20% at ripeness. The soluble sugars detected in ripened banana are mainly sucrose, glucose and fructose (Adao and Glória, 2005). The mean level of starch content in 'Prata' banana was reduced from 15.7 g/100 g to 3.40 g/100 g during ripening. As well total soluble sugar content was increased from 1.26g/100g to 14.3g/100g (Adao and Glória, 2005). Adewale*et al* (2013) reported that unripe banana (*Musa sapientum)* had highest amylase activity (3900 ± 310 Units/mg protein) and decreased rapidly to a very low value (100 ±15 Units/mg protein) when it will be fully ripened.

2.5 Pigments

The peel colour changes from green to yellow during ripening of banana fruit. The most important compounds responsible for the change in peel color are chlorophylls and carotenoids (Subagio*et al.,* 1996). Chlorophyll content decreases from green to yellow stage of banana fruit (Seymour *et al.,* 1987). The level of total carotenoids decreased to half the level at the colour break and subsequently again reached a level similar to that in green fruit. According Gross et al (1976) the major pattern of pulp carotenoids is α - carotene (31%), ß-carotene (28°/o) and lutein (33°/o) of total carotenoids. Banana peel contained 3-4 µg/g carotenoids content as lutein equivalent and ingredients were lutein, ß- carotene, α – carotene, violaxanthin, auroxanthin, neoxanthin, isolutein, ß-cryptoxanthin and α- cryptoxanthin.

2.6 Pectin

The inter-lamella layer in higher plant is composed of polysaccharides mainly pectin (Verma*et al.,* 2014). The nature of the pectic component in cell wall is associated with fruit softening. Pectin is a linear chain of α- (1→4)-linked D-galacturonic acid which are methyl esterified. The great strength of green fruit is due to the protopectin or water insoluble pectic which is partially esterified polygalacturonic acid (Joslyn, 1963). Increased solubility of pectic polysaccharides is one of the identified changes happening during fruit ripening. Pectic enzymes are related with the softening of fruits along with the increase in soluble pectins. The main enzymes involved in pectin degradation are Polygalacturonase (PG) and pectin methylesterase (PME). According to Tapre and Jain (2012) pectin content in banana (*Musa sp*. var 'Robusta') pulp increased from 0.37-0.66% significantly which was measured as calcium pectate. Smith *et al* (1989) reported the presence PG in banana tissue. Patil and Magar (1975) reported that PME activity is highest at colour stage 4 (greenish yellow) and fell down sharply in the advanced stages of ripening.

2.7 Organic acids

Vonloesecke (1950) reported the presence of malic, citric, oxalic, and tartaric acids in the banana fruit, with malic acid being the principal acid. Malic and Citric acids are responsible for tartness in the unripe banana while oxalic acid contributed to astringent taste of the fruit

(Seymour *et al.*, 1987). Further it has reported that malic acid concentration has been reported to vary between 0.8 and 7.5 meq/100g and it is increasing three to sevenfold during ripening. However, titratable acidity decreased gradually until the fruit reaches to full-ripe (stage-6) then increased at stage- 7 (Soltani*et al.*, 2010; Kulkarni *et al.*, 2011; Malakar*et al.*, 2015). According to Wyman and Palmer (1964) malic acid content was 1.36 meq/100g at the pre climacteric stage and it increased up to 5.37 at the climacteric stage and 6.2 meq/100g at the post climacteric stage while oxalic acid content was decreased from 2.33 to 1.37 meq/100g at pre climacteric stage to post climacteric stage. However total organic acid increased during ripening.

2.8. Astringency

Most fruits and vegetables are astringent in unripe stage and during ripening process astringency is reduced. Similarly banana which tastes astringent in unripe stage becomes palatable in the eating ripe stage. Astringency is claimed to be related to tannins which causes drying, puckering of the mouth epithelia giving astringency in oral sensation. Unripe fruit contained considerable level of tannin which reduces as ripening proceeds (Malakar*et al.*, 2015). However some earlier researches reported that tannin remained unchanged during ripening. According to Von loesecke (1950) there are many types of tannin and during ripening soluble tannin becomes insoluble. Barnell and Barnell (1945) explained that there are two types of tannin containing elements, latex vessels in both the pulp and peel and small scattered cells in the peel.

2.9. Volatile constituents

Very few researches have been published on aroma compounds and their changes during ripening. The unique aroma of bananas arises from set of volatile constituents including esters, alcohols, ketones, aldehydes and phenol esters (Seymour *et al.*, 1987). According to Pino and Febles (2013) The composition of banana (Musa spp., AAA group) fruit volatiles included 75 esters, 18 ketones, 14 phenols and derivatives, 7 aldehydes, 13 alcohols, 7 acids, and 12 miscellaneous compounds. As well isoamyl and isobutyl esters together with 2-pentanone are the major compounds found in banana volatile profile (Jordan *et al.*, 2001).

Mccarthy et al (1963) reported that characteristic banana flavour is due to the amyl esters of acetic, propionic and butyric acids. Jordan *et al* (2001) studied aromatic profile of fresh banana fruit paste and identified 26 volatile compounds including *E*-2-hexenal and hexanal as major aldehydes and 3-hydroxy-2-butanone, 3- methyl-1-butanol, 2-pentanol, isoamyl acetate, isoamylisobutyrate, and eugenol were also presented in high concentrations.

2.10. The role of enzymes in removal of toxic wastes

Phenols are poisonous and harmful to organisms even at low concentrations, and as a result detoxification of phenol has wide prospect. In addition to toxic effects, phenolic compounds also generate an oxygen demand in different sources of waters and pass on taste and smell to water with tiny concentrations of their chlorinated compounds. Ground waters are polluted by phenolics as a result of the constant release of these harmful compounds from petrochemical, coal conversion and phenol-generating industries. Owing to its harmful effects, the waste water containing phenolic compounds must be treated before discharging these compounds into the water streams (Roostaei and Tezel, 2004).

Methods to remove phenols from industrial wastewater are classified as conventional and advanced methods. Conventional methods include steam distillation, liquid–liquid extraction, adsorption, solid-phase extraction, wet air oxidation, catalytic wet air oxidation and biodegradation for the removal of phenols. Highly developed technologies are also there for treatment of phenol such as electrochemical oxidation, photo-oxidation, ozonation, Fenton reaction, membrane processes and microbial and enzymatic treatment (Mohammadi*et al*. 2015).Enzymes have several favourable characteristics. They have either narrowed or far-reaching specificity. Due to its specificity, enzymes show their functionality even in mixed compounds. Enzymes extensively alter structuraland toxicological properties of contaminants into mildinorganic harmless end products. Furthermore, enzymesmay present balance over traditional technologies andalso over microbial remediation. All these characteristics turn enzymes into eco-friendly catalysts, and enzymatic techniques are environmentally affable processes (Raoet al. 2010).

