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Why Coconut Water is both a Biostimulant and an Anti-cancer Agent

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Estefany Reyes Estévez
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2. Abstract

Coconut water (CW) naturally contains phytohormones called cytokinins, which exist in different forms like kinetin, kinetin riboside and zeatin. The bioactive cytokinins in plants are the zeatin-type, and these natural compounds exert many vital plant physiological effects. Nutrient media based on CW is effective in augmenting nutrition efficiency, abiotic stress tolerance and crop quality in soil-cultivated, as well as in micropropagated plants. Moreover, CW is increasingly being utilized as a natural biofertilizer in crop cultivation to obtain better produce. This is because the natural cytokinins in CW increase the total available pool of cytokinins for plant growth when added to the roots. In addition to photosynthesis, cytokinins guide the appropriate development of meristems and cell cycle rates according to the growth environment, utilizing minerals as building blocks.

Interestingly, these plant cytokinins also have important effects on human and other animal cells. Being rich in minerals (mainly potassium but also magnesium, calcium, and sodium), makes CW good for replenishing dehydration as well. Treatments with CW have presented other beneficial medical effects because of their specific cytokinin (kinetin and kinetin riboside) content. Targeting malignant cells and consequently acting anti-proliferatively, as well as inducing apoptosis, gives them the potential of becoming key therapeutic ingredients in treating and preventing various types of human cancers, thrombosis as well as signs of aging (cosmetics). Although, it is possible that, equivalent to plants, a conversion to zeatin-type cytokinins is required for the kinetin-type to have positive biological function in humans. This concludes that further research on the plant cytokinin-biosynthesis and metabolic pathways in humans is needed.

3. Popular scientific summary

- Coconut water is naturally rich in inorganic ions which serve as building blocks for plant and mammal general cellular growth. As replenishing electrolytes, they make coconut water a rehydrating beverage with lifesaving potential for acute dehydration.
- Kinetin-type cytokinins, which are signaling molecules present in coconut water, have demonstrated beneficial biomedical effects on mammalian cells (injected *in vitro*) with anti-cancer, anti-oxidative, anti-aging, cardioprotective, and DNA-repairing functions. Conversely, because of ethical reasons, there is no direct way of assessing these biomedical effects of coconut water when it is consumed orally by humans.
- Zeatin-type cytokinins, also present in coconut water, are the bio-active form of cytokinins in plants and are specifically driving growth and development in tissue cultures and biofertilizer. This is because they are signaling molecules that promote vital cellular processes (e.g., chloroplast formation) including appropriate cell cycle development and regulating key physiological functions (e.g., stomatal opening).
- Isopentenyladenine (iP), a cytokinin also present in coconut water, is a precursor to the biologically active form of cytokinin in plants and is also produced by bacteria.
- Kinetin-type and zeatin-type cytokinins, along with iP, all share the same biosynthesis and metabolic pathway.
- It is to date unknown what form of cytokinin is bioactive in humans, likewise for the start mechanism of the anti-cancer activity. It is possible that, just like in plants, the cytokinins need to be converted to the zeatin-type to have functionality in humans.
- I speculate that the reason behind coconut water's duality and overlapping functions on both plants and mammals, is due to both belonging to the eukaryotic domain and having conserved genetic codes.

Table of contents

4. List of technical terms and abbreviations

Apoptosis – Programmed cell death

ATP – Adenosine triphosphate

CEB – Carbohydrate-electrolyte beverage

CW – Coconut water (see introduction for definition)

In vitro – In an artificial environment (e.g., in a test tube or in a culture dish)

In vivo – In a living organism

IV – Intravenous

K – Kinetin

KR – Kinetin riboside

Micropropagation – Rapid vegetative propagation of plants under *in vitro* conditions

OGZ – O-glucosyl zeatin

Proliferation – Generation, growth or increase in numbers

Xenograft – Transplantation of tissue, organs or cells from one species to another

Z – Zeatin

ZR – Zeatin riboside

5. Introduction

The coconut tree, *Cocos nucifera*, is a member of the palm family, *Arecaceae*. They exist in several varieties, and these are principally distinguished by either the color of their fruits (yellow, orange, brown, red, or green) or the height of the tree (tall, dwarf or hybrid) (Debmandal and Mandal, 2011; Prades et al., 2012). The coconut tree is considered indispensable in many parts of the world. It is known for the versatility of uses of it; the leaves and the trunk are utilized as building material, and the roots are used for medicinal purposes. When it comes to the coconut fruit, every part of it can be put to good use too. The coconut fruit is, albeit its name, a stone fruit and not a nut (figure 1). It is composed by an outer skin, called the exocarp, followed by the fibrous mesocarp, also called the husk, which is used to make rope and is processed to e.g., make textiles and growing media. Inside of the husk is a wood-like endocarp which becomes high-quality activated charcoal when processed. Then, follows the testa (seed coat) which delimits the endocarp from the endosperm. Coconuts have two types of endosperm: liquid, and solid. When the coconut fruit starts to develop, the

endosperm is initially liquid and hardens throughout the ripening process from the testa toward the center. From a soft jelly consistency, it develops into a harder, white, fleshy layer that never fully fills the center cavity of the fruit. During the fruit development, the water content diminishes and causes the sugar content to rise, and the liquid becomes increasingly concentrated. This results in the formation of a solid endosperm, which is rich in fats and proteins, and a liquid endosperm, which is rich in minerals, sugars, vitamins, and phytohormones (Prades et al., 2012).

Figure 1: Cross-section of a young coconut. Source: (Keira Morgan, Flickr, 2012)

The water, juice, or milk, as some literature refer to the liquid endosperm (CW), from the young (6-7 months) coconuts are extensively consumed as a drink in tropical regions and increasingly so in the rest of the world (Mahidol University, 2021). CW has been associated with biomedical benefits such as being anti-bacterial, anti-fungal, anti-viral, anti-parasitic, anti-dermatophyte, antioxidant, anti-thrombotic, rehydrating, hypoglycemic, hepatoprotective, cardioprotective, anti-inflammatory, as well as immunostimulatory (Campbell-Falck et al., 2000; Anurag and Rajamohan, 2003; Ge et al., 2005; Debmandal and Mandal, 2011; Prathapan and Rajamohan, 2011; Mohamad et al., 2019).

5.1 Purpose

Is it possible to find a biochemical connection between our diet and plants? What is the fate of ingested plant phytohormones in humans (for a review, see Chanclud & Lacombe1, 2017)? The purpose of this essay is to assess how a phytohormone (or biostimulant) could be a drinkable beverage that also contains biomedically beneficial compounds in treating human tumours/cancers. The aim is also to explore the interface and identify potential research gaps in the literature about plant phytohormones in humans. The multifunctionality of the popular and globally used and also consumed CW is therefore of great interest.

5.2 Limitations

The focus of the essay is on CW's functionality in plant application, as well as in biomedicine. The analysis of key plant growth hormones of CW, from a biomedical perspective, is limited to cytokinins and excludes auxins and gibberellins. The cosmetic uses of CW, and the use of CW in plant tissue culture, are only briefly mentioned and discussed. The essay also mentions the different coconut varieties, but limitedly.

6. Methods

A literature study was conducted, utilizing online databases and libraries such as Google Scholar, Web of Science, and Primo.

