

**CONTROLLED RELEASE OF NUTRIENTS FROM
COCONUT WATER-BASED HYDROGELS**

VIKNESWARAN MUTHU

UNIVERSITI SAINS MALAYSIA

2019

CONTROLLED RELEASE OF NUTRIENTS FROM COCONUT WATER-BASED HYDROGEL

by

VIKNESWARAN MUTHU

**Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science**

August 2019

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my advisor Dr. Tan Thuan Chew for the continuous support of my master study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my master study.

Besides my advisor, I would like to thank the rest of my co-supervisors: Assoc. Prof. Dr. Ahmad Munir Che Muhamed and Prof. Dato' Dr. Azhar Bin Mat Easa, for their insightful comments and encouragement, but also for the hard question which incited me to widen my research from various perspectives.

I thank my fellow labmates in for the stimulating discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had in the last four years. Also I thank my lab assistants for their guidance.

Last but not the least, I would like to thank my family: my parents and to my brothers and sister for supporting me spiritually throughout writing this thesis and my life in general.

TABLE OF CONTENTS

ACKNOWLEDGEMENT	ii
TABLE OF CONTENT	iii
LIST OF TABLES	vi
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	x
ABSTRAK	xi
ABSTRACT	xiii
CHAPTER 1- INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Objectives	4
CHAPTER 2- LITERATURE REVIEW	5
2.1 Nutrition requirement in sports	5
2.1.1 Carbohydrate consumption during sports	5
2.1.2 Assessment of performance exercise test	11
2.1.3 Exhaustion and dehydration during exercise	12
2.1.4 Feasibility of coconut water in sports application	13
2.2 Pre-exercise supplementation	14
2.2.1 Energy gel	14
2.2.2 Variation in commercial gels	17
2.2.3 Maltodextrin	22
2.3 Coconut	23
2.3.1 Coconut water	23
2.3.2 Varieties of Coconut	25
2.3.3 Benefits of Coconut water	29
2.4 Hydrocolloids	32

2.4.1 Types of hydrocolloids	32
2.4.2 Gellan gum	37
2.4.3 Xanthan gum	40
2.4.4 Controlled release of <i>in situ</i> gels	42
2.5 In vitro study	
2.5.1 In vitro digestion system	44
2.5.2 In vitro digestion and enzymes	48
2.5.3 In vitro digestion and sample conditions	49
2.5.4 Digestion and transit time	49
2.5.5 <i>In vitro–in vivo</i> correlation	50
2.6 Concluding remark	50
CHAPTER 3- MATERIALS AND METHODS	53
3.1 Chemical and reagents	55
3.2 Young coconut water	56
3.2.1 Extraction of young coconut water	56
3.2.2 Sampling plan	57
3.3 Properties of Coconut Water	57
3.3.1 Measurement of pH	57
3.3.2 Total soluble solid	58
3.3.3 Sugar content	58
3.3.4 Mineral content	60
3.4 Preparation of gellan-xanthan sol gels	62
3.5 Dissolution of the sol gels	63
3.6 Preparation of gels	63
3.6.1 Preparation of coconut water-based hydrogel	63
3.6.2 Preparation of coconut water-based energy gel	64
3.6.3 Preparation of coconut water-based drink	64
3.6.4 Preparation of water-based energy gel	64
3.7 In vitro experiment	65

3.8 Sensory Evaluation	65
3.9 Performance Exercise Test	67
3.9.1 Subjects	67
3.9.2 Preparation of gels	67
3.9.3 Pre-experimental protocol	68
3.9.4 Research protocol	69
3.9.5 Measurement	70
3.9.5(a)Cardiorespiratory measurement	70
3.9.5(b) Subjective ratings	70
3.9.5(c) Haematological measurement	71
3.10 Statistical analysis	72
CHAPTER 4- RESULT AND DISCUSSION	74
4.1 Physiochemical properties of young coconut water	74
4.2 Dissolution of gellan-xanthan sol gels	76
4.3 <i>In vitro</i> nutrient release study of coconut water-based hydrogel	81
4.4 Formulation of coconut water-based energy gel	86
4.5 Effect of coconut water and gelling agents	94
4.6 Sensory evaluation	100
4.7 Performance Exercise Test	101
CHAPTER 5- CONCLUSION AND FUTURE	111
RECOMMENDATION	
5.1 Conclusion	111
5.2 Recommendations for future research	112
REFERENCES	114
APPENDICES	

LIST OF TABLES

		Page
Table 2.1	Studies of carbohydrate intake immediately before and/or during high-intensity exercise ~ 1 h duration	7
Table 2.2	Nutritional composition of a range of sports gels available in Australia	18
Table 2.3	Descriptive statistics for the carbohydrate gels for parameters related to serving size, energy density, energy content, carbohydrate content, free sugars content, fructose content and osmolality	20
Table 2.4	Nutritional data for coconut water per 100 g	25
Table 2.5	A simplified classification of coconut varieties	26
Table 2.6	Composition and physicochemical properties of coconut water obtained from immature (IMC), mature (MC), and overly mature coconut (OMC)	28
Table 2.7	Hydrocolloids and their characteristics	34
Table 2.8	Characteristics of gellan gum	38
Table 2.9	Characteristics of xanthan gum	41
Table 2.10	Several survey of in vitro digestion systems used to test various foods	46
Table 3.1	List of chemicals and reagents used for analysis	56
Table 3.2	HPLC conditions for the sugar analysis in coconut water	59
Table 3.3	Concentration range of the standard solution for the analysed minerals	61
Table 3.4	Formulation of gellan-xanthan sol gels	62
Table 3.5	Description of the 7-point Hedonic scale	66
Table 3.6	Rating of perceived exertion (RPE) scale	71
Table 4.1	Sugars and selected minerals contents in young coconut water.	76
Table 4.2	Consumers' preference on coconut water-based energy gel	101