Peroxidases are the enzymes that catalysethevariety of organic and inorganic compounds at the expenseof peroxide, usually H2O2.Peroxidases tarnish phenoliccompounds to simple

radical species, which spontaneouslyproduce insoluble oligo- or polymeric derivatives.These insoluble, polymerized products may be isolatedusing uncomplicated techniques like sedimentation andfiltration techniques (Regalado *et al.* 2004). Fruit wastes such as peels contain abundant phenolics, such as flavonoids, tannins and hydroxycinnamic acid esters. Besides theirrole in defence against pathogens and in host–plant resistanceto insects, phenolics are implicated in free radicalscavenging, inhibition of lipid peroxidation and defence against UV radiation. Two polyphenol oxidase enzymes, laccase (*o*- and *p*-diphenoloxidase, EC 1.10.3.2) and catechol oxidase (*o*-diphenoloxidase, EC 1.10.3.1), and POD (EC 1.11.1.7) oxidize alarge number of aromatic structures at the expense of O2orH_2O_2, respectively (Pourcel*et al*. 2007). Oxidized phenolics protect sensitiveplant tissues from biotic and abiotic stress (Duffey and Stout 1996).

3. MATERIALS AND METHODS

3.1. Description of study area

The study was conducted at debremarkos city and Haramaya University main campus, whicharelocated at about 285km north of addisababa and 510 km East of Addis Ababarespectively, HaramayaUniversity located between Dire Dawa and Harar cities. The laboratory experiment was conducted in Biotechnology lab under the school of biological sciences and Biotechnology, Haramaya University.

3.2. Research Design

The banana (*Musa sp*.) fruit sample was collected from Home garden in Debremarkos city. The enzyme extraction involved homogenization of the sample,filteration in cold acetone and then the filterate was homogenized in 0.1M sodium acetate buffer, pH 7. The protein content was determined by the Lowry assay methodusing bovine serum albumin (BSA) as a standard protein. Then enzymatic activity was determined by peroxidase, polyphenol oxidase and catalase assays. The optimum activities of the enzyme were determined from line curvesconstracted for the activities of each enzyme at different ph and temperature conditions. The antioxidant activities of the enzyme extract were also determined by deteremingthe DPPH and hydrogen peroxide free radical scavenging activities. The relationships among enzyme assay parameters were determined by correlation coefficient and cluster methods.

3.3. Experimental Material

The banana (*Musasp*.) fruit samplewas collected from Home garden in Debremarkos city, Ethiopia. The fruit samples were manually washed with distilled water and residual moisture was evaporated at room temperature. Afterwards, the pulp and peel samples were freeze dried and milled to fine powders in a grinder for 2 min, the process was stopped for 15sec to avoid heating of sample using the standard methods of the Association of Analytical Chemists (AOAC, 2000).

3.4. Enzyme Extraction and assay

Extraction of the enzyme was performed according to the modified method described by El-hilali*et al.* (2003). As the fruit peels contain pigments and essential oils, which may interfere with the measurement of enzyme activity, these compounds, were eliminated with cold acetone. Three grams (3g) samples were crashed, homogenized in cold acetone,filteration in cold acetone. The powder was then dried under vacuum and stored at (–20°C) for enzyme activity assay. Extraction procedure consisted of taking 0.3 g of the resulting white powder, grinding for 10 min in 0.1M sodium acetate buffer, pH 7 until a homogenous paste was obtained. The mixture was centrifuged for 30 min at 15000xg at 4°C. The supernatant was collected and used for enzyme activity assay and determination of total soluble proteins.

3.4.1. Determination Total soluble protein

The total soluble protein was determined by the modified method of Lowry (Lowry *et al.,* 1951) using bovine serum albumin (BSA) as a standard protein. In this assay, three reaction solutions were prepared as a Lowry solution (SolA + SolB + SolC) with a ratio of 100:1; 1 on volume basis, For SolA about 2.86 g of NaOH and 14.31 g of Na2CO3 were dissolved in 500 mL of deionized water. SolB was prepared by dissolving 1.42 g of CuSO4·5(H2O) in 100 mL of deionized water. SolC was prepared by dissolving 2.86 gofNa_2Tartrate·2(H_2O) in 100 mL of deionized water. Folin Reagent was freshly prepared every assay by mixing 5 mL of 2N Folin and Ciocalteu' s Phenol Reagent with 6 mL of deionized water. The assay was initiated by mixing 0.5 mL sample with 0.7 mL of Lowry solution. Then the sample mixture was incubated for 20 min at room temperature in dark condition. After 20 min of incubation, the sample mixture was immediately added to 0.1 mL of Folin Reagent and mixed vigorously. Subsequently, the mixture was incubated for 30 min at room temperature in dark condition again. After incubation, the mixture was vortexed briefly, and the absorbance was measured shortly. The protein content was estimated by measuring the absorbance at 750 nm using Varian Cary® 50 UV-visible spectrometers. A protein standard curve was established by serially diluting 2 mg ml-1 BSA protein standard.

$$\text{Conc of protein in sample soln (mg/ml)} = \frac{\text{OD of test sample} - \text{OD of blank}}{\text{OD of std} - \text{OD of blank}} \text{ x conc of std BSA}$$

3.4.2. Peroxidase Assay

Peroxidase activity was measured following the method described by Grzywnowicz*et al.* (1992). For POD activity, the phenol solution was used as an electron donor for oxidation. To 0.1ml of supernatant enzyme extract, 0.09 ml of 30mM H_2O_2, 50 µl of 1.5 mM of phenol and 2.75 ml of 0.1M phosphate buffer, pH, and 7 were added. The reaction mixture was incubated for 5 min at 25^0C after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 460nm. Enzyme activity was evaluated as follows:

$$\text{PODA (U/ml)} = \frac{\text{A460}}{\text{Vt}}$$

Where, PODA: peroxidase activity;

A460 = absorbance at 460 nm;

V = volume of the sample and

t = time of the reaction.

Peroxidase specific activity is obtained by dividing units of POD by total soluble protein in the sample.

$$\text{POD specific activity (U/mg)} = \frac{\text{PODA}}{\text{extracellular protein concentration}}$$

3.4.3. Polyphenoloxidase Assay

Five-milliliter assay mixture for polyphenoloxidase activity consisted of the same assay mixture as that of peroxidase without H_20_2. The absorbanceof the purpurogallin formed was taken at 420 nm. Both peroxidase and polyphenoloxidase activities were expressed in absorbancy units. An enzymatic activity unit was defined with an increase in absorbance (at 420nm) of 0.001 per minut is taken one unit of enzyme activity

Enzyme activity was evaluated as follows:

$$\text{PPOA} = \frac{\text{A420}}{\text{Vt}}$$

Where; PPOA-polyphenol oxidase activity;

A420 – absorbance at 420 nm;

V – Volume of the sample and

t – Time of the reaction.

