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Review Article

Potential and Simulation of Optimization Design for Ozonation Process on Commercial Sterility Level of Coconut Water Product: A Review

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ABSTRACT

Coconut (*Cocos nucifera* Linnaeus) water is a functional refresher drink because it contains sugar, minerals, amino acids, enzymes, organic acids, fatty acids, vitamins, and phenolic components. Thermal processing in coconut water can reduce its nutrients and sensory. Therefore, ozone plasma has the potential to be applied to this product because its composition is dominated by water. Another problem in coconut water is deterioration by polyphenol oxidase and peroxidase. Ozonation can inactivate bacterial spores and endogenous enzymes to maintain the quality of coconut water. This review aims to provide scientific information about the risk of postharvest coconut products, the potential of ozone for commercial sterility, and a simulation for adopting equivalence and optimizing the ozonation process using thermal process kinetics approach. The methods discussed for adoption in this work were based on thermal kinetic reduction of quality parameters (k and D values) plotted against temperature (Z-value). Temperature-time combination for process optimization were set reffering to D and Z values. For equivalence thermal and ozonation process optimization, the k; D; and Z values determination were adopted by conducting ozonation. It began with characterizing the ozone machine, determining the kinetics of inactivation of *Clostridium sporogenes* spores, polyphenol oxidase, and peroxidase activities during ozonation. The simulation results of adopting thermal process kinetics design can be used to design a commercial sterile and optimization of the ozonation process using ozonation process kinetics data. This review dramatically contributes to equivalence of the ozonation process in commercial sterilization.

***Keywords*:** bioactive compound, *Clostridium sporogenes*, coconut water, enzyme, ozone

Introduction

Coconut water is a pure drink made from coconuts consumed widely by the public because it is a very popular fresh drink. The distinctive coconut aroma, sweet taste, high functional value, and health protection properties have become a strong attraction for consuming fresh coconut water. Mahayothee *et al.* reported that coconut water contains 5-9% total dissolved solids, in which simple sugars dominate more than 80% of the total dissolved solids.[1] These sugars include 1.4% fructose, 1.36% glucose, and 0.06% sucrose.[2] Other important ingredients are minerals, amino acids, enzymes, organic acids, fatty acids, vitamins, and several phenolic components. The amount of minerals from these components is the most dominant, with a proportion of 0.4-1% of the total components that comprise other coconut water.[2] The most dominant mineral from coconut water is potassium, while other components including sodium, calcium, chlorine, magnesium, and phosphorus have also been reported to be present in coconut water. The composition of the minerals in coconut water can create the same osmotic pressure as in blood, so coconut water can be called an electrolyte fluid.[3]

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The functional properties of coconut water provided by the presence of this mineral do not cause plasma coagulation in the blood. It will reduce blood pressure and toxins and improve drug induction that causes toxicity due to overdosage. The presence of phenolic components in coconut water acting as antioxidant agent could prevent oxidative stress-related diseases.[4] However, flavonoid is one of polyphenol component that prevents cancer and degenerative diseases.[5] Both of phenolic and flavonoid components obstruct the destruction of tissues or organs of human body caused by degenerative diseases.[6] Coconut can assist in absorbing drugs in the blood.[3] The utilities of coconut tree's parts are available in Table 1.

Alchoubassi *et al*.[7] also added that these trace elements are necessary for normal growth, development, and human physiology, carrying out cellular functions, enzymatic activation, gene expression, and metabolism of amino acids, lipids, and carbohydrates. Coconut water contains a small number of organic acids such as malic acid, citric acid, succinic acid, and tartaric acid; small amounts of fatty acids such as palmitic acid and oleic acid; and vitamins such as thiamin (B1), riboflavin (B2), pantothenic acid (B5), and ascorbic acid.[2] The phenolic components found in coconut water include catechins, salicylic acid, 4-hydroxybenzoic acid, syringic acid, coumaric acid, gallic acid, and caffeic acid.[2] Biotin, folic acid, and phytohormones such as auxin, 1,3-diphenylurea, and cytokinins have been reported in coconut water.[3] Coconut water also contains free amino acids such as alanine, cysteine, and serine and enzymes such as polyphenol oxidase, peroxidase, and catalase.[2]

Thermal process using heat to process raw materials is the most recognized and widely used approach for food safety and preservation. Prades *et al*. explained that several coconut water derivative products had been processed using various techniques, including canning and

high-temperature techniques, in a short time.[8] This statement indicates that the thermal process is still a technique that coconut water producers pay attention to regarding food preservation and safety. The thermal process guarantees the safety of pathogenic microbial spores or spoilage microbes, which can be significantly reduced in number according to measurable targets, namely at least fulfilling the 12D concept. The 12D concept with *Clostridium botulinum* spore inactivation standard is a commercial sterility process validated in a thermal process. However, specifically for coconut water does have drawbacks for processing that uses a thermal process; namely, the taste produced is different from the taste before it is heated.[9] In addition, several heat-sensitive nutrients have been degraded during heating.[9]

The solution to decreasing the quality of taste and nutrients in coconut water processed by thermal processes is to use non-thermal processing. Naik *et al*. have stated that non-thermal technologies such as high-pressure processing, pulsed electric fields, ultraviolet, and ozone can be used as alternative technologies in processing coconut water without reducing taste quality and nutrients.[9] Because the chemical composition of coconut water is dominated by water, the ozone technique is very suitable to be selected as an alternative to coconut water treatment. Comparison of ozone with high-pressure processing is that high-pressure processing requires very high pressure, up to 1000 MPa, to inactivate bacterial spores.[10] This very high pressure certainly requires costly maintenance costs. In processing with ultraviolet and pulsed electric fields, it has a low lethal effect on bacterial spores.[11] During the ozonation process, ozone will naturally decompose into oxygen gas in the water and will not produce harmful by-products.[12] Ozone has Generally declared Recognized as Safe (GRAS) in 1997 and can be used for ozonation in liquid drinks or food.[13] The potential for the inactivation of spores by ozone will allow the ozonation process to be conceptually equivalent to commercial sterility with thermal processes.[14]

Published studies on the effect of ozone on the viability of microbial spores have been conducted since 1985 by Foeging. In 2001 there were experimental results from Khadre and Yousef. Meanwhile, in 2003 Sakurai *et al*. published the experimental results about micorbial spore inactivation using ozonation.[15] Then Aydogan and Gurol also conducted ozonation to inactivate microbial spore in 2006. Finally, in 2018, Torlak and Isik researched the inactivation of microbial spores by ozone. The experimental results from these researchers can be seen in Table 2. The potential for the inactivation of spores by ozone will allow the ozonation process to be conceptually equivalent to commercial sterilization using a thermal process.

On the other hand, Kanjanapongkul and Baibua stated that one of the most important problems in coconut water is colour damage due to enzymatic browning caused by polyphenol oxidase enzymes.[16] Together with this polyphenol oxidase enzyme, the peroxidase enzyme also experiences increased activity after direct exposure of coconut water with air. Peroxidase acted to oxidize both organic and inorganic compounds, especially phenolic components in coconut water.[17] Hence, the risk of rancidity in coconut water is high. Ozonation can inactivate microbes and endogenous enzymes, such as polyphenol oxidases and peroxidases, to maintain the quality and freshness of coconut water. The mechanisms of microbial and enzyme inactivation by ozone can be illustrated in Figure 1. In this case, the microbial parameter is a food safety parameter that must be achieved at certain targets. Standard inactivation of *Clostridium botulinum* spores is the main target for processing that aims for a long shelf life at room temperature with hermetic packaging.[18]

However, *Clostridium botulinum* is a very dangerous pathogenic bacterium; it is usually necessary to find a substitute for *Clostridium botulinum* which has similar characteristics to this bacterium. *Clostridium sporogenes* is a spore-producing bacterium that is similar to *Clostridium botulinum* in terms of the resistance of the spore wall to external influences, especially heat but is non-pathogenic, which is very suitable to replace *Clostridium botulinum* in designing an ozonation process that is equivalent to commercial sterility.[19] Then the quality parameters that are also attempted to provide information on processing limits that are carried out so as not to exceed the critical limits of the quality parameters that have been set are total phenols and flavonoids.

Table 1: The Utilities of Coconut Tree Parts

No	Parts	Utilities	Reference
1	Wood	Building materials: beams, trusses, posts, and floors	Wang *et al*.[77]
		Glued laminated timber	Kusnindar *et al*.[78]
		Cross-laminated timber	Srivaro *et al*.[79]
			Srivaro *et al*.[80]
2	Leaves	Handcrafts	Ignacio and Miguel[81]
		Primary packaging of "ketupat", Indonesian traditional food	Rianti *et al*.[82]
3	Husk	Carpets, geotextiles, rope and compressed wood	Ignacio and Miguel[81]
		As an inert sterile support medium for growing plants	
		A nutritional fiber source	
		As seat padding in vehicles such as trucks and trains	
4	Shell	Activated charcoal	Manaf *et al*.[83]
		Activated carbon	Sasono *et al*.[84]
5	Solid Endosperm	Copra	Rangana and Wickramasinghe[85]
		Refined, bleached, and deodorized (RBD) coconut oil	Amit *et al*.[86]
		Virgin Coconut Oil	Amit *et al*.[87]
		Desserts	Ignacio and Miguel[81]
6	Liquid Endosperm	Coconut milk	Thirukumaran *et al*.[88]
		Fresh coconut water	Aba *et al*.[89]
		"Nata de coco" product	Nguyen *et al*.[90]
		Intravenous hydration	Campbell-Falck *et al*.[91]

Table 2: Previous Studies on Inactivation of Microbial Spores by Ozone

No	Source	Process Condition	Results
1	Foegeding [47]	Evaluation at the range of ozone concentration 0-1.5 mg/L at pH 3 for 15 minutes	Resistance of *Clostridium botulinum* ATCC 12885A spore (highest) > *Clostridium perfringens* NCTC 8798 spore > *Bacillus cereus* T
		Evaluation at the range of ozone concentration 0-2.2 mg/L at pH 3 for 15 minutes	Resistance of *Bacillus stearothermophilus* ATCC 1518 spore > *Bacillus cereus* spore
2	Khadre and Yousef [49]	1.8 mg/L ozone at pH 3	Inactivate ≥95% *Bacillus cereus* T & *Bacillus cereus* F4810/72 spores from initial Inactivate *Bacillus stearothermophilus* ATCC 1518 spore for 37% from initial
		Dissolved ozone concentration of 11 µg/mL water at 22°C for 1 minutes	Reduce *Bacillus stearothermophilus* OSU24 spore → 1.3 log CFU/mL Reduce *Bacillus cereus* OSU11 spore → 6.1 log CFU/mL
3	Sakurai *et al*. [15]	Ozone concentration of 15,000 ppm, RH 90%, inactivate *Geobacillus stearothermophilus* strain ATCC 12980 or ATCC 7953 at biological indicators kit commercial Steris Co., Ltd.	D-value= 5.9-6.2 min at 15°C D-value = 4.6-5.3 min at 25°C D-value = 2.3-3.0 min at 35°C
4	Aydogan and Gurol [48]	*Bacillus subtilis* spore was dried on different material surfaces at RH 70-95%. Ozone concentration of 3 mg/L for 4 hours	Reduce 3 log spore
5	Torlak and Isik [14]	Three Paenibacillus larvae inoculated Pinewood dan *polyvinyl chloride* (PVC) strain spore mixes. Ozone concentration of 9.8 and 17.1 mg/L at room temperature for 120 minutes. Ozonation at 17.1 mg/L for 120 min could:	Reduce 4 log spores at PVC Reduce 2.3 log spores at pine wood.
6	Cheong *et al*. [92]	Inactivation of multidrug-resistant bacteria and *Clostridium difficile* spore by ozone generator using dielectric barrier discharge plasma reactor. Ozonation was conducted by 500 ppm for 15 minutes	Reduce 2 log of all bacterial in stainless steel, fabric and wood media. Reduce 1-2 log in glass and plactic for vancomycin-resistant *Enterococcus faecium* and carbapenem-resistant *Acinetobacter baumanii*. However, *Clostridium difficile* spore was the most resistant as compared to the other evaluated bacteria.

In order to initiate efforts to achieve the equivalence of commercial sterility of coconut water with this ozonation process, it is also necessary to characterize the ozone machine used to determine the ozone concentration in coconut water products as a function of ozonation time. This process design hopes to optimize the ozonation process in inactivating microbes, polyphenol oxidase enzymes, peroxidases, and their sensitivity to phenol and flavonoid content shown in the graph of the relationship between ozone concentration in the product and processing time.

Furthermore, a recommendation area for the ozonation process will be obtained with the parameters that have been set but fulfill the concept of commercial sterility. This review aims to explain the characteristics of coconut water and the kinetics of coconut water quality parameters, either in thermal or ozonation processes. An understanding of kinetics

is needed to design an optimization design for the coconut water ozonation process by knowing the character of the ozone machine in delivering ozone gas to all sides of the product; determine the kinetics of inactivation of *Clostridium sporogenes* spores, polyphenol oxidase, and peroxidase enzymes with the limitation of decreasing levels of phenol and flavonoids. The standard for *Clostridium sporogenes* spores inactivation is 12D, which equates the ozonation process with commercial sterility level.