7. Results

Appendix table 1 is a compilation of the currently known biochemical composition of coconuts at different stages of growth. During the literature review process, it was apparent that there are major gaps in CW research with reference to the varietal differences, age of coconuts, chemical analyses, and environmental influences (e.g., drought versus rainy season) on CW.

Among the compounds found in CW are phytohormones, which are signaling molecules that drive the cell cycle and important plant physiological functions (Yong et al., 2009). Because phytohormones are commonly present in trace concentrations below 30 μmol g^{-1} of fresh weight, it has become possible in recent years, with improved and more sensitive bioanalytical methods, for researchers to detect their presence in natural products (such as certain plant

species, biofertilizers, and food) and human cells, as well as quantifying their presence at more accurate and rapid rates (Rattan, 2002; Tarkowski et al., 2009). A combination of Mass Spectrometry, Gas or Liquid Chromatography and Capillary Electrophoresis, is regarded as the most precise method of analyzing cytokinins (Dobrev et al., 2017). Further, these methods are fast, resource efficient, and enhancing each technique's competence synergistically. With them, parallel analyses of multiple compounds are possible, giving researchers the possibility to follow phytohormones' metabolic pathways (Ge et al., 2005; Tarkowski et al., 2009; Dobrev et al., 2017).

Cytokinins are a major group of plant growth hormones that are principally vital for the functioning of the plant cell-cycle. They are further involved in regulatory functions like chloroplast formation, and opening stomata (Tarkowski et al., 2009; Voller et al., 2019). Derivatives of the nitrogenous base and purine, adenine, are the main building blocks of cytokinins in both plants and bacteria (figure 2) (Rattan, 2002; Sakakibara, 2006).

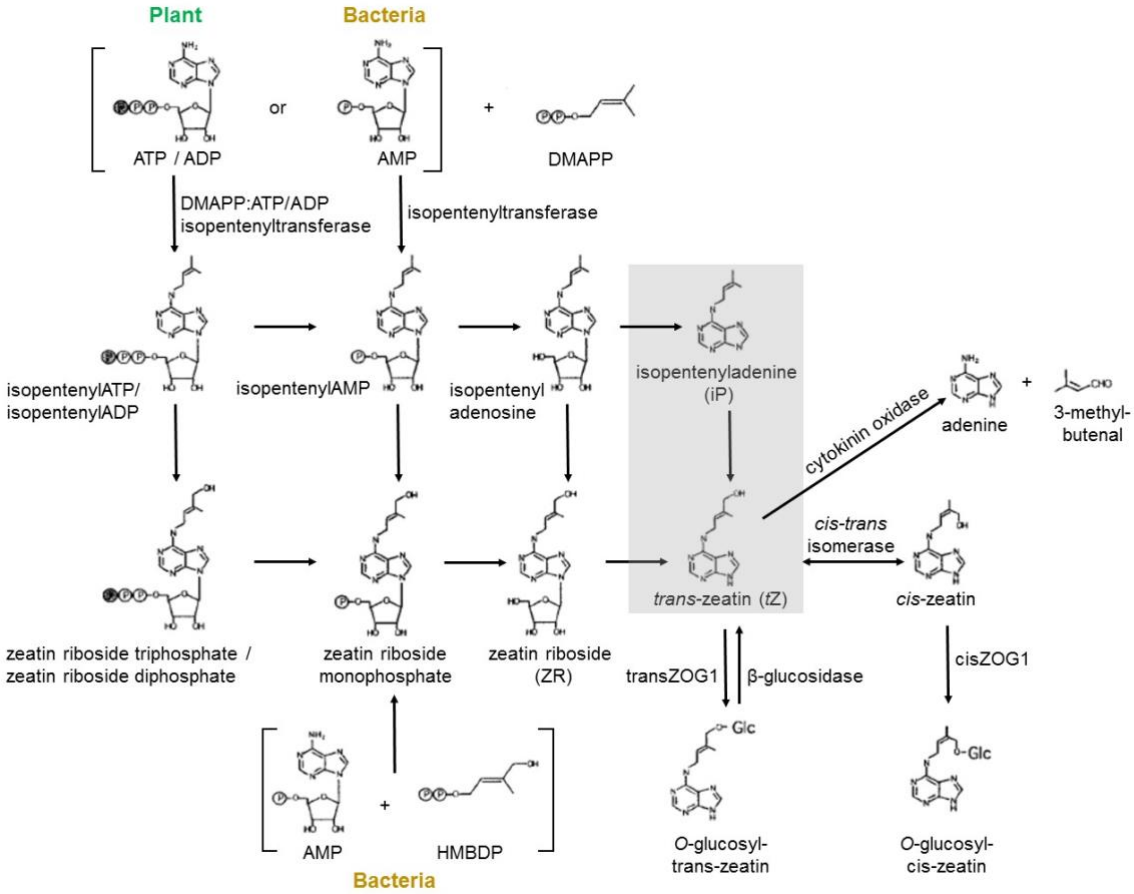

Figure 2: A model for the biosynthesis and metabolic pathways (cycle) of cytokinins in plants and bacteria. From 2015 with permission from Jean W. H. Yong.

In 1963, Stuart Letham was the first scientist to isolate a naturally occurring cytokinin, trans-zeatin, from maize (Letham, 1963) where the name zeatin was originally derived from *Zea mays*. Before that, this type of phytohormone was believed to not be found in nature, but as a byproduct of DNA chemical degradation (Miller et al., 1955; Miller et al., 1956). Kobayashi et al. (1995) and Ge et al. (2005) discovered naturally occurring kinetin (K) and kinetin riboside (KR) in the water of young, fresh coconuts. Zeatin (Z), K and its riboside, KR, have in recent years been identified in CW as significant components with great biomedical potential (table 1). CW's growing global importance in many applications has increased the need for even more rapid ways of analyzing the contents (Lakshmanan et al., 2020; Yong et al., 2009).

Table 1: Phytohormones and the quantities in which they are present in the CW of a young, green, coconut. From 2009 with permission from J.W.H. Yong.

Phytohormones in young green coconuts		Estimated original concentration ($x10^{-3}$ μM)
Cytokinins	isopentenyladenine	0.26
	dihydrozeatin	0.14
	trans-zeatin	0.09
	kinetin (N-6-furfuryladenine)	0.31
	ortho-topolin	3.29
	dihydrozeatin O-glucoside	46.6
	trans-zeatin O-glucoside	48.7
	trans- zeatin riboside	76.2
	kinetin riboside	0.33
	trans-zeatin riboside-5'-monophosphate	10.2
	14-O-(3-O-[β-D-galactopyranosyl-(1→2) -α-D- galactopyranosyl -(1→3)-α-L-arabinofuranosyl] -4-O-(α-L-arabinofuranosyl)-β-D-galactopyranosyl)- trans-zeatin riboside	Present
Gibberellins	gibberellin 1	16.7
	gibberellin 3	37.8
Auxin	indole-3-acetic acid	150.6

