2021 S.T. Yau High School Science Award (Asia)

Research Report

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Title of Research Report

Kombuchas from tannin-rich fruit skins as bio-disposables

Date

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Kombuchas from tannin-rich fruit skins as bio-disposables

LEUNG Wai Chung, TAM Kwan Chun Kenny, NG Ka Ho

Abstract

In the move from plastics disposables to biodegradable alternatives, roasted kombucha straws and cups made of fruit skins are plausive options. Roasted kombuchas of fruit skins are biodegradable and had good strength. Kombuchas are cellulose membranes (Cottet, 2020) which are fermented products of condensed tannins which are known for their polymerization chemistry. (Aduri, 2019)

In this investigation, roasted kombucha cups and straws were tested and certified based on GB 18006-2008 and ISO18188:2016. Actually 14% of lemons and 57% of oranges were disposed of as putrecibles in the form of peels ((Sagar, 2018)), roasted kombuchas of lemon skins which require a shorter roasting time are potential eco-friendly alternatives materials to replace plastic disposbles such as plastic straws.

The roasted kombucha cups and straws were tested and certified as follows.

1.1 When the time of roasting of kombuchas of lemon skins was between 0 to 15mins at 120°C, the ratio of absorbance of -OH to the absorbance of C=C in FTIR (measurement done using FTIR Spectrum II at Carmel Pak U Secondary School, HKSAR) dropped significantly from 1.65 to 1.24, the percentage by mass of water absorbed significantly dropped by 33% (from 2571% to 1716%) and the strength of the kombucha obtained was the strongest among other kombuchas (28N to pinch through 1mm thick kombucha). These results indicated that structural changes of kombuchas of lemon skins took place when kombuchas of lemon skins were roasted at 120°C for 0 to 15 minutes.

1.2 For complete biodegradation, roasted kombucha cups (lemon skins) took 2 weeks and straws took 3.5 weeks.

1.3 No bacterial colonies were present in drinking water kept overnight in roasted kombucha cups or soaked with kombucha straws, so they are safe for serving as biodegradable disposables.

1.4 Bio-disposables such as roasted kombucha cups remained intact after the drop test, no deformation, peelings or wrinkles in the hot-water resistance test, no water leakage in the water leakage resistance test and volume deviation of 8% which was only 3% higher than the requirement for plastic cups based on GB 18006-2008 and roasted kombucha straws showed no rupture and hence resistance to bending, no deformations and no fading of colour in the heat endurance test based on ISO18188:2016.

Roasted kombucha cups and straws should be eligible for marketing.

Fig.1.1 Roasted kombucha straws (left) and cups (middle & right) as bio-disposables

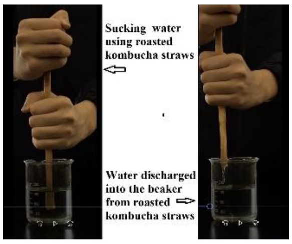

Fig.1.2 Sucking water using roasted kombucha straw

Keywords: kombucha, plastic alternative, tannin polymerization, fermentation, biodegradable, FTIR

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. Lam Chun Shing and Mr. Li Ka Chi, Laboratory Technicians, for his contribution and valuable technical support on this project.

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 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.

This research report has been submitted in the past or concurrently with the following competitions.

a. 23rd Hong Kong Youth Science & Technology Innovation Competition
b. Hong Kong Student Science Project Competition 2021
c. Young Professionals Exhibition & Competition 2021
d. City I&T Grand Challenge
e. "Digi-Science" Video Production Competition for Hong Kong Secondary Schools 2021

(Signatures of full team below)

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Table of Content

1. Theory

Plastic tableware such as plastic cups and straws are widely used and available. It is estimated that 500 million plastic straws and 120 billion single-use cups are used and disposed in the US alone every year. (Environmental facts, UKONSERVE https://ukonserve.com/pages/environmental-facts) The figure is big and it is posing a threat to the environment.

1.1 Problem of plastic tableware

Plastic tableware are both cheap and convenient. However, these benefits cause people to throw them away without much consideration. This leads to the deposition of plastic tableware into soil and waterways such as oceans, rivers and seas and caused them to be contaminated. To make the matter worse, this plastic tableware can neither decompose nor biodegrade easily. It will break down only after hundreds of years(Fig1.1.1).These plastic waste will end up in wildlife habitat and stay there for a long time only to leach toxic chemical. In the end, it will just threaten the life of animals. For example, the animals may mistake it as food or be stuck in the plastic waste(Fig 1.1.2).The consequence is disastrous since these small pieces of plastic can actually cause the death of many animals and endanger wildlife.

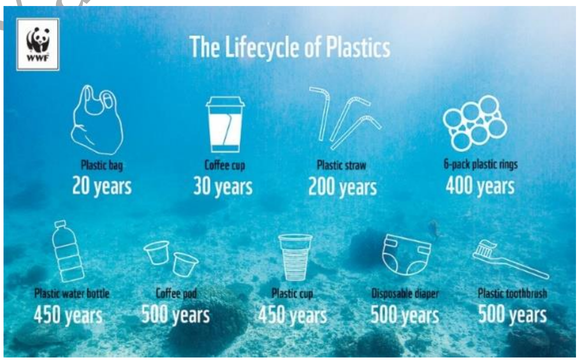


Fig 1.1.1 Life Cycle of plastics

(https://www.wwf.org.au/news/blogs/the-lifecycle-of-plastics)

Fig 1.1.2 Green Goblin, a green sea turtle that had a plastic straw stuck in its nostril

(https://www.pilotonline.com/life/wildlife-nature/article_fdf8053c-3cd3-11e8-811b-f3ca3c1bbee4.html)

1.2 Comparison of current methods

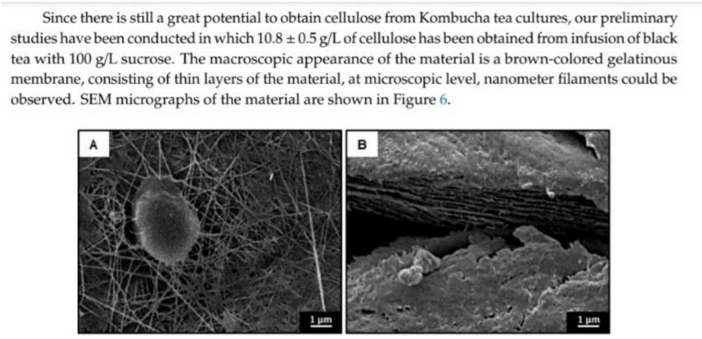
There are now two main ways to deal with the problem of plastic tableware. First it is about using reusable materials such as stainless steel, silicone and glass for tableware. These materials are mostly durable and can be reused many time. However, they might not be hygienic if these tableware are not properly washed. Second, it is about using biodegradable materials such as bamboo, paper and hay for tableware. They are made out of plant materials and therefore can break down naturally in a shorter time. However, most of these materials are not durable enough and cannot be reused. They also come with their own environmental cost. However, the above alternatives are still not popular because of their higher cost and inconvenience compared with plastic. Therefore, we come up with a new material which is cheap, convenient and biodegradable.

1.3 Kombucha as alternatives

Kombucha would be used as an alternative to plastic.

Microbial cellulose membrane of Kombucha could be obtained from tea fermentation, using a black tea infusion at static conditions, and room temperature.

Because of its microbial cellulose purity and biodegradability, bacterial cellulose is an attractive biopolymer for a number of applications including biotechnological food, biomedical etc. (Cottet, 2020) Kombucha is biodegradable and can be a excellent choice for replacing plastic in the future.


Since there is still a great potential to obtain cellulose from Kombucha tea cultures, our preliminary studies have been conducted in which 10.8 ± 0.5 g/L of cellulose has been obtained from infusion of black tea with 100 g/L sucrose. The macroscopic appearance of the material is a brown-colored gelatinous membrane, consisting of thin layers of the material, at microscopic level, nanometer filaments could be observed. SEM micrographs of the material are shown in Figure 6.

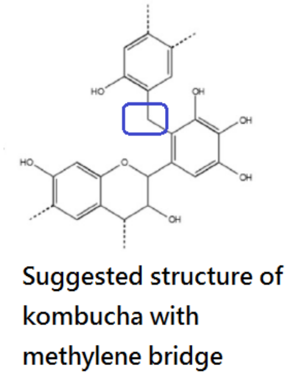


Fig 1.3.1 SEM observation of dried kombucha cellulose surface (7000X)(A)
and cellulose film(B) (Cottet, 2020)

Fig. 1.3.2 suggested structure of komubcha

1.4 Identification and quantification of the amount of tannin in fruit skins using vanillin-HCl method

Tannin content can be quantified in fruit skin by using vanillin-HCl method. During the vinylation of tannin using vanillin, a coloured adduct is formed which is absorbed at 500nm. In this reaction, vinylation involves the coupling of vanillin via the formation of –C=C- from the –CHO aldehyde group on vanillin. (Gade, 2018)

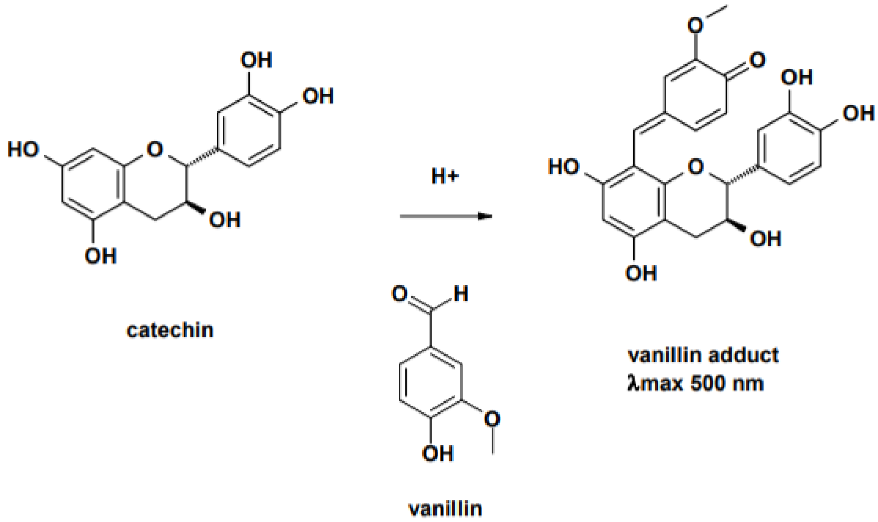


Fig 1.4.1 vinylation of tannin using vanillin

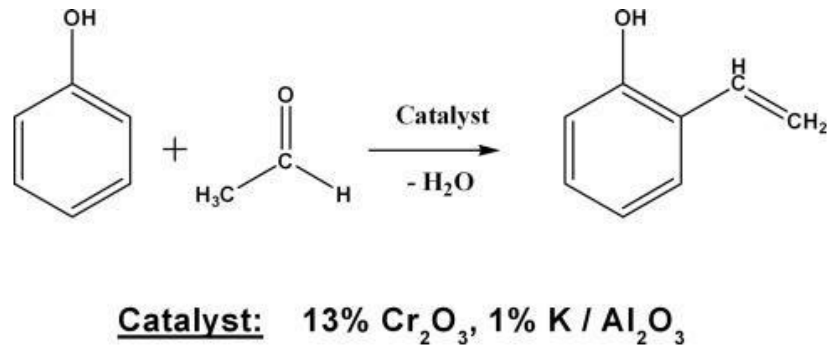


Fig 1.4.2 example of vinylation reaction

(http://www.users.miamioh.edu/hagermae/Vanillin%20Assay.pdf)

1.5 Structure determination using FTIR

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

The following FTIR Spectrum Two has been installed in Carmel Pak U Secondary School .

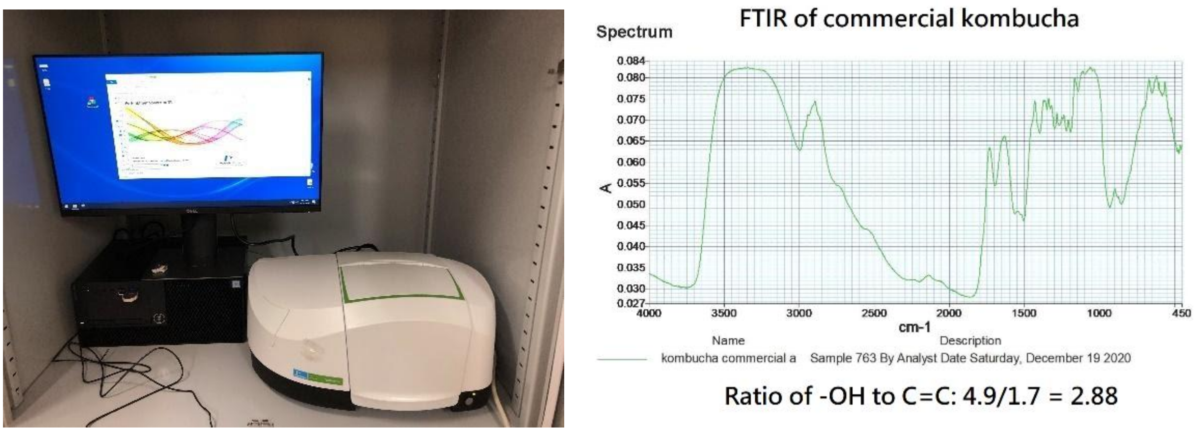


Fig 1.5.1 FTIR Spectrum Two (left) & FTIR of commercial kombucha (right)

Spectrum Two FT-IR spectrometers feature:

- Standard, high-performance, room-temperature $LiTaO_3$ (lithium tantalate) MIR (mid infra-red) detector with a SNR (signal to noise ratio) of 9,300:1
- Optional temperature-stabilized, high-performance DTGS (deuterated triglycine sulfate) MIR detector with a SNR of 14,500:1. Ideal for low-light, high throughput applications

- Standard optical system with KBr windows for data collection over a spectral range of 8,300 – 350 cm^{-1} at a best resolution of 0.5 cm^{-1}

1.6 Competing enzymatic actions between formation of kombucha and other enzymatic actions such as enzymatic peeling

1.6.1 Inhibition of formation of kombucha by tannins

It is known that enzymatic actions are inhibited by tannins as they can form precipitate with the enzymes. Less precipitate is formed at pH values lower than 2.0 (Goldstein, 1965). Besides, tannins can reduce the catalytic efficiency of enzymes by altering the chemical environment in which enzymes operate (Triebwasser, 2012). As the pH of oranges is calculated to be in the range of 3.69-4.34, whereas the pH of lemons is around 2-2.6, formation of kombucha which involved the polymerization of tannins in lemon should be less inhibited and should go faster. (https://foodstruct.com/compare/oranges-vs-lemon#:~:text=Lemons%20often%20taste%20more%20sour,%25%20in%20oranges%20(3).&text=The%20pH%20of%20lemon%20juice,the%20pH%20of%20a%20lemon)

1.6.2 Enzymatic peeling of citrus fruits

Enzymatic peeling is an example of enzymatic reactions with pH range between 3.5 and 4.5. The best temperature leading to enzymatic peeling of oranges was 35°C to 40ºC. (Pretel, 2008).

1.6.3 starting pH of formation of kombuchas

The starting pH of kombucha needs to be at or under 4.5. As fermentation carries on, microbial cellulose of kombuchas start to form and pH keeps on going down to even 2.5. (https://fermentaholics.com/why-is-ph-important-for-kombucha/#:~:text=The%20starting%20pH%20of%20kombucha,a%20higher%20pH%20at%203.5.)

By controlling the pH of kombucha solution, other enzymatic actions such as peeling should subside by keeping the pH between 2.5 to 3. In doing so, kombucha should be formed from fruit skins faster with a higher yield (i.e. higher percentage of kombucha formed).

1.7 Testing and certification

1.7.1 Testing and certification of roasted kombucha cups based on GB 18006-2008 (General requirement of disposable plastic tableware, National Standard of the People's Republic of China)

https://members.wto.org/crnattachments/2009/TBT/CHN/09_0814_00_et.pdf

We will work on the following characteristics of service performance (section 5.4) of disposable cups.

1.7.1.1 Volume deviation 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%.

1.7.1.2 Drop performance During the drop test of disposable tableware, there must be no cracks or splits to any of the three samples.

1.7.1.3 Hot-water resistance After the hot-water resistance test, there shall be no deformations, peelings or wrinkles to the disposable tableware.

1.7.1.4 Water leakage resistance For disposable tableware such as boxes, bowls or cups which have the function of containing liquid, after the water leakage test, there must be no water leakage.

1.7.2 Testing and certification of roasted kombucha straws based on ISO18188:2016 (Specification of polypropylene drinking straws)

https://cdn.standards.iteh.ai/samples/61726/3ede8ed7c91042edaf95570a82d77046/ISO-18188-2016.pdf

We will work on the following characteristics of disposable straws

1.7.2.1 Resistance to bending

When tested, the straws shall not rupture.

1.7.2.2 Heat endurance There shall be no deformations and/or colour fading after testing.

2. Methodology

The following materials are used in the experiments.

 Orange skins	 Lemon skins	 Tangerine skins	 Pomelo skins	 Commercial kombucha
 Biodegradable paper cups	 Paper cups (waxed)	 PE plastic cups	 PS plastic cups	 Paper plates (waxed)
 Biodegradable paper straws	 PLA (polylactic acid & bamboo) straws	 Plastic straws	 Buffer of pH2 and pH4 pH paper	

Table.2.1 Samples under investigation

2.1 Investigation of the content of tannin and amount of kombucha obtained from different fruit skins.

2.1.1 Investigation of the amount of tannin in different fruit skins using vanillin-

HCl method

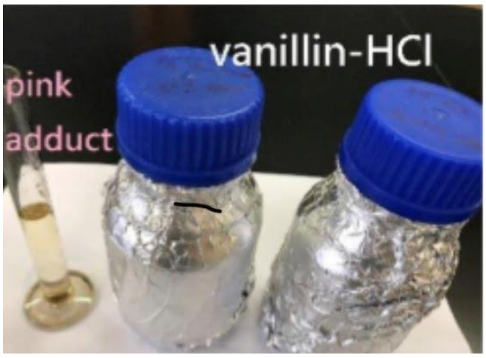


Figure 2.1.1.1 Vanillin-HCl method.