Polyphenol oxidase specific activity is obtained by dividing units of PPOA by total soluble protein in the sample.

$$\text{PPO specific activity (U/mg)} = \frac{\text{PPOA}}{\text{total soluble protein concentration}}$$

3.4.4. Catalase Assay

Catalase activity was assayed by the ability to decompose H_2O_2. For this purpose, four milliliters of assay mixture (50 mMTris buffer, pH 6.8, containing 5 mM H2O2) was added to 0.5 ml of the enzyme extract. After 10 min at 20^0C, the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The resulting solution was appropriately diluted and the residual H_2O_2 was determined by measuring the absorbance at 415 nm against a blank that contained all components except the extract. A control, in which the enzyme activity was stopped at "zero" time, was run at the same time. One unit of catalase activity is defined as the amount of enzyme that decomposed 0.01 mmol of H_2O_2 per minute under the assay conditions.

$$\text{CA} = \frac{\text{A415}}{\text{Vt}}$$

Where; CA: Catalsae activity;

A415 – absorbance at 415nm;

V – volume of the sample and t – time of the reaction.

$$\text{CASPa (U/mg)} = \frac{\text{PODA}}{\text{extracellular protein concentration}}$$

Where, CASPa: catalase specific activity.

3.5. Optimization of enzyme activity

3.5.1. Determination of pH optimum and pH stability

The purified enzyme was incubated for 5 min at different pH levels that was prepared using 0.1 M KH_2PO_4 buffer solution with different pH levels (pH3.0-9.0) and PH was adjusted with0.1N NaOH and 0.1 N HCl.Then, the peroxidase activity wasdeterminedby taking the absorbance at 460nm as in section 3.4.2.

3.5.2. Determination of temperature optimum

To determine the effect of temperature on the peroxidase activity, the purified enzyme was incubated for 5 min at different temperature ranging from 0 to 40 °C. All experiments were performed at optimum pH (predetermined) and during the experiments; temperature was controlled using a thermostatic water bath. Then peroxidase activity was determined at absorbance of 460nm as in section 3.4.2.

3.6. DPPH Radical Scavenging Activity

The radical scavenging activity (RSA) of the enzyme extractswasadopted to measure antioxidant activity using the DPPH method (Loizzo*et al.*, 2016)**.** Briefly, 2 mL of extract solution (1–100µg/mL) in methanol was added to 2mL of DPPH (0.1mM) solution. The mixtures was kept aside in a dark area for 30 min and absorbance was measured at λmax 517 nm against an equal amount of DPPH and methanol as a blank. The percentage of DPPH radical scavenging activity (RSA %) was estimated using the equation:

$$\text{DPPH radical scanveging activity}(\%) = \frac{(A_o - A_1)}{A_o} \text{x}100$$

Where A**0** is the absorbance of the control and A**1** is the absorbance in the presence of the sample.

3.7. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of individual enzyme extract was determined using the H2O2 method (Bozin*et al.*, 2008). Briefly, 2 mL of extract solution (10–100 _g/mL) in methanol was added to 4.0 mL of H_2O_2 (20 mM) solution in phosphate buffer (pH 7.4). After 10 min, the absorbance was measured at λ_{max} 230 nm against the phosphate buffer blank solution. The percentage scavenging of H_2O_2 was calculated using the equation:

% scavenging of H_2O_2 = [(A0 - A1)/A0] x100,

Where A0 = absorbance of the control (phosphate buffer with H_2O_2) and A1 = absorbance of the test extracts.

3.8. Data Analysis

All data were entered into Microsoft excel. Mean comparison and Analysis of variance (ANOVA) were carried out using SAS version 20 software package. Statistically significant differences were indicated by $p<0.05$ and $p<0.01$.

4. RESULTS AND DISCUSSION

4.1. Enzyme activity assay

The total soluble protein content and the activities of enzymes extracted from ripen banana peels and pulp is shown in Table 1. It was observed that significantly higher, total soluble protein (12.67mg/ml), peroxidase activity (PODA, 81.60U/ml), peroxidase specific activity (PODspa, 6.44U/mg), catalase activity (CATA, 86.50U/ml) and catalase specific activity (CATspa, 6.83U/mg), were recorded for pulp enzyme extract.in Contrast, significantly higher polyphenoloxidase activity (PPOA, 92.40U/ml) and polyphenoloxidase specific activity (PPOspa, 9.61U/mg) were observed in banana peel extract than in banana pulp extract.

Table 1.Activities of enzyme extract from banana (*Musa sp*.) fruit peel and pulp

Enzyme extract	Total protein (mg/ml)	PODA U/ml	PODspa U/mg	PPOA U/ml	PPOspa U/mg	CATA U/ml	CATspa U/mg
Peel	9.62±0.45b	39.40±0.57b	4.11±0.25b	92.40±0.57a	9.61±0.51a	43.20±1.70b	4.50±0.38b
Pulp	12.67.39a	81.60±1.70a	6.44±0.06a	50.90±1.27b	4.02±0.02b	86.50±3.54a	6.83±0.06a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. .PODA: peroxidase activity; PODspa: peroxidase specific activity; PPOA: polyphenol oxidase activity; PPOspa: polyphenol oxidase specific activity; CATA: catalase activity; CATspa: catalase specific activity.

4.2. Optimization of enzyme activity from banana fruit peel and pulp

4.2.1. The effect of pH

Assay of the peroxidase activity of ripen banana peel andpulp (Fig 1) indicated that banana fruit pulp peroxidase activity was higherthan fruit peel peroxidase activity. In both fruit pulp and peel, the peroxidase was most active at pH 6. ASimilarfinding was reported by Kalaiarasan and Palvannan(2014) who indicated that stabilized horse radish peroxidase displayedhighest activity at pH 4.2 indicating that the activity of peroxidase is highat acidic conditions. The differences in optimum pH forperoxidase activity depended on the various

parameters suchas plant sources, extraction etmethods, purity of the enzyme, buffers and substrates as reported by Seles-Marchart*et al*. (2006).

