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Phenolic Levels and Polyphenol Oxidase Activities of Eggplants (Solanum spp.) During Ripening

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ABSTRACT: Phenolic levels and polyphenol oxidase (PPO) activities were determined in various eggplants: *Solanum melongena* L. (round and oval varieties), *Solanum aethiopicum* L. and *Solanum gilo* L. (big and small varieties) during ripening. There were variations in the phenolic levels and polyphenol oxidase (PPO) activities in the eggplants during ripening; the pattern of variations was based on the type of variety. The phenolic levels determined by the Folin-Ciocaultau method increased in all the eggplants in their overripe stage expect in *S.gilo* (big variety) which showed a decrease. The activities of polyphenol oxidase (PPO) in the eggplants were determined using catechol and pyrogallol as substrates. PPO activities with catechol as substrate were higher than that of pyrogallol. Catechol was observed to be the preferred substrate for the enzyme in all the eggplants. PPO activities, using catechol as substrate increased in *S.melongena* (oval variety) that showed an increase; while the PPO activities using pyrogallol as substrate increased in overripe stage of all the eggplants except in *S.melongena* (round variety). Phenolic compounds are major antioxidants in human diet, therefore the increase observed in their levels in the overripe stage of the eggplants showed that nutritional benefit can be derived from their consumption. Futhermore the inverse relationship between phenolic levels and PPO activities in these eggplants except in *S.melongena* (oval variety) suggests that enzymatic browning decreases with ripening in these fruits.

Keywords: Phenolic level, Polyphenol oxidase, Eggplants, Catechol, Pyrogallol.

Introduction

Eggplants (Solanum spp.) are used as food sources. The leaves or fruits of some species are consumed due to their nutritive values. Edible fruits include Solanum melongena, Solanum aethiopicum, Solanum gilo and Solanum incanum. Eggplants are commonly eaten in tropical Africa. The unripe fruits of eggplants are mostly eaten raw or used to prepare sauce or stew (Grubben, 2004; Perez-Amador et al., 2007; Sodipo et al., 2011). Eggplants have considerable economic importance, since they serve as a source of income for farmers. They also have medicinal values in indigenous medicine (Stommel and Whitaker, 2003; Gajewski et al., 2009). Phenolic compounds are secondary metabolites of plant origin; they are aromatic compounds possessing one or more hydroxyl groups (Kamble et al., 2011). Phenolic compounds are major antioxidants in our diet (Scalbert et al., 2005). Antioxidants have been identified as free radicals or reactive oxygen species (ROS) scavengers (Rajurkar and Gaikwad, 2012). ROS are continuously produced in cells. Overproduction of ROS causes damage to valuable biomolecules resulting in an increased risk of cardiovascular diseases, cancer and other chronic diseases (Boskou, 2006). Phenolic compounds as antioxidants protect the body against these degenerative diseases. They also have anti-inflammatory, anti-microbial and anti-allergic activities (Borneo et al., 2008). In plants, phenolic compounds are involved in defense mechanisms such as enzymatic browning (Hayat et al., 2007; Manach et al., 2004). They are synthesized from phenylalanine which is produced via the shikimate pathway and have been found to contribute to color changes and fruit quality due to their involvement in astringency, bitterness, and flavor during ripening (Håkkinen, 2000). Phenolic compounds are classified based on their chemical structures (the type and number of phenolic ring) (Barket et al., 2012). Studies on phenolic contents of some fruits showed that apple, papaya and guava contained 21.53, 28.91, 115.35 mg gallic acid equivalent (GAE)/100 g fresh weight respectively. Polyphenol oxidase (PPO) is the name for the copper containing enzyme that catalyses the oxidation of phenolic compounds to form corresponding quinone intermediates such as o-dopa quinones, which polymerize to form melanin pigment on the surfaces of cut fruits (Yoruk and Marshall 2003; Arnok et al., 2010; Sambasiva et al., 2013). It is also known as catechol oxidase or odiphenol:oxidoredtuctase activity of this enzyme is present in some fruits (Yoruk and Marshall, 2003). PPO is mainly located in the thylakoid membrane in the chloroplast and its phenolic substrates are located in vacuoles and upon cell damage, the enzyme and substrate come in contact, leading to oxidation of the substrate (Chazarra et al., 2001). PPO together with its phenolic substrates are involved in defense mechanisms by forming brown pigments that could resist infection on injured surfaces of fruits. They are also involved in plants' resistance to stressful conditions like wounds and infections (Lundell et al., 2008). PPO activity has been observed to change during fruit ripening in olive fruit (Francisca et al., 2007). PPO has been characterized in strawberry (Marco et al., 2007), pawpaw (Caodi, 2007), tomatoes (Shahryar, 2012) and eggplant (Solanum melongena) (Bibhuti et al., 2012).