Figure 2.1.1.2 Spectrometer

1. About 5g of a dried sample were weighed.
2. $30cm^3$ of 80% ethanol was added for the extraction of tannin.
3. The mixture was microwaved for 2 min using medium low power.

   $1cm^3$ tannin extracted by 80% ethanol was added to $1cm^3$ vanillin and $1cm^3$ of 8%HCl solution.
4. A pink colour was developed after 3 min.
5. The absorbance of the reaction mixture was taken at 500nm and 510nm.
6. The above was repeated using $40cm^3$ 80% ethanol.

2.1.2 Investigation of the percentage by mass of kombucha obtained from different fruit skins

Figure 2.1.2 Production of kombucha

1. Brewing kombuchas using different fruit skins.
2. 100g of glucose was added to $500cm^3$ water to form a culture.
3. About 500g of dried fruit skins were added to the culture.

4. A piece of commercial kombucha was added as seeding to speed up the process.

5. A layer of new kombucha obtained a week later was dried and weighed.

6. The piece of commercial kombucha was reused next time.

2.2 Investigation of the structures of kombuchas before and after roasting using FTIR.

2.2.1 Investigating the structural change of kombuchas after roasting at different temperatures and different times using FTIR.

Fig.2.2.1.1 Roasted kombucha for different time (left)

Fig.2.2.1.2 Roasted kombucha at different temperature (right)

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.

2.2.2 Comparison of physical property of kombuchas

2.2.2.1. Measuring percentage water absorbance of different kombuchas

Measuring the percentage of water absorbed by different samples of kombuchas (lemon skins & orange skins)

Figure 2.2.2.1 Kombuchas in water

1. Weigh the dried samples of kombuchas.
2. Add excess water to the samples.
3. Weigh the wet samples after 1 day.

2.2.2.2 Measuring the force required to pierce through 1mm kombucha

Measuring the force applied to punch through samples of roasted kombuchas

Figure 2.2.2.2 Experiment of hardness test

1. Measure the thickness of the kombuchas using a caliper micrometer.
2. Record the force required to pierce through the kombuchas using a Newton balance.

2.3 Investigation of the effect of pH on competing enzymatic actions: the formation of kombuchas and other enzymatic actions such as enzymatic peeling

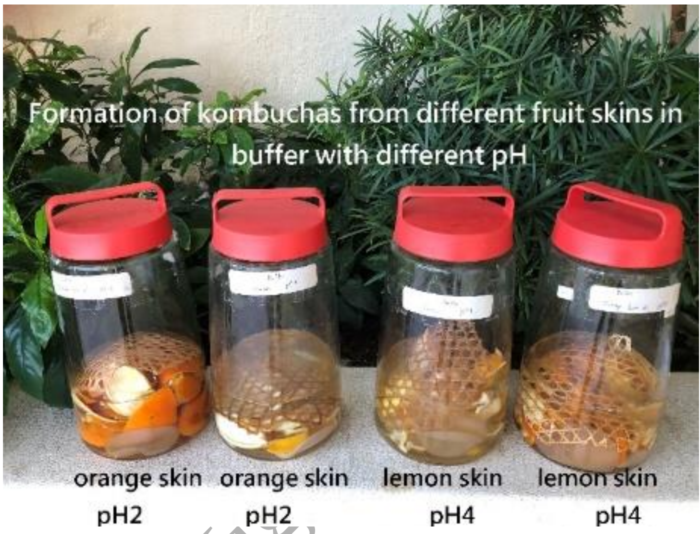


Fig. 2.3 Formation of kombucha from different fruit skins in buffer with different pH

1. 200cm^3 kombucha solution with komucha seedings, 500cm^3 buffer solution at pH2 and 500cm^3 sugar solution were added to a known mass of skins of lemon.
2. Weigh the dried kombucha formed after a week.
3. Measure the ratio of absorbance at 3400 cm^{-1} by -OH and absorbance at 1660 cm^{-1} by C=C using FTIR Spectrum II.
4. Repeat using 500cm^3 buffer at pH4 and skins of orange.

2.4 Comparing the biodegradability of different roasted kombuchas and disposables

Figure 2.4 Different roasted kombucha and disposables to be bio-degraded

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.

2.5 Investigating the feasibility of using roasted kombuchas of lemon skins as disposables

2.5.1 Designing kombucha disposables such as drinking straws and cups.

Figure 2.5.1 Examples of kombucha disposable, drinking straw(left), cups(right)

2.5.2 Comparing the performance of kombucha cups and paper cups using drinking water and hot water

1. Drinking water and hot water were put into kombucha cups and paper cups.
2. Time before leakage was recorded.

2.5.3 Counting bacterial colonies of drinking water kept in roasted kombucha cups and straw overnight

Counting bacterial colonies of drinking water kept in kombucha cups and straw overnight

Roasted kombucha (lemon skin) straw in drinking water overnight

Roasted Kombucha (lemon skin) cup holding drinking water overnight

Figure 2.5.2 experiment set up about studying the growth of oral bacteria in drinking water

1. Drinking water was kept in roasted kombucha cups and straw overnight.
2. 100 microlitre of the drinking water was spread over the agar of culture solution.
3. After 24 hours, bacterial colonies were counted.

2.6 Testing and certification

2.6.1 Testing and certification of roasted kombucha cups based on GB 18006-2008

(General requirement of disposable plastic tableware, National Standard of the People's Republic of China)

https://members.wto.org/crnattachments/2009/TBT/CHN/09_0814_00_et.pdf

We will work on the following characteristics of service performance (section 5.4) of disposable cups.

2.6.1.1 Volume deviation

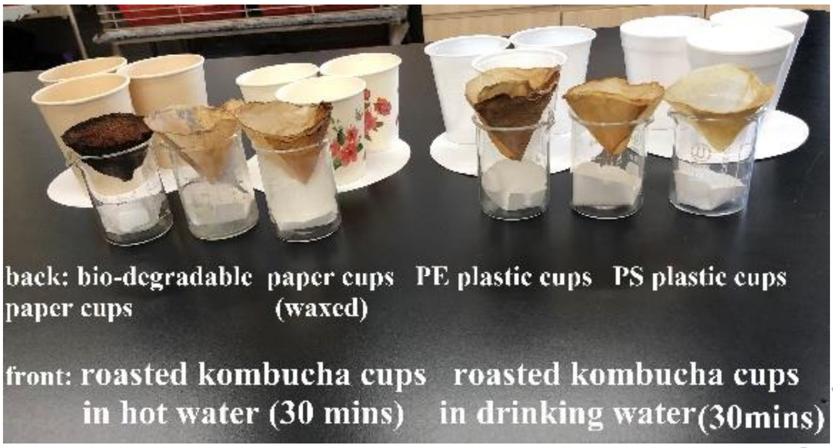


Fig.2.6.1.1 Samples of cups testing for volume deviation

1. Measure the volume of drinking water filled in a roasted kombucha cup before and after standing for 30 minutes.

2. Repeat using hot water.

3. Calculate the volume deviation (percentage change in volume)

2.6.1.2 Drop performance

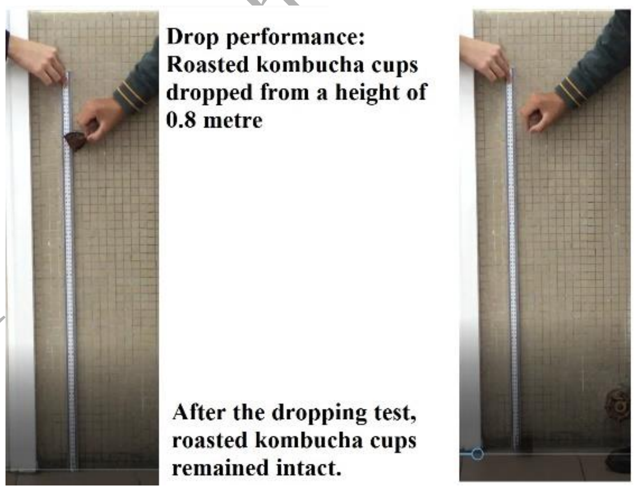


Fig.2.6.1.2 Testing drop performance

1. At a normal temperature, a roasted kombucha cup was allowed to drop freely, facing bottom-down, from a height of 0.8 m, onto a level cement floor.

2. The cup was examined and see if it was intact.

2.6.1.3 Hot-water resistance

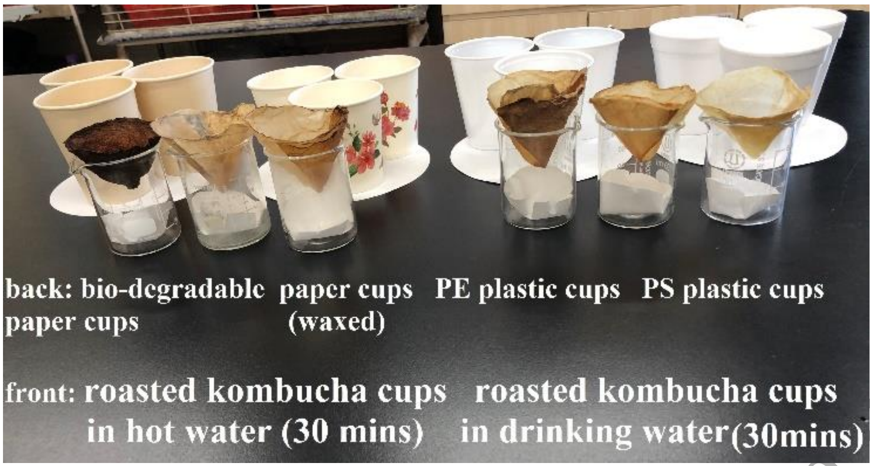


Fig.3.6.1.3 Testing hot-water resistance & water leakage resistance

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

2.6.1.4 Water leakage resistance

1. Place a piece of filter paper under a roasted kombucha cup.

2. Fill the roasted kombucha cup completely with drinking water, at a temperature of 23 ºC± 2ºC, leave the samples standing for 30 minutes.

3. Observe if there was leakage of water.

2.6.2 Testing and certification of roasted kombucha straws based on ISO18188:2016

https://www.iso.org/obp/ui/#iso:std:iso:18188:ed-1:v1:en

We will work on the following characteristics of service performance (section 5.3) of disposable straws.

2.6.2.1 Resistance to bending

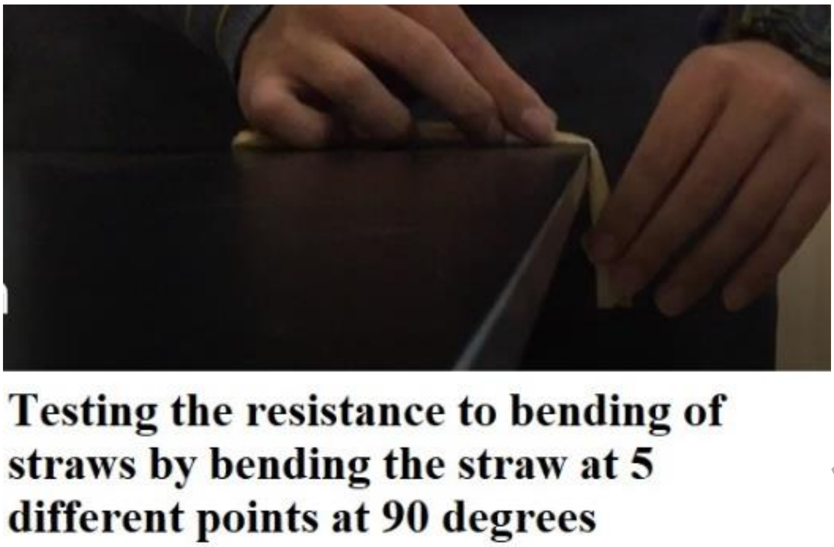


Fig.2.6.2.1 Testing the resistance to bending of straws

1. A roasted kombucha straw was bent at 90° and released at five different points along its length (including the flexible part of flexible straws).

2. The straw should be examined thoroughly for any rupture.

2.6.2.2 Heat endurance test

Fig. 2.6.2.2 Testing heat endurance of straws

1. A roasted kombucha straw was 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 straw was removed from the water, spreaded out and left at ambient temperature for 30 min. It was examined thoroughly for any visible deformations and colour fading.

3. Results

3.1 Investigation of the content of tannin and amount of kombucha obtained from different fruit skins.

3.1.1 Investigation of the amount of tannin in different fruit skins using vanillin-HCl method

Fruit skin	Percentage of tannin %
orange	6.28
lemon	3.05
tangerine 柑橘	3.35
pomelo 沙田柚	4.15
Black tea((Khasnabis, 2015)	13.36

Table 3.1.1.1 Percentage of tannin present in different fruit skin

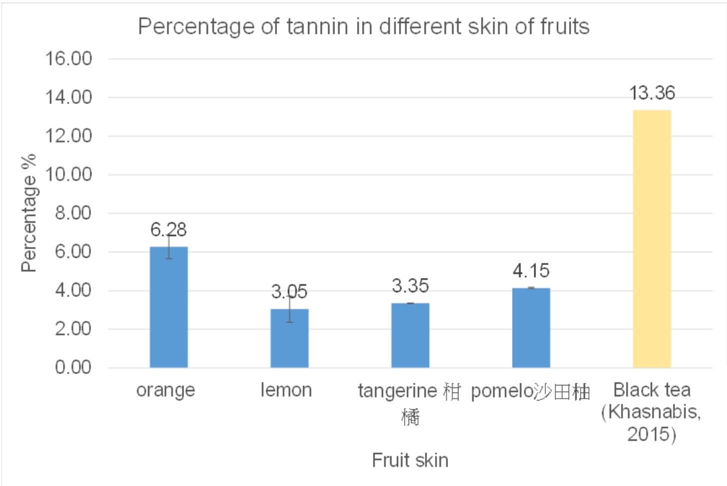


Fig. 3.1.1.2 Percentage of tannin present in different fruit skin

The experimental results show that orange skin had the highest tannin content of 6.28% among citrous fruit skins. Others also had considerable tannin content such as lemon skin had 3.05% while Black tea had the highest (13.36%) (Khasnabis, 2015).

3.1.2 Investigation of the amount of kombucha obtained from different fruit

skins

Fruit skin	Percentage by mass of kombucha (%)	Percentage of tannin %
lemon	2.59	3.05
orange	1.68	6.28

Table 3.1.2.1 Percentage by mass of kombucha and tannin obtained from different fruit skins

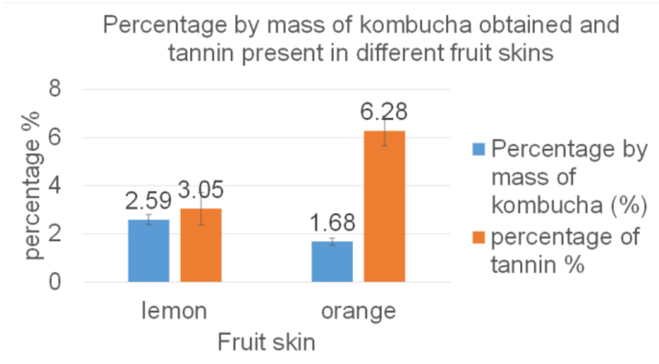
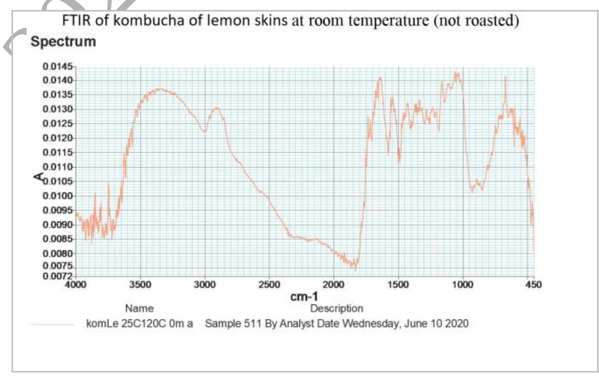


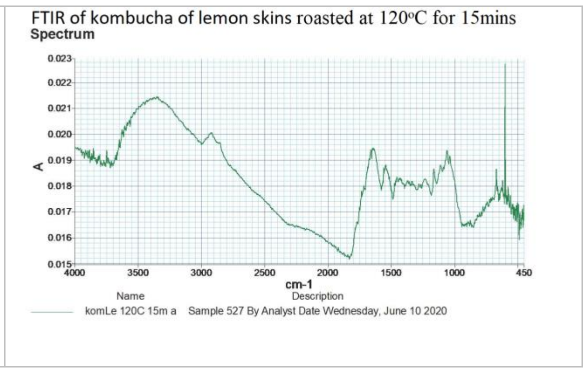
Fig. 3.1.2.2 Percentage by mass of kombucha and tannin obtained from different fruit skins

The percentage by mass of kombucha obtained from lemon was found to be 2.59% which was higher than that of orange (1.68%) though the percentage by mass of tannin in lemon was 3.05% which was lower than that of orange (6.28%).