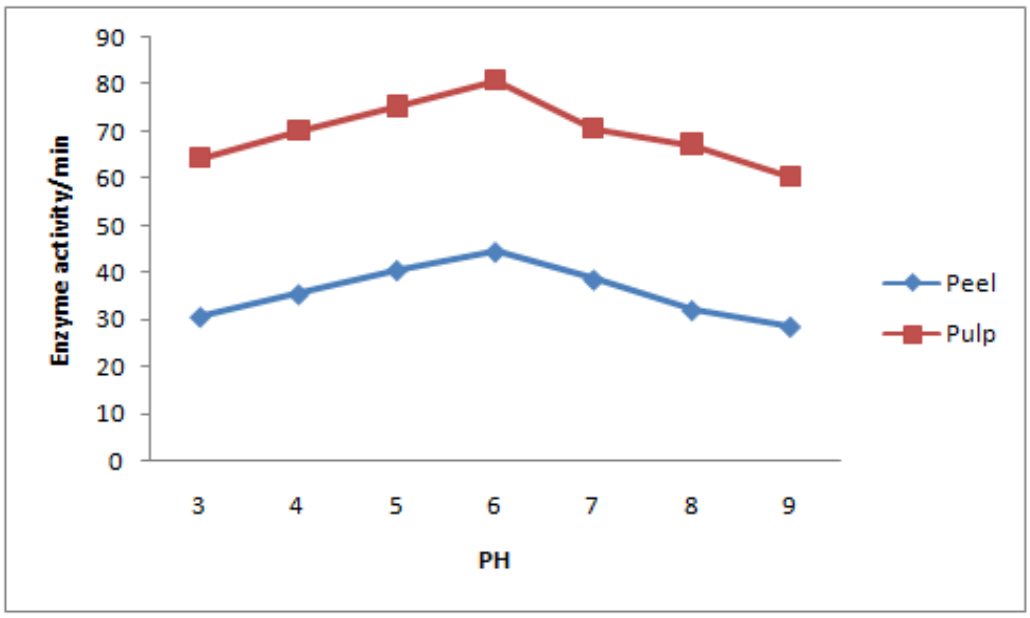


Figure 1.effect of pH onperoxidase activity of banana peel and pulp peroxidase activity

4.2.2. The effect of temperature

Assay of the peroxidase activity of ripen banana fruit peel and pulp (Fig 2) indicated that banana fruit pulp peroxidase activity was higher than its fruit peel. It was found that peroxidase activity of both fruit peel and pulp has presented a wide temperature range of activity (0 to 30^0C). This finding was in agreement with Gomez *et al* (2006) who suggested thatperoxidases have an extensive temperature range within which their activity is maintained.At − 20 °C the activity reached its peak. When the temperature increases, the activity began to decline, and inactivation was observed above room temperature. These results confirm that Zea Mays peroxidases are not heat resistant, indicating that heat treatments are not required for Zea Mays peroxidase (Lakshmi *et al*., 2018).

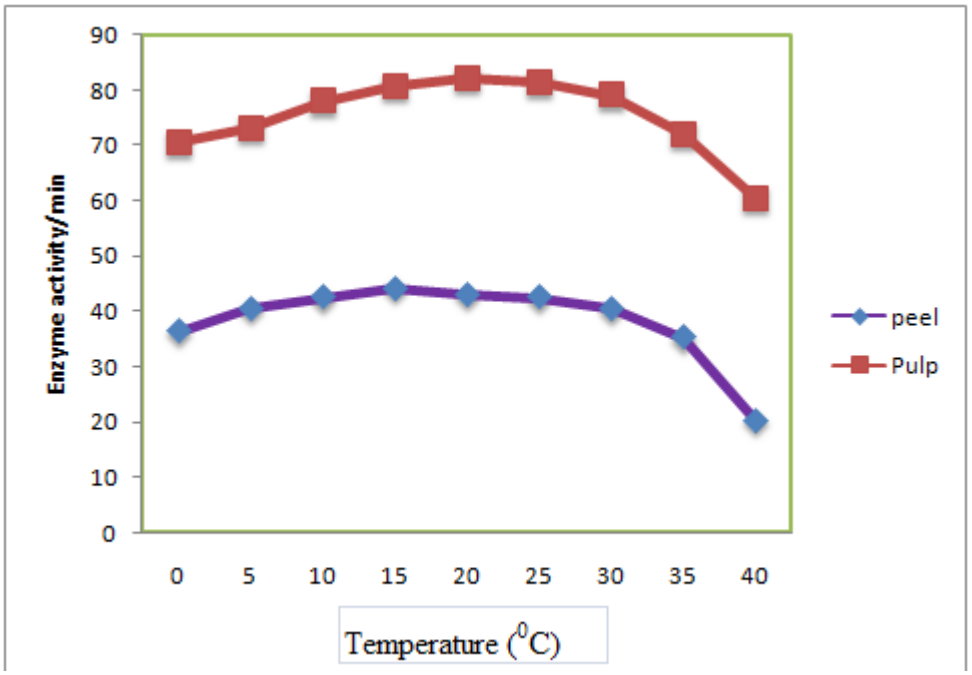


Figure 2.effect of temperatureonbanana peel and pulp peroxidase activity

4.3. Antioxidant activities of the enzymeextractes of banana peel and pulp

DPPH is often used to test how far compounds can act as free radical scavengers or hydrogen donors, and to quantify antioxidants in complex systems (Antolovich*et al*., 2002).DPPH is a very stable organic free radicalwith a deep violet color which givesabsorption maxima within the 515–528 nmrange. Accordingly, in the present study, the antioxidant activity of the enzyme extracts (Table 2) indicated that significantly higher DPPH radical scavenging activity (83.12±0.12) was observed for peel extract. However, significantly higher hydrogen peroxide scavenging activity (HPSA) was recorded for banana fruit pulp extract. It was observed that both DPPH and HPSA had antagonistic effects. Further, study need to be conducted to further elaborate the relationship between them.

Antioxidant refers to any substances in attendance at stumpy concentration in foodstuffs and capable of significantly averting oxidation by playing a responsibility in antioxidation as a free radical scavenger, chelator, reducing agent, and/or singlet oxygen scavenger. Herbs and spices are one of the important sources for search of natural antioxidants from safety point of

view (Nadeem et al., 2013). Antioxidants which inhibit oxidation of food also quench dreaded free radicals produced due toenvironmental and physiological stresses which lead to aging, atherosclerosis and cancer.Antioxidation and oxidation processes in plants are complex and therefore it is difficult tomeasure each antioxidant component separately (Jeya*et al.*, 2019).

Table 2. Antioxidant activities of enzyme extracts from banana (*Musa sp*.) fruit peel and pulp

Enzyme extract	DPPH	HPSA
Peel	83.12±0.12a	28.68±0.11b
Pulp	75.22±0.32b	62.10±0.07a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test.DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity.

4.4. Relationship among biological activity parameters of the enzyme extracts

The Pearson correlation coefficient of enzyme activity parameters and free radical scavenging activities as in Table 3 and Fig 3. It was found that protein content was significant and positively correlated with CATA, CATspa, PODA, PODspa, and HPsa. Catalase activity was significant and positively correlated with protein content, CATspa, PODA, PODspa and HPSA. Polyphenoloxidase activity (PPOA) was significant and positively correlated with PPOspa and DPPH.