Table 4.3	Time trial completed during 10 km after consuming coconut water-based energy gel, coconut water-based hydrogel, and water-based energy gel	108
-----------	--	-----

LIST OF FIGURES

		Page
Figure 2.1	Chemical structure of gellan gum	37
Figure 3.1	Flowchart of the study	54
Figure 4.1	Percentage of gel dissolution in simulated gastric fluid (SGF) (pH 1.22) for 60 min	79
Figure 4.2	Complete dissolution time of the remaining gels from SGF into simulated intestinal fluid (SIF) (pH 6.80)	80
Figure 4.3	<i>In vitro</i> release profile of glucose (▲) and fructose (■) from coconut water-based hydrogel (CWH) into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	83
Figure 4.4	<i>In vitro</i> release profile of into sodium (◆), potassium (▲), and calcium (■) from coconut water-based hydrogel (CWH) into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	84
Figure 4.5	HPLC chromatogram showing (A) the peaks obtained for young coconut water used to prepare the hydrogels and (B) the peaks obtained for <i>in vitro</i> study of coconut water-based hydrogel (CWH) at 45 min	86
Figure 4.6	<i>In vitro</i> release profile of maltodextrin (◆), sucrose equivalent (■), glucose (▲), and fructose (●) from CWE into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	88
Figure 4.7	<i>In vitro</i> release profile of potassium (■), sodium (◆), and calcium (▲) from CWE into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	90
Figure 4.8	Cumulative percentage of (A) maltodextrin, (B) sucrose equivalent, (C) glucose and (D) fructose released from	92

	CWE into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	
Figure 4.9	Cumulative percentage of (A) sodium, (B) potassium and (C) calcium released from CWE into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	93
Figure 4.10	<i>In vitro</i> release profile of (A) maltodextrin, (B) sucrose equivalent, (C) glucose, and (D) fructose from coconut water-based energy gel (◆), water-based energy gel (■), and coconut water-based energy drink (▲) into simulated gastric fluid (SGF) for first 60 min and then into simulated intestinal fluid (SIF) for 75 min	98
Figure 4.11	Comparison on the blood glucose responses during 60-minute exercise, 10km time trial and recovery phase after consuming coconut water-based energy gel (◆), coconut water-based hydrogel (■), and water-based energy gel (▲)	103
Figure 4.12	Comparison on the rating of perceived exertion during 60-minute exercise after consuming coconut water-based energy gel (◆), coconut water-based hydrogel (■), and water-based energy gel (▲)	104
Figure 4.13	Power output generated during 10 km time trial after consuming coconut water-based energy gel (◆), coconut water-based hydrogel (■), and water-based energy gel (▲)	105

LIST OF ABBREVIATIONS

CWE	Coconut water-based energy gel
CWH	Coconut water-based hydrogel
HPLC	High-performance liquid chromatography
RPE	Rating of perceived exertion
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
TSS	Total soluble solid
TT	Time trial
VO _{2max}	Maximal oxygen consumption

PELEPASAN TERKAWAL NUTRIEN DARIPADA HIDROGEL BERASASKAN AIR KELAPA

ABSTRAK

Kajian membuktikan bahawa air kelapa dapat menggantikan nutrien yang digunakan semasa latihan fizikal. Nutrien dalam air kelapa seperti gula dan elektrolit boleh diserap oleh badan kita dengan mudah dan cepat. Dengan merancang air kelapa menjadi sistem pelepasan terkawal, ia dapat memberikan nutrien kepada tubuh dengan cara yang berterusan. Oleh itu, tujuan penyelidikan ini adalah untuk membangunkan gel tenaga berasaskan air kelapa dengan sistem penggelan *in situ* dan pelepasan terkawal. Hidrogel berasaskan air kelapa yang diperbuat daripada gum gellan dan xanthan telah dirumuskan sebagai asas untuk membangunkan gel tenaga berasaskan air kelapa. Formulasi hidrogel dengan campuran 0.7% gellan dan 0.3% xanthan menunjukkan toleransi yang paling tinggi terhadap simulasi cairan gastrik dan usus selama tempoh satu jam semasa ujian kelarutan. Semasa kajian pelepasan *in vitro*, arah aliran profil pelepasan menunjukkan bahawa hidrogel mempunyai keupayaan untuk mengekalkan pelepasan nutrien dalam tempoh satu jam. Selepas 75 minit, arah aliran pelepasan itu statik menunjukkan jumlah gula dikeluarkan daripada hidrogel. Oleh kerana hidrogel tidak memberikan nutrien yang mencukupi, iaitu karbohidrat dan sodium yang diperlukan untuk senaman, maltodekstrin dan natrium klorida ditambah untuk menghasilkan gel tenaga berasaskan air kelapa. Ujian *in vitro* menunjukkan bahawa gel tenaga boleh melepaskan nutrien dalam cara terkawal. Arah aliran garisan datar peratusan kumulatif membuktikan bahawa pembebasan nutrien yang berterusan dari gel tenaga. Kesan bahan pembentuk gel dan air kelapa yang ditambahkan ke dalam