Phenolic levels and PPO activity have not been determined in various eggplants during ripening. The objective of this study was to determine the phenolic levels and PPO activity (diphenol and triphenol oxidase activities) in *Solanum melongena* L. (round and oval varieties), *Solanum aethiopicum* L. and *Solanum gilo* L. (big and small varieties) during ripening.

Materials and Methods Plant Material

Unripe fruits of the various eggplants; *S. melongena* (round and oval varieties), *S. aethiopicum* and *S. gilo* (big and small varieties) were purchased from New Benin market in Benin-City, Nigeria (6°19' N, 5°63' E). The fruits were allowed to ripen normally and

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samples were collected from the ripe (yellow) and overripe (red) stages for the analyses of phenolic level and polyphenol oxidase activity.

Extraction of phenolic compounds

The extraction was carried out according to the method of Ayub *et al.*, (2011). 4 g of each eggplant was homogenized with 8 ml of 50 % aqueous ethanol (v/v). The homogenate was allowed to stand at room temperature for 30 min with occasional agitation. The homogenate was centrifuged at 2000 g using a centrifuge (model 80-2) for 15 min and the supernatant was used as the extract for the determination of phenolic level.

Assay for phenolic compounds

The assay for phenolic compounds was carried out using Folin-ciocaltau's method (Rajurkar and Gaikwad, 2012). 1.5 ml Folinciocaulteu's reagent (1 in 10 dilutions) was added to 0.3 ml of the phenolic extract. 1.2 ml of sodium carbonate (7.5 % w/v) was added to the reaction mixture and allowed to stand for 30 min at room temperature. Absorbance was read at 765 nm and the phenolic level was determined from a gallic acid standard curve. The assay was carried out in triplicate and distilled water was used as control. Phenolic level was expressed as mg gallic acid equivalent (GAE)/g fresh weight.

Extraction of Polyphenol oxidase

Extraction of Polyphenol oxidase was done according to the method of Adamson and Abigor (1980) with slight modifications. 5 g of each eggplant was homogenized with 1.25 g of polyvinylpyrrolidone (PVP) in 20 ml of ice-cold 0.05 M sodium phosphate buffer pH 6.5. The homogenate was filtered through a double layer cheese cloth and centrifuged for 10min at 10,000 g using a centrifuge (model 80-2). The resultant supernatant was used as the enzyme extract.

Assay of Polyphenol oxidase activity

Assay was carried out according to the method of Adamson and Abigor (1980) with slight modifications. 1.25 ml of 10 mM substrate (cathechol or pyrogallol) was added to 1.25 ml of 0.1 M sodium phosphate buffer (pH6.5). 0.5 ml of enzyme extract was added to the reaction mixture. Absorbance was read at 420 nm for 3 min. Polyphenol oxidase activity was expressed as change in absorbance per minute per gram fresh weight (ΔA /min/gFW).

Statistical analysis

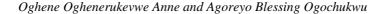
Data were subjected to analysis of variance (ANOVA) using statistical package for social sciences (SPSS) (Version 20). Tukey's honestly significant difference (HSD) was used to identify differences among means at the probability level of 1%.