Coconut (Cocos nucifera Linnaeus)

Coconut (*Cocos nucifera* Linnaeus) belongs to the family Arecaceae (Palmae), subfamily Cocoideae.[3] There are two main distinct groups of coconut trees: tall trees and dwarf trees. Varieties with tall trees grow slowly and produce fruit 6-10 years after planting the seedlings. This tall type of coconut tree dominates around 95% of the total coconut trees in the world.[2] This variety's copra, oil, and fiber products are good quality. Tall coconut trees are stronger and sturdier and can live up to 80-120 years. Since the male flowers of this type mature faster than the female ones, most are cross-pollinated. Coconut fruit maturity occurs in the 12th month after cross-pollination.[3] Another case is with varieties of dwarf coconut trees. This tree grows quickly and can harvest its fruit in the 4th or 5th year after planting the seedlings. Due to the overlap in the maturation times of male and female flowers on dwarf coconut trees, these trees pollinate independently. Farmers commonly harvested the coconut fruits that have a green colour.[20]

The colour of the coconut skin on the dwarf coconut tree is yellow, red, green, and orange for each tree. Dwarf coconut trees tend not to be as sturdy as tall types. This tree requires certain climatic conditions and soil types for better growth.[3] Coconut trees can grow well on clay soils with warm and humid conditions at RH above 60%. The best climatic conditions for growing coconut trees are in the temperature range of 27°C with an average rainfall of 1500-2500 mm per year. With good climatic conditions, the productivity of coconut trees will be optimal, with several 12-16 bunches per year. Each coconut bunch can produce 8-10 coconuts. During its lifetime, a coconut tree can produce up to 10,000 coconuts.[3] An example of a coconut fruits image can be seen in Figure 2.

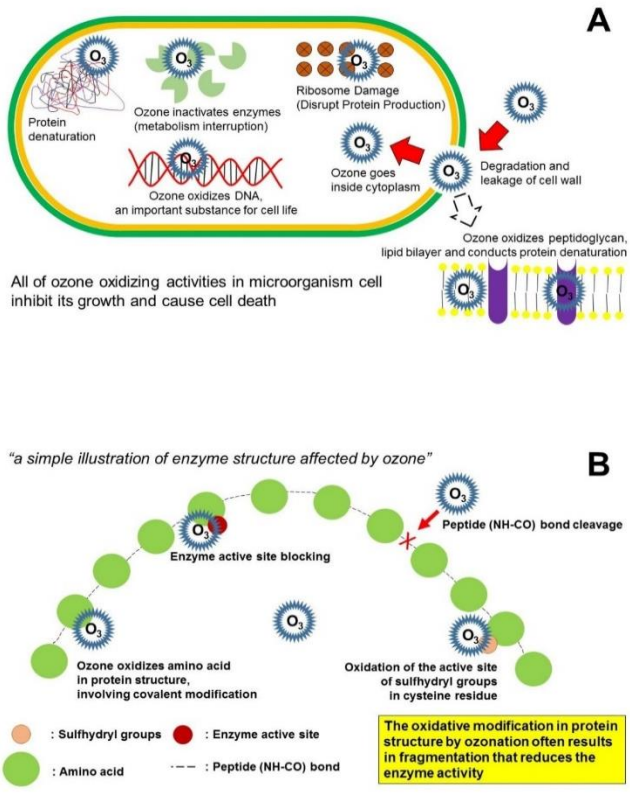

Figure 1. Mechanisms of Microbial (A) and Enzyme (B) Inactivations by Ozone

Figure 2: Coconut (*Cocos nucifera* Linnaeus) Fruits

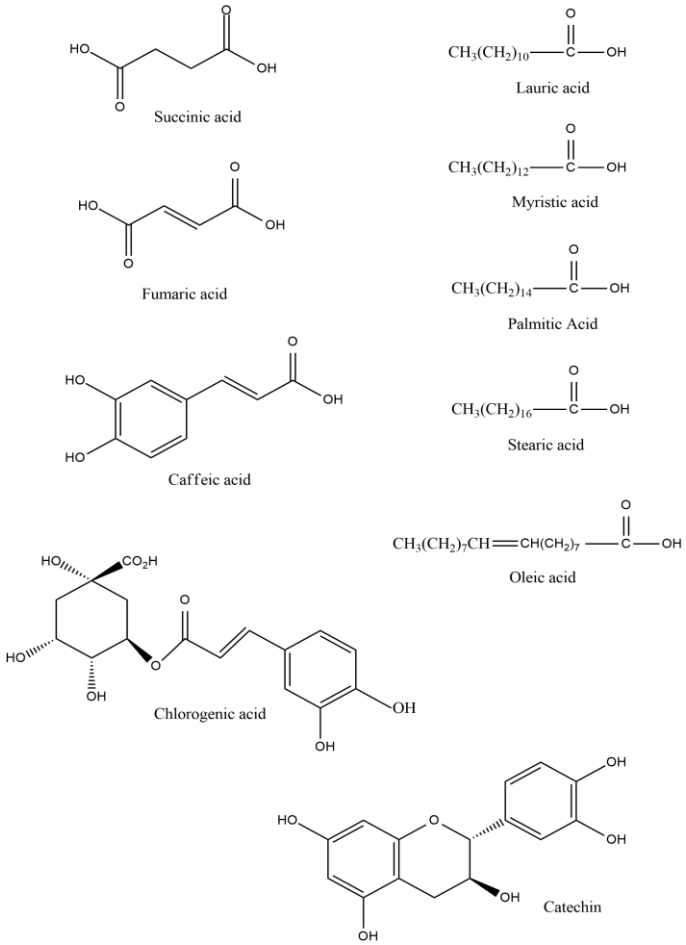

Figure 3: Chemical Structures of Dominant Organic Acids and Secondary Metabolites in Coconut Water

In recent years there has been an increase in interest in using coconut products commercially.[21] This is based on the taste, nutritional value, and glycemic index, which is quite low. Coconut (*Cocos nucifera* Linnaeus) is one of the important plant groups because it has provided various forms of food for both raw materials and food additives. Coconut is often found in tropical and subtropical regions. Mandal and Mandal stated that coconut trees have 12 different cultivation methods for coconuts, from flowering until the coconuts have matured.[3] According to the Indonesian Plantation Statistics Book from the Directorate General of Plantations, Ministry of Agriculture that in 2018 in Indonesia, there were four groups of coconut production areas, namely provinces with low, medium, high, and very high total coconut

production.[22] Based on the average coconut production per province in Indonesia in 2015-2020, ten provinces are the largest coconut-producing regions, with a total contribution of 66.18% to total coconut production in Indonesia. Indonesia's ten largest coconut-producing provinces are Riau, North Sulawesi, East Java, North Maluku, Central Sulawesi, Central Java, Jambi, Maluku, North Sumatera, and West Java.

In Indonesia, coconut tree species has 3 main varieties, namely dwarf coconut, tall coconut, and hybrid coconut.[23] Mardiatmoko and Ariyanti informed the well-known dwarf coconut in Indonesia i.e. "Kelapa Genjah" (*Cocos nucifera* L. var. *eburnea*), "Kelapa Raja" (*Cocos nucifera* L. var. *regia*), "Kelapa Puyuh" (*Cocos nucifera* L. var. *pumila*), "Kelapa Raja Malabar" (*Cocos nucifera* L. var. *pretiosa*).[24] Whereas, the tall coconut tree involves "Kelapa Hijau" (*Cocos nucifera* L. var. *viridis*), "Kelapa Merah" (*Cocos nucifera* L. var. *rubescens*), "Kelapa Kelabu Cokelat" (*Cocos nucifera* L. var. *macrocarpa*).[24] Indonesia also has an abnormal coconut fruits, but it is a favourite in term of coconut consumption. It is called "Kelapa Kopyor".[24]

In 2020, Indonesia produced coconut products with an estimated figure of 2,798,980 tons or 2.79 million tons in a total area of 3.37 million hectares of coconut plantations.[22] From these data, the estimated productivity of the coconut commodity is 828.74 kg/ha. From 2014 to 2020, coconut productivity in Indonesia fluctuated with an average growth rate of 0.37% per year. The highest coconut productivity was achieved in 2014, which was 1136 kg/ha. Based on data from the Food and Agriculture Organization (FAO), the total world production for the coconut commodity is approximately 60.7 million tons. Indonesia is the largest contributor to this commodity, followed by the Philippines and India.[2] Part of the coconut fruit includes the outer coconut husk or mesocarp; the middle is like a very hard brown shell or endocarp; and the inner part is the edible white soft endosperm.[2] The inner part of the coconut, usually known as the endosperm, consists of two parts: the solid white kernel and the clear liquid containing nutrients commonly known as coconut water.[25] Coconut has been a valuable product for the medical community because it is believed to be antiblenoragic, anti-bronchitis, antigingivitis, and has febrifugal activity.[3] Coconut downstream products such as coconut oil, coconut milk, coconut cream, and coconut water are also used to treat hair loss, burns, and heart disease.[3] Of the various downstream products, coconut water appears to be a very popular refreshing drink due to its distinctive coconut aroma, sweet taste, high functional value, and proven health protection properties.[26]

Chemical Composition of Coconut Water

The high demand of fresh coconut water product caused industries to develop several coconut water processing technologies. Because of that, canned coconut water was available in retail market.[8] Mahayothee *et al*. reported that coconut water contains 5-9% total dissolved solids, in which more than 80% of the total dissolved solids are dominated by the simple sugars' glucose, sucrose, and fructose.[1] Fructose and glucose are the highest sugar components in young coconuts, with 1.4% and 1.36%, respectively, followed by sucrose at 0.06%.[2] Other important constituents are minerals, amino acids, enzymes, organic acids, fatty acids, vitamins, and several phenolic components. The amount of minerals from these constituents is the most dominant with a proportion of 0.4-1% of the total coconut water.[2] The predominant mineral of coconut water is potassium, while other components, including sodium, calcium, chlorine, magnesium, and phosphorus, have also been reported to be present in coconut water. Coconut water also contains free amino acids such as alanine, cysteine, and serine and enzymes such as polyphenol oxidase, peroxidase, and catalase.[2] Coconut water contains a small number of organic acids such as malic acid, citric acid, succinic acid, and tartaric acid; small amounts of fatty acids such as palmitic acid and oleic acid; and vitamins such as thiamin (B1), riboflavin (B2), pantothenic acid (B5), and ascorbic acid.[2] The phenolic components in coconut water include catechins, salicylic acid, 4-hydroxybenzoic acid, syringic acid, coumaric acid, gallic acid, and caffeic acid.[2] Biotin, folic acid, phytohormones such as auxin, 1,3-diphenylurea, and cytokinins; Enzymes such as acid phosphatase, dehydrogenase, diastase, and RNA polymerase have been reported in coconut water.[3]

Even though young and mature coconut water has nutritional value, most consumers prefer to consume fresh coconut water that has just been split from the young coconut shell (kernel).[1] Water from ripe coconut has a taste that consumers do not like. Most consumers also believe that young coconut water has better nutrients and more functional properties when compared to water from mature coconuts.[27] According to Mandal and Mandal, coconut water has a calorific value of 17.4/100 g of coconut water.[3] Chemical constituents in coconut water can be seen in Table 3.

Table 3: Chemical Constituents in Coconut Water

No	Constituents	Values
1.	pH*	5.59
2.	Titratable Acidity (TA) (%)*	0.14
3.	Total Soluble Solids (°Brix)*	5.06
4.	Dry matter (%)*	5.04
5.	Moisture (%)*	94.96
6.	Protein (%)*	0.269
7.	Fat (%)*	0.172
8.	Carbohydrate (%)*	4.12
9.	Fiber (%)*	0.0210
10.	Ash (%)*	0.47
11.	Energy value (Kcal/100 mL)*	19.10
12.	Phosphorus (P) (mg/L)*	0.02
13.	Total phenolic content (µg/mL)**	83.5
14.	Vitamin C (mg/L)**	0.288
15.	Calcium (µg/mL)**	117
16.	Magnesium (µg/mL)**	60.4
17.	Iron (µg/mL)**	0.2905
18.	Zinc (µg/mL)**	0.4004
19.	Sodium (µg/mL)**	302.3
20.	Potassium (µg/mL)**	2163
21.	Copper (µg/mL)**	0.0013

Source: * Coulibaly *et al*.[25] ; ** Raj *et al*.[26]

The composition of the minerals in coconut water can create the same osmotic pressure as in blood, so coconut water can be called an electrolyte liquid.[3] Coconut water does not cause coagulation of plasma in the blood. The mineral potassium in coconut water has been evaluated for lowering blood pressure. This ion can also provide a cardioprotective effect for heart disease.[3] Coconut water can reduce toxins and improve drug-induced toxicity due to overdosage. The nature of coconut water, which contains electrolytes, also helps absorb drugs in the blood.[3] Free amino acids, L-arginine, and ascorbic acid can function as antioxidants. Trace elements are necessary for normal growth, development, and human physiology. Several metals, such as iron, copper, manganese, and zinc, are essential for cellular function, enzymatic activation, gene expression, and metabolism of amino acids, lipids, and carbohydrates.[7] The chemical structure of dominant organic acids and secondary metabolites in coconut water can be seen in Figure 3.

Alchoubassi *et al*. stated that coconut water accounts for approximately 25% of the fruit's weight and contains several essential minerals, especially potassium, and manganese.[7] Data on trace element concentrations in coconut water are scarce, but the concentrations obtained in a recent study on coconut water from Bangladesh have reported metal concentrations in the range of 0.3–1.5; 7.77-21.2; 0-0.71; 0-0.9; 0-0.2; 0.9-17.3; 0.1-0.9; 0-0.9; and 0-0.7 mg/L coconut water for Fe, Ni, Cu, Cd, Cr, Zn, Pb and Se, respectively.[28] Seeing the results of this evaluation, coconut water deserves to be truly valued as an

electrolyte drink with a wide range of functional properties that are, of course, very good for the body's daily metabolism.

Coconut Water Deterioration

As is well known, inside the endocarp of coconut fruit is a solid endosperm, commonly known as coconut meat, and liquid endosperm or coconut water. Young coconuts are 6-9 months old and are mostly in demand by consumers to consume the fruit flesh and water directly or can also be further processed into various coconut water-based drinks.[27] As a natural drink with high nutritional value, coconut water can replenish lost body fluids and reduce electrolyte imbalances. In an emergency, coconut water can be injected intravenously into patients for hydration.[27] For most coconut farmers, the popularity of coconut water as a health drink may be very supportive. However, observations have shown that young coconuts can only be stored for less than a month under cold storage conditions.