gel tenaga diperiksa. Ketiadaan agen penggelan (seperti yang ditunjukkan oleh minuman tenaga berasaskan air kelapa) menyebabkan pembebasan nutrien menjadi tidak terkawal, sementara ketiadaan air kelapa (seperti yang ditunjukkan oleh gel tenaga berasaskan air) tidak menyebabkan pembebasan gula ringkas dalam simulasi cecair gastrik. Penilaian sensori gel tenaga berasaskan air kelapa menunjukkan bahawa gel itu boleh diterima oleh panel. Ujian prestasi senaman menunjukkan bahawa hidrogel berasaskan air kelapa mampu meningkatkan prestasi berbasikal ~1 jam sebanyak 11.8% yang dimakan 30 minit sebelum latihan. Gel tenaga berasaskan air kelapa tidak menunjukkan peningkatan dalam ujian prestasi senaman. Lebih banyak penyelidikan diperlukan untuk menguji keberkesanan gel tenaga.

CONTROLLED RELEASE OF NUTRIENTS FROM COCONUT WATER-BASED HYDROGEL

ABSTRACT

Studies have proven that coconut water could replenish nutrients and rehydrate during physical exercise. Nutrients in coconut water, *i.e.* sugars and electrolytes, can be easily and rapidly absorbed by our body. By designing coconut water into a controlled-release system, it can provide nutrients release to the body in a sustained manner. Therefore, the aim of this study is to develop a coconut water-based energy gel with in situ gelling property. Coconut water-based hydrogel made of gellan and xanthan gelling polymers was formulated as a base to develop the energy gel. Hydrogel formulation with mixture of 0.7% gellan and 0.3% xanthan showed in situ gelling system that was able to retain over 1 h period in dissolution test before completely dissolved. During in vitro release study, the trend of release profile showed that the hydrogel had the ability to sustain nutrients release over a period of 1 h. After 75 min, the release trend was static, indicating that all the sugars were released from the hydrogel. Since the developed hydrogel does not provide sufficient nutrients, *i.e.* carbohydrate and sodium, required for exercise, maltodextrin and sodium chloride were added to produce coconut water-based energy gel. The in vitro test on the energy gel showed that it is capable fo releasing the nutrients in a controlled manner. The linear trend of cumulative percentage showed sustained nutrient release from the energy gel. The effect of gelling agents and coconut water being added to the energy gel was also examined using the same in vitro study. The absence of gelling agents (as demonstrated by coconut water-based energy drink) causes the release of nutrients to

be uncontrolled, whilst the absence of coconut water (as demonstrated by water-based energy gel) causes no release of simple sugars in simulated gastric fluid. The sensory evaluation of the coconut water-based energy gel showed that the gel is acceptable by the panelist. Exercise performance tests show that coconut water-based hydrogel can improve the ~ 1 hour cycling performance by 11.8% consumed 30 minutes before the training. Coconut water-based energy gel did not show enhancement in exercise performance test. More research needed to test the effectiveness of the energy gel.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The key element of sports success begins with enough energy intake to support calorie consumption and to promote the maintenance or development of strength, stamina, muscle frame and fitness. Insufficient energy consumption for calorie burning will decrease athletic performance and even reverse the benefits of physical training (Potgieter, 2013). The consequences of inadequate energy cause the body to lose fat and intramuscular adipose tissue as a source of food to the body. At the same time, insufficient blood sugar levels rise exhaustion and eventually reduce performance of the athletes. As time passes, this could affect the athlete's performance and the immune system, endocrine, and skeletal muscle (Kerksick & Kulovitz, 2013).

Energy gels are typically formulated on a fast release carbohydrate to deliver a source of energy that an athlete's body can take in easily and use. The source of carbohydrate, the amount and the concentration of the gel can all differ significantly. Carbohydrate gels and other supplements are usually consumed by athletes during a sports event to help replace carbohydrate levels in body. In a study conducted to test effectiveness of the carbohydrate gels on 26 athletes involved in sprint performance, results showed that endurance capacity of the athletes improves as the carbohydrate gel was taken before the event (Phillips *et al.*, 2012).

Coconut water is composed of sugars (fructose, glucose and sucrose), minerals (potassium, sodium, magnesium and calcium), and vitamins (vitamin C and B complex) (Prades *et al.*, 2012; Tan *et al.*, 2014). Studies have proven that coconut water have similar rehydration index as commercial rehydration drinks which can replenish the fluid and nutrients during physical activities (Saat *et al.*, 2002). Previous study showed that sodium enriched coconut water is effective as commercial sports drink for rehydration (Ismail *et al.*, 2007).

Theoretically, the delivery of the coconut water-based hydrogel into our body could be achieved by consuming coconut water-based hydrogels prepared using a wide-range of hydrocolloids, such as gelatine, agar and starch. In our opinion, this approach might not be the best way of delivery coconut water-based hydrogel, as the athletes need to spend time to masticate the hydrogels before swallowing it and at certain occasions, the hydrogels obtained would be unpalatable (Pelkman *et al.*, 2007). We believe that a coconut water-based energy gel (consisting of 2 hydrocolloids, namely gellan gum and xanthan gum) would be a more proper way of delivery of the hydrogel. Athletes will consume the gel which is in sol-gel and the coconut water-based gel will be formed later in the stomach. The gel is expected to maintain some integrity as it passes through the gastrointestinal tract from anecdotal observation of the gel formed by *in vitro* test. Assuming similar gel formation in the human digestive tract, the gel could slow fluid and nutrients absorption. Hence, providing a slow controlled-release system that could be of great advantage during exercise.

