Ripening behaviour of capsicum (*Capsicum annuum* L.) fruit

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Abstract

Fruit of *Capsicum annuum L.* (capsicum or pepper) are one of the major sources of red food colourant and pungency for spice production. In the spice production industry, fruit are mechanically harvested at different ripeness stages and fruit colour needs to be synchronised before being processed. However, even though capsicum ripens normally on the plant it often fails to ripen fully and turn red once harvested at the green stage. Attempts to promote ripening of harvested fruits have had limited success and the reason for this has been unclear. This project, therefore, investigated ripening behaviour on and off the plant of capsicum fruit grown in Australia and examined effects of pre- and postharvest applications on ripening of green harvested fruit.

To examine ripening behaviour on and off the plant, capsicum fruit from three different cultivars (a mild paprika type *cv.* "Papri Queen", a cayenne chilli *cv.* "Caysan", and a sweet type bell pepper *cv.* "Aries") were either allowed to ripen naturally on the plant or harvested at three different maturity stages: light green, deep green and breaker. Harvested fruit were stored individually at room temperature and several ripening characteristics including internal ethylene (C_2H_4) and carbon dioxide (CO2) concentration, extractable colour, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and oxidase activity, and total soluble solid content (TSSC) were studied during storage.

There was very limited involvement of C_2H_4 during ripening of capsicum and the change in ACC synthase and ACC oxidase (two enzymes in C_2H_4 biosynthesis pathway) activity was not closely related to that of C_2H_4. However, it appeared that colour development in *cv.* "Papri Queen" was closely associated with what C_2H_4 production did occur while a climacteric-like peak of C_2H_4 could be observed in all fruit from *cv.* "Caysan".

For all three cultivars, the level of internal CO_2 concentration, extractable colour and TSSC were greater in fruit ripened on the plant followed by fruit harvested at the breaker, deep green and light green stage, respectively. Fruit harvested at the light

green stage failed to change colour properly and had very low levels of internal CO_2 concentration and TSSC while fruit harvested from the breaker stage onwards ripened normally and developed sufficient colour for spice processing. This may suggest a role of external carbon-supply during ripening.

To study the effect of the external-carbon supply during ripening, the stem of fruit were cinctured when fruit reached the light green stage and fruit were left to ripen on the plant. Cincturing delayed colour development of fruit by approximately five days but cinctured fruit were still able to turn red and develop extractable colour higher than the acceptable level of 140 ASTA units. Cincturing did not significantly alter other ripening behaviour such as CO_2 concentration or TSSC. The lack of external carbon-supply is, therefore, unlikely to play a major role in the failure of green harvested fruit to ripen.

To study the effect of application of plant growth regulators (both pre- and postharvest), an effective method of solution application utilising cincturing was firstly developed. Different plant growth regulator solutions including ethephon, naphthalene acetic acid, abscisic acid, jasmonic acid, sucrose, and different combinations of these were applied to fruit at the light green stage to study preharvest effects on ripening parameters during storage. Only treatment with high concentrations of ethephon increased the extractable colour higher than the acceptable level of 140 ASTA units and induced the complete degradation of chlorophyll. To study effects of postharvest application, 10 µL of various plant growth regulators was dropped into the hole created on the stem of harvested fruit for ten consecutive days. Treatment with ethephon significantly increased extractable colour and degraded chlorophyll content of fruit. Pre- and postharvest ethephon treatment strongly up-regulated *Capsanthin-capsorubin synthase* (*Ccs)* gene expression in a manner similar to the up-regulation of *Ccs* observed in fruit ripened on the plant. This explains the effect of C_2H_4 on colour development and also indicates the possible reason for the failure of green harvested fruit to ripen. However, the *Ccs* gene expression and chlorophyll degradation induced by ethephon was not visible until 14 days after harvest which indicated it may not be a direct effect and other signal transduction factors may be involved. When fruit are ripened on the plant, colour development may, therefore, be induced by ripening-related

factors (other than C_2H_4) which is possibly inhibited or inactivated when fruit are harvested at the green stage. C_2H_4 application to fruit at this stage may help to reactivate or recover these factors which in turn induce colour development. Thus, although capsicum fruit show typical non-climacteric behaviour, C_2H_4 appears to be involved in some aspects of the ripening process.

Statement

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person except where due reference is made in the text.

I consent to this thesis being made available for photocopying and loan.

Pham Thi Ngoc Thang
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A	absorbance
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
AOAC	Association of Official Analytical Chemist
ASTA	American Spice Trade Association
B	breaker
BR	breaker red
^{o}C	Degree Celsius
Ccs	capsanthin-capsorubin synthase
CH_3COONa	sodium acetate
C_2H_4	ethylene
CO_2	carbon dioxide
cm	centimetre
DAA	days after anthesis
DAH	days after harvest
DG	deep green
DNA	deoxyribonucleic acid
DR	deep red
DR & D	deep red and partially dried
DW	dry weight
EDTA	ethylenediaminetetraacetic acid
ethephon	2-chloroethylphosphonic acid
FW	fresh weight
g	gram
Hepes	4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
h	hour
$HgCl_2$	mercury chloride
i.d.	internal diameter

JA	jasmonic acid
KOH	potassium hydroxide
Lcy	lycopene-β-cyclase
LG	light green
LR	light red
LSD	least significant difference
mg	milligram
mL	millilitre
mm	millimetre
mM	millimole
MOPS	4-morpholinopropanesulfonic acid
NaCl	sodium chloride
NaOCl	sodium hypo chloride
NaOH	sodium hydroxide
Na_2HPO_4	sodium phosphate dibasic
nL	nanolitre
nm	nanometre
NAA	naphthalene acetic acid
PVPP	poly(vinylpolypirrolidone)
RNA	ribonucleic acid
s	second
SAM	S-5’- adenosyl methionine
sds	sodium dodecyl sulphate
SE	standard error of the mean
SUC	sucrose
Tris	tris (hydroxymethyl) aminomethane
TSSC	total soluble solid content
UC	University of California (potting mix)
v/v	volume by volume
w/v	weight by volume
µL	microlitre
µmol	micromole
%	percent