Isolation and Characterization of Antimicrobial Peptides From Okara Waste

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***Abstract*— Higher amount of okara waste produced from soy bean curd manufacturing is becoming global concern due to big disposal and environment problem. Utilization of okara can be very beneficial due to their high nutritive value as study stated that okara have potential to exert biological activities such as antimicrobial and antioxidant. This approach are milestone steps in reducing okara waste in directly become a big potential to use as source of natural antibiotic. The increasing dependency on antibiotic resulted in emerging of bacteria resistant is becoming a global concern. There is urgent need for innovation, for producing new natural resource antibiotic to prevent or lessen the antibiotic dependency. From previous studies, okara contains mostly crude fiber composed of cellulose, hemi cellulose, and lignin, about 25% protein, 10& lipd and other nutrients. Due to their its functional ingredient composition and health-promoting attributes such as therapeutic activity proved by previous researchers, this research aim is to isolate purify peptides from chosen byproduct waste which is okara; a soybean residue that contain its own nutritive value using enzymatic hydrolysis. The research includes to determine antimicrobial activity and antioxidant activity of the isolated peptide bacteria using reverse-phase high performance liquid chromatography (HPLC) and Fourier-transform infrared (FTIR) were crucial strategic approaches. . Based on the results, it was found that okara contain nitrogen represent about 69.43 % of protein. While HPLC found four compounds (e.g. aspartame, glutamate, asparagine, serine) in each trial that have same functional group tested by FTIR. Functional group of alkene, alkyne, alkane, alcohol was found in all four of the compounds that have similar bands based on standard group band for after HPLC analysis.**

Keywords—antimicrobial peptide (AMP), okara, enzymatic hydrolysis, antioxidant peptide

I. INTRODUCTION

Okara, also known as *biji* (Korean) or *douzha* (Chinese), is a food processing byproduct derived from soybeans (*Glycine max*) and it is a soybean residue after soymilk production. Large amount of okara are produced especially in current high bean consumption countries such as in Hong Kong, Singapore, Canada and Australia [1]. However, despite large amount of okara generated, they will be discarded due to their high moisture content that lead to spoilage. Study stated that in Japan most is burnt as waste [2]. while in Hong Kong it is dumped in landfills, thus it is important to finds ways of reducing the amount of okara being wasted.

Much research have been taken that show okara contained high nutritive value that might be useful bioactive products. Utilization of okara can be very beneficial because okara contains about 50% fiber, 25% protein, 10% lipid, and other nutrients including isoflavones, lignans, phytosterols, etc [3]. Moreover, these compounds have various physiological and therapeutic function that exert health benefits on the basis of the bioavailability and bioactivity of the identified peptide [4] In many studies, bioactive peptides of okara are said to have antiobiotic and antimicrobial roles that are surely beneficial in increasing okara waste in protein usage field.

Recently, the usage of antibiotic become imminent in treatment until new resistance mechanism of bacteria are emerging and spreading globally that become pressing global health problems. Overuse of antibiotics are major factor which inhibit the uses of AMPs as essential therapeutic and indirectly accelerating the process of antibiotic resistance. It is important to finds ways of reducing the uses of antibiotic in medical treatment.

Okara waste has recently been shown to have abundance nutritive value that mainly high-quality proteins. Moreover, the compound in okara waste have various physiological and therapeutic functions, hence means the reuse of okara waste can be beneficial in wide field. bioactive peptides extracted okara waste may have potential to play various roles such as immunomodulatory, antimicrobial, antioxidative, antithrombotic, hypocholesterolemic, and antihy- pertensive function [5] A study stated that a diabetic mice that being fed with okara for 5 weeks, show that okara can decrease plasma glucose and protect kidney and liver [6]. Another study stated that okara capable of protecting ICR mice against obesity [7]

The objectives of this study were to isolate purify peptides from okara waste; a soybean residue that contain its own nutritive value using enzymatic hydrolysis with pepsin enzyme and to evaluate antimicrobial activity and antioxidant activity of the isolated peptide on bacteria to get a deeper understanding on what characteristic of peptides that may have potential antimicrobial and antioxidant activities toward the bacteria.

