2023 S.T. Yau High School Science Award (Asia)

Research Report

The Team

Registration Number: Bio-173

Name of team member: Yau Nam Choi

School: Carmel Pak U Secondary School

City, Country: HKSAR

Name of team member: Ka Hei So

School: Carmel Pak U Secondary School

City, Country: HKSAR

Name of team member: Kin Kwan Ng

School: Carmel Pak U Secondary School

City, Country: HKSAR

Title of Research Report

Antimicrobial Edible Bio-disposables of Kombucha of Fruit Skins with Chitosan Coating

Date

31 July 2023

Antimicrobial Edible Bio-disposables of Kombucha of Fruit Skins with Chitosan Coating

Choi Yau Nam[1], So Ka Hei[2], Ng Kin Kwan[3]
[1] Carmel Pak U Secondary School, HKSAR (willwillcyn@gmail.com)
[2] Carmel Pak U Secondary School, HKSAR (s2018091@cpu.edu.hk)
[3] Carmel Pak U Secondary School, HKSAR (s2018077@cpu.edu.hk)

Abstract

The plastic pollution problem worldwide has been deteriorating over decades. In 2020, 10809 tonnes of municipal solid waste (MSW) was disposed of at landfills everyday in HK. The situation has worsened as the use of non-biodegradable tablewares in takeaways spikes during lockdowns throughout the pandemic. To combat this problem, we aim to use edible ***kombucha of fruit skins with chitosan coating (KFC)*** *as antimicrobial bio-disposables to ease the burden. Kombuchas are cellulose membranes [1] which are formed when bacteria present in scoby secrete bacterial cellulose membranes using carbon-containing compounds like glucose as raw materials. [2] Chitosan is an edible dietary fibre that helps digestion and stabilizes blood sugar levels etc. [3] Chitosan has antibacterial and antifungal properties which qualify it for food preservation. In this investigation, edible roasted kombuchas with different chitosan coating were tested and certified based on GB 18006-2008 [4], ISO18188:2016 [5] and COMMISSION REGULATION (EU) 2017/2158 on the presence of acrylamide in food [6], so they should be edible and safe for consumption, biodegradable with high tensile strength, show good water proofness and would not cause any allergic effect. Our invention of antimicrobial edible bio-disposables of* ***Kombucha of fruit skins with chitosan coating (KFC)*** *signifies a brand-new breakthrough as they are not only eco-friendly alternative materials to replace plastics disposables such as plastic straws but also antimicrobial so that natural resources are saved from making wrapping materials for disposables. Besides, an innovative greener way that involves neither 2M nitric acid nor 16.7M NaOH but using vinegar only to obtain antimicrobial chitosan coating (16.7% by mass of black soldier fly* ***BSF*** *using vinegar**;** cf. 19.2% using 2M nitric acid & 16.7M NaOH) from* ***BSF*** *was discovered. The marketing of such antimicrobial edible bio-disposables of kombucha of fruit skins is not only a great leap forwards to a plastics-free society but also a more sustainable society and betterment of future generations.*

Figure 1 Antimicrobial edible bio-disposables of **kombucha of fruit skin with chitosan coating (KFC)** of orange skins of Fancl Supplement

Figure 2 Drinking using kombucha cup & straw and eating kombucha disposables

Keywords: kombucha, chitosan, antimicrobial, biodegradable, edible

Acknowledgement

I would like to express my very great appreciation to my supervising teachers Dr. Tsz Yeung WONG and Ms. Yuen Yu IP for their valuable and constructive suggestions during the planning and development of this research work.

I would also like to thank Mr. Chan Chi Hong, Mr. Suen Hui Keung and Mr. Li Ka Chi, Laboratory Technicians, for his contribution and valuable technical support on this project.

Declaration

This project was submitted to I2ASPO INDONESIA INTERNATIONAL APPLIED SCIENCE PROJECT OLYMPIAD 2023, KSEF Korean International Science Fair 2023, Hong Kong Student Science Project Competition 2023 and Hong Kong Youth Science & Technology Innovation Competition 2023.

Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where the we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA(Asia) is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA(Asia) is final and no appeal will be accepted.

(Signatures of full team below)

X
Name of team member: Yau Nam Choi

X
Name of team member: Ka Hei So

X
Name of team member: Ki Kwan Ng

X
Name of supervising teacher: Dr Wong Tsz Yeung

X
Name of supervising teacher: Ms Ip Yuen Yu

Noted and endorsed by

(signature)

Name of school principal: Mr Wong Wai Keung

1. Theory

Antimicrobial edible bio-disposables of **kombucha of fruit skins with chitosan coating (KFC)** are alternatives of plastic and other non-biodegradable disposables.

1.1 Plastic disposable problem worldwide

Since the 1950s, extensive use of plastic has resulted in its 200-fold production mainly because of its versatility, durability, and cost-effectiveness (Fig. 2.1). [7]

Figure 1.1 Global plastic production since 1950

However, 72% of plastic waste landfilled or discarded causes long-term pollution to both terrestrial and marine environments. In terrains, buried plastics will contaminate underground water, causing soil degradation; In the latter case, plastics are broken into tiny microplastic, which upon consumption by marine wildlife, pose a significant threat to their health and survival. [8] Plastic causes climatic change around the world and makes marine animals live under threat. In their stomach plastic tableware can easily be found. If we kept using plastic tableware, we would be in big trouble.

1.2 Plastic disposal problem being aggravated by covid-19 in Hong Kong

Hong Kong(HK), being the 10th largest plastic waste producer globally (2010), produces 0.398kg of plastic waste per person per day (Fig1.2.1) [7]. According to the Environment Bureau (EB) of Hong Kong, in 2020, 10809 tonnes of municipal solid waste (MSW) was disposed of at landfills everyday. Among these, around 21.4% (around 2312 tons) were plastic waste, in which 25% of them were dining wares (Fig 1.2.2) [9]. Citizens' preference of takeaways during Covid-19 lockdowns can lead to a potential rise in these figures [10].

	Country / Region	Year	Per capita plastic waste (kg/person/day)
#1	Kuwait	2010	0.686
#2	Antigua and Barbuda	2010	0.66
#3	Saint Kitts and Nevis	2010	0.654
#4	Guyana	2010	0.586
#5	Barbados	2010	0.57
#6	Saint Lucia	2010	0.522
#7	Germany	2010	0.485
#8	Ireland	2010	0.43
#9	Netherlands	2010	0.424
#10	***Hong Kong***	***2010***	***0.398***
#11	Bahamas	2010	0.39
#12	Macao	2010	0.368
#13	Seychelles	2010	0.358
#14	Sri Lanka	2010	0.357
#15	United States	2010	0.335

Figure 1.2.1 Plastic waste generation per capita in 2020 by country/region

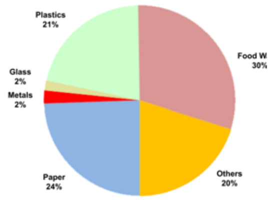


Figure 1.2.2 Composition of MSW disposed of at landfills in percentages in 2020

1.3 Current solution to plastic pollution

Table 1.3 Disadvantages to current solution to treat plastic [11] [12] [13] [14]

Methods	Disadvantages	Preferable?
Incineration	Produce side products such as Municipal Solid Waste Incineration Asd (MSWIBA), harms environment while leaking heavy metals; MSWIBA-HCl treatment and utilisation are cumbersome	No
Landfilling	Lack of aeration so no biodegrading process; Plastic produce greenhouse gases, CO_2 and CH_4, etc	No
Recycling	Plastic melting releases VOCs; rapid quality deterioration after 2-3 cycles	No

As all the above solutions to plastic solutions are not preferable, alternatives to plastic were invented. For tableware, alternatives like paper plates, paper cups, PLA cups, biodegradable paper straws and PLA straws are commonly used nowadays.

1.4 Edible antimicrobial bio-disposables: Kombucha and chitosan

1.4.1 Kombucha as a biodegradable alternative of plastics

Kombucha is a fermented tea beverage prepared as a result of the symbiotic nature of bacterial cultures and yeast, the so-called SCOBY (*Symbiotic Cultures of Bacteria and Yeasts*). Characterised by rich chemical content and healthy properties, kombucha includes organic acids, minerals and vitamins originating mainly from tea, amino acids, and biologically active compounds, particularly polyphenols. [15].
Microbial cellulose's purity and biodegradability makes bacterial cellulose appealing as a biopolymer for numerous applications including biomedics, etc. [1] Kombucha have some B-complex vitamins such as thiamin and niacin. [16]

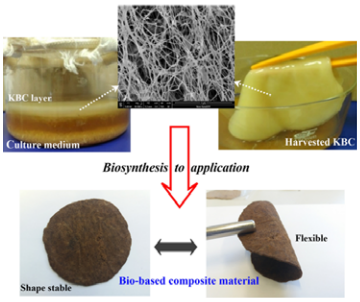


Figure 1.4.1 Biosynthesis to application of kombucha[17]

1.4.2 Kombucha the scoby as bacterial cellulose or tea fungus in a symbiotic system [18]

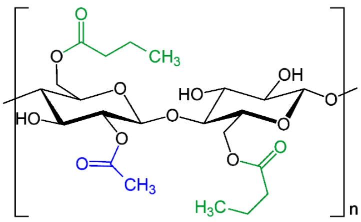


Figure 1.4.2 structure of bacterial cellulose [19]

1.4.3 Edible chitosan as antimicrobial coating

Chitosan, a dietary fibre source that helps aid digestion, can stabilise blood sugar levels, reduce cholesterol, normalise digestive function and maintain satiety after consumption. [3] Chitosan has antibacterial and antifungal properties which qualify it for food protection. Chitosan and its blends with other natural polymers such as starch, essential oils etc., could clay in the field of edible films for food protection. [20] The chitosan coating acts as a barrier to oxygen transfer which inhibits the growth of aerobic bacteria. [21].

1.4.4 Chitin-rich natural sources - crustaceans and *Hermetia illucens*, black soldier fly BSF

Chitin sources are not just from large crustaceans and crabs, but also insects and small crustaceans. As a new and promising raw material , Black Soldier Fly **BSF** (*Hermetia illucens L.*), with its chitinous shell, is domesticated and liable to breeding representatives of invertebrate animals. Their shells are obtained a sufficiently high percentage chitin yield at 21.3% (1281.2 g per year per m^3 of a cage), almost complete absence of residual protein (C = 0.98 µg/ml) and high adsorption ability (X = 156.6 mg/g) of the extracted polysaccharide. [22]

Table 1.4.4 Chitosan sources from numerous species

Material Type	Content of Chitin, %	Material Type	Content of Chitin, %
Crustaceans		Coleoptera Insects	
Crab shells	32.4	Elytra of:	
Shrimp shells	9.7	- Colorado potato beetle	32.2
Shells of crayfish	35.0	- Cock chafer	33.9
Dried Gammarus	26.6	- Common stag beetle	40.0
Frozen Gammarus	26.2	- Ground beetles	36.1
Antarctic krill	2.8-4.5	- Meal worm	29.0
Gladius of a squid	28.0-35.0	- Margined water beetle	34.5
		- Black waterleaf	32.4
		Cuticle of Coleoptera larvae of:	
		-Meal worm	33.0
		-Cock chafer	33.5

1.5 Biodegradability of kombucha and chitosan

1.5.1 Biodegradability of kombucha

A tannin-degrading strain of Bacillus sp. AB1 was isolated from garden soil in which tannase was found to be held responsible for the degradation of tannin. Biodegradability of roasted kombucha disposables in soil was probably due to enzymes such as tannase.

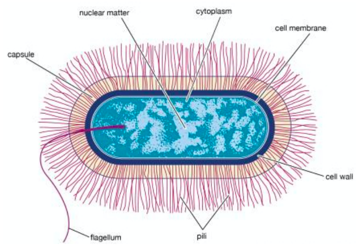


Figure 1.5.1 Structure of bacteria cell [23]

1.5.2 Biodegradability of chitosan

Lysozymes that were used in numerous in vitro experiments of chitosan degradation were regularly from hen egg-white. Human lysozyme has been proved to be more active than hen egg-white lysozyme. Lysozyme cleaves the glycoside bonds between the polysaccharide units in the polymer and degrades chitosan to oligosaccharides. [24]

1.6 Roasting of kombuchas and hydrogel of chitosan

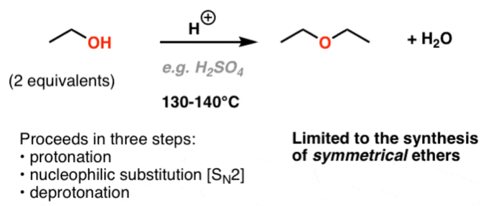


Figure 1.6 Ethers via acid-catalysed dehydration of alcohols [25]

1.7 Measurements of Acrylamide level in roasted kombucha bio-disposables

1.7.1 Testing & certification of food safety of antimicrobial edible bio-disposables of roasted KFC based on [26]

Acrylamide is a chemical substance formed when starchy foods, such as potatoes and bread, are cooked at high temperatures (above 120°C). It can be formed when foods are roasted. [27]
For non-starchy food such as hazelnuts, the only acrylamide formation was detected in treatment of the highest heat (170 °C) and longest time (30 min) with the value of 19 ± 2.5 µg/kg. [28]
The discovery of acrylamide in food is a public health concern because acrylamide is a potential carcinogen and genotoxicant. [29] The levels are 350 micrograms (µg) of acrylamide per kilogram for biscuits and cookies to 750µg per kilogram for potato crisps.
The method entails water extraction of acrylamide, sample enrichment and clean-up by solid-phase extraction cartridges followed by detection at 210 nm. [30] soluble at least to 40% (w/v) in water , and reportedly up to 215 g/100 mL in water at 30°C. [26]

1.7.2 UV-VIS spectrometer: Determination of the content of tannin and acrylamide

Figure 1.7.2 UV-VIS spectrometer [31]

The aforementioned levels [29] and temperature [27] to form acrylamide in starchy and non-starchy food can be measured by entailing water extraction of acrylamide, followed by detection at 210 nm. [30] Solubility is reported at least to 40% (w/v) in water, even 215 g/100 mL in 30°C water at 275 nm [32][26]
Kombuchas (non-starchy foods) do not form acrylamide at 120°C with measurements of UV-vis spectrometer at 210 nm and 275 nm.

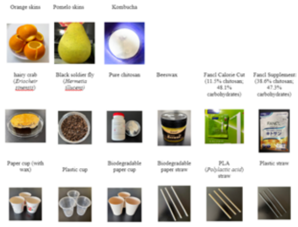
1.8 Detection of surface protein allergen using 3M Clean-Trace ALLTEC60

Muscle tropomyosin isoforms are involved in regulating interactions between actin and myosin in the muscle sarcomere and play a pivotal role in regulated muscle contraction. [32]
3M Clean-Trace ALLTEC60 was used to detect the presence of surface protein allergens on different roasted chitosan coatings.

Figure 1.8 3M Clean-Trace ALLTEC60 for the detection of the presence of surface protein allergens

2. Methodology

Table 2. Samples Used



2.1 Investigation of the structures of KFC before and after roasting using FTIR

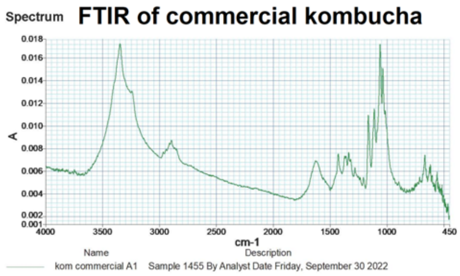
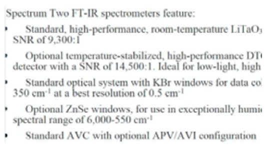


Figure 2.1 FTIR of commercial kombucha

2.1.1 Investigation on the structural change of KFC after roasting at different temperatures and different times using FTIR

Determination of the ratio of absorbance at 3400 cm^{-1} by -OH and absorbance at 1660 cm^{-1} by C=C to investigate the effect of roasting at different temperatures and for different time on the structural changes in kombuchas.