The aim of this study is to develop a new coconut water-based energy gel that is capable of providing continuous delivery (controlled-release) of nutrients to the individuals involved in strenuous physical activity about an hour. On top of that, the effect of important variables (percentage of gellan and xanthan gum) on both gelation and release mechanism will also be investigated, which will promote coconut water-based hydrogels to wider applications.

1.2 Problem Statement

Recently, sport nutrition industry focusing on “natural” beverages since some individuals prefer natural alternatives to the manufactured sport supplement. For example, many sport gels contain artificial flavours and sweeteners, and added electrolytes. According to previous researches, coconut water could play a great role in rehydration during physical exercise as it is naturally very rich in potassium, contains sodium, chloride, and carbohydrate. However, coconut water was chosen in this study not because it replenishes fluids well, but rather because its exercise-enhancing properties are not well understood. This study attempts to determine the effect of coconut water with added carbohydrate source in improving athlete’s performance. Consumption of coconut water has no controlled-release property and gives fast absorption of nutrients since it is in liquid form. Even if in the solid form, it would require mastication and would end up in the gel being broken down into smaller pieces and nutrient release would be fast. In response to this problem, our study proposes a mechanism in which a sol gel is consumed and later it forms a gel in the stomach which dissolves slowly and releases the nutrients in more controlled manner.

Incorporation of gellan and xanthan gum would give the coconut water a sol gel form which gives the *in situ* gelling property that has controlled-delivery system for nutrients release. The study also attempts to determine the effectiveness of the gel in enhancing athlete's performance.

1.3 Objectives

The objectives of this study are as follows:

- i. To formulate a coconut water-based hydrogel with appropriate amount of gellan and xanthan gum in order to develop an energy gel with controlled-release property.
- ii. To evaluate the nutrients release characteristic of the developed energy gel in order to determine its feasibility for endurance events.
- iii. To determine the potential of coconut water-based energy gel in enhancing performance during exercise.

CHAPTER 2

LITERATURE REVIEW

2.1 Nutrition requirement in sports

2.1.1 Carbohydrate consumption during sports

The key element of sports success begins with enough energy intake to support calorie consumption and to promote the maintenance or development of strength, stamina, muscle frame and fitness. Insufficient energy consumption for calorie burning will decrease athletic performance and even reverse the benefits of physical training (Potgieter, 2013). Age, gender, weight, and physical activity play the role in total energy requirement (Sygit, 2016). Consumption of proper and well-designed diet of macronutrients such as carbohydrates, proteins and fat enhances the athletes performance (Kerksick *et al.*, 2013). It is recommended that food intake should be done two hours prior to any physical activity specifically low fat, moderate protein and high carbohydrate meals. In the field of sports, carbohydrates are the key energy provider to athletes whereas protein is for muscle development (Indoria & Singh, 2016). One of the purpose athletes consume supplements is because they gives performance enhancement effect directly to them. Supplements contain more proper and complete set of nutrients whereas daily foods might lack of any of it (Castell *et al.*, 2009).

During prolonged, moderate to high-intensity exercise, carbohydrate (blood glucose, muscle and liver glycogen) is the principle substrate for the contracting muscle (Loon *et al.*, 2001; Romijn *et al.*, 1993). However, endogenous carbohydrate stores are limited, and potentially as a consequence, fatigue during endurance exercise often coincides with hypoglycaemia and the depletion of muscle glycogen stores (Coyle *et al.*, 1986). To maximize prolonged endurance performance, it is recommended that athletes should consume carbohydrate during endurance events (Goodson *et al.*, 2003).

The time of aerobic exercise and amount of work performed has been increased with the carbohydrate ingestion through pre-exercise, during exercise and post exercise has been proven. The carbohydrate supplementation related to the increase of blood glucose suggests enhanced aerobic performance during decrease of muscle glycogen utilization or using blood glucose as a main fuel supply as glycogen is exhausted. Table 2.1 listed studies that have been done previously with carbohydrate intake during 1 h exercise (Bronkhorst *et al.*, 2014).

Table 2.1. Studies of carbohydrate intake immediately before and/or during high-intensity exercise ~ 1 h duration.

Study	Subjects	Supplement protocol	Exercise protocol	Enhanced endurance/performance	Comments
Below et al., (1995)	Well-trained cyclists (8 M) Crossover design with carbohydrate +/- fluid replacement	~1.1 g/kg carbohydrate Treatments: 1.33 L of 6% carbohydrate or 200 mL of 40% carbohydrate drink vs. fluid only Intake: Ad libitum intake during trial	Cycling 50 min @ 80% $\text{VO}_{2\text{max}}$ + ~10 min TT	Yes	Overall, carbohydrate was associated with 6.3% enhancement of time trial (TT) performance compared with no carbohydrate replacement. No significant difference ($P < 0.05$) in physiological parameters between carbohydrate and no carbohydrate trials.
Carter et al., (2003)	Trained cyclists (8 M) Crossover design	~ 1 g/kg carbohydrate Treatments: 6% carbohydrate drink vs. placebo. Intake: 8 mL/kg immediately before trial + 3 mL/kg every 15 min	Cycling 73% $\text{VO}_{2\text{max}}$ to exhaustion	Yes	Time to exhaustion was increased by 13.5% with carbohydrate (60.6 ± 11.1 min) compared with flavoured placebo (50.8 ± 7.5 min). No significant difference ($P < 0.05$) between rate of rectal temperature rise, although trend to higher temp at exhaustion with carbohydrate. Blood glucose maintained in both trials, but slightly higher with carbohydrate.
Millard-Stafford et al., (1997)	Well-trained runners (10 M) Crossover design with 2 different types of	~ 0.7–1.0 g/kg: Treatments: 6 or 8% carbohydrate drinks vs. placebo	Running 15 km treadmill run: 13.4 km at steady state then	Yes	Both carbohydrate drinks enhanced TT performance (6% carbohydrate: 344 s)