Abscisic acid	abscisic acid	65.5

7.1 Coconut water as a biostimulant

In plants, zeatin-type (Z-type) cytokinins are bioactive and the most important generators of plant physiological effects (Sakakibara, 2006). Normal plant growth is driven by its own self-produced cytokinins and is enhanced by those produced by the symbiotic microbes associated with the plants, e.g., in the soil (Nihorimbere et al., 2010; Wong et al., 2016). Based on earthworm research, by Zhang et al. (2014), it was demonstrated that symbiotic bacteria like rhizobacteria and bacteria in the digestive system of animals such as earthworms (vermicompost) produce isopentenyladenine (iP)-type cytokinins, which are the precursors of Z-type cytokinins. Interestingly, these Z-type cytokinins are also available in CW (Aremu et al., 2015; Wong et al., 2016; Yong et al., 2009; Zhang et al., 2014). This evidence suggested an additional way for plants to obtain more cytokinins, namely by directly enriching the soil with a cytokinin containing product. As previously mentioned, CW is rich in cytokinins and contains a variety of the cytokinins (table 1). Functionally, cytokinins act as 'coordinating' signals to guide the plant through a developmental program in response to a given growth environment: for example, the appropriate spatial and temporal development of meristems and cell cycle completion in tandem with the growth environment, while utilizing minerals as building blocks (figure 3) (Miller et al., 1956; Wong et al., 2016). Because CW contains cytokinins, it therefore possesses a plant growth regulating potential that was adequately described by the European biostimulants industry council's (EBIC) general definition of biostimulants (EBIC, 2011; Sim et al., 2008; Wong et al., 2016):

"Agricultural biostimulants include diverse formulations of compounds, substances and other products that are applied to plants or soils to regulate and enhance the crop's physiological processes, thus making them more efficient. Biostimulants act on plant physiology through different pathways than nutrients to improve crop vigor, yields, quality and post-harvest shelf life/conservation."

A widely utilized plant growth enhancement method is the incorporation of legumes in crop rotations (Costa et al 2021; Jacoby et al., 2017). Because of their symbiotic relationship with rhizobacteria, legumes fix nitrogen in their nodules through biological nitrogen fixation in plant-available forms, consequently increasing the nitrogen concentration in the soil (Jacoby et

al., 2017; Oono et al., 2011; Yong et al., 2014). More importantly, during biological nitrogen fixation, it was demonstrated recently that the concomitant cytokinin production was higher in legumes with certain strains of *Rhizobium* (Yong et al., 2014). Thus, there is a close and synergistic association between biological nitrogen fixation activity and additional cytokinin production in legumes. This positive and symbiotic strategy is increasingly used widely and successfully in sustainable plant production systems using lesser external chemical fertilizer inputs (e.g., Costa et al 2021).

Studies have been made on test plants in both greenhouse and field, where biostimulants in the form of vermicompost or plant growth-promoting rhizobacteria, like legume-symbiotic bacteria, have been compared to chemical fertilizers (the biostimulants generally contained a lower amount of mineral nutrients whilst the chemical fertilizers had a high mineral nutrient value) (Arancon et al., 2004; Çakmakçi et al., 2006). The results demonstrated that the two methods could adequately stimulate growth in the test crops, respectively. These observations provided evidence that plant growth promotion is not reliant upon the sole availability of mineral nutrients, but rather from a combination of plant-growth stimulators and mineral nutrients serving as structural materials (Arancon et al., 2004; Çakmakçi et al., 2006; Wong et al., 2016).

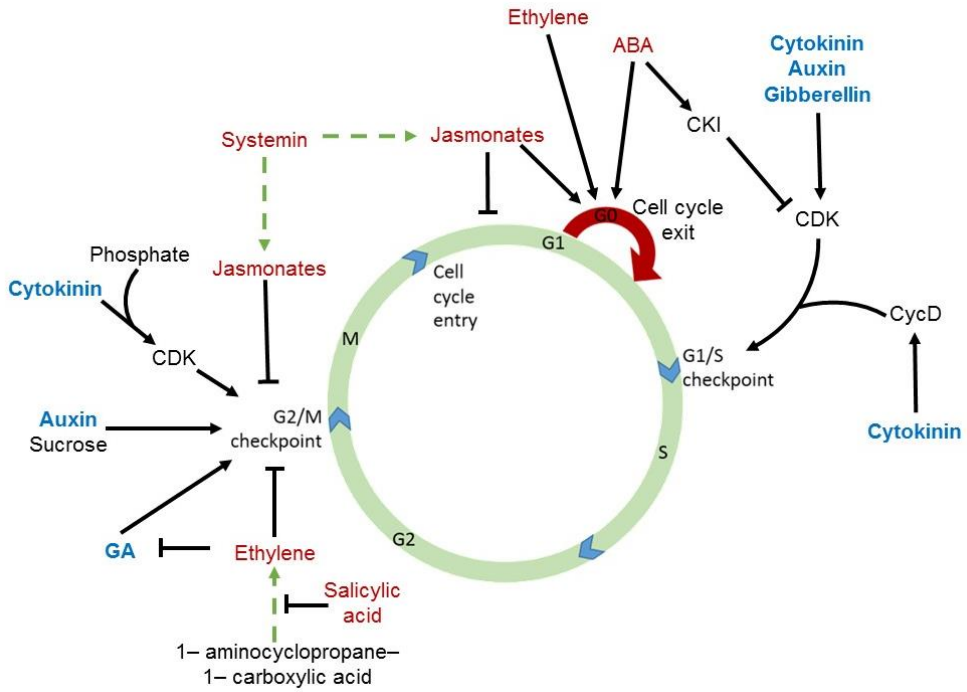

Figure 3: Conceptual picture of the plant cell cycle. From 2016 with permission from J.W.H. Yong.

CW is a cheap culture medium alternative to the conventional media used e.g., in large-scale production of *Bacillus thuringiensis* var. *israelensis*, relevant in plant protection. As CW is often a waste product in the coconut oil and copra (e.g. Santan) industries, utilizing it for micropropagation makes it a circular economy waste minimization choice (Prabakaran et al., 2008). Studies have shown that CW enhances plant quality (by regulating physiological and morphological processes) of treated plants better, in comparison to solely treating with synthetic cytokinins (Ang and Yong, 2005). This likely has to do with plants' need of other phytohormones, such as auxins and gibberellins, which are both present in CW and also crucial for co-regulating the progression of the cell cycle and contributing broadly to the hormonal homeostasis of plants (table 1; Yong et al., 2009). It is plausible that there are other undetected components (e.g., special proteins) in CW that, also, could be imperative for optimal development of the plant (Kobayashi et al., 1995). Ultimately, this points to potential synergistic effects of combinations of compounds (hormonal and non-hormonal growth promoting compounds; some are unknown) in CW that are useful in plant growth (Yong et al., 2009).

There is a major gap in research about the Z-type cytokinins, such as Z, zeatin riboside (ZR), and O-glucosyl zeatin (OGZ, a storage form), derived from CW (Sakakibara, 2006). Conversely, more work has been done on K and KR, as these are widely available in the biomedical research arena. In addition, the high cost of acquiring Z from specialized suppliers is another difficulty for researchers (Tarkowski et al., 2009). Although the quantified concentrations of Z-type cytokinins are higher than those of kinetin-type (K-type) in the reviewed literature (Yong et al., 2009), it is significant to bear in mind that the solute concentrations *per se*, could not define the *in vivo* efficacy of each cytokinin component of CW. While the absolute levels of Z may be higher than those of KR in coconuts (plant system), it may not imply that the biological efficiency rates are the same *in vivo* inside any human or animal systems. They are also different molecules with probably different *in vivo* biological functions. Z-type cytokinins might be the precursor of K-types, or vice versa, and they might convert into each other; none of this is known today as the associated enzymes have not been identified (Sakakibara, 2006; Voller et al., 2019). Further, some researchers have attributed plant physiological benefits, such as in leaf expansion and seed germination, to KR (Rajabi et al., 2012), as well as plant life prolonging effects due to K-linked delaying senescence (Rattan, 2002). Since the mentioned effects are typical for cytokinins in general, it cannot, in this case either, be confirmed which the functioning form/s of cytokinin/s is/are (for a review, see Voller

et al., (2019). More research is certainly needed to understand the fate of plant cytokinins in humans.

