学校编码：10384　　分类号＿＿＿密级＿＿＿
学号：200326096　　UDC ＿＿＿

厦门大学

硕士学位论文

# 马铃薯多酚氧化酶性质及抑制剂作用机理的研究

# Study on the Properties of Potato Polyphenol Oxidase and the Inhibition Mechanism of its Inhibitors

指导教师姓名：　陈清西　教授
专　业　名　称：生物化学与分子生物学
论文提交日期：2006 年 4 月 15 日
论文答辩时间：2006 年 5 月 20 日
学位授予日期：2006 年　月　日

答辩委员会主席：黄河清
评　　阅　　人：＿＿＿＿

2006 年　5 月

# 厦门大学学位论文原创性声明

兹呈交的学位论文，是本人在导师指导下独立完成的研究成果。本人在论文写作中参考的其他个人或集体的研究成果，均在文中以明确方式标明。本人依法享有和承担由此论文产生的权利和责任。

声明人（签名）：

年　　月　　日

# 厦门大学学位论文著作权使用声明

本人完全了解厦门大学有关保留、使用学位论文的规定。厦门大学有权保留并向国家主管部门或其指定机构送交论文的纸质版和电子版，有权将学位论文用于非赢利目的的少量复制并允许论文进入学校图书馆被查阅，有权将学位论文的内容编入有关数据库进行检索，有权将学位论文的标题和摘要汇编出版。保密的学位论文在解密后适用本规定。

本学位论文属于

1、保密（ ），在　　年解密后适用本授权书。

2、不保密（ ）

（请在以上相应括号内打“√”）

作者签名：　　日期：　　年　月　日

导师签名：　　日期：　　年　月　日

# 目 录

# Contents

# 摘　要

多酚氧化酶(EC.1.14.18.1)广泛存在于微生物、动植物中,是结构复杂的含铜氧化还原酶。它是果蔬褐变过程中起重要作用的一种酶，在果蔬的酶促褐变过程中，催化产生引起褐变的色素物质。因此对此酶的酶学特性,催化作用机理和抑制机理的研究将为果蔬的保鲜奠定基础。

以马铃薯为原料提取多酚氧化酶，进行了分离纯化，得到比活力为1928 U/mg，纯化倍数为9.4的部分纯化的马铃薯多酚氧化酶。研究马铃薯多酚氧化酶的理化性质，该酶作用的最适温度和pH分别是30℃和pH 6.0；酶在低于50℃和在pH 5.15～8.75范围内稳定；在pH 6.8、30℃条件下，酶催化L-DOPA氧化反应的$K_m$为7.24 mmol/L。

金属离子$Li^+$、$K^+$、$Ba^{2+}$和$Mg^{2+}$对酶活力没有影响，$Al^{3+}$、$Fe^{3+}$、$Cd^{2+}$、$Mg^{2+}$、$Co^{2+}$、$Pb^{2+}$、$Mn^{2+}$和$Zn^{2+}$有激活作用，而$Cu^{2+}$表现抑制作用。

测定了几种有机溶剂(包括醇、醛、酮、二甲亚砜和二氧六环)对马铃薯多酚氧化酶的影响，得出了它们的$IC_{50}$和抑制作用类型，并测定了抑制常数。

研究了苯甲酸,苯甲醛和18种苯甲酸衍生物对马铃薯多酚氧化酶的作用。从苯甲酸衍生物中筛选多酚氧化酶抑制剂，探讨各种效应物对马铃薯多酚氧化酶的影响。研究发现苯甲酸类衍生物的结构是它们的抑制作用强弱和抑制类型的基础。

对亚硫酸氢钠，偏重亚硫酸钠对酶的作用原理和效果进行了研究。发现它们对此酶存在不可逆的抑制作用，而且可以导致酶的迟滞时间增加。

选取亚硫酸氢钠，偏重亚硫酸钠，氯化钙为效应物，研究它们对马铃薯鲜切片护色效果。研究了处理时间和它们的浓度对马铃薯鲜切片护色效果的影响。以亚硫酸氢钠，偏重亚硫酸钠，热烫的时间和温度为因素，设计正交实验。正交实验的结果显示最佳热烫温度条件为85 ℃，最佳热烫时间为3 min，亚硫酸氢钠最佳浓度为30 mmol/L，偏重亚硫酸钠最佳浓度为30 mmol/L。

**关键词：**马铃薯；多酚氧化酶；抑制作用

# Abstract

Polyphenol oxidase (EC 1.14.18.1) is widely distributed in microorganisms, animals and plants.It is a copper-containing enzyme which has complex constructure. Polyphenol oxidase (PPO) acts as an important role during the process of enzymetic browning in fruit and vegetable,for the reason that it can catalyze the melanin Synthesis. The studies of the enzymetic characteristics, catalytic mechanism and inhibition mechanism of PPO were very important in anti-browning research field.

In this paper, we purified the PPO from potato and studied some physical and chemical properties of this enzyme.Optimum temperature and optimum pH were determined to be 30℃ and 6.0 respectively. $K_m$ was determined to be 7.24 mmol/L at 30℃ and pH 6.8. The enzyme activity was stable at the pH ranging from 5.15~8.75 and under 50℃.

The effects of metal ions on the PPO for the oxidation of L-DOPA were studied. The results showed that $Li^+$, $K^+$, $Ba^{2+}$ and $Mg^{2+}$ had no effect on the PPO activity, $Al^{3+}$, $Fe^{3+}$, $Cd^{2+}$, $Mg^{2+}$, $Co^{2+}$, $Pb^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ activated the activity of PPO. $Cu^{2+}$ could inhibit the activity of PPO.

The effects of some organic solvents, including alcohol compounds, aldehyde compounds, acetone, dimethylsulfoxide and dioxane， on the potato PPO for the oxidation of L-DOPA were studied. The $IC_{50}$ values and the inhibitory mechanisms were determined and compared.

Benzoic acid,benzladehyde and 18 benzoic acid family compounds were studied in order to screen the inhibitor of PPO. The results showed that all of the tested compounds had effects on potato PPO. The reactions of these compounds were reversible with remaining enzyme activity.The structures of these compounds were the foundation of inhibit ability and inhibit mechanism.

The mechanisms and effects of sodium bisulfite and sodium metabisulfite on the potato PPO were studied.The results showed that they have some unreversible inhibit on the enzyme,and they could increase the lag time of potato PPO.

On the basis of the study that were carried out in above part , We chosen sodium bisulfite, sodium metabisulfite and calcium chloride as inhibitors for the anti-browning research on fresh-cut potato slices. We studied the effects of immersing time on the

antibrowning ability of them.Also,we studied effects of their concentration on the antibrowning ability for potato slices. The study on the anti-browning effect of combination of sodium bisulfite, sodium metabisulfite and heating showed that the optimum heating temperature was 85℃, the optimum heating time was 3 min, the optimum concentration of sodium bisulfite solution was 30 mmol/L, the optimum concentration of sodium metabisulfite solution was 30 mmol/L.

**Key Words**: potato; polyphenol oxidase; inhibition.