Figure 2.1.1.1 FTIR spectrum II


Spectrum Two FT-IR spectrometers feature:

- Standard, high-performance, room-temperature $LiTaO_3$ SNR of 9,300:1
- Optional temperature-stabilized, high-performance DT detector with a SNR of 14,500:1. Ideal for low-light, high
- Standard optical system with KBr windows for data co 350 cm^{-1} at a best resolution of 0.5 cm^{-1}
- Optional ZnSe windows, for use in exceptionally humi spectral range of 6,000-550 cm^{-1}
- Standard AVC with optional APV/AVI configuration

Figure 2.1.1.2 Specifications of the FTIR spectrum II

2.1.2 Investigation on the structural change of chitosan after roasting at different temperatures and different times using FTIR

Determination of the degree of deacetylation DD% : For roasting at different temperature at different times for chitosan, the effect can be derived as follows:

$$DD[\%] = 100\% - \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \times 100\%\right)$$

Figure 2.1.2 Formula of calculating the degree of deacetylation [33]

1. A_{1655} (cm-1) = absorbance of C=O in amide
2. A_{3450} (cm-1) = absorbance of $-NH_2$ in amide

2.2 Comparing the water absorbance and strength of KFC of orange skins before and after roasting at different temperatures and for different times with paper disposables and other biodegradable disposables

2.2.1 Measuring percentage of water absorbance of different chitosan-coated kombuchas

1. Weigh the dried samples of chitosan-coated kombuchas.

2. Add excess water to the samples.

3. Weigh the wet sample after 1 day.

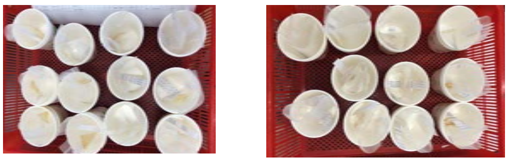


Figure 2.2.1 Dried samples of chitosan-coated kombucha of orange skin roasted at different temperature and different time

2.2.2 Measuring the force required to pierce through 1mm chitosan-coated kombucha

1. Measure the thickness of the chitosan-coated kombuchas using a calliper micrometre.

2. Record the force required to pierce through the chitosan-coated kombuchas using a spring balance.

Figure 2.2.2. Measuring tensile strength using calliper micrometer (left) amd spring balance (right)

2.3 Comparing the biodegradability of different roasted KFC and other disposables

1. Dry samples were weighed.

2. Samples were left in soil and water was added to keep the soil wet.

3. Wet samples were weighed two times every week.

Figure 2.3 Investigation of the biodegradability of different samples in a pot of soil

2.4 Investigating the feasibility of using roasted KFC of orange skins as edible bio-disposables

2.4.1.1 Designing antimicrobial edible bio-disposables of KFC such as drinking straws and cups.

with chitosan coating of crab shell chitosan

with chitosan coating of BSF shell chitosan

with chitosan coating of pure chitosan

with chitosan coating of Fancl Supplement

2.4.1.2 Investigating the use of untreated BSF as antimicrobial chitosan coating

1. 60cm^3 of vinegar was added to 10g of shells of **BSF** for demineralization of $CaCO_3$.

2. 30 cm^3 of vinegar was added to the residue to obtain hydrogel of chitosan of untreated **BSF**.

Figure 2.4.1.2 antimicrobial edible bio-disposables of **KFC** with chitosan coating of untreated **BSF** (without using 2M nitric acid & 16.7M NaOH)

2.4.2 Counting bacterial colonies of drinking water with oral bacteria soaking with different kinds of chitosan coating and commercial disposables

1. Drinking water with oral bacteria soaking with crab chitosan-coated kombucha was allowed to stand overnight.

2. 100 μL of each of the drinking water of dilution factors of 10^{-2}, 10^{-3} & 10^{-4} was spread over the agar of culture solution.

3.Number of bacterial colonies were counted, if any.

2.4.3 Counting bacterial colonies of drinking water kept in roasted cups and straws of KFC

1. Drinking water (without oral bacteria) soaking with roasted **KFC** cups and straws was allowed to stand for 30 mins.

2. 100 μL of the drinking water was spread over the agar of culture solution.

3.Number of bacterial colonies were counted, if any.

2.4.4 Investigation of the food safety of roasted KFC as edible bio-disposables by determining the concentration of acrylamide using UV-VIS spectrometer

1. Standard solutions of acrylamide were prepared by dissolving 5.0 μg/g acrylamide (safety limit based on COMMISSION REGULATION (EU) 2017/2158), 10.0μg and 15μg in 1.0 dm^3 distilled water.

2. About 5g of roasted chitosan-coated kombucha bio-disposables were soaked in 100.0 cm^3 distilled water.

3. Absorbance of each sample solution was recorded using a UV-VIS spectrometer at 210 and 275 nm.

Figure 2.4.4 UV-VIS spectrometer

2.4.5 Detection of surface protein allergens

3M Clean-Trace ALLTEC60 was used to detect the presence of surface protein allergens on different roasted chitosan coating.

Figure 2.4.5 3M Clean-Trace ALLTEC60 for the detection of the presence of surface protein allergens

2.5. Testing and certification

3.5.1 Testing and certification of antimicrobial edible cups of roasted KFC based on GB 18006-2008

(General requirement of disposable plastic tableware, National Standard of the People's Republic of China) [4]
Tasks investigating characteristics of service performance (section 5.4) of disposable cups:

For Volume deviation:

1. Measure the volume of drinking water filled in disposable cups before and after standing for 30 minutes.
2. Repeat using hot water.
3. Calculate the volume deviation i.e. percentage change in volume. The value shall be ≤5%.

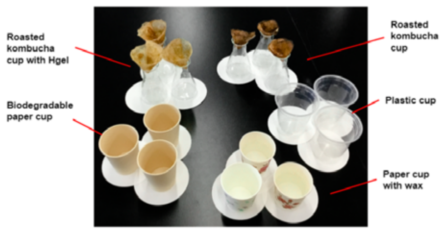


Figure 2.5.1 Samples of cups testing for volume deviation

For Drop performance:

1. At a normal temperature, disposable cups were allowed to drop freely, facing bottom-down, from a height of 0.8 m, onto a level cement floor.

2. The cups were examined to see if it was intact. Samples should have No cracks or splits during drop test of the disposable tableware.

For Hot-water resistance:

After doing the hot-water resistance test, observe if deformations, peelings or wrinkles appeared on the disposable cups.

For Microwave high-frequency heat test:

1. Place the samples in the microwave, heat for 3.5 minutes for the microwave at an output of 600W.
2. Remove the sample and cool it until room temperature.
3. Repeat the procedure. Observe any deformation, defect, leakage and abnormality.

For Water leakage resistance:

1. Place a piece of filter paper under disposable cups.
2. Fill the disposable cups completely with drinking water, at a temperature of 23°C± 2°C, leave the samples standing for 30 minutes.
3. Observe for any leakage of water.

For Microwave heat resistance:

1. Pour approximately 50% of sample volume of olive oil into samples.
2. Put the samples in the microwave until it reaches 200 °C.
3. Take the samples out and leave it until it reaches room temperature.
4. Observe any deformation, defect, leakage and abnormality.

For Hot-oil Resistance:

1. Pour the oil so that it covers the surface of the container.
2. Examine if the disposable tableware has any deformations, peelings or wrinkles.

3.5.2 Testing and certification of antimicrobial edible straws of roasted KFC based on ISO18188:2016 (Section 5.3)
(Specification of polypropylene drinking straws) [5]

For Bending Resistance Test:

1. Disposable straws were bent at 90° and released at five different points along its length (including the flexible part of flexible straws).
2. The straws should be examined thoroughly for any rupture.

For Heat endurance test:

1. Disposable straws were immersed entirely in a beaker filled with (95 ± 2) °C distilled water.
2. The beaker with the straw was put in an oven at a constant temperature of (50 ± 2)°C for 30 min.
3. The straws were removed from the water, spread out and left at ambient temperature for 30 min. It was examined thoroughly for any visible deformations and colour fading.

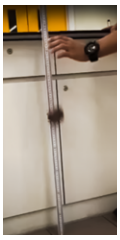


Figure 2.5.2 Drop test

Figure 2.5.3 Hot-oil Resistance

Figure 2.5.4 Samples under microwave high-frequency test

Figure 2.5.5 Bending test

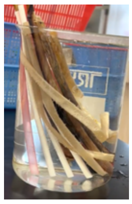


Figure 2.5.6 Samples under heat endurance test

Figure 2.5.7 Samples under cold endurance test

3.5.3 Testing and certification of antimicrobial edible straws of roasted KFC based on DJS348:2019
For Cold Endurance Test:

1. Immerse the straw fully in a beaker filled with (1±1)°C cold water for 30 minutes
2. Take the straw out and immediately bend it 90 degrees at five different points.
3. Examine for any cracks.

3. Results

3.1 Investigating the content of chitin, chitosan, DA% of chitin and DD % of chitosan using FTIR of different antimicrobial coating

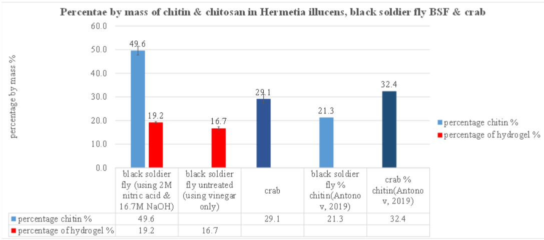


Figure 3.1.2 Percentage of mass of chitin & chitosan in *Hermetia Illucens*, black soldier fly BSF & crab

Conclusion: **BSF** has 49.6% chitin, followed by crab's 33.7%. They are consistent with the literature of 21.3% chitin and 32.4% chitin (Antonov, 2019) respectively.
BSF (treated with 2M nitric acid & 16.7M NaOH), **BSF** (untreated, using vinegar in 2 steps only) and crab have 19.2% and 16.7% respectively.

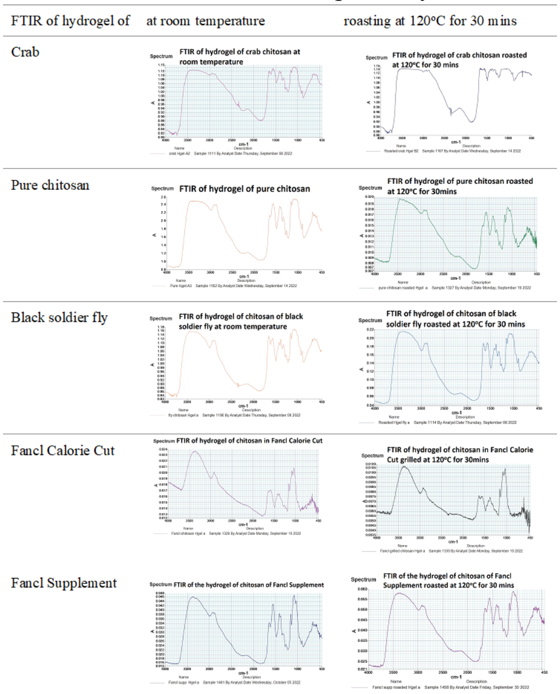


Figure 3.1.3 FTIR graphs of hydrogel and roasted hydrogel of chitosan of different sources

Table 3.1.4 Tensile strength, water absorbance, DD% of hydrogel and roasted hydrogel of chitosan of different sources

Antimicrobial coating	force/ N/mm	no. of times of mass of water absorbed	DD% of the hydrogel of chitosan	DD% of the roasted hydrogel of chitosan
roasted hydrogel of pure chitosan	5.00	4.90	78.5	75.7
roasted hydrogel of crab chitosan	5.12	10.83	71.6	65.4
roasted hydrogel of chitosan black soldier fly BSF	8.64	7.01	74.1	66.2
Fancl Calorie Cut roasted chitosan (100mg/873mg)	14.69	3.56	79.2	75.3
Fancl supplement roasted chitosan (310mg/804mg)	36.67	2.17	84.5	78.9

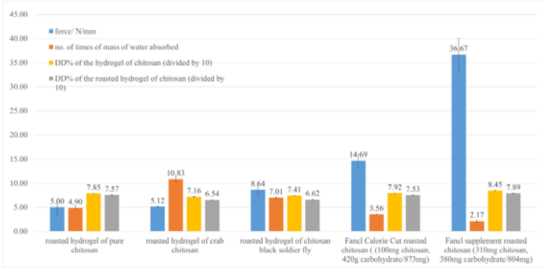


Figure 3.1.5 Tensile strength, water absorbance, DD% of the antimicrobial hydrogel of chitosan of crab, pure chitosan and *Hermetia Illucens*, black soldier fly BSF roasted at 120 degree Celsius

Conclusion: The tensile strength of Fancl Supplement (38.6% chitosan, 47.3% carbohydrates) was the strongest and the waterproofness was the best, followed by that of Fancl Calorie Cut (11.5% chitosan, 88.5% carbohydrates). The tensile strength of roasted hydrogels of Fancl Calorie Cut (14.7N/mm) was the 2nd highest, and then chitosan of **BSF** (8.64N/mm). The tensile strength of roasted hydrogels of crab chitosan and that of pure chitosan were more or less the same. (5.12N/mm & 5.00N/mm). The waterproofness of roasted hydrogels of Fancl Calorie Cut (11.5% chitosan: 88.5% carbohydrate; 3.6 times) was the best, followed by pure chitosan (4.90 times), **BSF** (7.01 times) and crab chitosan (10.83 times).
DD% of hydrogel of chitosan of crab (71.6%) **BSF** (74.1%), Fancl Calorie Cut (79.2%) and Fancl Supplement (84.5%) were consistent with that of pure chitosan (78.5%).
DD% of roasted hydrogel of chitosan of crab (65.4%) and **BSF** (66.2%) were smaller than that of pure chitosan (75.7%) and that of Fancl Calorie Cut (75.3%) and Fancl Supplement (78.9%) was consistent with that of pure chitosan (75.7%) showing that roasting was effective in causing structural changes for all hydrogels of different chitosans and very effective for that of crab chitosan (DD% dropped from 71.6% to 65.4%) and chitosan of **BSF** (DD% decreased from 74.1% to 66.2%).

3.2 Investigating the structural changes of KFC before and after roasting

3.2.1 Investigating the effect of time of roasting at 120°C

3.2.1.1 Roasting of KFC of orange skin with hydrogel of pure chitosan as antimicrobial coating at 120°C for different time

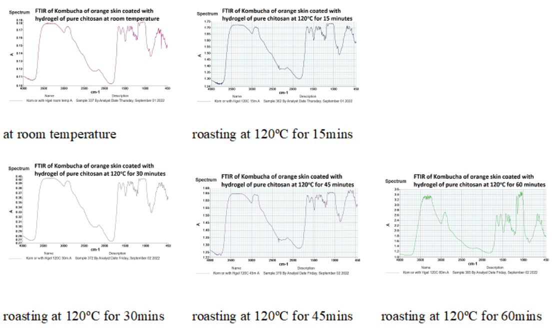


Figure 3.2.1.1.1 FTIR graphs of KFC of orange skin with hydrogel of pure chitosan as antimicrobial coating roasted at 120°C for different time

Table 3.2.1.1.2 Time of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating at 120°C and the ratio of absorbance of -OH γ3400 to C=C γ1600 of kombucha of orange skin)

Time of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating at 120°C /min	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombucha of orange skin)
0	2.33
15	2.39
30	2.07
45	2.12
60	2.14

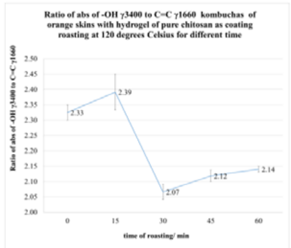


Figure 3.2.1.1.3 Ratio of -OH γ3400 to C=C γ1600 kombuchas of orange skins with hydrogel of pure chitosan as coating roasting at 120 degree Celsius for different time

Conclusion: The ratio of absorbance at γ3400 to that at γ1660 of the **KFC** of orange skin with antimicrobial coating of hydrogel of pure chitosan measured by FTIR Spectrum II dropped sharply (2.39 to 2.07) when the time of roasting was between 15 to 30 minutes, so structural changes should have taken place.