7.2 Coconut water's currently accepted biomedical function

Containing more than 4 times the amount of potassium as in a banana, the water of a coconut is more than just a refreshing beverage (Lakshmanan et al., 2020; Mahidol University, 2021; Pareek, 2016; Yong et al., 2009). CW's content of inorganic ions makes it effective in replenishing the dehydration and loss of electrolytes following physical activities (Prades et al., 2012). Studies on rehydration after exercise compared CW to plain water and a carbohydrate-electrolyte beverage (CEB) (Saat et al., 2002). CW and CEB had the same fluid retaining efficiency post consumption and were the superior replenishers. Despite CW's lower content of sodium, the participants of the studies preferred CW because more of it could be ingested faster, with less nausea and without their stomachs getting upset (Saat et al., 2002).

In 1942, Cuban researchers successfully conducted a clinical trial where they utilized CW as a temporary and sterile solution to treat patients with acute dehydration by injecting it into their veins (Campbell-Falck et al., 2000). Although CW has a similar electrolyte composition as intravenous (IV) fluid, it is usually slightly more acidic (Campbell-Falck et al., 2000). Because of this, it could not replace IV fluid completely but can serve as a short-term emergency solution, if injected in small doses. Today, CW serves this purpose in remote places where IV and sterile fluid is not accessible (Campbell-Falck et al., 2000).

In human cells, treatments with CW have presented beneficial medical effects because of their K and KR content; nevertheless, the synergistic effect of the other substances present in CW cannot be ruled out (Anurag and Rajamohan, 2003; Choi et al., 2008; Mohamad et al., 2019). K is a powerful antioxidant, naturally present in CW (Get el al., 2005; Rattan, 2002; table 1). Moreover, it affects the enzyme superoxide dismutase's superoxide radical dismutation activity (Tiedemann et al., 2008). Increasingly appearing as an ingredient in cosmetics to combat the signs of time and sun damage on the complexion (Minorsky, 2003), K has multiple ways of counteracting symptoms of senescence and light-induced aging in human cells. Long term studies on *in vitro* cultures of human dermal fibroblasts, which are important in the proliferation (generation) of new skin tissue to replace damaged skin during healing, treated with K, suggest that the component induces a retardation of the biochemical and morphological cellular aging processes (Rattan and Clark, 1994; Yoon et al., 2017). Examples of these are cell enlargements and irregular flattening of the cells' shape (Rattan and Clark, 1994). What is more, *in vitro*

treated cells showed minor reversions of aging-related changes, although not with an equal prevalence as when used continuously as a preventive measure (Rattan, 2002). Short-term pairing of K with other frequently used compounds in anti-aging cosmetics, such as retinol, glycolic acid, and serum growth factors, are suggested to subdue the cytotoxic effects of its fellow anti-aging agents, making the recombinant compound non-toxic to cell cultures of human skin cells (Rattan, 2002). *In vitro* studies have shown K's protective properties against the formation of detrimental mutations under protein oxidation and glycoxidation (Rattan, 2002).

In terms of cellular mechanism, K has been reported to stimulate the reparation of damaged DNA in human cells (Rattan, 2002). Presumably, CW's amino acid content could also be responsible for its improved fidelity (accuracy) during DNA transcription. Additionally, because K participates in signal transduction, it also stimulates tRNA synthesis (Rattan, 2002).

Experiments on rats treated with the heart stimulant and inducer of myocardial infarction, isoproterenol, revealed that when fed with CW, the rats' total concentration of cholesterol (high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein) as well as the numbers of triglycerides and phospholipids in the serum, heart, and aorta were reduced (Anurag and Rajamohan, 2003; Prathapan and Rajamohan, 2011; Bhagya et al., 2012). Further, studies carried out on fruit flies fed with K, which proved to expand the lifespan of the flies by 55-60%, although there are yet studies to be executed on understanding the K's potential effects on mammals' life expectancies (Rattan, 2002).

Like its free base form, KR is a hermetic compound; it stimulates apoptosis (programmed cell death) in cancer cells while it, in increasing concentrations, inhibits its own apoptogenic effect (Rajabi et al., 2012). KR functions as a potent anti-proliferative by virtue of its apoptogenic abilities on several types of tumors on plants and mammals; cell lines HCT-15 of human colon cancer, HepG2 liver cancer, and melanoma cells, are three of them (Cheong et al., 2009; Griffaut et al., 2004; Rajabi et al., 2012). Cytokinin ribosides in mammals are reportedly more efficient in suppressing malignant cells than any other corresponding forms of cytokinin (Wang et al., 2012). Comparing K and KR, Cheong et al. (2009) concluded that KR negatively impacts the ATP production in the mitochondria and thus its membrane potential in hepatoma cells while the free base K does not.

Human tumor xenograft models in which human leukemia, cervical cancer, as well as melanoma cells have been transplanted into mice, showed that KR can suppress their tumor growth (Choi et al., 2008; Rajabi et al., 2012). Further, *in vitro* analyses demonstrated that KR causes cell cycle arrest that leads to apoptosis on human hepatoma cells (Cheong et al., 2009).

Periodic treatments with a KR concentration of 2,5 μM have proved to inhibit tumorous cell cycles from progressing as well as to stopping further proliferation of malignant cells (Rajabi et al., 2012). Interestingly, studies suggested KR's ability to repress the overexpression of cyclin D1 and cyclin D2, which when individually overexpressed, would cause the tumors of multiple myeloma (bone marrow cancer) cancer of plasma cells (Tiedemann et al., 2008). By doing so, KR brings the tumorous cell cycle to a halt. Here, the precise mechanisms that induce apoptosis are also unclear but are speculated to be connected to the hindrance of tumor cell proliferation to retain vigor (Tiedemann et al., 2008).

To overcome fresh CW's limited shelf life, freeze-dried CW vinegar has been studied on 4T1 breast cancer cells (Mohamad et al., 2019). It was suggested that fermentation of CW did not change the activity of the present cytokinins, but rather its chemical structure and possibly, its conjugation (Mohamad et al., 2019). Treatments on breast cancer cells suggested that CW vinegar delayed the development of breast cancer and its spreading from the primary site (metastasis); thereby activating an "anti-tumor immunity" (Mohamad et al., 2019). Because of the displayed suppressive inhibitory abilities towards human breast cancer cells, it might be possible that the fermentation of CW causes cytokinins to transform into higher concentrations of KR (Cheong et al., 2009; Choi et al., 2008; Mohamad et al., 2019). Unfortunately, endogenous cytokinins were not measured in the study by Mohamad et al. (2019), and thus the reactions and compounds which fermentation generated are unclear.

