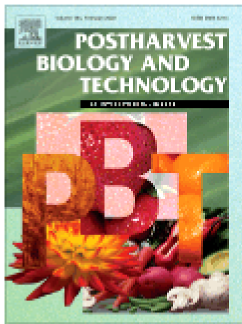
ELSEVIER

Search



ISSN: 0925-5214

Postharvest Biology and Technology

An International Journal

Publishing options: OA Open Access ↗ S Subscription ↗

↗ Guide for authors Track your paper ⌄ Order journal ⌄

Editor-in-Chief > Editorial board

C. Watkins

The journal is devoted exclusively to the publication of original papers, review articles and frontiers articles on **biological** and **technological postharvest research**. This includes the areas of postharvest storage, treatments and underpinning mechanisms, quality evaluation, packaging, handling and distribution of fresh horticultural crops including fruit, vegetables, flowers and nuts, but excluding grains, seeds and forages.

Papers reporting novel insights from fundamental and interdisciplinary research will be particularly encouraged. These disciplines include systems biology, bioinformatics, entomology, plant physiology, plant pathology, (bio)chemistry, engineering, modelling, and technologies for nondestructive testing.

Manuscripts on fresh food crops that will be further processed after postharvest storage, or on food processes beyond refrigeration, packaging and minimal processing will not be considered.

Benefits to authors

Feedback

We also provide many author benefits, such as free PDFs, a liberal copyright policy

We also provide many author benefits, such as free PDFs, a liberal copyright policy,

special discounts on Elsevier publications and much more. Please click here for more information on our author services.

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

Hide full aims & scope

Submit your paper

The Impact Factor of this journal is 5.537, ranking it 6 out of 91 in *Agronomy*

With this journal indexed in 5 international databases, your published article can be read and cited by researchers worldwide

View articles

CiteScore

7.9

Impact Factor

5.537

Acceptance Rate

19%

Top Readership

CN US TH

Review Time

6 weeks

View historical data and other metrics on Journal Insights.

Feedback

Recent Articles | Most Downloaded | Most Cited

Dynamic labelling reveals central carbon metabolism responses to stepwise decreasing hypoxia and reoxygenation during postharvest in pear fruit

K. Terzoudis, M.L.A.T.M Hertog, B.M. Nicolaï

Sorbitol immersion controls chilling injury in CA stored ‘Palmer’ mangoes

Alex Guimarães Sanches, Maryelle Barros da Silva and 6 more

A model for firmness and low temperature-induced storage breakdown disorder of ‘Hayward’ kiwifruit in supply chain

S.G. Gwanpua, M. Zhao and 3 more

› View all recent articles

Special Issues | PlumX Metrics | Open Access Articles

VIS-NIR-IR review

Kerry Walsh, Renfu Lu, Bart Nicolai

Alternatives to the use of synthetic fungicides for the control of postharvest decay of fruit and vegetables: current status and future perspectives

Pietro Tonutti, Samir Droby, Gianfranco Romanazzi

Optimal Controlled Atmosphere

R. Cavalieri, H.W. Peppelenbos

› View all special issues

News | Announcements

Recent changes in the Editorial team

January 16, 2015

Thank you to our reviewers

April 10, 2014

› View all news

Feedback

Editorial Board

Full editorial board

56 editors and editorial board members in 20 countries/regions

CN China (8) US United States of America (8) ES Spain (6) and 17 more

Editor-in-Chief
C. Watkins

› View editorial board

Conferences

4th Agriculture and Climate Change Conference

May 7 – 9, 2023 Dresden, Germany

› View all conferences

Mendeley Datasets

Data for: Development of deep learning method for predicting firmness and soluble solid content of postharvest Korla fragrant pear using Vis/NIR hyperspectral reflectance imaging

1 file (2018) Xinjie Yu, Huanda Lu, Di Wu

Data for: Predicting shelf life gain of fresh produce in Modified Atmosphere Packaging

1 file (2018) Valérie GUILLARD, Nathalie Gontard and 3 more

Tier-based Dataset: Musa-Acuminata Banana Fruit Species

1,191 files (2018) Eduardo Jr Piedad, Laura Vithalie Ferrer and 5 more

› View all Mendeley datasets

Abstracting and Indexing

Feedback

This journal has been indexed in:

- Current Contents - Agriculture, Biology & Environmental Sciences
- EMBiology
- CAB International

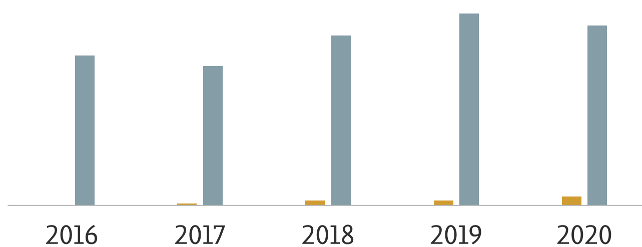
and 2 more

> View all indices

Supports Open Access

OA Open Access S Subscription

This journal offers authors two options (Open Access or subscription) to publish their research.



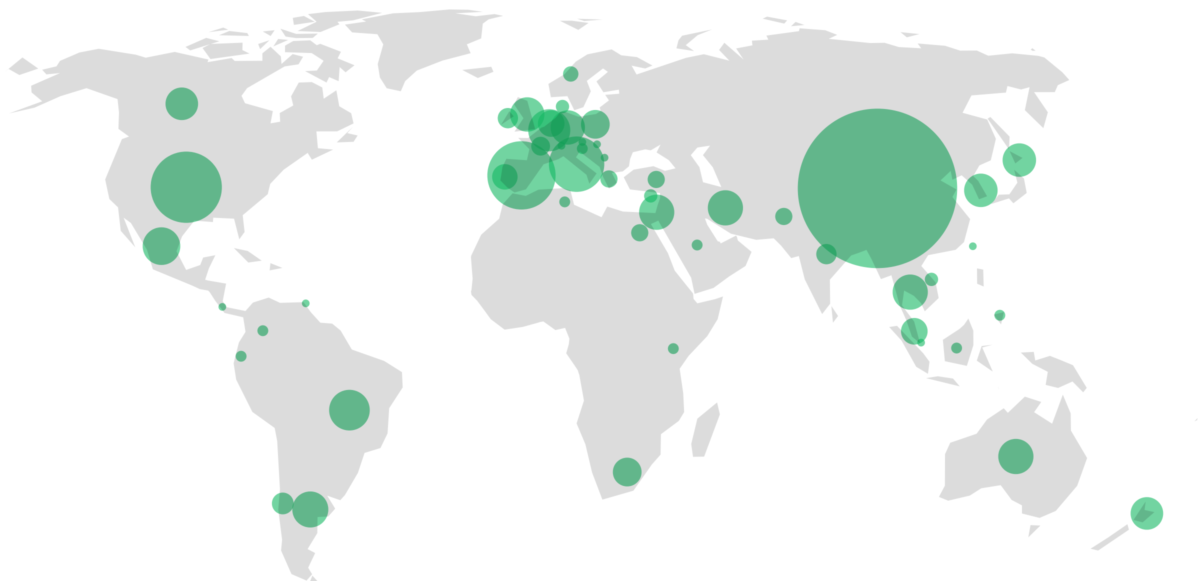
A total of 244 articles were published in 2020.

● 12 open access ● 232 subscription

↗ View all publishing insights

Authorship

The primary corresponding authors at country/region level in the last five years.



Top countries/regions Authors

#1 CN China 431

Feedback

	Top countries/regions	Authors
#2	US United States	86
#3	ES Spain	79

↗ View all countries

Author Resources

Whether you are planning a research project or working on your next manuscript, Elsevier's author resources are there to help.

Research & Preparation

↗ Researcher Academy

Writing

↗ Mendeley

↗ Guide for authors

↗ Language editing services

↗ Translation services

Submission & Publication

↗ Check submission status

↗ Track accepted paper

↗ View all resources and services

Your Research Data

Make your research data available, accessible, discoverable, and usable.

↗ Discover options for sharing your research data

↗ Publish your research output in a Research Elements journal

Feedback

United Nations Sustainable Development Goals

Is this the right home for your research?

JournalFinder uses smart search technology and field-of-research specific vocabularies to match your manuscript to Elsevier journals.

Simply insert your title and abstract and select the appropriate field-of-research for the best results.

Fill in details >

Postharvest Biology and Technology

Readers Authors > Librarians >

Feedback

View Articles

Volume/Issue Alert

Personalized Recommendations

Submit your Paper

Check Submitted Paper

Researcher Academy

Rights and Permissions

Elsevier Author Services

Support Center

Track Accepted Paper

Order Journal Personal

Order Journal Institutional

Editors >

Publishing Ethics Resource Kit

Support Center

Reviewers >

Reviewer Guidelines

Log in as Reviewer

Reviewer Recognition

Support Center

Copyright © 2021 Elsevier, except certain content provided by third parties

Cookies are used by this site. Cookie Settings

Terms and Conditions Privacy Policy Cookie Notice Sitemap

Feedback

Postharvest Biology and Technology

An International Journal

Editor-in-Chief
Prof. Bart Nicolai, Katholieke Universiteit Leuven, Box 2428, de Croylaan 42, B-3001 Leuven (Heverlee), Belgium

Associate Editors: Prof. Shi-Ping Tian, Chinese Academy of Sciences Key Lab of Plant Resources - Institute of Botany, Xiangshan Nanxincun 20, Haidian District, Beijing 100093, P.R. China
Prof. Pietro Tonutti, Scuola Superiore Sant'Anna, Institute of Life Science, Piazza Martiri Libertà 33, PISA 56127, ITALY
Dr. Peter M.A. Toivonen, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, CANADA
Prof. Chris Watkins, Cornell University School of Integrative Plant Science, Horticulture Section, 127 Plant Science Building Ithaca, NY 14853, USA

Founding Editor: G.E. Hobson, Angmering, Littlehampton, UK

Honorary Editors: R.P. Cavalieri, Washington State University, Pullman, Washington, USA
I.B. Ferguson, The New Zealand Institute for Plant & Food Research Ltd., Auckland, New Zealand

Editorial Advisory Board

G. Bengtsson, Ås, Norway
D. Brummell, Palmerston North, New Zealand
J. DeEll, Simcoe, ON, Canada
B. Defilippi, Santiago, Chile
M.I. Gil, Murcia, Spain
M.L.A.T.M. Hertog, Leuven, Belgium
K. Ichimura, Tsukuba, Japan
Y. Jiang, China
M.T. Lafuente, Valencia, Spain
A. Lichter, Bet Dagan, Israel
A. Macnish, Australia
O. Martín-Belloso, Lleida, Spain
D. O'Beirne, Limerick, Ireland
P. Perkins-Veazie, Lane, NC, USA
A. Plotto, FL, USA
D. Prusky, Bet Dagan, Israel
P. Ragaert, Gent, Belgium
G. Romanazzi, Ancona, Italy
A.D. Stead, London, England, UK
D. Sugar, Corvallis, OR, USA
L.A. Terry, Bedford, UK
S. Tian, Beijing, China
P. Toivonen, Summerland, BC, Canada
P. Tonutti, Pisa, Italy
P. Verboven, Leuven, Belgium
B. Whitaker, MD, USA
C-L. Xiao, Parlier, CA, USA

Processed at Thomson Digital, Gangtok (India)

VOLUME 125 (2017)

ELSEVIER

Amsterdam—Boston—London—New York—Oxford—Paris—Philadelphia—San Diego—St. Louis

doi:10.1016/S0925-5214(16)30708-6

Postharvest Biology and Technology 125 (2017) 77–90

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Postharvest control of litchi (*Litchi chinensis* Sonn.) pericarp browning by cold storage at high relative humidity after enzyme-inhibiting treatments

Mareike Reichel[a], Julia Wellhöfer[a], Rini Triani[a,1], Pittaya Sruamsiri[b], Reinhold Carle[a,c], Sybille Neidhart[a,*]

[a] *Institute of Food Science and Biotechnology, Chair of Plant Foodstuff Technology and Analysis, Hohenheim University, Garbenstrasse 25, 70599 Stuttgart, Germany*
[b] *Department of Crop Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand*
[c] *Biological Science Department, King Abdulaziz University, P.O. Box 80257, Jeddah 21589, Saudi Arabia*

ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 4 October 2016
Accepted 7 October 2016
Available online xxx

Keywords:
Enzymatic browning
Enzyme inhibition
Humidity
Pericarp color
Peroxidase
Polyphenol oxidase

ABSTRACT

Enzyme inhibitors were studied as adjuvant treatments in the control of litchi pericarp browning by protection against desiccation during reefer transport. Various organic acids (acetic, malic, citric, and oxalic acid) and inorganic salts (NaCl, $CaCl_2$) were investigated for *in vitro* inhibitory effects on the peroxidases (POD) and phenol oxidases (including laccase) extracted from litchi pericarp. Promising inhibitors were tested on 'Hong Huey' litchi fruit for their capability to prevent pericarp browning during cold storage (21 d, 5 °C, 90% relative humidity) with and without foil wrapping. An enzyme assay had been optimized for measuring the *in vitro* activities of phenol oxidases toward (−)-epicatechin, being the natural phenolic key substrate in litchi. Phenol oxidase activities were chiefly pH-dependent and completely inactivated at pH ≤ 3.5 by the organic acids used for buffering, whereby chelating agents performed best, especially oxalic acid. POD activity was stable over wider pH and ionic strength ranges, with inhibition being maximal (84%) in 0.25 M oxalic acid buffer (pH 3.5). $CaCl_2$ (0.25 mol L^{-1}) decreased POD activity by 68%, while rising doses increased the initial lag phase up to 2.5 min. In contrast to these *in vitro* enzyme-inhibiting effects, postharvest fruit treatments with these phenol oxidase and peroxidase inhibitors did not improve color retention during cold fruit storage, but proved ineffective or even favored pericarp browning compared to the control fruit. Pericarp color retention was maximal (96–97%) throughout cold storage of fruit in gas-permeable but moisture-retaining foil bags for at least two weeks, whether the fruit had been dipped into cold water (control) or into citrate (25 mmol L^{-1}) solution. Consequently, pericarp color retention only required an intact pericarp at harvest and postharvest protection against desiccation. Preventing water loss through preservation of cell compartmentation thus proved to be crucial and sufficient for the control of enzymatic browning under reefer conditions.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Enzymatic browning of the pericarp is one of the key factors limiting the marketability of fresh litchi (*Litchi chinensis* Sonn.) fruit, because the rapid loss of its attractive red color often creates a false impression of spoilage (Reichel et al., 2010, 2013). Due to enzyme inhibition and other protective effects, sulfur dioxide fumigation is still the current practice to prevent litchi pericarp browning (Liang et al., 2012), but undesirable effects on fruit quality and health concerns have led to consumer rejection and legal restriction (Sivakumar et al., 2010). This has necessitated the search for alternative processes (Bhushan et al., 2015). Prevention of pericarp browning has been understood to require measures that reduce the pericarp pH, but chiefly slow pericarp dehydration and weight loss (Joas et al., 2005). The aim is to improve pericarp appearance and freshness during distribution in reefer containers (Ducamp-Collin et al., 2008; Wang et al., 2010; Shafique et al., 2016) used for long distance transport. However, according to our previous study (Reichel et al., 2013), the rather high relative humidity (RH) of 90% could not prevent the loss of pericarp

* Corresponding author.
E-mail address: sybille.neidhart@uni-hohenheim.de (S. Neidhart).
[1] Present address: School of Life Science and Technology, Bandung Institute of Technology, Jalan Tamansari Nomor 64, Bandung 40116, Indonesia.

http://dx.doi.org/10.1016/j.postharvbio.2016.10.002
0925-5214/© 2016 Elsevier B.V. All rights reserved.

moisture and thickness upon cold storage (5 °C) without additional measures. Consequently, slow desiccation of fruits lying close together resulted in dark brownish-red pericarp due to prolonged oxidation of (−)-epicatechin and co-oxidation of anthocyanins. More rapid water loss of exposed pericarp favored surface scurf formation, yielding unattractive light brown fruit that showed much better anthocyanin retention but greater losses of flavanol oligomers (Reichel et al., 2013). Notable residual activities of polyphenol oxidases (PPO) and peroxidases (POD), which were found in the pericarp of those fruit despite declining enzyme activities with ongoing desiccation, suggested the additional application of enzyme inhibitors and/or further measures to improve moisture retention.

