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Enzymatic browning reduction in white cabbage (*Brassica oleracea*) using honey: Does honey color matter?

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ABSTRACT

Thirteen honey groups consisting of 66 samples from different geographic locations in Zambia were screened for total phenolics, total flavonoids and antioxidant activity, and their color parameters (L^*, a^* and b^*) were measured by transmittance. Total phenolic and flavonoid contents ranged from 479.2 ± 1.1 to 1383.9 ± 3.7 mg Gallic Acid Equivalents per kilogram of honey (mg GAE/kg) and from 85.5 ± 1.8 to 609.2 ± 3.7 mg Catechin Equivalents per kilogram of honey (mg CE/kg) respectively while total antioxidant activity ranged from 3.9 ± 0.5 to 7.8 ± 0.9 mmol (Fe^{2+})/kg. Enzymatic browning reductions increased with decreasing honey lightness (L^* value) and honey redness (a^* value) but were not found to be significantly affected by honey yellowness (b^* value) ($p > 0.05$), implying that darker honeys possess stronger ability to reduce enzymatic browning in white cabbage than lighter honeys. Furthermore, the effect of honey color on aroma and taste characteristics was not significant ($p > 0.05$). However, geographic location had an effect on flowery ($b = -0.21$, $t = -2.48$, $p < 0.05$) and acidic ($b = -0.52$, $t = -3.47$, $p < 0.01$) characteristics of honey, suggesting that honey aroma or taste is likely to be influenced by the location where honey is harvested rather than by its color.

1. Introduction

Cabbage (*Brassica oleracea* L. capitata) is a versatile food and is increasingly becoming an important vegetable in restaurants, dinning commons and fast food outlets because of its convenience and less requirement for washing, cutting or shredding (Watada & Qi, 1999). However, cabbage and a variety of fruits and vegetables, such as lettuce, potato, apple, and banana, are susceptible to enzymatic browning during processing and storage. Enzymatic browning in vegetables is often associated with undesirable brown colors, off-flavors and lower nutritional value (Sagar & Kumar, 2010). The browning reaction needs the presence of oxygen, polyphenol oxidases (PPO) and phenolic compounds and is generally triggered by the enzymatic oxidation of monophenols into *o*-diphenols and quinones, which further undergo non-enzymatic polymerization leading to the formation of pigments (Gao, Zhao, Duan, & Tao, 2014; Pizzocaro, Torreggiani, & Gilardi, 1993). Although enzymatic browning is beneficial to the color and flavor development of certain food items such as tea and coffee, it impairs the quality of fresh-cut produce (Lopez-Nicolas, Perez-Lopez, Carbonell-Barrachina, & Garcia-Carmona, 2007; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). In fact, approximately 30% loss of quality in post-harvest food commodities including vegetables in developing nations is due to enzymatic browning (FAO, 1989; Pedreschi et al., 2013).

Many studies have focused on browning control by targeting PPO, substrates (oxygen and phenols) or the end products of browning reaction (Lee & Whitaker, 1995; Queiroz, da Silva, Lopes, Fialho, & Valente-Mesquita, 2011; Złotek & Gawlik-Dziki, 2015). Prevention of undesirable browning reactions has primarily been achieved by use of synthetic and commercial products mainly reducing agents such as sulfites, citric acid and ascorbic acid (Šarić et al., 2012). But sulfites have been reported to trigger health problems in some people (Stevens, Kuczek, Burgess, Hurt, &

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Armold, 2011). A main disadvantage of using reducing agents is that they are usually incompatible with sanitizers (chlorine, ozone and chlorine dioxide) that are widely used in the fresh-cut industry to prevent pathogen contamination, because these sanitizers are oxidative in nature (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004; Lee, Hong, & Kim, 2014). In order to preserve quality of fresh-cut vegetables and fruits, a browning inhibitor that is readily available and compatible with the currently widely used sanitizing treatment is urgently needed.

Honey has been reported to have natural protective characteristics and recommendations to promote its use as a food preservative agent is growing (Jean & Zhao, 2005; Mundo, Padilla-Zakour, & Worobo, 2004; Nagai, Inoue, Kanamori, Suzuki, & Nagashima, 2006). There are many antioxidants present in honey that predispose its use as a protective agent and include alkaloids, ascorbic acid, catechins, cinnamic acid derivatives, flavonols, flavonoids and phenolic acids (Friedman, 1996; Saxena, Gautam, & Sharma, 2010). Sugars like arabinose, galactose, glucose and rhamnose are also present in honey (Mărghitaş et al., 2009). These components give honey its chemical characteristic and have been reported to slow down browning processes in vegetables and fruits (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000), to stop the spread of spoilage organisms in food (Mundo et al., 2004; Taormina, Niemira, & Beuchat, 2001) and to reduce oxidation in meat (Nagai et al., 2006). Quantification of biochemical components in honeys and detecting those with considerable ability to reduce enzymatic browning would enhance their commercial value as alternative or supplementary inhibitors especially among the poor communities and stimulate honey production in the region.