3.2.1.2 Roasting of KFC of orange skin using hydrogel of pure chitosan as antimicrobial at 120ºC for different time

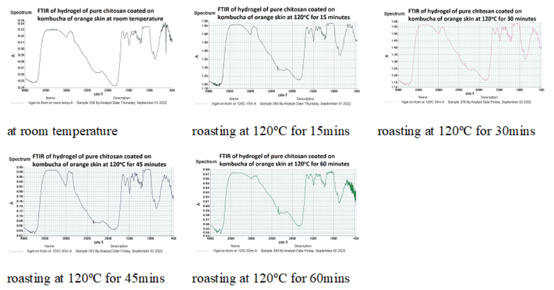


Figure 3.2.1.2.1 FTIR graphs for roasting of KFC of orange skin using pure chitosan as antimicrobial coating at 120ºC for different time

Table 3.2.1.2.2 Time of roasting hydrogel of pure chitosan coated on kombucha of orange skin at 120°C and DD% of coating of hydrogel of pure chitosan

Time of roasting hydrogel of pure chitosan coated on kombucha of orange skin at 120°C /min	DD% (1- ratio of abs v1660 to v3400) x100% of coating of hydrogel of pure chitosan
0	68.5
15	71.0
30	62.0
45	62.0
60	60.4

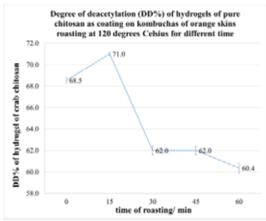


Figure 3.2.1.2.3 DD% of hydrogels of pure chitosan as coating on kombuchas of orange skins roasting at 120 degree Celsius for different time

Conclusion: The DD% of the antimicrobial coating of hydrogel of pure chitosan on the **KFC** of orange skin measured by FTIR Spectrum II dropped sharply (71.0 to 62.0) when the time of roasting was between 15 to 30 minutes, so structural changes should have taken place.

3.2.2 Investigating the effect of temperature of roasting for 30 minutes

3.2.2.1 Roasting of KFC of orange skin with hydrogel of pure chitosan as antimicrobial coating at different temperature for 30 minutes

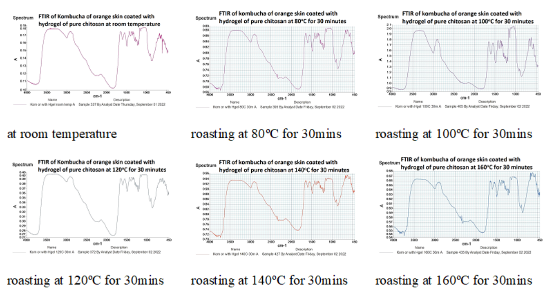


Figure 3.2.2.1.1 FTIR graphs of roasting of KFC of orange skin with hydrogel of pure chitosan as antimicrobial coating at different temperatures for 30 minutes

Table 3.2.2.1.2 Temperature of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating for 30min/°C and ratio of absorbance of -OH γ3400 to C=C γ1600 of kombucha of orange skin

Temperature of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating for 30 minutes / °C	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombucha of orange skin)
25	2.33
80	2.34
100	2.31
120	2.07
140	1.98
160	1.89

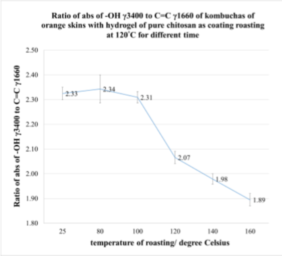


Figure 3.2.2.1.3 Ratio of absorbance of -OH γ3400 to C=C γ1600 of kombuchas of orange skins with hydrogel of pure chitosan as coating roasting at 120 degree Celsius at different time

Conclusion: γ3400 : γ1660 absorbance ratio of the **KFC** of orange skin with antimicrobial coating of hydrogel of pure chitosan measured by FTIR Spectrum II dropped sharply (2.31 to 2.07) with roasting taken place between 100 to 120°C for 30 minutes, so structural changes should have taken place.

3.2.2.2 Roasting of KFC of orange skin using hydrogel of pure chitosan as antimicrobial coating at different temperatures for 30 minutes

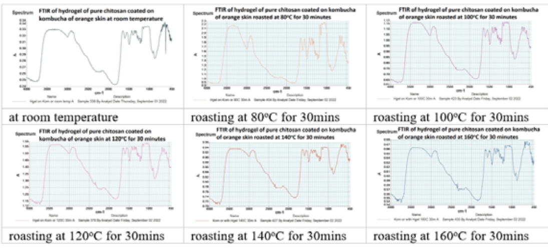


Figure 3.2.2.2.1 FTIR graphs of roasting KFC of orange skin of hydrogel of pure chitosan as antimicrobial coating at different temperatures for 30 minutes

Table 3.2.2.2.2 Temperature of roasting hydrogel of pure chitosan coated on kombucha of orange skins for 30mins and DD% of coating of hydrogel of pure chitosan

Temperature of roasting hydrogel of pure chitosan coated on kombucha of orange skin for 30 minutes / °C	DD% (1- ratio of abs v1660 to v3400) x100% of coating of hydrogel of pure chitosan
25	68.5
80	69.8
100	71.4
120	62.0
140	60.5
160	62.9

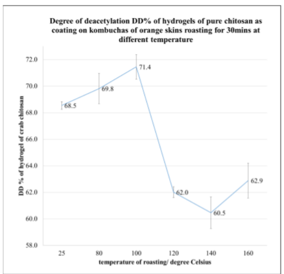


Figure 3.2.2.2.3 DD% of hydrogels of pure chitosan as coating on kombuchas of orange skins roasting for 30 minutes at different temperatures

Conclusion: The degree of deacetylation DD% of the antimicrobial coating of hydrogel of pure chitosan on the **KFC** of orange skin measured by FTIR Spectrum II dropped sharply (71.4 to 62.0) when the temperature of roasting was between 100 to 120ºC for 30 minutes, so structural changes should have taken place.

3.3 Comparing the water absorbance and strength of KFC of orange skins before and after roasting at different temperatures and for different times with paper disposables and other biodegradable disposables

3.3.1 Investigating the water absorbance of antimicrobial edible disposables of KFC before and after roasting

3.3.1.1 Investigating the effect of time of roasting at 120 °C

3.3.1.1.1 Hydrogel of pure chitosan as antimicrobial coating

Table 3.3.1.1.1.1 Time of roasting of kombucha of orange with hydrogel of pure chitosan as coating at 120°C/min and percentage by mass of water absorbed%

Time of roasting of kombucha of orange skin coated with hydrogel of pure chitosan as coating at 120°C/min	Percentage by mass of water absorbed%
0	283
15	299
30	113
45	120
60	80

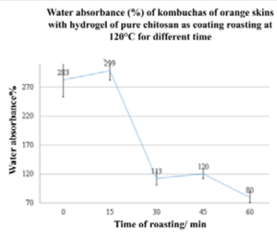


Figure 3.3.1.1.1.2 Water absorbance (%) of kombuchas of orange skins with hydrogel of pure chitosan as coasting roasting at 120 degree Celsius for different time

Conclusion: Water absorbance of **KFC** of orange skin coated with hydrogel of pure chitosan dropped from 3 times to 1 time with 120°C roasting between 15 to 30 minutes, so structural changes took place.

3.3.1.1.2 Beeswax as antimicrobial coating

Table 3.3.1.1.2.1 Time of roasting of kombucha of orange skin with beeswax as coating at 120°C and percentage by mass of water absorbed

Time of roasting of kombucha of orange skin with beeswax as coating at 120°C/min	Percentage by mass of water absorbed %
0	1633
15	1683
30	931
45	862
60	749

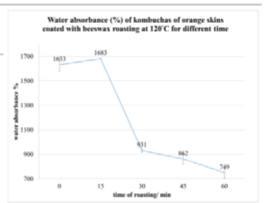


Figure 3.3.1.1.2.2 Water absorbance (%) of kombuchas of orange skins coated with beeswax roasting at 120 degree Celsius for different time

Conclusion: Water absorbance of kombucha of orange skin coated with beeswax dropped drastically from 17 times to 10 times with 120°C roasting between 15 to 30 minutes, representing structural changes that took place.

3.3.1.2 Roasting of KFC of orange skin with different antimicrobial coating at different temperature for 30 minutes

3.3.1.2.1 Hydrogel of pure chitosan as antimicrobial coating

Table 3.3.1.2.1.1 Temperature of roasting of kombucha of orange skin coated with hydrogel of pure chitosan as coating for 30 min/°C

Temperature of roasting of kombucha of orange skin coated with hydrogel of pure chitosan as coating for 30 minutes / °C	Percentage by mass of water absorbed %
25	283
80	214
100	223
120	113
140	133
160	129

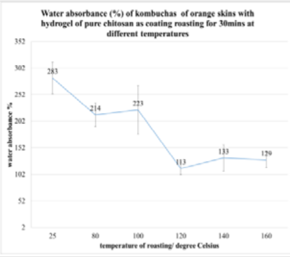


Figure 3.3.1.2.1.2 Water absorbance (%) of kombuchas of orange skins with hydrogel of pure chitosan as coating roasting for 30 minutes of different temperatures

Conclusion: Water absorbance of kombucha of orange skin coated with hydrogel of pure chitosan dropped from 2 times to 1 time with roasting between 100 to 120ºC for 30 minutes, causing Structural changes.

3.3.1.2.2 Beeswax as the antimicrobial coating

Table 3.3.1.2.2.1 Temperature of roasting of kombucha of orange skin coated with beeswax as coating for 30 min/°C

Temperature of roasting of kombucha of orange skin coated with beeswax as coating for 30 min/°C	Percentage by mass of water absorbed %
25	1633
80	1727
100	1189
120	647
140	818
160	661

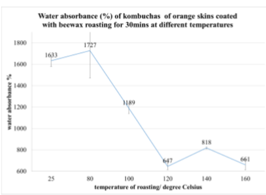


Figure 3.3.1.2.2.2 Water absorbance (%) kombuchas of orange skins coated with beeswax roasting for 30 minutes at different temperatures

Conclusion: Water absorbance of kombucha of orange skin coated with beeswax dropped from 12 times to 6 times with 30-minute roasting between 100 to 120ºC, so structural changes took place.

3.3.2 Investigation on the tensile strength of antimicrobial edible bio-disposables of KFC before and after roasting

3.3.2.1 Investigation on the effect of time of roasting at 120ºC

3.3.2.1.1 Hydrogel of pure chitosan as antimicrobial coating

Table 3.3.2.1.1.1 Time of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating at 120°C/min

Time of roasting of kombucha of orange skin with hydrogel of pure chitosan as coating at 120oC /min	average force to punch 1mm/ N per mm
0	39.2
15	40.3
30	68.8
45	32.4
60	34.7
kombucha only	4.4
roasted kombucha with dried hydrogel of pure chitosan	37.8
biscuit bowl	3.67
paper cup	11.6
biodegradable paper cup	5.6
PLA straw	1.4
biodegradable paper straw	80.6

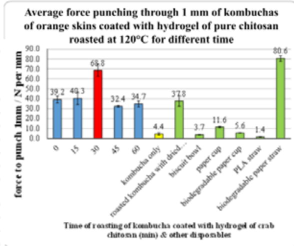


Figure 3.3.2.1.1.2 Average force punching through 1 mm of kombuchas of orange skins coated with hydrogel of pure chitosan roasted at 120 degree Celsius for different time

Conclusion: The tensile strength of **KFC** of orange skin coated with hydrogel of pure chitosan increased drastically from 40.3N/mm to 68.8N/mm with roasting at 120ºC between 15 to 30 minutes, so structural changes took place. The tensile strength was even much higher than paper cups (11.6N/mm), biodegradable paper cups (5.6N/mm) and PLA straws (1.4N/mm).

3.3.2.1.2 Beeswax as antimicrobial coating

Table 3.3.2.1.2.1 Time of roasting of kombucha of orange skin with beeswax as coating at 120°C/min and average force to punch 1mm/N per mm

Time of roasting of kombucha of orange skin with beeswax as coating at 120°C /min	average force to punch 1mm/ N per mm
0	6.4
15	6.4
30	9.6
45	6.8
60	6.4
kombucha only	4.4
roasted kombucha with dried hydrogel of pure chitosan	6.4
biscuit bowl	3.7
paper cup	11.6
biodegradable paper cup	5.6
PLA straw	1.4
biodegradable paper straw	80.6

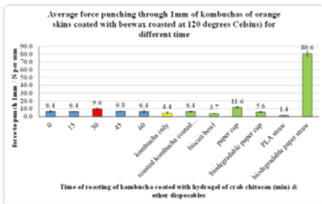


Figure 3.3.2.1.2.2 Average force punching through 1mm o kombuchas of orange skins coated with beeswax roasted at 120 degree Celsius for different time

Conclusion: The tensile strength of kombucha of orange skin coated with beeswax increased from 6.41N/mm to 9.64N/mm with roasting at 120ºC between 15 to 30 minutes, so structural changes took place. Such strength was comparable with paper cups (11.6N/mm), while stronger than biodegradable paper cups (5.6N/mm) and PLA straw (1.4N/mm).

3.3.2.2 Investigation on the change in tensile strength when kombuchas were roasted at different temperature for 30 minutes

3.3.2.2.1 hydrogel of pure chitosan as antimicrobial coating

Table 3.3.2.2.1.1 Time of roasting of hydrogel of pure chitosan-coated kombucha of orange skin and the average force to punch 1mm/ N per mm

Time of roasting of hydrogel of pure chitosan-coated kombucha of orange skin	average force to punch 1mm/ N per mm
25	39.2
80	41.9
100	41.7
120	68.8
140	33.5
160	37.5
kombucha only	4.4
roasted kombucha with dried hydrogel of pure chitosan	37.8
biscuit bowl	3.7
paper cup	11.6
biodegradable paper cup	5.6
PLA straw	1.4
biodegradable paper straw	80.6

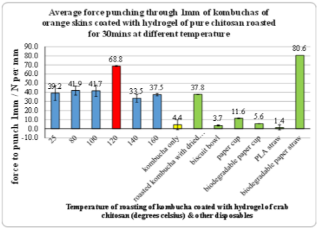


Figure 3.3.2.2.1.2 Average force punching through 1 mm of kombuchas of orange skins coated with hydrogel of pure chitosan roasted from 30 minutes of different temperatures

Conclusion:

The tensile strength of **KFC** of orange skin coated with hydrogel of pure chitosan increased drastically from 41.7N/mm to 68.8N/mm with 30-minute roasting was between 100 to 120ºC, so structural changes took place. The tensile strength was much higher than paper cups (11.6N/mm), biodegradable paper cups (5.6N/mm) and PLA straws (1.4N/mm).