Pericarp browning in litchi has mainly been ascribed to PPO (Jiang et al., 1997, 1999), showing the highest activity in the exocarp (Underhill and Critchley, 1995). Directly after harvest, PPO gene expression was induced by pericarp desiccation and rapidly upregulated until reaching an early maximum, in parallel to PPO activity (Wang et al., 2014). The greater the water loss or the storage temperature, the higher these maxima were found to be. High affinity to (−)-epicatechin, being the natural substrate due to its prevalence in the pericarp, was shown for litchi PPO (Liu et al., 2007; Reichel et al., 2011) and a recently described laccase (LAC) that was mainly found in vacuoles of mesocarp cells in intact litchi pericarp (Fang et al., 2015). While LAC gene expression was always notably high, LAC was secreted to the extracellular space after beginning pericarp browning and significantly contributed to the overall polyphenol oxidizing activity in litchi pericarp (Fang et al., 2015). Litchi POD, being specific for H_2O_2, accept a wide range of co-substrates and are thus nonspecifically involved in postharvest browning, chiefly through stress-induced surface scurf formation (Reichel et al., 2011, 2013). Litchi exocarp has been shown to have a dense palisade-like layer of elongated suberized cells directly below the epidermis (Riederer et al., 2015).

Dipping into organic-acid solutions is common to prevent enzymatic browning of fresh or minimally processed fruits and vegetables (Buta and Moline, 2001; Son et al., 2001). While acids inhibit enzyme proteins nonspecifically due to pH effects, chelating agents among the carboxylic acids additionally block central or stabilizing ions of their active sites (Yoruk and Marshall, 2003; Jiang et al., 2008). Likewise, effects of buffer concentration on conformation of the enzyme protein due to the resultant ionic strengths have to be considered (Laurenti et al., 2000). The latter also plays a role in the application of salt solutions. Halides being known to inhibit LAC (Morozova et al., 2007) have also been discussed as PPO inhibitors (Son et al., 2001; Liu et al., 2007). Their cations, such as Na^+ or Ca^{2+}, may also have an effect on the structural stability and activity of both PPO and POD (Rasmussen et al., 1998; Sun et al., 2008). The inhibitory effects of organic acids and halide salts described for isolated litchi pericarp PPO and POD (Gong and Tian, 2002; Jiang et al., 1997, 1999; Sun et al., 2008) should still be verified *in vitro* for crude enzyme extracts in the presence of the natural substrate for a deeper understanding of the potential that these inhibitors can have in view of the coacting browning enzymes *in vivo*.

High doses of organic acids in combination with chitosan coating (Joas et al., 2005) or Ca^{2+} application (Wang et al., 2010) were shown to support color retention efficiently, but they could only slow browning during cold storage for 8–10 d, which might be too short for long-distance transport. Concurrently, pericarp integrity was impaired, because acid impregnation required wetting (Wang et al., 2010) or pretreatments enhancing pericarp porosity (Caro and Joas, 2005). Inconsistent responses to treatments, including varietal differences (Ducamp-Collin et al., 2008), and the viscosity of coating solutions (Caro and Joas, 2005) have turned out to be other limiting factors. However, for acidic calcium sulfate (Wang et al., 2010) and $CaCl_2$ dipping (Kou et al., 2015), the slower activity decline of various oxygen-scavenging enzymes was found to contribute to browning prevention, thus suggesting more complex protective mechanisms.

Thus, the aim of this follow-up study of our work (Reichel et al., 2013) was to improve pericarp color retention upon cold and humid storage (5 °C, 90% RH), exploring adjuvant fruit treatments with moderate doses of organic acids and chloride salts alone and in combination with fruit packaging in gas-permeable but moisture-retaining foil bags. The inhibitor doses should preferably not enhance pericarp porosity, but become effective locally, where cell compartmentation was lost. Concurrent application of surfactants was thus to be avoided. The *in vitro* inhibitory effects of the organic acids and chloride salts on POD and the litchi phenol oxidases (PPO+LAC) were to be verified for crude enzyme extracts in the presence of the natural substrate (−)-epicatechin, considering the impact of pH, inhibitor concentration, and ionic strength.

2. Materials and methods

2.1. Chemicals used

Unless otherwise stated, reagents were from VWR International (Darmstadt, Germany). (−)-Epicatechin, polyvinylpolypyrrolidone (PVPP), and tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the *in vivo* trials, tripotassium citrate monohydrate and oxalic acid dihydrate were supplied by Ajax Finechem (Auckland, New Zealand) and sodium chloride by Lab-Scan (Bangkok, Thailand). Deionized water was used throughout.

2.2. Fruit material under study

The *in vitro* tests (*cf.* 2.3) required a large amount of homogenous *Litchi chinensis* Sonn. pericarp powder having high activities of browning enzymes. It was obtained from 'Chacapat' fruit, because this cultivar is known for its large fruit, which can yield a considerable amount of pericarp per unit due to notable pericarp thickness, while the pericarp is rather unsusceptible to browning directly after harvest despite sufficiently high enzyme activities (Reichel et al., 2010, 2013). Fully ripe 'Chacapat' fruit, which had a mean size of 37.7 ± 0.4 g, pericarp color values of $L^* = 38.5 \pm 0.5$, $a^* = 33.0 \pm 0.6$, and $b^* = 26.2 \pm 0.4$, and a litchi maturity index of 6.5 according to Reichel et al. (2010) (*LMI*, *cf.* Eq. (3) in 2.4.2), were bought on the local fresh-fruit market in Chiang Mai, northern Thailand, (02 June 2008) maximally 10 h after they had been harvested in Fang (~125 km north of Chiang Mai) and subsequently distributed in traditional baskets, which were lined with paper to protect the fruit against desiccation. The fruit were directly brought to the laboratory at Chiang Mai University in Chiang Mai, debranched, and shock-frozen in liquid nitrogen. The pericarp removed from 40 fruits was kept at −80 °C until lyophilization. Afterwards, it was vacuum-packed and sent deep-frozen (−40 °C) to Hohenheim University, Stuttgart, Germany. There, it was finely ground, vacuum-packed, and stored at −80 °C until use.

For the *in vivo* trials (*cf.* 2.4) of this follow-up study, fruit of the commercially most important Thai litchi cultivar 'Hong Huey' were used exemplarily, as before (Reichel et al., 2013). 'Hong Huey' and 'Chacapat' fruit were shown to be comparable in terms of the PPO and POD activities directly after harvest, but fruit of the former cultivar are more prone to rapid browning and surface scurf formation due to their specific pericarp morphology and polyphenol composition (Reichel et al., 2013). To ensure homogenous fruit quality, the 'Hong Huey' fruit were picked from two adjacent trees in a research orchard in Mae Sa Mai, northern Thailand (18.8° North

latitude, 820 m a.s.l.), on two consecutive days (~10 kg/d) in May 2008 (~105 d after anthesis). The fruit bunches were always instantly precooled by passive air-cooling between ice packs in styrofoam boxes and transported this way to the laboratory at Chiang Mai University within 1 h (Reichel et al., 2010). After debranching and sorting, only sound fruit were selected and characterized in terms of fruit size (by weight, $n = 100$), color (*cf.* 2.4.2), and maturity (*LMI*, *cf.* 2.4.2). The fruit picked on the first (batch 1) and the next day (batch 2) had mean sizes of 18.3 ± 0.3 and 17.0 ± 0.3 g, respectively. The pericarp color values were $L^* = 38.8 \pm 2.7$, $a^* = 32.3 \pm 3.3$, and $b^* = 32.7 \pm 1.9$ for batch 1 with a *LMI* of 6.4 ± 0.5 (means of 180 fruit) and $L^* = 38.4 \pm 2.5$, $a^* = 33.2 \pm 2.9$, and $b^* = 24.5 \pm 5.6$ for batch 2 with a *LMI* of 6.5 ± 0.4 (means of 140 fruit). Fruit treatments with browning inhibitor solutions (*cf.* 2.4.1) were always performed within a maximum of 10 h after harvest on the same day.

2.3. *In vitro tests*

2.3.1. *Preparation of crude enzyme extracts from litchi pericarp*

Fresh enzyme extracts from 'Chacapat' pericarp powder (*cf.* 2.2) were produced daily, as described by Reichel et al. (2010). For the extraction under stirring (2 h, 4 °C), pericarp powder (m_1, 2.0 g) was mixed with 25 g McIlvaine buffer [citric acid (0.1 mmol L^{-1})/ sodium dihydrogen phosphate (0.2 mmol L^{-1}), 3:7 (v/v), pH 6.5] that contained 0.7 g PVPP. Centrifugation of the slurry (25,000 × *g*) plus isolation of the supernatant through a filter yielded a filtrate that was brought to 20 g (m_2) with the buffer. The density of this enzyme extract ($\rho_{4°C}$, kg L^{-1}) was measured, using a DMA 48 density meter (Anton Paar, Graz, Austria).

2.3.2. *Peroxidase activity assay*

The enzyme extract was analyzed as detailed by Reichel et al. (2010) to quantitate enzyme activities of POD (phenolic donor: hydrogen-peroxide oxidoreductase; EC 1.11.1.7) in duplicate. Accordingly, an aliquot of the *K*-times diluted extract (v_1, 0.2 mL) was added to the substrate solution (v_2, 1.3 mL) containing tropolone (12 mmol L^{-1}) and H_2O_2 (3.3 mmol L^{-1}) in McIlvaine buffer (pH 6.5). The absorbance at 418 nm (*A*) was read every 7 s for 10 min (path length *d*, 0.01 m), using a spectrophotometer Cary 100 (Varian, Mulgrave, Australia). Based on the molar absorption coefficient ε_{418nm} of 207,500 L mol^{-1} m^{-1} (Baur et al., 2004), the POD activity of the pericarp powder (*POD*, kat kg^{-1}) was calculated according to Eq. (1) from the maximum linear increase in absorbance ($\dot{A}$, s^{-1}), which was corrected by subtracting the slopes of the time–absorbance functions that had been recorded for both an enzyme blank ($\dot{A}_E$) and a substrate blank ($\dot{A}_S$).

$$POD = \frac{(v_1 + v_2) \cdot (\dot{A} - \dot{A}_E - \dot{A}_S) \cdot K \cdot m_2}{\varepsilon_{418nm} \cdot d \cdot v_1 \cdot \rho_{4°C} \cdot m_1} \quad (1)$$

A separate *in vitro* experiment comprising two approaches was performed to verify the involvement of (–)-epicatechin in the POD-catalyzed reduction of H_2O_2. First, the aforesaid standard POD activity assay was modified by replacing the phenolic donor accordingly. As to the use of (−)-epicatechin in McIlvaine buffer of pH 6.5, the conditions were thus adapted to those of the standard PPO+LAC assay (*cf.* 2.3.3). The enzyme activity toward H_2O_2 (3.3 mmol L^{-1}) plus (–)-epicatechin (1.3 mmol L^{-1}) was quantitated and compared to the results of the H_2O_2-free standard PPO+LAC assay for the same (–)-epicatechin concentration. Second, the modified POD assay based on (–)-epicatechin and H_2O_2 was altered once more by changing the pH to acidic conditions (McIlvaine buffer, pH 3) that inhibited the phenol oxidases (*cf.* 3.1). For the two modified POD assays, which were performed in duplicate, the absorbance was measured at 390 nm directly after adding the H_2O_2 solution. The reading was corrected by subtracting the respective enzyme and substrate blanks. The enzyme activities were calculated, using the molar absorptivity for oxidized (−)-epicatechin (ε_{390nm}; *cf.* 2.3.3).

2.3.3. *Enzyme activity assay for polyphenol oxidases plus laccases*

The total activity of the phenol oxidases toward the litchi-specific main substrate was quantitated in duplicate, using the previously applied spectrophotometric assay based on (–)-epicatechin (Reichel et al., 2013). The assay conditions given below were the result of experiments on (1) the appropriate substrate and sodium dodecyl sulfate (SDS) concentrations, (2) the optimal wavelength, and (3) the respective molar absorptivity, as detailed in the Supporting information (Figs. S1–S4). Due to the use of crude enzyme extracts and the natural substrate, the assay cannot differentiate between PPO (1,2-benzenediol:oxygen oxidoreductase; EC 1.10.3.1) and LAC (benzenediol:oxygen oxidoreductase; EC 1.10.3.2) enzyme activities, but natural pericarp browning processes involving both types of phenol oxidases can be mimicked.

