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Internal discoloration of various varieties of Macadamia nuts as influenced by enzymatic browning and Maillard reaction

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ABSTRACT

Brown kernels or internal discolouration (IDC) in various varieties of Macadamia nuts (*Macadamia integrifolia* and *Macadamia tetraphylla*) occurred through three different pathways: (i) an enzymatic browning reaction, (ii) Maillard reaction and (iii) infection by microorganisms. The phenolic compounds and polyphenol oxidase (PPO) activities of macadamias were analysed. Brown sections of the same kernel had higher levels of bound phenolics compared to the white sections, indicating the participation of phenolic compounds in the formation of brown kernel. The Maillard reaction was studied by determining the sugar amount using HPLC. The reducing sugars during drying reacted with kernel proteins causing the formation of brown pigments. Among the various varieties studied, 'Daddow' variety showed the least degree of hydrolysis. When kernel was infected with *Penicillium aurantiogriseum*, the kernel turns brown. The internal discoloration in macadamias is the first report that has been explored in our study. The findings of this study have potential to improve the existing postharvest techniques used in the Macadamia processing industry.

1. Introduction

Macadamia nuts are packed with numerous health benefiting nutrients, minerals, antioxidants and vitamins that are essential for optimum health and wellness. Macadamia nuts have sweet taste and are rich source of monounsaturated oil like oleic acid (18:1) and palmitoleic acids (16:1) (Murano, 2003). They are widely grown in Australia, South Africa, Guatemala and United States of America, especially, in Hawaii (USDA, 2011). Only two species viz., *Macadamia integrifolia* and *Macadamia tetraphylla* and their hybrid varieties are commercially available as compared to other species, which have a bitter taste and unsuitable for consumption. Macadamia nuts contain high content of unsaturated fats, which are beneficial to health by reducing the low density lipoprotein cholesterol levels and improving the markers for oxidative stress, inflammation and clotting tendencies (Griel et al., 2008). From the review literature, high consumption of unsaturated fat could have some health benefits, including prevention of diabetes, control of body weight and prevention of cardiovascular disease (Lovejoy, 2005). Garg et al. (2007) reported that consumption of macadamia kernels resulted in significant reduction of oxidative stress and inflammation in human body. In addition, the high concentration of oleic acid in macadamias is beneficial for decreasing the coronary heart disease due to the high percentage of unsaturated fatty acids (Sinanoglou et al., 2014)

The development of browning is associated with Macadamia nuts, which usually occur during the thermal processing of postharvest treatment. Postharvest treatments are the steps performed after the nuts are no longer on the tree. Proper postharvest procedures are needed to prevent physical and chemical damage which can lead to loss of quality. There are generally six postharvest steps in macadamia nut processing; harvesting, de-husking, thermal processing, cracking, grading and packaging. The internal discolouration in macadamia nuts causes the formation of off-flavours and aroma, which in turn leads to economic loss and decrease the nut quality.The extent of this defect, is largely unquantified at this stage. As a consequence, it costs the industry an estimated loss of several million dollars per year due to kernel downgrading and processing inefficiency. More importantly, the occurrence of brown centers in nuts sold at the retail level has the potential to greatly undermine customer confidence and thus reducing repeat sales. The fundamental factors associated with this defect are still unknown. Since this problem occurs erratically, therefore the basic cause of this defect has been difficult to investigate (Lagadec, 2009). However, the internal discolouration or browning of the kernel cen-

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ter varies from light brown to dark brown depending on the shape of nuts which ranges from oval to unconformity. The discolouration occurs mostly at the center but could spread to any locations in the Macadamia kernel. In general, the colour of Macadamia nuts varies from white to creamy; therefore the discolouration in these nuts could be easily detected. Walton and Wallace (2015) reported that by reducing time period between harvest and dehusking processing Macadamia at low moisture content (10–12%) could improve the kernel quality. Whilst, Macadamia nuts stored at high moisture content and elevated temperature with limited air circulation showed increased occurrence of brown center (Walton et al., 2013). Other study conducted by Phatanayindee et al., (2012) showed that reduction of drying time using heat pump dryer combined with tunnel drying resulted in improved kernel quality and reduced IDC in Macadamia nuts. This indicated that brown kernels could be found during all postharvest treatments from harvesting to packaging stage. In general, the kernel quality is influenced by internal and external factors, processing steps and environmental conditions. Furthermore, these factors could be interrelated, for example the polyphenol oxidase enzyme could be inactivated during drying, leading to reduced susceptibility to enzymatic browning. Moreover, brown kernels could be found among fresh, dried and roasted samples. Therefore, it was hypothesized that browning in Macadamia kernel could be triggered by enzymatic and non-enzymatic browning reactions. These two biochemical reactions could form brown pigment as their end product. In addition, microorganisms may also influence brown kernel development via the production of extracellular enzymes.