3.3.2.2.2 Beeswax as antimicrobial coating

Table 3.3.2.2.2.1 Average force required to pierce through beeswax-coated kombucha of orange skin roasted at different temperature/ N per mm

Time of roasting of Beeswax -coated kombucha of orange skin	average force to punch 1mm/ N per mm
25	6.4
80	6.3
100	3.2
120	9.6
140	5.8
160	5.6
kombucha only	4.4
roasted kombucha with dried hydrogel of pure chitosan	6.4
biscuit bowl	3.7
paper cup	11.6
biodegradable paper cup	5.6
PLA straw	1.4
biodegradable paper straw	80.6

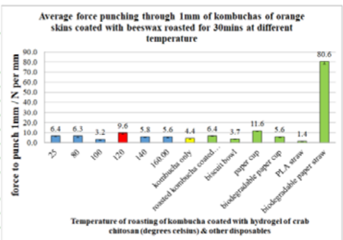


Figure 3.3.2.2.2.2 Tensile strength of beeswax-coated of orange skins roasted at different temperatures and other disposables

Conclusion:

The tensile strength of kombucha of orange skin coated with beeswax increased from 3.21N/mm to 9.64N/mm with 30-minute roasting between 100 to 120ºC, so structural changes took place. The tensile strength was stronger than biodegradable paper cups (5.6N/mm) and PLA straw (1.4N/mm) while comparable with that of paper cups (11.6N/mm).

3.4 Investigating the possibility of using untreated (without using 2M nitric acid & 16.7M NaOH) BSF shells as the source for KFC antimicrobial coating

3.4.1 Investigating the content of chitin and chitosan in untreated BSF (without using 2M nitric acid & 16.7M NaOH), and comparing with that of BSF and crab shells

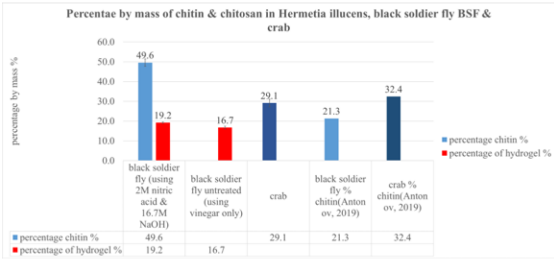


Figure 3.4.1 Comparison of the percentage by mass of chitin and chitosan in BSF and crab shells

Conclusion: When BSF shells were treated with 2 steps of vinegar only (which was much greener than using 2M nitric acid that is strongly oxidizing and 16.7M NaOH which is highly corrosive for deacetylation), the percentage of chitosan was 16.7% which was about half of the % of chitosan obtained after deacetylation (19.2%).

3.4.2 Investigating the DA% of chitin, tensile strength, water absorbance & DD% of hydrogel and roasted hydrogel of chitosan of Black Soldier Fly BSF (untreated: without using 2M nitric acid & 16.7M NaOH)

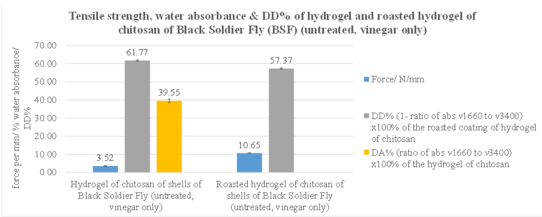
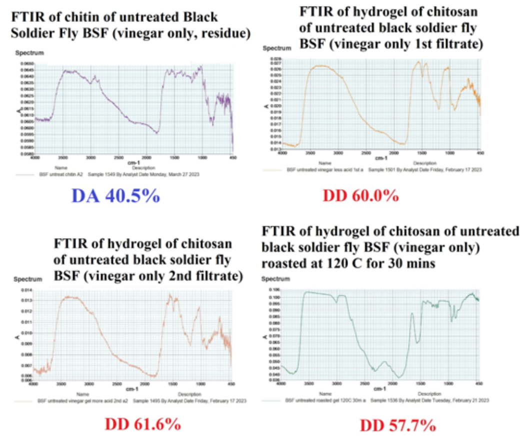


Figure 3.4.1 Tensile strength & DD% of hydrogel and roasted hydrogel of chitosan of Black Soldier Fly BSF (untreated: without using 2M nitric acid & 16.7M NaOH)

Table 3.4.2 FTIR graphs of hydrogels and roasted hydrogels of untreated (without using 2M nitric acid & 16.7M NaOH) BSF without using 2M nitric acid & 16.7M NaOH)



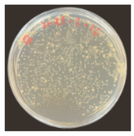
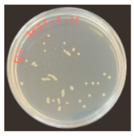
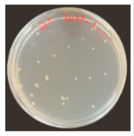
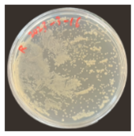
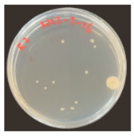
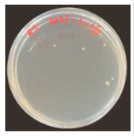
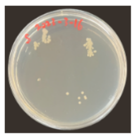
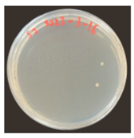
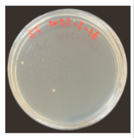
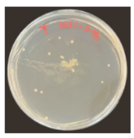
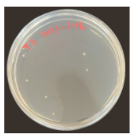
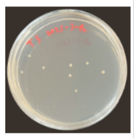
Conclusion:

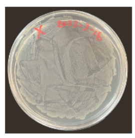
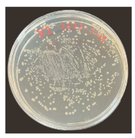
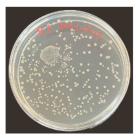
The tensile strength of hydrogel of chitosan of untreated (without using 2M nitric acid & 16.7M NaOH) shells of **BSF** increased from 3.52 N/mm to 10.65 N/mm after roasting, indicating that the tensile strength of hydrogel of chitosan of untreated (without using 2M nitric acid & 16.7M NaOH**)** shells of **BSF** could be increased by roasting.

The DA% of chitin of untreated **BSF** was 39.55%. DD% of hydrogel of chitosan of untreated shells of **BSF** (61.77%) and roasted hydrogel of chitosan of untreated shells of **BSF** (57.35%) showing that even without being treated with 2M nitric acid and 16.7M NaOH for deacetylation, the hydrogel of chitosan of untreated shells of **BSF** shows a high DD% which means a greener way that involves neither strong oxidizing agents such as 2M nitric acid nor corrosive chemicals such as 16.7M NaOH to obtain chitosan for making **KFC** coating is discovered.

3.4.3 Investigation of the antimicrobial effect of untreated BSF shell as antimicrobial chitosan coating materials

Table 3.4.3 No. of bacterial colonies of drinking water with oral bacterial soaked with hydrogel and roasted hydrogel of chitosan of untreated shell of BSF after 2 steps using vinegar only (control: with oral bacteria only)

Count of bacterial colonies after soaking 0.1g of hydrogel with anti-microbial coating in water with oral bacteria	Without Dilution	Dilution factor 1/100	Dilution factor 1/1000
Black Soldier Fly Hydrogel (untreated) After 1st vinegar			
No. of bacterial colonies	N.A	54	24
Roasted Black Soldier Fly Hydrogel (untreated) After 1st vinegar			
No. of bacterial colonies	N.A.	14	6
Black Soldier Fly Hydrogel (untreated) After 2nd vinegar			
No. of bacterial colonies	53	2	3
Roasted Black Soldier Fly Hydrogel (untreated) After 2nd vinegar			
No. of bacterial colonies	48	7	8

Oral bacteria only			
No. of bacterial colonies	N.A	N.A	246

Conclusion: The no. of bacterial colonies found in water with oral bacteria on drinking bottle diluted by 10^2 times and 10^3 times:

Hydrogel of chitosan of **BSF** (untreated) after the first step using vinegar (N.A., 54, 24); Roasted

Hydrogel of chitosan of **BSF** (untreated) after the first step using vinegar (N.A., 14, 6).

Hydrogel of chitosan of **BSF** (untreated) after second step using vinegar (53, 2, 3); Roasted Hydrogel of chitosan of **BSF** (untreated) after the second step using vinegar (48, 7, 8).

(cf. no. of bacterial colonies of control with oral bacteria only: N.A., N.A., 246 w.r.t. dilution factors of 10^{0}, 10^{-2} & 10^{-3}).

It showed that hydrogel of chitosan of **BSF shell** after 2-step vinegar only demonstrated good antimicrobial effect. As the chitosan content after the second step of using vinegar increased, the antimicrobial effect also increased. This showed that it was feasible to use untreated shells of **BSF** as an antimicrobial coating. In fact, it was an innovative greener way of using 2-step vinegar only to obtain antimicrobial chitosan-coating as the antimicrobial coating obtained was edible and ready for direct consumption as only vinegar was used (neither 2M HNO_3 & nor 16.7M NaOH were used for deacetylation)

3.5 Comparing the biodegradability of different roasted kombucha, roasted KFC and other disposables

Table 3.5.1 The percentage change in mass of different cups after immersed in soil every 3 days

Percentage change in mass%	Day 1	Day 4	Day 7	Day 11	Day 14	Day 17	Day 21	Day 24	Day 28	Day 31	Day 35	Day 38	Day 41	Day 35
Roasted kombucha of orange skin	0	-2.5	+ 1	+4	+ 6	-5	-16	-46	-74	-89	-100	-100	-100	-100
Roasted Chitosan-coated kombucha of orange skin (roasted at 120°C)	0	-8	-8	-12	-36	-56	-80	-100	-100	-100	-100	-100	-100	-100
Biodegradable paper straw	0	-7	11	27	28	31	-27	-52	-56	-63	-79	-86	-91	-95
PLA straw	0	2.4	-2	-1	2.4	5.4	2.2	3.1	-2	-10	-12	-13	-17	-25
Plastic straw	0	-0	-0	0.1	-1	-4	-2	-3	-4	-5	-5	-5	-5	-5

Table 3.5.2 The percentage decrease in mass of different disposables in 4 weeks

Samples of straws	Percentage decrease in mass in 4 weeks (%)
Roasted kombucha of orange skin	74
Roasted chitosan-coated kombucha of orange skin	100
Biodegradable paper straw	56
PLA straw	2
Plastic straw	4

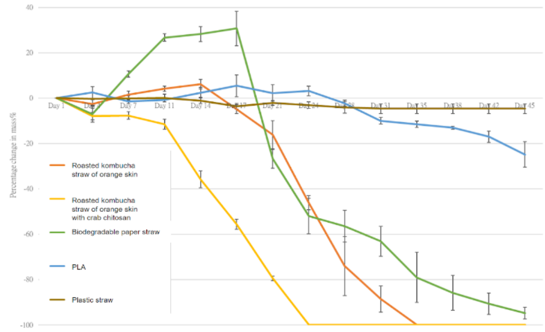


Figure 3.5.3 Percentage change in mass of the tested samples after biodegrading

Conclusion:

Roasted **KFC** straws (of orange skin) were biodegraded completely in 4 weeks and roasted Kombucha straws (of orange skin) were completely biodegraded in 5 weeks, so they are more biodegradable and environmentally friendly than biodegradable paper.
Biodegradable paper straw (biodegraded by 95% in 6 weeks) and PLA straws (25% in 6 weeks). Plastic straws were not biodegradable.

3.6 Investigating the feasibility of using roasted KFC of orange skins as edible bio-disposables

3.6.1 Investigation of the antimicrobial effect of coating materials on kombucha of orange skin

Table 3.6.1.1 No. of bacterial colonies of drinking water with oral bacteria soaked with roasted kombucha of orange skin roasted with hydrogel of crab chitosan coated one side, both sides, and no coating

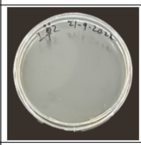
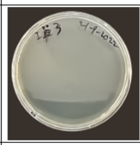
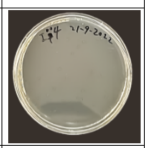
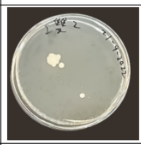
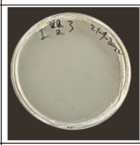
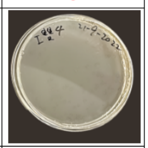
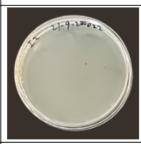
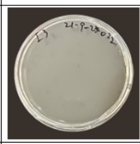
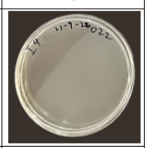
Count of bacterial colonies after soaking 0.1g of kombucha with anti-microbial coating in water with oral bacteria (Roasting done at 120°C for 30 mins)	Dilution factor 1/100	Dilution factor 1/1000	Dilution factor 1/10000
Roasted kombucha of orange skin with hydrogel of crab chitosan (one side coated only)			
No. of bacterial colonies	0	0	0
Roasted kombucha of orange skin with hydrogel of crab chitosan (both sides coated)			
No. of bacterial colonies	3	0	0
roasted hydrogel of crab chitosan			
No. of bacterial colonies	0	0	0

Table 3.6.1.2 No. of bacterial colonies of drinking water with oral bacterial soaked with roasted kombucha of orange skin roasted with hydrogel of chitosan of black soldier fly and pure chitosan (control: with oral bacteria only)

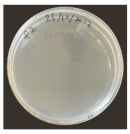
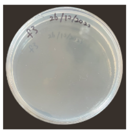
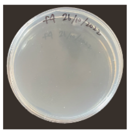
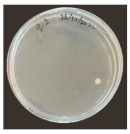
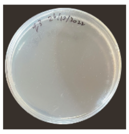
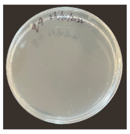
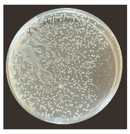
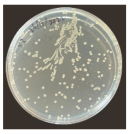
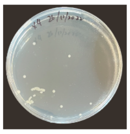
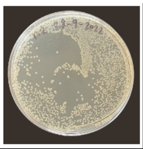
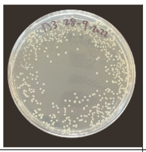
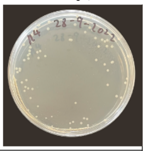
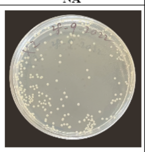
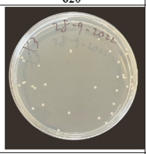
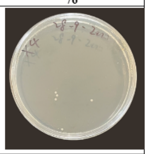
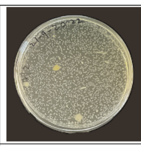
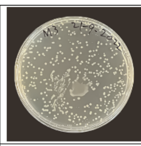
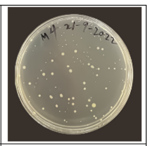
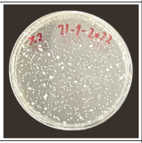
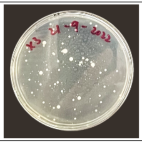
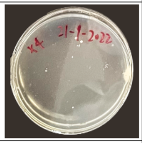
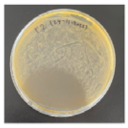
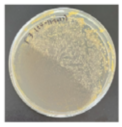
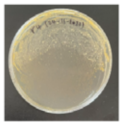
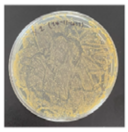
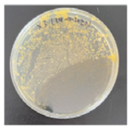
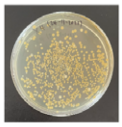
roasted hydrogel of chitosan of black soldier fly			
No. of bacterial colonies	0	0	0
roasted hydrogel of pure chitosan			
No. of bacterial colonies	0	0	0
oral bacteria only			
No. of bacterial colonies	NA	160	40

Table 3.6.1.3 No. of bacterial colonies of drinking water with oral bacterial soaked with biodegradable cup, PLA straw and biscuit cutlery (control: with oral bacteria only)

PLA straw			
No. of bacterial colonies	NA	620	76
oral bacteria only			
No. of bacterial colonies	376	40	6

biodegradable paper cup			
No. of bacterial colonies	NA	585	45
oral bacteria only			
No. of bacterial colonies	NA	150	20

Biscuit cutlery			
No. of bacteria colonies	NA	NA	NA
Oral bacteria only			
No. of bacteria colonies	NA	NA	NA

Conclusion: The no. of bacterial colonies found in water with oral bacteria on drinking bottle diluted by 10^2 times, 10^3 times and 10^4 times:

Roasted kombucha with a hydrogel of crab chitosan coated on one side only (0, 0, 0), that with coating on both sides (3, 0, 0) and roasted hydrogel of crab chitosan only (0, 0, 0). It showed that kombucha was made antimicrobial with roasted hydrogel of crab chitosan as coating on either side of kombucha bio-disposables. (cf. no. of bacterial colonies of control with oral bacteria only: NA, 150, 20 w.r.t. dilution factors of 10^{-2}, 10^{-3} & 10^{-4}).