3.2 Investigation of the structures of kombuchas before and after roasting at different temperature/time using FTIR

3.2.1 FTIR graphs of kombuchas of lemon skins before and after roasting at 120°C for different time





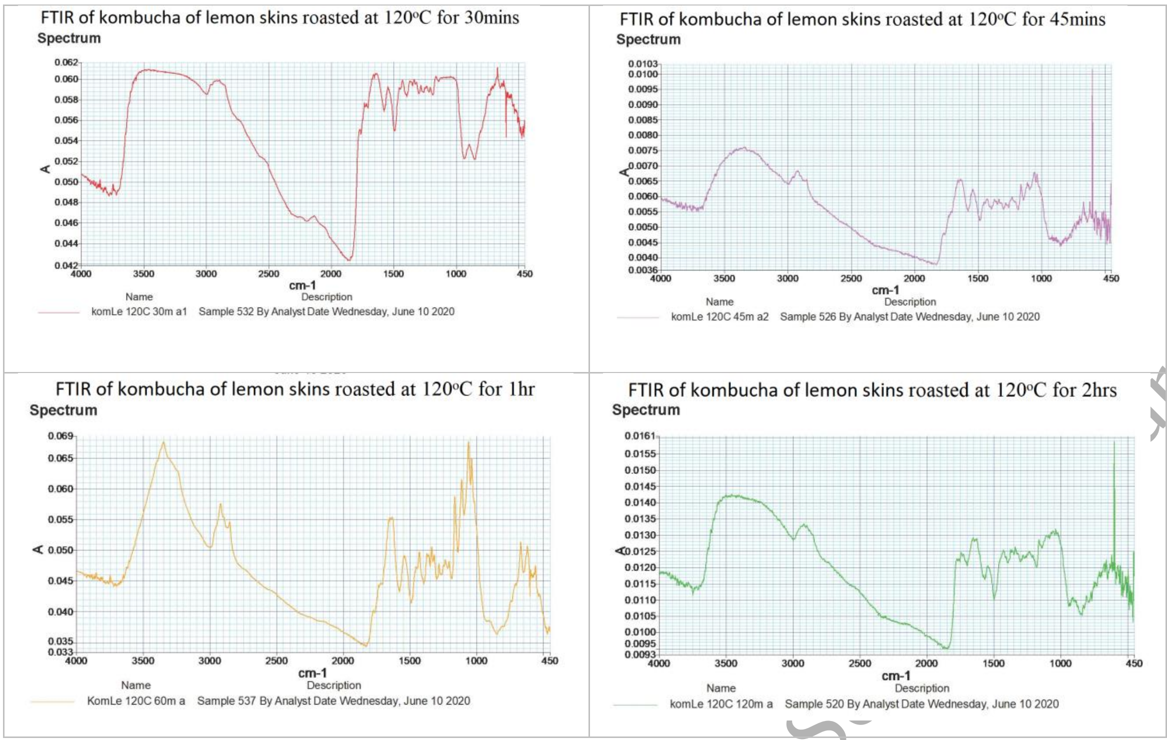


Table 3.2.1.1 FTIR graph of roasted kombuchas of lemon skins for different time

Time of roasting of kombuchas of lemon skins at 120°C /min	**Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of lemon skins)**
0	**1.65**
15	1.24
30	1.32
45	1.29
60	1.40
120	1.34

Table 3.2.1.2 Ratio of of abs of -OH γ3400 to C=C γ1660 of roasted kombuchas of lemon skins for different time

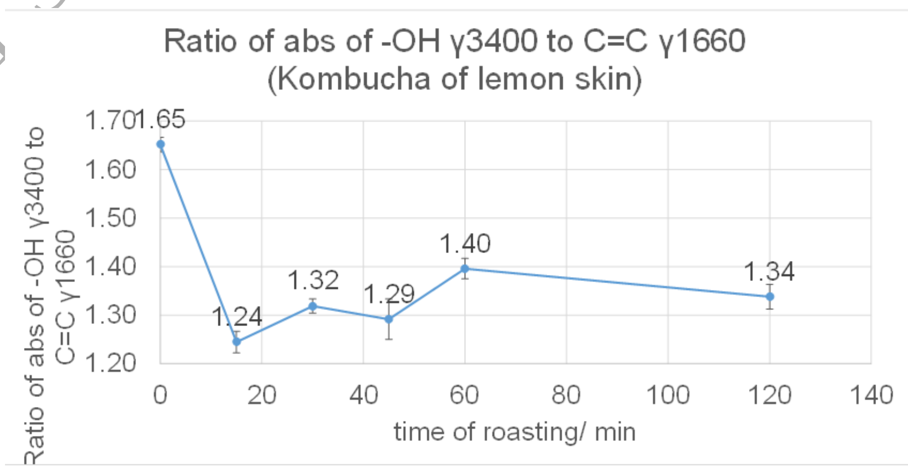


Fig. 3.2.1.3 Ratio of absorbance of –OH at 3400cm^{-1} to C=C at 1660cm^{-1} of kombuchas of lemon skins

Ratio of absorbance of -OH to the absorbance of C=C dropped significantly from 1.65 to 1.24 between 0 - 15 min, indicating that structural changes of kombuchas of lemon skins took place at 120℃ during roasting. Unroasted Kombucha at room temperature has the highest ratio of absorbance of -OH to the absorbance of C=C (1.65) while Kombucha roasted at 120℃ for 15 min has the lowest ratio of absorbance of -OH to the absorbance of C=C (1.24).

3.2.2 FTIR graphs of kombuchas of orange skins before and after roasting for different time

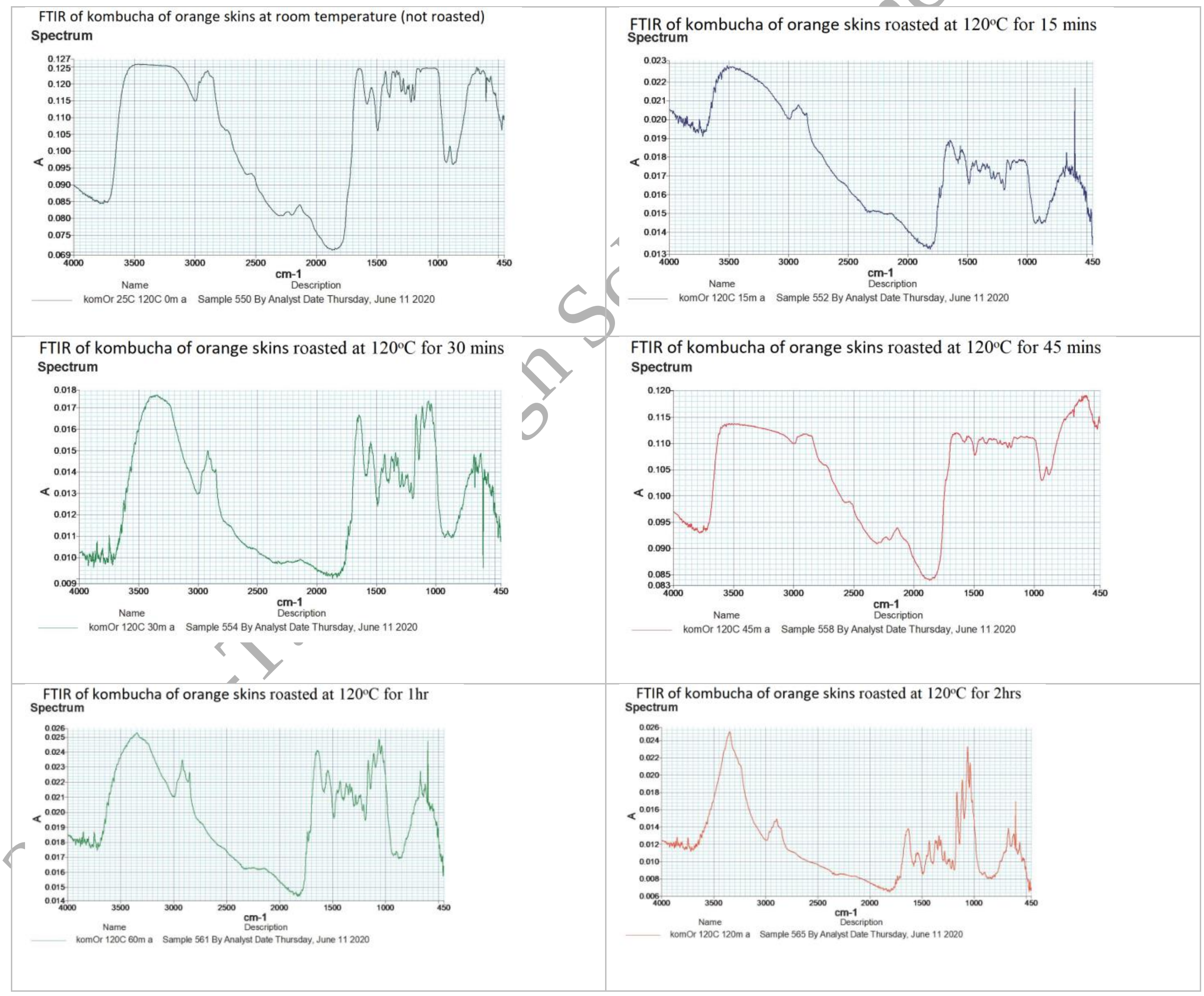


Table 3.2.2.1 FTIR graph of roasted kombuchas of orange skins for different time

Time of roasting of kombuchas of orange skins at 120°C /min	**Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of orange skins)**

0	**1.82**
15	1.78
30	1.52
45	1.58
60	1.44
120	1.37

Table 3.2.2.2 Ratio of absorbance of –OH at 3400cm^{-1} to C=C at 1660cm^{-1} of kombuchas of orange skins

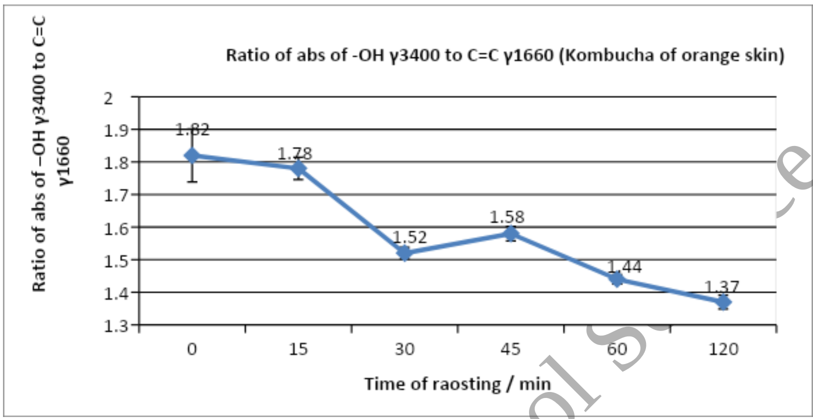
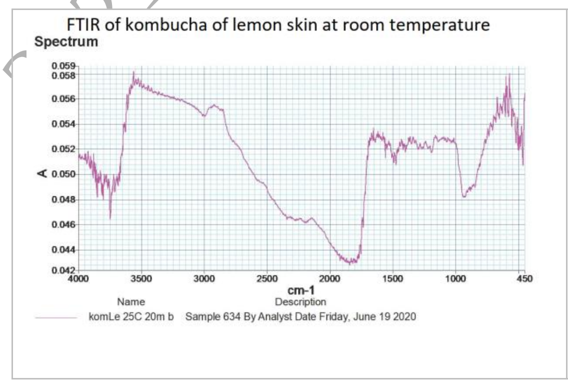
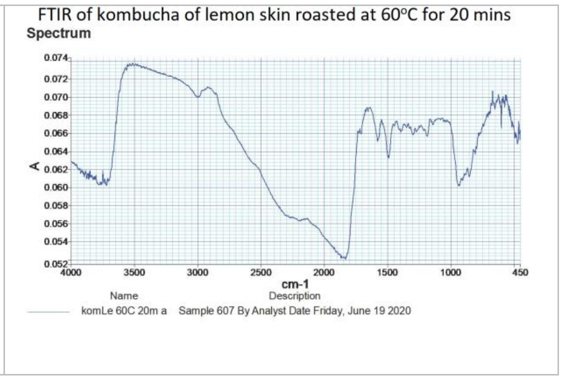


Fig. 3.2.2.3 Ratio of absorbance of –OH at 3400cm^{-1} to C=C at 1660cm^{-1} of kombuchas of orange skins

Ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 1.78 to 1.52 between 15- 30 min, indicating that structural changes of kombuchas of orange skins took place during roasting at 120°C. Unroasted Kombucha at room temperature has the highest ratio of absorbance of -OH to the absorbance of C=C (1.82) while Kombucha roasted at 120°C for 120 min has the lowest ratio of absorbance of -OH to the absorbance of C=C (1.37).

3.2.3 FTIR graphs of kombuchas of lemon skins before and after roasting at different temperature for 15 minutes





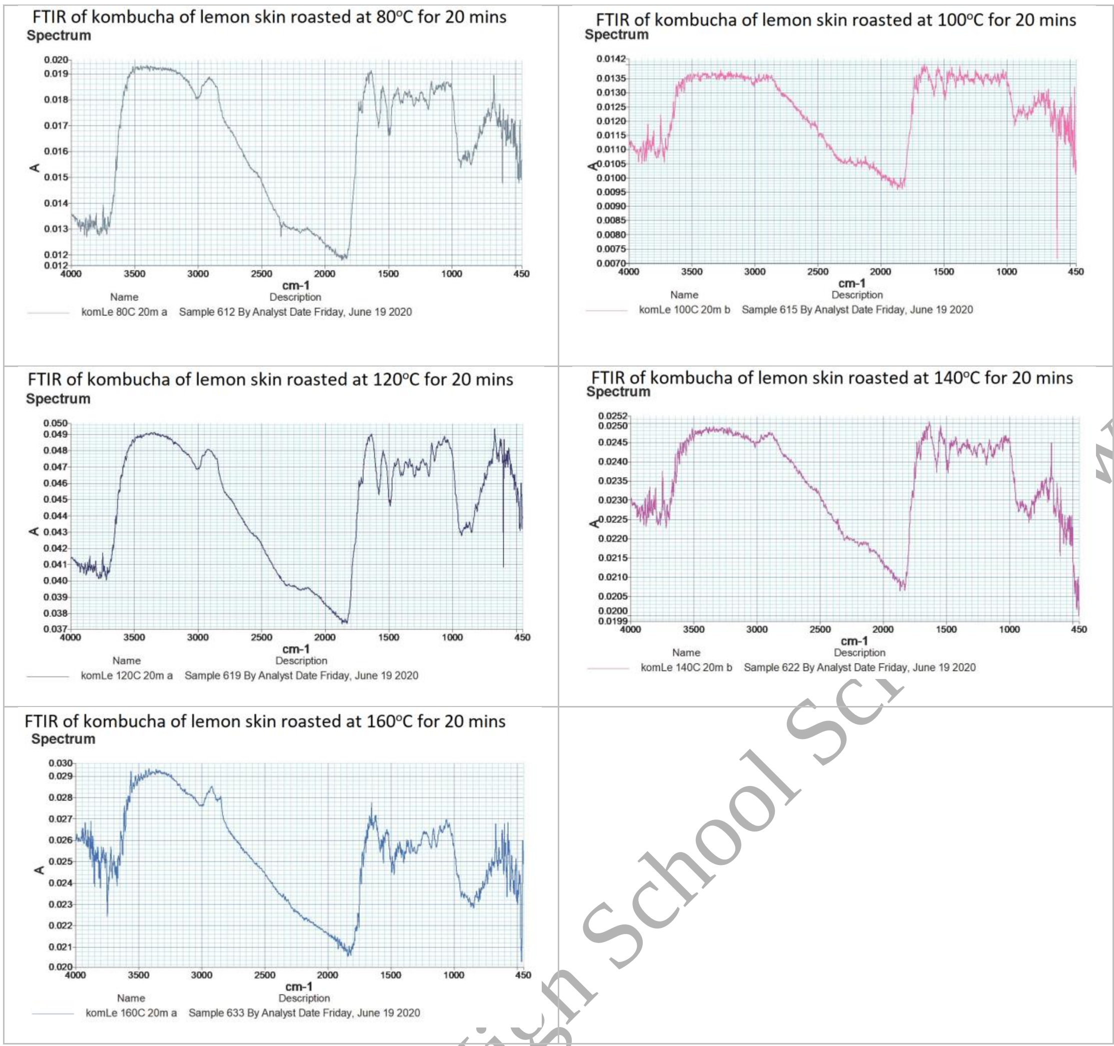


Table 3.2.3.1 FTIR graph of roasted kombuchas of lemon skins at different temperature

Temperature of roasting of kombuchas of lemon skins for 15mins/ °C	**Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of lemon skins)**
room temperature	1.74
60	1.81
80	1.85
100	1.78
120	1.47
140	1.37
160	1.39

Table 3.2.3.2 Ratio of absorbance of –OH at 3400cm^{-1} to C=C at 1660cm^{-1} of kombuchas of lemon skins at different temperature

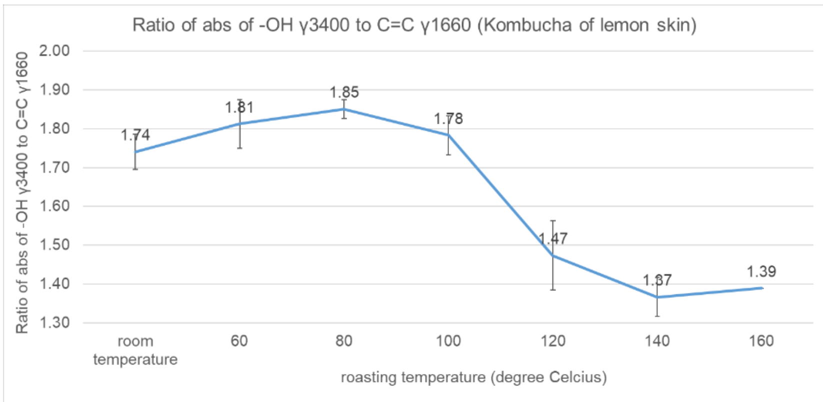
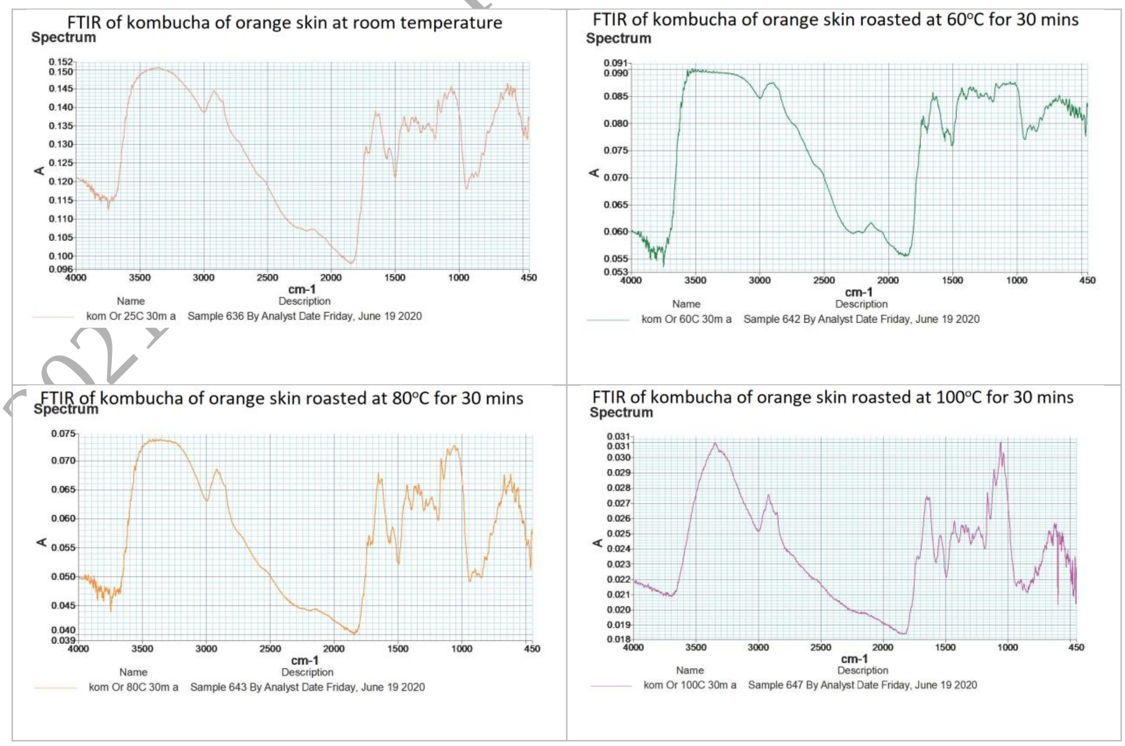


Fig. 3.2.3.3 Ratio of absorbance of –OH at 3400cm^{-1} to C=C at 1660cm^{-1} of kombuchas of lemon skins at different temperature

Ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 1.78 to 1.47 between 100°C to 120°C, indicating that structural changes of kombuchas of lemon skins took place during 15 minutes of roasting. Kombucha roasted at 80°C for 15 min has the highest ratio of absorbance of -OH to the absorbance of C=C (1.85) while Kombucha roasted at 140°C for 15 min has the lowest ratio of absorbance of -OH to the absorbance of C=C (1.37).