Fresh coconut water is still sterile, so there is another reason for the spoilage problem. Flavor compounds in young coconut water can be formed from the degradation of fatty acids, which may be caused by oxidative metabolism involving mechanisms such as p-oxidation and lipoxygenase pathways.[27] During storage, nonanal and octanal levels, which may indicate an off flavor, increased significantly in the coconut water from extracted commercial young coconuts. Furthermore, young coconuts stored at normal temperatures showed a wrinkled exocarp shape, then weight loss increased and started browning and loss of original colour in the exocarp.[27] Relatively high transportation costs and a very short storage period have limited the development of young coconut products as commercial products. Several Asian countries and Brazil produce coconut water in cans, bottles, or tetra packs, but the taste differs from fresh coconut water. Only now, extending the shelf life of coconut water without changing its taste and nutritional properties remains a challenge.

Unripe young coconuts are the best and ideal way to preserve coconut water, but they cannot be stored at room temperature for more than six days. Young coconuts can be kept fresh and safe for three weeks at 13-15°C and 70% RH.[8] As with many other tropical fruits, storage below 12°C causes chilling injury, so the coconut skin quickly turns brown. In intact coconuts without any treatment, curing for 28 days at 12°C or 17°C gave the best results.[8] The results were equivalent to or even better for whole coconuts wrapped in film, namely 28 days at 12°C with PE film and 30 days at 12°C with PVC film.[8] So far, it has been done that the longest shelf life for whole young coconuts is with paraffin wrapping, which can be stored for 49 days at 12°C. However, the fibers that have been trimmed will quickly turn brown and reduce the commercial value of the fruit even though the coconut water in it is still good.

Without packaging or treatment, partially peeled coconuts can only be stored for seven days at 17°C.[8] Indeed, to overcome the browning problem, it can be soaked in a solution of an anti-browning agent such as sodium metabisulfite with a concentration of around two g/L for 5-10 minutes.[8] With anti-browning treatment and film wrapping, the shelf life of coconut is estimated to last up to 24 days at 5°C.[8] On the other hand, soaking coconut in carnauba wax emulsion can maintain the freshness of partially peeled young coconuts for 30 days at 12°C.[8] According to Prades *et al*., the main problem with the stabilization of coconut water does not appear to be from the aspect of microbiological or chemical damage but from the aspect of endogenous enzyme activity, which needs to be deactivated to stabilize the colour and taste of the coconut water.[8] Like many fruit extracts or juices, polyphenol oxidase and peroxidase enzymes are also present in young coconut water. The consequence of the activity of the both enzymes in coconut water is a colour change.[8] Discoloration of yellow, brown, or pink in coconut water can occur a few minutes or several hours after being taken from the coconut shell. Discoloration can also occur after several weeks of storage of treated coconut water.

Coconut Water Processing

It has been explained that the colour change and spontaneous fermentation process will occur after the coconut water is extracted from the shell. This colour change is mainly influenced by the activity of polyphenol oxidase and peroxidase enzymes, which are naturally present in coconut water. In order to inactivate the polyphenol oxidase and peroxidase enzymes in coconut water, thermal treatment can be an alternative to deactivating these enzymes. The process can be done using pasteurization, sterilization, or microwave heating. Prades *et al*. stated that research conducted by Campos *et al*. with heat treatment at 90°C, total inactivation was obtained after 550 seconds for the polyphenol oxidase enzyme and 310 seconds for the peroxidase enzyme.[8,29] Heating at 139°C for 10 seconds and combined with the addition of 200 mg/L ascorbic acids, the polyphenol oxidase enzyme will be completely inactivated, while the peroxidase enzyme still has an activity level of 40% of its initial activity.[30] Cao *et al*. also explained from other paper studies that polyphenol oxidase enzymes are more resistant than peroxidase enzymes during pasteurization.[31] In addition, Abreu and Faria informed that the peroxidase enzyme is more thermostable during the sterilization process.[30] At temperatures below 90°C, the peroxidase enzymes in coconut water are less thermostable when compared to the polyphenol oxidase enzymes.[32] Aliberti *et al*. evaluated the thermal behavior of the polyphenol oxidase and peroxidase enzymes analyzed and calculated during the pasteurization process.[33]

Mathematical equations was developed to predict the thermal resistance of the two enzymes using the same first-order model by two-component[33]. The thermal resistance of peroxidase enzymes can be estimated by the D-value, which is the time required to reduce the enzyme's activity to 10% (1 log) of its initial value. The temperature required for the D-value is 5 minutes, namely 81.2°C for the peroxidase enzyme from coconut water.[34] In addition to pasteurization and sterilization, microwave techniques have been used to deactivate polyphenol oxidase and peroxidase enzymes.[35] With the combination of sugar and salt, fructose damages the peroxidase enzyme more than the inactivation of the polyphenol oxidase enzyme. Salt significantly affects the stability of polyphenol oxidase enzymes and peroxidase enzymes. At temperatures above 77°C, polyphenol oxidase enzymes were found to be more heat resistant from microwave heating when compared to peroxidase enzymes.

The presence of salt in the simulated solution and microwave heating reduced the enzymatic activity to undetectable levels. In addition, the thermal behavior of natural coconut water, when compared to the simulated solution, shows that the natural enzymes in coconut water are more resistant to heating temperatures than commercial enzymes in the simulated solution.[34] Matsui *et al*. used a first-order kinetic model to describe the experimental results and determine the parameter D-value for the polyphenol oxidase enzyme.[34] The result was that the D-value at 92.2°C was 52 seconds, while the D-value for the peroxidase enzyme at 92.2°C was 16 seconds. The investigation results stated that the thermal inactivation of the two enzymes was faster by heating using microwaves when compared to pasteurization or sterilization. In Taiwan, sterilization is usually used as a thermal treatment to stabilize coconut water. Indeed, it usually causes the liquid to brown due to a non-enzymatic browning reaction.

Karmakar and De also stated that fresh coconut water has a shelf life of around 24 hours.[36] Due to the presence of sugars and several endogenous enzymes, polyphenol oxidase and peroxidase enzymes, young coconut water strongly tends to undergo biochemical changes and taste deterioration after the fruit is harvested from the tree.[36] Adding food additives can worsen the parameters of taste and quality of coconut water. Heating, usually carried out between 60°C and 100°C, removes bacteria and degrades the entire flavor quality profile.[36] Chutia *et al*. also stated that among the various endogenous enzymes, polyphenol oxidase and peroxidase enzymes, which have more stable properties, are responsible for damage to coconut water through biochemical reactions and are used as indicators of the effectiveness of thermal processes in coconut water.[37] This enzyme is known to change sensory properties, nutrients, and texture which can reduce product quality. Polyphenol oxidase enzymes are responsible for browning and discoloration, while peroxidase enzymes catalyze peroxidation reactions in which the final product has an unpleasant taste and is not liked by consumers.[37]

Kanjanapongkul and Baibua also stated that one of the most important problems for coconut water producers is colour damage due to enzymatic browning, polyphenol oxidase enzymes, and peroxidase

enzymes that occur after coconut water is extracted and exposed to air.[16] Heat treatment is widely used to pasteurize fruit juices, including coconut water.[16] Tan *et al*. found that the D-values of the polyphenol oxidase and peroxidase enzymes in coconut water processed at a heating temperature of 83.8°C were 500 and 140 seconds, respectively, indicating that the heat stability of the polyphenol oxidase enzymes was much higher than that of the peroxidase enzymes.[38] Similar results were also reported by Thaisakornphun and Tongchitpakdee who found that the peroxidase enzyme was completely inhibited after heating at 85°C for 3 minutes.[39] In contrast, the activity of the polyphenol oxidase enzyme was still detectable even after heating at 90°C for 14 minutes. Aliberti *et al*. informed that the polyphenol oxidase enzyme in coconut water consists of two isoforms: heat labile and heat stable.[33] Thus, the heat-stable polyphenol oxidase enzymes may respond to the physicochemical changes in pasteurized coconut water during cold storage and limit the shelf life of the coconut water product.

Ozonation

Maintaining young coconut water with its original characteristics remains a challenge, so non-thermal processing techniques can be an alternative process to maintain the quality and stability of young coconut water. The use of ozone gas to reduce microbial contamination and maintain fruit juice quality has been widely evaluated. Ozone is a triatomic allotrope of oxygen that decomposes quickly and leaves no residue in food.[40] The Food and Drug Administration (FDA) has recognized ozone as GRAS, and the US FDA has approved it as an antimicrobial agent.[40] Ozone is a strong oxidant with oxidizing potential in alkaline solutions, so that it can be used as an effective antimicrobial agent.[40] Ozone is known to affect proteins, enzymes, and nucleic acids resulting in the inactivation of microorganisms. Then it can also oxidize the lipid components of the cell wall, which causes cell lysis.[40] Rajashri *et al*. also evaluated the character of coconut water with ozone and sonication treatment compared to the heating treatment during the storage process in the refrigerator.[40] Young coconut water is taken from the shell and immediately filtered using a double-layer cheese filter. Then for treatment with ozonation, Rajashri *et al*. used ozone gas with a concentration of 20 mg/L with a gas flow rate of 1 L/minute.[40] The gas is passed through a bubble column with a length: diameter ratio of 10:1 with an ambient temperature of 25°C at various ozonation treatment times of up to 10 minutes. Ozone generator system including its dielectric barrier discharge reactor chamber and corona discharge reactor chamber can be seen in Figure 4.

From the results of Rajashri *et al*. after ozone treatment with the addition of nisin, it was found to reduce the ascorbic acid content of young coconut water by 85%, while the reduction in total phenols and flavonoids was 36% and 30%, respectively.[40] At the end of storage, almost 94% of ascorbic acid, 53% of total phenols, and 20% of total flavonoids decreased in ozone treatment with nisin in young coconut water. Ozone treatment can cause the oxidation of ascorbic acid, flavonoids, and phenolics.[41] Alothman *et al*. also evaluated the effect of ozone treatment for 10 minutes, which reduced the content of polyphenols, flavonoids, and antioxidant activity in guava fruit.[42] Although there was a significant decrease in total phenol in treated young coconut water compared to fresh coconut water without treatment, no significant difference in the phenolic content of non-thermal treated coconut water and heat treatment was observed during storage in the refrigerator.[40] There was a significant decrease in flavonoids in the heating and sonication treatments with the addition of nisin. However, the flavonoid content in young coconut water treated with ozone and added nisin was statistically similar to fresh young coconut water without treatment.[40] The decrease in the antioxidant capacity of the samples in the experiments of Rajashri *et al*. may be associated with a decrease in the phenol and flavonoid levels of young coconut water given to various treatments.[40] The experimental results of Rajashri *et al*. showed that no polyphenol oxidase and peroxidase enzyme activity was observed in young coconut water during storage, both storage day 0 in the ozonation treatment and the addition of nisin.[40] This has confirmed the inactivation of both enzymes in the presence of ozone and nisin treatment.

Rico *et al*. also reported that ozone treatment could reduce the activity of polyphenol oxidase enzymes and peroxidase enzymes in fresh-cut lettuce immersed in water containing one ppm ozone for 5 minutes.[43] The results of experiments conducted by Zhang *et al*. on celery immersed in water containing ozone at a concentration of 0.03, 0.008, and 0.018 ppm for 5 minutes also showed a decrease in polyphenol oxidase enzyme activity.[44] The activity of the enzyme phenylalanine ammonia-lyase was also significantly reduced by the nisin-added ozone treatment in Rajashri *et al*.'s experiments.[40] The investigation results of Baur *et al*. also showed that celery washed with water containing one ppm ozone for 2 minutes could significantly reduce phenylalanine ammonia-lyase activity.[45] Rajashri *et al*. made observations on young coconut water with the ozonation treatment and the addition of nisin, which turned out to be able to reduce bacteria and destroy *E. coli*.[40] However, after three weeks of storage, fungal growth was seen in the stored samples. Patil *et al*. reported that the effectiveness of ozone for the inactivation of *Listeria* for a reduction of 5 log cycles was carried out at concentrations of 75–78 µg/mL ozone between 5.05 and 8.44 in orange juice.[46] Regarding sensory test quality, young coconut water treated with ozonation and the addition of nisin had a sensory score acceptable to the panelists and not much different from the sensory quality of fresh young coconut water.[40] Likewise, after being stored for the first three weeks, it did not show a significant change in taste quality and was able to maintain panelist acceptance as on day 0 of storage.[40] The sensitivity of *Bacillus* and *Clostridium* spores to ozone at medium pH 3, 4, and 5 were evaluated by Foegeding.[47]

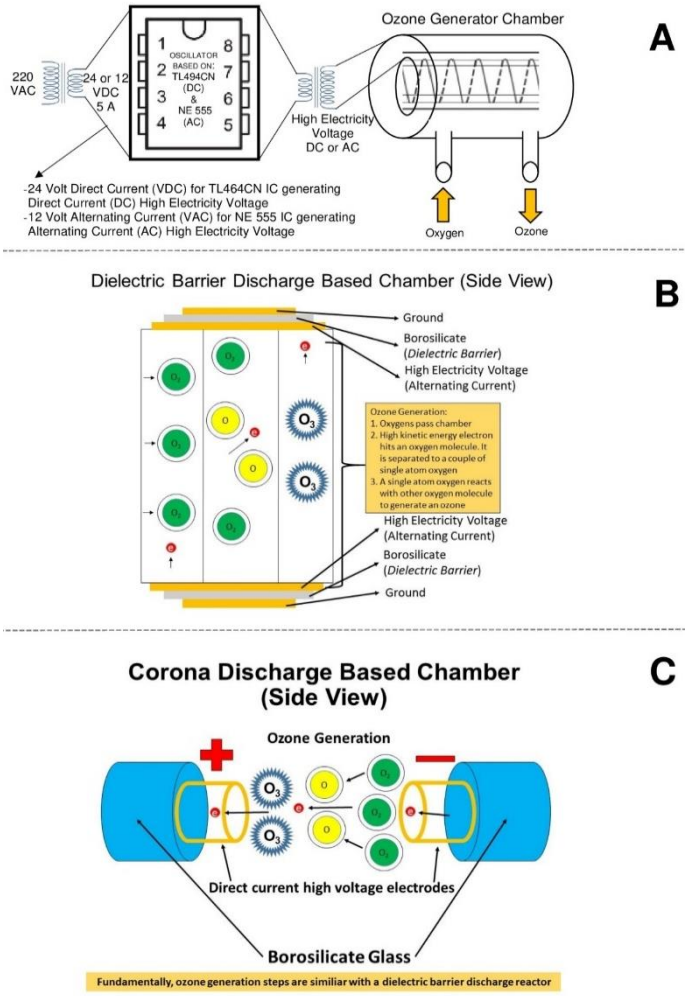

Figure 4: Ozone Generator System (A) including its Dielectric Barrier Discharge Reactor Chamber (B) and Corona Discharge Reactor Chamber (C)

From the results of his research, the spores of *Bacillus stearothermophilus* ATCC 1518 had a higher resistance to ozone exposure compared to the spores of several subspecies of *Bacillus cereus* at an initial spore count of 1×10^7.

