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The effect of different ripening stages of durian *Durio zibethinus* fruit on zinc content in liver of rats loaded with cholesterol

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Abstract: *The effect of different ripening stages of durian Durio zibethinus fruit on zinc content in liver of rats loaded with cholesterol*. Durian *Durio zibethinus* is an exotic, climacteric fruit, rich in dietary fiber, polyphenols, saccharides, vitamins and minerals. Postharvest changes in durian fruit influence its physical and chemical quality, which determined its health protective effect – antioxidant and hypocholesterolemic. Durian is a good source of zinc. The aim of this study was to investigate the zinc content in lyophilisate of durian fruit and in the diets supplemented with durian fruits at different stages of maturity. The *in vivo* experiment was designed to determine the influence of these diets on zinc content in the liver of rats loaded with cholesterol. The zinc content in durian fruit, in the diets, and in rats livers was examined using a flame atomic absorption spectrophotometry (Perkin-Elemer 1100B). The zinc content in durian fruits ranged from 8.1 to 12.3 mg/kg. The zinc content amounted 45.0, 44.2, 47.1, 47.3 and 48.0 mg/kg in C, ch, chDM, ch DR and chDOR diet, respectively. The content of zinc in the liver in all animal groups was within the normal limits and amounted from 25.0 to 33.0 mg/kg fresh weight. Diet supplemented with durian fruits at different stages of ripening increases accumulation of zinc in the liver of rats loaded with cholesterol. Durian fruits, especially ripe ones, could be used as a natural supplementation of zinc in the diet and might be helpful in prevention of diseases or disorders related to zinc deficiency.

Key words: durian fruit, climacteric, zinc, rat, liver

INTRODUCTION

The exotic durian fruit *Durio zibethinus* is a precious source of bioactive compounds like dietary fiber, polyphenols, oligosaccharides, vitamins and minerals (Arancibia-Avila et al. 2008). It is one of the most important tropical fruit crops in Thailand, however the lack of an established technology for handling, transport and storage makes durian available abroad only frozen or lyophilised. Durian is a climacteric fruit what means that respiratory activity and ethylene production is rising also after the harvest and many enzymatic processes occur, which cause changes in content of saccharides and bioactive compounds (Ketsa and Daengkanit 1998). Ketsa and Pangkool (1994, 1995) and Ketsa and Daengkanit (1998) reported that temperature and humidity, and also ethylene and carbon dioxide production have particular importance in ripening process. They also

indicated that these parameters determined physical and chemical properties of durian, its consumption quality. Precise knowledge of the chemical composition of durian fruits during ripening is very important because durian is consumed in different stages of maturity. An unripe durian may be cooked as a vegetable, a ripe one is consumed mainly fresh, of course, where it is available. Overripe durian has a strong odour that attracts carnivorous animals and could be used as a dietary supplement which improves palatability and feed intake. Postharvest changes in durian fruit influence its physical and chemical quality, which determined its health protective effect – antioxidant and hypocholesterolemic. One of the most popular cultivar of durian is Mon Thong. The flesh, botanically called aril, is creamy, mild sweet with relatively moderate smell and have a higher content of polyphenols and antioxidant potential than in Chanee and Kan Yao cultivars (Leontowicz et al. 2008), which are also widely available at the Thai markets. Leontowicz et al. (2008) suggested that durian fruits cultivar Mon Thong, especially ripe ones, can be used like a dietary supplement for humans with metabolic disorders and in consequence can help people who suffer from cardio-vascular diseases.

Durian is a good source of zinc, however its concentration may differ depending on cultivar, stage of maturity, and ripening conditions (Haruenkit et al. 2007, 2010, Leontowicz et al. 2008, 2011, Poovarodom et al. 2010). Zinc is an essential mineral for multiple aspects of metabolism required for the catalytic activity of more than 200 enzymes (Osredkar and Sustar 2011). Zinc is also critical to tissue growth, wound healing, immune system function, prostaglandin production, bone mineralization, proper thyroid function, blood clotting, and normal functioning of the brain and central nervous system (Bhowmik et al. 2010). So far, studies on the ripening of durian fruits have been mainly related to physicochemical changes in its respiration, solids, starch, firmness, activities of polygalacturonase and pectinesterase, content of polyphenols, and antioxidant potential (Imsabai et al. 2002, Toledo et al. 2008). To the best of our knowledge no results of comparative studies describing the influence of diets supplemented with durian fruits at different stages of ripening (mature, ripe and overripe) on zinc content in liver of rats have been published.