II. METHODOLOGY

A. Chemicals

All chemicals used were obtained at level 6 Chemical Laboratory, Faculty of Chemical Engineering, UiTM. 75 g of raw okara, 30 g of pepsin enzyme, 1M of sodium hydroxide (NaOH), 1M of sodium hydroxide (HCI), distilled water. 0.1% 1mL/L of TFA, 1 L of acetonitrile were used as eluents for HPLC analysis.

B. Preparation of Raw Material

Raw okara was filtered from soybean milk residue after process of grinding. Then 75g of raw okara were homogenized with 75g of distilled water followed (okara: water ratio 1:1) and blended within 3 minutes. Then the homogenate were thawed overnight in freezer.

C. Proximate Analysis

The proximate analysis of the samples includes the determination of protein content based on nitrogen concentration using Elemental Analyzer (CHNS/O) modeled Flashea 1112 and pH determination. Both two are determined in laboratory and carried out by adopting the procedures.

I. Protein Content

The protein content determination is carried out using Elemental Analyzer (CHNS/O) modeled Flashea 1112 to determine amount

of protein based on calculating nitrogen concentration on the samples. From this analysis, amount of raw okara needed were determined for the enzymatic hydrolysis. The procedure began with 3 mg of dried samples in powder was weighed into tin containers of 8 x 5 mm using analytical microbalance. The weighed sample then loaded into elemental analyzer. The analysis done for 4 minutes without interruption by blank and recalibration chemicals. The data produced then transferred in computer with dedicated software for further processing. Protein content is expressed by nitrogen concentration from the data obtained in the software. Finally calculation were done to obtain amount of protein followed equation below:

$$\% \text{ Protein} = \% N / 0.16$$

II. pH Analysis

pH of the prepared sample was determined using the pH meter. The protein sample was diluted with distilled water where the ratio of the sample to distilled water is at ratio (1:3). A beaker was used as the benchmark for the ratio determination. Then, the probe of the pH meter was inserted into the liquid portion of the slurry. The pH values was recorded and repeated measurements with new slurry will be done in order to assure a proper reading.

D. Okara Protein Hydrolysates Preparation

The procedure started with , 75 g of raw okara that thawed overnight were The homogenate heated using thermostatistically controlled shaking water bath up to T=85ºC for duration of 30 minutes. The sample need to be homogenized in shaking water bath using magnetic stirrer in order to adjust pH by following optimum condition for pepsin enzyme. After reaching desired temperature, the pH was adjusted to 8.3 with 1M NaOH or 1M HCI and maintained throughout the hydrolysis by checking using pH probe. The enzymatic hydrolysis was done in shaking water bath set up at T=60ºC. After the mixture was adjusted to optimum condition for pepsin enzyme, the enzyme was added to the mixture in an enzyme: substrate 10g: 100g ratio where 7.5g of pepsin added. Next, the temperature was increased to T=90°C for enzyme inactivation for enzyme pepsin. These process took 10 minutes only, then the sample was left out of the water bath at room temperature. The mixture was then centrifuged at 6000 rpm for 15 minutes in order to separate precipitate phase with lipid and fiber. Supernatant were collected. Then okara protein hydrolysates was further purified by reducing moisture with freezedryer. The freeze-dried okara protein hydrolysates obtained was subjected to assays.

E. Analysis of Components using HPLC

High Performance Liquid Chromatography (HLPC branded Perkin Elmer was used to analyze compounds in raw material. Solvent A and b for elution of proteins were prepared that consists of water and aqueous acetonitrile with 60:40 by volume each containing of 0.1% 1mL/L of TFA. To elute the proteins from the column, 15 minutes was used in that process. The flow rate was 1.0 ml/min, detection at 280 nm and sample loaded was 20µL. The sample was filtered first before loaded into the column due to large particles. The elution waste was collected at each elution peak recorded.