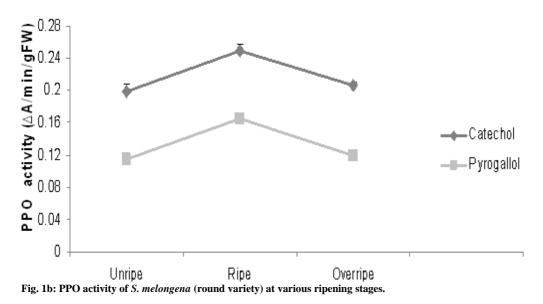
Results

Phenolic level of *S. melongena* (round variety) decreased significantly (P<0.01) by 1.37 fold (26.75 %) from the unripe to the ripe stage; while it increased significantly (P<0.01) by 1.17 (17.97 %) from the ripe to the overripe stage (Fig 1a). PPO activity in *S. melongena* (round variety) was higher with catechol as substrate than with pyrogallol (Fig 1b). There was an inverse relationship between the phenolic level and PPO activity in *S. melongena* (round variety) during ripening. PPO activity increased significantly (P<0.01) by I.25 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by 1.20 from the ripe to the overripe stage, when catechol was used as substrate (Fig 1b). On the other hand PPO activity increased significantly (P<0.01) by I.44 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by I.44 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by I.44 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by I.44 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by I.44 fold from the unripe to the ripe stage.



Fig. 1a: Phenolic level of S. melongena (round variety) at various ripening stages.





In *S. melongena* (oval variety), the phenolic level increased non-significantly (P>0.01) in all the ripening stages (Fig 2a). Phenolic level increased by 1.04 fold (3.54 %) from the unripe stage to the ripe stage and 1.28 (28.09 %) from the ripe to the overripe stage. PPO activity in the same variety increased in all the ripening stages, when both catechol and pyrogallol were used as substrates (Fig 2b). PPO activity in *S. melongena* (oval variety) was higher with pyrogallol as substrate than with catechol (Fig 2b). There was a linear relationship between the phenolic level and PPO activity in *S. melongena* (oval variety) during ripening. The increase in PPO activity was 1.11 fold from the unripe to the ripe stage, it was non-significant (P>0.01) and 3.08 fold from the ripe to the overripe stage which was significant (P<0.01), when catechol was used, 1.44 fold increase in PPO activity from the unripe to the ripe stage was significant (P<0.01) and the 1.04 fold increase from the ripe to the overripe stage was non-significant (P>0.01) (Fig 2b).

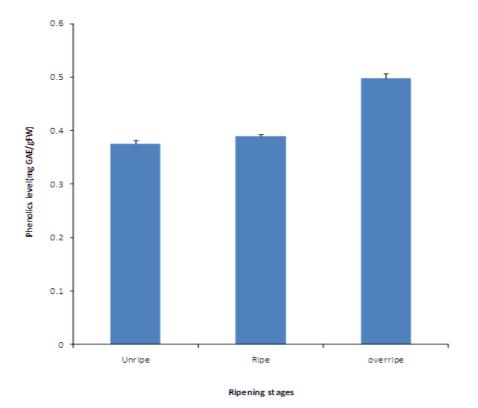
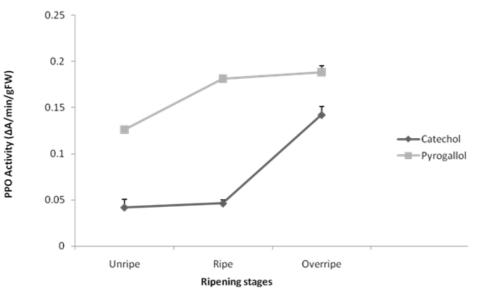
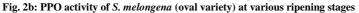


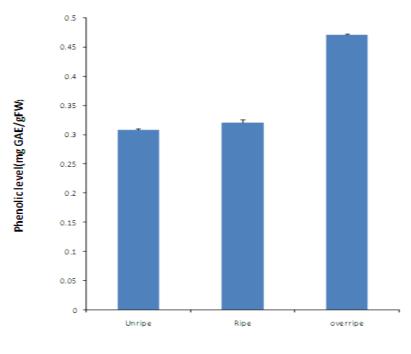
Fig. 2a: Phenolic level of S. melongena (oval variety) at various ripening stages