The aim of this study was to investigate the zinc content in lyophilisate of durian fruit and in the diets supplemented with durian fruits at different stages of maturity. The *in vivo* experiment was designed to determine the influence of these diets on zinc content in the liver of rats loaded with cholesterol. The results of the investigation of durian at different stages of maturity *in vivo* on rats loaded with cholesterol would advances the use of this fruit in human nutrition.

MATERIAL AND METHODS

Samples preparation

In this investigation samples of Mon Thong cultivar of durian fruits at different stages of ripening were studied. All durian samples were harvested in May 2008, from a 25-year old commercial durian orchard, in Chantaburi province,

eastern Thailand. Harvesting and determination of maturity were carried out by Thai workers using the following techniques: day count, character of fruit spines, tapping the fruit, colour and shape of the fruit (Yaacob and Subhadrabandhu 1995). To get mature durian flesh with firm texture and no odour samples were left for 1 day at room temperature. Other fruits were left for another 4 days to ripen until their flesh became soft and overripe samples – having strong odour were obtained when fruits were left for another 3 days. All fruits were cleaned, weighed, chopped and homogenised in a high-speed blender (Hamilton Beach Silex professional model) for 1 min and lyophilised for 48 h (Virtis model 10--324).

Rats, diets and management

The study was conducted in the Department of Physiological Sciences, Faculty of Veterinary Medicine Warsaw University of Life Sciences – SGGW. The results of plasma lipids, antioxidant activity, liver enzymes and histopathology of the aorta and liver of rats were presented in the previous publication (Leontowicz et al. 2011). The experimental model comprised the male Wistar rats (n = 30) loaded with dietary cholesterol (1%) for 6 weeks. The body weight of rats at the beginning of the experiment was 95.4 ±3.0 g. The animals were randomly divided into 5 groups: control (C), control with cholesterol (ch) and with mature (chDM), ripe (chDR) and overripe (chDOR) durian fruits. The rats of all 5 groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures (Leontowicz et al. 2011). Control group (C) was fed the BD only, 1% of cholesterol was added to the BD of the ch group, and BD of three other groups: chDM, chDR, chDOR was supplemented with 1% of cholesterol and 5% durian fruits (as freeze-dried powder) at the mentioned above different stages of ripening. All rats were fed *ad libitum* and had free access to water. The diets were offered once a day. The feed intake was monitored daily.

Before the section the rats were not fed for 24 h. At the end of the experiment, the rats were anaesthetized using Narcotan® (Zentiva) for inhalation, and from the liver of each experimental rats left lateral lobe were dissected. The Animal Care Committee of the Warsaw University of Life Sciences – SGGW, Poland approved this study (No. 10/2007).

Zinc analysis

Approximately 0.5 g of lyophilized durian fruits in different stages of ripening, 0.5 g of five diets (C, ch, chDM, chDR and chDOR) and 1.0 g of liver were mineralized in microwave oven Milestone 900 with 5 ml 65% HNO_3 (Merck 1.00441) and 1 ml 30% H_2O_2 (Merck 1.07298) and the content of zinc was determined using a flame atomic absorption spectrophotometer (Perkin-Elmer 1100B), at 210 nm. The method provides a linearity in the concentration range of 0–1 mg/l with a detection limit of 1 µg/l. The standards of 0.5 and 1.0 mg/l were prepared using 9953 Titrisol Zinc standard (Merck).

Statistical analysis

One-way ANOVA analysis of variance (Duncan's test) was performed using Statistica 9 software. Differences were considered significant at $P \leq 0.05$. Data are

presented in figure as means ±standard deviation.

