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Physicochemical and bioactive properties of some Sri Lankan traditional rice varieties

A thesis submitted for the Degree of Doctor of Philosophy

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ABSTRACT

Rice (*Oryza sativa* L.) is the dietary staple over half of the world's population and wide range of rice varieties are available worldwide with varying physicochemical and bioactive properties. Rice bran is a by-product of the rice milling process and currently numerous applications worldwide in functional foods and nutraceutical industries. In Sri Lanka, rice is the dietary staple and reported to have many Sri Lankan traditional rice varieties. In the traditional knowledge of Sri Lanka, some of these varieties are claimed to have varying physicochemical and functional properties. However, there is no single comprehensive study conducted in the country to date, to evaluate physicochemical and bioactive properties of these varieties. This study therefore, was undertaken to investigate the selected physicochemical and bioactive properties for selected Sri Lankan traditional rice varieties.

Twenty three (23) red and white rice varieties were selected for the study. The selected varieties were studied for grain size, shape, color, moisture, crude protein, crude fat, ash, total carbohydrate, amylose content, gelatinization temperature and gel consistency as physicochemical properties of the rice grain. These properties were studied for the whole grains or the brown rice (endosperm and bran). As bioactivities anti-diabetic, anti-inflammatory, anticancer, anti-oxidant and anti-hypertension properties were studied using wide variety of enzymatic, non enzymatic and cell based bioassays *in vitro*. Anti-hyperglycemic activity also studied using *in vivo* rat model (1g/kg body weight) as an anti-diabetic property. Screening of selected varieties was performed based on anti-diabetic property (α -amylase inhibitory activity and anti-glycation activity) and rest of the bioactivities were conducted only for the highly biologically active varieties selected at the screening process. Seventy percent ethanolic extract of the rice bran (concentration range: 1 - 400 μ g/ml) was used in all the bioactivities. Anti-inflammatory and anti-cancer properties were studied also for rice bran fractions, by partitioning 70 % ethanolic extracts using hexane, dichloromethane and butanol. Effect of rice bran protein hydrolysates (concentration range: 250 - 1200 μ g/ml) were studied only for anti-hypertension and anti-cancer properties.

Physicochemical properties studied indicated that out of 23 varieties 20 varieties are red rices and size and shape were mostly short bold (56 %) and medium bold (30 %). Moisture, crude protein, crude fat, ash and the total carbohydrate contents ranged from $10.42 \pm 0.25 - 12.33 \pm 0.02$ %, $10.59 \pm 0.12 - 13.27 \pm 0.32$ %, $2.18 \pm 0.10 - 4.12 \pm 0.28$ %, $1.30 \pm 0.14 - 1.92 \pm 0.05$ % and $81.42 \pm 0.25 - 85.66 \pm 0.24$ % respectively and varied significantly ($p < 0.05$) among the varieties. Among the varieties studied 95 % of the varieties were high amylose rices (> 25 %). Gelatinization properties studied indicated that gel consistency was mostly medium (48 %) and soft (39 %) and gelatinization temperature was mostly intermediate (65 %). The highly biologically active varieties selected at the screening process were Sudu Heeneti, Goda Heeneti, Masuran and Dik Wee and these four varieties are red rices. These four varieties had anti-diabetic, anti-oxidant, anti-inflammatory and anti-cancer properties. Anti-diabetic property of these varieties was mediated by multiple mechanisms which include α -amylase inhibitory activity, anti-glycation activity, glycation reversing ability and acetyl and butyrylcholinesterase enzyme inhibitory activities. Further, Masuran, Sudu Heeneti and Dik Wee varieties had low *in vitro* starch digestion rate indicating that these varieties may be low glycemic index rices. Brans of Masuran variety also showed *in vivo* anti-hyperglycemic activity. Anti-inflammatory activity of selected varieties was too mediated by multiple mechanisms. These mechanisms include

reduction of oxidative burst of human whole blood, and isolated immune cells including polymorphonuclear cells and macrophages, inhibition of nitric oxide and proinflammatory cytokines, TNF- α and IL-1 β without mediating cytotoxicity to normal cells. As anti-cancer property the selected four varieties showed cytotoxicity on human lung cancer cell line NCI-H460 and human cervical cancer cell line HeLa. All these varieties had high glutathione S-transferase enzyme inhibitory activity and indicate its potential to be used as adjuvant in managing cancer patients during chemotherapy. Anti-oxidant property of these varieties are multifaceted and mediated by presence of polyphenolic antioxidants, scavenging of free radicals, DPPH, ABTS, superoxide and nitric oxide and ferric reducing antioxidant power. However, all these varieties did not show anti-hypertension activity tested via ACE inhibition. Seventy % ethanolic extracts showed significantly high activity compared to rice bran protein hydrolysates in different bioactivities tested in this study. Fractionation results indicated that synergistic as well as antagonistic effect. However, generally non polar fractions were more biologically active compared to polar fractions of the rice bran. Among the bioactivities studied α -amylase inhibitory activity, anti-glycation activity, glycation reversing ability and oxidative burst inhibitory activity showed potent activity while other bioactivities showed moderate activities compared to standard drugs used in the study and other rice varieties worldwide.

In conclusion, Sri Lankan traditional rice possesses desirable physicochemical and bioactive properties. In terms of physicochemical properties both red and white rice had desirable properties. However, in terms of biological activities red rice is superior to white rice. Brans of selected red rice showed the potential of isolating active compounds for developing promising novel drugs for managing diabetes, cancer and inflammatory diseases. It also shows the possibility of using rice and rice bran in functional foods and nutraceuticals industries. Collectively, this research added value to the rice bran, a cheap source and a byproduct of the rice milling industry. Finally, the findings of this study may essentially help to promote Sri Lankan traditional rice especially red rice as a healthy staple food for Sri Lankans and thought out the world and make it a profitable industry to the country.