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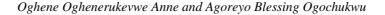


Phenolic level increased in all the ripening stages of *S. aethiopicum* (*Fig 3a*). The phenolic level increased non-significantly (P>0.01) by 1.04 fold (3.88 %) from the unripe stage to the ripe stage and increased significantly (p<0.01) by 1.47 fold (46.73 %) from the ripe to the overripe stage. PPO activity in *S. aethiopicum* was higher with catechol as substrate than with pyrogallol (Fig 3b). There was an inverse relationship between the phenolic level and PPO activity in *S. aethiopicum* when catechol was used as substrate and a linear relationship when pyrogallol was used. PPO activity decreased significantly (P<0.01) in all the ripening stages of *S. aethiopicum* when catechol was used (Fig 3b). It decreased by 1.09 fold from the unripe to the ripe stage and by 3.92 fold from the ripe to the overripe stage. The increase in PPO activity was 1.01 fold from the unripe to the ripe stage which was non-significant (p>0.01) and 1.32 fold from the ripe to the overripe stage which was significant (p<0.01), when pyrogallol was used (Fig 3b).



Ripening stages

Fig. 3a: Phenolic level of S. aethiopicum at various ripening stages



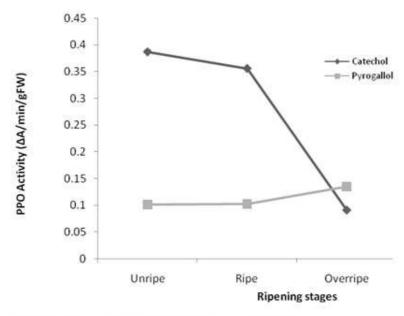


Figure 3b: PPO activity of S. aethiopicum at various ripening stages Fig. 3b: PPO activity of S. aethiopicum at various ripening stages

In *S. gilo* (small variety) the phenolic level decreased significantly (P<0.01) by 1.18 fold (15.58 %) from the unripe stage to the ripe stage and increased significantly (P<0.01) by 1.23 fold (23.44 %) from the ripe to the overripe stage (Fig 4a). Activity of PPO in *S. gilo* (small variety) was also higher with catechol as substrate than with pyrogallol (Fig 4b). There was an inverse relationship between the phenolic level and PPO activity in *S. gilo* (small variety) when catechol was used as substrate and a linear relationship when pyrogallol was used. PPO activity in *S. gilo* (small variety) by 1.52 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by 1.53 fold from the ripe to the overripe stage, when catechol was used (Fig 4b). PPO activity decreased significantly (P<0.01) by 1.26 fold from the ripe to the overripe stage and increased significantly (P<0.01) by 1.26 fold from the ripe to the overripe stage, when pyrogallol was used (Fig 4b).

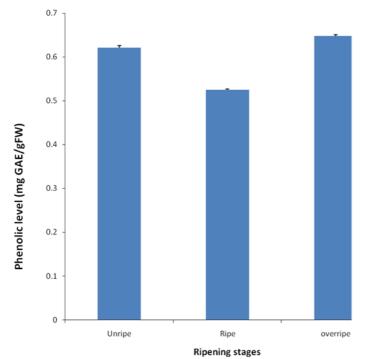
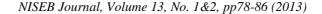