Foegeding reported that 1.8 mg/L ozone at pH three would inactivate approximately ≥95% of *Bacillus cereus* T and *Bacillus cereus* F4810/72 spores of the initial spore count.[47] Meanwhile, with the same level of ozone exposure, *Bacillus stearothermophilus* ATCC 1518 was only inactivated by approximately 37% of the initial number of spores. Foegeding also added that comparing the resistance of *Clostridium perfringens* NCTC 8798 and *Clostridium botulinum* ATCC 12885A spores to *Bacillus cereus* T spores had a significant level of resistance.[47] *Clostridium botulinum* ATCC 12885A has the highest level of resistance, followed by *Clostridium perfringens* NCTC 8798 and *Bacillus cereus* T.

To determine the role of the bacterial spore layer in protecting *Bacillus cereus* T spores from exposure to ozone, the spore population was treated to remove the protein coating on the spores and exposed to 0.2 or 0.6 mg/L ozone at pH 3.[47] Exposure to 5×10^6 CFU/mL at 0.2 and 0.6 mg ozone/L at pH 3 resulted in spore inactivation of 99.99% and 99.999% within 5 minutes. Spore inactivation did not appear during the first 30 minutes when exposed to a pH three environment.[47] The number of *Bacillus cereus* T spores without intact spore coating removal treatment will be more resistant, with an inactivation percentage of only around 0-90% within 15 minutes of exposure to ozone.[47] Spores lacking an intact mantle exhibit ozone resistance comparable to that of vegetative cells of the same species. These cells were grown for 24 hours in TSB at 30°C, washed, and suspended in saline for exposure to ozone.[47]

Research on the effectiveness of ozone tested on *Bacillus subtilis* spores was also carried out by Aydogan and Gurol.[48] This study used spores that were dried on the surface of different carrier materials and exposed to ozone in the range of 500–5000 ppm at a relative humidity (RH) of 70–95%. Ozone was highly effective against *Bacillus subtilis* spores at a concentration of 3 mg/L (1500 ppm) with an inactivation rate of 3 logs after 4 hours of exposure.[48] The inactivation curve consists of a short lag phase followed by an exponential decrease in the number of surviving spores. Prehydration of bacterial spores has been confirmed to eliminate the initial lag phase.[48] The inactivation rate increases with increasing ozone concentration but not more than 3 mg/L. The rate of inactivation also increases with increasing RH.[48] Inactivation of *Geobacillus stearothermophilus*, formerly classified as *Bacillus stearothermophilus* with strain ATCC 12980 or strain ATCC 7953 on a commercial Bls (biological indicators) kit from Steris Co., Ltd was evaluated by Sakurai *et al*.[15] In this study, an investigation was carried out on the D-value of the *Geobacillus stearothermophilus* culture (commercial kit) with results ranging from 5.9 to 6.2 minutes at 15°C; 4.6-5.3 minutes at 25°C; and 2.3-3.0 minutes at 35°C.[15]

One of the bacterial spores from *Paenibacillus larvae* can cause American Foulbrood disease, a highly contagious disease of honey bee bacteria.[14] In this study, Torlak and Isik evaluated the effectiveness of ozone gas in inactivating *Paenibacillus larvae* spores on wood and artificial nests.[14] Pinewood and polyvinyl chloride (PVC) plastic stems inoculated with a mixture of spores from three strains of *Paenibacillus larvae* were treated by exposure to ozone gas at two different concentrations, 9.8 and 17.1 mg/L, at room temperature for 120 minutes. Ozonation at 17.1 mg/L for 120 minutes reduced 4 logs of spores on PVC stems, while 2.3 logs occurred on pine logs.[14] In the study of Khadre and Yousef looking at the ability of ozone sporicide against *Bacillus* spp. spores.[49] Ozone in water with a concentration of 11 µg/mL at 22°C for 1 minute can reduce spores by 1.3-6.1 logs CFU/mL, depending on the species of bacteria tested.[49] The highest resistance of spores to ozone was in spores of *Bacillus stearothermophilus* OSU24 (1.3 logs CFU/mL), and the lowest was in *Bacillus cereus* OSU11 (6.1 log CFU/mL). Khadre and Yousef reported that *Bacillus stearothermophilus* spores were suitable for testing the ability of ozone to kill bacterial spores.[49] Electron microscopic studies of *Bacillus subtilis* spores exposed to ozone showed that the outer layer of the spore, which is the mantle, is the point of action of ozone for sporicidal.[49]

Kinetic of Quality Parameter Reduction of Coconut Water during Ozonation

Enzymes are substances that are sensitive to the presence of oxidizing agents. Ozone is one of the cold plasma produced in atmospheric environment. Polyphenol oxidase, one of enzyme presented in coconut water, was reported to have a reduction on its activity during ozonation treatment by atmospheric cold plasma method.[50] Still in atmospheric condition, peroxidase enzyime activity was also decreased during non-thermal plasma treatment.[51] That non-thermal plasma treatment was conducted by dielectric barrier discharge method in atmospheric environment which produced ozone during treatment. Not only the polyphenol oxidase and peroxidase affected by ozone treatment, but phenolic and flavonoid components are also degraded by the presence of ozone molecule. Phenolic component was informed to have a reduction during ozonation treatment.[52] Flavonoid was also degraded by the ozone treatment.[53]

Since coconut water deteriorates after extraction due to the activity of polyphenol oxidase enzymes and peroxidase enzymes, these two enzymes are one of the parameters that need to be considered in maintaining the quality of coconut water. Polyphenol oxidase enzymes and peroxidase enzymes will increase their activity and cause a decrease in the quality of coconut water, such as a change in colour to brown and the appearance of a rancid aroma in coconut water.[16] Putnik *et al*. also stated that the activity of endogenous enzymes, especially polyphenol oxidases and peroxidases present in vegetable and fruit products, would decrease the quality of these foodstuffs.[54] Various thermal and non-thermal processing methods have carried out attention to the inactivation of these two enzymes.

Particularly in non-thermal processing using the ozonation process, Panigrahi *et al*. inactivated these two enzymes in sugarcane juice as raw material.[55] Panigrahi *et al*. stated that ozone is a gas that can oxidize various compounds, including enzymes.[55] Its hyperactivity causes protein oxidation in the form of ozone or its derivatives, such as superoxide anion radicals and hydroxyl radicals. The mechanism of enzyme inhibition by ozone can be explained by the presence of an ozone oxidation process on the active site of the sulfhydryl group on the cysteine residue.[56] The process of protein oxidation by ozone is also capable of fragmenting protein polymers, which causes a decrease in enzyme activity.[57] The need for a measurable decrease in the activity of the polyphenol oxidase and peroxidase enzymes has been evaluated through experiments on the inactivation kinetics of the two enzymes.

The inactivation of polyphenol oxidase enzymes and peroxidase enzymes using the ozonation process has been evaluated for kinetics using first order. The results showed that the polyphenol oxidase enzyme exposed to ozone at a dose of 0.45 mg/minute mL had a coefficient of decrease in enzyme activity (k) of 0.043 units/minute with an R-square of 0.963.[55] Peroxidase enzymes exposed to ozone of 0.45 mg/minute mL had a coefficient of decrease in enzyme activity (k) of 0.055 units/minute with an R-square of 0.964.[55] This data can be seen in Table 4. As bioactive components in coconut water, it is hoped that the phenol and flavonoid groups will not experience a significant decrease during processing. The resulting coconut water tends to be browner in colour in the thermal process. The degradation of phenol with polyphenol oxidase enzymes causes this. Ozone, as a non-thermal alternative technology, is expected to maintain the unique properties of coconut water after the ozonation process. It is undeniable that ozone is a strong oxidizing agent, so it is necessary to evaluate how much the phenol and flavonoid components are degraded by ozone during the ozonation process.

Fonseca *et al*. evaluated the degradation kinetics of phenol and flavonoid components in distilled water added with butanol.[58] Butanol is used to inhibit the activity of ozone derivatives so that only ozone will be evaluated in degrading phenols and flavonoids. The degradation rate of coumaric acid as a component of phenol and quercetin as a component of flavonoids can be plotted properly using a first-order reaction. The coefficient for the degradation of coumaric acid using the first order reaction was 0.3393 ppm/minute, while the quercetin degradation coefficient using the first order reaction was 0.0682 ppm/minute.[58] Both components were exposed to ozone at 300 mg/minute concentrations. The coefficient values of changes in the activity of polyphenol oxidase enzymes, peroxidase enzymes, and degradation of coumaric acid and quercetin during the ozonation process can be seen in Table 4.

Effect of Ozonation on Other Food Chemical Compounds

Ozone was declared GRAS in 1997, making it safe for food processing.[13] Even so, this safety level of ozone is stated to be completely decomposed into oxygen gas again and leaves no residue if ozone is used to treat mineral water. The pure water component in mineral water is the best medium for the ozonation process, which will not produce residues left over from the ozone oxidation process. It should be noted that most foodstuffs contain macro components, namely carbohydrates, proteins, and lipids. It is also necessary to know the effect of ozonation on these macro components during the processing of foodstuffs using the ozonation process.

Research conducted by Lima *et al*. used an ozonation process exposed for 30 minutes in a starch solution medium from the root vegetable *Arracacia xanthorrhiza*.[59] In Table 5, the results are obtained to state that there is a change in the carbohydrate component. The initial amylose content was 34.5%, reduced to 27.5% during ozonation. The initial reducing sugar content, which was originally 2.5 mg/g starch, was reduced to 9.8 mg/g starch. While the carbonyl group with an initial number of 0.08 increased to 0.13 groups/100 glucose units. The increase in carbonyl groups indicates an oxidation reaction on the hydroxyl groups of glucose monomers, especially carbon atoms 2, 3, and 6. Seeing this, ozone can affect the presence of starch carbohydrates in foodstuffs. Ozone is also reactive to protein. In Table 5, research by Fang *et al*. reported that the amino acid cysteine was reduced by 53.67% in the ozonation process for 10 minutes in silver carp myosin media.[60] The amino acid tyrosine decreased by 93.17%.[60] Nickhil *et al*. informed that the ozonation process at 20 minutes/day for five days on pea media caused a decrease in total essential amino acids from initial levels of 349.83 to 258.89 mg/g protein.[61] Total non-essential amino acids also decreased from initial levels of 519.42 to 341.88 mg/g protein.[61]

Oil and fat become reactive components in structures prone to oxidation processes, such as fatty acid molecules. For oxidation to occur due to the presence of ozone. In Table 5, research conducted by Alameda *et al*. reported that the ozonation process for 60 minutes in lard media caused a decrease in oleic acid levels from 45.1% to 34.8%.[62] The same thing also happened to linoleic acid. The initial level of linoleic acid was originally 7.8%, reduced to 4.3%.

Table 4: Coefficient Value of The Change of Quality Parameter during Ozonation

Quality Parameter	Ozonation	k (min^{-1})	Order	Source
Polyphenol oxidase Activity Reduction	0.45 mg/mL (dissolved ozone)	0.043	1	Panigrahi *et al*.[55]
Peroxidase Activity Reduction		0.055		
Komaric Acid Degradation	300 mg/min (ozone gas)	0.3393		Fonseca *et al*.[58]
Quercetin Degradation		0.0682		

Table 5: Change of Food Chemical Compounds during Ozonation

Chemical Compound	Change	Note	Source
Antioxidant	Reduce 62%	60 minutes ozonation in cantaloupe juice	Fundo *et al*.[64]
Ascorbic Acid	Reduce 14%		
Dehydroascorbic Acid	Reduce 75%		
Carotene	Reduce 83%		
Amylose	Decrease from 34.5% to 27.5%	30 minutes ozonation in starch solution from the root vegetable *Arracacia xanthorrhiza*	Lima *et al*.[59]
Reduction Sugar	Decrease from 2.5 to 9.8 mg/g starch		
Carbonyl Groups	Decrease from 0.08 to 0.13 group/100 unit glucose.		
Cysteine	Reduce 53.67%	10 minutes ozonation in silver carp myosin	Fang *et al*.[60]
Tyrosine	Reduce 93.17%		
Essential amino acids	Decrease from 349.83 to 258.89 mg/g protein	20 minutes/day for five days at peas	Nickhil *et al*.[61]
Non-essential amino acids	Decrease from 519.42 to 341.88 mg/g protein		
Saponification number	Decrease from 162.7 to 172.2	3 hours ozonation in mustard oil	Tripathi dan Agrawal[63]
Degree of unsaturation	Decrease from 0.68 to 0.48	60 minutes ozonation in lard	Alameda *et al*.[62]
Oleic Acid	Decrease from 45.1% to 34.8%		
Linoleic Acid	Decrease from 7.8% to 4.3%		

Meanwhile, the degree of unsaturation also decreased from 0.68 to 0.48.[62] The saponification number parameter also decreased from the initial value of 162.7 to 172.2 during the 3 hours of the ozonation process in mustard oil media.[63] Apart from the macronutrient components, several other chemical components were also identified as experiencing a decrease. In Table 5, it is known that for 60 minutes, the ozonation process carried out on cantaloupe juice media will cause a decrease in antioxidant activity, total ascorbic acid, dehydroascorbic acid, and total carotenoids amounting to 86%, 21%, 47%, 53% respectively.[64] This is because ozone compounds are very reactive and can oxidize organic compounds contained in foodstuffs. Therefore, paying special attention to selecting food ingredients suitable for processing with ozonation is necessary.