Roasted kombucha with a hydrogel of chitosan of **BSF** (0, 0, 0), and that of pure chitosan (0, 0, 0). It showed that kombucha was made antimicrobial with roasted hydrogel of chitosan of **BSF** or pure chitosan as coating of kombucha bio-disposables. (cf. no. of bacterial colonies of control with oral bacteria only: 376, 40, 6 w.r.t. dilution factors of 10^{-2}, 10^{-3} & 10^{-4}).

The no. of bacterial colonies found in water with oral bacteria on the drinking bottle diluted by 10^2 times, 10^3 times and 10^4 times with biodegradable paper cups were NA, 585 & 45 and that of PLA straw were NA, 620 & 76 and that of biscuit cutlery were all NA showing that they were not antimicrobial at all.

Table 3.6.1.4 No. of bacterial colonies in drinking water kept in roasted kombucha cups of orange skin coated with pure chitosan, black soldier fly BSF chitosan, crab chitosan, and chitosan from Fancl Calorie Cut

S

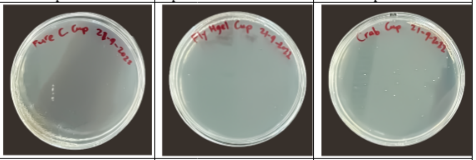
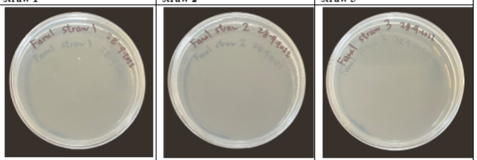
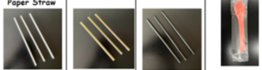
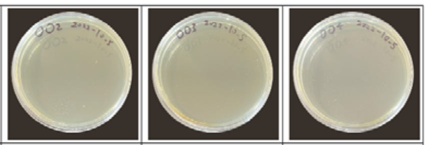
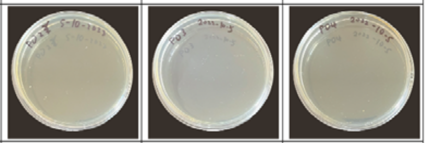
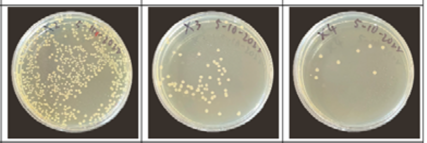
	roasted kombucha (orange skin) with hydrogel of pure chitosan cup	roasted kombucha (orange skin) with hydrogel of fly Hgel cup	roasted kombucha (orange skin) with hydrogel of crab chitosan cup
Disposable soaked in drinking water for 30 mins			
No. of bacterial colonies	0	0	0
	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 1	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 2	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 3
Disposable soaked in drinking water for 30 mins			
No. of bacterial colonies	0	0	0

Table 3.6.1.5 No. of bacterial colonies in drinking water soaked with different disposables (standing in air for a week without wrapping material) for 30 mins

drinking water without oral bacterial	Roasted hydrogel of chitosan of Fancl Calorie Cut 11.5% chitosan	Roasted hydrogel of chitosan of Fancl Supplement 38.6% chitosan	Black Soldier Fly Hydrogel (untreated) After 2-step vinegar	Biodegradable Paper Straw	PLA Straw	Plastic Straw	Biscuit cutlery
							
Disposables standing in air without wrapping for a week							
No. of bacteria colonies	0	3	2	110	39	0	N.A.

Conclusion: No or few bacterial colonies were found in drinking water kept in antimicrobial edible cup of **KFC** using different chitosan coating such as Fancl Calorie cut and untreated **BSF** obtained from the innovative green method of 2-step vinegar and plastic straws that had been standing in air even without wrapping material, so antimicrobial edible bio-disposables of **KFC** were more hygienic than other bio-disposables such as PLA straws, biodegradable paper straws and biscuit spoon, and could save natural resources in making wrapping materials.

Table 3.6.1.6 No. of bacterial colonies of drinking water with oral bacteria soaked with roasted hydrogel of chitosan of Fancl Calorie Cut and Fancl supplement (control: with oral bacteria only)

Roasted hydrogel of chitosan of Fancl Calorie Cut (100mg/873mg)			
No. of bacterial colonies	0	0	0
Roasted hydrogel of chitosan of Fancl Supplement (100mg/873mg)			
No. of bacterial colonies	6	2	1
Oral bacteria only			
No. of bacterial colonies	886	65	9

Conclusion: Roasted hydrogel of chitosan of Fancl Calorie Cut (0, 0, 0), and that with Fancl Supplement (6, 2, 1) w.r.t. dilution factors of 10^{-2}, 10^{-3} & 10^{-4}). It showed that chitosan coating was made antimicrobial with chitosan from current commercial available products (Fancl Calorie Cut and Fancl Supplement). (cf. no. of bacterial colonies of control with oral bacteria only: 886, 65 & 9 w.r.t. dilution factors of 10^{-2}, 10^{-3} & 10^{-4}).

3.6.2 Investigation of the food safety of roasted KFC as edible bio-disposables by determining the concentration of acrylamide using UV vis spectrometer

Figure 3.6.2.1 Standard acrylamide solutions

Table 3.6.2.2 Mass of acrylamide with their absorbance at 210 nm and 275 nm

mass of acrylamide in μg/ml	absorbance at 210nm	absorbance at 275nm
0.10	0.011	0.022
0.08	0.010	0.019
0.05	0.005	0.012
0	0	0

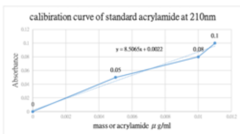


Figure 3.6.2.3 Calibration curve of standard acrylamide at 210nm

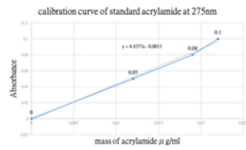


Figure 3.6.2.4 Calibration curve of standard acrylamide at 275nm

Table 3.6.2.5 Mass of acrylamide (μg/kg) in different treated kombucha and safety limits in some food

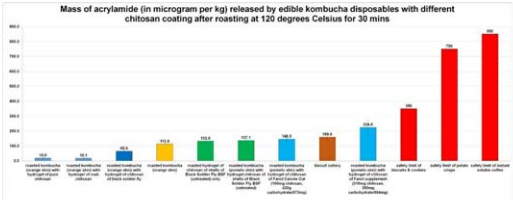


Figure 3.6.2.6 Mass of acrylamide (μg/kg) released by edible kombucha disposables with different chitosan coating after roasting at 120 degree Celsius for 30 minutes

Conclusion: In 1kg of sample, 19.0μg (pure chitosan), 18.1μg (crab chitosan), 65.8μg (black soldier fly **BSF** chitosan), 113.6μg (roasted kombucha only), 137.1μg (untreated shells of BSF:without using 2M nitric acid & 16.7M NaOH) of acrylamide (a carcinogen) per kilogram were found in drinking water soaked with edible **KFC** of orange skin roasted at 120°C for 30 mins.

146.5μg was found when using Fancl Calorie Cut (11.5% chitosan, 48.1% carbohydrates) as edible chitosan-coating and 224.8μg/ kg when using Fancl Supplement (38.6% chitosan, 47.3% carbohydrates) as edible chitosan-coating on roasted kombucha of pomelo skin. 159.6μg/ kg was found in biscuit disposables. These values were within the safety limit of acrylamide: 350μg of acrylamide per kg for biscuits and cookies to 750μg/ kg for potato crisps and 850μg/ kg for instant soluble coffee based on the presence of acrylamide in food [6].

3.6.3 Detection of the presence of surface protein allergens in different chitosan-coating

Figure 3.6.3 Surface protein allergens absent when different roasted hydrogel of chitosan were tested using 3M Clean-Trace ALLTEC60

Conclusion: As surface protein allergens were absent in the roasted hydrogel of chitosan of **BSF** (both untreated using vinegar only and deacetylated using HNO_3 & NaOH), crab, pure chitosan, Fancl Calorie Cut and Fancl supplement, it was unlikely that allergens such as tropomyosin would be present. [32]

3.7 Testing and certification

3.7.1 Testing and certification of antimicrobial edible cups of roasted KFC based on GB 18006-2008

Table 3.7.1 Testing and certification of disposable cups based on GB18006-2008

Section no.	GB 18006-2008	Roasted kombucha cup	Roasted Kombucha of Fruit skin with Chitosan coating (KFC) cup	Biscuit bowl	Bio-degradable paper cup	Plastic cup	Paper cup (waxed)
5.4.1 Volume deviation Standard error stated in ( )	Less than 5%	12.0%(4.0)	5.0%(1.0)	13.0%(3.5)	3.7%(0.3)	4.5%(0.5)	4.5%(0.5)
5.4.3 Drop performance (from 0.8m)	intact	intact	intact	broken into pieces	intact	intact	intact
5.4.5.1 Hot-water resistance (hot water for 30 mins)	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles	deformation with peelings	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles
5.4.5.2 Hot-oil resistance	no deformations, peelings or wrinkles of tableware; no smears or leakages of oil	no deformations, peelings or wrinkles of tableware; no smears or leakages of oil	no deformations, peelings or wrinkles of tableware; no smears or leakages of oil	bowl charred; no smears or leakages of oil	cup softened; no smears or leakages of oil	cup melted; Smears and leakages of oil	cup softened; no smears or leakages of oil
5.4.6 Water leakage resistance	no leakage	no leakage	no leakage	no leakage	no leakage	no leakage	no leakage
5.4.7.1 Microwave high-frequency heat performance	No defects, odour or abnormality	No defects, odour or abnormality	No defects, odour or abnormality	charred	charred	No defects, odour or abnormality	charred

Volume deviation:

Table 3.6.1.1.2 Volume deviation% of different cups after keeping drinking water for 30mins

drinking water 30 mins	volume deviation %
roasted kombucha (orange) cup	12.0
roasted kombucha of orange skin with hydrogel of crab chitosan cup	5.0
biscuit bowl	13.0
bio-degradable paper cup	3.7
plastic cup	4.5
paper cup (waxed)	4.5
plastic cup GB18006-2008	5.0

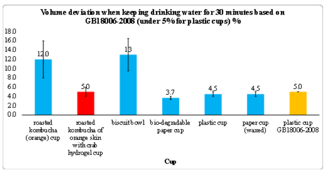


Figure 3.7.1 Volume deviation when keeping drinking water for 30 minutes based on GB18006-2008

Most commercially available disposable cups and roasted chitosan-coated kombucha cups (~5%) met the standard except biscuit bowl (13.0%), so roasted chitosan-coated kombucha cups should be suitable for serving as disposable cups based on GB 18006-2008.

N.B. For tableware such as disposable food boxes, bowls, cups, jars and pots etc. which function as a vessel, the volume deviation thereof shall not be greater than 5% based on GB 18006-2008.

Drop performance:

Most commercially available disposable cups and roasted chitosan-coated kombucha cups passed the drop test with no cracks or splits and remain intact except biscuit bowls (broken into pieces) based on GB 18006-2008.
N.B. During the drop test of disposable tableware, there must be no cracks or splits to any of the three samples based on GB 18006-2008.

Hot-water resistance:

All commercially available disposable cups and roasted **KFC** cups passed the hot-resistance test with no deformations, peelings or wrinkles based on GB 18006-2008.

Water leakage resistance:

All commercially available disposable cups and roasted **KFC** cups passed the water leakage resistance test with no water leakage based on GB 18006-2008.

Microwave high-frequency heat test:

All **KFC** cups and plastic cups passed the microwave high-frequency heat performance with no defects, odour or abnormality based on GB18006-2008. Biscuit bowl, biodegradable paper cup, and waxed paper cup are all charred.

Hot-oil Resistance:

All **KFC** cups passed the hot-oil resistance with no deformations, peelings or wrinkles, and no smears or leakages of oil based on GB18006-2008. Most commercially available disposable products are either softened, charred or melted.

3.7.2 Testing and certification of antimicrobial edible straws of KFC based on ISO18188:2016 (Section 5.3) and DJS 348:2019 (Section 5)

Table 3.7.2. Testing and certification results of roasted chitosan-coated kombucha straws based on ISO18188:2016 and DJS 348:2019

Section no.	ISO18188:2016	Roasted kombucha straw	Roasted Kombucha of Fruit skin with Chitosan coating (KFC) straw	Bio-degradable paper straw	PLA straw	Plastic straw
5.3.1 resistance to bending	no rupture	no rupture	no rupture	no rupture	2.7 ruptures out of 5 points (Standard error: 0.3)	no rupture
5.3.2 heat endurance (in 95°C hot distilled water in an oven at 50°C for 30mins)	no deformations; no fading of colour	no deformations; no fading of colour	no deformations; no fading of colour	no deformations; no fading of colour	bent immediately; no fading of colour	no deformations; no fading of colour
	DJS 348:2019 (Bamboo straw)					
5.4.2 cold endurance test (in 1°C ice water for 30mins; bending at 5 points)	no cracks	no cracks	no cracks	no cracks	5 cracks out of 5 points (standard error: 0)	no cracks

Bending Resistance Test:

Table 3.6.2.1.2 No. of rupture of the tested straws

bending test at 90°C; 5 points	resistance to bending: no. of rupture
roasted kombucha (orange) straw	0
roasted kombucha of orange skin with crab hydrogel straw	0
biodegradable paper straw	0
PLA straw	2.7
plastic straw	0

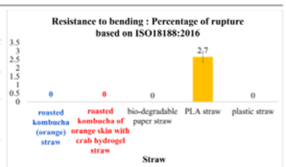


Figure 3.7.2.3 Resistance to bending: percentage of rupture based on ISO18188:2016

All commercially available disposable straws (except PLA & bamboo straws; 2.7 ruptures out of 5) and roasted **KFC** straws showed no rupture in the bending test based on ISO18188:2016.

Heat endurance:

All commercially available disposable straws and roasted chitosan-coated kombucha straws showed neither deformations nor colour fading after testing. Only PLA straws bent immediately when immersed in hot water of 93°±2°C probably due to the uneven expansion of the polylactic acid and bamboo materials used to make the straws based on ISO18188:2016.

Cold endurance:

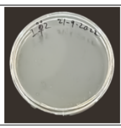
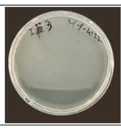
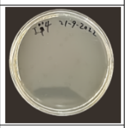
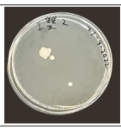
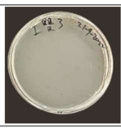
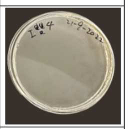
Most commercially available disposable straws and roasted chitosan-coated kombucha straws showed neither deformations nor colour fading after testing. Only all PLA straws cracked when bended at 90º at all five points after immersing in ice water at 1ºC for 30 minutes. probably due to the uneven expansion of the polylactic acid and bamboo materials used to make the straws based on DJS 348:2019.