Fig. 4a: Phenolic level of S. gilo (small variety) at various ripening stages



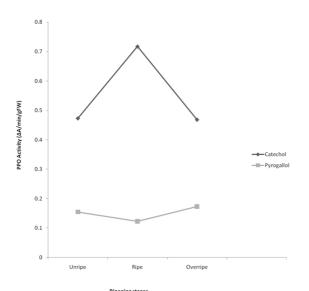


Fig. 4b: PPO activity of *S. gilo* (small variety) at various ripening stages

In *S. gilo* (big variety), the phenolic level decreased significantly (P<0.01) in all the ripening stages by 1.36 fold (26.45 %) from the unripe stage to the ripe stage and 1.15 fold (13.14 %) from the ripe to the overripe stage (Fig 5a). Activity of PPO in *S. gilo* (big variety) was also higher with catechol as substrate than with pyrogallol (Fig 5b). There was an inverse relationship between the phenolic level and PPO activity in *S. gilo* (big variety) when catechol and pyrogallol were used as substrates although there was a linear relationship only in the overripe stage, when catechol was used as substrate. PPO activity increased significantly (P<0.01) by 1.83 fold from the unripe to the ripe stage and decreased significantly (P<0.01) by 2.11 fold from the ripe to the overripe stage, when catechol was used (Fig 5b). PPO activity increased non-significantly in all the ripening stages (P>0.01) by 1.03 fold from the unripe to the ripe to the overripe stage, when pyrogallol was used (Fig 5b).

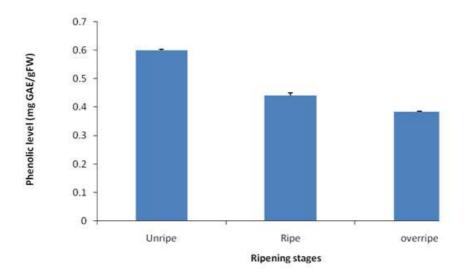


Fig. 5a: Phenolic level of S. gilo (big variety) at various ripening stage

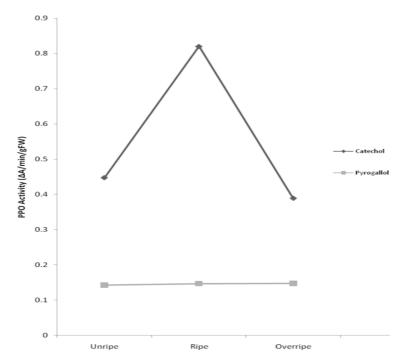


Fig. 5b: PPO activity of S. gilo (big variety) at various ripening stages.

Discussion

In this study phenolic levels increased in all the eggplants in their overripe stage expect in *S. gilo* (big variety) which showed a decrease. This increase in phenolic levels correlates with the report of Casado *et al.* (2004) who observed phenolic levels increased during ripening in loquat fruits, but this is not in agreement with the report from Onuoha and Nwagbara (2011) who observed that phenolic compound decreased in the overripe stage in banana and plantain. They attributed the loss of phenolic compounds to the polymerization insolubilisation rather than decomposition of these phenolic compounds. This result suggests that the overripe stage of most eggplants contain high phenolic levels. Newilah *et al.* (2010) reported that the accumulation of phenolic compounds varies in relation to the physiological state of the fruit as result of equilibrium between biosynthesis and further metabolism including catabolism. Comparison of the phenolic levels among the eggplants showed that in the unripe stage, the two varieties of *S. gilo* had the highest phenolic levels, followed closely by *S. melongena* (round and oval varieties) and *S. aethiopicum*, which had the lowest phenolic level.

In the ripe stage of the eggplants, S. gilo (small variety) had the highest phenolic level followed by S. gilo (big variety), S. melongena(oval variety), S. melongena(round variety) and S. aethiopicum respectively.

In the overripe stage, S. gilo (small variety) also had the highest phenolic level, followed by S. melongena(oval variety), S. aethiopicum, S. melongena(round variety), and S. gilo(big variety) respectively. This result showed that S. gilo (small variety) has the highest phenolic level in all the ripening stages. This result also suggests that phenolic levels in eggplants vary according to species and varieties.