F. Analysis of Functional Group using FTIR

The FTIR system branded Perkin Elmer/Spectrum One was used to identified functional group of samples. First step conducted with cleaning the plate by wiping it with acetone and filling the diamond crystal area with samples. Once the acetone was evaporated from the plates, put drops of samples onto the plate and the reading was taken. Any 'noise' need to be avoided to prevent disturbance toward the spectrum band formed as those made it difficult to interpret the spectrum, that was, to distinguish between noise and sample of absorption bands. The procedure were repeated with other samples. The results of transmission will be compared between sample before and after went through analysis of HPLC. The type of functional group is identified based on standard detected wavelength from previous studies.

III. RESULTS AND DISCUSSION

Proximate Analysis

Protein Content

The protein content determination was carried out using Elemental Analyzer (CHNS/O) modeled Flashea 1112 to determine amount of protein based on calculating nitrogen concentration on the samples. Protein content is expressed by nitrogen concentration from the data obtained in the software. The principle of CHNS/O analyzer is to get simultaneous determination of percentage composition of carbon, nitrogen, oxygen, sulphur and hydrogen contained in organic or inorganic materials are generally combustible at 1800o C. From the analyzer, the content found in the okara was written and shown below:

Table 4.1: Percentage Composition in Okara Sample 1

Element Name	***Percentage (%)***	***Retention Time (s)***
Nitrogen	14.1490	92
Carbon	71.2387	130
Hydrogen	7.7164	1197

Table 4.2: Percentage Composition in Okara Sample 2

Element Name	***Percentage (%)***	***Retention Time (s)***
Nitrogen	13.3770	96
Carbon	75.2132	135
Hydrogen	8.7157	1155

Table 4.3: Percentage Composition in Okara Sample 3

Element Name	***Percentage (%)***	***Retention Time (s)***
Nitrogen	14.2815	97
Carbon	74.7735	135
Hydrogen	96.7125	1196

In our case, the percentage of nitrogen concentration are expressed as protein content in the raw material. Average percentage of nitrogen are calculated to be 13.9358 %. Calculation were done to obtain amount of protein followed equation below:

$$\% \text{ Protein} = \% \text{ N}/0.2$$

$$= \frac{13.9358}{0.16}$$

$$= 69.679\ \%$$

As to undergo the elemental analysis, the sample need to be oven-dried in order to get powdered okara. The dried okara after extract remaining water was placed in muslin cloth to be heated at T=60°C. If moisture is reduced to very low levels, then residual and its soluble components will also reduce [8] . This when the amount of water phase extracted from okara or whether water is added to extract residual extractable components will determine the proximate composition of okara. Thus, the calculated amount of protein based on nitrogen concentration might be affected because of the physical manner in which soy milk or water is removed from

okara. To strengthen the fact, [9] discovered that the protein extracted depends on whether the okara has been treated.

pH Analysis

The purpose of this analysis was to evaluate the pH of raw material in order to know the best condition for pH for next enzymatic hydrolysis. From the analysis, it found that the okara is actually slightly alkaline.

Table 4.4: pH Analysis

Sample	***Trial 1***	***Trial 27.05***	***Trial 3***
Okara	7.05	7.06	7.03

Analysis of Components in Okara using HPLC

The RP-HPLC was used to isolate and analyze components contained in okara protein hydrolysates produced with pepsin. Analyzation are based on elution times and peak area of the RP-HPLC profiles. The elution order of solutes in HPLC is governed by polarity. In reverse phase HPLC the retention time of a compound increases with decreasing polarity of the particular species [10]

With the flow rate was 1.0 ml/min, detection at 280 nm and for elution of proteins were prepared that consists of water and aqueous acetonitrile with a linear gradient of 60:40 by volume each containing of 0.1% 1mL/L of TFA. RP-HPLC is generally carried out with an acidic mobile phase, with TFA the most commonly used additive because of its volatility [11]. While for detection of peptides and proteins in RP-HPLC, generally involves detection between 210 and 220 nm, which is specific for the peptide bond, or at 280 nm, which corresponds to the aromatic amino acids tryptophan and tyrosine. When the filtered sample were analyzed by RP-HPLC, several distinct elution peak were observed for each batches.