Studies to quantify phenolics, flavonoids and antioxidant activity of honeys are voluminous in the literature and include honeys from Africa (Beretta, Granata, Ferrero, Orioli, & Facino, 2005; Serem & Bester, 2012), Americas (Isla et al., 2011; Sant'Ana, Buarque Ferreira, Lorenzon, Berbara, & Castro, 2014), Australasia (Yao et al., 2003), Asia (Liu, Ye, Lin, Wang, & Peng, 2012; Saxena et al., 2010) and Europe (Kuś et al., 2014; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2011). Although it is generally agreed that honey contains chemical components that reduce browning in food, there are very few experimental studies on the use of honey to reduce enzymatic browning in vegetables (Chen et al., 2000). However, whether this ability is related to honey color is not clear. Thus, the aims of this study were; (i) to quantity biochemical contents in the Zambian honeys, (ii) to assess the ability of these honeys in reducing enzymatic browning in white cabbage, and (iii) to determine whether variation in enzymatic browning reduction is linked to honey color, and (iv) to evaluate sensory characteristics of these honeys. We used Folin–Ciocalteu assay for the total phenolic content (TPC), colorimetric method for total flavonoid content and the ferric reducing antioxidant power (FRAP) assay for total antioxidant activity. Honey color was characterized using the CIE (Commission Internationale de l'Eclairage) $L^*a^*b^*$ method. To our knowledge this is the first study to analyze biochemical contents, efficacy in enzymatic browning and color of honeys in Zambia.

2. Materials and methods

2.1. Study area and honey samples

Samples of honey used in this study were collected from three different geographic regions (Kabwe, Kabompo and Kitwe) in Zambia between January and December 2012. These regions are dominated by Miombo woodland, which is part of Zambezian phytoregion with a large presence of legume tree species in the *Julbernadia, Brachystegia, Isoberlinia* and *Marquesia* genera (Chidumayo, 1997). Miombo tree species are characterized by a brief deciduous flowering period and is consistent with the bee foraging seasons (Chidumayo, 1997).

We collected 66 honey samples from different beekeepers, apiaries and the market. Based on their geographic source and then on beekeepers or traders, honey samples were grouped into 13 groups (ZMH 1 to ZMH 13) (see Table 1 for specific number of samples collected per group). Groups ZMH 1, ZMH 5 and ZMH 7 were obtained from professional beekeepers in Kabwe-central Zambia; groups ZMH 2, ZMH 6, ZMH 11 and ZMH 12 were bought from the market in Kabompo-northwest Zambia; and groups ZMH 3, ZMH 4, ZMH 8, ZMH 9, ZMH 10 and ZMH 13 from the apiaries at Zambia Forestry College in Kitwe-northern Zambia (Table 1). All honey samples were stored at 4 °C in the dark at School of Natural Resources, Copperbelt University, Zambia before processing.

2.2. Total phenolic content (TPC)

Folin-Ciocalteu assay was used to estimate TPC (Saxena et al., 2010). A 25 μl of a 100 g/l honey solution was added to each well of a 96 well micro plate followed by 50 μl Folin-Ciocalteu and 50 μl of a 75 g/l Na_2CO_3 solution, thoroughly mixed and the absorbance was read at 765 nm. To correct for any color interference for each sample, a blank consisting of 25 μl of a 100 g/l honey solution with 100 μl sterile water was run. Measurements were performed in triplicate. TPC of each honey sample was expressed as mg Gallic Acid Equivalents per kilogram of honey (mg GAE/kg).

2.3. Total flavonoid content (TFC)

We estimated TFC using a colorimetric method (Saxena et al., 2010) with some modification. A 25 μl of a 100 g/l honey solution was added to each well of a 96 well micro plate followed by 20 μl of a 25 g/l $NaNO_3$, 20 μl of a 25 g/l $AlCl_3$ and 100 μl of a 20 g/l NaOH.

Table 1
Samples of honey used in this study, collection date and their floral sources.

Honey groups	N[a]	Location	Date	Floral source
ZMH 1	5	Kabwe	July	Mixed natural forest dominated by *Julbernadia* and *Isoberlinia* species
ZMH 2	4	Kabompo	July	Mixed natural forest dominated by *Julbernadia* species
ZMH 3	5	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species
ZMH 4	5	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species
ZMH 5	6	Kabwe	December	Mixed natural forest dominated by *Julbernadia* and *Isoberlinia* species
ZMH 6	5	Kabompo	January	Mixed natural forest dominated by *Julbernadia* species
ZMH 7	5	Kabwe	December	Mixed natural forest dominated by *Julbernadia* and *Isoberlinia* species
ZMH 8	5	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species
ZMH 9	6	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species
ZMH 10	6	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species
ZMH 11	3	Kabompo	January	Mixed natural forest dominated by *Julbernadia* species
ZMH 12	5	Kabompo	January	Mixed natural forest dominated by *Julbernadia* species
ZMH 13	6	Kitwe	July	Mixed natural forest dominated by *Julbernadia, Brachystegia* and *Isoberlinia* species

[a] Number of samples collected per group.