Polyphenol oxidase (PPO) activities in this study were higher with catechol as substrate than pyrogallol. Catechol was observed to be the preferred substrate for the enzyme in all the eggplants except in *S. melongena* (oval variety) in which the preferred substrate for the enzyme was pyrogallol. This result is in agreement with the report of Serap *et al.* (2005) on artichoke (*Cynara scolymus* L.) in which they observed that PPO showed more affinity for catechol than pyrogallol. Similar result was also reported by Jianhui *et al.* (2006), who characterized PPO from mango pulp (*Mangifera indica* 1. Cv. "tainong") and found that the enzyme had higher affinity for catechol. This higher affinity for catechol than pyrogallol could be an indication of the presence of isoforms of PPO with different endogenous concentrations or it might indicate the presence of one isoform of the enzyme with catechol as its preferred substrate. The presence of different isoforms of PPO has been reported (Jianhui *et al.*, 2006; Bibhuti *et al.*, 2012). Comparison of the PPO activity in the eggplants in each ripening stage showed varietal differences, Podsedek *et al.* (2000) observed similar results in apple cultivars. Comparison of the activity of PPO in each ripening stage with catechol as a substrate showed that in the unripe stage, *S. gilo*(small variety) had the highest PPO activity followed respectively by *S. gilo* (big variety), *S. aethiopicum*, and *S. melongena* (round and oval varietes).

In the ripe stage, S. gilo(big variety), had the highest PPO activity, followed by S. gilo (small variety) S. aethiopicum, and S .melongena (round and oval varieties) respectively.

In the overripe stage, *S. gilo* (small variety), had the highest activity, followed by *S. gilo* (big variety), *S. melongena* (round variety), *S. melongena* (round variety), and *S. aethiopicum* respectively. Comparison of the activity of PPO in each ripening stage with pyrogallol as substrate also showed that in the unripe stage, *S. gilo* (small variety) had the highest activity, followed by *S. gilo* (big variety), *S. melongena* (round variety), *S. melongena* (round variety) and *S. aethiopicum* respectively. In the ripe stage, *S. melongena* (oval variety) had the highest activity and *S. aethiopicum* had the least activity. Lastly in the overripe stage, *S. melongena* (oval variety) had the highest activity and *S. melongena* (round variety) had the least PPO activity. Changes in PPO activities with ripening may be due to an increased or decreased synthesis of a particular isoform in response to change in concentration of its preferred endogenous phenolic substrate. In this study, PPO activities showed inverse relationship with phenolic levels in *S. melongena* (round variety) when both

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catechol and pyrogallol were used as substrates. However, inverse relationship was only observed in *S. gilo* (small variety) and *S. aethiopicum* and when catechol was used as substrate. When pyrogallol was used as substrate, only *S. gilo* (big variety) showed inverse relationship. This result correlates with the report of Adriano *et al.* (2005) who observed that PPO activity in peaches increased during ripening and total phenolic level decreased during storage. Nguyen *et al.* (2003) also reported inverse relationship in PPO activity and total phenolic level in banana peel at low temperature storage. The inverse relationship between phenolic levels and PPO activities in these eggplants suggests that enzymatic browning decreases with ripening in these eggplants.

Linear relationship between PPO activity and phenolic level was observed in *S. melongena* (oval variety) when both catechol and pyrogallol were used as substrates. However, linear relationship was only observed in *S. gilo* (small variety) and in *S. aethiopicum* when pyrogallol was used as substrate. This result correlates that of Sejong and Kyung (2011), who reported that linear relationship occured in PPO activity and total phenolic level in persimmon (*Diospyros kaki* Thunb.). Linear relationship between PPO activity and phenolic level when pyrogallol was used as substrate in *S. gilo* (small variety) and in *S. aethiopicum* may not suggest an increase in enzymatic browning in these species during ripening due to the low activity of PPO with pyrogallol.

From the results obtained in this study phenolic compounds were observed to increase in the overripe stage of almost all the eggplants. Phenolic compounds are major antioxidants in human diet, therefore the increase observed in their levels in the overripe stage of the eggplants showed that nutritional benefit can be derived from their consumption. Also the inverse relationship between phenolic levels and PPO activities in these eggplants except in *Solanum melongena* (oval variety) suggests that enzymatic browning decreases with ripening in these fruits.