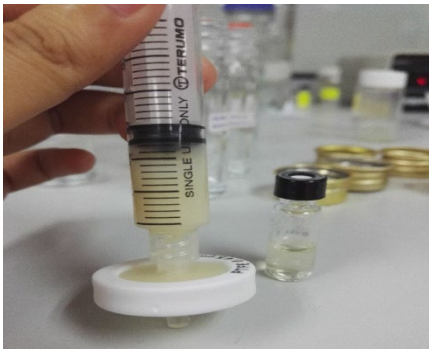


Figure 4.1: Filtered samples before loading into the column

Based on the two trial that was conducted, it was found that both of profile depicted almost similar elution time and peak area. The seven peaks was found between elution times of 1.0 to 4.5 minutes. Both elution profiles projected slightly different but almost the same in all elution time and area. Basically, the retention times expressed as the type of compound in samples while the area of peak represents concentration of compound there thus it were four compounds (e.g. glutamate, aspartame, serine and asparagine) in the samples. Based on Table 4.5, it was observed that Trial 1 have longer retention times at all peaks and larger area compared to Trial 2. These indicated that the concentration of compounds in Trial 1 was more than Trial 2. The graphs and tables were shown below.

Table 4.5: Analysis Report of Elution Profile Trial 1 for Okara Batch 1

Peak	**Time (min)**	**Height (mV)**	**Area (mV*min)**
	Trial 1		
1	1.266	200	253.2
2	1.525	540	823.5
3	1.666	515	857.99
4	1.805	330	595.65
5	2.034	425	864.45
6	2.921	90	262.89
7	4.094	10	40.94

Table 4.6: Analysis Report of Elution Profile Trial 2 for Okara Batch 1

Peak	**Time (min)**	**Height (mV)**	**Area (mV*min)**
	Trial 2		
1	1.300	190	247
2	1.512	535	808.92
3	1.650	525	866.25
4	1.740	400	696
5	2.018	435	877.83
6	2.894	85	245.99
7	4.032	5	20.16

*100 mV to 1 unit scale – y-axis

Based on the two trial that was conducted, it was found that both of profile depicted almost similar elution time and peak area. Five elution peaks were interpreted from the graph that showed between times of 1.0 to 4.5 minutes. It can be concluded that both trial for batch obtained almost the same retention time and peak area that might represent same compounds as Batch 1. But however, for number of elution peaks, it was discovered that Batch 1 succeed to have more peaks than Batch 2, which means that the hydrolysis process of Batch 1 was more succeed than Batch 2. The tables of Batch 2 were shown below.

Table 4.7: Analysis Report of Elution Profile Trial 1 for Okara Batch 2

Peak	**Time (min)**	**Height (mV)**	**Area (mV*min)**
	Trial 1		
1	1.558	780	1215.24
2	1.666	1030	1715.98
3	2.073	620	1285.26
4	3.009	90	270.81
5	4.286	80	342.88

*200mV to 1 unit scale – y-axis

Table 4.8: Analysis Report of Elution Profile Trial 2 for Okara Batch 2

Peak	Time (min)	Height (mV)	Area (mV*min)
	Trial 2		
1	1.525	800	1220
2	1.636	1200	1963.2
3	2.041	520	1061.32
4	2.949	120	353.88

*200 mV to 1 unit scale – y-axis

Despites having almost same pattern of peaks however, it can be shown that the retention time was a bit drift from Trial 1 and Trial 2 for both Batch 1 and Batch 2. The differentiation might occurred for few reasons. Firstly, because of filtration methods went wrong. Before the samples were injected into column, the samples need to be filter through a 0.22µm filter to remove particulates that could block solvent line or the column thus effect the retention time [11]. Some of the drifting retention time might be cause of temperature. According to the rule of thumb which the retention times change by about 1% to 2% per 1°C shows that there are possibility that temperature can cause fluctuation too.

