

## **Inhibition of Aldehyde Dehydrogenase Enzyme by Durian (*Durio zibethinus* Murray) Fruit Extract**

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Abbreviations: ALDH, aldehyde dehydrogenase; DE, durian extract; DER, disulfiram-ethanol reaction;  $K_m$ , Michaelis constant  $\beta$ -NAD or NAD, nicotinamide-adenine dinucleotide; Rf, retardation factor;  $V_0$ , initial velocity;  $V_{max}$ , maximum velocity

## ABSTRACT

The scientific basis of the adverse, or at times lethal, effect of ingesting durian (*Durio zibethinus* Murray) while imbibing alcohol has not been established. Symptoms are reminiscent of the Disulfiram-Ethanol Reaction (DER) arising from the inhibition of aldehyde dehydrogenase (ALDH). Cognizant of the inhibitory effect of sulfur compounds like disulfiram on ALDH and the rich sulfur content of durian, the influence of durian fruit extract on the ALDH-mediated oxidative metabolism of acetaldehyde was investigated. We report a dose-dependent inhibition of yeast ALDH (yALDH), at most 70% at 0.33 ppm (mg extract/L assay mix), by dichloromethane:pentane extracts. Sulfur-rich TLC fruit extract fractions that eluted farthest from the origin effected the greatest inhibitory action. yALDH assay using diethyl disulfide as internal standard further supports the role of durian's sulfury constituents in the fruit's ALDH-inhibiting property. Insight into the etiology of DER-like symptoms felt upon simultaneous durian and alcohol consumption is hereby presented.

## 1. Introduction

Compelling scientific evidence support the beneficial effects of fruit and vegetable consumption (WHO, 2003), and moderate alcohol intake (Hines and Rimm, 2001) on cardiovascular health. Thus, dietary policies in countries like the US support the inclusion of these food items as part of disease preventive diets (USDA/HHS, 2005). However, risky interactions with ethanol were observed in some vegetables (Desager *et al.*, 2002; Lindros *et al.*, 1995).

Durian (*Durio zibethinus* Murray) is an important nutritional resource for Southeast Asian diets (FAO, 2003a). Its increasing popularity even in distant markets as the US and EC ensued the intensification of research to inform and familiarize consumers on the characteristics of the fruit (FAO, 2003b). Novel findings on its bioactive composition and health benefits (Charoensiri, *et al.*, 2009; Mahattanatawee *et al.*, 2006) point to its potential use in disease preventive diets (Leontowicz, *et al.*, 2007). However, anecdotal accounts on the interaction of durian with ethanol suggest an unsafe combination.

Reports on the adverse effects, which include cardiac episodes or deaths, in patients drinking alcohol with durian persists (Fuller, 2007; Croft, 1981; Singh, 1941). The symptoms have been described as being very unpleasant with clinical manifestations such as facial flushing, palpitation, drowsiness, vomiting and nausea. Similar effects have been associated with alcohol aversion therapy using disulfiram (tetraethylthioram disulfide), a sulfur-containing drug (Lipsky *et al.*, 2001; Veverka *et al.*, 1997). Disulfiram is known to inhibit aldehyde dehydrogenase (ALDH), causing the accumulation of alcohol-derived acetaldehyde. Although the possible

contribution of alcohol itself to the unpleasant physiological effects has been discussed in several studies (Duranceaux *et al.*, 2006; Jensen and Faiman, 1986), acetaldehyde appears to have a central role in mediating the adverse reactions referred to as the Disulfiram-Ethanol Reaction or DER (IARC, 1999; Brien and Loomis, 1985; Inoue *et al.*, 1984).

The inhibitory effects of sulfur-containing compounds from natural products on ALDH have been proposed by several groups (Veverka *et al.*, 1997; Kitson and Weiner, 1996; Lindros *et al.*, 1995; Brien and Loomis, 1985). Durian is rich in sulfur compounds (Voon *et al.*, 2007a,b; Weenan *et al.*, 1996; Wong and Tie, 1995; Moser *et al.*, 1980; Baldry *et al.*, 1972), but there is yet no study demonstrating the inhibitory effects of durian or its sulfur constituents on ALDH. This study provides information on the possible link between some components of durian and DER-like effects.

## **2. Materials and Methods**

### *2.1. Chemicals*

Inhibition studies were conducted using potassium-activated ALDH enzyme from baker's yeast (*Saccharomyces cerevisiae*) in lyophilized powder form (Sigma-Aldrich, St. Louis, Missouri, USA) with  $\beta$ -NAD<sup>+</sup> (Oriental Yeast Corporation, Osaka, Japan) as cofactor. Dichloromethane, pentane, and authentic diethyl disulfide were procured from Wako Pure Chemical Industries, Ltd. (Osaka City, Japan). All chemicals and solvents used were of analytical grade and purity.