Mitochondria make a significant contribution to cell death by apoptosis (Cheong et al., 2009). Because they control ATP synthesis, these malfunctioning mitochondria can interrupt the ATP energy generating process, and consequently cause the organism to die (Cheong et al., 2009). Similar to K, KR reduces the potential of the mitochondrial membrane by lowering the amount of ATP, eventually leading to cell death, possibly by ATP depletion (Choi et al., 2008; Rajabi et al., 2012). In the intrinsic pathway of apoptosis of mammals, cell death is induced by changes in the mitochondria (Cheong et al., 2009; Rajabi et al., 2012). Bad and Bak are pro-apoptogenic proteins and Bcl-2 and Bcl-x are anti-apoptogenic genes that are located in the mitochondrial membrane (Wang et al., 2012). In normally functioning cells, the two types of proteins will bind and block out each other's actions. Natural apoptosis can be triggered by cells causing the anti-apoptogenic proteins to be blocked out, changing the potential in the mitochondrial membrane and facilitating leakage of mitochondrial substances, such as cytochrome-c and apoptosis-inducing factors, into the cytoplasm (Wang et al., 2012). Further, complexes activate a cascade of caspases in which each amplify another and break down cellular materials (Cheong et al., 2009). In tumors, the anti-apoptogenic genes are often overexpressed, causing an

accumulation of malignant cells instead of these being naturally eliminated (Choi et al., 2008). Treatments with KR on human leukemia cells have displayed the ability to upregulate the pro-apoptogenic genes while selectively suppressing the anti-apoptogenic genes of malignant cells, making it a specified cytotoxicity (Choi et al., 2008)

Other types of cytokinins have also been reported to induce apoptosis in cancer cells, presumably through the mitochondria/caspase pathway (Voller et al., 2010; Wang et al., 2012). One of them is ortho-topolin riboside and has a strong anti-cancer effect (Wang et al., 2012). Although it is not present in CW, its base form, ortho-topolin, was reported to be present (table 1; Yong et al., 2009). It could be speculated that ortho-topolins are part of the cytokinin cycle, but research has not been done to confirm it.

Although not being cytokinins, glucocorticosteroids have also demonstrated anti-cancer potential (Tiedemann et al., 2008). KR and glucocorticosteroids have a synergistic cytotoxicity effect when treating myeloma (Tiedemann et al., 2008). Additionally, it is one of the most effective treatments for this type of cancer (Rajabi et al., 2012; Tiedemann et al., 2008). Moreover, this could mean that research on the combination of glucocorticosteroids, and CW, which is rich on KR (table 1), could be of interest.

8. Discussion

The research on the many benefits of CW is well under way in many countries and so are the synergistic combinations of CW and other compounds such as glucocorticosteroids, retinols, and the processing of CW e.g., fermentation (Cheong et al., 2009; Choi et al., 2008; Lakshmanan et al., 2020; Mohamad et al., 2019; Rattan, 2002).

CW is a holistic biostimulant as it is rich in three imperative phytohormones that contribute to plants' normal cell growth and hormonal homeostasis; cytokinins, auxins and gibberellins (Ang and Yong, 2005; Yong et al., 2009). Research has specifically attributed the cytokinins Z, K and KR, present in CW, with numerous benefits; from growth biostimulation of plants, due to the phytohormones' crucial participation in driving of the plant cell cycle (Sakakibara, 2006; Wong et al., 2016; Yong et al., 2009), to treating dehydration and loss of electrolytes in humans with its content of inorganic ions (Campbell-Falck et al., 2000; Prades et al., 2012; Saat et al., 2002), working against oxidative damage and aging of both human and plant cells (Ge et al., 2005; Minorsky, 2003; Rattan, 2002; Rattan and Clark, 1994; Tiedemann et al., 2008; Yoon

et al., 2017) and selective suppression of human cancer cells (Cheong et al., 2009; Choi et al., 2008; Griffaut et al., 2004; Rajabi et al., 2012; Wang et al., 2012).

An interesting and paradigm shifting concept is the fact that the intercropping of legumes enhances plant growth because the cytokinins produced by the nodule-rhizobacteria coordinate and guide the developmental programme, while utilizing minerals as building blocks. The addition of CW (a waste product in some countries) to support plant cultivation and growth promotion with cytokinins, through the same biostimulatory pathway as in legumes and earthworms, is exciting (Arancon et al., 2004; Aremu et al., 2015; Çakmakçi et al., 2006; Miller et al., 1956; Wong et al., 2016; Yong et al., 2009; Zhang et al. 2009).

This study has identified a big research gap in whether K and KR have the same anti-cancer and anti-aging properties when CW is consumed as a beverage compared to when it is injected into an organism. The studies investigating CW's anti-cancer effects have all been performed by injecting CW *in vitro* and on human tumor xenograft models on mice which have suggested that CW successfully can select and suppress tumor cells and could therefore have similar effect on humans (Anurag and Rajamohan, 2003; Choi et al., 2008; Mohamad et al., 2019; Rajabi et al., 2012). Unfortunately, because of ethical reasons, trials on humans with active diseases, such as cancer, have not been performed. In order to really test CW's potential to prevent and combat cancer, it would be optimal to carry out studies on tumors *in-situ* in order to demonstrate its true specified cytotoxic potential on humans (Anurag and Rajamohan, 2003; Choi et al., 2008; Mohamad et al., 2019), and so would studies on the potential of CW when it is ingested by cancer patients. However, since such human-linked studies are unethical, the expansion of human tumour xenograft models to primates could therefore be a possible next step from mice, in order to be phylogenetically closer to the human organism.

The complexities of trace level cytokinin biochemistry and cellular responses have hampered the wider research effort in this area. It could be that researchers refer to K and KR as separate compounds when in fact they might be referring to one, both, or another compound within the cytokinin cycle (Voller et al., 2019). It is also possible that, equal to plants, a conversion to Z-type cytokinin is required for the K-type phytohormones to have biological functionality (Rajabi et al., 2012). Like the targeted apoptosis onset mechanism, the specific chemical structure of the functioning cytokinin type in humans, when ingested orally, is unknown (Choi et al., 2008; Rajabi et al., 2012; Tiedemann et al., 2008; Voller et al. 2019).

To conclude, it is possible that the reason why cytokinins from plant cells, KR in particular, have the ability to repair human cells is because humans and plants have similar genetic blueprints (Yoshiyama et al., 2013). Presumably, CW's amino acid content could be

responsible for its improved fidelity (accuracy) of DNA transcription (Rattan, 2002). This could be due to humans and plants belonging to the same eukaryotic domain; their cells and cellular responses are phylogenetically conserved (Yoshiyama et al., 2013).

From the perspective of biomedical research investment by profit-driven companies in general, simple, effective, and widely available anti-cancer compounds like cytokinin-related compounds may not be attractive to companies as they would not be able to protect their market share by owning exclusive or unique molecules, unlike the ubiquitous cytokinins.

9. Suggestions for future research

- Chemical compositions of CW from different coconut varieties
- Chemical compositions of CW throughout the different stages of maturity of coconuts
- Soil and environmentally affected chemical compositions of CW
- Breeding coconut plants for specific chemical compositions and benefits of CW
- The plant cytokinin cycle in humans and profiling cytokinin metabolism during cancer and anti-cancer trials.

10.References

10.1 Literature references

ANG, S. & YONG, J. 2005. A protocol for *in vitro* germination and sustainable growth of two tropical mistletoes. *Plant Cell, Tissue and Organ Culture,* 80**,** 221-228.

ANURAG, P. & RAJAMOHAN, T. 2003. Cardioprotective effect of tender coconut water in experimental myocardial infarction. *Plant Foods for Human Nutrition,* 58**,** 1-12.