For the standard PPO+LAC assay, a reaction mixture (2 mL) containing (–)-epicatechin (1.3 mmol L^{-1}), SDS (0.6 mmol L^{-1}), and the enzyme extract (0.05 L L^{-1}) was incubated for 10 min at 25 °C in McIlvaine buffer (pH 6.5). According to consecutive absorption spectra for the enzymatic oxidation of (–)-epicatechin at this pH (Fig. S2 of the Supporting information), spectrophotometric analysis was performed at 390 nm in intervals of 7 s for 10 min. Based on the respective enzyme and substrate blanks and the molar absorptivity ε_{390nm} (702,173 L mol^{-1} m^{-1}; *cf.* Fig. S3B), the total activity of the phenol oxidases (PPO+LAC, kat kg^{-1}) was calculated as described in 2.3.2 for *POD*, using Eq. (1) by analogy after adaptation.

2.3.4. *Modified enzyme inhibition assays for testing organic acids*

Since oxalic and citric acid were reported to serve as chelating agents and browning inhibitors for fruit (Ducamp-Collin et al., 2008; Ruíz-Jiménez et al., 2014), they were included in the *in vitro* trials. Because malic acid is the major acid in litchi fruit (Reichel et al., 2010), it was considered as well. For the comparison with a monocarboxylic acid of low molar mass, acetic acid was chosen. Except oxalic acid, the substances are commonly used as food additives and are generally recognized as safe (GRAS).

The standard assays for POD and the phenol oxidases (*cf.* 2.3.2–2.3.3) were adapted to study the inhibiting effects that acetic, citric, malic, and oxalic acid might have on the enzyme activities, by replacing the previously used McIlvaine buffer (pH 6.5) accordingly. Both the tropolone/H_2O_2 solution for the POD assay and the SDS solution for the PPO+LAC assay were thus prepared in acetic acid/acetate, citric acid/citrate, malic acid/malate, and oxalic acid/oxalate buffer, respectively. The ionic strength *I*, which depends on the concentration c_i and the valency z_i of all *j* kinds of ions *i* in the buffer solution, was adjusted to $I = 0.98$ mol L^{-1} according to Eq. (2) to equalize any effects of this type on the enzymes.

$$I = \frac{1}{2} \sum_{i=1}^{j} (c_i \cdot z_i^2) \quad (2)$$

To study the pH-induced effects on the POD and the total PPO +LAC activity in the range of pH ~2.5–7, the two assays were performed in acetic acid/acetate buffer (0.98 mol L^{-1}) at 8 pH levels between pH 3.5 and 6.9, in citric acid/citrate buffer (0.16 mol L^{-1}) at 6 pH levels between pH 2.5 and 5.5, in malic acid/malate buffer (0.33 mol L^{-1}) at 7 pH levels between pH 2.4 and 5.3, and in oxalic acid/oxalate buffer (0.33 mol L^{-1}) at pH 3.5 and 3.9, respectively. Different phosphate buffers (0.16 mol L^{-1}) of the same ionic strength were used for assays in the range of pH 6 to 7.5 to

include neutral to slightly alkaline conditions. For comparison, McIlvaine buffers were applied, covering the whole range from pH 3 to 7.8.

Furthermore, the influence of the acid concentration on the enzyme activities was studied by varying the buffer concentration of the assays (25, 50, 100, 250, and 500 mmol L^{-1}) at two constant pH levels (pH 3.5 ± 0.1; pH 4.9 ± 0.1). For these buffers, suitable solutions of trisodium citrate and citric acid were mixed at ratios (w/w) of 0.63:1 (pH 3.57 ± 0.07) and 2.97:1 (pH 5.00 ± 0.17). The respective ratios (w/w) were 0.17:1 (pH 3.51 ± 0.06) and 4.82:1 (pH 4.92 ± 0.03) for sodium acetate and acetic acid, 0.78:1 (pH 3.56 ± 0.07) and 5.80:1 (pH 4.91 ± 0.11) for disodium malate and malic acid, and 2.26:1 (pH 3.56 ± 0.09) for disodium oxalate and oxalic acid. Due to low solubility, oxalate buffer at pH 4.9 was unavailable.

All assay variants were carried out in duplicate, using a fresh enzyme extract per day. The buffer in question was also applied to both the enzyme blank and the substrate blank that were needed for each assay variant. The enzyme activities were calculated as described in 2.3.2–2.3.3. Residual enzyme activities (in %) were computed relative to the activities that the standard assays according to 2.3.2–2.3.3 on the basis of McIlvaine buffer (pH 6.5, I = 1.02 mol L^{-1}) had yielded on the same day for the same enzyme extract.

2.3.5. Modified enzyme inhibition assays for testing chloride salts

Likewise, the standard assays of 2.3.2–2.3.3 were modified to study the effects of sodium chloride and calcium chloride on the POD and the PPO+LAC activities. Instead of the McIlvaine buffer, acetate buffer (0.1 mol L^{-1}) of pH 5.5 (pH value according to Fig. 1 in 3.1.1) was used to prepare the substrate solution for the POD assay and the SDS solution for the PPO+LAC assay in order to avoid any pH-dependent or chelating effects (*cf.* 3.1.1). The salt type studied was added to these solutions, with the final concentrations being 10, 25, 50, 100, and 250 mmol L^{-1} of total assay volume. The modified assays were carried out in duplicate. The corresponding enzyme blank and the substrate blank also contained the relevant salt concentration. Residual enzyme activities (in %) were relative to the activity found in acetate buffer on the same day for the same extract, but without salt addition.

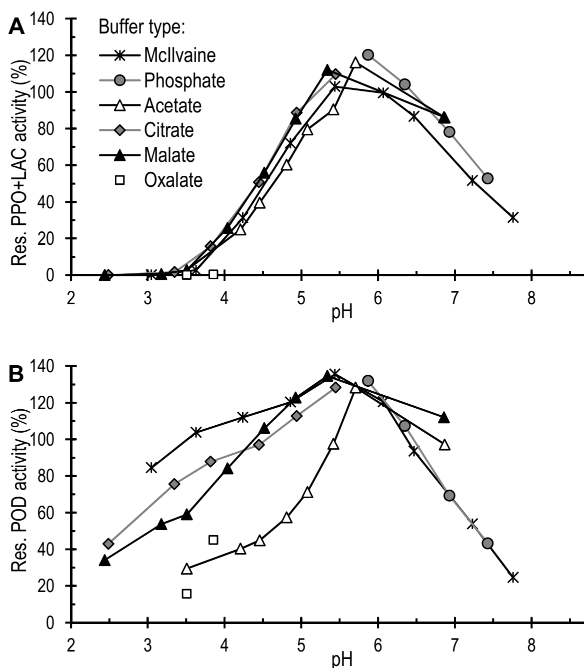


Fig. 1. *In vitro* inhibition of (A) the phenol oxidases [*i.e.*, polyphenol oxidase plus laccase (PPO+LAC)] and (B) peroxidases (POD) in crude enzyme extracts from litchi pericarp by organic acids that were applied in the form of the corresponding buffer systems replacing the reference buffer: Residual enzyme activities at different pH values and ionic strengths (I) of 0.98 mol L^{-1} relative to the respective reference activities (100%) in McIlvaine buffer of pH 6.5 (I = 1.02 mol L^{-1}). McIlvaine buffers of pH 3.05–7.76 (I = 0.72–1.17 mol L^{-1}) and phosphate buffers (I = 0.98 mol L^{-1}) were tested by analogy for comparison.

2.4. *In vivo* screenings of browning inhibitors

2.4.1. Treatments of fruit with browning inhibitors plus subsequent storage

Seven browning inhibitors were tested, each at three concentration levels. Dip solutions for these variants were prepared, using ultrapure water (Elgastat Optima 30 plus Elgastat Maxima; Elga, High Wycombe, UK). Solutions of 5, 25, and 50 mmol L^{-1} were produced from citric, oxalic, and malic acid, and from potassium citrate. For sodium chloride, calcium chloride, and hydrochloric acid, the three dip solutions always covered higher inhibitor concentrations (50, 100, and 200 mmol L^{-1}). Final pH values were measured (Professional Meter PP-50; Sartorius, Göttingen, Germany). All acidic inhibitor solutions were adjusted to pH 2.9 with NaOH (~750 g L^{-1}). Solutions representing the two lower concentration levels of a browning inhibitor were filled in portions of 0.3 L into impermeable polyamide/polyethylene (PA/PE) plastic bags (250 mm × 250 mm), which were sealed with a film welding machine NT 42 (Boss Verpackungsmaschinen, Bad Homburg, Germany) and stored at 4 °C, until they were used for the treatment of one fruit lot (10 fruits) per packaging unit. From solutions corresponding to the maximal inhibitor concentration level, bags containing 0.6 L were prepared by analogy for the later treatment of two lots (2 × 10 fruits) per pouch at the same time.

'Hong Huey' fruit of batch 1 (*cf.* 2.2) were treated with carboxylic acids or citrate on the first harvest day (16 variants). Chlorides were applied to 'Hong Huey' fruit of batch 2 (*cf.* 2.2) on the next day (12 variants). On each day, two fruit lots (controls) were treated with ultrapure water instead of an inhibitor solution. For each variant, 10 fruit were chosen randomly. Each fruit was marked with a number from 1 to 10, before its pericarp color was recorded (color value of the fresh fruit on day 0 before the treatment ["day 0_f"]; *cf.* 2.4.2).

In each case, fruit were first washed in drinking water (5–8 °C). Surplus water was removed by centrifugation in salad spinners. Mechanical peel damage was prevented by filling the fruit into textile bags for the spinning action. For the treatment, the 10 fruits of a lot were completely immersed into the cooled inhibitor solution in the prepared bag, which was immediately resealed and kept in a water bath (5 °C) for 15 min. Surplus solution was removed in the salad spinner, with textile bags protecting the fruit from mechanical peel damage. After passive drying at room temperature (~20 min), weight and pericarp color of each fruit were determined again to characterize the fruit on day 0 directly after the treatment ("day 0_t").

Subsequently, the ten fruit of a lot (treatments or controls) were loosely spread on a plastic plate that was packed into a 250 mm × 250 mm polyamide pouch 35 PA 40 (Amcor Flexibles Europe, Bristol, UK) having a film thickness of 35 μm and a permeability (5 °C) of 0.025 nmol m^{-2} s^{-1} Pa^{-1} for O_2 and 0.030 nmol m^{-2} s^{-1} Pa^{-1} for CO_2. This type of foil bag was chosen, because we found in preliminary experiments that gas permeability was high enough to afford protection against desiccation without modifying the atmosphere. The bag was not sealed; its open side was just folded down and placed under the plate to facilitate frequent sampling. Fruit were stored for 21 d (5 °C, 90% RH) in a climate simulator CAT 610/620 (Contherm Scientific, Lower Hutt, New Zealand). On day 13, the foil bag was removed to continue fruit storage in the climate

simulator without packaging. Pericarp color was monitored throughout storage (*cf.* 2.4.2).

In addition, the ten fruit of the second lot, which had resulted from treatments with an inhibitor at its maximal concentration level, and those of the other control lot of each harvest day were stored for 9 d under the same conditions, but without packaging in foil bags.

2.4.2. Color measurement

The pericarp color of each fruit was determined on day 0 both before ("day 0_f") and after the treatment ("day 0_t"). Initial changes in pericarp color upon storage were covered by daily monitoring until day 5, long-term browning effects by further analyses on the days 9, 13, 17, and 21. CIELab color values were measured on three fixed points along an equatorial line around the fruit, using a colorimeter CR 300 (Minolta, Osaka, Japan) set to the D65 illuminant and 2° standard observer angle. The litchi maturity index (*LMI*, Eq. (3)) was calculated from the color values of each lot on the day of harvest before the treatment ("day 0_f") according to Reichel et al. (2010). The L^*, a^*, and b^* color values that had been obtained for each fruit lot at a time ($n = 10 \times 3 = 30$ records per type) were subjected to outlier detection. For this purpose, nearest-neighbor clustering was applied to these three-dimensional data sets. The CLUSTER procedure of SAS 9.1 (method option SINGLE) was customized to exclude 5 outliers (~15%; trim = 15) by means of the density function. The 25 color value sets that had the closest distance to their 25 nearest neighbors ($k = 25$) out of 30 were thus identified. These data sets yielded the postharvest litchi color index (*PLCI*, Eq. (4); Reichel et al., 2010) per variant and storage day. *PLCI* differences revealing pericarp browning during storage were computed for each lot relative to the *PLCI* value on day 0 before the treatment of those fruits ("day 0_f").

$$LMI = 10\frac{a^*}{\sqrt{(a^*)^2 + (L^*)^2}} \quad (3)$$

$$PLCI = \sqrt{(a^*)^2 + (L^*)^2} \quad (4)$$

2.5. Statistical analyses

Significance ($P \le 0.05$) of treatment effects *in vitro* and *in vivo*, respectively, was detected by means of Tukey's multiple comparison tests, using SAS 9.1 (SAS Institute, Cary, NC). Least significant differences (Tukey $HSD_{0.05}$) and Tukey grouping are indicated in Tables 1–4 for each effect. Replications, repeats, and the numbers of observations were as detailed in 2.3 for the *in vitro* tests and in 2.4 for the *in vivo* trials.

Table 1
Effects of different organic acids on the activities of litchi pericarp phenol oxidases [*i.e.*, polyphenol oxidase plus laccase (PPO+LAC)] and peroxidases (POD) at pH 3.5 and pH 4.9 after *in vitro* application in the form of buffer solutions of varying concentration (c_i) and ionic strength (I_i).