References

- Aberoumand A and Deokul, S: Comparison of phenolic compounds of some edible plants from Iraq and India. *Pakistan Journal of Nutrition* **8**:26-31, 2008.
- Adamson I and Abigor R Transformation associated with cathecholase in *Dioscorea alata* during storage. *Phytochemistry* **19**:1593-1595, 1980.
- Adriano B and Carlos HGL: Polyphenol oxidase activity, Browning potential and Phenolic content of Peaches during Postharvest Ripening. *Journal of Food Biochemistry* 26(6): 624-637, 2005.
- Arnok P, Ruangviriyachai C, Mahachai R, Techawongstien S and Chanthai S: Optimization and determination of polyphenol oxidase and peroxidase activities in hot pepper (*Capsicum Annuum* L.) pericarb. *International Food Research Journal*, **17**: 385-392, 2010.
- Ayub MA, Nayan V, Victoria KC, Lalsanglura R and Inaotombi D: Antioxidant activity of fruits available in Aizawi market of Mizoram, India. World Journal of Agricultural Sciences, 7(3): 327-332, 2011.
- Barket A,Shafiqa M and Niyaz AW: Effect of catechol, gallic acid and pyrogallic acid on the germination, seedling growth and the level of endogenous phenolics in cucumber (*Cucumis sativus* L.). International Journal of Life Science Biotechnology and Pharma Research, 1(3): 50-55, 2012.
- Bibhuti BM, Satyendra G and Arun S: Purification and characterization of Polyphenol oxidase (PPO) from eggplant (*Solanum melongena*). Food chemistry, **134**:1855-1861, 2012.
- Borneo R, Leon AE, Aguirre A, Ribotta P and Cantero JJ: Antioxidant Capacity of medicinal plants from the province of CÓrdoba (Argentina) and their invitro testing in model food system. *Food Chemistry*, **112**:664-670 2008.
- Boskou D: Sources of natural phenolic antioxidants. Trends in Food Science & Technology, 17: 505-512, 2006.
- Caodi F: Characterization of polyphenol oxidase and antioxidants from pawpaw (Asimina tribola) fruit. University of Kentucky Master's Theses, 2007.
- Casado VJ, Sellés Marchart S, Gómez Lucas I and Bru Martínez R: Evolution of phenolics and polyphenoloxidase isoenzymes in relation to physical-chemical parameters during loquat (*Eriobotrya japonica* cv. Algerie) fruit development and ripening. International Centre For Advanced Mediterranean Agronomic Studies- Mediterranean Agronomic Institute of Zaragoza, pp151-198, 2003.
- Chazarra S, Garci`a-Carmona F, Cabanes J: Evidence for a tetrameric form of Iceberg lettuce (*Lactuca sativa L.*) Polyphenol oxidase: purification and characterization. *Journal of Agricultural and Food Chemistry*, **49**: 4870-4875, 2001.
- Francisca O, Santos B, Ángeles Peinado M and Juan P: Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'Picual' trees during fruit ripening. *Tree Physiology*, 28: 45–54, (2007).
- Gajewski M, Katarzyna K and Bayer M: The influence of post harvest storage on cultivars. *Notulae Botanicae Horti Agrobotanici*, **37**: 200-205, 2009. quality characteristics of fruit of eggplant
- Grubben GH: Plant Resources of Tropical Africa. Vegetables(PROTA2), Pp.472, 2004.
- Häkkinen S Flavonols and Phenolic Acids in Berries and Berry Products. Kuopio University Publications D. Medical Sciences, 221: 17-18, 2000.
- Hayat S, Ali B and Ahmad A: "Salicylic Acid: Biosynthesis, Metabolism and Physiological Role in Plants", in Hayat S and Ahmad S (Eds.) Salicyclic Acid: A Plant Hormone, Springer, The Netherlands, pp.1-14, 2007.
- Jianhui W, Weibo J, Baogang W, Shijian L, Zhengli Ĝ and Yunbo L: Partial Properties of Polyphenol Oxidase In Mango (*Mangifera* Indica L. Cv. "Tainong") Pulp. Journal of Food Biochemistry, **31**:45–55, 2006.
- Kamble GS, Torane RC, Mundhe KS, Deshpande NR and Salvekar JP: Evolution of free radical scavenging potential of *Embelia* basal. Journal of Chemical And Pharmaceutical Research, **3**(2):465-471, 2011.
- Lundell TK, Mäkelä MR and Hildén K: Lignin-modifying enzymes in filamentous basidiomycetes ecological, functional and phylogenetic review. *Journal of Basic Microbiology*, **50**(1) 5–20, 2010.
- Manach C, Scalber A, Morand C, Rémésy C and Jimenez L: "Polyphenols: Food Sources and Bioavailability". *The American Journal* of Clinical Nutrition, **79**: 727-747, 2004.
- Marco C, Riccardo NB and Giovanni S: Characterization of polyphenol oxidase and perioxidase and influence on browing of cold stored strawberry fruit. *Journal of Agricultural and Food Chemistry*, **59**(9): 3469-3481, 2007
- Newilah NG, Brat P, Tomekpe K, Alter P, Fokou E and Etoa FX: Effect of Ripening on Total Polyphenol Contents of *Musa* Hybrids and Cultivars Grown in Cameroon. *Acta Horticulturae*, **879**: 413-418. (2010).
- Nguyen TT, Ketsa S, Wouter GV: Relationship between browning and the activities of polyphenol oxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. *Postharvest Biology and Technology*, **30**:187-193, 2003.