3.2.4 FTIR graphs of kombuchas of orange skins before and after roasting at different temperature for 30 minutes



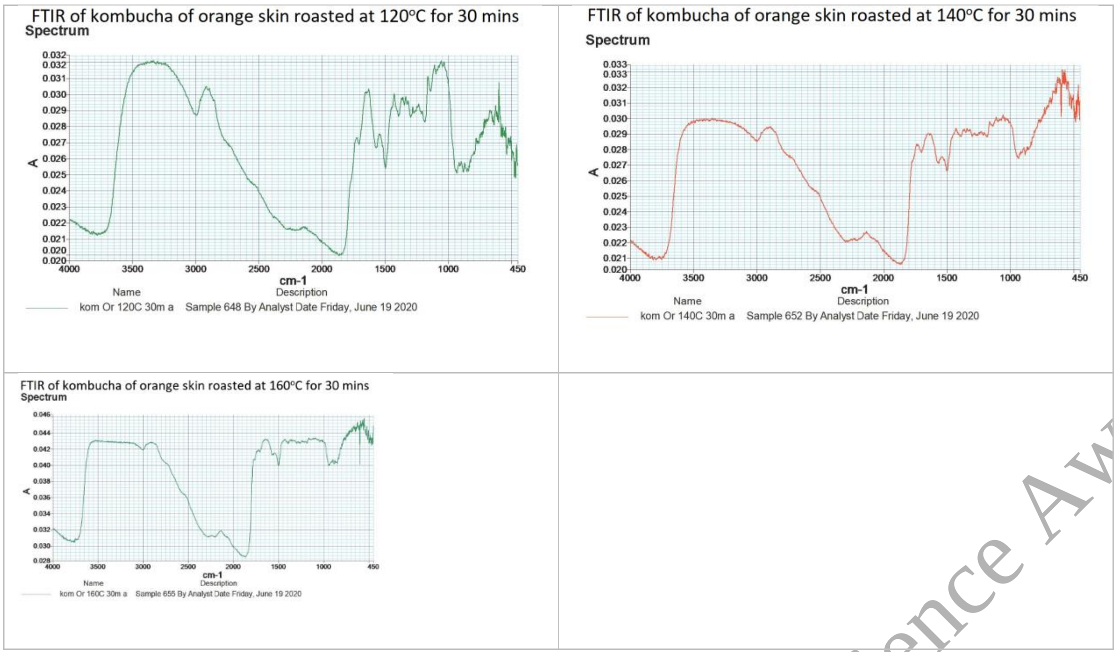


Table 3.2.4.1 FTIR graph of roasted kombuchas of orange skins at different temperature

Temperature of roasting of kombuchas of orange skins for 15mins/ °C	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of orange skins)
room temperature	2.24
60	2.28
80	2.16
100	2.10
120	1.86
140	1.87
160	1.88

Table 3.2.4.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of orange skins

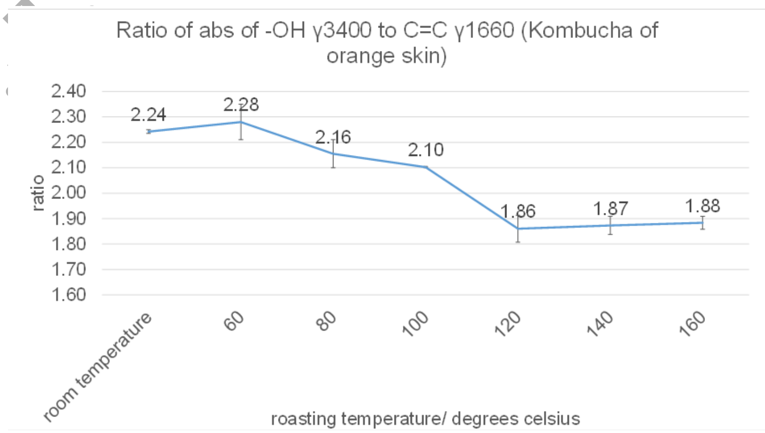


Fig. 3.2.4.3 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of orange skins

Ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 2.10 to 1.86 between 100°C to 120°C, indicating that structural changes of kombuchas of orange skins took place during 30 minutes of roasting. Kombucha roasted at 60°C for 30 min has the highest ratio of absorbance of -OH to the absorbance of C=C (2.28) while Kombucha roasted at 120°C for 30 min has the lowest ratio of absorbance of -OH to the absorbance of C=C (1.86).

3.3 Comparison of physical properties of kombuchas

3.3.1 Measuring percentage water absorbance of kombuchas of lemon skins roasting at 120°C for different time

Time of roasting of kombuchas of lemon skins at 120°C /min	Percentage by mass of water absorbed by kombuchas (lemon skins) after 1 day %
0	2571
15	1716
30	1770
45	1783
60	1593
120	1602

Table 3.3.1.1 Percentage by mass of water absorbed by kombuchas of lemon skins for different roasting time

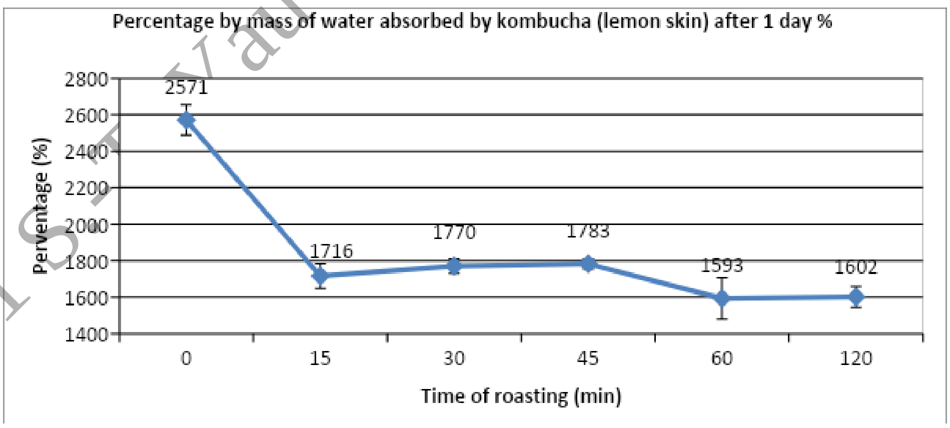


Fig. 3.3.1.2 Percentage by mass of water absorbed by kombuchas of lemon skins for different roasting time

Unroasted Kombucha at room temperature has the highest water absorbance (2571%) while Kombucha roasted at 120°C for 60 min has the lowest water absorbance (1593%). Percentage by mass of water absorbed significantly dropped by 33% (from 2571% to 1716%) when kombuchas of lemon skins were roasted for 0 to 15 min at 120℃, indicating that structural changes took place during roasting.

3.3.2 Measuring percentage water absorbance of kombuchas of orange skins roasting at 120℃ for different time

Time of roasting of kombuchas of Orange skins at 120ºC /min	Percentage by mass of water absorbed by kombuchas (orange skins) after 1 day %
0	985
15	942
30	745
45	741
60	735
120	601

Table 3.3.2.1 Percentage by mass of water absorbed by kombuchas of orange skins for different roasting time

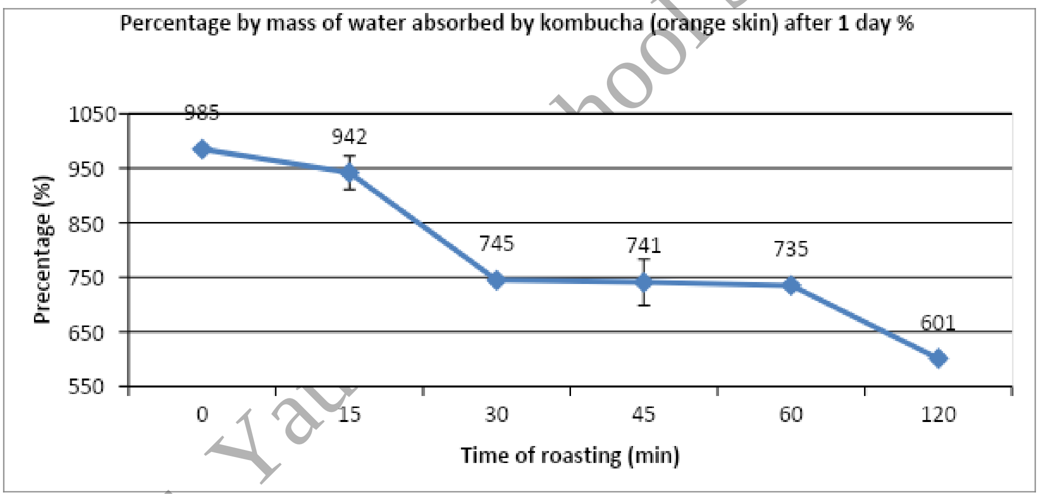


Fig. 3.3.2.2 Percentage by mass of water absorbed by kombuchas of orange skins for different roasting time

Percentage by mass of water absorbed dropped significantly from 942% to 745% when kombuchas of orange skins were roasted for 15 to 30 min at 120℃, indicating that structural changes took place during roasting. Unroasted Kombucha at room temperature has the highest water absorbance (985%) while Kombucha roasted at 120℃ for 120 min has the lowest water absorbance (601%)

3.3.3 Measuring percentage water absorbance of kombuchas of lemon skins

roasting for 15 minutes at different temperature

Temperature of roasting of kombuchas of lemon skins for 15 mins/ °C	Percentage by mass of water absorbed by kombuchas (lemon skins) after 1 day %
room temperature	2770
60	2958
80	2812
100	3100
120	1235
140	1103

Table 3.3.3.1 Percentage by mass of water absorbed by kombuchas of lemon skins of different roasting temperature

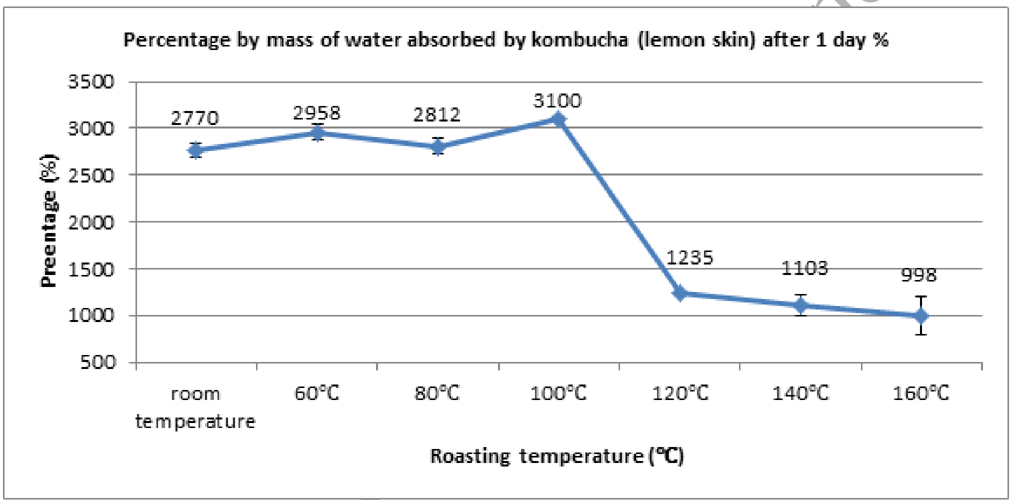


Fig. 3.3.3.2 Percentage by mass of water absorbed by kombuchas of lemon skins of different roasting temperature

Percentage by mass of water absorbed dropped significantly from 3100% to 1235% when kombuchas of lemon skins were roasted between 100°C to 120°C for 15 min, indicating that structural changes took place during roasting. Kombucha roasted at 100°C for 15 min has the highest water absorbance (3100%) while Kombucha roasted at 160°C for 30 min has the lowest water absorbance (998%)

3.3.4 Measuring percentage water absorbance of kombuchas of orange skins roasting for 30 minutes at different temperature

Temperature of roasting of kombuchas of orange skins for 30 mins/ °C	Percentage by mass of water absorbed by kombuchas (orange skins) after 1 day %

room temperature	2945
60	2975
80	2756
100	2522
120	1451
140	1444

Table 3.3.4.1 Percentage by mass of water absorbed by kombuchas of orange skins of different roasting temperature

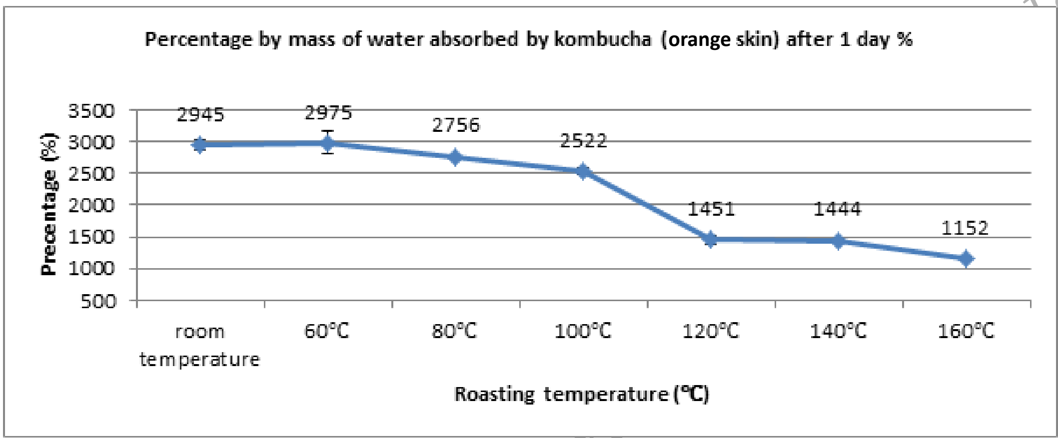


Fig. 3.3.4.2 Percentage by mass of water absorbed by kombuchas of orange skins of different roasting temperature

Percentage by mass of water absorbed dropped significantly from 2522% to 1451% when kombuchas of orange skins were roasted between 100°C to 120°C for 30 min, indicating that structural changes took place during roasting. Kombucha roasted at 60°C for 30 min has the highest water absorbance (2975%) while Kombucha roasted at 160°C for 30 min has the lowest water absorbance (1152%)

3.3.5 Measuring the force applied to pierce through 1mm kombuchas of lemon skins roasting at 120°C for different time and other disposables

Time of roasting /min	Force applied per 1mm thick kombucha (lemon skin)
0	7.3
15	12.7
30	11.6
45	11.2
60	7.3
120	5.1

Table 3.3.5.1 Force required to pierce through 1mm thick of kombucha roasted for different time and other samples

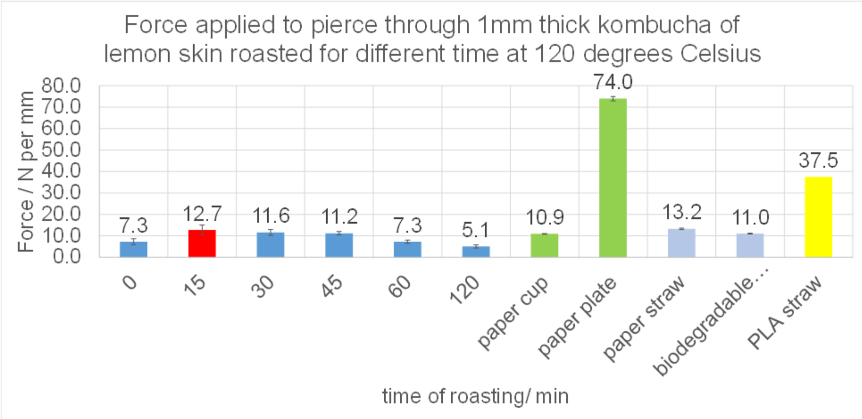


Fig. 3.3.5.2 Force required to pierce through 1mm thick of kombuchas roasted for different time and other samples

Paper plate (74.0N) has shown the strongest strength. Kombuchas of lemon skins roasted for 0 to 15 minutes at 120°C (12.7N) was the strongest among other Kombucha. Its strength was comparable with those of paper cup (10.9N), paper straw (13.2N) and biodegradable paper cups (11.0N). The results indicated that structural changes took place during the roasting.