### *2.2. Durian Fruit Extract Preparation*

The pulp of table-ripe durian fruit (*Durio zibethinus* Murray *cv.* Monthong) purchased from a supermarket in Tsukuba City, Japan was mixed with dichloromethane:pentane (1:1 v/v) (Weenan *et al.*, 1996) in a 1:2 proportion (1 kg:2 L solvent mix) for 48 h at 4 °C. The clear liquid was decanted and the solvent was evaporated under vacuum ( $27.5 \pm 2.5$  inHg) at 50 °C and 50 rpm in an RE52 Rotavaporator set-up (Yamamoto, Tokyo, Japan). The fruit extract (1.7 ml/kg pulp) was sealed in a glass container and kept at -20 °C.

### *2.3. Fractionation by TLC*

TLC fractions of durian extract (DE) and durian extract spiked with authentic diethyl disulfide (55 mM) (DE+DD) were developed in 0.33 mm Chromato sheets (2 cm x 7 cm) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) using water, 2-butanol and 28% ammonia (1:1:1) as solvent. Eight sheets each for DE and DE+DD were spotted with 10  $\mu$ l sample (1 cm above the base) and developed in a chamber at 25 °C for 45 mins until the solvent front reached 1 cm below the tip of the sheets. Chromatograms were dried in a fumehood at 25 °C, trimmed (0.5 cm base and top), cut into six equal parts (where Rf 0 encloses the spot; Rf 1 the solvent front). Each part was soaked with 150  $\mu$ l 1 M Tris-HCl buffer, and centrifuged in Beckman GS-15R Centrifuge (Beckman Inst. Inc., Palo Alto, California) at 5000 rpm for 5 min, 10 °C. Fractions from the same Rf values of DE and DE+DD were pooled ( $\cong$  1.2 ml/Rf), placed in ice bath and immediately used in inhibition assay.

#### 2.4. *yALDH* Assay

Enzymatic assay was monitored by following the rate of NADH accumulation indicated by the increase in  $A_{340}$  using a thermostat-equipped JASCO V-550 spectrophotometer (Tokyo, Japan) at 25 °C for 3 min (Sigma, 1996). A standard 3 ml final reaction mixture contained: 103 mM Tris HCl buffer, pH 8.0; 0.67 mM  $\beta$ -NAD<sup>+</sup>; 0.25-0.5 units *yALDH* solution (diluted in 100 mM Tris HCl buffer with 0.02%(w/v) BSA); 100 mM-KCl; 10 mM 2-mercaptoethanol; 10 mM acetaldehyde; filled to volume with deionized water. Reactions were commenced by adding 0.10 ml  $\beta$ -NAD<sup>+</sup> to an otherwise complete reaction mixture.

Assay in test samples: authentic diethyl disulfide (1.1 ppm, final concentration); and filtered (PTFE 0.2  $\mu$ m Millipore Millex-LG syringe-driven filter) durian extract (0.03, 0.07, 0.16, 0.33, 0.65, & 1.63 ppm, mg extract/L assay mix) and TLC fractions of DE and DE+DD (55 mM) followed the conditions described above except that reactions were commenced by adding a mix of the test sample and  $\beta$ -NAD<sup>+</sup>.

Initial velocity studies in 0.33 ppm durian extract (mg/L assay mix) at 1, 2, 5, 10 & 50 mM acetaldehyde followed the reaction conditions described above. Computed kinetic parameters (*i.e.*  $K_m$  &  $V_{max}$ ) were compared to that obtained without the extract at 2, 5, 7, 10, 30, & 50 mM acetaldehyde.

Determinations were done in triplicate *vis-à-vis* an almost identical solution (blank) that contains the enzyme diluent in *lieu* of the *yALDH* solution. Order of enzyme, substrate, cofactor and test sample addition was maintained in all enzymatic assay conducted. Results were subjected to a *t*-test ( $P > 0.05$ ).

## 2.5. Sulfur Test

Collected TLC fractions (300  $\mu$ l) from the durian extract were mixed with 600  $\mu$ l 20% (w/v) sodium formate solution and heated to liberate bound sulfur as hydrogen sulfide that manifests as dark discolorations in the lead acetate paper upon contact with tainted fumes (adopted from FAO, 1996).

## 3. Results and Discussion

The durian-alcohol anecdote has been sheltered with over 250 years of belief. Few attempts to validate the interaction have vaguely disproved the alleged antagonism between durian and ethanol (Nilvises and Saengsirinavin, 1986; Techapaitoon and Sim, 1973; Ogle and Teh, 1969; 1971). None of these *in vivo* studies, however, considered the possible interference of some components in durian on the enzymatic system for detoxifying alcohol. To our knowledge, this is the first attempt to explore the molecular basis of the durian-alcohol anecdote and no other literature has yet linked a fruit to adverse reactions that may arise upon interaction with alcohol.