Oxidation Product Potentially Harmful to Health

All foods containing lipids have the potential to undergo lipid oxidation even though their composition of unsaturated fatty acids is low.[65] Consumption of lipid oxidation products can potentially harm health. Since there is a processing process with ozonation techniques, the oxidation reaction of oxygen compounds to nutrients needs to be a concern. Coconut water, which is prone to rancidity after extraction, is thought to be caused by the oxidation of the lipid components in the material. The ozonation process in coconut water needs to review the effect of ozone oxidation on the lipid components in coconut water. Lipid oxidation produces potentially toxic products that have shown a correlation with inflammatory diseases, cancer, atherosclerosis, and aging.[65] These potentially toxic products can enter the body through food. Oxidation products can be absorbed into the blood and, in some cases, distributed to tissues. Lipid oxidation products that are most often studied because they are the most reactive oxidation products are acrolein, 4-hydroxy-transnonenal, 4-hydroxy-trans-hexanal, crotonaldehyde, and malondialdehyde.[65] Without realizing it, consuming foods containing lipids can experience lipid oxidation and produce compounds such as lipid hydroperoxides, oxidized sterols, and fatty acid decomposition products such as aldehydes, ketones, and epoxides.[65] Lipid oxidation products and their derivate products can be seen in Table 6. Jiang *et al*.[66] also informed that acrolein was the degradation product of glycerol. Acrolein by 5 mg/kg dose could promote the reduction of memory and learning ability of male SD rats.[67] It was happened because acrolein disturbs the glutamate releasing as neurotransmitter excitatory.[67] Crotonaldehyde was included in harmful substance product. It could make a injury on testicular cells and reduce the enzyme working for testosterone production.[68] Li *et al*.[69] explained that crotonaldehyde caused apoptosis and lung damage. It was caused by damaging of mitochondrial. However, the lipid oxidation product could raise and accelerate some cardiovascular deseases.[70]

These compounds can cause various biological responses through food consumption and their production in the body. Compounds derived from lipid oxidation that is considered harmful to health can be seen in Table 6. However, complex antioxidant systems in the human body, such as antioxidants from food, metal-binding proteins, and antioxidant-producing enzymes, are usually sufficient to prevent damage from lipid oxidation products consumed.[65] However, in some cases, lipid oxidation products can lead to situations that lead to biological abnormalities that will lead to disease.[65] Lipid oxidation is a complex process that produces lipid hydroperoxides as the main product. This product can be decomposed into oxygenated and aliphatic fatty acid degradation products. These decomposition products are found in food. Linoleic acid hydroperoxides have been reported to break down into secondary lipid oxidation products in the gastric lumen. Oxidized lipids can enter the portal vein system or be transported to the liver.[65] Oxidized lipids can also enter the circulation via the lymph as chylomicrons. Because lipid hydroperoxides do not appear to be absorbed into the plasma, observations of the toxicity of lipid oxidation products are usually focused on secondary lipid oxidation products, particularly aldehydes.[65]

One of the most reactive groups of lipid oxidation products is the unsaturated *α-β*-aldehydes. These compounds are electrophiles susceptible to nucleophilic attack on *β*-carbons.[65] Nucleophiles that unsaturated *α-β*-aldehydes can modify include nucleic acids, proteins, membranes, and sulfhydryl-containing compounds such as glutathione. Modified aldehyde molecules are advanced lipoperoxidation end products that have been postulated to be involved in diseases such as diabetes, kidney disease, cardiovascular disease, and cancer. Examples of unsaturated *α-β*-aldehydes in oxidized lipids include acrolein, crotonaldehyde, and 4-hydroxyalkyl.[65] Another product of lipid oxidation that can be produced is malonaldehyde. Malonaldehyde is a dialdehyde that can act as a crosslinking agent that reacts with proteins and DNA amino groups.[65] Malonaldehyde is more mutagenic, whereas 4-hydroxy-trans-2-nonrenal tends to be more toxic. Malonaldehyde is one of the more abundant aldehydes from the decomposition of fatty acids in food. Malonaldehyde has been found bound to lysine residues in food proteins. Malonaldehyde can be released during digestion and absorbed from the intestine into the blood. Malonaldehyde levels were higher in old human red blood cells than in young red blood cells. This indicates increased oxidative damage as red blood cells age.[65]

Sensory Evaluation on Product Treated by Ozonation

The presence of compounds resulting from ozone oxidation in organic components of foodstuffs is thought to affect the sensory quality of products. Although compounds resulting from ozone oxidation in organic components are still a concern in terms of health, validation is needed that the quality of products produced by non-thermal processing such as ozonation can still maintain sensory quality. It is undeniable that to carry out this sensory test, and special considerations are needed considering that the ozone dissolved in coconut water will be able to be in the coconut water for up to 24 hours. This means that the ozonation process is still relatively vulnerable to sensory evaluation considering the high concentration of dissolved ozone, which will later be consumed in the body.

Rajashri *et al*. tried to perform a sensory evaluation of coconut water subjected to an ozonation process.[40] Methodically, this evaluation is classified as safer because sensory evaluation is carried out after a storage period of one week, except for sensory evaluation at week 0. From the results, fresh coconut water is consistent with a watery liquid with a cloudy or opaque appearance.

Table 6: Lipid Oxidation Products and Its Derivates

Lipid Oxidation	Derivatives of Lipid Oxidation Products that are Allegedly Dangerous
Aldehyde	Acrolein
	4-Hydroxy-Trans-Nonenal
	4-Hydroxy-Trans-Hexanal
	Crotonaldehyde
	Malondialdehyde
Lipid Hydroperoxide	-
Oxidized Sterols	-
Ketones	-
Epoxide	-

Source: Vieira *et al*.[65]

Coconut water has the desired sweet-sour score and relatively high enjoyment, with an optimal sweet and moderate sour taste. Fresh coconut water was very acceptable, with a good sensory score.[40] Fresh coconut water, ozone, and nisin-treated coconut water and heat-treated coconut water were subjected to sensory evaluation immediately after the ozonation treatment. All samples of coconut water processed by ozonation were acceptable with sensory scores above 9.0 out of a maximum score of 15.[40] After the first and second weeks of storage, all samples of coconut water treated with ozonation did not show a significant change in taste quality and were still as acceptable as those treated at the beginning of the 0th week of storage. Treatment of coconut water with ozone and nisin and heat treatment is sensory acceptable for up to 3 weeks of storage.[40] Usually, coconut water that is not subjected to heat treatment will turn a pink colour. This is probably caused by the oxidation of phenolic compounds into O-quinone or melanin pigments by the residual activity of browning enzymes.[71] The colour density accumulates with increasing storage time. Coconut water treated with ozone with nisin was damaged due to visible fungal growth in the fourth week of storage. However, heat-treated coconut water was unacceptable at the end of the fourth week of storage because the sweet and sour taste had disappeared.[40]

The Concept of Optimization Design of Ozonation by Commercial Sterility Standard

Standards for commercial sterility equivalence always begins with the characterization of the process machine used, in this case, the ozone machine for processing coconut water. Characterization is needed to determine the ability of the machine to inactivate *Clostridium botulinum* spores or their substitutes. Target spore inactivation was carried out by knowing the remaining number of active spores as a function of time. In this stage, the kinetics of target spore inactivation will be known. At this stage, the equivalence of the ozonation process with commercial sterility is known. Kinetic data of targeted spore inactivation could explain the time required to reduce 12 decimal logs of that targeted spore. Commercial sterility needs to achieve 12 decimal logs inactivation of *Clostridium botulinum* spore. Based on that explanation, the spore inactivation kinetic data is needed to determine the required time to inactivate 12 decimal logs of targeted spore (*Clostridium botulinum*) as minimum standards of commercial sterility prerequisite. However, with the quality parameters of endogenous enzyme activity and bioactive components, the equivalence stage needs to be continued up to the final stage, namely optimizing the ozonation process with commercial sterility standards. In order to reach the optimization step, the kinetics of inactivation of the polyphenol oxidase enzyme is required; peroxidase enzymes; degradation of phenolic compounds; and degradation of flavonoid compounds. After all kinetic testing data has been collected, then data analysis is carried out by determining the coefficient of the quality parameter change (k-value). Because several ozonation research results on these quality parameters have been validated using first order, the k-value can be converted to a D-value as in the thermal process concept. By knowing the D-value, then with differences in variations in the ozonation process (the concentration of dissolved ozone in the product), a Z-value will also be obtained as in the thermal process. D and Z-values are used to design the optimization of the ozonation process in coconut water products.

Characterization of Ozone Generator Machine

In the process of inactivating bacterial spores and endogenous enzymes, their effectiveness depends on the amount of ozone the coconut water product receives. It is expected that the more the amount of ozone in the product, the more ozone molecules will be able to inactivate endogenous spores and enzymes. The amount of ozone gas contained in a unit volume of carrier air varies greatly depending on the ozone gas that is capable of being produced by the ozone generator machine, so each ozone generator machine has its ozone production capability. Therefore, before testing the kinetics of bacterial spore inactivation and endogenous enzymes, it is necessary to characterize the ozone machine in producing ozone gas until coconut water products receive it.

The ozone machine was characterized by measuring the ozone concentration received by the coconut water product as a function of the ozonation time at a certain oxygen gas flow rate. The ozone concentration in the product is calculated during the ozonation time. At a certain time, the concentration of ozone gas in the product will stabilize. From the results of plotting these two variables in the graph, the slope on the side of the linear slash is the character of the ozone machine in carrying out the ozonation process on coconut water according to the oxygen gas flow rate used. Then the ozone machine characterization test was carried out similarly but with different oxygen gas flow rates. In term of using a Dielectric Barrier Discharge Reactor, the higher oxygen gas flow rate, the slope between the ozone gas concentration variable in the product as the y-axis and the ozonation time as the x-axis will also increase. This means that the higher the flow rate of oxygen gas, the faster the penetration of ozone gas into the product is marked by an increase in the slope value and the higher the concentration of ozone gas in the product. The ozone machine characterization design can be seen in Figure 5.

However, coconut water products have limitations in accepting ozone gas. This is because ozone gas will react with molecules from bacterial spore constituents and endogenous enzymes, decomposing itself into oxygen gas in and out of the coconut water through the surface. Referring to this, the concentration of ozone gas in the product will reach a maximum and reach a stable point. So, for the next ozonation process, it is at this point that will be used as the conditions for the future ozonation process, namely the oxygen gas flow rate, the come-up time to reach stability, and the concentration of ozone gas in the product when it starts to reach stability. This is, of course, greatly influenced by the volume factor of the coconut water products that are ozonated. The smaller the volume of the product, the faster the maximum and stable point is reached for the concentration of ozone gas in the product. Pure oxygen gas or gas from the surrounding environment will always be supplied to pass through the ozone generator chamber so that the ozone from the generator will always flow into the product continuously. The method for dissolving ozone gas in the product can be done by passing it through a fine porous diffuser which will produce ozone gas with a relatively small size. The product is not supplied continuously, but with a batch system, a certain volume is subjected to agitation during ozonation. Agitation will help break up the tiny ozone bubbles so that the ozone will easily dissolve in the product.

Kinetic of Bacteria Spore Inactivation during Ozonation

Spores of *Clostridium sporogenes* can be used in microbiological parameters according to parameters in commercial sterilizers. The sporulation method to obtain spores can be used, which can be modified according to laboratory needs.[72] Concisely, stock culture needs an activation prior to sporulation. The sporulation was conducted in appropriate media. Reinforced Clostridial Medium is a famous media for *Clostridium sporogenes* incubation. After sporulation, the remaining vegetative cells was inactivated by heating on 85°C for 15 minutes. Spore suspension was ready to be used after washing by a sterile aquadest. For the spore inactivation experiment, an amount of 10^8 CFU spore was prepared. The kinetic study method for the inactivation of *Clostridium sporogenes* spores in coconut water media can be carried out using modifications of González-Angulo *et al.*[73]

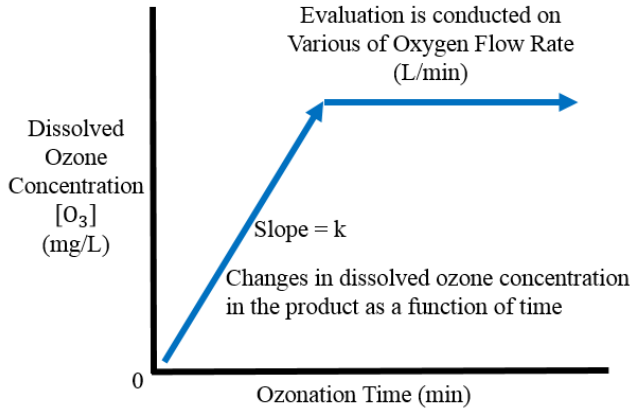

Figure 5: Ozone Machine Characterization Design

A certain volume of sterile coconut water is poured into the ozonation chamber, then added with spore suspension with a concentration of 10^8 spores/mL. On the results of the characterization of the ozone machine, an ozonation process was carried out with different levels of acceptance of ozone gas in the product, which was accompanied by testing for total spores at certain time intervals. The number of spores in the log is plotted in the spore inactivation equation with an first order approach so that the D-value will be obtained as the inactivation character of *Clostridium sporogenes* spores on the ozone machine.