Buffer type	c_i	I_i	Residual PPO+LAC activity [%][a]						Residual POD activity [%][a]					
	[mmol L^{-1}]		pH 3.5 ± 0.1			pH 4.9 ± 0.1			pH 3.5 ± 0.1			pH 4.9 ± 0.1		
Citric acid/citrate	25	150	25.5	b[b]	B[c]	111.2	a	A	111.7	a	B	132.4	a	A
	50	300	17.4	c	B	101.1	b	AB	100.8	b	BC	127.3	ab	A
	100	600	9.9	d	C	96.6	b	A	92.7	c	A	117.4	abc	A
	250	1500	4.3	e	B	88.6	c	A	66.4	d	C	111.5	bc	A
	500	3000	1.7	f	B	77.3	d	B	48.7	e	B	104.9	c	B
$HSD_{0.05}$[b]				2.0			4.6			7.1			17.7	
Acetic acid/acetate	25	25	33.1	b	A	101.8	a	B	124.1	a	A	146.3	a	A
	50	50	24.1	c	A	94.0	bc	B	110.6	b	A	136.6	a	A
	100	100	15.5	d	A	92.7	c	B	96.8	c	A	120.8	b	A
	250	250	6.9	e	A	88.5	cd	A	77.1	d	A	110.5	bc	A
	500	500	5.3	e	A	86.4	d	A	55.3	e	AB	99.7	c	B
$HSD_{0.05}$[b]				5.5			6.1			6.9			13.4	
Malic acid/malate	25	75	27.2	b	AB	103.4	a	B	104.8	a	B	134.3	ab	A
	50	150	18.1	c	B	104.5	a	A	104.8	a	AB	136.7	a	A
	100	300	12.2	d	B	93.7	c	AB	95.5	b	A	118.9	c	A
	250	750	5.0	e	B	89.1	d	A	70.8	c	B	119.0	c	A
	500	1500	1.7	f	B	79.8	e	AB	57.6	d	A	129.3	b	A
$HSD_{0.05}$[b]				1.8			2.3			5.8			5.6	
Oxalic acid/oxalate	25	75	1.8	b	C				108.2	a	B			
	50	150	0.2	b	C				92.8	c	C			
	100	300	0.03	b	D				53.4	d	B			
	250	750	0.02	b	C				16.5	e	D			
$HSD_{0.05}$[b]				3.0						6.5				
$HSD_{0.05}$[c]	25				6.9			4.3			9.2			21.6
	50				1.6			7.2			8.6			13.1
	100				1.3			3.0			7.1			3.6
	250				1.3			3.2			3.1			17.0
	500				0.8			6.8			6.7			14.7

[a] Residual enzyme activities ($n = 2$) are expressed as a percentage of the respective reference activities that were yielded by the standard activity assays for the same crude enzyme extract in McIlvaine buffer (pH 6.5; ionic strength: 1020 mmol L^{-1}).
[b] Residual enzyme activities, which differed ($P \le 0.05$) by not less than the honestly significant difference ($HSD_{0.05}$) from the reference activity (control, 100%) and from each other for a given buffer type, pH value, and enzyme due to the concentration of this buffer, are marked by different lower case letters (a–f) vertically for this buffer type.
[c] Residual enzyme activities, which differed ($P \le 0.05$) from each other by not less than the honestly significant difference ($HSD_{0.05}$) due to the buffer type for a given concentration and pH, are marked by different upper case letters (A–D) vertically for the 3–4 analogous solutions of the four buffer types.

Table 2
Effects of fruit treatments with enzyme inhibitors (maximal doses) on pericarp color ($n = 30$) during subsequent storage (5 °C, 90% RH) *without* foil wrapping.

Treatment	Fresh fruit directly after harvest[e] (day 0_f)	PLCI [][a,b,c,d] Storage time (d) after the fruit treatment with inhibitor solution: 0_t	1	2	3	4	5		$HSD_{0.05,PLCI}$[b]
Fruit batch 1 (size: 18.3 ± 0.3 g/fruit, litchi maturity index (*LMI*): 6.4 ± 0.5 at harvest)									
H_2O (control 1a), pH 5.5	51.2 A[b] (100)	45.2 B (88) b[c] α[d]	48.9 A (96) abc α	45.0 B (88) a α	38.4 C (75) a β	35.5 D (70) a β	35.1 D (69) a β	34.6 D (68) a β	2.8
Malic acid, pH 2.9 (50 mmol L^{-1})	50.8 A (100)	51.4 A (102) a α	47.3 B (94) bc α	39.3 C (78) b β	34.9 D (69) ab β	34.9 D (69) a β	34.8 D (69) a β	35.8 D (71) a β	3.0
Oxalic acid, pH 2.9 (50 mmol L^{-1})	50.8 A (100)	50.5 A (99) a α	46.1 B (91) c β	39.7 C (78) b β	34.0 D (67) b β	33.2 D (65) a β	33.0 D (65) a β	33.9 D (67) a β	2.5
Citric acid, pH 2.9 (50 mmol L^{-1})	50.5 A (100)	51.1 A (101) a α	49.9 A (99) a β	44.4 B (88) a β	36.8 C (73) ab β	35.5 CD (70) a β	32.9 E (65) a β	34.3 DE (68) a β	2.2
Citrate, pH 9.1 (50 mmol L^{-1})	49.7 A (100)	48.4 A (98) a α	48.3 A (97) ab β	43.7 B (88) a β	36.5 C (74) ab β	34.4 D (70) a β	32.7 D (66) a β	32.8 D (66) a β	2.1
$HSD_{0.05,rel.PLCI}$[c]		(4.7)	(5.5)	(7.1)	(6.8)	(5.1)	(4.4)	(4.9)	
Fruit batch 2 (size: 17.0 ± 0.3 g/fruit, *LMI*: 6.5 ± 0.4 at harvest)									
H_2O (control 2a), pH 5.4	49.7 A (100)	48.3 A (97) a α	41.8 B (84) c β	38.8 CD (78) bc β	36.4 E (73) b β	36.9 DE (74) a β	37.2 DE (75) a β	39.7 BC (80) a β	2.1
NaCl, pH 6.1 (200 mmol L^{-1})	51.0 A (100)	49.4 A (97) a α	47.1 B (92) a α	46.4 B (91) a β	40.3 C (79) a β	35.7 D (70) a β	34.1 D (67) b β	35.5 D (70) b β	2.1
$CaCl_2$, pH 5.6 (200 mmol L^{-1})	51.0 A (100)	48.8 A (96) a α	44.6 B (88) bc β	38.5 C (75) c β	35.1 D (69) c β	36.1 D (71) a β	35.4 D (69) b β	36.2 CD (71) b β	2.3
HCl, pH 2.5 (200 mmol L^{-1})	51.7 A (100)	50.7 A (98) a α	46.7 B (90) ab β	41.4 C (80) b β	38.0 D (74) b β	38.2 D (74) a β	38.6 CD (75) a β	39.3 CD (76) a β	2.9
$HSD_{0.05,rel.PLCI}$[c]		(3.3)	(4.7)	(4.6)	(4.3)	(4.1)	(4.0)	(4.1)	

[a] Postharvest litchi color index (*PLCI*, Eq. (4)) of freshly harvested fruit on day 0 both before (0_f) and after (0_t) immersion into different inhibitor solutions and after 1, 2, 3, 4, 5, and 9 d of storage (in parentheses: this index as a percentage of the *PLCI* level of the fresh fruit before (0_f) the treatment of the respective fruit batch subsample).
[b] *PLCI* values, which differed ($P \leq 0.05$) from each other by not less than the honestly significant difference ($HSD_{0.05,PLCI}$) due to the storage time of a given subsample, are marked by different upper case letters (A–E) horizontally.
[c] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other between subsamples at a given storage time due to the type of inhibitor solution (treatment) by not less than the honestly significant difference ($HSD_{0.05,rel.PLCI}$), are marked by different lower case letters (a–c) vertically (for the variants of the same fruit batch).
[d] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other by not-less than the honestly significant difference ($HSD_{0.05}$ not shown) for a given treatment and storage time due to the kind of fruit packaging during storage [*i.e.*, either non-application (this Table 2) or application of a foil pouch (Table 3 for fruit batch 1, Table 4 for fruit batch 2)], are marked in the present table by different Greek letters in italics (*α*, *β*).
[e] In this column, every *PLCI* value refers to the fruit of that subsample, which was used per batch for the respective treatment.

3. Results and discussion

3.1. In vitro *enzyme inhibition by carboxylic acids*

3.1.1. The optimum pH levels enabling maximal enzyme activities

The selection of the reference buffer (McIlvaine buffer, pH 6.5) for enzyme extraction (*cf.* 2.3.1) was consistent with the reported long-term stability of PPO in the range of pH 6–8 (Liu et al., 2007). By its use for the standard activity assays (2.3.2–2.3.3), the improved stability of the phenolic reaction products under slightly acidic conditions, which might prevent (−)-epicatechin from autoxidation (Mochizuki et al., 2002), was considered. Substitution of the reference buffer at constant ionic strength by a buffer of pH 5.8 ± 0.1, on the basis of either acetic acid/acetate or mono- and dihydrogen phosphate, still increased the total PPO+LAC activity of pulverized pericarp to the maximal level of 567 ± 13 μkat kg^{-1} (118% of the reference value; Fig. 1A). For litchi LAC, optimum pH values have not been described yet, but (−)-epicatechin degradation by LAC from avocado pericarp infected by *Colletotrichum gloeosporioides* has been shown to be maximal at similar pH (Guetsky et al., 2005). In contrast, pericarp PPO of different litchi cultivars have been reported to be most active between pH 6.5 and pH 7.5, depending on the substrate and detection method used (Jiang et al., 1997; Liu et al., 2007; Sun et al., 2008). However, these authors also described a secondary peak activity between pH 5–6. Whether the PPO or the LAC activities prevailed in the crude enzyme extract of the present study is unknown, but enzymatic (−)-epicatechin oxidation was obviously favored under slightly acidic rather than neutral conditions (Fig. 1A). For comparison, the vacuolar pH of pericarp had been concluded to be ~pH 4 for fully ripe litchis harvested 103 d after anthesis (Underhill and Critchley, 1992).

Irrespective of the buffer system, maximum POD activity of the pulverized pericarp (626 ± 21 μkat kg^{-1}) was at pH 5.6 ± 0.2. This peak of activity was 34% higher than the reference value at pH 6.5 (Fig. 1B). Maximum activity of partially purified POD was stated to be at pH 6.8 (Gong and Tian, 2002) or pH 6.5 (Mizobutsi et al., 2010). In general, the activities found for crude enzyme extracts result from different POD isoenzymes that coexist in the plant tissue, but are active within their specific pH ranges (Gaspar et al., 1985). Soluble tomato POD, which was reported to play a major role in wound-induced cell wall rigidification, also had an optimum around pH 5 (Loukili et al., 1999).

3.1.2. Enzyme inhibition as a function of pH at constant ionic strength

Acidic and slightly alkaline conditions beyond the optimal range of pH 5.6–5.8 reduced the POD and the PPO+LAC activities (Fig. 1). Compared to the standard assay at pH 6.5, the residual PPO+LAC activity was only 31%, when the pH of the McIlvaine buffer was changed to pH 7.8 or 4.2 (Fig. 1A). Indeed, underestimation of the activity could not be excluded for the most acidic and the alkaline conditions due to probably increasing amounts of undetectable reaction products that chiefly absorb near 280 and 440 nm, respectively (Guyot et al., 1995, 1996; *cf.* Supporting information). However, pH changes generally influence the structural conformation of proteins and thus the catalytic activity of the enzymes. The first step of PPO catalysis is particularly sensitive to pH changes, because the unprotonated form of the enzyme with a bridging hydroxide between the two copper ions of the active site is needed (Siegbahn, 2004), while the dicopper core

Table 3
Effects of fruit treatments with enzyme inhibitors (various doses of organic acids or their salts) on pericarp color ($n = 30$) during subsequent storage (5 °C, 90% RH) *with* additional foil wrapping.

Treatment (fruit batch 1)		*PLCI* [][a,b,c,d] At harvest[e] (day 0_f)	Storage time (d) after the fruit treatment with inhibitor solution 0_t	1	2	3	9	13	17	21	$HSD_{0.05, PLCI}$[b]	a^* [][a,b] At harvest[e] (day 0_f)	Storage time (d) after the fruit treatment 0_t	1	2	13	17	21	$HSD_{0.05, a^*}$[b]
Malic acid, pH 2.9	0 mmol L^{-1}	51.8 A[b]	42.6 BC	44.6 B	42.8 BC	42.0 BC	43.0 BC	42.1 BC	36.8 D	30.9 E	2.7	34 A[b]	26 B	26 B	27 B	27 B	22 C	17 D	2.4
	(control 1b: H_2O)[f]	(100)	(82) b[c]	(86) c	(83) c	(81) c	(83) c	(81) c	(71) a	(60) b									
	5 mmol L^{-1}	50.8 A	48.8 AB	49.7 AB	49.0 AB	48.9 AB	48.4 AB	48.1 B	34.0 C	33.7 C	2.7	34 A	33 A	32 A	33 A	32 A	20 B	18 B	2.8
		(100)	(96) a α[d]	(98) ab α	(97) a α	(96) a α	(95) a α	(95) a α	(67) a α	(66) a α									
	25 mmol L^{-1}	51.1 A	49.9 AB	51.1 A	49.6 ABC	49.1 BCD	47.9 CD	47.4 D	33.7 E	33.1 E	1.9	33 A	32 AB	32 AB	31 ABC	29 D	18 E	16 E	2.3
		(100)	(98) a α	(100) a α	(97) a α	(96) a αβ	(94) ab α	(93) a αβ	(66) a αβ	(65) a α									
	50 mmol L^{-1}	52.4 A	49.5 B	49.6 AB	48.3 BC	47.5 BC	46.8 BC	45.9 C	34.4 D	32.8 D	2.9	35 A	32 B	31 B	31 B	29 B	20 C	17 D	3.0
		(100)	(94) a β	(95) b γ	(92) b β	(91) b β	(89) b β	(88) b γ	(66) a αβ	(63) ab γ									
	$HSD_{0.05,rel.PLCI}$[c]		(4.5)	(4.5)	(4.2)	(3.9)	(4.8)	(4.7)	(5.8)	(4.3)									
Oxalic acid, pH 2.9	0 mmol L^{-1}	51.8 A	42.6 BC	44.6 B	42.8 BC	42.0 BC	43.0 BC	42.1 BC	36.8 D	30.9 E	2.7	34 A	26 B	26 B	27 B	27 B	22 C	17 D	2.4
	(control 1b: H_2O)[f]	(100)	(82) b	(86) b	(83) b	(81) b	(83) b	(81) b	(71) a	(60) b									
	5 mmol L^{-1}	50.6 A	49.6 ABC	50.4 AB	49.6 ABC	48.8 ABC	47.5 BC	47.4 C	35.9 D	32.3 E	2.9	32 A	32 AB	31 ABC	31 ABC	29 BC	20 D	17 E	2.7
		(100)	(98) a α	(100) a α	(98) a α	(97) a α	(94) a α	(94) a α	(71) a α	(64) ab α									
	25 mmol L^{-1}	51.5 A	50.5 AB	51.3 A	50.3 AB	49.7 AB	48.2 BC	46.6 C	32.3 D	31.1 D	2.8	35 A	35 A	34 AB	34 AB	31 B	19 C	18 C	2.9
		(100)	(98) a α	(100) a α	(98) a α	(97) a αβ	(94) a α	(91) a β	(63) b β	(60) b β									
	50 mmol L^{-1}	52.2 AB	52.2 A	51.0 ABC	49.7 BCD	48.5 DE	48.7 CDE	46.4 E	33.1 F	34.1 F	2.5	34 AB	34 A	33 ABC	32 ABC	30 C	19 D	17 D	2.8
		(100)	(101) a α	(98) a βγ	(96) a αβ	(93) a αβ	(94) a αβ	(89) a βγ	(64) b β	(66) a βγ									
	$HSD_{0.05,rel.PLCI}$[c]		(3.9)	(4.5)	(4.5)	(5.0)	(5.6)	(5.2)	(6.7)	(4.2)									
Citric acid, pH 2.9	0 mmol L^{-1}	51.8 A	42.6 BC	44.6 B	42.8 BC	42.0 BC	43.0 BC	42.1 BC	36.8 D	30.9 E	2.7	34 A	26 B	26 B	27 B	27 B	22 C	17 D	2.4
	(control 1b: H_2O)[f]	(100)	(82) b	(86) b	(83) b	(81) b	(83) b	(81) b	(71) a	(60) b									
	5 mmol L^{-1}	49.2 A	48.4 AB	49.7 A	48.2 AB	47.4 AB	47.1 AB	46.2 B	34.3 C	32.3 C	2.8	30 A	31 A	31 A	32 A	29 A	19 B	17 C	2.8
		(100)	(98) a α	(101) a α	(98) a α	(97) a α	(96) a α	(94) a α	(70) a α	(66) a α									
	25 mmol L^{-1}	50.7 AB	50.2 ABC	51.2 A	49.4 ABC	49.3 ABC	48.8 ABC	48.1 C	34.8 D	34.9 D	2.5	30 A	31 A	31 A	30 A	29 A	19 B	18 B	2.5
		(100)	(99) a α	(101) a α	(97) a α	(97) a α	(96) a α	(95) a αβ	(69) a α	(69) a α									
	50 mmol L^{-1}	48.7 ABC	49.2 AB	49.9 A	47.8 ABCD	47.5 BCDE	46.3 DE	45.4 E	34.0 F	33.1 F	2.3	30 ABC	32 A	32 AB	31 AB	28 C	19 D	17 D	2.4
		(100)	(101) a α	(103) a α	(98) a α	(98) a α	(95) a α	(93) a αβ	(70) a α	(68) a αβ									
	$HSD_{0.05,rel.PLCI}$[c]		(4.2)	(4.1)	(3.9)	(4.7)	(4.5)	(4.7)	(6.0)	(4.0)									
Citrate, pH 8.4–9.1	0 mmol L^{-1}	51.8 A	42.6 BC	44.6 B	42.8 BC	42.0 BC	43.0 BC	42.1 BC	36.8 D	30.9 E	2.7	34 A	26 B	26 B	27 B	27 B	22 C	17 D	2.4
	(control 1b: H_2O)[f]	(100)	(82) b	(86) b	(83) b	(81) b	(83) b	(81) b	(71) a	(60) c									
	5 mmol L^{-1}	50.0 A	48.8 AB	50.0 A	49.4 AB	48.3 AB	47.1 B	47.2 B	33.7 C	31.8 C	2.6	31 A	31 A	31 AB	31 A	28 B	19 C	17 C	2.7
		(100)	(98) a α	(100) a α	(99) a α	(97) a α	(95) a α	(95) a α	(68) a α	(64) bc α									
	25 mmol L^{-1}	49.4 A	47.7 AB	49.1 AB	46.9 AB	46.1 B	46.5 AB	47.3 AB	34.3 C	32.1 C	3.1	32 A	31 A	31 A	30 A	29 A	20 B	17 B	3.4
		(100)	(97) a α	(100) a α	(95) a α	(93) a β	(94) a α	(96) a α	(69) a α	(65) b α									