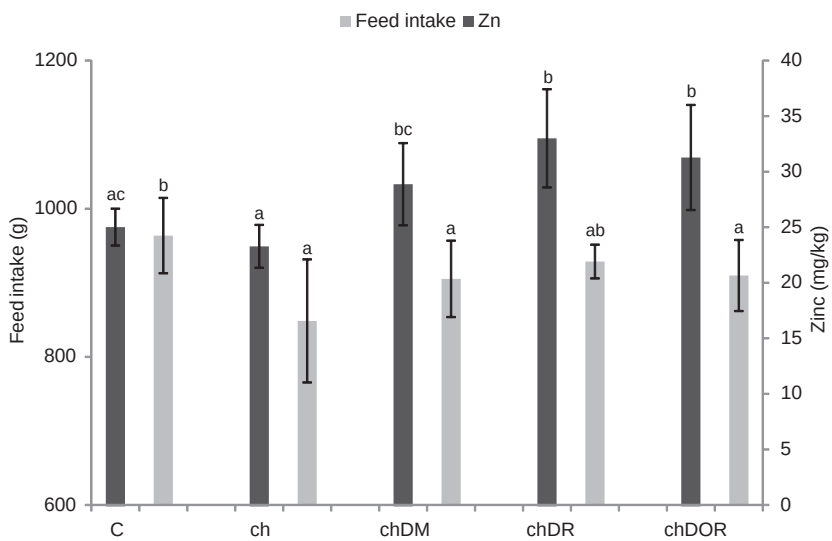
RESULTS AND DISCUSSION

Fruits species vary widely in zinc concentration. The factors affecting the nutritional quality of crops can be assigned to two groups: pre-harvest like soil type, climate, disease, insects, harvest date, and post-harvest: storage conditions, post-harvest processing. It has been shown that out of four investigated exotic fruits: dragon fruit, star fruit, Longgong and durian, the last fruit cultivar Chanee has the highest (0.27 mg/100 g) content of zinc in fresh flesh (Charoensiri and Kongkachuichai 2006). Leontowicz et al. (2008) have reported, that the content of zinc in all three studied cultivars of durian: Mon Thong, Kan Yao and Chanee was comparable ($P > 0.05$) and ranged from 49.5 to 78.4 μg/100 g fresh weight. Our study indicates that the content of zinc ranged from 8.1 to 12.3 mg/kg in durian fruit Mon Thong harvested in 2008. It must be noted that analytical determination was made in lyophilisate of durian. Our previous studies, conducted since 1994, have confirmed that freeze-drying process (lyophilisation) does not lead to loss of valuable components.

Zinc is an important component of carotenoids. Costa et al. (2011) have published that no significant variation was found in the Zn, P, Na and K concentrations during the tomato ripening after harvest, but have indicated that zinc concentration were higher in red tomatoes than in green ones. The increase in zinc content and reached a peak at the ripe stage was noted in banana *Musa* ssp. fruits (Adeyemi and Oladiji 2009). During ripening after harvest, green pigment of unripe banana is converted to carotenoids in ripe banana. Zinc requirements for most young domestic animals and poultry range from approximately 40 to 100 ppm in the diet (McDowell 1992). In our study all rats were fed basal diet containing 45 mg/kg zinc. The other four experimental diets with 1% of cholesterol and 5% of fruits in different stage of maturity were not significantly different ($P > 0.05$) in zinc content and amounted 44.2, 47.1, 47.3 and 48.0 mg/kg in ch, chDM, ch DR and chDOR diet, respectively. Various dietary components may change zinc absorption. Puls (1994) indicates that increased dietary cholesterol reduces serum zinc and high density lipoproteins which may result in development of coronary heart disease. Most zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye (Osredkar and Sustar 2011). The concentrations of zinc in most mammalian tissues ranged from 10 to 100 μg/g fresh weight (30–250 μg/g dry weight), with little variation among species (McDowell 1992). The liver plays a central role in zinc homeostasis and vice versa, zinc plays an important role in the therapy for several liver diseases (Sidhu et al. 2004, Tian et al. 2014). In our previous work (Leontowicz et al. 2011) we concluded that durian at different stages of ripening, especially ripe one, constitutes an excellent source of effective natural compounds with antioxidant and health-protective activity in general, and liver and heart-protective effect in cholesterol fed rats in particular. Hepatic tissue morphology of rats fed basal diet with 1% of cho-

lesterol and with unripe durian revealed high contents of fat inside hepatic cells in contrast to rats fed diets with ripe durian, where hepatic tissue shows small number of large fat droplets. Furthermore somatic index of liver increased significantly in all rats receiving diet supplemented with cholesterol. This stress could affect zinc levels, despite the fact that durian fruits are rich in various minerals and other bioactive compounds (Leontowicz et al. 2011). The conducted research was designed to answer if diet supplementation with durian at different stages of maturity influences the liver zinc content in rats loaded with cholesterol. As shown at Figure 1, despite the highest feed intake in the control group (C), the zinc content is not reflected in the liver.