We used yALDH as a model protein because its active region is 95-100% homologous to that of the mammalian ALDHs (Saigal *et al.*, 1991). Veverka *et al.* (1997) also used yALDH for studies on the mechanism of inhibition by disulfiram for the same reason.

yALDH significantly lost ( $P < 0.05$ ) enzymatic activity in the presence of durian fruit extract at all concentrations used (Fig.1, line graph). The intensity of inhibition increases with fruit extract concentration to a maximum of 70% at 0.33 ppm extract (final concentration). At higher concentrations (0.67ppm and 1.67ppm), the inhibitory effect weakened. Several studies reported the potent inhibitory action of sulfur compounds on ALDH (Park, 2003; Lindros *et al.*, 1995; Desager *et al.*, 2002; Brien and Loomis, 1985). Similarly, the non-polar organic constituents (Rf 0.6, 0.8 and 1.0) of the durian fruit extract that also registered positive results in the sulfur-test elicited significant inhibitory effects on ALDH (Fig.1, shaded bar graph). The degree of inhibition progressively declined from Rf 1.0 to Rf 0.4 with increasing polarity of these fractions. Veverka *et al.* (1997) reported a complete inhibition of yALDH activity upon treatment with 20  $\mu$ M disulfiram by oxidizing proximal cysteines in the active site to form a disulfide bridge. With this mechanism, they suggested that sterically suitable compounds containing a disulfide bridge may potentially inhibit ALDH. In durian, diethyl disulfide is the most abundant sulfur compound that contains a disulfide bridge (Voon *et al.*, 2007a; Laohakunjit, *et al.*,

2007). Assay in 1.1 ppm authentic diethyl disulfide (final concentration) lowered the activity of yALDH by 81.5%. The contribution of diethyl disulfide to the durian extract-elicited inhibition of yALDH activity is hereby supported.

Authentic diethyl disulfide added to the durian fruit extract (55 mM) and subjected to planar TLC fractionation enhanced the inhibition elicited by the fraction with a calculated Rf value of 0.8 (Fig.1, unshaded bar graph). The possible involvement of other non-polar sulfury constituents of durian to the observed inhibition, as our results imply, merits further investigation since the resulting fractions from TLC may be broad and the contents of Rf 0.8 may have spread out to adjoining fractions.

Addition of the contents of Rf 0 and 0.2 enhanced yALDH activity (Fig.1). Consistent with the fading inhibitory effect of durian extract at concentrations beyond 0.33 ppm earlier observed, enhanced yALDH activity in these polar fractions may have resulted from the increase in substrate concentration contributed by the endogenous aldehyde content of the durian fruit (Voon *et al.*, 2007a). Fractions obtained from Rf 0.4 elicited insignificant alteration ( $P>0.05$ ) of the enzyme's activity.

<Insert Figure 1 here>

Assay in 0.33 ppm crude extract lowered both the  $K_m$  and  $V_{max}$  values of yALDH (Fig.2). Increasing the substrate concentration did not alter the activity of the enzyme as suggested by the low coefficient of determination ( $r^2=0.2941$ ) (Fig.2 insert). Based on the kinetic parameters, components of the durian fruit extract appear to elicit a mixed type of inhibitory action on yALDH. However, as this study involved the use of crude extract, further investigation of the inhibitory constituents is required to provide a conclusive characterization of the mechanism of inhibition.

<Insert Figure 2 here>

#### **4. Conclusion**

This study provides evidence that some component(s) of durian fruit inhibit(s) yALDH *in vitro*. Inhibitory action of the non-polar sulfury constituents of the fruit like diethyl disulfide on yALDH, with further investigation, offers a novel rationale to the etiology of the durian-alcohol anecdote. As such, work on this

theme is currently undertaken to confirm the bioactive component(s) in the durian fruit, and validate the consistency of the findings hereby reported in mammalian models *in vivo*.

An important implication of studies along this line lies in strengthening science-based food safety precautions serving as impetus for issuing reliable toxicological disclaimer on the durian-alcohol interaction in a similar fashion to that of the watercress-alcohol. Such information should be useful to health care professionals or even the general consumer to avoid risky food-beverage interactions.

### **Acknowledgements**

Gratitude is hereby extended to Dr. Alex Buchanan for his seminal contribution in developing the perspective of this research; Dr. Norie Anne B. Nolasco, Department of Food Science and Nutrition, College of Home Economics, University of the Philippines, for her masterful critiquing; Dr. Custer C. Deocar, Japan National Institute for Advanced Industrial Science and Technology, for insights on the paper.

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## Figure Legends

**Fig. 1.** yALDH inhibition by durian extracts (line graph), non-polar TLC fractions of durian extracts (DE, shaded bar graph) that yielded positive on the sulfur test (Rf 0.6, 0.8, & 1.0), and non-polar fractions of durian extract added with diethyl disulfide (DE + DD, unshaded bar graph). Values are reported as % inhibition of “control” yALDH activity, which was determined in the absence of any inhibitor. Data are means  $\pm$  sd of three replicates. Graphs with similar letters indicate insignificant differences ( $P > 0.05$ ).

**Fig. 2.** Double reciprocal plot of  $V_0$  vs. [Acetaldehyde] in 0.33 ppm durian fruit extract ( $\Delta$ ) and control ( $\circ$ ) (regressed values). *Insert*, plot of  $V_0$  as a function of [Acetaldehyde].