3.3.6 Measuring the force applied to pierce through 1mm kombuchas of lemon skins roasting for 15 minutes at different temperature and other disposables

Roasting temperature/ degrees Celsius	Force applied per 1mm thick kombucha (lemon skin)
Room temperature	16.1
60	22.9
80	19.6
100	21.2
120	28.0
140	17.0
160	9.0
paper cup	10.9
paper plate	74.0
biodegradable paper cup	11.0
PLA straw	37.5

Table 3.3.6.1 Force applied to pierce through 1mm thick of kombucha roasted at different temperature for

15mins and other biodegradable disposables

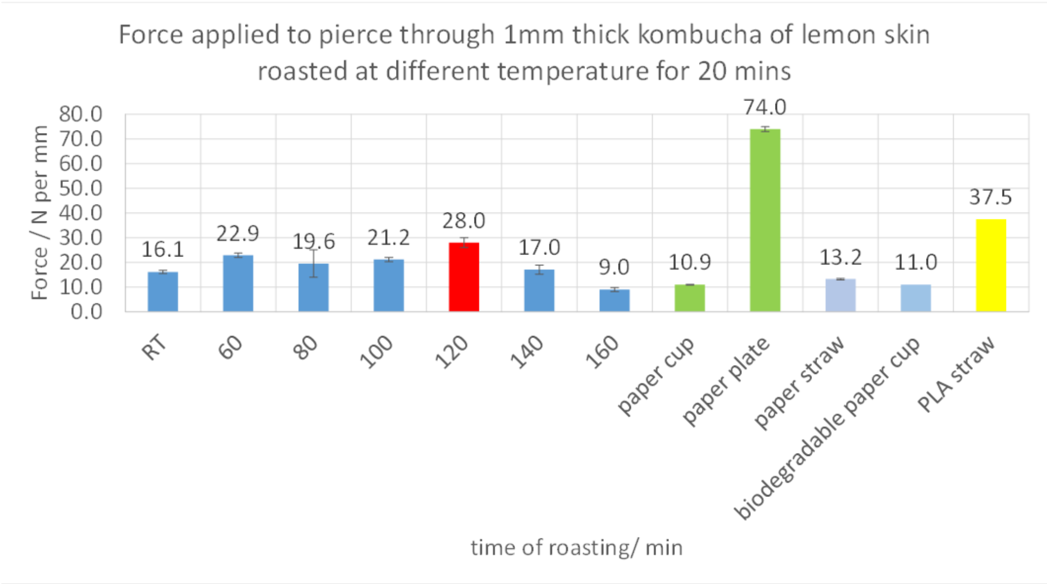
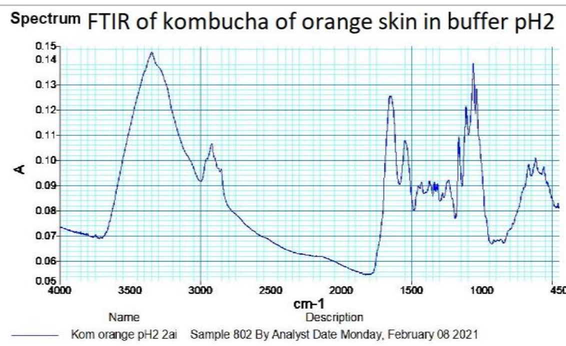


Fig. 3.3.6.2 Force applied to pierce through 1mm thick of kombucha roasted at different temperature for 15mins and other biodegradable disposables

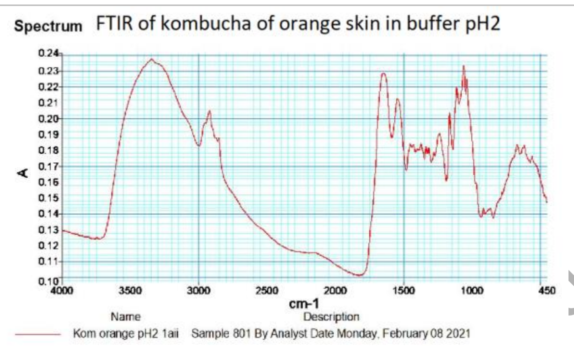
Paper plate (74.0N) has shown the strongest strength. Kombuchas of lemon skins roasted between 100°C to 120°C for 15 minutes (28.0N) showed the strongest strength among other kombuchas. It was found to be stronger than paper cups (10.9N), paper straws, biodegradable paper cups (11.0N) and has a comparable strength with PLA straws (37.5N). The results were consistent with the hypothesis that there are differences between structures of kombuchas of lemon skins and kombuchas of orange skins.

3.4 Effect of pH on the competing enzymatic actions: formation of kombucha and other enzymatic actions

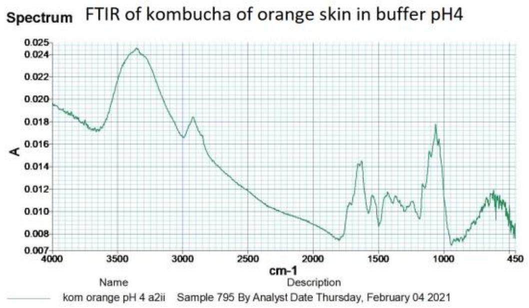
3.4.1 Enzymatic actions in orange skins



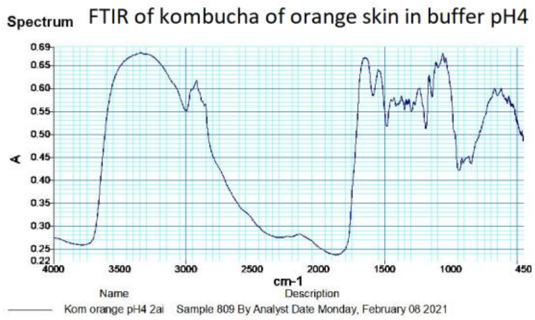
pH2 Ratio of absorbance of γ3400 to γ1660: 1.50



pH2 Ratio of absorbance of γ3400 to γ1660: 1.70



pH4 Ratio of absorbance of γ3400 to γ1660: 1.43



pH4 Ratio of absorbance of γ3400 to γ1660: 1.88

Fig.3.4.1.1 FTIR of kombuchas of orange skin formed in buffer solutions at pH 2 and pH 4

Ratio of absorbance of γ3400 to γ1660 of kombuchas of orange skins	**Buffer pH2 (pH value of solution=3)**	**Buffer pH4**
1	1.50	1.43
2	1.54	1.48
3	1.58	1.53
4	1.63	1.64
5	1.65	1.69
6	1.70	1.88

Table 3.4.1.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of orange skins in different buffer

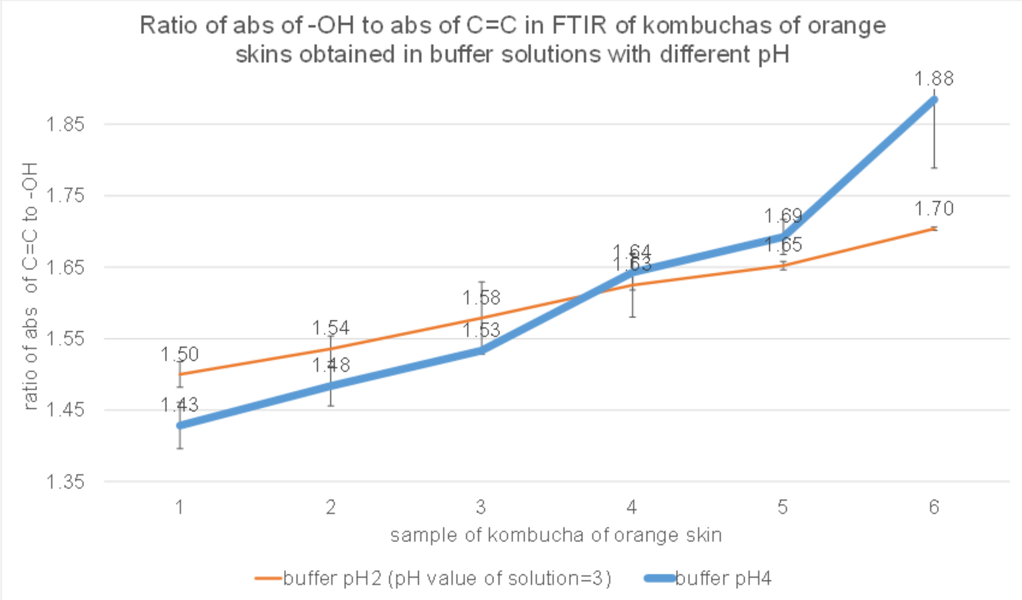


Fig. 3.4.1.3 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of orange skins in different buffer

The structure of kombuchas obtained in buffer pH4 showed a wider range of ratios of absorbance γ3400 to γ1660 of orange skins from 1.43 to 1.88 than that of buffer pH2 (from 1.50 to 1.70). The pH of the buffer pH4 solution changed to pH=3 during the cause.

3.4.2 Enzymatic actions in lemon skins

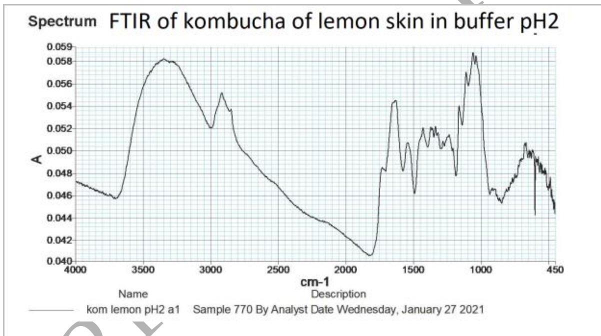
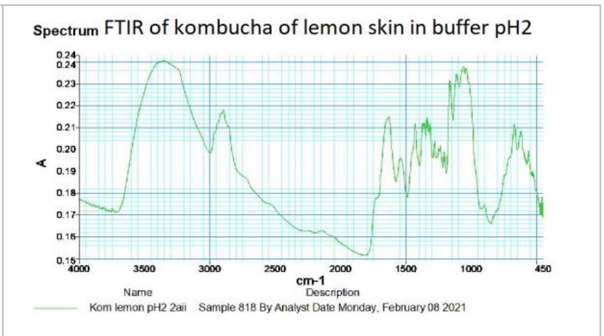
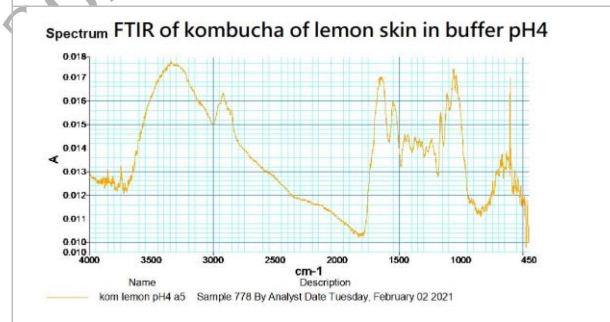
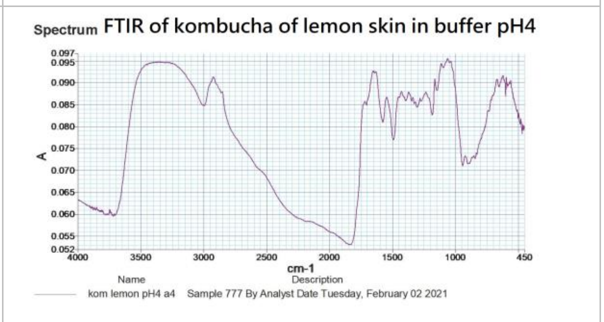
	
pH2 Ratio of absorbance of γ3400 to γ1660: 1.31	pH2 Ratio of absorbance of γ3400 to γ1660: 1.70
	
pH4 Ratio of absorbance of γ3400 to γ1660: 1.22	pH4 Ratio of absorbance of γ3400 to γ1660: 1.83

Fig. 3.4.2.1 FTIR of kombuchas of lemon skin formed in buffer solutions at pH 2 and pH 4

Ratio of absorbance γ3400 to γ1660 of kombuchas of lemon skins in FTIR	**Buffer pH2**	**pH4 (pH value of solution=3)**
1	1.31	1.22
2	1.32	1.27
3	1.39	1.37
4	1.62	1.70
5	1.68	1.82
6	1.70	1.83

Table 3.4.2.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins in different buffer

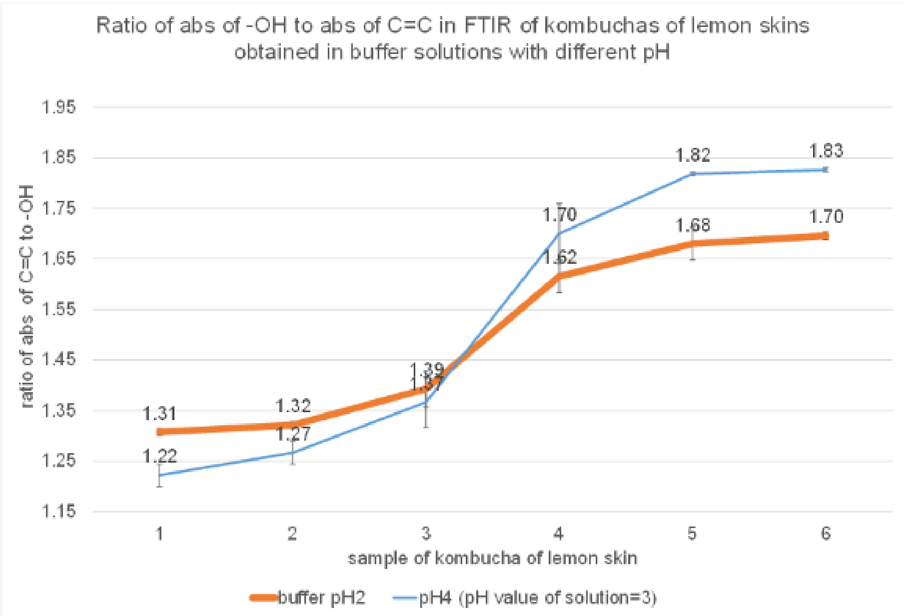


Fig. 3.4.2.3 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins in different buffer

The structure of kombuchas obtained in buffer pH4 showed a wider range of ratios of absorbance γ3400 to γ1660 of lemon skins from 1.22 to 1.83 than that of buffer pH2 (from 1.31 to 1.70). The pH of the buffer pH2 solution changed to pH=3 during the cause.

	Percentage of kombucha obtained in buffer pH2	**Percentage of kombucha obtained in buffer pH4**
orange skin	0.38	0.89
lemon skin	2.70	1.13

Table 3.4.2.4 Percentage of kombucha obtained buffer and the ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins and orange skins in different buffer

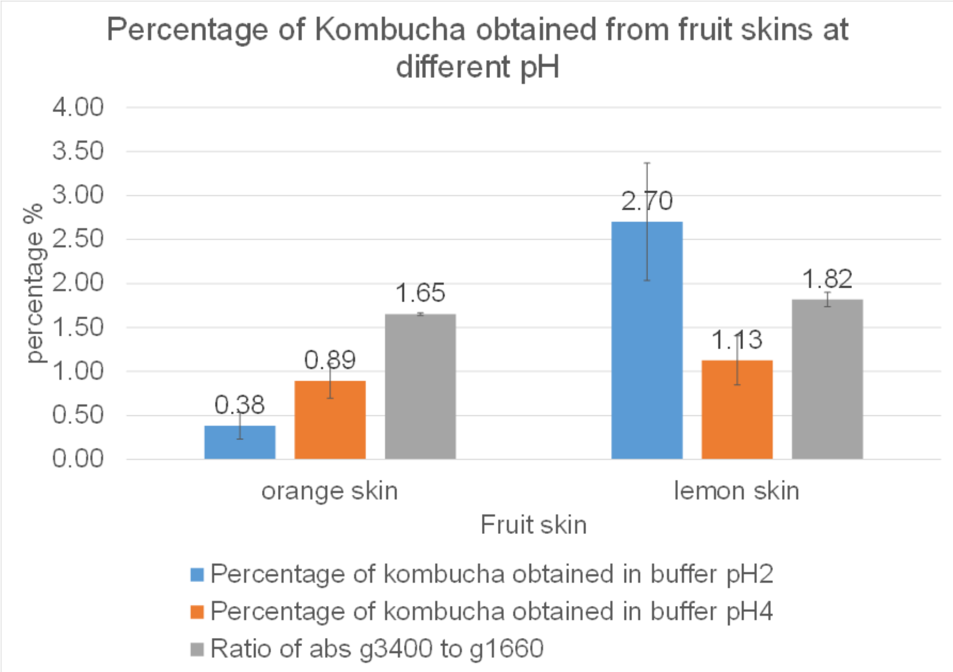
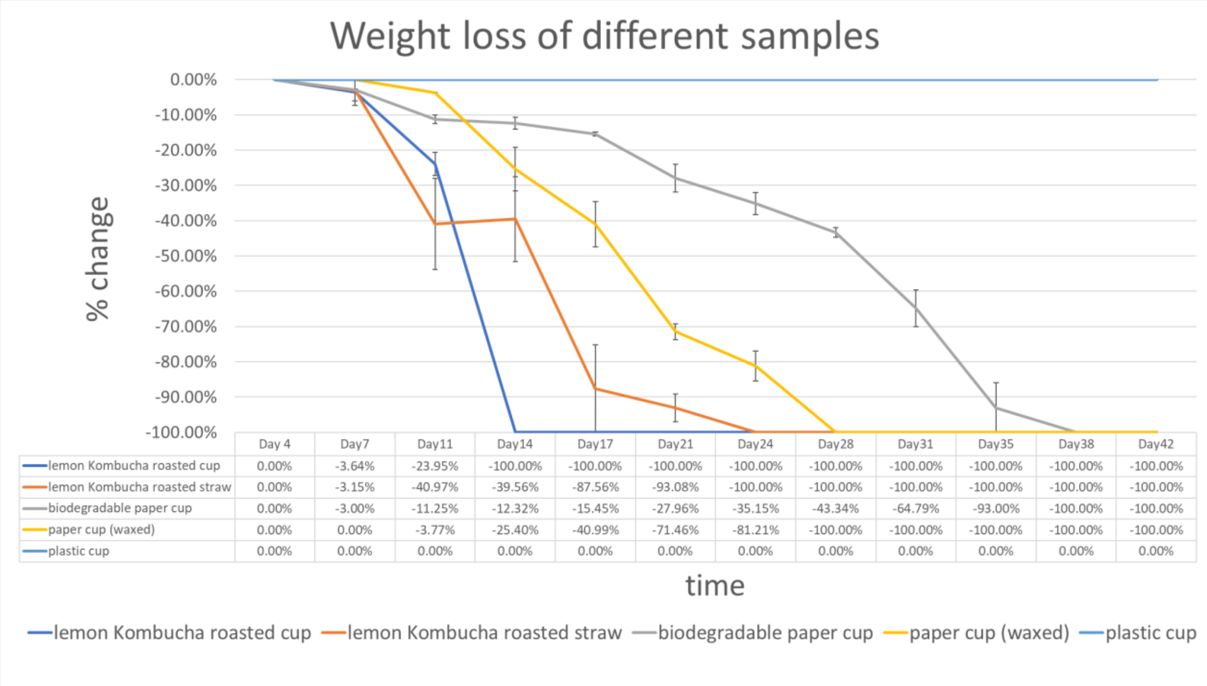