	carbohydrate drink	Intake: 1 L before running, ad libitum during run	1.6 km TT			for 1.6 km TT; 8% carbohydrate 341 s) compared to flavoured placebo (358 s), no significant difference ($P < 0.05$). Cool/moderate environment or environment not stated
Neufer et al., (1987)	Well-trained cyclists (10 M) Crossover design with solid and liquid carbohydrate	~0.6 g carbohydrate Treatments: solid carbohydrate, carbohydrate drink vs. placebo Intake: immediately before exercise	Cycling 45 min @ 77% $\dot{V}O_{2max}$ then TT	Yes		Greater work done with solid and liquid forms of carbohydrate (~175 kNm) compared with placebo (159 kNm), no significant difference ($P < 0.05$). No glycogen sparing with carbohydrate intake.
Anantaramen et al., (1995)	Trained subjects (5 M) Crossover design with pre-carbohydrate and pre+ during carbohydrate	~ 0.5 or 2 g/kg carbohydrate Treatments: 10% carbohydrate drink vs. placebo Intake: 300 mL immediately before, or 300 mL before and 300 mL every 15 min	Cycling 60 min protocol starting at 90% $\dot{V}O_{2max}$ and declining as necessary	Yes		Workload maintained in all trials until 40–60 min where greater drop off in power with placebo trial. Total work done during trial was higher in both pre- CHO trial (619 ± 234 kJ, and Pre + during carbohydrate trial (599 ± 235 kJ) compared with placebo (560 ± 198 kJ), No significant difference ($P < 0.05$).
Jeukendrup et al., (1997)	Well-trained cyclists (19 M+F) Crossover design	1.1 g/kg carbohydrate Treatments: 8% carbohydrate drink vs. placebo	Cycling TT to complete work expected to last about	Yes		TT performance with carbohydrate (58.74 ± 0.52 min; mean power = 297 W) was enhanced compared with performance with a flavoured placebo

		Intake: 14 mL/kg consumed in equal portions immediately before and each 25% of TT	~1 h		(60.15 ± 0.65 min, mean power = 291 W), $P < 0.001$
Kovacs et al., (1998)	Well-trained cyclists (15 M) Crossover design	~ 1 g/kg carbohydrate Treatments: 7% carbohydrate drink vs. placebo Intake: 14 mL/kg consumed as 8 mL/kg immediately before and 3 mL/kg at ~20 and 40 min of TT.	Cycling TT lasting ~1 h	No	Similar times to complete TT in carbohydrate trial (62.5 ± 1.3 min) and placebo (61.5 ± 1.1 min), NS.
Nikopoulos et al., (2004)	Well-trained cyclists (8 M) Crossover design	~1 g/kg carbohydrate Treatments: 6.4% carbohydrate drink vs. placebo Intake: 14 mL/kg consumed as 8 mL/kg immediately before and 2 mL/kg at 15 min intervals.	Cycling Time to fatigue @ 85% V_{O2max}	Probably	Strong trend for increase in endurance: 13% increase in time to exhaustion with carbohydrate (58.54 ± 8.48 min) compared with flavoured placebo (51:18 ± 5:54 min:sec), NS. Surface EMG same for first 30 min in each trial; after 45 min and at fatigue, electromyographic activity lower in CHO trial. Suggests neural mechanism for CHO effect
Desbrow et al., (2004)	Well-trained cyclists/triathletes (9 M) Crossover design	0.8 g/kg Treatments: 6% CHO drink vs. placebo	Cycling TT lasting ~1 h	No	No difference in TT performance between carbohydrate (62:34 ± 6:44 min:sec) and placebo trials (62:40 ±

		Intake: 14 mL/kg consumed as 8 mL/kg immediately before and 2 mL/kg at ~25, 50, 75% of TT.			5:35 min:sec). No differences ($P > 0.05$) in post-TT blood glucose concentrations between trials.
Van Nieuwenhoven et al., (2005)	Trained to well-trained runners (90 M + 8 F) Crossover design	~ 0.6 g/kg Treatments: 7% carbohydrate drink vs. water Intake: 600 mL consumed in equal portions before and at 4.5, 9, and 13.5 km of race	Running 18 km road race	No	No differences in performance of whole group between water (78:03 ± 8:30 min) and carbohydrate trials (78:23 ± 8:47 min:sec), or for 10 fastest runners (63:50 vs. 63:54 min:sec for water and carbohydrate, respectively).
Burke et al., (2005)	Highly-trained distance runners (18M) Crossover design	1.1 g/kg Treatments: carbohydrate gel vs. placebo Intake: dose split between immediately before, and at 7 km and 14 km	Running Half-marathon (field study)	No	Differences in half-marathon time trivial: 73.56 vs. 73.35 min for placebo and CHO (difference = 0.3%, No significant difference ($P < 0.05$)).

