**UNIVERSITI TEKNOLOGI MARA**

# ISOCRATIC RP-HPLC METHOD FOR QUANTIFICATION OF L-CITRULLINE CONTENT IN 100% WATERMELON [*Citrullus lanatus* (THUNB.) MATSUM. AND NAKAI] JUICES AND ITS ROLE IN IMPROVING SWIMMING PERFORMANCE

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# ABSTRACT

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) contains high L-citrulline content that may beneficial to maximize exercise performance. The present study aimed to assess L-citrulline status in local 100% watermelon juices and investigate the role of 100% flesh and rind watermelon juices supplementation for 14 days to improve swimming performance in rats. Determination and quantification of L-citrulline in 100% flesh and rind watermelon juices were performed by isocratic RP-HPLC method on Gemini $C_{18}$ using 0.1% $H_3P0_4$ as mobile phase at flow rate of 0.5 mL/min and detection wavelength at 195 nm. The method was validated in terms of linearity, sensitivity, accuracy and precision. L-citrulline was determined at retention time, 5.7 minutes. High concentration of L-citrulline was found in flesh and rind of 100% watermelon juices, 2.46 g/L and 3.13 g/L, respectively. The validated method showed a good linearity (>0.99), low values of LOD and LOQ, recoveries within 101.94% – 103.38% and RSD less than 2%. For *in vivo* study, twenty four male Sprague-Dawley rats were randomly divided into four groups consisting of Cx group of rats supplemented with filtered tap water (negative control), L-cit group of rats supplemented with L-citrulline (positive control), FR group of rats supplemented with 100% flesh watermelon juice and RR group of rats supplemented with 100% rind watermelon juice. Each group supplemented for 14 days *ad libitum* prior to swimming exercise protocol. Rats performed swimming exercise for 3 days and the swimming time until exhaustion was measured. Plasma samples were collected to measure lactate concentration, ammonia concentration and nitric oxide production. Rats supplemented with 100% flesh watermelon juice demonstrated significant prolongation of swimming time until exhaustion, reduction in lactate and ammonia concentration and increment in nitric oxide production. These findings postulate that 100% flesh watermelon juice supplementation for 14 days may improve swimming exercise performance owing to high L-citrulline content.

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# CHAPTER ONE
# INTRODUCTION

## 1.1 Research Background

A well-known concept in exercise sciences holds mild to intense exercises lead to exhaustion when the cardiorespiratory system supply oxygen to exercising muscles fails to meet their demand inducing anaerobic metabolism (Noakes, 2000). The heat and metabolites generation from anaerobic metabolism subsequently exhaust energy stored leading to muscle strain (Ament & Verkerke, 2009). The urge to avert such consequences demand an effective alternative of health supplements to improve exercise performance.

Literature has demonstrated that L-citrulline supplementation may improve exercise performance and involved in removal of excess metabolites from body system (Takeda et. al., 2011). Perez-Guisado reported supplementation of citrulline malate (8 g) able to reduced muscle soreness at 24 and 48 hours of anaerobic exercise. Meanwhile, *in vivo* study by Meneguello, Mendonça, Lancha, & Costa Rosa, (2003) on mice supplemented with single dose of 0.26 g/kg body weight of L-citrulline in mixture containing 0.4 g/kg body weight of L-arginine and 0.2 g/kg body weight of L-ornithine resulted in suppression of blood ammonia accumulation after exercise and prolonged the time until exhaustion in swimming exercise. These findings suggest the beneficial function of L-citrulline in improving endurance performance, accelerating metabolites wastes removal and faster recovery after exercise.

Awareness have been raised that dietary L-citrulline supplementation may cause excess production of L-arginine. Evans, Fernstrom, Thompson, Morris, & Kuller, (2004) study has demonstrated gastrointestinal discomfort, nausea and diarrhea in individuals after L-arginine consumption, making it unsuitable for daily intake. These unfavourable side effects may be due to rapid and elevated nitric oxide (NO) production by gastrointestinal tract leading body discomfort (Wu & Meininger, 2000). Hence, emerging research is focusing on exploratory safer alternative nutritional regimens to improve exercise performance that contains L-citrulline with other nutrients (Tarazona-Díaz, Alacid, Mart, & Aguayo, 2013; Cutrufello, Gadomski, & Zavorsky, 2015; Jayaprakasha & Patil, 2016). Evidences remarkably thrive to