Table 3 (*Continued*)

Treatment		*PLCI* [][a,b,c,d]									$HSD_{0.05,PLCI}$[b]	*a** [][a,b]							$HSD_{0.05,a^*}$[b]
(fruit batch 1)		At harvest[e] (day 0_f)	Storage time (d) after the fruit treatment with inhibitor solution									At harvest[e] (day 0_f)	Storage time (d) after the fruit treatment						
			0_t	1	2	3	9	13	17	21		(day 0_f)	0_t	1	2	13	17	21	
	50 mmol L^{-1}	49.4 A (100)	48.0 AB (97) a αβ	49.3 A (100) a αβ	48.0 AB (97) a α	47.2 B (96) a α	46.8 B (95) a α	46.9 B (95) a α	32.7 C (66) a αβ	34.3 C (70) a α	2.1	32 AB	32 A	31 AB	30 AB	28 C	18 D	17 D	2.0
	$HSD_{0.05,rel.PLCI}$[c]		(4.8)	(4.2)	(4.0)	(3.9)	(5.1)	(5.1)	(6.3)	(4.4)									
$HSD_{0.05,rel.PLCI}$[d]	5 mmol L^{-1}		(4.2)	(4.7)	(4.5)	(4.7)	(5.1)	(4.2)	(6.8)	(4.4)									
	25 mmol L^{-1}		(4.4)	(4.2)	(4.0)	(3.7)	(4.2)	(4.8)	(5.4)	(4.4)									
	50 mmol L^{-1}		(4.8)	(4.3)	(4.9)	(5.1)	(5.0)	(5.2)	(4.7)	(4.0)									

[a] Postharvest litchi color index (*PLCI*) and the CIE color value *a** (redness) of freshly harvested fruit (batch 1: 18.3 ± 0.3 g/fruit, *LMI* = 6.4 ± 0.5) on day 0 both before (0_f) and after (0_t) immersion into different inhibitor solutions and after 1, 2, 3, 9, 13, 17, and 21 d of storage (in parentheses: this index as a percentage of the *PLCI* level of the fresh fruit before (0_f) the treatment of the respective fruit batch subsample). After the fruit analyses on day 13, fruit storage was continued until day 21 without foil wrapping.

[b] Values (*PLCI* and *a**, respectively), which differed ($P \leq 0.05$) from each other by not less than the honestly significant difference ($HSD_{0.05,PLCI}$ and $HSD_{0.05,a^*}$, respectively) due to the storage time of a given subsample, are marked by different upper case letters (A–F) horizontally.

[c] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other between subsamples for a given inhibitor type and storage time due to the concentration of the inhibitor solution by not less than the honestly significant difference ($HSD_{0.05,rel.PLCI}$), are marked by different lower case letters (a–c) vertically within an inhibitor group.

[d] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other for a given inhibitor concentration and storage time due to the type of inhibitor by not less than the honestly significant difference ($HSD_{0.05,rel.PLCI}$), are marked by different Greek letters (α–γ) for the four analogous samples within the same column.

[e] In this column, every value (*PLCI* or *a**) refers to the fruit of that fruit batch subsample, which was used for the respective treatment.

[f] Control treatment at pH 5.5 (control 1b).

is stabilized by six histidine ligands (Eicken et al., 1999). LAC, which contains three different copper centers in its active site, even requires interaction of four copper ions being stabilized by ten histidine imidazoles in total (Morozova et al., 2007). When the imidazole group of the basic amino acid histidine is protonated at pH << 6, the active sites of these enzymes are destabilized. This may cause irreversible deactivation at pH ≤ 4.5 (Liu et al., 2007). The curve described by Jiang et al. (1997) for the cultivar 'Mauritius' was similar to those of Fig. 1A, but revealed complete inactivation already at pH 4.2 in the presence of the substrate 4-methylcatchol. In contrast, partially purified PPO of Brazilian 'Bengal' litchi showed activity toward the same substrate over a wider range from pH 3 to 9 (Mizobutsi et al., 2010). The residual activity toward (−)-epicatechin was even ≥ 60% at pH 4 and pH 8 for partially purified PPO of the Chinese cultivar 'Guiwei' (Sun et al., 2008). However, (−)-epicatechin degradation by LAC extracted from avocado pericarp after fungal infection was more than halved already at pH 4.8 (Guetsky et al., 2005). For PPO and LAC from pericarp of the Thai 'Chacapat' litchis, complete inactivation (insignificant residual activity ≤ 3%) was observed at pH ≤ 3.5 ± 0.1 in all buffer systems, except for the oxalate buffer (Fig. 1A). When the latter was used, inactivation was already found at pH 3.9. All in all, the type of the buffer – unlike its pH value – hardly had an effect on the total PPO+LAC activity at constant ionic strength.

As known for horseradish POD (Bovaird et al., 1982), the activity of litchi pericarp POD was markedly affected by both the pH and the type of the buffer at constant ionic strength (Fig. 1B). For the 0.33 M malate and the 0.16 M citrate buffers, the pH-dependent effects were similar. Accordingly, POD might still be active (~34–43%) around pH 2.5. Unlike the phenol oxidases, POD was not inactivated completely. The lowest residual activity (16% of the reference value) occurred in 0.33 M oxalate buffer at pH 3.5 (Fig. 1B). However, a slight increase to pH 3.9 for the same buffer raised the residual POD activity to 45%, which was already found at pH 4.5 for 0.98 M acetate buffer. Given constant ionic strengths, the acetate buffers were the most concentrated solvents. Acetate may replace water in the active site of POD (Berglund et al., 2002) and thus interfere with the catalytic cycle. A broad pH profile for litchi pericarp POD as shown in Fig. 1B was also found by Gong and Tian (2002), who detected residual activity even at pH 1. In contrast, Mizobutsi et al. (2010) reported complete inactivation of partially purified POD at pH values ≤ 2.5 and ≥ 8.5. The active site of these class III secretory plant POD consists of a central iron(III) ion that is coordinated to a porphyrin ring (heme b) and to one proximal histidine residue (Berglund et al., 2002; Hiner et al., 2002). The structure depends on an extensive hydrogen bond network, which maintains the active conformation of the domains above and below the heme plane and can be perturbed by pH changes (Welinder, 1985). The catalytic cycle is also pH-dependent, because an acid-base mechanism operates during the formation of the first intermediate, which is the POD compound I containing an oxyferryl heme iron center [Fe(IV)=O] and a heme porphyrin π-cation radical (Hiner et al., 2002). However, the distal histidine in the active site can act as an acid-base catalyst over a wide pH range, because it is stabilized and assisted by the arginine of the distal pocket (Hiner et al., 2002). Consistently, POD was highly active over a wide acidic pH range in McIlvaine buffer (at pH 3.1 ~85% of the reference value), but pH values > 6 caused notable deactivation until a residual activity of 25% at pH 7.8 (Fig. 1B). Different activities as a function of the buffer also occurred around the neutral point, as shown by the findings for malate, acetate, and phosphate buffer at pH 6.9. Strong inactivating effects of the bulky hydrogen phosphate ion may be due to distortions in the heme pocket geometry and binding to cationic sites of the enzyme such as the distal arginine (Laurenti et al., 2000).

Table 4
Effects of fruit treatments with enzyme inhibitors (various doses of chloride salts or HCl) on pericarp color ($n = 30$) during subsequent storage (5 °C, 90% RH) *with* additional foil wrapping.

Treatment		*PLCI* [][a,b,c,d]									$HSD_{0.05,PLCI}$[b]	*a** [][a,b]							$HSD_{0.05,a^*}$[b]
(fruit batch 2)		At harvest[e] (day 0_f)	Storage time (d) after the fruit treatment with inhibitor solution									At harvest[e] (day 0_f) (day 0_f)	Storage time (d) after the fruit treatment						
			0_t	1	2	3	9	13	17	21			0_t	1	2	13	17	21	
NaCl, pH 5.7–6.1	0 mmol L^{-1}	49.9 A[b]	48.9 A	48.6 A	49.8 A	48.6 A	48.2 A	48.3 A	35.5 B	33.7 B	2.6	33 A[b]	33 A	32 AB	31 AB	31 AB	20 C	17 C	2.9
	(control 2b: H_2O)[f]	(100)	(98) b[c]	(97) ab	(100) ab	(98) bc	(97) a	(97) a	(71) ab	(68) a									
	50 mmol L^{-1}	50.2 AB	50.3 AB	49.4 ABC	50.9 A	50.0 ABC	48.4 BC	47.8 C	35.6 D	33.2 E	2.3	34 A	34 A	33 A	34 A	32 A	22 B	20 B	2.6
		(100)	(101) ab α[d]	(99) a α	(102) a α	(100) ab α	(97) a α	(95) a α	(71) ab β	(66) a α									
	100 mmol L^{-1}	49.6 ABC	51.4 A	49.2 ABCD	50.8 AB	50.6 AB	47.8 CD	47.1 D	37.9 E	33.7 F	2.4	32 A	33 A	32 A	32 A	30 A	23 B	18 C	2.4
		(100)	(104) a α	(99) a α	(103) a α	(102) a α	(96) a α	(95) a α	(77) a α	(68) a α									
	200 mmol L^{-1}	52.5 A	50.5 ABC	49.0 BCD	50.9 AB	50.0 ABC	47.0 D	48.8 BCD	35.0 E	35.4 E	2.6	34 AB	35 A	33 ABC	33 ABC	34 AB	21 D	20 D	2.5
		(100)	(96) b α	(93) b α	(97) b α	(95) c α	(90) b α	(93) a α	(67) b β	(67) a α									
	$HSD_{0.05,rel.PLCI}$[c]		(4.7)	(4.3)	(4.5)	(4.3)	(3.5)	(4.8)	(6.1)	(4.1)									
$CaCl_2$, pH 5.5–5.6	0 mmol L^{-1}	49.9 A	48.9 A	48.6 A	49.8 A	48.6 A	48.2 A	48.3 A	35.5 B	33.7 B	2.6	33 A	33 A	32 AB	31 AB	31 AB	20 C	17 C	2.9
	(control 2b: H_2O)[f]	(100)	(98) a	(97) a	(100) a	(98) a	(97) a	(97) a	(71) ab	(68) a									
	50 mmol L^{-1}	50.1 A	48.1 AB	47.2 ABC	48.6 AB	47.6 ABC	44.8 C	44.6 C	38.8 D	33.3 E	3.0	31 A	31 A	30 A	30 A	29 A	23 B	18 C	3.6
		(100)	(96) a β	(94) a β	(97) a β	(95) a β	(90) b β	(89) b β	(78) a α	(67) a α									
	100 mmol L^{-1}	51.3 A	49.2 ABC	48.8 ABC	49.9 AB	49.3 ABC	45.8 D	47.7 BCD	34.7 E	32.4 E	2.6	34 A	34 A	33 AB	32 AB	31 B	22 C	19 D	2.9
		(100)	(96) a β	(95) a α	(97) a β	(96) a β	(90) b β	(93) ab α	(68) b β	(64) a $\alpha\beta$									
	200 mmol L^{-1}	50.6 A	48.7 ABC	48.4 BCD	49.8 AB	47.7 BCDE	44.6 F	45.9 EF	36.3 G	33.6 H	2.1	33 AB	34 A	33 AB	32 AB	29 D	22 E	19 F	2.5
		(100)	(96) a α	(96) a α	(99) a α	(94) a $\alpha\beta$	(88) b α	(91) b α	(72) ab α	(66) a α									
	$HSD_{0.05,rel.PLCI}$[c]		(4.1)	(3.5)	(3.6)	(4.0)	(4.7)	(4.6)	(7.1)	(4.3)									
HCl, pH 2.5–3.4	0 mmol L^{-1}	49.9 A	48.9 A	48.6 A	49.8 A	48.6 A	48.2 A	48.3 A	35.5 B	33.7 B	2.6	33 A	33 A	32 AB	31 AB	31 AB	20 C	17 C	2.9
	(control 2b: H_2O)[f]	(100)	(98) a	(97) a	(100) a	(98) a	(97) a	(97) a	(71) a	(68) ab									
	50 mmol L^{-1}	51.6 A	49.9 AB	49.2 ABC	50.1 AB	49.0 ABC	47.1 C	48.6 BC	37.1 D	35.8 D	2.5	32 A	32 A	32 AB	31 AB	30 AB	21 C	18 D	2.9
		(100)	(97) a $\alpha\beta$	(95) a $\alpha\beta$	(97) a β	(95) ab β	(92) b β	(94) ab α	(72) a $\alpha\beta$	(70) a α									
	100 mmol L^{-1}	51.6 A	49.2 ABCD	49.5 ABC	50.1 AB	48.0 BCD	46.4 D	46.8 CD	36.8 E	32.3 F	3.0	34 AB	34 A	34 A	33 ABC	30 C	23 D	19 E	3.1
		(100)	(95) a β	(96) a α	(97) a β	(93) b γ	(90) b β	(91) b α	(71) a $\alpha\beta$	(63) c β									
	200 mmol L^{-1}	51.6 A	50.0 A	49.2 ABC	49.6 AB	47.3 BCD	46.1 D	46.7 CD	35.4 E	32.9 E	2.7	34 AB	35 A	34 AB	33 ABC	29 D	22 E	18 F	2.7
		(100)	(97) a α	(96) a α	(96) a α	(92) b β	(89) b α	(91) b α	(69) a $\alpha\beta$	(64) bc α									
	$HSD_{0.05,rel.PLCI}$[c]		(4.5)	(3.8)	(4.1)	(4.1)	(4.8)	(5.4)	(6.9)	(4.6)									
$HSD_{0.05,rel.PLCI}$[d]	50 mmol L^{-1}		(4.2)	(3.4)	(3.6)	(4.1)	(4.0)	(3.8)	(6.4)	(4.1)									
	100 mmol L^{-1}		(4.0)	(4.1)	(4.2)	(3.1)	(4.3)	(4.8)	(6.8)	(4.5)									
	200 mmol L^{-1}		(3.4)	(3.7)	(3.7)	(3.0)	(3.9)	(4.2)	(4.3)	(3.7)									