Therefore, the aim of this study was to determine the possible mechanisms of kernel browning and study various factors that are likely related with quality deterioration based on different cultivars. The information obtained from this study will contribute to understand the internal discolouration (IDC) and could lead to preventive measures for maintaining nut quality.

2. Material and methods

2.1. Materials

Macadamia samples were obtained from various plantations in different seasons located in New South Wales (Deenford plantation, Lismore) and Queensland. The samples included were various commonly grown varieties including 'A 38', '246', '816', '842', and 'Daddow'. Samples were sorted, graded and cracked. The nuts obtained were graded into 4 categories (1) nuts with smooth texture and light colour; (2) nuts with colour defects, uneven browning or off-colour; (3) nuts with brown centers; (4) dark shriveled nuts. The nuts were classified based on the definitions of Prichavudhi and Yamamoto (1987). Category 1 nuts were used in all the experiments, whilst category 2 nuts were used in brown kernel experiments only. Due to the insufficient quantity of brown nuts, the brown kernel samples were also obtained from colour sorter at Macadamia processing company (MPC Ltd, Lismore, NSW). The belt type colour sorter machine (Alstonville, Australia) is used during processing step to separate brown colour kernel from white colour kernel. Therefore, brown nuts used in this study were procured from both plantations as well as from processor.

2.2. Chemicals and reagents

1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, phenolic compounds (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid and syringic acid) and fatty acids standard C12-C20 were purchased from Sigma–Aldrich (Sydney, Australia). HPLC grade methanol acetonitrile and other solvents were obtained from Merck and Honeywell (Sydney, Australia). All chemicals and reagents used in the study were of analytical grade.

2.3. Kernel segmentation

The segmentation of kernels was performed on fresh brown nuts using sharp knife to mechanically separate the non-brown and brown sections. The concentration and types of phenolic compounds present in both brown and non-brown sections were determined separately. Prior to the analysis, the non-brown and brown section was carefully placed in different tubes (1.5 mL). The brown kernels samples were divided into 7 groups (A–G) randomly. Each group included the average data of 10 brown Macadamia nuts.

2.4. Processing

2.4.1. Drying

Drying of nuts were carried out through a series of experiments using an in-house built cabinet dryer, followed by heat pump dryer (Greenhalgh, Australia) and finally with vacuum oven (Croydon, England). Samples were dried in the form of nut in shell (NIS). The drying conditions in the cabinet varied from 30 to 50 °C and RH range was 20–40%. Heat pump dryer was operated at 30 °C and 20% RH. Vacuum oven drying was performed at 35 °C at 3.29 kPa. The drying time of each experiment was 8 days.

2.4.2. Roasting

The roasting experiments were carried out in a convection oven (Contherm, Australia) at 30, 60, 90, 120 and 150 °C for 30 min.

2.5. Chemical analysis

2.5.1. Moisture content and pH determination

The moisture contents of kernels and shells were determined by a vacuum oven method 934.01 (18) G (AOAC, 1995). Macadamia kernels were ground with a coffee grinder and placed in pre-dried aluminium dishes and dried in vacuum oven at 75 °C for 24 h at 3.29 kPa. The ground Macadamia nut was mixed with MilliQ water at 1:4 ratio and was used for pH determination using a pH meter at 25 °C.

2.5.2. Enzyme extraction and analysis of polyphenol oxidase (PPO)

The extraction and analysis method of PPO were performed according to Srichamnong et al., (2012). Macadamia nuts (10 g) were defatted twice with 100 mL of hexane and10 mL of petroleum ether. Defatted sample (5 g) was transferred to a 250 mL conical flask and mixed with 100 mL phosphate buffer (pH 6.8) containing 5% polyvinylpyrrolidone (PVPP). Extraction time was 8 h at 4 °C. Samples were then filtered through Whatman No. 41 filter paper and the filtrate was centrifuged at 4 °C at 20,000 rpm for 30 min. Supernatant was collected and diluted with acetone (4 × volume) to precipitate protein. Any remaining liquid was evaporated by nitrogen flushing. The acetone precipitates were kept at −18 °C for further analysis. Prior to purification, protein powder was mixed with 1 mL of 0.1 M phosphate buffer (pH 6.8) and passed through a syringe filter (0.22 μm) and injected into a Bio-Rad (BioLogic LP) chromatography system with LP Data view v1.03 software. Chromatography conditions for purification were programmed with gradient elution; flow rate was ramped up from 1 to 2 mL/min. Mobile phase was a binary system: mobile phase A was phosphate buffer (pH 6.8) and mobile phase B was phosphate buffer (pH 6.8) with 1 M NaCl. Total run time of 1 cycle was 113 min. Fractions were collected at 2.5 min interval and subjected to enzyme activity analysis. Absorbance measurements were recorded with a Spectramax