Kinetics of Endogenous Enzyme Activity Reduction during Ozonation
Coconut water naturally contains endogenous enzymes, namely polyphenol oxidase enzymes and peroxidase enzymes. These two enzymes are quality parameters that must be considered in optimizing the coconut water ozonation process. Polyphenol oxidase and peroxidase enzyme solutions with a certain volume and activity are mixed in an ozonation chamber containing coconut water to obtain coconut water containing polyphenol oxidase and peroxidase enzymes with certain activities. The mixture is ozonated according to the level of acceptance of ozone gas in the product with its variations. The ozonation process is accompanied by testing the activity of polyphenol oxidase enzymes and peroxidase enzymes at certain time intervals. The activity of the polyphenol oxidase and peroxidase enzymes was plotted in the equation for the inactivation of polyphenol oxidase and peroxidase enzyme activities with an order of 0 or 1 so that a k-value or D-value would be obtained as a character of the decrease in the activity of the polyphenol oxidase enzyme and peroxidase enzyme in the ozone machine used.

Kinetics of Total Phenols and Flavonoids Reduction during Ozonation
As a compound that is a strong oxidizing agent, ozone has the potential to oxidize the bioactive components present in coconut water during the ozonation process. Compounds that have the potential to be disrupted are phenols and flavonoids which have a relatively dominant amount in coconut water. Therefore, the phenolic and flavonoid components are the limiting parameters in the coconut water ozonation. This means that the dissolved ozone concentration in product and minimum ozonation time required to achieve commercial sterility should be optimized to limit phenol and flavonoid degradation. The design of the kinetics test was carried out by ozonating coconut water with a certain volume. Ozonation is carried out according to the maximum acceptable level of ozone gas in the product with various variations. During the ozonation process, total phenol and flavonoid tests were carried out at certain intervals. The total phenols and flavonoids were plotted in the equation for the degradation of total phenols and flavonoids with an approach of order 0 or 1 so that the value of k or D will be obtained as a character of the decrease in the concentration of phenols and flavonoids in the ozone generator used. Graph of the evaluation design for inactivation (decrease) of quality parameters, including inactivation of *Clostridium sporogenes* spores, polyphenol oxidase enzymes, peroxidase enzymes, degradation of phenols and flavonoids during the ozonation process can be seen in Figure 6.

Determination of k, D, and Z-Values on Kinetic of Quality Parameter Changes
All kinetics of quality parameter changes during the ozonation process (total spores, polyphenol oxidase enzyme activity, peroxidase enzyme activity, total phenol, and total flavonoids) are calculated for the k or D-values as changes in the time required to change 1 log value of the amount or concentration of each quality parameter. The value of the test results for each quality parameter for each predetermined ozonation process time is plotted in the graph as the y-axis. In contrast, the time for the ozonation process is plotted on the x-axis. For quality parameters, the values are changed in the log first, then the regression equation of each quality parameter is determined by first order model. The slope of each regression equation curve is a 1/D-value, so the D-value can be calculated. In determining the coefficient of change in quality parameters (kinetics), although from some literature the ozonation process can be explained well-using first order, it is possible to plot it also in equations of order 0, order 2, or other kinetic equations that have a higher R-square value.

After obtaining the kinetic values for the quality parameters, the sensitivity to changes in the amount of ozone dissolved in the product can be evaluated by calculating the value of Activation Energy (Ea) or the Z-value if it can be fitted using first order model. The calculation of Z-value, which is interpreted as the sensitivity of spores or the sensitivity of quality parameters to changes in the concentration of ozone received by the product, can be calculated by adjusting the flow rate of ozone gas, which gives the difference in the concentration of ozone that the product will receive. The Z-value is obtained by plotting the log of the D-value as the y-axis and the range of ozone concentrations received by the product as the x-axis for each quality parameter. Then look for the regression equation from the plot results on each quality parameter to obtain the slope value as a value of 1/Z. Then the Z-value can be calculated. The Z-value represents the change in the ozone concentration received by the product, which can change 1 log in the D-value. In this case, each quality parameter will have a Z-value, which is the sensitivity to changes in the ozone concentration received by the product.

Design of Optimization for Ozonation by Commercial Sterility Standard
Design of optimization in this work uses a method described by Deeth.[74] That paper used a thermal process which could be adopted in ozonation process. Design of optimization is carried out in several stages. The first is to evaluate the activity of the initial polyphenol oxidase and peroxidase enzymes and determine the initial total phenol and total flavonoids in coconut water. This can be seen in the kinetic evaluation at minute 0, except for the activity of the enzyme because usually, its biological activity tends to be below the limit of detection, then the pure enzyme is added. Once these four things are known (the initial values of polyphenol oxidase activity; peroxidase activity; total phenol; and total flavonoid), the time the ozonation process takes to reduce the activity of the polyphenol oxidase and peroxidase enzymes can be calculated to pass 0 U/mL/h, or it can be said to be an over-zonation process. The length of time used for the ozonation process can also be expressed as a D-value, which, if using the ozonation process until there is a decrease of three decimal places or 3 logs from the activity value of the polyphenol oxidase and peroxidase enzymes, can be expressed as a 3D process. The process is carried out by treating the maximum ozone concentration received by the product so that the D-value used is the D-value for the maximum ozone concentration treatment received by the product by the characterization of the ozone machine. The D-value can be found using the regression equation on the graph of the Z-value of each of the kinetics of decreasing the activity of the polyphenol oxidase and peroxidase enzymes. This Z-value equation can be written as follows:

$$\mathrm{Log\ D_2} = \left(\frac{[\mathrm{O_3}]_1 - [\mathrm{O_3}]_2}{\mathrm{Z}}\right) + \mathrm{Log\ D_1}$$

The Log D_2 means the log of D-value at certain/expected dissolved ozone concentration $[O_3]_2$. D_1 is D-value at $[O_3]_1$. The Z-value means the change of dissolved ozone concentration which is needed to change 1 log D-value. With changes in the concentration of ozone received by the product, which is used to evaluate the kinetics of total phenols and flavonoids, these two parameters can be determined as limiting quality parameters for the ozonation process so that it can be designed a process that can reduce the activity of polyphenol oxidase enzymes and peroxidase enzymes as optimally as possible while limited by the degradation of phenols and flavonoids to a minimum in the length of processing time and the concentration of ozone received by the product, given the conditions the process will operate, unlike the case with the determination of the length of time on the activity parameters of polyphenol oxidase enzymes and peroxidase enzymes.
Both parameters need to be clearly defined to reduce their activity to 0 U/mL/h using the D-value and Z-value. However, for the degradation of phenols and flavonoids, it is necessary to calculate the D-value at the ozone concentration received by the product, which is used for coconut water sterilization by using the Z-value equation. After finding the D-value, which corresponds to the concentration of ozone received by the product in the sterilization process, then look for the value of k (quality degradation rate constant) for the decrease in phenol and flavonoid

concentrations with the equation (if the kinetics of degradation concentrations of phenols and flavonoids follow first order):

$$D = \frac{2.303}{k}$$

Until now, some have been able to model by linking the degradation coefficient as a function of ozone concentration following a first-order reaction.[75] In a good processing process, quality parameters that are degraded must have a minimum decrease so that it can be determined that the limit of the ozonation process carried out is maximum to give a degradation effect on total phenols and total flavonoids, for example 20% of the initial concentration. Furthermore, as explained by Fonseca *et al*. stated, the reduction rate of total phenols and flavonoids could use the first-order reaction equation.[58] However, the data obtained can still be plotted with an order 0, order 2, or another kinetic equation approach if the R-square value is much higher than the first order equation. Therefore, to find the length of time for the ozonation process with a Limitation of degradation of total phenols and flavonoids can follow the following equation:

$[Q]_t = -k * t + [Q]_0$ → Zero Order Model

$Ln[Q]_t = -k * t + Ln[Q]_0$ → First Order Model.

Note $[Q]_t$ is the concentration of quality parameters when total phenols and total flavonoids are degraded by for example 20%, $[Q]_0$ is the initial concentration of quality parameters of total phenols and flavonoids, t is the length of time the process that causes a decrease in the concentration of quality parameters from $[Q]_0$ to $[Q]_t$, and k is the rate constant for the decrease in the concentration of quality parameters under certain conditions, in this case, the treatment of the concentration of ozone gas received by the product in a certain amount. After obtaining the t value for each degradation of the total phenol and flavonoid quality parameters, the overall length of time for the 3D process and the concentration of ozone gas received by the product on the parameters of inactivation of polyphenol oxidase enzymes and peroxidase enzymes, as well as the treatment time and concentration of ozone gas obtained. The product received to achieve maximum degradation of 20% of the initial concentration of total phenols and flavonoids is plotted on a graph of the relationship between time as the y-axis and the concentration of ozone received by the product as the x-axis. Each quality parameter has a point of pair of time and ozone concentration received by the product. Then based on the Z-values for all parameters, a re-investigation is carried out on the graph either for the addition or reduction of the ozone concentration received by the product. The second point is given by the combination (change in Z-value as x: change of 1 log time as y).

After each parameter has 2 points, then a line is drawn. Do the intersection of the lines if there is a sharp enough slope of the curve. The optimal process design, or in this case, the combination of ozone gas concentration received by the product and the length of time for the ozonation process, is given in the area on the graph above the parameter line for the inactivation of polyphenol oxidase enzymes and peroxidase enzymes and the area below the parameter line for phenol and flavonoid degradation parameters. Finally, the area must be delimited by the target spore inactivation parameter according to the commercial sterility concept, namely 12D, which can be expressed in terms of the area above the spore inactivation line. The point on the x-axis in the spore inactivation parameter is chosen according to the conditions of the ozonation process, which gives the maximum concentration of ozone gas the product receives. With the Z-value of the spore inactivation parameter, one more point can be determined according to the change in the Z-value on the known spore inactivation Z-value curve. The y-axis, as the time of the ozonation process, adjusts to changes in the Z-value given with a change of 1 log. Then the main boundary lines in the parameter of spore inactivation will be obtained on the process optimization graph (time of the ozonation process as the y-axis and the concentration of ozone received by the product as the x-axis). The design of optimizing the ozonation process in coconut water can be seen in Figure 7.

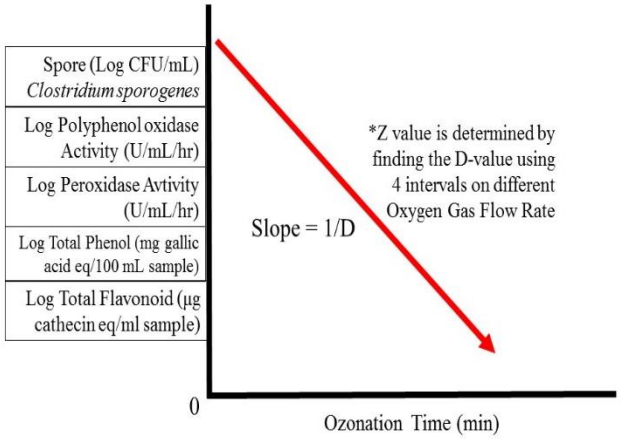

Figure 6: Design for The Change of Quality Parameters in the Coconut Water Ozonation Process

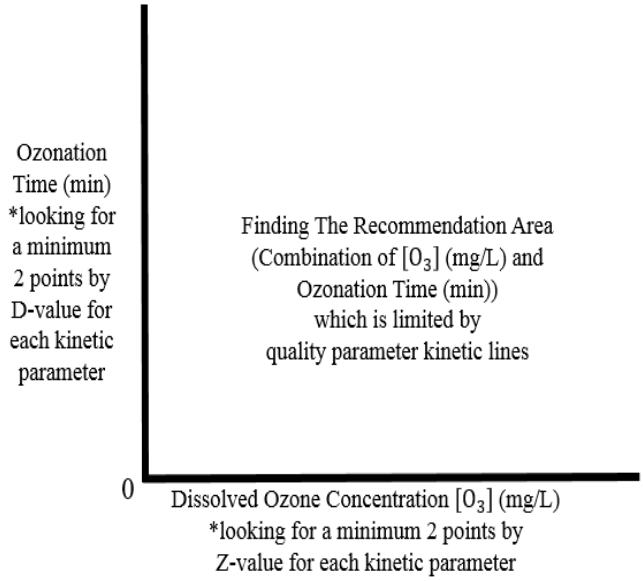

Figure 7: Design of Coconut Water Processing Optimization by Ozonation

Verification of Optimization for Ozonation Result using Commercial Sterility Standard

After obtaining process recommendations on the combination of ozone gas concentration in the product and length of time for ozonation, a verification process is carried out. This process is carried out by testing a sample of coconut water product which is processed with one of the combinations of ozone gas concentration in the product and the length of time of ozonation recommended for process optimization. After the ozonation process, the total spore bacteria, polyphenol oxidase enzyme activity, peroxidase enzyme activity, total phenol, and total flavonoids were tested. The success of the kinetics test was based on the adequacy of food safety parameters equivalent to commercial sterility, the absence of polyphenol oxidase and peroxidase enzyme activity, and the total phenol and flavonoids remaining at least 80% of the initial amount.