Fig.3.4.2.5 Percentage of kombucha obtained buffer and the ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins and orange skins in different buffer

The percentage of kombucha obtained from lemon skin in buffer pH2 was 2.70% with a smaller variation in structure (range of ratios of absorbance γ3400 to γ1660 of lemon skins from 1.31 to 1.70 pH2< 1.22 to 1.83 in pH4). The percentage was higher than that from orange skin (0.89% buffer pH4 with a larger range of ratios of absorbance γ3400 to γ1660 of orange skins 1.22 to 1.83). The structures of the kombuchas obtained from lemon skin were different from those from orange skins the ratio of absorbance γ3400 to γ1660 in FTIR was mainly 1.65 but that from orange skin was mainly 1.82. Obviously, formation of kombuchas of lemon skins was much favourable at pH about 2 to 3. The structures of kombuchas of lemon skins had smaller and narrower range of ratios of absorbance γ3400 to γ1660 (mainly 1.65; 1.31-1.70) than that of orange skins (mainly 1.82; 1.43 to 1.88). This might also explain a shorter time of roasting of kombuchas of lemon skins (15 mins at 120°C) than that of orange skins (30 mins at 120°C) for the condensation of -OH groups to take place in order to attain the best waterproofness and the strongest tensile strength.

3.5 Comparing the biodegradability of different roasted kombuchas and disposables



	Day 4	Day7	Day11	Day14	Day17	Day21	Day24	Day28	Day31	Day35	Day38	Day42
lemon Kombucha roasted cup	0.00%	-3.64%	-23.95%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%
lemon Kombucha roasted straw	0.00%	-3.15%	-40.97%	-39.56%	-87.56%	-93.08%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%
biodegradable paper cup	0.00%	-3.00%	-11.25%	-12.32%	-15.45%	-27.96%	-35.15%	-43.34%	-64.79%	-93.00%	-100.00%	-100.00%
paper cup (waxed)	0.00%	0.00%	-3.77%	-25.40%	-40.99%	-71.46%	-81.21%	-100.00%	-100.00%	-100.00%	-100.00%	-100.00%
plastic cup	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Fig. 3.5.1 Percentage change in mass of samples in soil during bio-degradation

Samples of cups	Time required for complete degradation (no. of week)
lemon Kombucha roasted cup	2
lemon Kombucha roasted straw	3.5
biodegradable paper cup	5.5
paper cup (waxed)	4
plastic cup	>6

Table 3.5.2 Time required for complete degradation

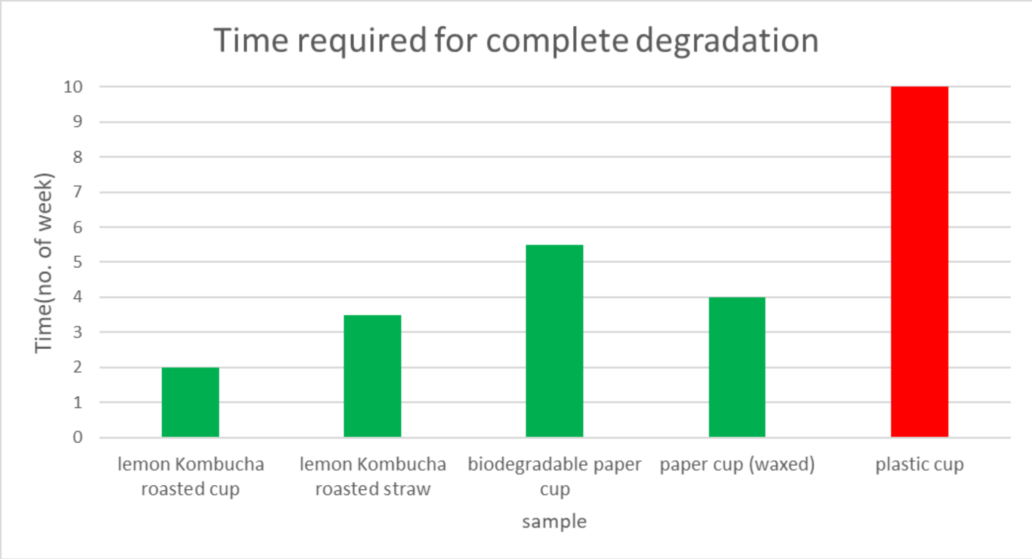


Fig. 3.5.3 Time required for complete degradation

For complete bio-degradation, roasted kombucha cups (lemon skins) took 2 weeks and roasted kombucha straws (lemon skins) took 3.5 weeks which were better than waxed paper cup (which took 4 weeks) biodegradable paper cup (which took 5.5 weeks). Plastic cups were not bio-degradable(not degraded within the experimental time).

3.6 Investigating the feasibility of using roasted kombuchas of lemon skins as disposables

3.6.1 Designing kombucha disposables such as drinking straws and cups

Kombuchas of lemon skins could be roasted to make cups and drinking straws.

Fig.3.6.1 straw(left) & cups(right) made of roasted kombuchas of lemon skins

3.6.2 Comparing the performance of roasted kombucha cups and paper cups holding drinking water and hot water

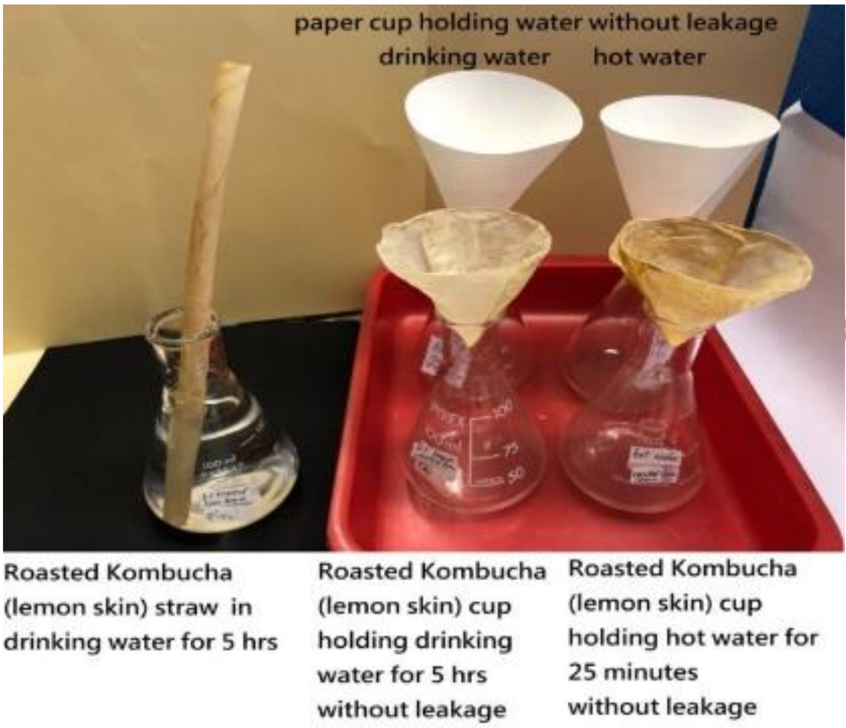


Fig.3.6.2.1 Performance of roasted kombucha cups and paper cups holding drinking water and hot water without leakage

Time	Drinking water	Hot water
Roasted kombucha cup (lemon skins)	5 hours	25 minutes
Paper cup	24 hours	24 hours

Table 3.6.2.2 Performance of roasted kombucha cups and paper cups holding drinking water and hot water without leakage

Roasted kombucha cups (lemon skins) could keep drinking water for 5 hours and hot water for 25 minutes without leakage, so roasted kombucha cups and straws could be biodegradable disposables.

3.6.3 Counting bacterial colonies of drinking water with different roasted kombucha disposables(lemon skin) overnight

No bacterial colonies were present in drinking water kept overnight in roasted kombucha cups(lemon skin) or soaked with kombucha straws (lemon skins), so roasted kombucha cups and straws are safe for serving as biodegradable disposables.

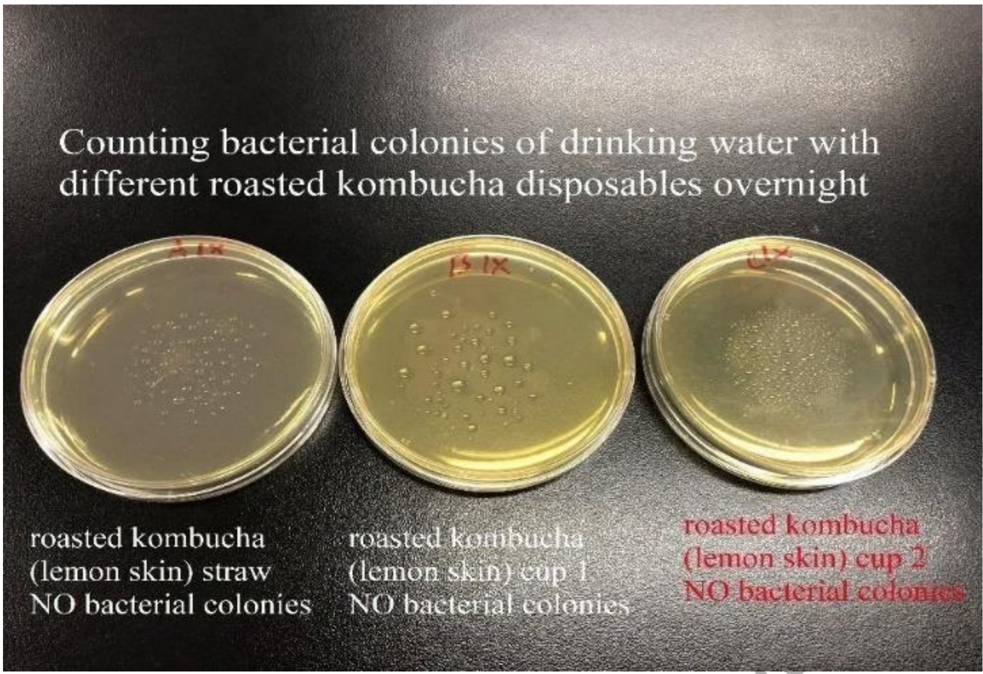


Fig.3.6.3 Counting bacterial colonies of drinking water kept in roasted kombucha straw (lemon skins)(left) and soaked with kombucha cups(lemon skins)(middle & right) overnight

3.7 Testing and certification

3.7.1 Testing and certification of roasted kombucha cups based on GB 18006-2008

3.7.1.1 Volume deviation

Holding drinking water 30 mins	Volume deviation %
roasted kombucha cup	8.0
bio-degradable paper cup	2.7
PS plastic cup	0.7
PE plastic cup	0.0
paper cup (waxed)	0.0

Table 3.7.7.1.1 Volume deviation of different cups

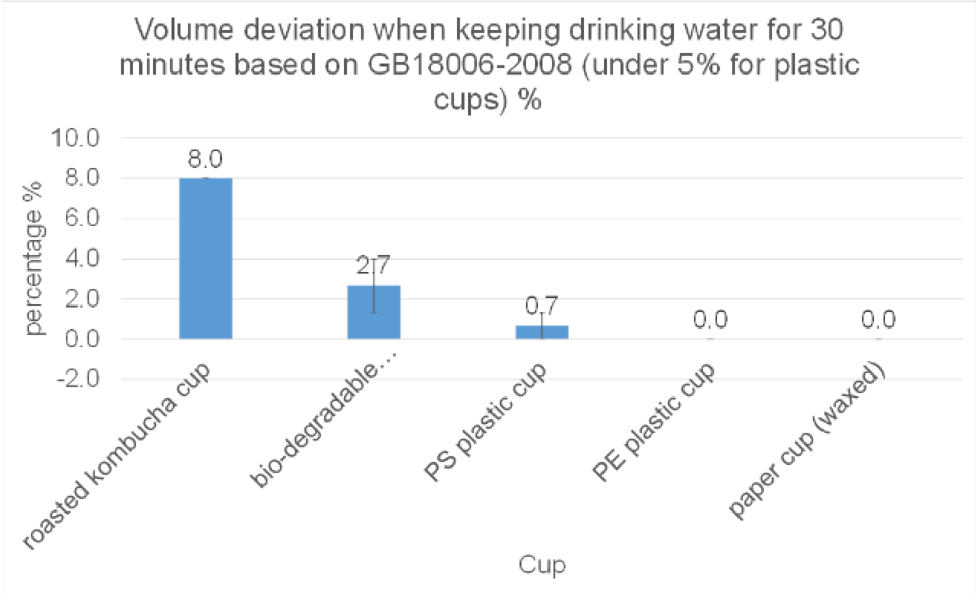


Fig. 3.7.7.1.2 Volume deviation of different cups

Plastic cups (0%) including PS cups and PE cups (0%) both met the volume deviation when keeping drinking water for 30 minutes) GB18006-2008 standard on plastic cups. Bio-degradable paper cups (2.7%) and paper (waxed) cups (0%) also met the standard. Roasted kombucha cups showed a deviation of 8% which was only 3% more than the standard for plastic cups, so Roasted kombucha cups should be suitable for serving as disposable cups.

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%.

3.7.1.2 Drop performance

Roasted kombucha cups, bio-degradable cups, PS plastic cups, PE plastic cups and paper cups (waxed) all pass the drop test with no cracks or splits and remained intact.

During the drop test of disposable tableware, there must be no cracks or splits to any of the three samples.

3.7.1.3 Hot-water resistance

Roasted kombucha cups, bio-degradable cups, PS plastic cups, PE plastic cups and paper cups (waxed) all pass the hot-resistance test with no deformations, peelings or wrinkles.

Fig. 3.7.1.3 Testing of hot-water resistance and water leakage resistance

3.7.1.4 Water leakage resistance

Roasted kombucha cups, bio-degradable cups, PS plastic cups, PE plastic cups and paper cups (waxed) all pass the water leakage resistance test with no water leakage.

3.7.2 Testing and certification of roasted kombucha straws based on ISO18188:2016 (Section 5.3)

3.7.2.1 Resistance to bending

Bending test at 90 degrees; 5 points	Resistance to bending: no. of rapture
roasted kombucha straw	0
bio-degradable paper straw	0
PLA straw	40
plastic straw	0

Table 3.7.2.1.1 Percentage rupture of different kinds of straws based on ISO18188:2016

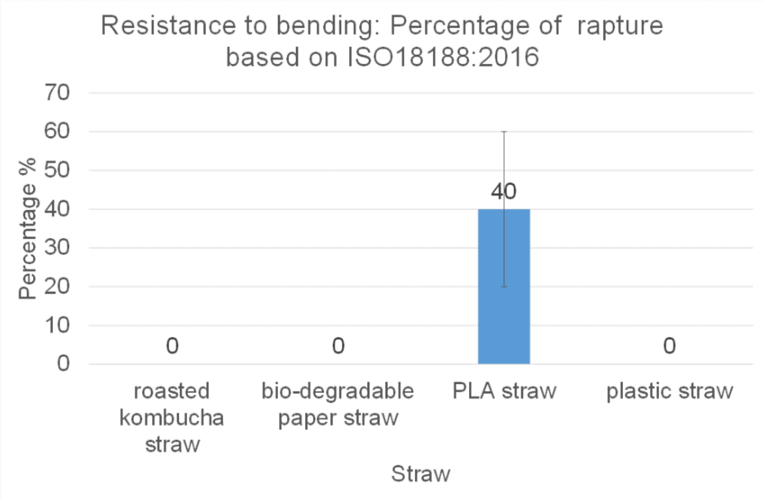


Fig. 3.7.2.1.2 Percentage rupture of different kinds of straws based on ISO18188:2016

Roasted kombucha straws, bio-degradable paper straw, Plastic straws all showed no rapture in the bending test. PLA straws bent immediate when hot water of 93°±2°C probably due to the uneven expansion of the polylactic acid and bamboo materials used to make the straws.

3.7.2.2 Heat endurance

Roasted kombucha straws, bio-degradable paper straw, Plastic straws all neither showed deformations nor colour fading after testing. Only PLA straws bent immediate when hot water of 93°±2°C probably due to the uneven expansion of the polylactic acid and bamboo materials used to make the straws. PLA straws showed no colour fading.