Note. M represents male; F represents female; TT represents time trial; V_{O2max} represents maximal oxygen uptake

(Adapted from Burke *et al.*, 2005)

2.1.2 Assessment of performance exercise test

Exercise performance can be evaluated by determining: (i) the period for subjects to exercise to fatigue at a pre-set work rate; (ii) the time required to complete a pre-set work task; or (iii) the quantity of work completed in a specified period. Most of the studies has been performed using cycle ergometry since it is easier for consuming samples and data collection without interfering with the exercise (Coombes *et al.*, 2000). Maximal oxygen uptake (VO_{2max}) is considered as the most important predictor of physical performance and cardiovascular fitness and a vital, independent predictor of cardiovascular health (Ekblom-Bak *et al.*, 2014). VO_{2max} is suitable for any aerobic exercise that takes for at least 20–30 min. For experienced endurance athletes, there are higher demands on the intensity and duration of the exercise to achieve a further increase in VO_{2max} (Björkman, 2017).

VO_{2max} determines the capability of an athlete, power measures the real strength being generated by an athletic motion. Cycle ergometers possibly could mimic in a laboratory what is happening during actual competition (Joyner *et al.*, 2008). Rating perceived exertion (RPE) represents perception of training stress which can include both physical and psychological stress whereas Borg's scale is considered as a global indicator of exercise intensity including the physiological and psychological factor. Perceived exertion reflects the interaction between the mind and body. Used together, RPE and heart rate gives a more complete picture of fitness and overall health (Shaikh *et al.*, 2017). Time trial is the maximum running velocity attained for at least 1 min before completion of the test. Obtaining VO_{2max} is, however, labour intensive and time

consuming. An indirect alternative is mean running velocity based on time-trial performance. Time trials are clearly easier, quicker, and more cost effective than laboratory testing (Lorenzen *et al.*, 2009).

2.1.3 Exhaustion and dehydration during exercise

In Oxford English dictionary fatigue is defined as 'lassitude or weariness resulting from either bodily or mental exertion' or 'a condition of muscles, organs, or cells characterized by a temporary reduction in power or sensitivity following a period of prolonged activity or stimulation' (Bridge, 2002). The consequences of inadequate energy cause the body to lose fat and intramuscular adipose tissue as a source of food to the body. At the same time, insufficient blood sugar levels raise exhaustion and eventually reduce performance of the athletes. As time passes, this could affect the athlete's performance and the immune system, endocrine, and skeletal muscle (Kerksick *et al.*, 2013). Dehydration also occurs during a physical exercise since exercise produces high sweat and electrolytes loss especially in a hot weather. If the scenario persists, it could further lead to excessive dehydration which can decrease the athlete's performance and increase risk of heat sickness. Sweat contains water and electrolytes (Carlton & Orr, 2015). The electrolytes should be replaced properly to avoid dehydration and hyponatremia. The requirement for these different components (carbohydrate and electrolytes) will depend on the precise sports (strength and period) and environments. Sodium and potassium help to restore electrolytes loss where sodium stimulate thirstiness and carbohydrate gives energy. These nutrients can be

consumed by nonfluid sources such as gels, energy bars, and other techniques (Sawka *et al.*, 2007).

2.1.4 Feasibility of coconut water in sports application

Previously studies showed that coconut water has the capability to replace the fluid and nutrient loss during an exercise as rehydration beverages in the market (Ismail *et al.*, 2007). Another study found that the rehydrating properties of a carbohydrate-electrolyte sport beverage and coconut from natural source, concentrated, non-concentrate and bottled water are alike (Kalman *et al.*, 2012).

Go Coco coconut water and a sports beverage in the market showed no significance difference between them as their capability to rehydrate after a dehydrating exercise were compared and participants feel sicker when consuming a sports drink compared to coconut water (Leishman, 2015). The outcomes demonstrated that coconut water (*Cocos nucifera*) brought down the pulse recurrence superior to the isotonic beverage (Syafriani *et al.*, 2014). A research suggested that Dwarf coconut water might be suitable as isotonic beverage regular crude material that was the nearest to the quality standard of isotonic beverage (Kailaku *et al.*, 2015). Similarly, another study aim to compare coconut water, sodium enriched coconut water and carbohydrate electrolyte sport drink and sodium enriched coconut drink on measures of hydration and physical performance of athletes. The study concluded that the sodium enriched coconut drink has a better effect on the performance as compared to other drinks (Chaubey *et al.*, 2017).

Coconut water showed fluid retention similar to a sports drink and is well tolerated (Pérez *et al.*, 2010). A study demonstrate that previous ingestion of coconut water improves exercise capacity in the heat and provide a reduced urine output in comparison to plain water and flavoured drink. Also there is no evidence for gastrointestinal distress (Laitano *et al.*, 2014).

2.2 Pre-exercise supplementation

2.2.1 Energy gel

Energy gels are typically formulated on a fast release carbohydrate to deliver a source of energy that an athlete can take in easily and use. The source of carbohydrate, the amount and the concentration of the gel can all differ significantly. Carbohydrate gels and other supplements are usually consumed by athletes during a sports event to help replace carbohydrate levels in body. A study was conducted to test effectiveness of the carbohydrate gels on 26 athletes where they involved in sprint performance. The study showed that endurance capacity of the athletes improves as the carbohydrate gel was taken before the event (Phillips *et al.*, 2012). Guidelines suggest that carbohydrate intake should be 3 to 12 g/kg per day depending on the intensity and period of exercise (Sousa *et al.*, 2016).