Adoption of Ozonation Optimization Design Using Thermal Process Model Simulation

The equivalence of the ozonation process with commercial sterilizers uses the 12D concept with the D-value obtained from the ozonation process at a certain dissolved ozone concentration. The concept of D-value can be used if target spore inactivation can be validated using the first order. Target 12D remains inactivated *Clostridium botulinum* spores. However, substitute bacteria can be used for testing, namely *Clostridium sporogenes*. The ozonation process that will be carried out

on coconut water will allow oxidation of the phenolic and flavonoid components to occur. During the ozonation process, the conditions that occur are attempts to inactivate target spores and endogenous enzymes but are also accompanied by oxidation of the bioactive components of phenols and flavonoids. Seeing this trend, it is necessary to optimize the ozonation process, namely, to inactivate the spores and enzymes according to the target. However, the degradation of bioactive compounds with a certain amount of reduction limits the process. In the process optimization design, data on the kinetics of the inactivation of target spores, endogenous enzymes, and degradation of bioactive components of phenols and flavonoids by the ozonation process are required. Optimization of the ozonation process is based on the equivalent of the ozonation process with commercial sterility in the thermal process. Optimizing the ozonation process can also be simulated using kinetic data on the thermal process. We still use the kinetics data of the ozonation process for laboratory testing, but the steps taken are the same as for optimizing the thermal process.

This paper will review these steps to serve as a reference for researchers on whether to optimize the thermal process or the ozonation process and other non-thermal processes equivalent to commercial sterility. The parameters are also flexible. They can be changed according to the company's research or business interests. In this simulation, we still use *the Clostridium botulinum* spore inactivation standard in process optimization calculations as a commercial sterility standard. Calculation simulations use thermal process kinetics data, but these data can be changed with ozonation process kinetics data, so the formulas for the calculations can also be adopted in optimizing the ozonation process if all kinetic parameters can be properly validated using the first order. Quality parameter of phenol and flavonoid degradation kinetics by thermal process uses data from Turturica *et al*.[76] The kinetics of the inactivation of polyphenol oxidase and peroxidase enzymes during the thermal process uses data from Cao *et al*.[31] Both sources use first-order reaction equations, as seen in Table 7. *Clostridium botulinum* spores use standard commercial sterility.

For the damage limit for each quality parameter, phenol, and flavonoid, it is determined that the damage limit that can be tolerated is half of the initial concentration of the two quality parameters. If the damage to this quality parameter exceeds half of the initial concentration, or in the sense that the concentration of the quality parameter after the heating or ozonation process is less than half of the initial concentration, the product will be rejected. Because the combination of temperature and heating time for the 12D concept is well known, we must first find the Z-value for the parameters of the degradation of phenols and flavonoids, as well as the degradation of polyphenol oxidase and peroxidase enzymes. Because from data sources show that the rate of phenol and flavonoid degradation reactions, as well as the inactivation of polyphenol oxidase and peroxidase enzymes, follow a first-order reaction.

If the equation $\mathbf{Log(Q)_t = -\left(\frac{k}{2.303}\right)t + Log(Q)_0}$ dips plotted on the graph with the x-axis as t and the y-axis as Log (Q)t, then the graph will be the same as the graph of the relationship between heating duration and the log amount of each -each quality parameter. The t value can be adopted at the time of ozonation. In this case, the slope $-\left(\frac{k}{2.303}\right)$ in the equation $\mathbf{Log(Q)_t = -\left(\frac{k}{2.303}\right)t + Log(Q)_0}$ ad is the same as the slope in the graph of the relationship between length heating with the log number of each quality parameter, namely $-\left(\frac{1}{D}\right)$. The D-value in each quality parameter reduction reaction at certain heating temperatures (already known in the table) can be known. After knowing the D-value at certain heating temperatures, as shown in the table, calculate the Z-value for each decrease in quality parameters by making a graph and plotting certain heating temperatures as shown on the x-axis and the Log D-value each on the y-axis. The heating temperatures can be adopted at the dissolved ozone concentration. Z-value can be obtained from the slope of the resulting line equation. Data on D-values and Z-values can be seen in the following table. While the graph of the relationship between heating temperature and Log D-value for quality parameter reduction can be seen in Figure 8. On the graph, the heating temperature can be adopted on the dissolved ozone concentration.

Table 7: Kinetic and Arrhenius Data for Enzyme Inactivation and Bioactive Compound Degradation

Quality Parameters	Temperature (°C)	k (min^{-1})	D-Value (minute)	Log D-Value	Z-Value (°C)	1/T (°K)	Ln k	Arrhenius Slope	Intercept
Phenol Degradation	70	0.007*	329	2.52	70.1	0.00292	-4.96	-4270.08	7.4
	80	0.009*	255.89	2.41		0.00283	-4.71		
	90	0.01*	230.30	2.36		0.00275	-4.61		
	100	0.014*	164.50	2.22		0.00268	-4.27		
	110	0.029*	79.41	1.90		0.00261	-3.54		
Flavonoid Degradation	70	0.012*	191.92	2.28	124.4	0.00292	-4.42	-2440.17	2.7
	80	0.015*	153.53	2.19		0.00283	-4.2		
	90	0.019*	121.21	2.08		0.00275	-3.96		
	100	0.022*	104.68	2.02		0.00268	-3.82		
	110	0.025*	92.12	1.96		0.00261	-3.69		
Polyphenol Oxidase Inactivation	55	0.0152**	151.51	2.18	14.26	0.00305	-4.19	-18445.31	52.04
	60	0.0329**	70	1.85		0.003	-3.41		
	65	0.0888**	25.93	1.41		0.00296	-2.42		
	70	0.1789**	12.87	1.11		0.00292	-1.72		
	75	0.3696**	6.23	0.79		0.00287	-0.1		

Peroxidase Inactivation	55	0.0018**	1279.44	3.11	9.31	0.00305	-6.32	-28378.34	81
	60	0.0311**	74.05	1.87		0.003	-3.47		
	65	0.0783**	29.41	1.47		0.00296	-2.55		
	70	0.2226**	10.35	1.01		0.00292	-1.50		
	75	0.3252**	7.08	0.85		0.00287	-1.12		

Source: *Turturica *et al.*[76] ; **Cao *et al.*[31]

Table 8: Kinetic Coefficient Values and Half-Life of Enzymes and Bioactive Compounds at 121.1°C

Parameter	Processing Temperature (°C)	1/T (°K)	Ln k	D-Value (minute)	$t_{(1/2)}$ (minute)	$t_{(1/1,11)}$ (minute)
Phenol Degradation	121.1	0.0025	-3.4711	-	22.3	3.36
Flavonoid Degradation	121.1	0.0025	-3.4775	-	22.44	3.38
Polyphenol oxidase Inactivation	121.1	0.0025	5.2445	0.0121	-	-
Peroxidase Inactivation	121.1	0.0025	8.9957	0.0002	-	-

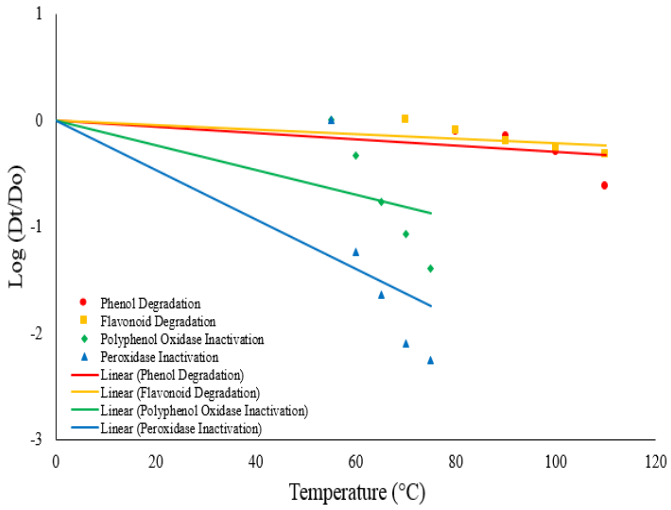

Figure 8: The Change of Log D-value for Quality Parameters as a Function of Temperature Change

The Z-value of this quality parameter reduction will later be used in determining the temperature factor in the combination of temperature and heating time in optimizing the thermal process. The temperature factor for decreasing quality parameters during the heating process is known, now is the time to calculate the time factor for each heating temperature, so that later a combination of temperature and heating time will be obtained that meets the criteria of 12D inactivation of *Clostridium botulinum*, 6D inactivation of polyphenol oxidase and peroxidase enzymes, and the decrease in the concentration of phenols and flavonoids is less than half of their initial concentrations. In adopting the ozonation process, the temperature factor is a factor of dissolved ozone concentration. To determine this, it is necessary to calculate the Arrhenius equation first from the five-reaction rate constant data (k) at each product's heating temperature. Arrhenius equation at linear form is consisted of ln.k as y-axis and (1/T) as x-axis. It could follow the model below:

$$\ln k = -\frac{Ea}{R}\left(\frac{1}{T}\right) + \ln A$$

Through the equation $\mathbf{\ln k = -\frac{Ea}{R}\left(\frac{1}{T}\right) + \ln A}$, the temperature (in Kelvin) of the table on the x-axis in position $\left(\frac{1}{T}\right)$. Then plot the k-value in the table (changed to the ln k-value first) on the y-axis at the ln k position. Then the line equation will be obtained, as shown in Figure 9. From the graph, it can be seen that the data of the Arrhenius equation for the slope and intercept of each degradation parameter is shown in the Table 7. The Arrhenius equation will calculate the ln k-value at 121.1°C for each degradation parameter because the product will be conditioned to be heated at 121.1°C. This temperature of 121.1°C will later become the dissolved ozone concentration in the ozonation process which is set as the standard operating process. This ln k-value will later be used to calculate how long the product lasts at 121.1°C with a time limit until the phenol and flavonoid concentrations reach half of the initial concentration. In ln, the inactivation of polyphenol oxidase and peroxidase enzymes will be used to find the D-value at an operating temperature of 121.1°C. This D-value will later be used to determine the length of time factor at the temperature and heating process time combination for standard inactivation of polyphenol oxidase and peroxidase in the amount of 6D. The results of Rajashri *et al*. stated that the activity of the polyphenol oxidase enzyme in fresh coconut was 1,200 U/mL/h, while the activity of the peroxidase enzyme was 8,400 U/mL/h.[40] As an effort to completely inactivate the activity of the polyphenol oxidase and peroxidase enzymes, the two enzymes will undergo an inactivation process of 6D. By knowing the D-value of the two enzymes at 121.1°C, it is only necessary to multiply the D-value by 6 to achieve total inactivation of polyphenol oxidase and peroxidase enzyme activity. Next, plotting the temperature value of 121.1°C in each of the Arrhenius equations for the four degradation parameters is necessary. From the Arrhenius equation for each degradation parameter by entering a temperature value of 121.1°C in 1/T Kelvin, the ln k-value is obtained at 121.1°C as shown in Table 8.

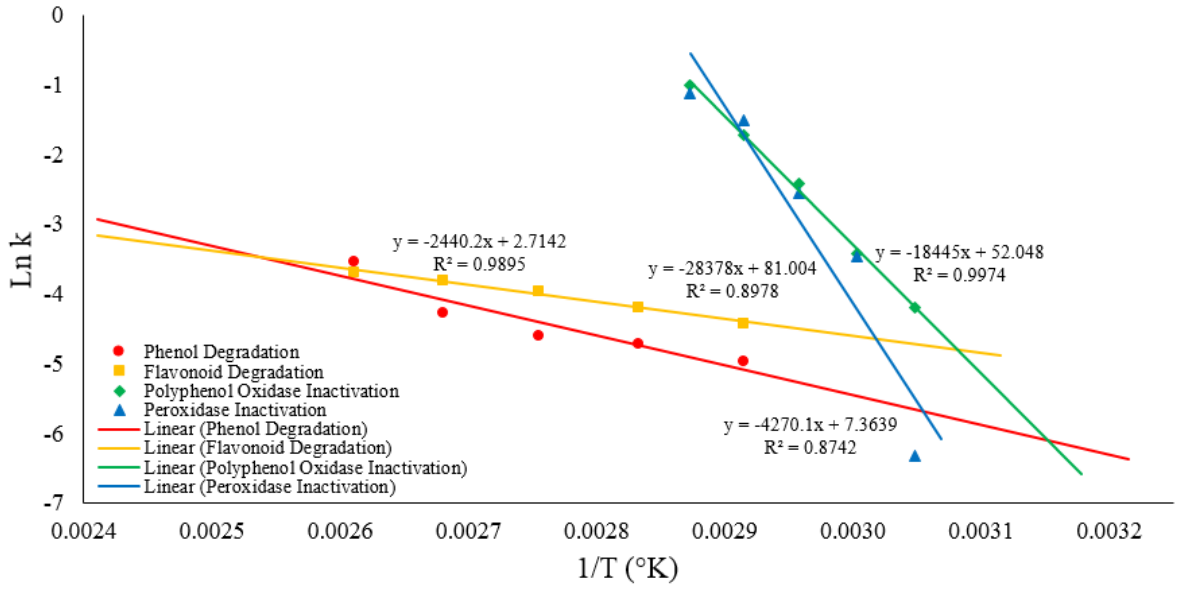

Figure 9: Arrhenius Plot for Quality Parameters

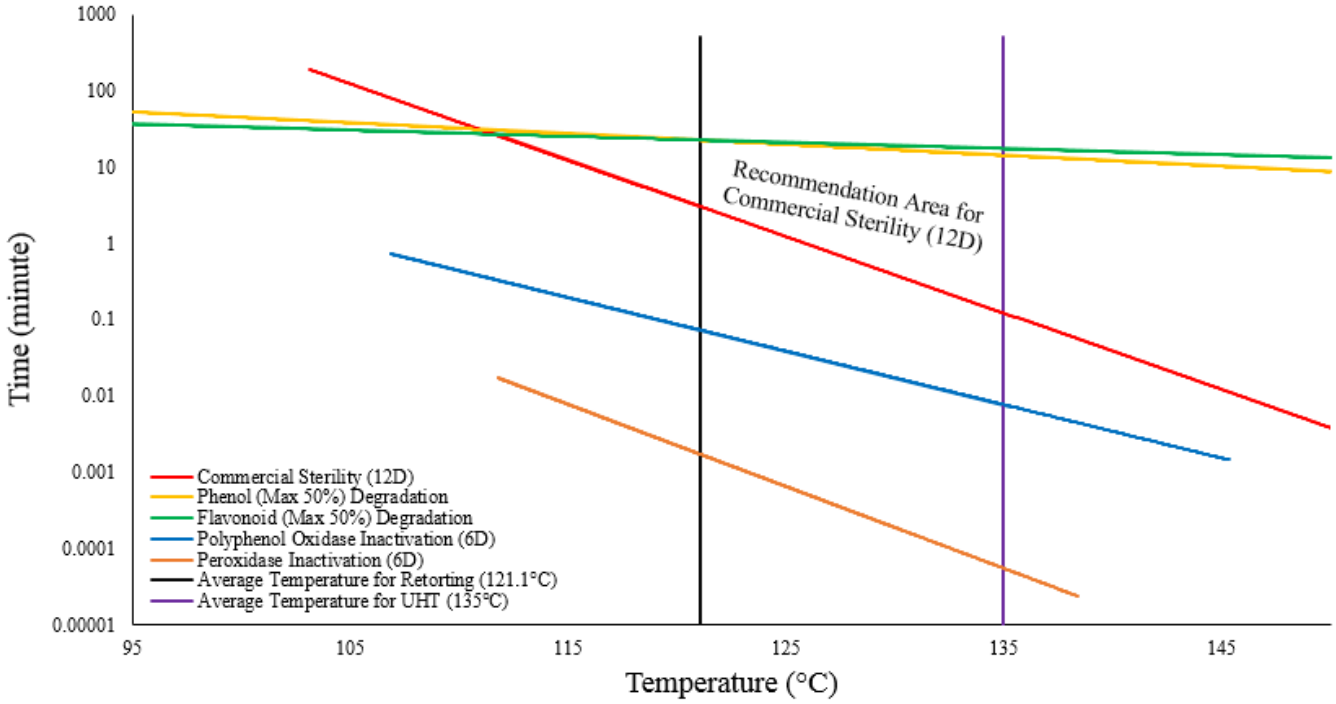

Figure 10: Optimization of Commercial Sterility with a Maximum of 50% Degradation for Bioactive Compounds