4. Findings

4.1 Edible antimicrobial bio-disposables of KFC

4.1.1 Value-added roasted bio-disposables of kombucha with roasted hydrogel of crab chitosan coating as antimicrobial agents on KFC

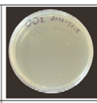
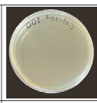
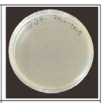
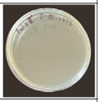
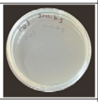
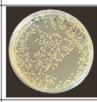
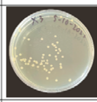
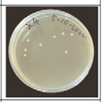
Table 4.1.1 No. of bacterial colonies of roasted kombucha with roasted hydrogel of crab chitosan coating for dilution factors 10^{-2}, 10^{-3} , and 10^{-4}

Count of bacterial colonies after soaking 0.1g of kombucha with anti-microbial coating in water with oral bacteria (Roasting done at 120°C for 30 mins)	Dilution factor 1/100	Dilution factor 1/1000	Dilution factor 1/10000
Roasted kombucha of orange skin with hydrogel of crab chitosan (one side coated only)			
No. of bacterial colonies	0	0	0
Roasted kombucha of orange skin with hydrogel of crab chitosan (both sides coated)			
No. of bacterial colonies	3	0	0

Roasted kombuchas of orange skin with roasted hydrogel of crab chitosan coating on either side and both sides were found to be antimicrobial as no or few bacterial colonies (3 in roasted hydrogel crab chitosan coated on both sides at dilution factor 10^{-2} ; none found for the rest). Such breakthrough implied that kombucha bio-disposables would go antimicrobial by simply applying hydrogel of crab chitosan coating before roasting and still edible bio-disposables of kombuchas of fruit skins would retain ALL the virtues and strengths.

4.1.2 Value-added roasted bio-disposables of kombucha with roasted hydrogel of chitosan of Fancl Supplement & Fancl Calorie Cut as effective antimicrobial agents on KFC

Table 4.1.2 No. of bacterial colonies of roasted kombucha with roasted hydrogel of chitosan of Fancl Calorie Cut, Fancl Supplement, and of oral bacteria of dilution factors 10^{-2}, 10^{-3} , and 10^{-4}(control: with oral bacteria only)

Roasted hydrogel of chitosan of Fancl Calorie Cut (100mg/873mg)			
No. of bacterial colonies	0	0	0
Roasted hydrogel of chitosan of Fancl Supplement (100mg/873mg)			
No. of bacterial colonies	6	2	1
Oral bacteria only			
No. of bacterial colonies	886	65	9

Conclusion:

Not only roasted kombucha with crab chitosan was proved to be antimicrobial according to **4.1.1**, but also roasted hydrogel of chitosan of Fancl Calorie Cut and that of Fancl Supplement. (N.B. no. of bacterial colonies of oral bacteria of roasted hydrogel of chitosan of Fancl Calorie Cut (0, 0, 0), and that of Fancl Supplement (6, 2, 1) while the no. of bacterial colonies of control with oral bacteria only: 886, 65 & 9 w.r.t. dilution factors of 10^{-2} , 10^{-3} & 10^{-4}). Probably, even with a low concentration of chitosan at around only 10%, such chitosan coating would exhibit antimicrobial effect.

Such breakthrough of using Fancl Calorie Cut (11.5% chitosan) and Fancl Supplement (38.6% chitosan) as antimicrobial agents implied that they could be applied as edible antimicrobial agents on edible bio-disposables of kombucha of fruit skins.

4.1.3 Pure chitosan coating as a better antimicrobial coating than beeswax coating

Although both hydrogel of pure chitosan coating and beeswax coating perform a structural change at roasting with 100°C to 120°C between 15 to 30 minutes, pure chitosan coating had a better performance than beeswax coating in experiments.
In terms of water absorbance, according to **3.3.1.1** and **3.3.1.2,** the percentage by mass of water absorbed by hydrogel of pure chitosan coating is much lower than that of beeswax no matter in any roasting time and temperature. This implied that chitosan-coating demonstrated better water-proof property.
In terms of tensile strength, according to **3.3.2.1** and **3.3.2.2**, the average force to punch 1mm of hydrogel of pure chitosan coating is much greater than that of beeswax no matter in any roasting time and temperature.
Therefore, pure chitosan coating is a better coating than beeswax coating in terms of water proofness and tensile strength.

Table 4.1.3 Comparison of water absorbance and tensile strength of kombucha with different antimicrobial coating

Kombucha roasted at 120°C for 30 mins with different antimicrobial coating	Water absorbance/ times	Force applied to punch through 1mm/ N per mm
Pure chitosan	1 time	68.8
Beeswax	6 times	9.6

4.2 Food safety of roasted KFC as edible antimicrobial bio-disposables

4.2.1 Content of acrylamide within safety level

Table 4.2.1 Mass of acrylamide formed when KFC (orange skin) with different hydrogel of chitosan coating were roasted at 120°C for 30 minutes

Chitosan coating on kombucha of orange skin		Mass of acrylamide (µg / kg)
Absent		113.6
Chitosan of lower molecular weight	Pure chitosan [40]	19.0
	Crab chitosan (140-kDa)	18.1
Chitosan of higher molecular weight	chitosan of black soldier fly (680-kDa)	65.8
Edible chitosan-containing commercial products	chitosan of Fancl Calorie Cut (100mg/873mg or 11.5% chitosan, 48.1% carbohydrates)	146.5
	chitosan of Fancl Supplement (310mg/804mg or 38.6% chitosan, 47.3% carbohydrates)	224.8

N.B. safety limit of acrylamide are 350 micrograms (µg) of acrylamide per kilogram for biscuits and cookies to 750µg per kilogram for potato crisps and 850µg per kilogram for instant soluble coffee based on the presence of acrylamide in food. [6]acrylamide

The content of acrylamide of both roasted kombucha with hydrogel of pure chitosan (19.0 µg/kg), crab chitosan (18.1µg/kg) and back soldier fly (65.8 µg/kg) were within 100 µg per kg while that of Fancl Calorie Cut (11.5% chitosan, 48.1% carbohydrates) and Fancl Supplement (38.6% chitosan, 47.3% carbohydrates) were 146.5µg/kg and 224.8µg/kg respectively. All of them were within the safety level of acrylamide. As starch is a main source of the formation of acrylamide, [27] when being roasted, starchy sources of chitosan (Fancl Calorie Cut and Fancl Supplement) have a relatively higher amount of acrylamide, but still within the safety limit of acrylamide. [6] All antimicrobial **KFC** of orange skin were safe for consumption.
Besides, **KFC** such as that coated with pure chitosan (19.0 µg/kg), crab chitosan (18.1 µg/kg) and chitosan of black soldier fly **BSF** (65.8 µg/kg) seemed to demonstration a reduction in the amount of acrylamide formed during roasting. (cf. roasted kombucha without coating: 146.5 µg/kg) This would be discussed in **5.4.3**.

4.2.2 Real-life application of antimicrobial edible bio-disposables of KFC on the quality of drinking water

Table 4.2.2.1 No. of bacterial colonies in drinking water kept with bio-disposables of kombucha of fruit skin with different chitosan-coating for 30 minutes

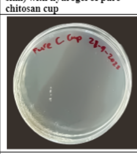
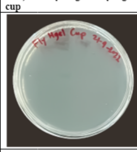
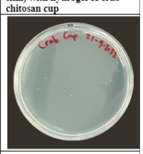
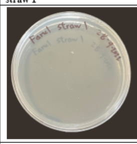
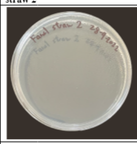
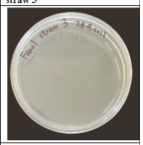
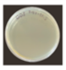
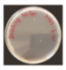
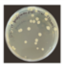
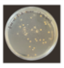
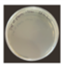
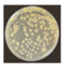
	roasted kombucha (orange skin) with hydrogel of pure chitosan cup	roasted kombucha (orange skin) with hydrogel of fly Hgel cup	roasted kombucha (orange skin) with hydrogel of crab chitosan cup
Disposable soaked in drinking water for 30 mins			
No. of bacterial colonies	0	0	0
	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 1	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 2	roasted kombucha (orange skin) with hydrogel of chitosan of Fancl Calorie Cut straw 3
Disposable soaked in drinking water for 30 mins			
No. of bacterial colonies	0	0	0

Table 4.2.2.2 No. of bacterial colonies in drinking water soaked with different disposables (standing in air for a week without wrapping material) for 30 mins

drinking water without oral bacterial	Roasted hydrogel of chitosan of Fancl Calorie Cut 11.5% chitosan	Roasted hydrogel of chitosan of Fancl Supplement 38.6% chitosan	Black Soldier Fly Hydrogel (untreated) After 2-step vinegar	Biodegradable Paper Straw 	PLA Straw 	Plastic Straw 	Biscuit cutlery 
Disposables standing in air without wrapping for a week							
No. of bacteria colonies	0	3	2	110	39	0	N.A.

As shown in the above, bacterial colonies were present in drinking water soaked with disposables without wrapping material such as PLA straws (110), biodegradable paper straws (39) and biscuit spoon (too many). On the other hand, no bacterial colonies were found when soaked with plastic straws and antimicrobial edible straw of **KFC** cup showing that the chitosan coating of Fancl Calorie Cut (11.5% chitosan, 48.1% carbohydrates) and chitosan of untreated **BSF** were effective antimicrobial agents. This also showed that even a low concentration of chitosan would be enough for carrying out an excellent antimicrobial performance and hence save natural resources for making wrapping materials.

In **3.4.3**, an innovative greener way to obtain antimicrobial chitosan-coating of untreated **BSF** by the 2-step vinegar only method demonstrated good antimicrobial effect. As the chitosan content after the second step using vinegar increased, the antimicrobial effect also increased. This showed that it was feasible to use untreated shells of black soldier fly **BSF** as an antimicrobial coating. The antimicrobial coating obtained was edible and ready for direct consumption as only vinegar was used (neither 2M HNO_3 & nor 16.7M NaOH were used for deacetylation)

Moreover, in **3.5.1**, antimicrobial ability of roasted **KFC** was again confirmed as no bacterial colonies were found in water with oral bacteria on the drinking bottle soaked with roasted **KFC** with all sorts of chitosan coating including chitosan in crab shell, **BSF** shell, Fancl Calorie Cut and Fancl Supplement overnight.

Such breakthrough in applying chitosan coating on edible kombucha of fruit skins as antimicrobial agent would bring a revolutionary change to the concept of function of cutlery as using chitosans as antimicrobial agents were safe for consumption and edible and at the same time keep cutleries from bacterial action during storage and provision of food and catering services. That means the use of antimicrobial edible bio-disposables of **KFC** would save lives and natural resources such as wrapping paper.

4.2.3 Absence of surface protein allergens

Muscle tropomyosin isoforms are involved in regulating interactions between actin and myosin in the muscle sarcomere and play a pivotal role in regulated muscle contraction. [32] In **3.5.3**, surface protein allergens were found absent in the roasted hydrogel of different sources of chitosan (crab shell, both untreated (using vinegar only) and deacetylated (using HNO_3 & NaOH) **BSF** shells, pure chitosan, Fancl Calorie Cut and Fancl Supplement) using 3M Clean-Trace ALLTEC60. As the allergic effect is caused by proteins such as tropomyosin, absence of proteins in the above chitosan coatings confirmed that they would not cause any allergic effect. In particular, shells of black soldier flies **BSF** separate from the insect itself during the process of ecdysis when the pupae turn into adults in their life cycle. Therefore, antimicrobial edible bio-disposables of kombuchas of chitosan coating should be non-allergic and safe for consumption.

Figure 4.2.3 Surface protein allergens absent when different roasted hydrogel of chitosan were tested using 3M Clean-Trace ALITEC60

4.3 Biodegradability of antimicrobial edible bio-disposables of roasted KFC

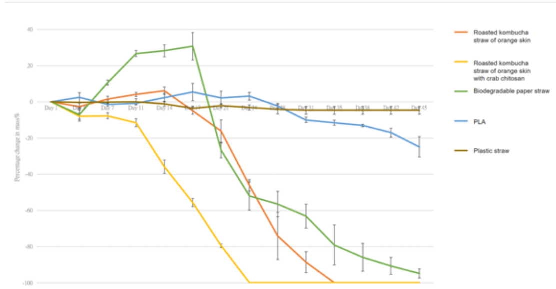


Figure 4.3 Percentage change in mass of different samples after immersing into soil

Our antimicrobial edible roasted bio-disposables of **KFC** showed excellent biodegradability for both with and without chitosan coating. Roasted kombucha without chitosan coating was completely biodegraded in 5 weeks while **KFC** (with roasted chitosan coating) even completely biodegraded in a span of 4 weeks, which in application can produce zero waste swiftly, when compared with the biodegradation of biodegradable paper straws which was slow and incomplete.

4.4 Black soldier fly BSF as a promising chitosan-rich source of antimicrobial agent

Shells of black soldier fly **BSF** are excellent sustainable sources of chitosan as antimicrobial agents. The application of the chitosan coating of **BSF** not only makes kombucha bio-disposables antimicrobial but also enhances the tensile strength, waterproofness and biodegradability of **KFC** bio-disposables.
The tensile strength of roasted hydrogels of chitosan of **BSF** was 8.64N/mm which was even stronger than that of crab chitosan (5.12N/mm) and that of pure chitosan (5.00N/mm).

The waterproofness of roasted hydrogels of **BSF** was 7.01 times which was also better than that of crab chitosan (10.83 times).
Moreover, the DD% of hydrogel of chitosan of **BSF** (74.1%) was similar to that of crab (71.6%), Fancl Calorie Cut (79.2%) and pure chitosan (78.5%).
DD% of hydrogel of chitosan of untreated **BSF** obtained from an innovative greener way of using 2-step vinegar only (neither using HNO_3 nor NaOH) was 61.8% and the tensile strength of the respective roasted hydrogel was 10.7N/mm. The percentage of yield of chitosan was 6.1% (cf. 19.2% after using HNO_3 & NaOH).
Shells of **BSF** were obtained with a sufficiently high percentage chitin yield at 21.3% (1281.2 g per year per m^3 of a cage), almost complete absence of residual protein (C = 0.98 μg/ml) and high adsorption ability (X = 156.6 mg/g) of the extracted polysaccharide. [22] As there is an increase in entomophagy, i.e, eating insects to provide our swelling population with all the same proteins, fats, amino acids, vitamins, and minerals as traditional livestock, but without the strain on our natural resources, applying chitosan-coating of **BSF** to cutlery such as edible bio-disposables of **KFC** would be another breakthrough in the production of edible antimicrobial bio-disposables.

4.5 ISO18188:2016 and DJS 348:2019

Table 4.5 Testing & certification of characteristics of roasted kombucha of Fruit Skin with Chitosan coating (KFC) straws based on section no. 5.3.1 & 5.3.2 in ISO18188:2016 & DJS 348:2019

Section no.	ISO18188:2016 5.3.1 resistance to bending	ISO18188:2016 5.3.2 heat endurance (in 95°C hot distilled water in an oven at 50°C for 30mins)	DJS 348:2019 5.4.2 cold endurance test (in 1°C ice water for 30mins; bending at 5 points)
Roasted Kombucha of Fruit Skin with Chitosan coating (KFC) straw	**no rupture**	**no deformations; no fading of colour**	**no cracks**

Antimicrobial edible bio-disposables of **KFC** straws were eligible for marketing.

4.6 GB 18006-2008

Table 4.6 Testing & certification of characteristics of antimicrobial edible cups of roasted KFC based on section no. 5.4.1, 5.4.3, 5.4.5.1, 5.4.5.2, 5.4.6 and 5.4.7.1 in GB 18006-2008

Section no.	5.4.1 Volume deviation Standard error stated in ( )	5.4.3 Drop performance (from 0.8m)	5.4.5.1 Hot-water resistance (hot water for 30 mins)	5.4.5.2 Hot-oil resistance	5.4.6 Water leakage resistance	5.4.7.1 Microwave high-frequency heat performance
GB 18006-2008	Less than 5%	intact	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles of tableware; no smears or leakages of oil	no leakage	No defects, odour or abnormality
Roasted Kombucha of Fruit skin with Chitosan coating (KFC) cup	**5%(1.0)**	**intact**	**no deformations, peelings or wrinkles**	**no deformations, peelings or wrinkles of tableware; no smears or leakages of oil**	**no leakage**	**No defects, odour or abnormality**

Antimicrobial edible bio-disposables of **KFC** cups were eligible for marketing.