Roasted kombucha straws **bio-degradable paper straws** **PLA straws** **plastic straws**

Fig. 3.7.2.2 Testing heat endurance of straws

4. Findings

4.1 Competing enzymatic actions: Formation of kombucha from tannin-rich substrates such as fruit skins and other enzymatic actions such as enzymatic peeling

	Percentage of kombucha obtained in buffer pH2	Percentage of kombucha obtained in buffer pH4	Ratio of abs γ3400 to γ1660	Percentage of tannin %
orange skin	0.38	0.89	1.65	6.28
lemon skin	2.70	1.13	1.82	3.05

Table 4.1.1 Percentage of kombucha & tannin obtained and the ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins and orange skins in different buffer

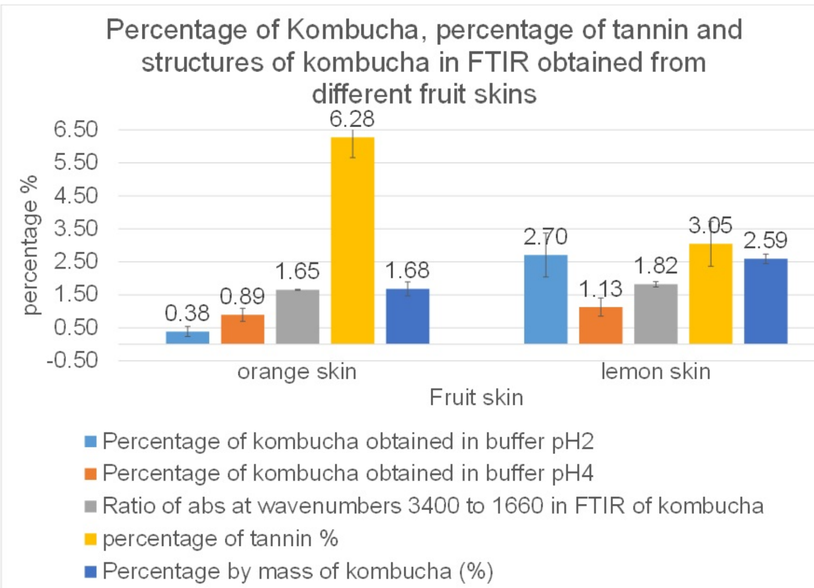


Fig. 4.1.2 Percentage of kombucha & tannin obtained and the ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} of kombuchas of lemon skins and orange skins in different buffer

Although the percentage by mass of tannin in lemon skin was 3.05% which was lower than that of orange (6.28%), the percentage by mass of kombucha formed from lemon skin was 2.70% (in buffer pH2) which was far greater than that of orange skin (0.89% kombucha in buffer pH4). At the same time, pH values of buffer pH2 increased from 2 to about 3 in the presence of orange skins suggesting that there were competing enzymatic actions taking place. There was competition between formation of kombucha which took place usually at a lower pH of 2 to 3 and other enzymatic actions such as peeling which took place at a higher pH of 3.5-4.5 (Pretel, 2008). Other enzymatic actions such as peeling was probably more prevalent in orange skins than formation of kombucha, so the pH value of buffer pH2 changed from 2 to 3 even though kombucha solution was present. On the other hand, the formation of kombuchas of lemon skins was more prevalent at a lower pH value of 2 to 3, so the rate of formation of kombucha was faster (on Day3 am in buffer pH2) than that of orange skin (on Day3 pm in buffer pH4) and the percentage of kombucha obtained was larger (2.70%) than that of orange (0.89%).

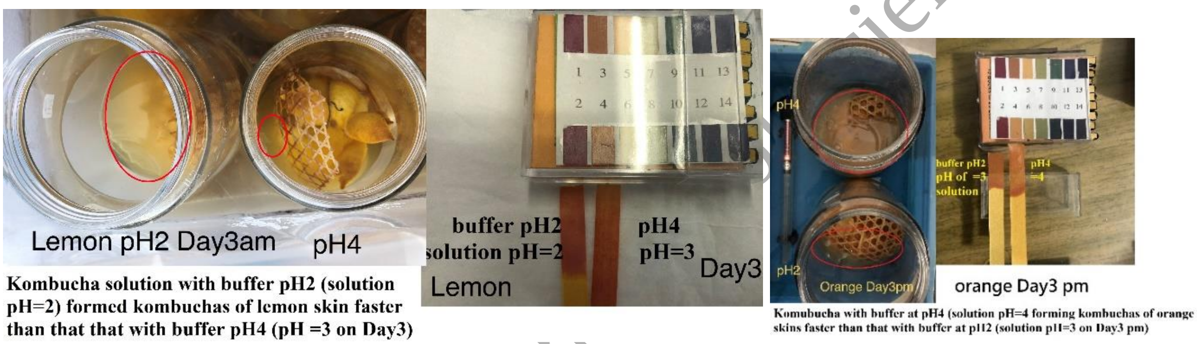


Fig. 4.1.3 Kombucha and pH values of lemon skins (left) & orange skins (right) in different buffer

4.2 Condensation of hydroxyl groups -OH in kombuchas by roasting at 120°C

4.2.1 Time for roasting of kombuchas of lemon skin at 120°C to attain the best waterproofness and the strongest strength

Time of roasting of kombuchas of lemon skins at 120°C /min	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of lemon skins)	Percentage by mass of water absorbed by kombuchas (lemon skins) after 1 day %	Force applied per 1mm thick kombuchas (lemon skins)
0	1.65	2571	7.3
15	1.24	1716	12.7
30	1.32	1770	11.6
45	1.29	1783	11.2

60	1.40	1593	7.3
120	1.34	1602	5.1

Table 4.2.1.1 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1}, percentage of water absorbed and strength of kombuchas of lemon skins roasted at 120°C for different time

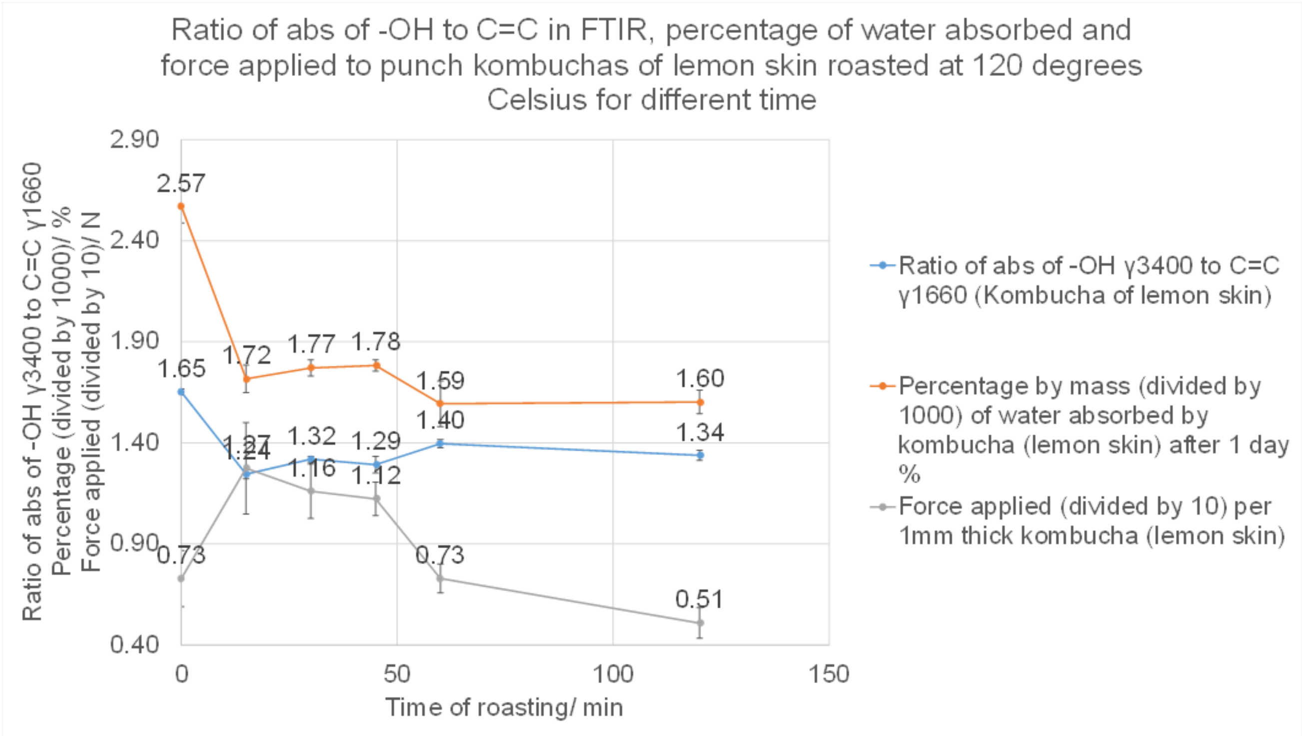


Fig. 4.2.1.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} , percentage of water absorbed and strength of kombuchas of lemon skin roasted at 120°C for different time

When the time of roasting of kombuchas of lemon skins was between 0 to 15mins at 120°C, the ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 1.65 to 1.24, the percentage by mass of water absorbed significantly dropped by 33% (from 2571% to 1716%) and the strength of the kombucha obtained was the strongest among other kombuchas (12.7N to pinch through 1mm thick kombucha). These results indicated that structural changes of kombuchas of lemon skins took place when kombuchas of lemon skins were roasted at 120°C for 0 to 15 minutes.

During the roasting of kombuchas, probably condensation of hydroxyl groups -OH took place. As the formation of kombuchas of lemon skins took place at pH about 2 to 3 and the structures of kombuchas obtained had smaller and narrower range of ratios of absorbance γ3400 to γ1660 (mainly 1.65; 1.31-1.70) than that of orange skins (mainly 1.82; 1.43 to 1.88), a shorter time of roasting of kombuchas of lemon skins (15 mins at 120°C) than that of orange skins (30 mins at 120°C) for the condensation of -OH groups to take place in order to attain the best waterproofness and the strongest tensile strength.

4.2.2 Temperature of roasting of kombuchas of lemon skin for 15 minutes to attain the best waterproofness and the strongest strength

Temperature of roasting of kombuchas of lemon skins for 15mins/ °C	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of lemon skins)	Percentage by mass of water absorbed by kombuchas (lemon skins) after 1 day %	Force applied per 1mm thick kombucha (lemon skin)
25	1.74	2770	16.1
60	1.81	2958	22.9
80	1.85	2812	19.6
100	1.78	3100	21.2
120	1.47	1235	28.0
140	1.37	1103	17.0
160	1.39	998	9.0

Table 4.2.2.1 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} , percentage of water absorbed and strength of kombuchas of lemon skins roasted for 15 mins at different temperature

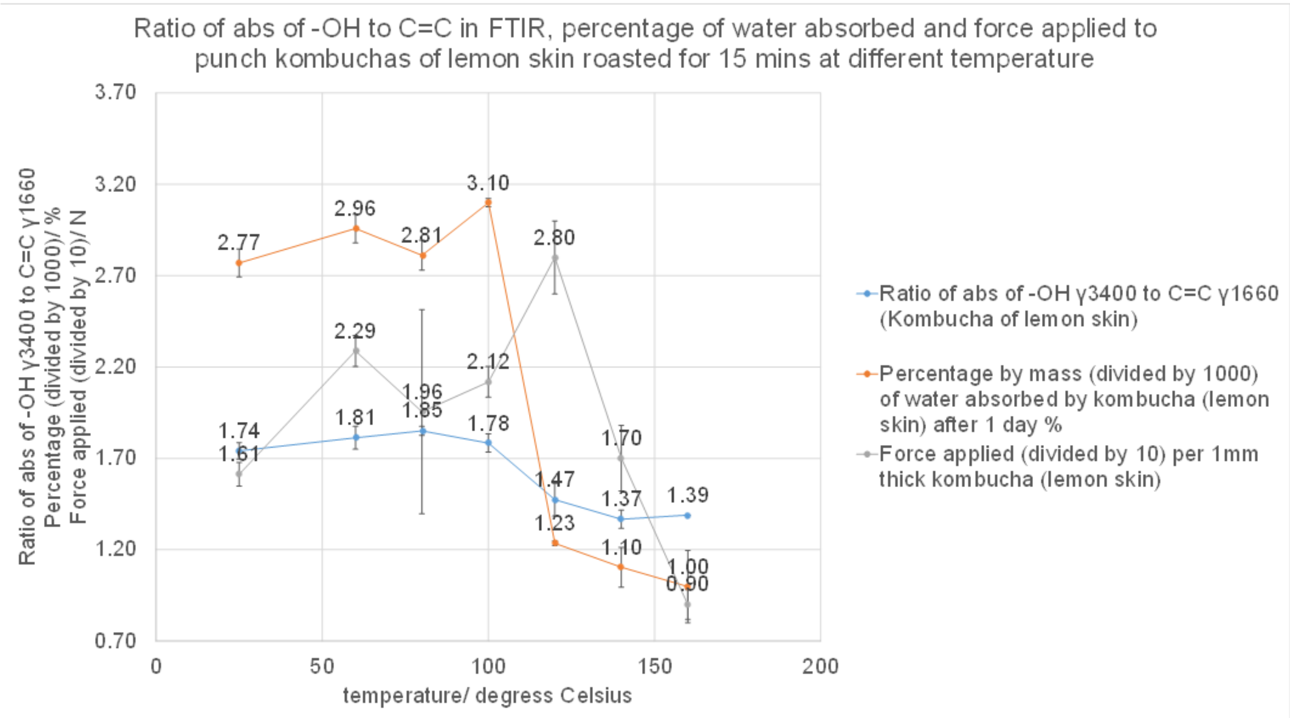


Fig. 4.2.2.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} , percentage of water absorbed and strength of kombuchas of lemon skin roasted for 15 mins at different temperature

When the temperature of roasting of kombuchas of lemon skins was between 100°C to 120°C for 15mins, the ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 1.78 to 1.47, the percentage by mass of water absorbed significantly dropped from 3100% to 1235% and the strength of the kombucha obtained was the strongest among other kombuchas (28.0N to pinch through 1mm thick kombucha). These results indicated that structural changes of kombuchas of lemon skins took place when kombuchas of lemon skins were roasted between 100°C to 120°C for 15 minutes.

4.2.3 Time for roasting of kombuchas of orange skin at 120°C to attain the best waterproofness

Time of roasting of kombuchas of orange skins at 120°C /min	**Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of orange skins)**	**Percentage by mass of water absorbed by kombuchas (orange skins) after 1 day %**
0	**1.82**	985
15	1.78	942
30	1.52	745
45	1.58	741
60	1.44	735
120	1.37	601

Table 4.2.3.1 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} and percentage of water absorbed by kombuchas of orange skins roasted at 120°C for different time

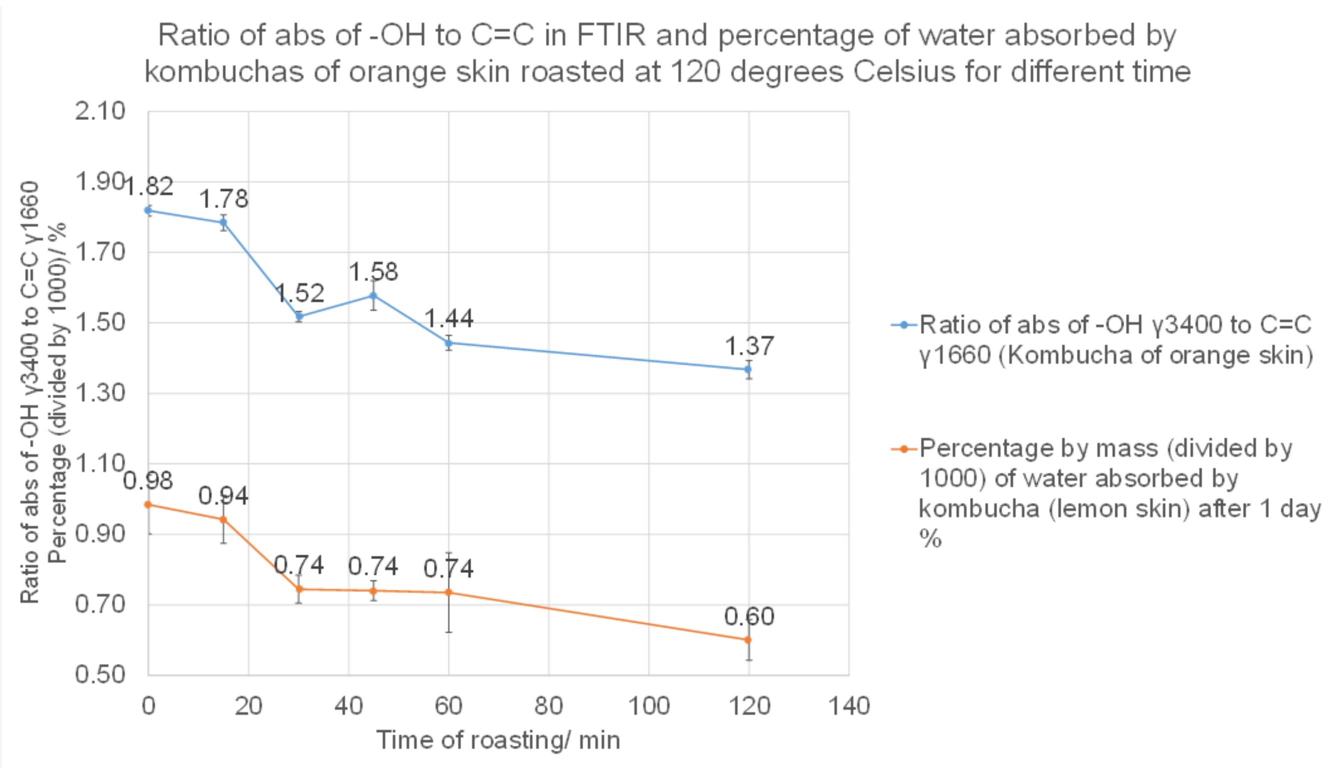


Fig. 4.2.3.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} and percentage of water absorbed by kombuchas of orange skin roasted at 120°C for different time

When the time of roasting of kombuchas of orange skins was between 15 to 30mins at 120°C, the ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 1.78 to 1.52 and the percentage by mass of water absorbed significantly dropped from 942% to 745%. These results indicated that structural changes of kombuchas of orange skins took place when kombuchas of lemon skins were roasted at 120°C for 15 to 30 minutes.

