Stability and Esterification of Lutein in Bread Wheat during Post Harvest Storage in Comparison with Banana

Submitted by

Fauziah Tufail Ahmad

This thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Plant and Food Science

School of Agriculture, Food and Wine

Faculty of Sciences

The University of Adelaide

February 2013

Special to Mum (Zabidah Biku) and Dad (Tufail Ahmad Hj. Ashtul)

-for continuous support and pray-

Lutein is an important micronutrient for humans as well as being the primary contributor to the pale creamy to yellow colour of bread wheat and durum based products but tends to be unstable against heat and UV light. During post harvest storage of bread wheat grain some of the lutein may be converted to mono- and di-fatty acid esters that appear to be more stable forms of lutein.

The aims of the work presented in this thesis were: to study the effects of temperature on lutein esterification; to compare the relative stability of free lutein and lutein esters in grain stored under wide temperatures and conditions; to confirm that esterification is an enzymic process; to examine the genetic control mechanisms; to attempt to identify the enzyme and the endogenous substrate source of fatty acids; and finally to compare esterification in wheat grain with the same process in banana fruit tissues. This study utilised a high lutein, ester forming bread wheat, *Triticum aestivum* L. cv DM5685*B12, a non-ester forming bread wheat cv Haruhikari and a high lutein durum wheat, *Triticum durum* L cv Kamilaroi, that like many durum cultivars does not form lutein esters. Reverse phase high pressure liquid chromatography (RP-HPLC) was used to quantify the lutein and lutein ester concentrations.

Lutein esterification was strongly favoured by low relative humidity (8% RH) and followed a first order reaction rate. The maximum rate of lutein esterification was at ≈80°C, however the optimum temperature for maximum synthesis with minimum degradation was between 50 and 60°C. No ester synthesis was observed at temperature higher than 120°C. These data were consistent with an enzyme participating in the esterification reaction. Lutein ester was found to be more stable than free lutein with a substantially longer shelf life at a temperature of 60°C.

An attempt to establish a bioassay system to study esterification was only partially successful since only very low levels of esterification were achieved in reconstituted samples. Further investigation would be required to optimise the process. The limited

data did provide suggestive evidence that free fatty acids were probably not involved, rather the fatty acids were more likely to be derived from phospholipids via an acyltransferase reaction. A hexane-soluble fraction derived from a non-ester forming durum, Kamilaroi, was the only substrate that in the presence of a crude enzyme extract and free lutein gave a significant formation of lutein ester.

As esterification appeared to be was enzymatically controlled, the genetic control of ester synthesis was investigated. Lutein esterification was compared in a series of nullisomic-tetrasomic Chinese Spring lines and a Haruhikari (zero ester)//Sunco/Indis.82 (high ester) doubled haploid population. Lutein esterification was controlled by a locus, designated *Lute*, located on the short arm of chromosome 7D closely linked with the marker loci *gwm295*, *wPt-1163* and *wPt-3727*.

In addition to wheat, esterification in banana, *Musa acuminata* Colla cv Cavendish group was also investigated. Compared to wheat, different patterns of esterification were observed in banana during the ripening with ester synthesis occurring in both banana peel and flesh during post harvest ripening. Esterification in banana occurred under higher moisture content than in wheat and offers another tissue model for the study of the esterification mechanism.

This thesis contributes valuable new information on the formation and genetic control of lutein ester formation in wheat grain and will be of value to manufacturers of wheat products seeking to retain lutein in end-products for delivery to costumers.