For this results, supposedly peak of proteins were identified by comparing retention times of the authentic standards. Okara that extracted from soybean were basically contained variety of bioactive compound since soybean is a type of plant. In plants, phenolic and isoflavones are important groups of biologically active compound found there [12]. Based on previous studies, phenolic and flavonoids components can be identified based on standard using high performance liquid chromatography (HPLC) but however due to the its diversity and complexity, some said it was rather difficult to characterize every compound [13] However due to error that we made throughout the process of identifying, enabled us to find out what compounds in the okara. It might be affected by our shorter duration of running time, T=15 minutes enabled the compounds that need longer running time to be detected. The concentrations of these bioactive compounds some might be affected by processing condition techniques such as enzymatic hydrolysis [14].

As in the final step in HPLC analysis, the elution wastes were collected at four of major elution peak for each batches represent compounds to be tested for its functional group by using FTIR analysis.

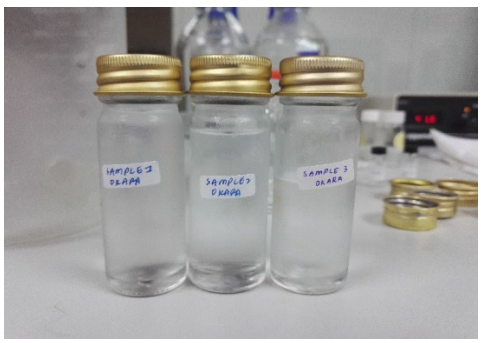


Figure 4.5: Some of the elution collected for next FTIR analysis

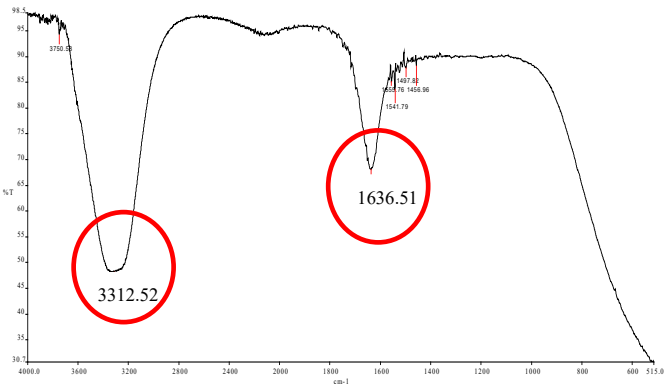
Analysis of Functional Group in Okara using FTIR

Fourier-transform Infrared Spectroscopy (FTIR) is an established tool that offers quantitative and qualitative analysis for organic and inorganic samples. The technology provided mostly information about the structural characterization of proteins based on absence or presence of certain functional group which was triggering molecular vibrations through irradiation with infrared spectrum [15] [16]. FTIR spectra were characterized by several typical peaks that could be related to the specific contribution of molecular groups. By knowing the functional groups presence in the samples, the inhibition properties which okara possesses can be studied. As previous studies believed that okara is incredibly nutritive compositions due to its functional properties and numerous health benefits, some functional group have been found to contribute to their antioxidant and antimicrobial properties. There were few of samples that have been tested on FTIR including acetonitrile buffer as benchmark, samples before and after HPLC analysis for two batches. From all the data we collected, the comparison from the data can be observed.