ARANCON, N. Q., EDWARDS, C. A., BIERMAN, P., WELCH, C. & METZGER, J. D. 2004. Influences of vermicomposts on field strawberries: 1. Effects on growth and yields. *Bioresource technology,* 93**,** 145-153.

AREMU, A. O., STIRK, W. A., KULKARNI, M. G., TARKOWSKÁ, D., TUREČKOVÁ, V., GRUZ, J., ŠUBRTOVÁ, M., PĚNČÍK, A., NOVÁK, O., DOLEŽAL, K., STRNAD, M. & VAN STADEN, J. 2015. Evidence of phytohormones and phenolic acids variability in garden-waste-derived vermicompost leachate, a well-known plant growth stimulant. *Plant growth regulation,* 75**,** 483-492.

BHAGYA, D., PREMA, L. & RAJAMOHAN, T. 2012. Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pacific journal of tropical medicine,* 5**,** 270.

CAMPBELL-FALCK, D., THOMAS, T., FALCK, T. M., TUTUO, N. & CLEM, K. 2000. The intravenous use of coconut water. *American Journal of Emergency Medicine,* 18**,** 108-111.

CHANCLUD, E. & LACOMBE, B. 2017. Plant Hormones: Key players in gut microbiota and human diseases? *Trends in Plant Science,* 22, 754-758.

CHEONG, J., GOH, D., YONG, J. W. H., TAN, S. N. & ONG, E. S. 2009. Inhibitory effect of kinetin riboside in human heptamoa, HepG2. *Molecular Biosystems,* 5**,** 91-98.

CHOI, B. H., KIM, W., WANG, Q. C., KIM, D. C., TAN, S. N., YONG, J. W. H., KIM, K. T. & YOON, H. S. 2008. Kinetin riboside preferentially induces apoptosis by modulating Bcl-2 family proteins and caspase-3 in cancer cells. *Cancer Letters,* 261**,** 37-45.

COSTA, N. R., CRUSCIOL, C. A. C., TRIVELIN, P. C. O., PARIZ, C. M., COSTA, C., CASTILHOS, A. M., SOUZA, D. M., BOSSOLANI, J. W., ANDREOTTI, M., MEIRELLES, P. R. L., MORETTI, L. G. & MARIANO, E. 2021. Recovery of ^{15}N

fertilizer in intercropped maize, grass and legume and residual effect in black oat under tropical conditions. *Agriculture, Ecosystems and Environment*, 310.

DEBMANDAL, M. & MANDAL, S. 2011. Coconut (*Cocos nucifera* L.: *Arecaceae*): in health promotion and disease prevention. *Asian Pacific journal of tropical medicine,* 4**,** 241.

DOBREV, P. I., HOYEROVA, K. & PETRASEK, J. 2017. Analytical Determination of Auxins and Cytokinins. *Auxins and Cytokinins in Plant Biology: Methods and Protocols,* 1569**,** 31-39.

GE, L., YONG, J. W. H., GOH, N. K., CHIA, L. S., TAN, S. N. & ONG, E. S. 2005. Identification of kinetin and kinetin riboside in coconut (*Cocos nucifera* L.) water using a combined approach of liquid chromatography-tandem mass spectrometry, high performance liquid chromatography and capillary electrophoresis. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences,* 829**,** 26-34.

GRIFFAUT, B., BOS, R., MAURIZIS, J. C., MADELMONT, J. C. & LEDOIGT, G. 2004. Cytotoxic effects of kinetin riboside on mouse, human and plant tumour cells. *International Journal of Biological Macromolecules,* 34**,** 271-275.

JACOBY, R., PEUKERT, M., SUCCURRO, A., KOPRIVOVA, A. & KOPRIVA, S. 2017. The Role of Soil Microorganisms in Plant Mineral Nutrition - Current Knowledge and Future Directions. *Frontiers in Plant Science*. 8, 1617.

KOBAYASHI, H., MORISAKI, N., TAGO, Y., HASHIMOTO, Y., IWASAKI, S., KAWACHI, E., NAGATA, R. & SHUDO, K. 1995. IDENTIFICATION OF A MAJOR CYTOKININ IN COCONUT MILK. *Experientia,* 51**,** 1081-1084.

LAKSHMANAN, J., ZHANG, B., WRIGHT, K., MOTAMENI, A. T., JAGANATHAN, V. L., SCHULTZ, D. J., KLINGE, C. M. & HARBRECHT, B. G. 2020. Tender coconut water suppresses hepatic inflammation by activating AKT and JNK signaling pathways in an *in vitro* model of sepsis. *Journal of Functional Foods,* 64.

LETHAM, D. S. 1963. ZEATIN, A FACTOR INDUCING CELL DIVISION ISOLATED FROM ZEA-MAYS. *Life Sciences***,** 569-573.

MILLER, C. O., SKOOG, F., OKUMURA, F. S., VON SALTZA, M. H. & STRONG, F. M. 1955. STRUCTURE AND SYNTHESIS OF KINETIN 1. *Journal of the American Chemical Society,* 77**,** 2662-2663.

MILLER, C. O., SKOOG, F., OKUMURA, F. S., VONSALTZA, M. H. & STRONG, F. M. 1956. ISOLATION, STRUCTURE AND SYNTHESIS OF KINETIN, A

SUBSTANCE PROMOTING CELL DIVISION. *Journal of the American Chemical Society,* 78**,** 1375-1380.

MOHAMAD, N. E., YEAP, S. K., ABU, N., LIM, K. L., ZAMBERI, N. R., NORDIN, N., SHARIFUDDIN, S. A., LONG, K. & ALITHEEN, N. B. 2019. *In vitro* and *in vivo* antitumour effects of coconut water vinegar on 4T1 breast cancer cells. *Food & Nutrition Research,* 63.

NIHORIMBERE, V., ONGENA, M., SMARGIASSI., M. & THONART., P. 2010. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnology, Agronomy, Society and Environment.* 5, 327-337.

OONO, R., ANDERSON, C., G. & FORD DENISON., R. 2011. Failure to fix nitrogen by non-reproductive symbiotic rhizobia triggers host sanctions that reduce fitness of their reproductive clonemates. *Proceedings of the royal society B.* 278, 2698-2703.

PAREEK, S. 2016. Chapter 3 - Nutritional and Biochemical Composition of Banana (Musa spp.) Cultivars. *Nutritional Composition of Fruit Cultivars,* 48-81.

PRABAKARAN, G., HOTI, S. L., MANONMANI, A. M. & BALARAMAN, K. 2008. Coconut water as a cheap source for the production of delta endotoxin of Bacillus thuringiensis var. israelensis, a mosquito control agent. *Acta Tropica,* 105**,** 35-38.

PRADES, A., DORNIER, M., DIOP, N. & PAIN, J. P. 2012. Coconut water uses, composition and properties: a review. *Fruits,* 67**,** 87-107.

PRATHAPAN, A. & RAJAMOHAN, T. 2011. ANTIOXIDANT AND ANTITHROMBOTIC ACTIVITY OF TENDER COCONUT WATER IN EXPERIMENTAL MYOCARDIAL INFARCTION. *Journal of Food Biochemistry,* 35**,** 1501-1507.