[a] Postharvest litchi color index (*PLCI*) and the CIE color value *a** (redness) of freshly harvested fruit (batch 2: 17.0 ± 0.3 g/fruit, *LMI* = 6.5 ± 0.4) on day 0 both before (0_f) and after (0_t) immersion into different inhibitor solutions and after 1, 2, 3, 9, 13, 17, and 21 d of storage (in parentheses: this index as a percentage of the *PLCI* level of the fresh fruit before (0_f) the treatment of the respective fruit batch subsample). After the fruit analyses on day 13, fruit storage was continued until day 21 without foil wrapping.

[b] Values (*PLCI* and *a**, respectively), which differed ($P \leq 0.05$) from each other by not less than the honestly significant difference ($HSD_{0.05,PLCI}$ and $HSD_{0.05,a^*}$, respectively) due to the storage time of a given subsample, are marked by different upper case letters (A–F) horizontally.

[c] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other between subsamples for a given inhibitor type and storage time due to the concentration of the inhibitor solution by not less than the honestly significant difference ($HSD_{0.05,rel.PLCI}$), are marked by different lower case letters (a–c) vertically within an inhibitor group.

[d] Relative *PLCI* values, which differed ($P \leq 0.05$) from each other for a given inhibitor concentration and storage time due to the type of inhibitor by not less than the honestly significant difference ($HSD_{0.05,rel.PLCI}$), are marked by different Greek letters (α–γ) for the three analogous samples within the same column.

[e] In this column, every value (*PLCI* or *a**) refers to the fruit of that fruit batch subsample, which was used for the respective treatment.

[f] Control treatment at pH 5.4 (control 2b).

3.1.3. Enzyme inhibition at different buffer concentrations

Variation of the buffer concentration between 25 and 500 mmol L^{-1} for two pH levels (Table 1) confirmed that inhibition of the phenol oxidases chiefly depends on the pH value, as illustrated by Fig. 1A. Increasing ionic strength and the type of the buffer were less important. Regardless of whether pH 4.9 had been adjusted by means of acetate, citrate, or malate buffer, even buffer concentrations of 500 mmol L^{-1} reduced the PPO+LAC activity only by 14–23% relative to the reference value. By contrast, only 25 mmol L^{-1} was needed for these types of buffer at the lower pH value (pH 3.5) to achieve 67–75% inhibition (Table 1). At each pH level, increasing buffer concentrations enhanced inhibition of the phenol oxidases for all three buffer types, as indicated by the lower case letters in Table 1. Only oxalate buffer (pH 3.5) inhibited these enzymes completely (insignificant residual activity) already at the lowest concentration (25 mmol L^{-1}). Otherwise, complete deactivation at pH 3.5 required 20 times higher concentrations (500 mmol L^{-1}), when citrate or malate buffer was used. The corresponding acetate buffer still retained a residual activity of ~5% (Table 1). The greater inhibitory potential of malic, citric, and notably oxalic acid presumably resulted from their chelating capacity, and thus from the ability to block the di- and multicopper cores of PPO and LAC, respectively (Guetsky et al., 2005; Jiang et al., 1999; Yoruk and Marshall, 2003).

POD inhibition was always enhanced, when the buffer concentration was raised (Table 1). At pH 3.5, the strongest decrease in POD activity was caused by oxalate buffer, followed by citrate buffer. However, compared with the standard assay (McIlvaine buffer at pH 6.5 and the ionic strength of 1.02 mol L^{-1}), POD was still more active in the pH 3.5-buffers of low concentration (25 mmol L^{-1}; I = 0.025–0.150 mol L^{-1}). POD inhibition by 42–51%, attended with (almost) complete deactivation of the phenol oxidases, required either 100 mM oxalate buffer (I = 0.3 mol L^{-1}) or a 500 mM buffer based on malate, acetate, or citrate (I = 0.5–3 mol L^{-1}) for the adjustment to pH 3.5 (Table 1).

3.2. *In vitro* enzyme inhibition by chloride salts

According to the results of the buffer tests (*cf.* 3.1, Fig. 1), the McIlvaine buffer (pH 6.5) of the standard assays was replaced by 0.1 M acetate buffer (pH 5.5) for the *in vitro* trials concerning the effects of neutral salts on the enzyme activities (Fig. 2), in order to minimize chelating effects. With regard to eventual *in vivo* applications, only chlorides with GRAS status were tested (NaCl,

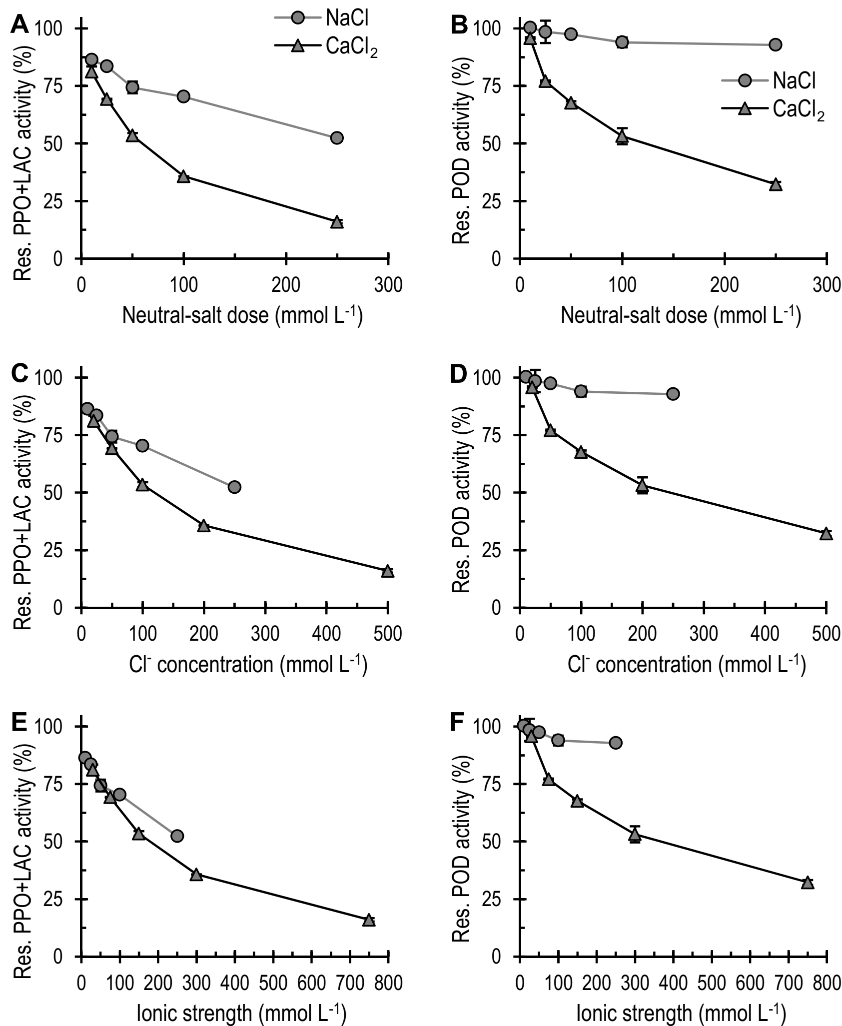


Fig. 2. *In vitro* inhibition of (A/C/E) the phenol oxidases [*i.e.*, polyphenol oxidase plus laccase (PPO+LAC)] and (B/D/F) peroxidases (POD) in crude enzyme extracts from litchi pericarp by chloride salts (NaCl, $CaCl_2$). Residual enzyme activities relative to the respective reference activities (100%) in the same buffer (acetate buffer, pH 5.5) without neutral salt were plotted against (A/B) the salt dose (*i.e.*, the effective concentrations of the halide salt cations), (C/D) the respective concentrations of the chloride anions, and (E/F) the resultant ionic strength.

$CaCl_2$). The enzyme activities were plotted against the ionic strength (Fig. 2E/F), the concentration of the chloride anion (Fig. 2C/D), and that of the respective cation (Na^+, Ca^{2+}) (Fig. 2A/B) to differentiate between these factors.

When applied in the same doses, $CaCl_2$ always caused greater reduction of total PPO+LAC activity than NaCl did at equal cation concentration (Fig. 2A). In terms of the absolute chloride concentrations in the assay, the differences between the two salts were less pronounced (Fig. 2C). Cl^- anions might interact with the dicopper center of PPO and replace the solvent ligand bridging the copper ions (Eicken et al., 1999). Litchi pericarp PPO has also been reported to be moderately inhibited by other halides such as fluoride (Liu et al., 2007), while such small inorganic anions are known to be LAC inhibitors (Morozova et al., 2007). The findings of Sun et al. (2008), who ascribed slight inhibition rather to Na^+ than to Cl^-, was not confirmed. According to the similar curves in Fig. 2E, however, the inhibiting effects of the two salts were likely to be caused by the associated ionic strength to a great extent.

In contrast, such an unspecific effect of the ionic strength could be excluded for POD (Fig. 2F). POD inhibition by chloride anions was also unlikely (Fig. 2D), because NaCl hardly showed any inhibitory effect on the activity of this enzyme. According to Gong and Tian (2002), NaCl did not inhibit litchi pericarp POD, even at doses >0.5 mol L^{-1}. Tolerance against ionic strength and NaCl was also reported for horseradish peroxidase (Bovaird et al., 1982), where Cl^- anions can enter the active site, but only bind to the heme core under acidic conditions (Laurenti et al., 2000). Being unbound, they were assumed to be too small to exert relevant constraints on the active-site geometry (Laurenti et al., 2000). Only $CaCl_2$ seemed to inhibit POD-catalyzed oxidation of tropolone (Fig. 2B). Being essential to maintain the conformation of active plant POD, calcium ions also promote the binding of this enzyme to membranes and play a regulatory role on its secretion and activation *in vivo* (Gaspar et al., 1985). According to Rasmussen et al. (1998), chloride ions are able to bind to the central iron of Ca^{2+}-activated barley peroxidases, resulting in a six-coordinate heme iron(III) ion, which decreased the formation rate of POD compound I with increasing Cl^- concentration. Reversible blocking of this first step of the catalytic cycle may have caused the initial lag phase found for POD, since rising $CaCl_2$ concentrations increased its duration by 0.6 s $mmol^{-1}$ L (R^2 = 0.95).

3.3. Involvement of (−)-epicatechin in reactions catalyzed by POD

For the pericarp phenol oxidases of five litchi cultivars (originally ascribed to PPO only), high affinity to (−)-epicatechin has previously been demonstrated by 12–77 times higher activities of crude enzyme extracts toward this natural key phenolic compound than toward 4-methylcatechol (Reichel et al., 2013). In view of (−)-epicatechin contents up to 19 g kg^{-1} (dry weight) in litchi pericarp (Reichel et al., 2011), involvement of this compound in the POD-catalyzed reduction of H_2O_2 may be of interest with respect to pericarp browning and the microcrack-induced formation of light brown surface scurf *in vivo* (Reichel et al., 2013). Similarly, the crude enzyme extracts used for the *in vitro* trials contained PPO, LAC, and POD. However, the substrate of the standard POD assay (tropolone) concomitantly inhibits phenol oxidases through copper chelation for quantitation of the sheer POD activity (Kahn and Andrawis, 1985). Conversely, in the absence of H_2O_2, the total activity of the oxygen-dependent phenol oxidases (PPO+LAC) is obtained exclusively.