The content of zinc in the liver in all animal groups was within the normal limits and amounted from 25.0 to 33.0 mg/kg fresh weight. The lowest zinc content and feed intake were noticed in control rats (ch) fed diet with cholesterol. Rats receiving atherogenic diet with ripe durian have the highest content of zinc in the liver, significantly different from C and ch group. The various components of the diet and the durian fruit itself may change absorption of zinc and affect its bioavailability. Durian fruit is a good source of Fe, Mn and Cu, which may compete or/and inhibit of Zn uptake into intestinal cells. This fruit is also rich in dietary fiber that binds cholesterol and decreases its content in the blood. It is important to mention that the amount of



a–b – columns marked with different letter differ at $P \leq 0.05$;
values are means ±SD (n = 6);
C – control group; ch – control with 1% of cholesterol; chDM – group with 5% of mature durian and cholesterol; chDR – group with 5% of ripe durian and cholesterol; chDOR – group with 5% of ripe durian and cholesterol durian fruits.

FIGURE 1. Zinc content in the liver (fresh weight) and total feed intake of rats fed diets with durian in different stages of maturity and with cholesterol

alanine and aspartate aminotransferases were in normal range in blood plasma of rats fed atherogenic diet supplemented with durian (Leontowicz et al. 2011). Koo et al. (1986) have reported that nutritional status of zinc is an important factor influencing the intestinal absorption and subsequent metabolism of dietary cholesterol.

CONCLUSIONS

Diet supplemented with durian fruits at different stages of ripening increases accumulation of zinc in the liver of rats loaded with cholesterol. Durian fruits, especially ripe ones, could be use as a natural supplementation of Zn in the diet and might be helpful in prevention of diseases or disorders related to zinc deficiency.

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Streszczenie: *Wpływ różnych faz dojrzałości owocu durianu Durio zibethinus na zawartość cynku w wątrobie szczurów obciążonych cholesterolem.* Durian *Durio zibethinus* to egzotyczny, klimakteryczny owoc, bogaty we włókno pokarmowe, polifenole, oligosacharydy, witaminy i minerały. Zmiany następujące po zbiorze owoców durianu wpływają na ich fizyczne i chemiczne parametry determinujące ich właściwości zdrowotne – przeciwutleniające i hypocholesterolemiczne. Durian jest cennym źródłem cynku. Celem badania było określenie zawartości cynku w owocach durianu, w dietach z dodatkiem durianu w różnych fazach jego dojrzałości. Badanie *in vivo* miało na celu określenie wpływu tych diet na zawartość cynku w wątrobie szczurów obciążonych cholesterolem. Zawartość cynku w owocach durianu, w dietach i wątrobach oznaczono metodą płomieniowej absorpcji atomowej (Perkin-Elmer 1100B). Zawartość cynku w liofilizacie owoców durianu mieściła się w zakresie 8,1–12,3 mg/kg. Zawartość cynku w dietach: C, ch, chDM, ch DR and chDOR wynosiła odpowiednio 45,0, 44,2, 47,1, 47,3 i 48,0 mg/kg. W wątrobie szczurów wszystkich grup zawartość cynku mieściła się w zakresie wartości referencyjnych i wynosiła od 25,0 do 33,0 mg/kg świeżej masy. Diety z dodtkiem owoców durianu w różnej fazie dojrzałości zwiększają akumulację cynku w wątrobie szczurów obciążonych cholesterolem. Owoce durianu, w szczególności dojrzałe, mogą być stosowane jako naturalny suplement cynku w diecie i być pomocne w prewencji chorób lub stanach związanych z niedoborem cynku.

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