RAJABI, M., GORINCIOI, E. & SANTANIELLO, E. 2012. ANTIPROLIFERATIVE ACTIVITY OF KINETIN RIBOSIDE ON HCT-15 COLON CANCER CELL LINE. *Nucleosides Nucleotides & Nucleic Acids,* 31**,** 474-481.

RATTAN, S. I. S. 2002. N-6-furfuryladenine (kinetin) as a potential anti-aging molecule. *Journal of Anti-Aging Medicine,* 5**,** 113-116.

RATTAN, S. I. S. & CLARK, B. F. C. 1994. KINETIN DELAYS THE ONSET OF AGING CHARACTERISTICS IN HUMAN FIBROBLASTS. *Biochemical and Biophysical Research Communications,* 201**,** 665-672.

SAKAKIBARA, H. 2006. Cytokinins: Activity, biosynthesis, and translocation. *Annual Review of Plant Biology,* 57**,** 431-449.

SAAT, M., SINGH, R., SIRISINGHE, R. & NAWAWI, M. (2002). Rehydration after Exercise with Fresh Young Coconut Water, Carbohydrate-Electrolyte Beverage and Plain Water. *Journal of physiological anthropology and applied human science,* 21, 93-104.

SIM, G., GOH, C. & LOH, C. 2008. Induction of *in vitro* flowering in Dendrobium Madame Thong-In (Orchidaceae) seedlings is associated with increase in endogenous N 6 -(Δ 2 -isopentenyl)-adenine (iP) and N 6 -(Δ 2 -isopentenyl)-adenosine (iPA) levels. *Plant Cell Reports,* 27**,** 1281-1289.

TARKOWSKI, P., GE, L. Y., YONG, J. W. H. & TAN, S. N. 2009. Analytical methods for cytokinins. *Trac-Trends in Analytical Chemistry,* 28**,** 323-335.

TIEDEMANN, R. E., MAO, X. L., SHI, C. X., ZHU, Y. X., PALMER, S. E., SEBAG, M., MARLER, R., CHESI, M., FONSECA, R., BERGSAGEL, P. L., SCHIMMER, A. D. & STEWART, A. K. 2008. Identification of kinetin riboside as a repressor of CCND1 and CCND2 with preclinical antimyeloma activity. *Journal of Clinical Investigation,* 118**,** 1750-1764.

VOLLER, J., BÉRES, T., ZATLOUKAL, M., DŽUBÁK, P., HAJDÚCH, M., DOLEŽAL, K., SCHMÜLLING, T. & MIROSLAV, S. 2019. Anti-cancer activities of cytokinin ribosides. *Phytochemistry Reviews,* 1-13.

VOLLER, J., ZATLOUKAL, M., LENOBEL, R., DOLEŽAL, K., BÉREŠ, T., KRYŠTOF, V., SPÍCHAL, L., NIEMANN, P., DŽUBÁK, P., HAJDÚCH, M. & STRNAD, M. 2010. Anticancer activity of natural cytokinins: A structure–activity relationship study. *Phytochemistry,* 71**,** 1350-1359.

WANG, L., SUN, C., WANG, Z. H. & GUO, G. Q. 2012. Mechanism of apoptotosis induced by ortho-topolin riboside in human hepatoma cell line SMMC-7721. *Food and Chemical Toxicology,* 50**,** 3384-3384.

WONG, W. S., TAN, S. N., GE, L., CHEN, X., LETHAM, D. S. & YONG, J. W. H. 2016. The importance of phytohormones and microbes in biostimulants: mass spectrometric evidence and their positive effects on plant growth. *II World Congress on the Use of Biostimulants in Agriculture,* 1148**,** 49-59.

WONG, W. S., TAN, S. N., GE, L., CHEN, X. & YONG, J. W. H. 2015. The Importance of Phytohormones and Microbes in Biofertilizers. In: MAHESHWARI, D. K. (ed.) Bacterial Metabolites in Sustainable Agroecosystem. *Sustainable Development and Biodiversity,* 12, 105-158. Cham: Springer International Publishing.

YONG, J. W. H., GE, L. Y., NG, Y. F. & TAN, S. N. 2009. The Chemical Composition and Biological Properties of Coconut (*Cocos nucifera* L.) Water. *Molecules,* 14**,** 5144-5164.

YONG, J. W. H., LETHAM, D., WONG, S. C. & FARQUHAR, G. 2014. Rhizobium-induced elevation in xylem cytokinin delivery in pigeonpea induces changes in shoot development and leaf physiology. *Functional Plant Biology,* 41, 1323-1335.

YOON, J.-Y., PARK, C.-G., PARK, B.-S., KIM, E.-J., BYEON, G.-J. & YOON, J.-U. 2017. Effects of Remifentanil Preconditioning Attenuating Oxidative Stress in Human Dermal Fibroblast. *Tissue Engineering and Regenerative Medicine,* 14**,** 133-141.

YOSHIYAMA, K., O., SAKAGUCHI, K. & KIMURA, S. 2013. DNA Damage Response in Plants: Conserved Response Compared to Animals. *Biology,* 2, 1338-1356.

ZHANG, H., TAN, S., WONG, W., NG, C., TEO, C., GE, L., CHEN, X. & YONG, J. W. H. 2014. Mass spectrometric evidence for the occurrence of plant growth promoting cytokinins in vermicompost tea. *Biology and Fertility of Soils,* 50**,** 401-403.

ÇAKMAKÇI, R., DÖNMEZ, F., AYDIN, A. & ŞAHIN, F. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry,* 38**,** 1482-1487.

10.2 Internet references

European biostimulants industry council (EBIC). 2011. Biostimulants definition agreed. Available at: http://www.biostimulants.eu/2011/10/biostimulants-definition-agreed/ [2019-06-09]

Mahidol University. 2021. Young coconut water: The functional drink from nature. Available at: https://pharmacy.mahidol.ac.th/en/knowledge/article/362/YoungCoconutWater/ [2021-01-27]

10.3 Picture references

Keira Morgan. 2012. Coconut. Flickr. Available at: https://www.flickr.com/photos/keiramellis/8427723703/in/photolist-dQJhD6-5DaPwi-8zVdck-2fqknga-6c4Na4-aeJ53r-dCq95x-9zNUSm-oZPEv3-dQ4eG9-KwvJdS-7CRtpA-4doVeW-7CMDxF-q1Ho9b-b8L24k-8jYCyw-3vngS9-47NGTW-7CMGzc-7CRvNW-r1tLv-d4Een9-dxRcv6-47NFaQ-6647nK-UxU2tF-afEVzr-47NJnE-6x73LZ-BoVE-47NCBq-

5vtAr5-7CMFgt-GN1ci-eYSbq-6nS76x-aYpsJ2-q2uCuH-5HY5z7-47JG82-5gTzgQ-nrTfUi-7CMCQM-7CME74-wTbzoH-mYTUv-47NEhA-5iDZZL-5Paaza/ [2019-08-14]

11. Appendix

Table 1: The chemical composition of CW. From 2009 with permission from J.W.H. Yong.