When tropolone was replaced by (−)-epicatechin for comparative *in vitro* POD trials (*cf.* 2.3.2) at the flavanol concentration of the standard PPO+LAC assay (*cf.* 2.3.3), the total enzymatic conversion rate for (−)-epicatechin was 367 ± 12 μkat kg^{-1} of pericarp in the presence of H_2O_2. It was only 9% higher than the PPO+LAC activity (336 ± 5 μkat kg^{-1}) found in the absence of H_2O_2 for the same buffer (pH 6.5). According to this difference, the POD activity toward (−)-epicatechin as the phenolic donor was 31 μkat kg^{-1}. Likewise, the redox reaction of (−)-epicatechin and H_2O_2 involved a POD activity of 38 ± 2 μkat kg^{-1}, when the phenol oxidases were completely inhibited by lowering the pH of the McIlvaine buffer to pH 3 (*cf.* Fig. 1A in 3.1.2). Thus, (−)-epicatechin having a molar mass (M) of 290 g mol^{-1} may act as the phenolic donor in the catalytic cycle of litchi pericarp POD, but it is a co-substrate of limited importance for this enzyme. For comparison, the respective POD activities toward tropolone (M = 122 g mol^{-1}) were 14 and 10 times higher at pH 6.5 (437 μkat kg^{-1}) and pH 3 (390 μkat kg^{-1}), respectively. According to Loukili et al. (1999), the co-substrate provoking the highest activity of neutral tomato POD was coniferyl alcohol (M = 180 g mol^{-1}), followed by guaiacol (M = 124 g mol^{-1}) and pyrogallol (M = 126 g mol^{-1}). High affinity to guaiacol, followed by catechol and pyrogallol, has also been described for litchi POD (Pang et al., 2004). The preference of simple phenolic donors to C_6-C_3-C_6 polyphenols, such as (−)-epicatechin, is consistent with the greater involvement of POD in lignification and wound-induced cross-linking of cell wall compounds compared to its role in polyphenol oxidation (Reichel et al., 2013).

3.4. *In vivo* effects on color

Prevention of pericarp browning *in vivo* is far more complex than the direct *in vitro* interaction of pericarp enzymes, substrates, and inhibitors. Directly after harvest, mesocarp and aril, the two water reservoirs of the fruit, are protected against desiccation by the cuticles on the outer and inner pericarp surfaces (Riederer et al., 2015). Intact epidermis cells form a natural barrier for every protective treatment (Underhill and Critchley, 1992). Conditions have to be chosen in a way that active substances can enter injured surface tissues or form a depot on the surface for long-term protection. Concurrently, such treatments must not damage intact tissue or inhibit other enzymes that are vital for the fruit (Saengnil et al., 2006). In the *in vivo* trials, fruit were thus incubated for a rather long time (15 min) to enable sufficient absorption of active agents (Ketsa and Leelawatana, 1992), while temperature was only 5 °C to slow the metabolism and respiratory activity of the fruit. Only moderately concentrated inhibitor solutions, which contained the tested acid at pH 2.9 or the salt at pH 5.5–6.1 (chlorides) to pH 8.4–9.1 (citrate) (Tables 2–4), were chosen to maintain vital pericarp functions and to avoid damage of cell wall polymers (Carle et al., 2001; Saengnil et al., 2006). In this way, pH effects were to be set apart from more specific impacts by comparing moderately acidic and non-acidic application forms for one inhibitor type of each group. The pH of the acidic solutions was just below the hydration constants (pK_H; Stintzing et al., 2002) of the major pericarp anthocyanins (Reichel et al., 2013). At pH 2.9, the red flavylium cation of cyanidin 3-*O*-glucoside (pK_H 3.01, Stintzing et al., 2002) should thus prevail (>56%) compared to its colorless hemiketal. Acetic acid was not tested *in vivo*, since its vinegary smell might create the impression of decay.

3.4.1. Immediate effects of postharvest fruit treatments on pericarp color

Having mean sizes of 18.3 ± 0.3 and 17.0 ± 0.3 g/fruit, respectively, the fruit of the 'Hong Huey' batches 1 and 2 picked for these trials on the first and the next day (*cf.* 2.2) were much smaller than fruit from the same orchard a year earlier (21.3 g/fruit; Reichel et al., 2010). For small fruit, the risk of tension-induced pericarp microcracks provoking browning is lower (Underhill and Critchley, 1992). Consistently, extraordinary pinkish-red pericarp color was documented by high litchi maturity indices (*LMI*; Eq. (3)) of 6.4 ± 0.5 and 6.5 ± 0.4 for batch 1 and 2, respectively. In contrast,

the mean *LMI* was only 5.5 at the fully ripe, red stage for the larger 'Hong Huey' fruit of the year before (Reichel et al., 2010).

For the control lots 1a (Table 2) and 1b (Table 3), dipping into water and natural drying caused a *PLCI* drop by 12–18% on the same day after this placebo treatment (*cf.* values for 0_t versus 0_f). Accordingly, the fruit of batch 1 suffered early browning, which was associated (Eq. (4)) with a marked initial decline of the CIE red value a^* (Table 3). This also applied to one of the two lots dipped into 50 mM malic acid (Table 3). However, all other treatments with organic acids or citrate did not provoke such initial browning (Tables 2 and 3). The inhibitors might have directly permeated through microcracks that existed or developed upon immersion. Unlike the controls for fruit batch 1, the two control lots 2a (Table 2) and 2b (Table 4) of batch 2, which were harvested and treated on the next day, displayed *PLCI* and a^* values being similar before (day 0_f) and directly after the placebo treatment (day 0_t). For fruit batch 2, a very intact pericarp could thus be assumed, in conformity with the smaller fruit size. Consistently, initial constancy of *PLCI* and a^* was also found for the other lots of batch 2, which had been treated with chloride salts or HCl.

3.4.2. Impact of browning inhibitors on the pericarp color of unpacked fruit during cold storage

In concert with earlier studies (Reichel et al., 2010, 2011, 2013), the declining *PLCI* of fruit stored without foil bag reflected rapid and intense browning within the first 3 d despite the cold and damp climate of the storage chamber (5 °C, 90% RH), as indicated by upper case letters for the control lots 1a and 2a in Table 2. Without foil wrapping during storage, fruit dipping into organic acids was irrelevant for color retention, as denoted per day by lower case letters in Table 2 for the five lots of fruit batch 1. Citric acid and citrate retained the color only for one day, but resulted in higher *PLCI* levels until day 2 than malic and oxalic acid did. Oxalic acid, causing a *PLCI* decline by 9% until day 1 and by 33% until day 3, even accelerated browning compared to the control. On the contrary, Saengnil et al. (2006) noted that immersion of 'Hong Huey' fruit into highly concentrated oxalic acid (5–15%, pH ≤ 1) could improve color stability and reduce PPO and POD activities, but at the expense of membrane destruction, fruit softening, and inner browning. In the presence of surfactant, also low doses of oxalic acid (1–4 mmol L^{-1}) were shown to delay pericarp browning and to lower the activities of these enzymes (Shafique et al., 2016). Similarly, oxalic acid was reported to be a more potent browning inhibitor for apple slices than malic and citric acid (Son et al., 2001). Conversely, oxalic acid (1 or 5 mmol L^{-1}) applied to peaches without further manipulation of outer cell layers increased PPO and POD activities (Zheng et al., 2007). Due to its strong chelating properties, oxalic acid might remove ions from the pericarp and solubilize ionically bound POD (Loukili et al., 1999).

Structural (*i.e.*, wall- or membrane-bound) calcium plays a great role in stabilizing cell walls (Carle et al., 2001) and in retarding senescence of fresh litchi fruit (Huang et al., 2005). However, preharvest sprays of $CaCl_2$ on the fruit surface did not increase the structural calcium of litchi pericarp (Huang et al., 2005). Similarly, in terms of color retention, dipping into $CaCl_2$ was as ineffective as the control treatment (Table 2). Nonetheless, almost the same treatment (2%, 15 min) alleviated brown spot disorder of Japanese pear (*Pyrus pyrifolia* (Burm. f.) Nakai) efficiently, while increasing the contents of soluble and bound calcium in the peel (Kou et al., 2015). For litchis treated with HCl at pH 0.2, the subjectively rated color was reported to be superior to the control after 2 d of storage at room temperature (Ketsa and Leelawatana, 1992). However, the *PLCI* differences observed between the control and fruit treated with HCl at pH 2.5 were insignificant (Table 2). The only treatment that improved *PLCI* retention during the first 3 d without foil wrapping was dipping into 200 mM NaCl. Due to the higher enzyme-inhibiting potential of $CaCl_2$ *in vitro* (Fig. 2), these effects of NaCl may be attributed to causes other than inhibition of POD and the phenol oxidases.

3.4.3. Impact of browning inhibitors on pericarp color during cold storage in foil bags

As expected (Sivakumar et al., 2010), color retention was always better, when the fruit were packed in gas-permeable foil bags (35 PA 40, *cf.* 2.4.1) for storage (days 0_t–13 in Tables 3 and 4). Packed and unpacked fruit of otherwise equally treated lots differed in *PLCI* as from day 1, 2, or 3, as shown in Table 2 by italicized Greek letters for the unpacked lot. After removal of the foil bags on day 13, the fruit developed rapid browning upon further storage until day 21, irrespective of the dip solution (Tables 3 and 4). Thus, protection against desiccation was the most effective measure to prevent litchi pericarp browning. In particular, formation of light brown scurf on the fruit surface (Reichel et al., 2013) was suppressed in the foil bags, as indicated by consistently high a^* values until day 13 (Tables 3 and 4). For the two control lots 1b (Table 3) and 2b (Table 4) and for 11 of the 21 lots treated with an inhibitor (acid or salt) of varying concentration, the changes in a^* were insignificant as long as the fruit were in the foil bags.

Unlike a^*, *PLCI* values are more appropriate to indicate PPO- and LAC-related browning of litchi pericarp (Reichel et al., 2013). Equal consideration of L^* and a^* (Eq. (4)) makes this index sensitive to the associated reduction of brightness, beyond scurf-induced hue changes. Earlier than a^* did, the slight decline of *PLCI* within the first 13 d turned out to be significant for the plastic-wrapped fruit of many variants, as indicated in Tables 3 and 4 by capital letters. However, the *PLCI* values were constant until day 13 for the packed fruit of the control lots 1b (Table 3) and 2b (Table 4). Nonetheless, due to its exceptional *PLCI* drop by 18% directly after the placebo treatment (*cf.* 3.4.1), the former control always displayed a much lower *PLCI* than all other lots of the same fruit batch did after they had been immersed into an organic acid or citrate (*cf.* small letters in Table 3). For fruit batch 1, *PLCI* retention in foil bags until day 13 was maximal (96%) after dipping into 25 mM citrate (Table 3). Other citrate doses (5 or 50 mmol L^{-1}), lower pH (5–50 mM citric acid), and low doses of other acids, such as malic (5–25 mmol L^{-1}) or oxalic acid (5 mmol L^{-1}), resulted in similarly high *PLCI* retention (93–95%) under these conditions. These findings might be seen as first evidence of retarded pericarp browning *in vivo* due to treatments with organic acids in conjunction with foil wrapping. In contrast to the indistinguishable concentration effects in Table 3, Jiang et al. (1999) had to increase the citric acid concentration of the dip solution from 10 to 100 mmol L^{-1} to reduce PPO activity and to retard browning during 6 d of storage in PE pouches at room temperature.

Nonetheless, also for fruit in foil bags, the inhibitor immersion baths contributed little to the prevention of pericarp browning during cold storage, since the best *PLCI* retention until day 13 was found for the control of fruit batch 2 (97% for control lot 2b, Table 4). Storage in foil bags for this period after dipping into NaCl (50–200 mmol L^{-1}), 100 mM $CaCl_2$, or 50 mM HCl led to almost the same *PLCI* retention (93–95%). Regardless of the type and concentration of the active agent and the pH of the dip solution, *PLCI* retention until day 13 always ranged at 88–96% for fruit in foil bags (Tables 3 and 4). Concurrently, retention of pericarp redness (83–100%) relative to the a^* value at harvest was equal or superior to that found by Wang et al. (2010) after 15 d (5 °C) following dipping into 1.25–5% acidic calcium sulfate. After further storage without foil bags, the *PLCI* values only amounted to 31–36 on day 21 for all lots, including the controls. Thus, none of the immersion baths created a depot effect on the surface or in the tissue for sustained efficacy of the inhibitor in dehydrated pericarp. As shown by the notably good performance of control lot 2b (Table 4),

fruit quality at harvest (*cf.* 3.4.1) was essential, in addition to protection against desiccation.

4. Conclusion

Oxalic acid proved to be the most potent *in vitro* inhibitor of POD and the phenol oxidases, as shown for pH < 4. Other organic acids required much larger concentrations for similar effects at a given pH in this range, while those that were also complexing agents performed best, in particular citric acid. Whereas the total PPO+LAC activity was chiefly pH-dependent, pH tolerance of POD in the acidic range was greater and more inhibitor-specific. None of the tested conditions inhibited POD completely, unlike the acid-labile phenol oxidases. Next to oxalic acid, another efficacious POD inhibitor was $CaCl_2$, being even superior to citric acid at equal doses of 250 mmol L^{-1}. In contrast, suppression of the PPO+LAC activities by $CaCl_2$ and NaCl was largely indistinguishable from associated ionic strength effects. Unlike the phenol oxidases, litchi pericarp POD displayed only low affinity to (−)-epicatechin.

Contrary to the *in vitro* findings, NaCl treatment resulted in the best color retention (91% of the initial *PLCI*) after 2 d of cold storage for unpacked fruit, whereas oxalic acid was least effective (22% *PLCI* loss). Hence, unknown effects on pericarp integrity were more essential *in vivo* than sheer inhibition of browning enzymes. Even during cold storage at rather high relative humidity (90% RH), enhanced protection against desiccation by packaging in gas-permeable foil bags (35 PA 40) was the only way to avoid microcracks and surface scurf. This was consistent with high negative correlation between browning levels and pericarp moisture content (Yang et al., 2015a) and reported changes of both PPO gene expression and PPO activity (Wang et al., 2014; Yang et al., 2015b). Provided that the pericarp is intact (as in case of the small fruit of control 2), immediate cooling between ice packs for transport from the orchard and subsequent cold storage in foil bags creating a microclimate near the dew point without waterlogging proved sufficient to prevent browning for at least two weeks through preservation of tissue integrity and cell compartmentation.

Additional treatments with organic acids or chloride salts for local inhibition of PPO, LAC, and POD in freshly damaged parts of the tissue (and, as the case may be, for pigment stabilization at ~pH 3) turned out to be ineffective or even detrimental. Such negative effects might be ascribed to serious disturbance of plant defense and repair mechanisms, by which damaged pericarp surface would otherwise be sealed for protection against water loss. Color retention merely due to uninterrupted cold chain at high humidity near the dew point still has to be confirmed for cultivars of varying susceptibility to microcracking and surface scurf formation (Reichel et al., 2013) and for the final retail turnaround times at ambient temperature (Yun et al., 2016). Antioxidants or radical scavengers (Zhang et al., 2015) may then be considered as well for improved membrane stability (Bhushan et al., 2015; Kumari et al., 2015). However, logistic-technical approaches maintaining the initially high pericarp moisture content of high-quality, small-sized fruit throughout cold and humid storage without the risk of handling-induced tissue damage appear to be most promising.