5. Discussion

5.1 Application of edible antimicrobial bio-disposables of KFC straws in food and catering services

Nowadays, straws are provided by eateries worldwide and available for free in restaurants. Disposable straws are usually placed and stored in containers or drawers without covering, so bacterial action would take place resulting in contamination of the rest of the stock of straws. Customers are alarmed and there is a global rise in hygienic concerns about the above issue especially during the pandemic. The situation is worsened as some eateries even provide straws with individual packaging to tackle this hygienic issue producing more wastes of wrapping paper, plastic wrap etc. thus intensifying plastic pollution.

Table 5.1 Comparison of antimicrobial effect, tensile strength, water absorbance and mass of acrylamide formed of different bio-disposables of **KFC**

	As antimicrobial agent in drinking water with oral bacterial: no. of bacterial colonies w.r.t. dilution factor of 10^{-2}, 10^{-3} & 10^{-4}).	No. of bacterial colonies in drinking water for 30mins	Force applied to punch through 1mm/ N per mm	Water absorbance/ times	Mass of acrylamide formed/μg per kg @
Roasted kombucha without coating	many	N.A.	3.92	9	113.6
Crab chitosan	1,0,0, (cf. control many, 150,20)	0	5.12	11	18.1
Fancl Calorie Cut#	0,0,0 (cf. control 886,65,9)	0	14.7	4	146.5
Fancl supplement*	6,2,1 (cf. control 886,65,9)	0	36.7	2	224.8
Roasted chitosan of untreated **BSF** (vinegar only; neither HNO_3 nor NaOH used)	48,7,8 w.r.t. dilution factor of no dilution, 10^{-2}, & 10^{-3}) (cf. control many, many, 246)	N.A.	10.7	N.A.	133.6

#100mg/873mg or 11.5% chitosan, 48.1% carbohydrates
*310mg/804mg or 38.6% chitosan, 47.3% carbohydrates
@ safety limit of acrylamide: 350 micrograms (μg) of acrylamide per kilogram for biscuits and cookies to 750μg per kilogram for potato crisps and 850μg per kilogram for instant soluble coffee based on the presence of acrylamide in food. [32]

As a seemingly appealing alternative to plastic problems, stainless steel straw has a high recyclability, hardness and reusability. Yet, their fluctuating quality range and possible release of heavy metal has proven to be extremely damaging to our health. Chromium is especially infamously abundant due to its anti-rusting ability applied to stainless steel straw, while it can cause occupational asthma, eye irritation and damage, perforated eardrums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer, skin irritation, and erosion and discoloration of the teeth. Worse still, taking in too much manganese may cause loss of appetite, slowed growth, anaemia, reproductive issues, etc. Severe symptoms are suggested for liver patients, like headache, insomnia, memory loss, irreversible nerve damage which may cause Parkinson's Disease, and reproductive problems.

With our roasted edible **KFC** straw, as it has strong antimicrobial ability, there is no need for extra individual packaging. As proteins were neither present in crab shells nor shells of black soldiers fly

BSF, **KFC** bio-disposables should be non-allergic. The content of acrylamide formed during roasting of hydrogel of **KFC with** crab chitosan was 18.1μg/kg which was way below safety limits [6] probably due to the inhibition of Maillard reaction exhibited by crab chitosan. [34] Marketing of antimicrobial edible bio-disposables of **KFC** is a brand-new breakthrough as they are edible and ego-friendly, so less or even no wastes would be produced. Even some used edible bio-disposables of **KFC** are not consumed, they would be biodegraded in 3.5 weeks in soil and hence help preserve the environment.

5.2 Versatility of chitosan coating

From **3.3**, it was obvious that roasted bio-disposables of **KFC** perform better after coating them with chitosan, as the coating greatly enhanced the water proofness (with coating: absorb 1 time of water; without coating: absorb 9 times of water) and tensile strength of **KFC** (with coating: 68.8N/mm; without coating: 4.4N/mm). Besides the fact that **KFC** could be biodegraded in 3.5 weeks and it is antimicrobial, chitosan coating can also be applied to other commercial disposables like biodegradable paper straws to strengthen them. During the use of biodegradable paper straws, water is absorbed and the straws will soften fast. Beeswax coating on roasted kombucha was also tested. However, results showed that the tensile strength of kombucha bio-disposables remained more or less the same. Chitosan coating could be applied to the kombucha bio-disposables and other commercial disposables. Investigation could be done in further studies.

5.3 Nutritional values of edible antimicrobial bio-disposables of KFC

It was found that no bacterial colonies were present in drinking water soaked with roasted **KFC** cups and straws. As the nutritional values of both kombucha solution and kombuchas scoby are high, further studies could be done on the possibilities of treating diseases such as AIDS, ageing, anorexia, arthritis, atherosclerosis, cancer, constipation, and diabetes using antimicrobial edible bio-disposables of roasted **KFC** of orange skin. Also, chitosan is used as the antimicrobial coating in our project. Some studies show that chitosan helps reduce fat and cholesterol the body absorbs from foods (Cristina Moraru et al. #). Hence chitosan has the ability to deal with high blood pressure, high cholesterol, obesity and wound healing.

5.4 Antimicrobial edible bio-disposables of KFC as alternative to make disposables

5.4.1 Using Antimicrobial edible bio-disposables of KFC to replace the use of plastics in making bio-disposable straws and cups

Antimicrobial edible bio-disposables of **KFC** straws and cups have comparable performance with higher biodegradability than those made of plastic, so they should be eligible alternatives to the environmentally unfriendly plastic. Ultimately a plastic-free society would be achieved. Our roasted **KFC** straws completely biodegraded in 3.5 weeks, which was much faster than that of biodegradable paper straws. When plastic disposables in takeaways are to be phased out in the 4th season, 2023, according to Policy Address (HKSAR government, 2022), our antimicrobial edible bio-disposables of roasted **KFC** can be alternatives of plastic disposables and meet the demand of customers.

5.4.2 Antimicrobial edible bio-disposables of KFC as alternative to edible disposables

Other edible disposables such as biscuit cutlery [35] and eco-friendly and biodegradable edible utensils made of flours having about 65% w/w or above carbohydrate content [36] are basically food shaped into disposables.

For the cookie straw, its tube body is made of cookies. However, sustainability cannot be achieved in this case, as starch, a food source, is involved. Together with its hygienic concerns, our antimicrobial edible straw of **KFC** has a landslide advantage over the cookie straw.

The biodegradable antimicrobial straw with PLA, PBS, plasticiser, etc, is another patented product that may be an alternative to plastic straw. Unfortunately, with the addition of plasticizer to strengthen PLA, it is not environmentally friendly. Also, the degrading progress of PLA takes a much longer time than our edible bio-disposables of roasted **KFC**. It can be seen that antimicrobial edible bio-disposables of **KFC** are better solutions to the current plastic pollution compared to the similar patented products.

The investigation and marketing of antimicrobial edible bio-disposables of **KFC** is an innovation and probably a better alternative than existing edible cutlery as **KFC** is a new material and its strength and water-proofness could be enhanced by roasting. It can be seen that our antimicrobial edible bio-disposables of **KFC** surpassed all the other competitors such as PLA disposables and biodegradable paper disposables in our experiments. Above all, with the antimicrobial ability of the chitosan coating, hygiene can be ensured. When antimicrobial edible bio-disposables of **KFC** were shaped into cups and straws, they fulfilled many requirements based on GB 18006-2008, ISO18188:2016 and COMMISSION REGULATION (EU) 2017/2158 on the presence of acrylamide in food [6]. Hence antimicrobial edible bio-disposables of **KFC** are better bio-disposables and hence should be eligible for marketing.

Table 5.4.2 Comparison Between Antimicrobial Edible Bio-Disposables of KFC and similar properties patented products

	Antimicrobial edible bio-disposables of chitosan-coated kombucha	**Antibacterial disposable absorbent article**	**Cookie straw**	**Biodegradable antibacterial straw**
Description	A bio-disposable consisting of edible kombucha obtained from fruit skins, and an antimicrobial edible chitosan coating that can: a) prevent quality deterioration as a result of unhygienic storage or usage.	A disposable article comprising a coating of an antimicrobial composition on a surface within the disposable article, wherein the antimicrobial composition comprises: a) a carrier comprising an aliphatic alcohol and a poly (alkylene dioxy) polymer b) 5-20% by weight of an antibacterial agent	A cookie straw with an oblique cutting opening, which includes a tube body having a thin long tube structure. Two ends of the tube body respectively have an opening. The tube body is made of edible cookies.	Biodegradable antibacterial straw using acid soluble chitosan. It comprises the following components PBS, PLA, inorganic filler, chitosan, coupling agent, plasticizer, antioxidant and nucleating agent.
Reference	/	[37]	[38]	[39]
Strengths	Antimicrobial Edible Biodegradable Social sustainable	Antimicrobial	Edible	Biodegradable Antibacterial
Weaknesses	Nil	Not edible Not biodegradable Not social sustainable as it is alcohol-based	Not biodegradable Not social sustainable as it used starch as raw material Not antimicrobial	No edible Addition of Plasticizer to strengthen PLA

5.4.3 Acrylamide-inhibiting ability of chitosan during roasting of KFC

Acrylamide inhibition takes place when chitosans, especially of lower molecular weight, are present during the roasting process. [34] Chitosan inhibits the formation of acrylamide by interrupting and intercepting the Maillard reaction. Those of lower molecular weight, such as shrimp(280 kDa) and crab shells(140 kDa) [40], show a larger effect in inhibiting acrylamide than those of higher molecular weight, such as **BSF** (approximately 680 kDa). [41]

Table 5.4.3.1 Mass of acrylamide formed of roasted KFC (orange skin) with different roasted hydrogel of chitosan coating

Chitosan coating on kombucha of orange skin		Mass of acrylamide/μg per kg
Without coating		113.6
Chitosan of lower molecular weight	Pure chitosan (100-200 kDa) [42]	19.0
	Crab chitosan (140-kDa)	18.1
Chitosan of higher molecular weight	Chitosan of black soldier fly (680-kDa)	65.8
Edible chitosan-containing commercial products	Chitosan of Fancl Calorie Cut #	146.5
	Chitosan of Fancl Supplement *	224.8

#100mg/873mg or 11.5% chitosan, 48.1% carbohydrates

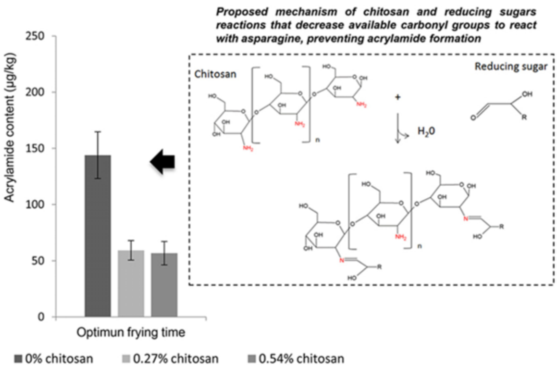
*310mg/804mg or 38.6% chitosan, 47.3% carbohydrates

@ safety limit of acrylamide: 350 micrograms (μg) of acrylamide per kilogram for biscuits and cookies to 750μg per kilogram for potato crisps and 850μg per kilogram for instant soluble coffee based on the presence of acrylamide in food. [6]

Table 5.4.3.2 Molecular weight ranges of different types of chitosan

Type of Chitosan	Molecular Weight Range
Oligochitosan	4.7 kDa
Low molecular weight chitosan (LMW)	~10 kDa 22 kDa 120 kDa 13 kDa 4.8 kDa 3.69 kDa 50–90 kDa 50–190 kDa
Medium molecular weight chitosan (MMW)	250 kDa 190–310 kDa 190–310 kDa
High molecular weight chitosan (HMW)	64.8–170 kDa 340 kDa 310–375 kDa

Acrylamide formation is also affected by the amount of carbohydrates present. Glucose was found to give less acrylamide than fructose during roasting.



\

Figure 5.4.3.3 Mechanism for chitosan to inhibit acrylamide

As asparagine, an amino acid that is responsible for the formation of acrylamide, is present in fruit skin (though at low concentration), roasting of kombucha bio-disposables would form 113.6 µg/kg acrylamide. The fact that chitosan coating would inhibit the formation of acrylamide when both chitosan coating and kombucha bio-disposables are roasted together give us more choices of antimicrobial chitosan agents for coating kombucha bio-disposables, ranging from bio-disposables of **KFC** with lower molecular weight such as pure chitosan (19.0µg/kg) and crab chitosan (18.1µg/kg) to that of higher molecular weight such as chitosan of **BSF** (65.8 µg/kg). Moreover, chitosan at low percentages in starch dietary fibres such as that in Fancl Calorie Cut (11.5% chitosan; 48.1% carbohydrates) and Fancl Supplement (38.6% chitosan; 47.3% carbohydrates) seemed to exhibit inhibition of formation of acrylamide when coated on kombucha bio-disposables as 146.5 µg/kg and 224.8 µg/kg acrylamide were formed respectively which were well below the safety limit of 350 micrograms (µg) of acrylamide per kilogram for biscuits and cookies to 750µg per kilogram for potato crisps and 850µg per kilogram for instant soluble coffee based on the presence of acrylamide in food. [6] Application of chitosan coating as an antimicrobial agent to edible bio-disposables of **KFC** definitely was a brand-new breakthrough.

5.5 Sustainable development

Bio-disposables of **KFC** meet the 12th sustainable development goal (SDG) of the United Nations - Ensure sustainable consumption and production patterns, in 2 ways.

Firstly, roasted **KFC** can replace plastic as disposable. Hence achieving the **Target 12.5** by substantially reducing waste generation through plastic prevention. Together with its biodegradability, antimicrobial edible bio-disposables of **KFC** can ensure sustainable development with no doubt.

Secondly, the source of chitosan used in antimicrobial coating can be extracted from **Black Soldier Fly BSF** using the innovative green method of 2-step vinegar only. In the life cycle of **BSF** (**Figure 5.4.1**), the **BSF** shells are disposed of after **BSF** has reached adulthood and become mature. To make our antimicrobial chitosan coating, the shells of **BSF** will be converted into chitosan using vinegar only but neither strongly oxidising nitric acid nor highly corrosive NaOH, hence reducing waste production. Crab shell, as another source of chitosan to make the coating can also be well used. As a result, they won't be disposed of as waste. **Target 12.2** can therefore be achieved.

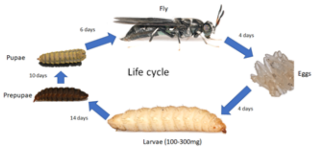


Figure 5.5.1 Life cycle of Black Soldier Fly BSF

Figure 5.5.2 12th SDG

Figure 5.5.3 Target 12.5

Figure 5.5.4 Target 12.2

Making use of **BSF** also supports the development of the circular economy and nothing will be wasted during the entire process. Nowadays, **BSF** larvae are used to consume food wastes. They will then undergo prepupae, pupae, and adult stage. When the **BSF** pupae mature to adults, they will undergo ecdysis and the shells will separate from the insects themselves without manual operation. The shells can then be used in many circumstances as it contains a high percentage of chitin and can be further converted into chitosan, in this case, the chitosan coating on our **KFC**s. The adults will then reproduce offspring and die soon. Since **BSF** is a high value animal protein, the dead or excess individuals can be used as fish feed or food for livestock. Apart from being used as a protein source, **BSF** can also be used as a fertiliser for soil amendments. These can potentially help promote other industries' growth. If the users do not want to ingest the **KFC**s, they can put them in the soil to allow the products to biodegrade itself. This enhances the nutrient content in the soil thus boosting the growth of the plants. More plants brings more food waste for the **BSF** larvae to consume. Leading this whole process self-sustainable.