4.2.4 Temperature of roasting of kombuchas of orange skin for 30 minutes to attain the best waterproofness

Temperature of roasting of kombuchas of orange skins for 15mins/ °C	Ratio of abs of -OH γ3400 to C=C γ1660 (Kombuchas of orange skins)	Percentage by mass of water absorbed by kombuchas (orange skins) after 1 day %
25	2.24	2945
60	2.28	2975
80	2.16	2756
100	2.10	2522
120	1.86	1451
140	1.87	1444
160	1.88	1152

Table 4.2.4.1 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} and percentage of water absorbed by kombuchas of orange skins roasted for 15 minutes at different temperature

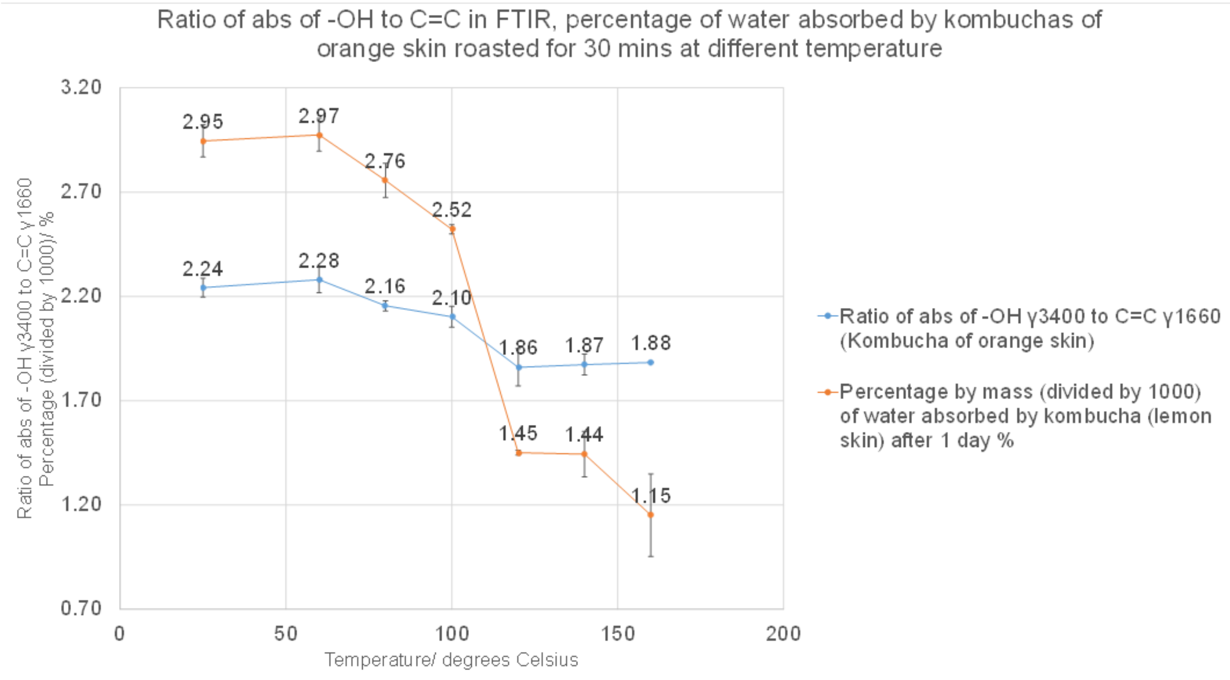


Fig. 4.2.4.2 Ratio of absorbance of –OH at 3400 cm^{-1} to C=C at 1660 cm^{-1} and percentage of water absorbed by kombuchas of orange skin roasted for 15minutes at different temperature

When the temperature of roasting of kombuchas of orange skins was between 100ºC to 120ºC for 15mins, the ratio of absorbance of -OH to the absorbance of C=C in FTIR dropped significantly from 2.10 to 1.86 and the percentage by mass of water absorbed significantly dropped from 2522% to 1451%. These results indicated that structural changes of kombuchas of lemon skins took place when kombuchas of lemon skins were roasted between 100ºC to 120ºC for 30 minutes.

4.3 Roasted kombuchas of lemon skin as bio-disposables

4.3.1 strength of roasted kombuchas

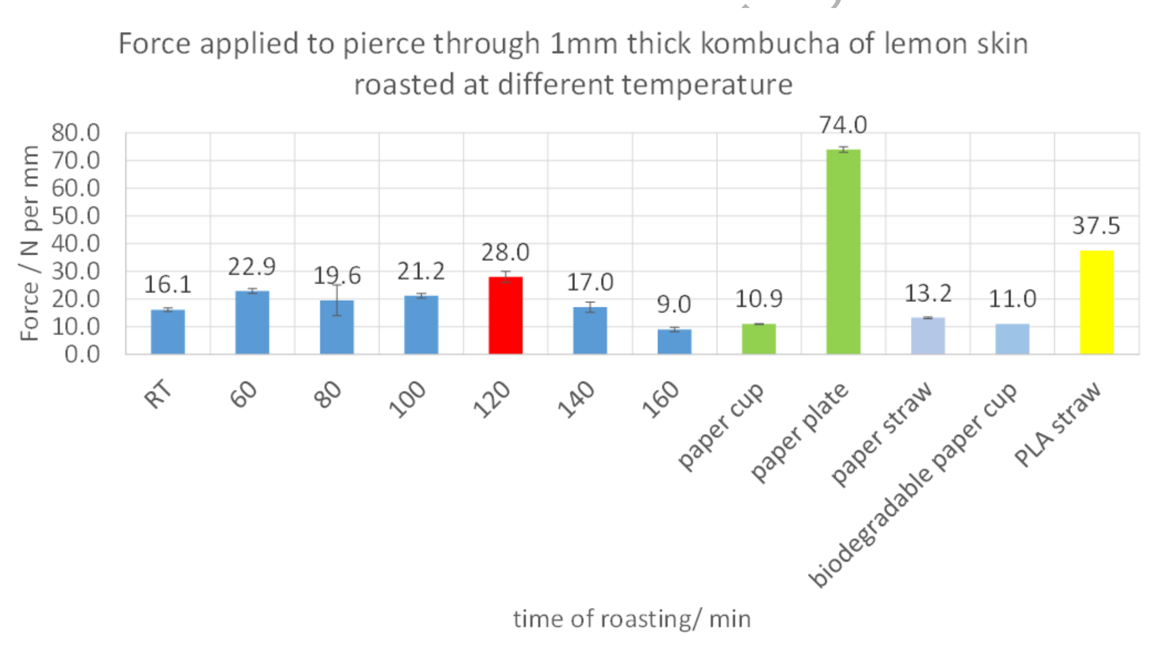


Fig. 4.3.1 Force applied to pierce through kombuchas of lemon skin roasted for 15 minutes at different temperature

Paper plates (74.0N) have shown the strongest strength. Kombuchas of lemon skins roasted between 100ºC to 120℃ for 15 minutes (28.0N) showed the strongest strength among other kombuchas. It was found to be stronger than paper cups (10.9N), paper straws, biodegradable paper cups(11.0N) and has a comparable strength with PLA straws (37.5N).

4.3.2 Performance of roasted kombuchas cups and drinking straws

Roasted kombucha cups (lemon skins) could keep drinking water for 5 hours and hot water for 25 minutes without leakage, so roasted kombucha cups and straws could be biodegradable disposables.

4.3.3 Health concerns about using roasted kombucha cups and straws

No bacterial colonies were present in drinking water kept overnight in roasted kombucha cups(lemon skin) or soaked with kombucha straws (lemon skins), so roasted kombucha cups and straws are safe for serving as biodegradable disposables.

4.3.4 Impact of the disposal of roasted kombucha cups and straws on the environment

For complete bio-degradation, roasted kombucha cups (lemon skins) took 2 weeks and roasted kombucha straws (lemon skins) took 3.5 weeks which were way better than biodegradable paper cup (which took 5.5 weeks). Paper cups (waxed) and plastic cups were not bio-degradable.

4.4 Testing and certification of roasted kombucha cups and straws

4.4.1 Testing & certification of characteristics of roasted kombucha cups based on GB 18006-2008

Section no.	GB 18006-2008	Roasted kombucha cup	Bio-degradable paper cup	PE Plastic cup	PS plastic cup	Paper cup (waxed)
4.4.1 Volume deviation Standard error stated in ( )	Less than 5%	**8%(0)**	2.7(1.3)	0.7(0.67)	0(0)	0(0)
4.4.3 Drop performance (from 0.8m)	intact	**intact**	intact	intact	intact	intact
4.4.5.1 Hot-water resistance (hot water for 30 mins)	no deformations, peelings or wrinkles	**no deformations, peelings or wrinkles**	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles	no deformations, peelings or wrinkles
4.4.6 Water leakage resistance	no leakage	**no leakage**	no leakage	no leakage	no leakage	no leakage

4.4.2 Testing & certification of characteristics of roasted kombucha straws based

on ISO18188:2016

Section no.	ISO18188:2016	Roasted kombucha straw	Bio-degradable paper straw	PLA straw	Plastic straw
4.3.1 resistance to bending	no rupture	**no rupture**	no rupture	2 ruptures out of 5 points (Standard error: 1)	no rupture
4.3.2 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	bent immediately; no fading of colour	no deformations; no fading of colour

Roasted kombucha straws met the standard required in ISO18088:2016 the resistance to bending test and heat endurance, so roasted kombucha straws should be certified to be disposable straws.

Roasted kombucha cups met the standard required in GB 18006-2008 (plastic cups) the drop performance, hot-water resistance and water leakage resistance. The volume deviation was 8% which was only 3% more than the requirement for plastic cups. (cf. bio-degradable paper cup 2.7%), so roasted kombucha cups should be certified to be disposable cups.

5. Discussion

5.1 Roasted kombucha cups and straws on the environment as bio-disposables

For complete bio-degradation, roasted kombucha cups (lemon skins) took 2 weeks and roasted kombucha straws (lemon skins) took 3.5 weeks which were better even than biodegradable paper cup (which took 4 weeks). Paper cups (waxed) and plastic cups were not bio-degradable.

5.1.1 Testing and certification of roasted kombucha cups based on GB 18006-2008 (plastic cups) and roasted kombucha straws based on ISO18188:2016

Roasted kombucha cups remained intact after the drop test, no deformations, peelings or wrinkles in the hot-water resistance, no water leakage in the water leakage resistance and volume deviation of 8% which was only

3% higher than the requirement for plastic cups.

Roasted kombucha straws showed no rupture and hence resistance to bending, no deformations and no fading of colour in the heat endurance test.

Roasted kombucha cups and straws are eligible for marking as bio-disposables.

5.1.2 Health concerns about using roasted kombucha cups and straws

No bacterial colonies were present in drinking water kept overnight in roasted kombucha cups(lemon skin) or soaked with kombucha straws (lemon skins), so roasted kombucha cups and straws are safe for serving as biodegradable disposables.

Roasted kombucha cups and straws are safe to serve as bio-disposables.

5.2 Cost effectiveness in the production of roasted kombuchas of lemon skins for a shorter time of 15 minutes instead of 30 minutes

To increase the waterproofness and strength of kombuchas, condensation of hydroxyl groups done by roasting was carried out at 120ºC and 15 minutes for kombuchas of lemon skins but a longer time of roasting of 30 minutes for that of orange skins.

Thus, the use of lemon skins as raw materials for the production of kombuchas would lower the production cost of manufacturing roasted kombucha bio-disposable.

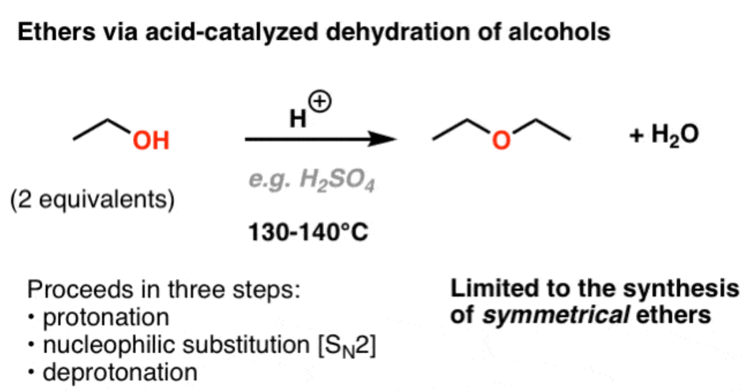


Figure 5.2.2 removal of hydroxyl group

(https://www.masterorganicchemistry.com/2014/11/14/ether-synthesis-via-alcohols-and-acid/)

5.3 Controlling pH to monitor competing enzymatic actions

The percentage of kombucha obtained from lemon skin in buffer pH2 was 2.70% that was higher than that without buffer (2.59%). Probably different enzymatic actions are favoured at different pH values. Formation of kombucha is more prevalent at lower pH values between 2 to 3 and other enzymatic actions such as enzymatic peeling is more prevalent at higher pH values between 3.5 to 4.5. As the optimum temperature for both enzymatic actions are 35ºC (Pretel, 2008), by controlling pH, we can manipulate competing enzymatic actions and make the most out of them.

6. Limitations

6.1 Testing and certification of disposables

The following testing and certification could not be done during the investigation due to lack of time, details such as GB/T 462 not available on the internet free of charge etc.

6.1.1 Roasted kombucha cups: Testing and certification based on GB 18006-2008 (Section 5.4-5.6)

6.1.1.1 Microwave-safe test

6.1.1.2 Microwave high-frequency heat performance

There shall be no electric sparks, no defects, odour or abnormality. There must be no defects, odour or abnormality with either test sample.

6.1.1.3 Microwave temperature-resistant performance

There shall be no deformations, defects, leakages or abnormality. There must be no deformations, defects, leakages or abnormality with all three samples.

6.1.1.4 Moisture content

For disposable tableware made of natural materials such as plant fibre moulding disposable tableware etc., the moisture content must not exceed 7% in accordance with GB/T 462.. The determination of moisture content only applies to disposable tableware made of natural materials. For disposable tableware made of other materials, the determination of moisture content is not required.

6.1.1.5 Biodegradability

The biodegradability for degradable disposable tableware shall meet the requirements of biodegradability specified in GB/T 20197-2006. (Section 5.4)

6.1.1.6 Load performance test

Load-bearing performance

$$w = \frac{H_0 - H}{H_0} \times 100 \quad (1)$$

in which,

W – the variation rate of the weight load of the sample, as a percentage (%);

H_o – the height before weight loaded, in millimetres (mm);

H – the height after weight loaded, in millimetres (mm).

6.1.2 roasted kombucha straws: Testing and certification based on ISO18188:2016 (Section 5.3)

6.1.2.1 Cold endurance (free details of the criteria are not available on the Internet.)

6.2 Effect of controlling pH on competing enzymatic action using different citrous fruit skin

Due to the limitation of time and space, only kombuchas of lemon skins and orange skins were under investigation.

7. Further studies

7.1 Testing and certification of disposables

7.1.1 Roasted kombucha cups: Testing and certification based on GB 18006-2008 (Section 5.4-5.6)

More testing and certification could be done as follows.

7.1.1.1 Microwave-safe test

Microwave high-frequency heat performance

There shall be no electric sparks, no defects, odour or abnormality. There must be no defects, odour or abnormality with either test sample.

7.1.1.2 Microwave temperature-resistant performance

There shall be no deformations, defects, leakages or abnormality. There must be no deformations, defects, leakages or abnormality with all three samples.

7.1.1.3 Moisture content

For disposable tableware made of natural materials such as plant fibre moulding disposable tableware etc., the moisture content must not exceed 7%. The determination of moisture content only applies to disposable tableware made of natural materials. For disposable tableware made of other materials, the determination of moisture content is not required.

7.1.1.4 Biodegradability

The biodegradability for degradable disposable tableware shall meet the requirements of biodegradability specified in GB/T 20197-2006. The biodegradability determination only applies to disposable tableware claimed or indicated or marked to be degradable.

7.1.1.5 Load-bearing performance

The shape of roasted kombucha cups should be modified for the Load-bearing performance as the cups used in our investigation did not have a flat bottom.(Fig. 7.1.1.5)

Fig. 7.1.1.5 samples of cups

7.1.2 roasted kombucha straws: Testing and certification based on ISO18188:2016 (Section 5.3)

7.1.2.1 Cold endurance (free details of the criteria are not available on the Internet.)

7.2 Effect of controlling pH on competing enzymatic action using different citrous fruit skin

As different citrous fruit skins have different surface pH, the effect of controlling pH should shed idea on how formation of kombucha and other enzymatic actions such as peeling would take place and the extent of theses actions. In doing so, the best roasting conditions including acidity and the time of roasting could be investigated to attain the highest yield and strongest roasted kombuchas.

8. Conclusion

The plastic pollution has affected globally for decades, especially the increasing demand for disposable straws and cups, alternatives have to be found in order to solve this dilemma. Bio-disposables such as roasted kombucha cups remained intact after the drop test, no deformations, peelings or wrinkles in the hot-water resistance, no water leakage in the water leakage resistance and volume deviation of 8% which was only 3% higher than the requirement for plastic cups based on GB 18006-2008 and roasted kombucha straws showed no rupture and hence resistance to bending, no deformations and no fading of colour in the heat endurance test based on ISO18188:2016. Besides, no bacterial colonies were present in drinking water kept overnight in roasted kombucha cups or soaked with kombucha straws, so roasted kombucha cups and straws are safe for serving as biodegradable disposables. The production cost for the production of roasted kombuchas using lemon skins was lower as only 15 to 15 minutes were required for the roasting of lemon skins (cf. orange skins: 30 mins) at 120^oC in increasing waterproofness and strength. By controlling pH at a lower value such as 2 to 3, the percentage of kombucha obtained was 2.70% that was higher than that without buffer (2.59%). Bio-disposables such as roasted kombucha straws and cups can upgrade our quality of life and save our planet.

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