Acknowledgments

Being a part of the Special Research Program 'Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia' (The Uplands Program), this research was funded by Deutsche Forschungsgemeinschaft (DFG) (DFG), Bonn, Germany: Project SFB 564–E2.3.

The authors thank Sabine Korhummel (Hohenheim University) for her excellent technical assistance. Special thanks go to Stefanie Kienzle, who performed preliminary experiments on the suitability of foil bags for the storage of Sapindaceae fruit in the scope of her diploma thesis (2007) at the Institute of Food Science and Technology of Hohenheim University as a part of project SFB 564–E2.3. Her unpublished results concerning the retention of pericarp moisture and the development of the O_2 and CO_2 contents inside the bags during fruit storage enabled the selection of the foil bag type for this study.

Appendix A. Supplementary data

Supplementary data associated with this article (Figs. S1–S4; *cf.* 2.3.3) can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2016.10.002.

References

Baur, S., Klaiber, R., Koblo, A., Carle, R., 2004. Effect of different washing procedures on phenolic metabolism of shredded, packaged iceberg lettuce during storage. J. Agric. Food Chem. 52, 7017–7025.

Berglund, G.I., Carlsson, G.H., Smith, A.T., Szöke, H., Henriksen, A., Hajdu, J., 2002. The catalytic pathway of horseradish peroxidase at high resolution. Nature 417, 463–468.

Bhushan, B., Pal, A., Narwal, R., Meena, V.S., Sharma, P.C., Singh, J., 2015. Combinatorial approaches for controlling pericarp browning in litchi (*Litchi chinensis*) fruit. J. Food Sci. Technol. 52, 5418–5426.

Bovaird, J.H., Ngo, T.T., Lenhoff, H.M., 1982. Optimizing the *o*-phenylenediamine assay for horseradish peroxidase: Effects of phosphate and pH, substrate and enzyme concentrations, and stopping reagents. Clin. Chem. 28, 2423–2426.

Buta, J.G., Moline, H.E., 2001. Prevention of browning of potato slices using polyphenoloxidase inhibitors and organic acids. J. Food Qual. 24, 271–282.

Carle, R., Borzych, P., Dubb, P., Siliha, H., Maier, O., 2001. A new process for firmer canned cherries and strawberries. Food Aust. 53 (8), 343–348.

Caro, Y., Joas, J., 2005. Postharvest control of litchi pericarp browning (cv. Kwai Mi) by combined treatments of chitosan and organic acids. II. Effect of the initial water content of pericarp. Postharvest Biol. Technol. 38, 137–144.

Ducamp-Collin, M.-N., Ramarson, H., Lebrun, M., Self, G., Reynes, M., 2008. Effect of citric acid and chitosan on maintaining red colouration of litchi fruit pericarp. Postharvest Biol. Technol. 49, 241–246.

Eicken, C., Krebs, B., Sacchettini, J.C., 1999. Catechol oxidase – structure and activity. Curr. Opin. Struct. Biol. 9, 677–683.

Fang, F., Zhang, X., Luo, H., Zhou, J., Gong, Y., Li, W., Shi, Z., He, Q., Wu, Q., Li, L., Jiang, L., Cai, Z., Oren-Shamir, M., Zhang, Z., Pang, X., 2015. An intracellular laccase is responsible for epicatechin-mediated anthocyanin degradation in litchi fruit pericarp. Plant Physiol. 169, 2391–2408.

Gaspar, T., Penel, C., Castillo, F.J., Greppin, H., 1985. A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiol. Plant. 64, 418–423.

Gong, Q.-Q., Tian, S.-P., 2002. Partial characterization of soluble peroxidase in pericarp of litchi fruit. Prog. Biochem. Biophys. 29, 891–896.

Guetsky, R., Kobiler, I., Wang, X., Perlman, N., Gollop, N., Avila-Quezada, G., Hadar, I., Prusky, D., 2005. Metabolism of the flavonoid epicatechin by laccase of *Colletotrichum gloeosporioides* and its effect on pathogenicity on avocado fruits. Phytopathology 95, 1341–1348.

Guyot, S., Cheynier, V., Souquet, J.-M., Moutounet, M., 1995. Influence of pH on the enzymatic oxidation of (+)-catechin in model systems. J. Agric. Food Chem. 43, 2458–2462.

Guyot, S., Vercauteren, J., Cheynier, V., 1996. Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. Phytochemistry 42, 1279–1288.

Hiner, A.N.P., Raven, E.L., Thorneley, R.N.F., García-Cánovas, F., Rodríguez-López, J.N., 2002. Mechanism of compound I formation in heme peroxidases. J. Inorg. Biochem. 91, 27–34.

Huang, X.-M., Wang, H.-C., Yuan, W.-Q., Lu, J.-M., Yin, J.-H., Luo, S., Huang, H.-B., 2005. A study of rapid senescence of detached litchi: roles of water loss and calcium. Postharvest Biol. Technol. 36, 177–189.

Jiang, Y.-M., Zauberman, G., Fuchs, Y., 1997. Partial purification and some properties of polyphenol oxidase extracted from litchi fruit pericarp. Postharvest Biol. Technol. 10, 221–228.

Jiang, Y., Fu, J., Zauberman, G., Fuchs, Y., 1999. Purification of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. J. Sci. Food Agric. 79, 950–954.

Jiang, Y., Li, J., Shi, J., 2008. Enhanced effect of L-cysteine and citric acid combination on browning inhibition and quality maintenance in harvested litchi fruit. J. Food Sci. Technol. 45, 75–77.

Joas, J., Caro, Y., Ducamp, M.N., Reynes, M., 2005. Postharvest control of pericarp browning of litchi fruit (*Litchi chinensis* Sonn. cv. Kwaï Mi) by treatment with

chitosan and organic acids. I. Effect of pH and pericarp dehydration. Postharvest Biol. Technol 38, 128–136.
Kahn, V., Andrawis, A., 1985. Inhibition of mushroom tyrosinase by tropolone. Phytochemistry 24, 905–908.
Ketsa, S., Leelawatana, K., 1992. Effect of pre- and post-storage acid dipping on browning of lychee fruits. Acta Hortic. 321, 726–731.
Kou, X., Wu, M., Li, L., Wang, S., Xue, Z., Liu, B., Fei, Y., 2015. Effects of $CaCl_2$ dipping and pullulan coating on the development of brown spot on 'Huangguan' pears during cold storage. Postharvest Biol. Technol. 99, 63–72.
Kumari, P., Barman, K., Patel, V.B., Siddiqui, M.W., Kole, B., 2015. Reducing postharvest pericarp browning and preserving health promoting compounds of litchi fruit by combination treatment of salicylic acid and chitosan. Sci. Hortic. 197, 555–563.
Laurenti, E., Suriano, G., Ghibaudi, E.M., Ferrari, R.P., 2000. Ionic strength and pH effect on the Fe(III)-imidazolate bond in the heme pocket of horseradish peroxidase: an EPR and UV-visible combined approach. J. Inorg. Biochem. 81, 259–266.
Liang, Y.S., Chen, N.L., Ke, L.S., 2012. Influence of dipping in sodium metabisulfite on pericarp browning of litchi cv. Yu Her Pau (Feizixiao). Postharvest Biol. Technol. 68, 72–77.
Liu, L., Cao, S., Xie, B., Sun, Z., Li, X., Miao, W., 2007. Characterization of polyphenol oxidase from litchi pericarp using (−)-epicatechin as substrate. J. Agric. Food Chem. 55, 7140–7143.
Loukili, A.-E., Limam, F., Ayadi, A., Boyer, N., Ouelhazi, L., 1999. Purification and characterization of a neutral peroxidase induced by rubbing tomato internodes. Physiol. Plant. 105, 24–31.
Mizobutsi, G.P., Finger, F.L., Ribeiro, R.A., Puschmann, R., de Melo Neves, L.L., da Mota, W.F., 2010. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. Sci. Agric. 67, 213–217.
Mochizuki, M., Yamazaki, S.-I., Kano, K., Ikeda, T., 2002. Kinetic analysis and mechanistic aspects of autoxidation of catechins. Biochim. Biophys. Acta 1569, 35–44.
Morozova, O.V., Shumakovich, G.P., Gorbacheva, M.A., Shleev, S.V., Yaropolov, A.I., 2007. "Blue" laccases. Biochemistry (Moscow) 72, 1136–1150.
Pang, X., Duan, X., Zhang, Z., Xu, F., Ji, Z., 2004. Purification and some properties of peroxidase from pericarp of litchi (*Litchi chinensis* Sonn.). J. Trop. Subtrop. Bot. 12, 449–454.
Rasmussen, C.B., Hiner, A.N.P., Smith, A.T., Welinder, K.G., 1998. Effect of calcium, other ions, and pH on the reaction of barley peroxidase with hydrogen peroxide and fluoride. J. Biol. Chem. 273, 2232–2240.
Reichel, M., Carle, R., Sruamsiri, P., Neidhart, S., 2010. Influence of harvest maturity on quality and shelf-life of litchi fruit (*Litchi chinensis* Sonn.). Postharvest Biol. Technol. 57, 162–175.
Reichel, M., Carle, R., Sruamsiri, P., Neidhart, S., 2011. Changes in flavonoids and nonphenolic pigments during on-tree maturation and postharvest pericarp browning of litchi (*Litchi chinensis* Sonn.) as shown by HPLC-MS^n. J. Agric. Food Chem. 59, 3924–3939.
Reichel, M., Triani, R., Wellhöfer, J., Sruamsiri, P., Carle, R., Neidhart, S., 2013. Vital characteristics of litchi (*Litchi chinensis* Sonn.) pericarp that define postharvest concepts for Thai cultivars. Food Bioprocess Technol. 6, 1191–1206.
Riederer, M., Arand, K., Burghardt, M., Huang, H., Riedel, M., Schuster, A.-C., Smirnova, A., Jiang, Y., 2015. Water loss from litchi (*Litchi chinensis*) and longan (*Dimocarpus longan*) fruits is biphasic and controlled by a complex pericarpal transpiration barrier. Planta 242, 1207–1219.
Ruíz-Jiménez, J.M., Zapata, P.J., Serrano, M., Valero, D., Martínez-Romero, D., Castillo, S., Guillén, F., 2014. Effect of oxalic acid on quality attributes of artichokes stored at ambient temperature. Postharvest Biol. Technol. 95, 60–63.
Saengnil, K., Lueangprasert, K., Uthaibutra, J., 2006. Control of enzymatic browning of harvested 'Hong Huay' litchi fruit with hot water and oxalic acid dips. Sci. Asia 32, 345–350.
Shafique, M., Khan, A.S., Malik, A.U., Shahid, M., 2016. Exogenous application of oxalic acid delays pericarp browning and maintain fruit quality of litchi cv. "Gola". J. Food Biochem. 40, 170–179.
Siegbahn, P.E.M., 2004. The catalytic cycle of catechol oxidase. J. Biol. Inorg. Chem. 9, 577–590.
Sivakumar, D., Terry, L.A., Korsten, L., 2010. An overview on litchi fruit quality and alternative postharvest treatments to replace sulphur dioxide fumigation. Food Rev. Int. 26, 162–188.
Son, S.M., Moon, K.D., Lee, C.Y., 2001. Inhibitory effects of various antibrowning agents on apple slices. Food Chem. 73, 23–30.
Stintzing, F.C., Stintzing, A.S., Carle, R., Frei, B., Wrolstad, R.E., 2002. Color and antioxidant properties of cyanidin-based anthocyanin pigments. J. Agric. Food Chem. 50, 6172–6181.
Sun, J., Shi, J., Zhao, M., Xue, S.J., Ren, J., Jiang, Y., 2008. A comparative analysis of property of lychee polyphenoloxidase using endogenous and exogenous substrates. Food Chem. 108, 818–823.
Underhill, S.J.R., Critchley, C., 1992. The physiology and anatomy of lychee (*Litchi chinensis* Sonn.) pericarp during fruit development. J. Hortic. Sci. 67, 437–444.
Underhill, S.J.R., Critchley, C., 1995. Cellular localization of polyphenol oxidase and peroxidase activity in *Litchi chinensis* Sonn. pericarp. Aust. J. Plant Physiol. 22, 627–632.
Wang, C.Y., Chen, H., Jin, P., Gao, H., 2010. Maintaining quality of litchi fruit with acidified calcium sulfate. J. Agric. Food Chem. 58, 8658–8666.
Wang, J., Liu, B., Xiao, Q., Li, H., Sun, J., 2014. Cloning and expression analysis of litchi (*Litchi chinensis* Sonn.) polyphenol oxidase gene and relationship with postharvest browning. PloS ONE 9 (4), 1–9. doi:http://dx.doi.org/10.1371/journal.pone.0093982 (e93982).
Welinder, K.G., 1985. Plant peroxidases—their primary, secondary and tertiary structures, and relation to cytochrome *c* peroxidase. Eur. J. Biochem. 151, 497–504.
Yang, Y.-C., Sun, D.-W., Wang, N.-N., 2015a. Rapid detection of browning levels of lychee pericarp as affected by moisture contents using hyperspectral imaging. Comput. Electron. Agric. 113, 203–212.
Yang, Y.-C., Sun, D.-W., Wang, N.-N., Xie, A., 2015b. Real-time evaluation of polyphenol oxidase (PPO) activity in lychee pericarp based on weighted combination of spectral data and image features as determined by fuzzy neural network. Talanta 139, 198–207.
Yoruk, R., Marshall, M.R., 2003. A survey on the potential mode of inhibition for oxalic acid on polyphenol oxidase. J. Food Sci. 68, 2479–2485.
Yun, Z., Qu, H., Wang, H., Zhu, F., Zhang, Z., Duan, X., Yang, B., Cheng, Y., Jiang, Y., 2016. Comparative transcriptome and metabolome provides new insights into the regulatory mechanisms of accelerated senescence in litchi fruit after cold storage. Sci. Rep. 6, 1–16. doi:http://dx.doi.org/10.1038/srep19356 (19356).
Zhang, Z., Huber, D.J., Qu, H., Yun, Z., Wang, H., Huang, Z., Huang, H., Jiang, Y., 2015. Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. Food Chem. 171, 191–1199.
Zheng, X., Tian, S., Meng, X., Li, B., 2007. Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature. Food Chem. 104, 156–162.