Because the maximum tolerance for the degradation of phenols and flavonoids is determined to be half of the initial concentration, the half-life equation will be able to calculate how long it will take for the concentration of phenols and flavonoids to reduce to half of the initial concentration as a result of degradation during the heating process at 121.1°C. Following the first-order reaction, this half-life equation can also be adopted for the kinetic constants resulting from the ozonation process. The half-life equation can be given as follows:

$$t_{\frac{1}{2}} = \frac{0.693}{k}$$

By plugging in the k-value from the ln k-value obtained for a heating temperature of 121.1°C in the half-life equation, the heating time at 121.1°C is obtained, which results in the degradation of half the amount of phenol from the initial concentration was 22.3 minutes. In comparison, the heating time at 121.1°C, which resulted in the degradation of flavonoids in half of the initial concentration, was 22.44 minutes. Meanwhile, the D-values for the inactivation of the polyphenol oxidase and peroxidase enzymes at 121.1°C were 0.01215 and 0.00029 minutes, respectively.

After obtaining one combination of temperature and heating time for each quality degradation parameter, two or three more combinations of temperature and heating time are needed for each quality degradation parameter. This is done so that the two or three combinations of temperature and heating time can be plotted on a graph and drawn in a straight line, resulting in an area that can be used as various combinations of temperature and heating time, which is still in the region of decreasing concentrations of phenol and flavonoids amounting to half of the initial concentration and above the standard line inactivated polyphenol oxidase and peroxidase enzymes in the amount of 6D. Determining the points between temperature and process time can use the concept of D-value and Z-value. First, determine the combination of temperature 121.1°C and 3 minutes as a benchmark that

this temperature is the reference temperature used in commercial sterility processes to apply the concept 12D followed by 3 minutes as Fo at 121.1°C to apply the 12D concept, which is the reduction of *Clostridium botulinum* by 12 decimal places. After the combination of temperature and time is used as a minimum benchmark for the heating process, both inactivation of the enzymes polyphenol oxidase, peroxidase, phenol, and flavonoids will also use the temperature of 121.1°C as a benchmark in determining the combination of temperature and heating time. It is known that the Z-value is the temperature change used to change the amount of one log D-value. The D-value is the time required to change one log of the concentration of quality parameters. Choose a heating temperature of 121.1°C, 121.1°C minus the Z-value, and 121.1°C plus the Z-value for the inactivation of the enzymes polyphenol oxidase, peroxidase, phenol, and flavonoids. It will get a long heating time pair for all quality parameters, as in Table 9.

It should be remembered that the heating process uses the 12D concept, which means following the concept of reducing 12 decimal places in *Clostridium botulinum* with 3 minutes of F_0 at the reference temperature for commercial sterility, which is 121.1°C. Considering that the Z-value for *Clostridium botulinum* is 10°C, if 10°C increases the heating process temperature of 121.1°C, the heating time will decrease by one decimal log from 3 minutes to 0.3 minutes. Vice versa, if the temperature of the heating process is 121.1°C lowered by ten °C, the heating time will increase by one decimal log from 3 minutes to 30 minutes. This condition also applies to all quality parameters because it uses the concepts of D-value and Z-value. These changes are a combination of temperature and length of heating time that can be used for product heating processes that still have a commercial sterilizing effect, namely the reduction of *Clostridium botulinum* in the amount of 12 decimal. The recommended combination of temperature and length of heating time is in the area above the commercial sterility line 12D, the polyphenol oxidase and peroxidase enzyme inactivation line a few 6D, and below the tolerance limit for phenol and flavonoid degradation. Then the area is also limited by the temperature of the retorting and UHT processes, namely 121.1 and 135°C. The heating process recommendation area that can be used to determine the combination of temperature and heating time can be seen in Figure 10.

Table 9: Combination of Processing Time and Temperature by 50% Reduction of Bioactive Compounds

Parameter	Processing Temperature (°C)	Fo by Z-value (minute)
Clostridium botulinum Inactivation for Commercial Sterility Standard (12D)	111.1	30
	121.1	3
	131.1	0.3
Phenol (Max 50%) Degradation	51	222.96
	121.1	22.3
	191.2	2.23
Flavonoid (Max 50%) Degradation	121.1	22.44
	245.5	2.24
Polyphenol Oxidase Inactivation (6D)	106.84	0.72908
	121.1	0.07291
	135.36	0.00729
Peroxidase Inactivation (6D)	111.79	0.01713
	121.1	0.00171
	130.41	0.00017

Table 10: Combination of Processing Time and Temperature by 10% Bioactive Compounds Reduction

Parameter	**Processing Temperature (°C)**	**Fo by Z-Value (minute)**
Phenol (Max 10%) Degradation	51	33.58
	121.1	3.36
	191.2	0.34
Flavonoid (Max 10%) Degradation	121.1	3.38
	245.5	0.34

Suppose the heating process is carried out at a combination of temperature and time, as in the recommended area in the figure above. The product will be safe from *Clostridium botulinum* contamination and degradation in that case. Phenols and flavonoids are still maintained at or above the maximum damage standard, which is damaged by half of the initial concentration so that the product can be accepted. It is different if the product is heated at 121.1°C but with a length of time outside the recommendation area, so more than 50% damage to phenols and flavonoids occurs, even though the concept of 12D reduction is well applied. However, the product cannot be accepted because the quality damage has exceeded the tolerance limit of more than 50%. Suppose the product is processed at a temperature and time below the standard commercial sterility line. In that case, it will be very dangerous because it does not comply with the 12D commercial sterility rules and has the potential to germinate *Clostridium botulinum* spores. Products with these conditions will be rejected because they do not meet the food safety requirements and quality standards that have been set.

Regarding business needs, the market will be more flexible in assessing and liking products. Unsurprisingly, the industry continues to conduct research in product development. Existing models will be more helpful for process optimization in varying industrial needs, especially in minimizing the degradation of functional compounds. If, in this

optimization, it is desired that the maximum damage to the degradation of phenols and flavonoids is 10%, then the half-life equation will define that the tolerance given is the maximum concentration given after the heating process is 90% of the initial concentration of phenols and flavonoids. This calculation also applies to modeling with an ozonation process that follows the first order. From this it can be derived the half-life equation which has the final concentration of phenols and flavonoids after the heating process an amount of 90% of each of the initial concentrations as follows:

$$t_{\frac{1}{1.11}} = \frac{0.10436}{k}$$

By entering the ln k-value at a heating temperature of 121.1°C (changed from ln k to k-value beforehand) in the equation $t_{\frac{1}{1.11}} = \frac{0.10436}{k}$, then the heating time will be obtained, which will have a damaging effect on phenols and flavonoids a maximum of 10% of the initial concentration so that the concentration of phenols and flavonoids after the heating process is a number 90%. The formula $t_{\frac{1}{1.11}}$ shows the time needed by phenols and flavonoids to be degraded by 10% at a certain heating temperature which is contained in the value of k in the equation $t_{\frac{1}{1.11}} = \frac{0.10436}{k}$. Table 10 is the calculation result of $t_{\frac{1}{1.11}}$; the result of the combination of temperature and heating time for the degradation of phenols and flavonoids is a maximum of 10%.

From the results of the combination plot of temperature and heating time for each parameter, both from the adequacy parameter of commercial sterility with 12D and 6D inactivation of polyphenol oxidase and peroxidase enzymes or the damage parameter of phenol and flavonoids in a maximum amount of 10%, a graph is obtained as shown in the figure below. The area tends to be narrower when compared to the 50% phenol and flavonoid damage requirement. This is because the damage requirements for the two bioactive compounds are lowered by up to 10%, thus making the calculation results for the length of time also tend to be even shorter. As seen in Figure 11, the process temperature cannot be reached at exactly 121.1°C because of the sensitivity of phenols and flavonoids. The amount that must be maintained is 90% of the initial concentration. This simulation also applies equally to the process optimization adopted by ozonation. Not only limited to the ozonation process but all food processing processes that can be validated properly using first order, it would be very good to follow this thermal process optimization modeling. This contribution of modeling from kinetic data will greatly help researchers and industry predict process conditions in food processing that has been running.

Conclusion

The thermal process kinetics has successfully described the commercial sterility simulation for the ozonation process. The characterization of an ozone machine can use the characterization of the distribution of heat penetration from the thermal process conducted for a similar perception between the coldest point on the product inside the machine and the amount of ozone received by the product. Inactivation kinetics of *Clostridium sporogenes* spores, polyphenol oxidase, peroxidase, and degradation of phenol and flavonoid compounds by the thermal process can be well-adopted ozonation if it can be well validated using first-order reactions. The lines in the thermal process optimization design can be adapted to optimize the ozonation process. Simulation of the design for the ozonation process by adopting the thermal process will greatly contribute to the equivalence of the commercial sterility by the ozonation process and the process optimization, including the quality parameters.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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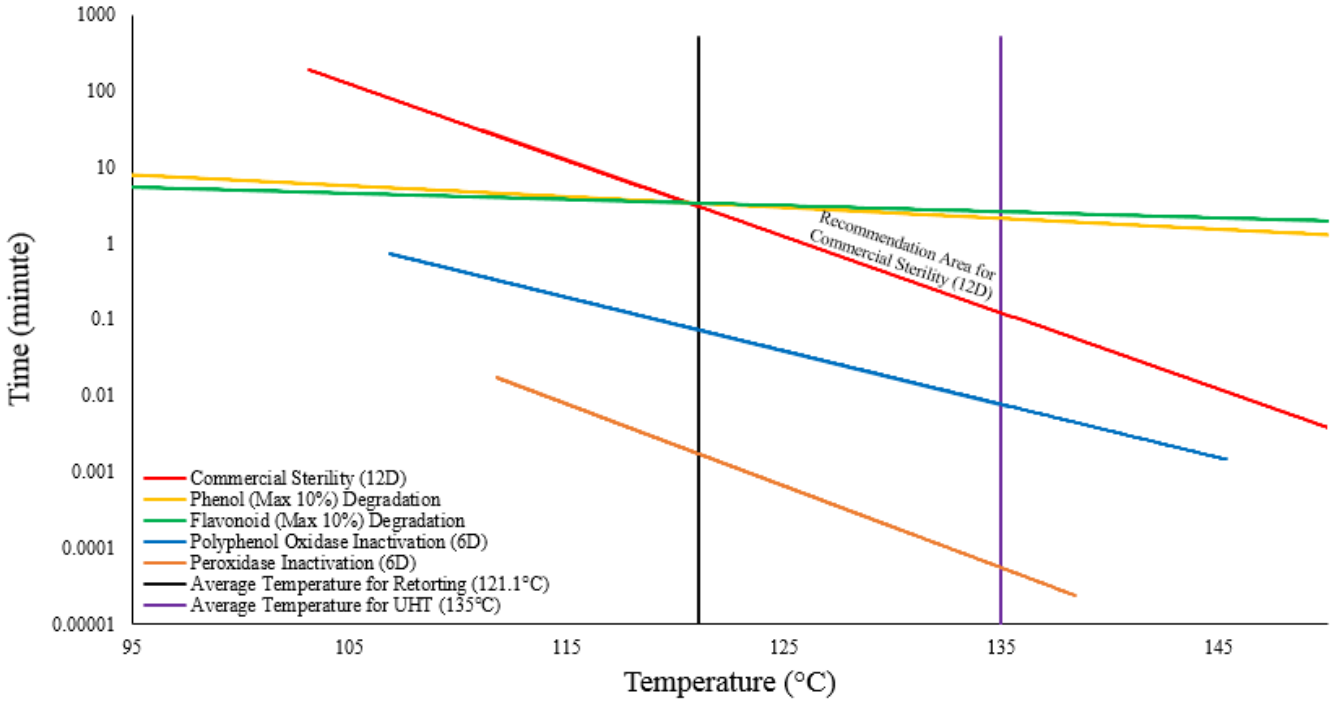

Figure 11: Optimization of Commercial Sterility with a Maximum of 10% Degradation for Bioactive Compounds

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