- Onuoha CI and Nwagbara EC: Comparative Studies Of Polyphenolic Concentration Of Plantain And Banana Of Different Post Harvest Ages. International Science Research Journal, 3: 82 – 84, 2011.
- Pérez-Amador MC, Muñoz Ocotero V, García Castañedal JM and González Esquinca AR: Alkaloids in Solanum torvum Sw (Solanaceae). International journal of Experimental Botany, 76: 39-45, 2007.
- Podsedek A, Wilska-Jeszka J, Anders B, and Markowski J: Compositional characterization of some apple varieties. *European Food Research and Technology*, **210**:268–272, 2000.
- Rajurkar NS and Gaikwad K: Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves. *Journal of Chemical and Pharmaceutical Research* **4**(1): 365-374, 2012.
- Sambasiva Rao KRS, Tripathy NK, Srinivasa Rao DI and Prakasham R: Production, Characterization, Catalytic and Inhibitory activities of Tyrosinase. *Research Journal of Biotechnology*, **8**(1): 87-99, 2013.
- Scalbert A, Manach C, Morand C and Reměsy C: Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science And Nutrition, 45: 287-306, 2005.
- Se Jong K and Mi Jung K: Effects of the PPO(Polyphenol oxidase) Activity and Total Phenolic Contents onBrowning and Quality of Dried-Persimmon According to Maturity Degree of Astringent Persimmon (Diospyros kaki Thunb.). 2nd International Conference on Biotechnology and Food Science (International Proceedings of Chemical, Biological and Environmental Engineering), 7: 115-118, 2011.
- Serap D,Yusuf T, Hatibe E and Oktay A: Characterization and Purification of Polyphenol Oxidase from Artichoke (*Cynara scolymus* L.). Journal of Agricultural And Food Chemistry, **53**: 776-785, 2005.
- Shahryar S: Demonstration and Modification of the pH Profile of Polyphenol Oxidase in *Solanum lycopersicum*. *International Journal of Bioscience, Biochemistry and Bioinformatics*, **2**(6): 385-388, 2012.
- Sodipo OA, Abdulrahman FI, Sandabe UK and Akinniyi JA: Effects of the aqueous fruit extract of *Solanum macrocarpum* L. on the hematological parameters of triton-induced hyperlipidemic rats. *African Journal of Pharmacy and Pharmacology*, **5**(5): 632-639, 2011.
- Stommel JR and Whitaker BD: Phenolic acid content and composition of eggplant fruit in a germplasm core subset. *Journal Of The American Society For Horticultural Science*, **128**(5):704-710, 2003.
- Yoruk R and Marshall MR Physicochemical properties and function of plant polyphenol oxidase: A review. *Journal of Food Biochemistry*, **27**: 361-422, 2003.