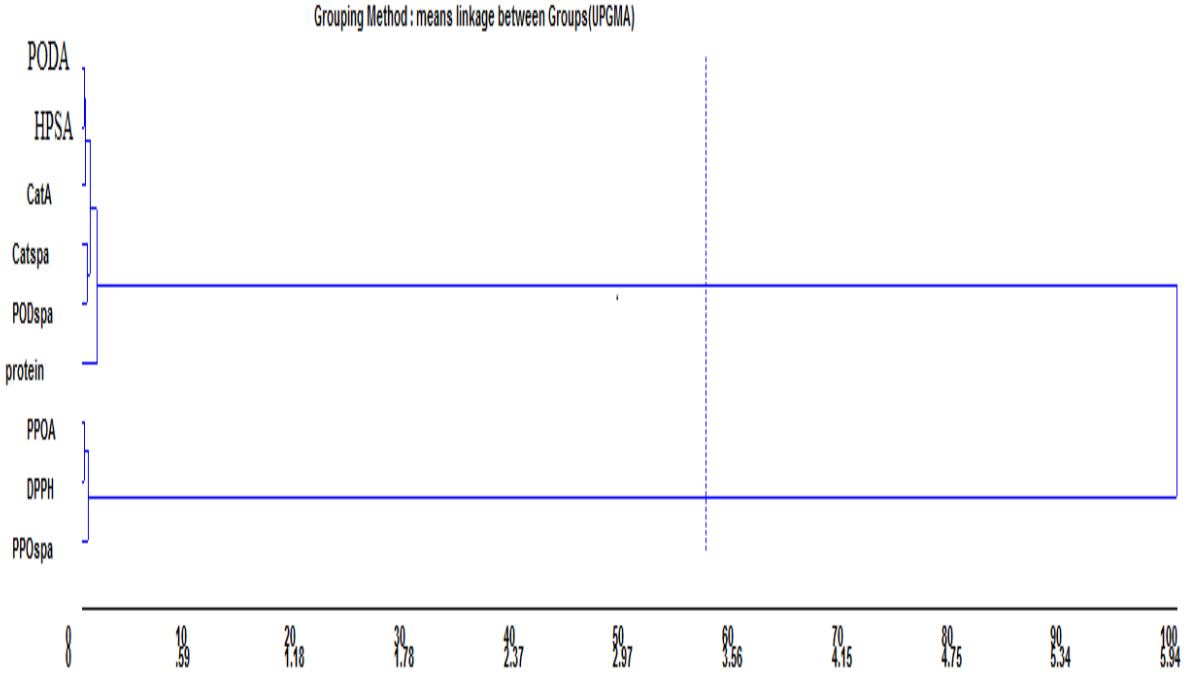
The DPPH free radical scavenging activity was found to be significant and positively correlated with PPOA and PPOspa. By contrast, hydrogen peroxide scavenging activity (HPSA) was significant and positively correlated with CATA, CATspa, PODA, PODspaand protein content. Both DPPH and HPSA were significant and negatively correlated suggesting that they have opposite effects.

Table 3. Pearson correlation matrix for association among enzyme activity parameters and free radical scavenging activities

Parameters	Protein	CATA	CATspa	PODA	PODspa	PPOA	PPOspa	DPPH	HPSA
Protein	1.00	0.982*	0.948*	0.984*	0.957*	-0.979*	-0.991**	-0.973**	0.982*
CATA		1.00	0.991**	0.999**	0.992**	-0.992**	-0.989**	-0.992**	0.996**
CATspa			1.00	0.989**	0.997**	-0.982*	-0.968*	-0.987*	0.986*
PODA				1.00	0.993**	-0.997**	-0.994**	-0.997**	0.999**
PODspa					1.00	-0.993*	-0.980*	-0.996**	0.994**
PPOA						1.00	0.996**	0.999**	-0.999**
PPOspa							1.00	0.994**	-0.996**
DPPH								1.00	-0.999**
HPSA									1.00

PODA: peroxidase activity; PODspa: peroxidase specific activity; PPOA: polyphenol oxidase activity; PPOspa: polyphenol oxidase specific activity; CATA: catalase activity; CATspa: catalase specific activity; DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity.

The dendrogram for clustering of enzyme activity parameters and free radical scavenging activities (Fig 3) grouped the parameters into two clusters. The first cluster includes PODA, HPSA, CATA, CATspa, PODspa, and protein content while PPOA, DPPH &PPOspa were grouped in the second cluster.those parameters grouped in the same cluster are positively correlated. And the determination of one parameter used to predict other positively correlated parameters.



disadvantages of , and these features limit industrial applications of it for this resean furthere investigation is the need

fo the day

. Figure 3.Dndrogram for Enzyme activity parameters and free radical scavenging activity.

PODA,PODspa,PPOA, PPOspa,CATA,CATspa,DPPH,HPSA and Total soluble protien

Peroxidases are widly distributed in plants, more than 150 plants have examined to determine Peroxidase activity. Althuogh,they have been isolated and purified from larg number of sources,the main source of comercialy available Peroxidase is horse radish roots (yntalet al., 2015).Horse radish Peroxidase has been studied for severaldecades and its highly investigated member ofperoxidase family.However,low thermostablity, low reactivity andhigh cost purification from the sourse(Lai and Lin,2005) are the main disadvantages and these features limit industril applications of it.for this resean further investigation for different source Peroxidase is the need of the day.

tion is the need of the day

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

The idea of utilizing fruit by-products mainly the peels which in some fruits represent almost 30% of the total weight, have slowly gaining popularity especially when researchers found that peels possessed better biological activities than other parts of the fruit. With the increasing interest for natural sources of bioactive compounds and the popularity of the concept of functional foods, food products enriched with fruit peels are been developed. However the potential application of fruit peels in food supplementation depends strongly on their chemical composition.The aim of the present study was to characterize enzyme extracts from ripen banana fruit peel and pulp with respect to enzymatic and antioxidant activities.

The study was conducted to investigate characteristics of enzyme extracts from banana fruit peel and pulp. The banana (*Musa sp*.) fruit sample was collected from Home garden in Debremarkos city. The enzyme extraction involved homogenization of the sample and filterate in cold acetone and then the filteratewas homogenized in 0.1M sodium acetate buffer, pH 7. The protein content was determined using Lowry assayusing bovine serum albumin (BSA) as a standard protein. Then enzymatic activity was determined by peroxidase, polyphenol oxidase and catalase assays. The optimum activity of the enzyme was done using PH and temperature. The antioxidant activities of the enzyme extract was conducted by using DPPH and hydrogen peroxide free radical scavenging activities. The relationships among enzyme assay parameters were determined by correlation coefficient and cluster methods.