Another study presented that carbohydrate was the most vital nutrient when working out for an endurance event. Sports drink provides carbohydrates and aid to

rehydrate the athletes too, but they are not as energy dense as gels and might be too sweet to consume. Generally, gels or gummies holds anywhere between 23–28 g of carbohydrate. Athletes could easily reach the adequate amount of carbohydrate during the sports since gels are energy dense (Osowski, 2014). Solid (bar) and semisolid (gel) carbohydrate is digested as efficiently as a carbohydrate solution in endurance studies with water consumption. Carbohydrate gels have also been shown to improve endurance capacity during intermittent high-intensity exercise (Baker *et al.*, 2015).

Carbohydrate–electrolyte gel ingestion raised blood glucose concentrations and improved dribbling performance during the extra-time period of simulated soccer match-play (Harper *et al.*, 2016). Energy gels are formulated to replace carbohydrate stores that are used during exercise since carbohydrate can only store a limited quantity in the muscles. The body can supply about 90 min of muscle glycogen when running at half marathon pace which is about 2 h (Sports gel technical document, 2009).

The gel's carbohydrate is not always used up by the working muscles. This is because carbohydrate is kept in both muscles and blood. The body usually consume the glycogen stored in the muscle on the event day. For glycogen to make its way to the muscles, it must first be digested, make its way through the intestinal wall, and then absorbed by the muscles. This process takes time and is not very effective. Therefore, gels play an important role here as it induces the body because our brain only functions on the glucose stored in the blood. A brain supplied with less glucose starts to be ineffective since the muscles absorb more blood glucose. So, the gel keeps the mind to stay active (Seifert *et al.*, 2012).

Gels have different absorption rates depend on the capability of the stomach to digest it. During a running event, the body often diverts blood away from the digestive track to help the legs continue to move forward. The body sometimes shuts the stomach down entirely while other times it just slows down to prevent the runners from throwing up fluids or gels right after consuming them late in the race. Therefore, it is acceptable to consume the gels before the race. A gel with different type of carbohydrates is preferred since the simple sugars from the energy gels will first be absorbed into the blood stream as glucose. Continuing to pump sugar into the blood stream can lead to sickness from too much sugar (Sports gel technical document, 2009).

Two studies were conducted to investigate gastrointestinal tolerance of high carbohydrate intakes during intense running. The first study investigated tolerance of a carbohydrate gel delivering glucose plus fructose at different rates. The second study investigated tolerance of high intakes of glucose vs. glucose and fructose gel. In conclusion, despite high carbohydrate gel intake, and regardless of the blend (glucose vs. glucose + fructose), average scores for gastrointestinal symptoms were at the low end of the scale, indicating predominantly good tolerance during a 16-km run (Pfeiffer *et al.*, 2009)

2.2.2 Variation in commercial gels

Energy gels have become a blooming sector of the sports nutrition market since the 1990s. A wide variety of tastes, textures and styles have evolved, driven in some cases by improved understanding of energy requirements during exercise and in others, a desire to provide a variety wide enough to suit every taste. However, a basis for choosing one product over another, as based on information provided to the athlete on packaging and marketing material, is often not immediately evident (Zhang *et al.*, 2015). Table 2.2 shows some information on the products available in the market.

Table 2.2. Nutritional composition of a range of sports gels available in Australia.

Gel/Brand	Size	Energy (kJ)	Carbohydrate (g)	Sodium (mg)
PowerBar (US and Australia)	41 g	462-504	27-28	200
PowerBar PowerGel Fruit (US and Europe)	41 g	454	27	300
Endura (Australia)	35 g	444	26	14
Gu (US)	32 g	420	20-25	40-65
Gu Roctane (US)	32 g	420	20-25	90-125
SIS Go Isotonic Gel (London)	60 mL	368	25	9
Carboshotz (Australia)	45 g	495	30	36
Body Science Energy Gel (Australia)	35 g	418	24	110
High 5 Energy Gel (UK)	30 mL	385	23	20
High 5 Energy Gel Plus (UK)	30 mL	385	23	20

(Adapted from AIS Sports Supplement Framework of Australian Sports Commission, 2014)

Gels are considered a convenient approach to carrying energy and fuelling while in movement without the need to carry excessive fluid intake. To meet the theoretical criteria for consumption during exercise, gels should therefore be considered practical to carry, contain high glycaemic carbohydrates, and should maximise carbohydrate absorption whilst also minimising gastrointestinal discomfort (Kreider *et al.*, 2010). Table 2.3 shows serving size, energy density, energy content, carbohydrate content, free sugars content, fructose content, and osmolality of 31 types of energy gels in the market.

Table 2.3. Descriptive statistics for the carbohydrate gels for parameters related to serving size, energy density, energy content, carbohydrate content, free sugars content, fructose content and osmolality.