Source information	**[20]**	**[31]**	**[29]**				**[30]**	
Coconut type		**young**	**young green**	**mature green**	**mature**	**mature (autoclaved)**	**young**	**mature**
Average Weight of Coconut (g)		206 (water)					565	393
Age of coconut							6 months	12 months
Source of coconut			Deerfield Beach, FL		Dominican Republic			
Proximates		*(g/100g)*					*(g/100g)*	
Water		94.99					94.18	94.45
Dry		5.01					5.82	5.55
Energy value		19 kcal (79 kJ)						
Protein		0.72					0.12	0.52
Total lipid (fat)		0.2					0.07	0.15
Ash		0.39					0.87	0.47
Carbohydrate, by difference		3.71					4.76	4.41
Fiber, total dietary		1.1					ND*	ND*
Sugars	*(mg/mL)*	*(g/100g)*		*(mg/mL)*			*(g/100g)*	
Total		2.61	9.16	21.68	13.87	15.20	5.23	3.42
Sucrose	9.18		0.93	9.18	8.90	10.70	0.06	0.51
Glucose	7.25		3.93	7.25	2.46	2.02	2.61	1.48
Fructose	5.25		4.30	5.25	2.51	2.48	2.55	1.43
Sugar alcohols	Present [a]			*(mg/L)*				
Mannitol	0.8			0.80				
Sorbitol	15 [d]			15.00				
Myo-inositol	0.01			0.01				
Scyllo-inositol	0.05			0.05				

Table 1: Cont.

Inorganic ions	*(mg/100g)*	*(mg/100g)*	*(mg/100g)*	*(mg/100g)*	
Calcium, Ca		24		27.35	31.64
Iron, Fe	0.01	0.29	0.01	0.02	0.02
Magnesium, Mg	30	25	30	6.40	9.44
Phosphorus, P	37	20	37	4.66	12.77
Potassium, K	312	250	312	203.70	257.52
Sodium, Na	105	105	105	1.75	16.10
Zinc, Zn		0.1		0.07	0.02
Copper, Cu	0.04	0.04	0.04	0.01	0.03
Manganese, Mn		0.142		0.12	0.08
Selenium, Se		0.001			
Chlorine, Cl	183		183		
Sulfur, S	24		24	0.58	
Aluminium, Al				0.07	0.06
Boron, B				0.05	0.08
Vitamins	*(mg/mL)*	*(mg/100g)*	*(mg /L)*	*(mg /100 dm ^{3})*	
Vitamin C, total ascorbic acid		2.4		7.41	7.08
Thiamin (B1)		0.03	Trace	Trace	0.01
Riboflavin (B2)		0.057	0.01	0.01	0.01
Niacin (B3)		0.08	0.64	ND*	ND*
Pantothenic acid (B5)	0.52	0.043	0.52		
Pyridoxine (B6)		0.032	Trace	ND*	ND*
Folate, total		0.03			
Folic acid	0.003	0	0.003		
Folate, food		0.003			
Folate, Dietary Folate Equivalent (DFE)		3 (µg_DFE)			
Biotin	0.02		0.02		
Nicotinic acid (Niacin)	0.64		0.64		

Table 1: Cont.

Lipids		*(g/100g)*					*(g/100g)*	
Total		0.2					0.0733	0.1482
Fatty acids, total saturated		0.176					0.03	0.1
6:00		0.001						
8:00		0.014					ND*	ND*
10:00		0.011					0.0007	0.0028
12:00		0.088					0.002	0.0274
14:00		0.035					0.0023	0.019
16:00		0.017					0.0219	0.032
17:00							0.0009	0.0016
18:00		0.01					0.0039	0.0108
20:00							0.0016	0.0033
Fatty acids, total monounsaturated		0.008					0.03	0.02
16:1 undifferentiated		0					0.0011	0.0007
18:1 undifferentiated		0.008					0.0194	0.015
20:1 undifferentiated							0.0049	0.0019
22:1 undifferentiated							0.0011	0.0023
Fatty acids, total polyunsaturated		0.002					0.0128	0.0054
18:2 *n-6*undifferentiated		0.002					0.0114	0.0032
20:4 *n-6*							0.0014	0.0022
Amino acids	*(µg/mL)*	*(g/100g)*		*(µg/mL)*			*(mg/g defatted sample)*	
Alanine	312	0.037	16.40	127.30	177.10	198.00	1.13	3.88
β-Alanine	12							
γ-Aminobutyric acid	820		1.90	34.60	168.80	173.20		
Arginine	133	0.118	14.70	25.60	16.80	20.70	0.13	0.81
Asparagine and glutamine	*ca.* 60							
Aspartic acid	65	0.07	11.30	35.90	5.40	11.40	1.60	0.76
Asparagine			17.10	10.10	10.40	25.30		
Cystine	0.97-1.17 [b]	0.014					0.00	0.00

Table 1: Cont.

Glutamic acid	240	0.165	9.40	70.80	78.70	104.90	3.44	3.75
Glutamine			80.00	45.40	13.40	2.00		
Glycine	13.9	0.034	1.30	9.70	13.90	18.00	0.43	0.11
Homoserine	5.2		ND*	ND*	5.20	8.80		
Histidine	Trace [a]	0.017	3.50	6.30	Trace [a]	Trace [a]	0.39	0.67
Isoleucine	18	0.028					0.26	0.27
Leucine	22	0.053	6.20	37.30	31.70	33.00	0.66	0.58
Lysine	150	0.032	4.40	21.40	22.50	13.00	4.72	3.41
Methionine	8	0.013	3.50	16.90	Trace [a]	Trace [a]	0.22	0.21
Ornithine	22							
Phenylalanine	12	0.037	ND*	ND*	10.20	Trace [a]	0.26	0.00
Pipecolic acid		Trace [a]						
Proline	97	0.03	4.10	31.90	21.60	12.90	0.52	0.95
Serine	111	0.037	7.30	45.30	65.80	85.00	0.64	1.06
Tyrosine	16	0.022	0.90	6.40	3.10	Trace [a]	0.00	0.00
Tryptophan	39	0.008					0.00	0.00
Threonine	44	0.026	2.90	16.20	26.30	27.40	0.20	0.33
Valine	27	0.044	5.60	20.60	15.10	15.50	0.91	0.82
Dihydroxyphenylaline	Present [a]							
Hydroxyproline	Trace [a]		Trace [a]	4.10	Trace [a]	8.20		
Pipecolic acid	Present [a]	Trace [a]						
Nitrogeneous compounds	*µmol/mL*							
Ethanolamine	0.01							
Ammonia	Present [a]							
Organic acids	*(meq/mL)*			*(meq/mL)*			*(mg /100 DM)*	
Tartaric							1.6	2.4
Malic	34.31		9.36	34.31	11.98	14.08	317	307
Citric	0.37			0.37	0.31	0.38	ND*	24.8

Table 1: Cont.

Acetic							ND*	1.3
Pyridoline	0.39 mg/mL		0.43	0.39	0.18	0.27		
Succinic					0.28	0.18		
Shikimic and quinic acids, etc.	0.57							
Enzymes								
Acid phosphatase	Present [a]							
Catalase	Present [a]							
Dehydrogenase	Present [a]							
Diastase	Present [a]							
Peroxidase	Present [a]							
RNA polymerases	Present [a]							
Phytohormones	*(mg/mL)*			*(mg/L)*				
Auxin	0.07			0.07				
1,3- Diphenylurea				5.8				
Cytokinin	Present [a]							
Miscellaneous								
Leucoanthocyanin	Present [a]							
Phyllococosine	Present [a]							
Chemical properties								
pH			4.6 to 5.6				4.7±0.1	5.2±0.1

* ND = Non detectable; [a] No units given; [b] Units: g/100g dried protein; [d] Units: mg/mL; [e] Due to contamination.