The result of enzymatic assay of ripen banana peel and pulp recorded significantly higher, total soluble protein, peroxidase activity (PODA), peroxidase specific activity (PODspa), catalase activity (CATA) and catalase specific activity (CATspa), were recorded for pulp

enzyme extract. Contrastingly, significantly higher polyphenol oxidase activity (PPOA) and polyphenol specific activity (PPOspa) were observed for the banana peel extract.

The effects of PH on peroxidase activity of ripen banana peel and pulp indicated that banana fruit pulp peroxidase activity was higher than fruit peel peroxidase activity. In both fruit pulp and peel peroxidase was most active at PH 6.

The effect of temperature on peroxidase activity of ripens banana fruit peel and pulp indicated that banana fruit pulp peroxidase activity was higher than its fruit peel. It was found that peroxidase activity of both fruit peel and pulp has presented a wide temperature range of activity (0 to 30^0C).

The antioxidant activity of the enzyme extracts indicated that significantly higher DPPH radical scavenging activity (83.12±0.12) was observed for peel extract. However, significantly higher hydrogen peroxide scavenging activity (HPSA) for banana fruit pulp extracts.

The Pearson correlation coefficient of enzyme activity parameters and free radical scavenging activities demonstrated that the DPPH free radical scavenging activity was found to be significant and positively correlated with PPOA and PPOspa. By contrast, hydrogen peroxide scavenging activity (HPSA) was significant and positively correlated with CATA, CATspa, PODA, PODspaand protein content. Both DPPH and HPSA were significant and negatively correlated suggesting that they have opposite effects.

The dendrogram for clustering of enzyme activity parameters and free radical scavenging activities grouped the parameters into two clusters. The first cluster includes PODA, HPSA, CATA, CATspa, PODspa, and protein content while PPOA, DPPH &PPOspa were grouped in the second cluster.

5.2. Conclusion

The present study has demonstrated the antioxidant activity of enzymatic extracts of ripen banana peels and pulp. The correlation coefficient among enzyme and antioxidant activities can have application in food, beverage, cosmetic industries and removal of toxic phenolic

compounds from industrial wastes. Enzymatic method is highly efficient and low cost compared to physical, chemical and microbial methods.Suchcost-effective alternative for phenol treatment in effluent water, especially in industries located close to agricultural lands, because the crude phenol-degrading enzyme (most likely, peroxidase and polyphenol oxidase, can be isolated from fruit wastes and therefore hasimmense potential in the complete degradation of phenol.

5.3. Recommendation

The present study characterized Peroxidase, Polyphenol oxidase and Catalase activities and test their correlation with DPPH and free radical scavenging activities. Since enzyme activity can be affected by the method of extraction, climatic conditions, the type of buffer and substrate used. Therefore further studies need to be conducted:

- To assess enzymes with low cost of purification, high thermo stability and high reactivity in organic media.
- To determine optimization of enzyme activities considering various parameters like substrate concentration and others.
- The biological activities need to be conducted using anti oxidant activity;
- Toxicological property, bio pesticides and other biological control mechanisms of the enzyme need to be conducted
- Purification of specific enzymes using gel electrophoreses and chromatographic method needed to be conducted

6. REFERENCES

Abdel-Hameed, E.S.S. 2009. Total phenolic contentsand free radical scavenging activity of certain Egyptian *Ficus*species leaf samples. *Food Chemistry*, 144(4); 1271-1277.

Adao R. C., Glória M. B. 2005.Bioactive amines and carbohydrate changes during ripening ofPrata'banana (*Musa acuminata*× *M. balbisiana*).*Food Chemistry*. 90(4):705-11.

Adewale I. O, Adefila A., Adewale T. B. 2013.Changes in Amylase Activity, Soluble Sugars and Proteins of Unripe Banana and Plantain during Ripening.*Annual Review & Research in Biology*.34:678-685.

Agravante J. U., Matsui T., Kitagawa H. 1990. Starch breakdown in ethylene-and ethanol-treated bananas: Changes in phosphorylase and invertase activities during ripening. *Nippon Shokuhin Kogyo Gakkaishi*.37 (11):911-5.

Ali Z. M., Chin L. H, Lazan H. 2004.A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits.*Plant Science.*167(2):317-27.

Altunkaya, A., Hedegaard, R. V., Brimer, L., Gökmen and Skibsted L. H. (2013) Antioxidant capacity versus chemical safety of wheat bread enriched with pomegranate peel powder, *Food Function*, 4, 722-727.

Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., Robards, K., 2002: Methods for testing antioxidant activity. Analyst 127, 183-198.

Anupama S, Pradeep NV, and Hampannavar US (2013. Anaerobic followedby aerobic treatment approaches for Spentwash using MFC andRBC.*Sugar Tech* 15(2):192–202.

AOAC, (2000).Official Methods of Analysis of the AOAC International (2000).7th Edition, Volume II. Gaithersburg, Maryland, USA.

Ashoush, I. S. and Gadallah, M. G. E. (2011) Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit, *World Journal of Dairy and Food Sciences*, 6 (1), 35-42.

Azmi N. A. 2010.Extraction of Antioxidant Activity, Phenolic Content and Minerals in Banana Peel [Thesis]. Malaysia: Faculty of Chemical and Natural Resources Engineering Universiti Malaysia Pahang; p. 9-12.

Babiker, W. A. M., Sulieman, A. M. E., Elhardallou, S. B. and Khalifa, E. A. (2013) *Physicochemical properties of wheat bread supplemented with orange peel by-products, International Journal of Nutrition and Food Sciences*, 2(1), 1-4.

Barnell H. R., Barnell E. 1945. Studies in tropical fruits: XVI. The distribution of tannins within the banana and the changes in their condition and amount during ripening. *Annals of Botany*. 9(33):77-99.

Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Goran, A.; Igic, R. 2008.Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae).*Food Chem*. 111: 925–929.

Busca G, Berardinelli S, Resini C, Arrighi L (2008) Technologies forthe removal of phenol from fluid streams: a short review of recentdevelopments. *J Hazard Mater* 160:265–288.

*Coriandrumsativum*L. Seed essential oil. American J. E. O. N. P., 7(2): 06-10.

Duffey SS, Stout MJ (1996) Antinutritive and toxic components ofplant defense against insects. *Arch Insect Biochem*. 32:3–37.

El-hilali F., Ait-Oubahou A., Remah A., Akhayat O. 2003.Chilling injury and peroxidase activity Changes in “fortune” mandarin fruit during low temperature storage.*Bulg. J. Plant Physio*l., 29(1–2), 44–54.

