A. Inspiration

Plastics and paper cups have brought convenience to humans' life. Yet, these non-biodegradable or non-recyclable material are harming the ecosystem, especially the marine lives. The condensed tannins are known for their polymerization chemistry. (Tondi, 2017) Therefore, kombucha, a form of condensed tannin, is a possible solution to the aforementioned problem.

Kombucha is an elastic, water-proof and bio-degradable polymers obtained from tannin-rich putrescible such as tea leaves and fruit skins. Moreover, kombucha is edible and being consumed worldwide because of its suggested benefits to human body. (Javabalan, 2014) In this study, method of producing quality kombucha and its properties are being investigated.

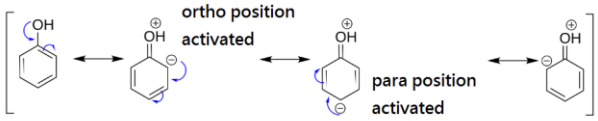
B. Theory

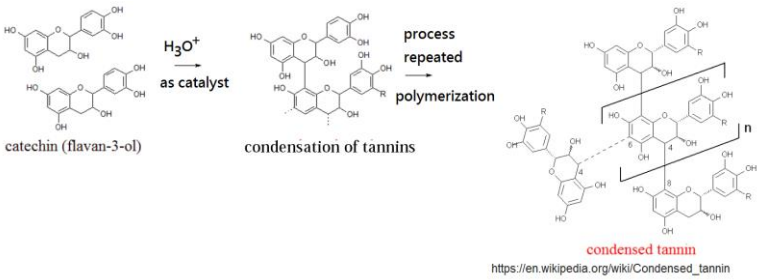
As a chalcogen, oxygen plays an important role in the detection of tannin and the formation of kombucha from tannin-rich samples such as tea leaves, coffee and fruit skins.

1.1 Lone pair(s) of electrons on oxygen are readily available for resonance in benzene rings making -OH a good ring activator in tannin/ condensed tannin.

Condensed tannins are polymers formed by the condensation of flavans such as flavan-3-ol in catechin which are commonly found in plants. They are all polyphenols with the functional group $C{=}C(OH)$. Condensation usually takes place at the para- or ortho (2- or 4-) position in the phenolic ring as the para and ortho positions are activated by the enol group.

The oxygen in the −OH group in phenol has unpaired electrons that are capable of being donated to the ring. Although oxygen is electronegative and does exhibit some negative inductive effects, the electron sharing that oxygen is capable of (shown by when drawing the resonance structures) has a stronger effect and results in –OH being a good ring activator. Resonance structures are shown below.

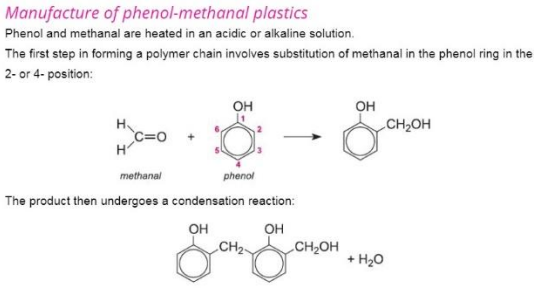




And the suggested process of the condensation of tannin is as above.

1.2 Lone pair of electrons on oxygen in carbon-oxygen double bond enables aldehydes to become electrophiles and undergo electrophilic aromatic substitution followed by condensation with phenols in tannins/condensed tannin.

During the reaction between methanal (electrophile: searching for electron-rich centre) and phenol (electron-rich centre due to ring activation by -OH), electrophilic aromatic substitution and takes place and then condensation as follows.



http://www.essentialchemicalindustry.org/polymers/methanal-plastics.html

Bridges are also formed between tannins using aldehydes. The condensed tannins or proanthocyanidins are already known for their polymerization chemistry (Tondi, 2017) with various hardeners, for example, aldehydes. Similarly, glucose which contains functional group –CHO can form bridges between tannins giving kombucha which is a polymer.

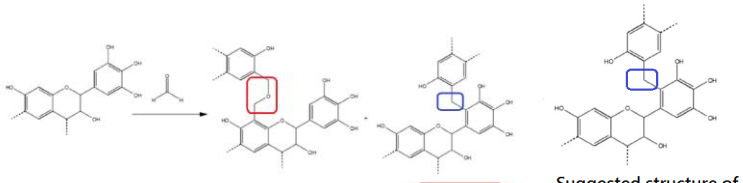


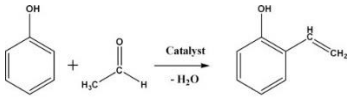
Figure 4. Polymerization reaction of Mimosa tannin with formaldehyde. Methylene-ether and methylene bridges.