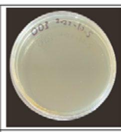
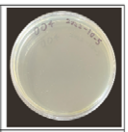
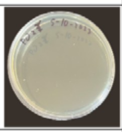
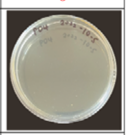
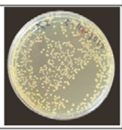
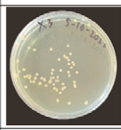
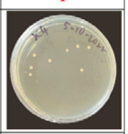
Chitosan extracted from the **BSF** shells are used as the coating on the **KFC**s. This is a new use of chitosan as it was more well-known for being used in the pharmaceutical industry. It was also used in fat loss products since it has the ability to reduce the fat and cholesterol absorbed by the body. It was also used in the development of antimicrobial films for food packaging. Considering all its benefits, edible, antimicrobial, biodegradable, we came up with the idea to coat it on to these disposables to improve them. Creating a new application of chitosan.

Figure 5.5.5 Circular economy operated by black soldier fly BSF

5.6 Eligibility for marketing of antimicrobial edible bio-disposables of KFC

Fancl Calorie Cut (11.5% chitosan; 48.1% carbohydrates) patented in Japan (no. 6633173) and Fancl Supplement (36.8% chitosan; 47.3% carbohydrates) made in Japan (Manufacturer Fancl company 4908049022037) are commercial products available on shelves currently. They have been tested and certified based on international standards, so they are safe for human consumption. Usually starch is added to bind different ingredients and starch is non-allergic. Edible kombucha bio-disposables of fruit skins with these chitosan coating as antimicrobial agents would still be edible and safe for consumption.
From our experimental results, 146.5µg was found when using Fancl Calorie Cut as edible chitosan-coating and 224.8µg/ kg when using Fancl Supplement as edible chitosan-coating on roasted **KFC** of pomelo skin. These values were within the safety limit of acrylamide in food. [6]

Table 5.6 No. of bacterial colonies in drinking water with oral bacteria soaked with roasted hydrogel of chitosan of different Fancl products (control: with oral bacteria only)

Roasted hydrogel of chitosan of Fancl Calorie Cut (100mg/873mg)			
No. of bacterial colonies	0	0	0
Roasted hydrogel of chitosan of Fancl Supplement (100mg/873mg)			
No. of bacterial colonies	6	2	1
Oral bacteria only			
No. of bacterial colonies	886	65	9

Besides, the antimicrobial effect was significant as no oral bacterial colonies were found with Fancl Calorie Cut as chitosan coating and few with that of Fancl Supplement (6,2,1 w.r.t. of Dilution factors of 10^{-2}, 10^{-3} & 10^{-4}).
Therefore, antimicrobial edible bio-disposables of **KFC** should be eligible for marketing.

6. Limitations

6.1 Testing and certification of disposables

Table 6.1 Tasks currently not performed

GB18006-2008 (Roasted KFC Cups T&C)
Moisture content Load performance test

6.2 Laboratory analysis of acrylamide

In practice, laboratory analysis of acrylamide [29] is conducted using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique, while UV-vis spectrometry is only an alternative workable method.

7. Further Studies

7.1 Incorporation of LC-MS

Acrylamide is normally not found in food with lower carbohydrate content. To further confirm and proof the safety of **KFC**, the following laboratory analysis of content of acrylamide (a carcinogen) are recommended by the Hong Kong Centre for Food Safety (HKCFS) [29]:

- Laboratory analyses to be conducted by the Food Research Laboratory (FRL) of the FEHD.

- The edible portion of the food sample was homogenised and an appropriate test portion (1-4 g) was taken. The acrylamide in the test portion was extracted by water and cleaned up by solid phase extraction, followed by determination using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique. Acrylamide-1,2,3-C13 was used as a surrogate for the analysis. The test method was validated by single-laboratory validation. The limits of detection and quantification were 3µg/kg and 10µg/kg respectively.

7.2 Adaptation of Chitosan coating to Commercial Products

7.2.1 Chitosan Coating to introduce Antimicrobial ability

This investigation had confirmed the antimicrobial ability, waterproofness etc of different chitosan coating. These chitosan coatings are not just promising alternatives to wax, which is neither biodegradable nor environmentally-friendly but also feasible to replace wax that is used as coating in paper disposables. Further study could be performed to reassure the applicability of chitosan coating as antimicrobial agent on other disposables which out of the scope of this investigation as the focus of this investigation was on the testing and certification of antimicrobial edible biodegradable **KFC** cups and straws made of Kombucha SCOBY.

7.2.2 Chitosan Coating to reduce Acrylamide Content

This investigation showed Chitosan coating, regardless of its sources used, showed excellent ability in reducing acrylamide to <20% of EU standard acrylamide safety limit for biscuits and cookies: 350μg per kg to **BSF** chitosan's 65.8 μg/kg. Further comprehensive Studies of coating chitosan to starchy utensils or any edible functional disposables can be carried out, as chitosan coating currently is designed for roasted kombucha straws and cups.

8. Conclusion

To conclude, antimicrobial edible bio-disposables of **KFC** represent a brand-new breakthrough to combat plastic pollution. They met different international standards like GB18006-2008 [4], ISO18188:2016 [5] and Commission Regulation (EU) 2017/2158 on the presence of acrylamide in food [6]. Chitosan coatings obtained from both treated **BSF** (using 2M HNO_3 & 16.7M NaOH) and untreated **BSF** (an innovative green way of using 2-step vinegar only) on kombucha bio-disposables were excellent antimicrobial agents as no or few oral bacterial colonies were found when bio-disposables of **KFC** with different chitosan coatings were soaked in drinking water with oral bacteria of different dilution factors. With the application of chitosan coating to edible kombucha bio-disposables, antimicrobial kombucha bio-disposables are not only edible and safe for consumption but also greatly reducing natural resources used in catering services. The content of acrylamide of all bio-disposables of roasted **KFC** with chitosan from different sources were as follows: pure chitosan (19.0μg/kg), crab chitosan (18.1μg/kg), **BSF** chitosan (65.8μg/kg), untreated (without using 2M nitric acid & 16.7M NaOH) **BSF** chitosan (137.1μg), Fancl Calorie Cut (146.5μg/kg) and Fancl Supplement (224.8μg/kg) which were all within the safety limit of acrylamide of food. Antimicrobial edible cups of **KFC** met the standards based on GB18006-2008, so antimicrobial edible cups of **KFC** are suitable for serving as disposable cups. Antimicrobial edible straws of **KFC also** met the standards ISO18188:2016 [5], so antimicrobial edible straws of **KFC** are suitable for serving as disposable straws. Surface protein allergens were not found on roasted hydrogel of chitosan of Fancl Calorie Cut, Fancl Supplement, **BSF**, crab, and pure chitosan using 3M Clean-Trace ALLTEC60 showing that antimicrobial edible bio-disposables of **KFC** are non-allergic in nature. Adding a simple production process and the use of eco-friendly raw materials like fruit skins, antimicrobial edible bio-disposables of **KFC** cups and straws are eligible for marketing. Sustainable consumption and development could also be achieved as **SDG Target 12.2** and **12.5** are met. Moreover, with our discovery that even **BSF** shells without being treated with 2M nitric acid and 16.7M NaOH for deacetylation, but only vinegar, the percentage of chitosan obtained (16.7%) is still enough to perform the antimicrobial ability . Together with the high DD% (57.7%-62.4%) of the untreated (without using 2M nitric acid & 16.7M NaOH) **BSF** shells, it is concluded that an innovative greener way that involves neither strong oxidizing agents such as 2M nitric acid nor corrosive chemicals such as 16.7M NaOH but a 2-step vinegar only to obtain antimicrobial chitosan as a source for making our **KFC** coating is found. This eco-friendly alternative with high tensile strength, good waterproofness, the promising marketing of antimicrobial edible bio-disposables of **KFC** would be a great leap forwards to a plastic-free society, ultimately a more sustainable society in the future.

9. Reference

[1] Cottet C., Ramirez-Tapias Y. A., Delgado J. F., de la Osa O., Salvay A. G., & Peltzer M. A. (2020). Biobased Materials from Microbial Biomass and Its Derivatives. Materials, 13(6), 1263.

[2] Priyanka, A., Kokolu, A.R., Areeba, F., Priyanka, K., & Shalini, A. (2019). Study of biodegradable packaging material produced from scoby. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 5(3), 389-404.

[3] Karst K. (n.d.). Chitosan, a source of dietary fibre from shellfish that has been shown to help improve weight loss. Retrieved from http://www.someaddress.com/full/url/

[4] Standardization Administration of the People's Republic of China. (2009). General requirement of disposable plastic tableware. (GB 18006-2008)

[5] International Organization for Standardization. (2021). Specification of polypropylene drinking straws (ISO Standard No. 18188:2016). https://www.iso.org/standard/61726.html

[6] Commission Regulation (EU). (2017). Establishing mitigation measures and benchmark levels for the reduction of the presence

of acrylamide in food. Official Journal of the European Union, 304, 24-44.

[7] Ritchie H. & Roser M. (2018). Plastic Pollution. Retrieved from https://ourworldindata.org/plastic-pollution

[8] Yaqoob L., Noor T., & Iqbal N. (2022). Conversion of Plastic Waste to Carbon-Based Compounds and Application in Energy Storage Devices. ACS Omega, 7(16), 13403-13435.

[9] Statistics Unit, Environmental Protection Department. (2021). Monitoring of Solid Waste in Hong Kong Waste Statistics for 2020. Retrieved from https://www.wastereduction.gov.hk/sites/default/files/msw2020.pdf

[10] Hong Kong Public Opinion Research Institute. (2022/07/06). 香港民意研究所 管制外賣即棄塑膠餐具意見調查. Retrieved from https://www.pori.hk/wp-content/uploads/2022/07/3_GP_takeaway_freq_rpt_v1_pori.pdf

[11] Xuan D., Tang P., & Poon C. S. (2018). Limitations and quality upgrading techniques for utilization of MSW incineration bottom ash in engineering applications – A review. Construction and Building Materials, 190, 1091-1102.

[12] BioteCH4. (27/5/2022). The negative effects of landfills. Retrieved from https://biotech4.co.uk/news/2022/05/27/the-negative-effects-of-landfills/#:~:text=It%20needs%20more%20space%2C,take%20decades%20to%20break%20down.

[13] Andreas. (n.d.). Advantages & Disadvantages of Plastic Recycling. Retrieved from https://environmental-conscience.com/plastic-recycling-pros-cons/

[14] Jang M., Yang H., Park S. A., Sung H. K., Koo J. M., Hwang S. Y., Jeon H., Oh D. X. & Park J. (2022). Analysis of volatile organic compounds produced during incineration of non-degradable and biodegradable plastics. Chemosphere, 303, 134946.

[15] Jakubczyk K., Kałduńska J., Kochman J., & Janda K. (2020). Chemical Profile and Antioxidant Activity of the Kombucha Beverage Derived from White, Green, Black and Red Tea. Antioxidants, 9(5), 447.

[16] Lehman M. Shereen. (2022/1/30). Kombucha Nutrition Facts and Health Benefits. Retrieved from https://www.verywellfit.com/kombucha-nutrition-facts-4136745#:~:text=Like%20many%20other%20tea%20varieties%2C%20kombucha%20may%20lack,has%20some%20caffeine%2C%20but%20the%20amount%20can%20vary.

[17] Nguyen, H.T., Saha, N., Ngwabebhoh, F.A., Zandraa, O., Saha, T., & Saha, P. (2021). Kombucha-derived bacterial cellulose from diverse wastes: a prudent leather alternative. Cellulose, 28(14), 9335-9353

[18] Soares, M.G., de Lima, M., & Reolon Schmidt, V.C. (2021). Technological aspects of kombucha, its applications and the symbiotic culture (SCOBY), and extraction of compounds of interest: A literature review. Trends in Food Science & Technology, 110, 539-550.

[19] PNGEGG. (n.d.). Kombucha Cellulose Chemical structure. https://www.pngegg.com/en/png-scscc

[20] Elsabee M. Z., & Abdou E. S. (2013). Chitosan based edible films and coatings: a review. Materials science & engineering. C, Materials for biological applications. 33(4), 1819–1841.

[21] Siripatrawan U. & Noipha S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocolloids, 27(1), 102-108.

[22] Antonov A., Ivanov G., Pastukhova N., & Bovykina G. (2019). Production of chitin from dead Hermetia Illucens. IOP Conference Series: Earth and Environmental Science, 315(4), 042003.

[23] Encyclopædia Britannica, Inc. (n.d.). Schematic drawing of the structure of a generalized bacterium. https://www.britannica.com/science/bacillus-bacteria/images-videos#/media/1/47965/19717

[24] Roman D. L., Ostafe V., & Isvoran A. (2020). Deeper inside the specificity of lysozyme when degrading chitosan. A structural bioinformatics study. Journal of molecular graphics & modelling, 100, 107676.

[25] Ashenhurst J. (n.d.). Ethers via acid-catalyzed dehydration of alcohols. https://www.masterorganicchemistry.com/2014/11/14/ether-synthesis-via-alcohols-and-acid/

[26] Selby G. (2018/4/11). New EU acrylamide legislation comes into force. Retrieved from https://fif.cnsmedia.com/a/ZF08p6giX1s=

[27] Food Standards Agency. (2018/1/9). Acrylamide, information on the risks of acrylamide and how you can reduce the chances of being harmed by it. Retrieved from https://www.food.gov.uk/safety-hygiene/acrylamide

[28] Tepe Y., Cebi A., & Aydin H. (2020). Acrylamide content and color formation of hazelnuts roasted at different processing temperatures and times. European Food Research and Technology, 246(8), 1543-1549.

[29] Centre for Food Safety. (2003). Chemical Hazard Evaluation, acrylamide in Food. Retrieved from https://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_fc_01_10_acrylamide.html

[30] Wang H., Feng F., & Guo Y. (2013). HPLC-UV quantitative analysis of acrylamide in baked and deep-fried Chinese foods. Journal of Food Composition and Analysis, 31(1), 7-11.

[31] HINOTEK. (n.d.). UV-VIS spectrometer. https://www.hinotek.com/lab/759s-uv-visible-spectrophotometer/

[32] Lee, A. J., Gerez, I., Shek, L. P., & Lee, B. W. (2012). Shellfish allergy--an Asia-Pacific perspective. Asian Pacific journal of allergy and immunology, 30(1), 3–10.

[33] Moosa A. A., Ridha A. M., & Kadhim N. A. (2016). Use of Biocomposite Adsorbents for the Removal of Methylene Blue Dye from Aqueous Solution. American Journal of Materials Science, 6(5), 135-146.

[34] Sansano M., Castelló M., Heredia A., & Andrés A. (2016). Protective effect of chitosan on acrylamide formation in model and batter systems. Food Hydrocolloids, 60, 1-6.

[35] EdiblePRO Edible Cutlery. Retrieved from https://ediblepro.com/

[36] Durr A. K., Rayapudi R. D., & Peespapty N. Eco-friendly and biodegradable edible utensils including cutlery and chopsticks and methods of making them. International Publication No. WO2012098448A1. WIPO. 2012/7/26.

[37] Sung W.C., Chang Y.W., Chou Y.H. & Hsiao H.I. (2018).The functional properties of chitosan-glucose-asparagine Maillard reaction products and mitigation of acrylamide formation by chitosans. Food Chemistry, 43, 141-144.

[38] Lee Y. H., Kim S. C., Nam K. D., Kim T. H., Jung B. O., Park Y.I., Synytsya A. & Park J. K. (2022). Chitosan isolated from black soldier flies Hermetia illucens: Structure and enzymatic hydrolysis Process Biochemistry, 118, 171-181.