	Mean \pm SD	Median	Range	Comments
Serving size (g)	50 \pm 22	45	29–120	20 out of 31 products are offered in packages below 45 g. Only 2 product ranges are packaged over 100 g
Energy density (kcal/g)	2.34 \pm 0.70	2.60	0.83–3.40	Only one product has an energy density less than 1 kcal/g. The most popular range of energy densities is 2–3 kcal/g, although a significant number of products (7 out of 31) do offer energy densities more than 3 kcal/g.
Energy (kcal)	105 \pm 24	100	78–204	The most popular energy range is between 100 and 120 kcal/gel. 25/31 products fall within this range, the majority falling within 100–110 kcal/gel.
Total carbohydrate (g)	25.9 \pm 6.2	24.6	18–51	The most popular carbohydrate range is 20–30 g/gel. 25/31 products fall into this range, with the majority of those (14) containing less than 25 g. Only 3 products offer less than 20 g, and only 3 offer more than 30 g.
Free Sugars (g)	9.3 \pm 7.0	7.9	0.6–26.8	Only one product has free sugars less than 1 g/gel. Of the remainder, the majority, <i>i.e.</i> 15 products, provide 5–15 g free sugar/gel. Four products provide more than 20 g free sugar/gel.
Free Sugars (% of Total carbohydrate)	35 \pm 25	33	3–95	Only 2 products have free sugars less than 10% total carbohydrate. 20/31 products have free sugars more than 20% of total carbohydrate, and of these nearly half (9) have free sugars more than 50% of total carbohydrate.

Fructose Content	Unknown	Unknown	0 – >20% carbohydrate	Out of 31 products, only 3 do not contain fructose in some form. Exact amounts present cannot be quantified from the ingredient labels.
Osmolality (mmol/kg)	4,424 ± 2,883	4722	303–10,135	Only one product range is isotonic. 27 out of 31 products have osmolality more than 1,000 mmol/kg.

(Adapted from Zhang *et al.*, 2015)

2.2.3 Maltodextrin

Maltodextrins are a class of carbohydrates obtained from a variety of plant sources. Technically they are formed by enzymatic or acid hydrolysis of starch, followed by purification and spray drying. Maltodextrin is available mostly in white powders which are of high purity and microbiological safety. They are used in a wide range of food and beverage products, including baked goods and sports drinks (Hofman *et al.*, 2016).

Maltodextrin is suitable for endurance athletes as it maintains a low osmolarity giving fast absorption and allowing slower digestion (Hornsby, 2011). It is crucial to take note on the type of carbohydrate supplementation. Glucose polymer solutions (maltodextrin) are desirable to isocaloric glucose solutions as a source of consumed carbohydrate before and during exercise because of the lower osmolalities. In fact, several studies have proven that the rates of gastric emptying for glucose polymer solutions are faster than those of isocaloric glucose solutions (Bronkhorst *et al.*, 2014). In addition, it has been suggested that glucose feedings (75 g of glucose solutions) 30 to 45 min before exercise in cyclists might impair exercise performance by causing a sudden drop in blood glucose and an accompanying acceleration of muscle glycogen oxidation (Bronkhorst *et al.*, 2014). Acceleration of muscle glycogen oxidation causes depletion in glycogen content where the athletes will experience a decrease in energy capacity and fatigue as well as an increased risk for overtraining and muscle damage (Bogdanis, 2012).

2.3 Coconut

2.3.1 Coconut water

Coconut water comprises of many electrolytes, some bicarbonates and albumins. Coconut water is acidic (Ajibogun *et al.*, 2013) and this align with the study done by Yong *et al.*, (2009). The interaction of variety and stage of maturity of the fruit appeared to be different on the chemical composition of the coconut water (Jackson *et al.*, 2004) . Priya and Ramaswamy (2014) claimed that the biochemical composition of coconut is affected by maturity stage, soil, and environmental conditions. Meanwhile, a study conducted in Brazil demonstrated that physical properties of coconut water were affected by varying nitrogen and potassium application (Yong *et al.*, 2009)

Vigliar *et al.* (2006) found that as the dwarf coconut matured, the concentrations of fructose and glucose are increased but there is a reduction in sucrose. The dwarf coconut palms planted in non-coastal regions were observed and Vigliar *et al.* (2006) found that the biochemical profile varied as the coconuts matured, observing reductions in the concentration of potassium, calcium, magnesium, chloride and osmolarity.

Other compounds such as kinetin, cytokinin, zeatin-O-glucoside, gibberellins, abscisic acid are also reported (Ge *et al.*, 2005). It is benefit by inhibits platelet aggregation in human platelets when stimulated by an agonist (Duszka *et al.*, 2009)

and could therefore help to prevent blood clots (Heo *et al.*, 2002). It also has anti-ageing, anti-carcinogenic and anti-thrombotic properties (Ajibogun *et al.*, 2013)

The concentration of natural electrolytes found in coconut water generates an osmotic pressure similar to that observed in blood and does not affect plasma coagulation (Uphade *et al.*, 2017). The high potassium content in coconut water is found to lower the blood pressure (Loki *et al.*, 2003) and gives cardioprotective effects in myocardial infarction (DebMandal *et al.*, 2011). Coconut water has antimicrobial property because of its high lauric acid content that has been used as medications for certain oral infections such as mouth sores. Some studies have reported that sucrose monolaurate and glycolipid component present in coconut has anti-caries properties (prevent dental plaque which causes tooth decay). This effect is probably due to condensed glycolysis and sucrose oxidation in a non-competitive method affected by *Streptococcus* mutants and hence inhibits in-vitro dental plaque (Rukmini *et al.*, 2017). The various saturated fatty acid chains in coconut water allows them to be directly absorbed from the intestine and sent to liver to be rapidly metabolized for energy production (DebMandal *et al.*, 2011). Table 2.4 shows the nutritional composition of coconut water.