Fatemeh, S.R.; Saifullah, R.; Abbas, F.M.A.; Azhar, M. E. 2012.Total phenolics, flavonoids and antioxidant activity of banana pulp and peelflours: influence of variety and stage of ripeness. International Food Research Journal,Serdang, v.19, n.3, p.1041-1046.

Figuerola, F., Hurtado, M.L., Estevex, A.M., Chiffelle, I. and Asenjo, F. (2005): Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food chemistry*.9: 395-401.

Finney E. E., Ben-Gera I. T, Massie D. R. 1967. An objective evaluation of changes in firmness of ripening bananas was using a sonic technique. *Journal of food science.* 32(6):642-6.

Gomez JL, Bódalo A, Gómez E, Bastida J, Hidalgo AM, GómezM (2006) Immobilization of peroxidases on glass beads: animproved alternative for phenol removal. *Enzyme MicrobTechnol*39(5):1016–1022.

Gowen S. 1995.Bananas and plantains.Springer Netherland.

Gross J., Carmon M., Lifshitz A., Costes C. 1976. Carotenoids of banana pulp peel and leaves. *Food Science and Technology*.9:211-4.

Grzywnowicz, K., Brzyska, M., Lobarzewski, J., Greppin, H., (1992).Changes in activityof soluble and immobilized cabbage peroxidase after treatment with seven different proteases.*J.Mol. Catal*,.77 ; 365–376.

Hailu M., Workneh T. S., Belew D. 2013. Review on postharvest technology of banana fruit. *African Journal of Biotechnology*, 12(7).

Hassan, H. F., Hassan, U. F., Usher, O. A., Ibrahim, A. B. and Tabe, N. N. 2018. Exploring the Potentials of Banana (*Musa Sapietum*) Peels in Feed Formulation.International Journal of Advanced Research in Chemical Science (IJARCS.) 5(5): 10-14.

Hirimburagama K. andGamage N. 2015.In vitro multiplication of local cultivars of banana (*Musa* spp.) through shoot-tip culture.*Journal of the National Science Foundation of Sri Lanka*,, 24(1).

Jeya KR, Veerapagu M and Sangeetha V. 2019. Antimicrobial and antioxidant properties of

Jordán M. J., Tandon K., Shaw P. E., Goodner K. L. 2001. Aromatic Profile of Aqueous Banana Essence and Banana Fruit by Gas Chromatography- Mass Spectrometry (GC-MS) and Gas Chromatography− Olfactometry (GC-O).*Journal of agricultural and food chemistry*. 2001; 49(10):4813-7.

Joslyn M. A. 1963. The chemistry of protopectin: a critical review of historical data and recent developments. *Advances in food research.* 11:1-07.

Kalaiarasan E, Palvannan T (2014) Removal of phenols from acidicenvironment by horseradish peroxidase (HRP): aqueous thermostabilizationof HRP by polysaccharide additives. *J Taiwan InstChemEng* 45(2):625–634.

Kojima K. 1996.Softening of banana fruit- Relationship between firmness and chemical composition.*Japan Agricultural Research Quarterl* 30:269-274.

Kulkarni S. G., Kudachikar V. B., Prakash M. K. 2011.Studies on physico-chemical changes during artificial ripening of banana (*Musa sp*) variety ‘Robusta’.*Journal of Food Science Technology*, 48(6):730-734.

Lai Y. C., .Lin S. C. 2005. Application of immobilized horseradishperoxidase for the removal of p-chlorophenolfrom aqueous solution.*Process Biochemistry*, 40: 1167- 1174.

Lakshmi S.,Shashidhara G. M.,· Madhu G. M., Muthappa R.,Vivek H. K., Prasad M. N.N. 2018. Characterization of peroxidase enzyme and detoxification of phenols using peroxidase enzyme obtained from *Zea mays* L.waste. *Applied Water Science*, 8:207.

Loizzo, M.R., Bonesi, M., Menichini, F., Tenuta, M.C., Leporini, M., Tundis, R., 2016: Antioxidant and carbohydrate-hydrolysing enzymes potential of *Sechiumedule*(Jacq.) Swartz (Cucurbitaceae) peel, leaves and pulp fresh and processed. *Plant Food Hum. Nutr.* 71: 381-387.

Lowry, O. H., Rosebrough N. J., Farr A. L.andRondal R. L. (1951) Proteinmeasurement with the folinphenolreagent, *J. Biol Chem*., 193, 265–73.

Malakar S, Roy S, Kumar N. 2015.Evaluation of Changes in Physico-Chemical Parameters of Banana (*Musa Spp.)* during Ripening in Storage.*Journal of Agricultural Engineering and Food Technology*. 2:100-103.

Mandal S, Yadav S., Nema R. K. 2009. Antioxidants: A review. *J Chem Pharm Res* 1(1):102-4.

McCarthy A. I., Palmer J. K., Shaw C. P., Anderson E. E. 1963.Correlation of Gas Chromatographic Data with Flavor Profiles of Fresh Banana Fruit.*Journal of Food Science*. 28(4):379-384.

Mohammadi S, Kargari A, Sanaeepur H, Abbassian K, Najafi A, Mofarrah E (2015) Phenol removal from industrial wastewaters: a short review. *Desalin Water Treat* 53:2215–2234.

Mohapathra D., Mishra, S., Suter, N. 2010. Banana and its byproducts: an overview. *J. of Scientific and Industrial Research* 69: 323-329.

Mohapatra D., Mishra S., Sutar N. 2010. Banana and its by-product utilisation: an overview. *J. of Scientific and Industrial Research*.69:323-329.

Nadeem M., Faqir Muhammad Anjum F. M., Khan M. I., and Tehseen S., El-Ghorab A., Sultan J. I. 2013.Nutritional and medicinal aspects of coriander (*Coriandrumsativum* L.)A review. *British Food Journal*, 115(5): 743-755.

Oliveira, D. A. S. B.; Müller, P. S.; Franco-Kotovicz V.; Waszczynskyj, N. 2015. Avaliação da qualidade de pão com adição de farinha e purê da banana verde. *RevistaBrasileira de Fruticultura, Jaboticabal,* v.37, n.3, p.699-707.

Patil D. L., Magar N. G. 1975. Correlation between bananas fruits ripening, its texture and pectin- methyl esterase activity. In: Proceedings of the Indian Academy of Sciences-Section B.; 81(3):127-130.

Pino J. A., Febles Y. 2013.Odour-active compounds in banana fruit cv. Giant Cavendish. *Food chemistry*.141(2):795-801.

Rao MA, Scelza R, Scotti R, Gianfreda L (2010) Role of enzymes inthe remediation of polluted environments.*J Soil Sci Plant Nutr* 10(3):333–353.