Suggested structure of kombucha with methylene bridge

Similarly, kombucha is formed as a result of electrophilic aromatic substitution between glucose and condensed tannin. Then condensation takes place. The suggested kombucha structure is shown as above.

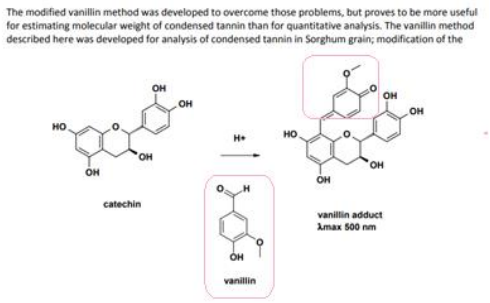
1.3 Detection of presence of tannin using vinylation between vanillin and tannin as a result of ring activation of -OH in tannins/condensed tannins

An example of vinylation reaction is as follows.



Catalyst: 13% Cr_2O_3, 1% K / Al_2O_3

(Parfenov, 2016)



(Hagerman, 2002)

Similarly, vinylation takes place when of tannin reacts with vanillin. A colour adduct is formed which absorb light at 500nm. In this reaction, vinylation involves the coupling of vanillin via the formation of –C=C- from the –CHO aldehyde group on vanillin. Thus, the amount of tannin can be determined by the amount of absorbance with a light spectrometer at 500nm. (Formagio, 2014)

C. Methodology

1. Determining the amount of 80% ethanol and the power of microwave used to extract tannin

1. About 2.5g of a sample were weighed.
2. 30cc of 80% ethanol was added for the extraction tannin.
3. The mixture was microwaved for 2 min using medium low power.
4. The absorbance of the reaction mixture was taken every 30sec at 500nm, 510nm and 700nm.
5. The experiment was repeated using medium power and 20cc 80% ethanol.

Fig. 4.1.1 Extraction of tannin using microwave (700W)

2. Extraction of tannin from tea bags, coffee and fruit skins

Fig. 4.2 Extraction of tannin using 80% ethanol at medium low power for 2min in a microwave(700W)

Fig.4.1.2 Spectrometer for the absorbance at 500nm and 510nm

3. Investigation of the amount of tannin in tea, coffee and fruit skins using vanillin-HCl

method

1. 1cc tannin extracted by 80% ethanol was added to 1cc vanillin and 1cc of 8%HCl solution.
2. A pink colour was developed after 3 min.
3. The absorbance of the mixture was measured using a spectrometer at 500nm and 510nm.

Fig. 4.3 Pink colour developed between tannin in Olong tea and vanillin-HCl

4. Production of kombucha

1. About 2.5g of tannin-containing sample was put into a plastic bag.
2. 100cc water and 7g of glucose was added.
3. Kombucha will be formed a week later.

Fig. 4.4.1 Production of Kombucha using zipper bags Fig. 4.4.2 Production of Kombucha using trays

5. Comparison between the performance of kombucha cup and paper cup

1. Kombucha from black tea was cut and folded into a cup shape.
2. The cup was roasted in oven at 150^{o}C for 30min to increase its rigidity and water-proof property.
3. Tap water, iced water and hot water were poured into each cup respectively.
4. A beaker was placed under each cup to hold any leakage.

Fig. 4.5 Paper cups (top) and kombucha (from black tea) cups(bottom)

D. Results and conclusion

1 Tannin in different substances are as follows:

1.1 In general, black tea (15.6%) and coffee (13.6%) contained significant amount of tannin.

1.2 Satisfactory amount of tannin was found in various fruit skin (raw avocado (22.6%), orange (8.78%), plum (4.6%), banana (3.14%), apple (2.97%), grape (2.62%), pomegranate (2.32%), cantaloupe (1.92%), raw mango (1.91%), kiwi fruit (1.34%) and pear (1.22%))

1.3 The skin from the raw fruits was found to contain more tannin than their ripe counterparts. (Avocado (raw: 22.6%, ripe: 10.1%) and mango (raw:1.91%, ripe1.26%))

2 Tannin in black tea was found to dissolve in hot water after 5 minutes and those in coffee was found to dissolve in hot water in 3 minutes.

3 Significant amount of tannin was capable to form kombucha. (Black tea (15.6% tannin, 11.0% kombucha) . Roasted coffee (1.9%, 1.3%) fruit skins of pineapple (0.072%, 0.065%), cantaloupe (1.9%, 0.23%), lemon (0.24%, 0.069%) and pomelo (0.45%, 0.028%))

4 Cups made from kombucha roasted in oven at 150^{o}C for 30min showed comparable water resistance with paper cup. (no leakage in water for more than 3 hours, iced water and boiled water for 30 min)

Putrescible such as fruit skins were found to contain significant amount of tannin which could then be processed to give kombucha. In doing so, landfill burden would be alleviated. Using kombucha cups to replace paper cups offered an alternative which was greener and more sustainable.