Rebello, L.P.G.; Ramos, A.M.; Pertuzatti,P.B.; Barcia, M.T.; Castillo-Munõz, N.;Hermosín-Gutiérrez, I. 2014.Flour of banana(*Musa* AAA) peel as a source of antioxidant phenoliccompounds.*Food Research International*, New York, v.55, p.397-403.

Regalado C, Garcia-Almendarez B, Duarte-Vasquez M (2004) Biotechnologicalapplications of peroxidases.*Phytochemistry* 3:243–256.

Rodriguez-Ambriz, S. L., Islas-Hernandez, J. J., Agama-Acevedo, E.;Tovar, J.; Bello-Pérez, L.A. 2008.Characterizationof a fibre-rich powder prepared by liquefaction ofunripe banana flour.*Food Chemistry,*.107, (.4), .1515-1521.

Rolfes, S.R., Pinna, K. and Whitney, E. (2009): "Understanding normal and clinicalnutrition".*J.Sci. Food*.36: 118- 122.

Roostaei N, Tezel FH (2004) Removal of phenol from aqueous solutionsby adsorption. *J Environ Manag* 70:157–164.

Salunkhe D. K., Kadam S. S. 1998.*Handbook of Vegetable Science and Technology: Production, Composition, Storage, and Processing*. CRC press, p.19.

Selles-Marchart S, Casado-Vela J, Bru-martinez R (2006) Isolation ofa latent polyphenol oxidase from loquat fruit (*Eriobotryajaponica*Lindl.)Kinetic characterization and comparison with the activeform.*ArchBiochemBiophys* 446:175–185.

Seymour GB, Thompson AK, John P. 1987. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. *Annals of applied biology.*110 (1):145-51.

Shalini, R. and Gupta, D. K. (2010), *Utilization of pomace from apple processing industries: a review, Journal of Food Science and Technology*, 47 (4), 365-371.

Shian, T.E.; Abdulliah, A.; Musa, K.H.;Maskat, M.Y.; Ghani, M.A. 2012. Antioxidant properties of three banana cultivars (*Musa acuminata*'Berangan', 'Mas' and 'Raja') Extracts. *SainsMalaysiana, Bangi,* .41, (.3), .319-324.

Smith N. J., Seymour G. B., Jeger M. J., Tucker G. A. 1989. Cell wall changes in bananas and plantains. In Symposium on Tropical Fruit in International Trade 269, 283-290.

Soltani M, Alimardani R, Omid M. 2010.Comparison of some chromatic, mechanical and chemical properties of banana fruit at different stages of ripeness.*Modern Applied Science.* 4(7):34.

Subagio A, Morita N, Sawada S. 1996. Carotenoids and their fatty-acid esters in banana peel. *Journal of nutritional science and vitaminology*. 42(6):553-66.

Tapre A. R., Jain R. K. 2012. Study of advanced maturity stages of banana. *International Journal of Advanced Engineering Research and Studies.*1 (3):272-274.

Varsha YM, Naga Deepthi CH, Chenna S (2011) An emphasis on xenobioticdegradation in environmental clean up. *J Bioremediation Biodegrad S.* 11:1

Veitch N. C., 2004. Horseradish peroxidase: amodernviewof a classic enzyme, *Phytochemistry,*65, 249-259.

Vergara-Valencia, N.; Granados-Pérez , E.; Agama-Acevedo, E.; Tovar, J.; Ruales,J.; Bello-Pérez, L.A. 2007.Fibre concentrate frommango fruit: Characterization, associated antioxidantcapacity and application as a bakery productingredient. LWT - *Food Science and Technology*,..40, .4), (.722-729.

Verma C., Singh R. K., Mishra S. 2014. Biochemical Characterization of Pectin Methylesterase from Musa acuminataReferring to Delayed Ripening.*IOSR Journal of Pharmacy and Biological Sciences*.9:42-47.

Von Loesecke H. W. 1950. Bananas: chemistry, physiology, technology. Interscience Publishers, Inc.

Wanasundara P. K., Shahidi F. 2005 Antioxidants: Science, Technology, and Applications [e-Book]. **6**thed. New York: John Wiley & Sons, Inc. p. 431-74.

Wyman H., Palmer J. K. 1964.Organic acids in the ripening banana fruit.*Plant physiology.* 39(4):630

Yang S. F., Hoffman N. E. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual review of plant physiology*. 35(1):155-89.

Ynal S., Kalyn R., Ozdemir N., Ozdemir H. 2015. Purification and characterization of red beet (*beta vulgaris*) peroxidase. *BCAMBL*, 1(1): 037-049.

Yunovan N. 2014. Isolasi Dan IdentifikasiSenyawaPenghambatEnzim α-glukosidasePisang Kepok (Musa Paradisiaca ABB) [Skripsi]. Jakarta: FakultasFarmasiUniversitas Pancasila.

7. APPENDICES

Appendix Table 1. Data for total soluble proteins and enzyme activities

Catalase activity				
Enzyme extract	rep	CA	Total protein	Catspa
Peel	1	44.4	9.30	4.77
Peel	2	42	9.94	4.23
Pulp	1	89	12.94	6.88
Pulp	2	84	12.39	6.78
Peroxidase activity				
Enzyme extract	rep	PODA	Total protein	Catspa
Peel	1	39.8	9.30	4.28
Peel	2	39	9.94	3.92
Pulp	1	82.8	12.94	6.40
Pulp	2	80.4	12.39	6.49
Polyphenoloxidase activity				
Enzyme extract	rep	PPOA	Total protein	Catspa
Peel	1	92.8	9.30	9.97
Peel	2	92	9.94	9.26
Pulp	1	51.8	12.94	4.00
Pulp	2	50	12.39	4.03

Appendix Table 2. Data for antioxidant activities

DPPH							
Enzyme extract	rep	Ac	As	Ac-As	Ac-As/Ac	%	DPPH
Peel	1	0.79	0.134	0.656	0.83	100	83.04
Peel	2	0.78	0.131	0.649	0.84	100	83.21
Pulp	1	0.729	0.179	0.55	0.76	100	75.45
Pulp	2	0.72	0.18	0.54	0.75	100	75.00
HPSA							
Enzyme extract	Rep	Ac	As	Ac-As	Ac-As/Ac	%	HPSA
Peel	1	0.381	0.272	0.109	0.28	100	28.61
Peel	2	0.379	0.27	0.109	0.28	100	28.76
Pulp	1	0.224	0.085	0.139	0.63	100	62.05
Pulp	2	0.214	0.081	0.133	0.62	100	62.15

Appendix Figure 1.extraction of Crude enzymes in the laboratory

Appendix Figure 2. Determination of enzyme activity

Appendix Figure 3. Optimization of enzyme activity