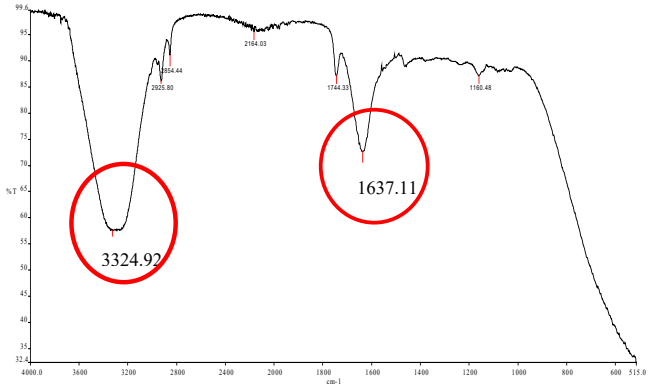
Samples before Conducting HPLC Analysis

Based on the results obtained, two distinct and major functional group that presence in acetonitrile which were in group band of 3350-3310 cm-1 and 1662-1626 cm-1. They both represent secondary amine and alkene respectively that showed medium intensity for their spectrum. However, still there were minor functional group presence in acetonitrile such as alcohol, Arene, nitro compound, and aromatic. Acetonitrile was tested too because we assumed to make acetonitrile as a benchmarks for comparing both before and after HPLC samples of okara.

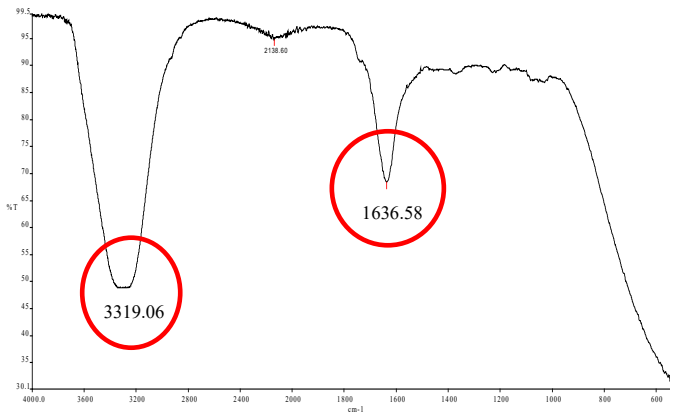
Next, based on results, it was found that sample Batch 1 and Batch 2 before conducting HPLC analysis, both of them have quite similar spectrums. Both of them have two major functional group same as acetonitrile. Batch 1 have wave number of 3324.92 cm-1 that represent secondary amine while Batch 2 have 3319.06 cm-1. In addition, Batch 1 have wave number of 1631.11 cm-1 represent alkyne while Batch 2 have 1636.58 cm-1 It shown the stretching of N-H bond and C=C bond of Batch 1 was more strong that Batch 2. Furthermore, from Figure 4.5 (b) and (c), we can compared the functional group detected by infrared light in Batch 1 was more than Batch 2 such as amine salts, esters, sulfone, and thiocyanate. The differentiation that occurred might be caused by the process of enzymatic hydrolysis it selves



(a)



(b)



(c)

Figure 4.6: (a) Graph of Acetonitrile (b) Graph of Okara Batch 1 before HPLC (c) Graph of Okara Batch 2 before HPLC

Table 4.9: Functional Group of Acetonitrile (Anonymous, 2014)

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3750.58	3700-3584	O-H stretch	Alcohol	Medium
3312.52	3350-3310	N-H stretch	Secondary Amine	Medium
1636.51	1662-1626	C=C stretch	Alkene	Medium
1555.76	1600-1450	C=C	Arene	Strong
1541.79	1550-1500	N-O stretch	Nitro Compound	Strong
1497.82	1600-1400	C=C	Aromatic	Weak
1456.96	1600-1400	C=C	Aromatic	Weak

Table 4.10: Functional Group of Okara Batch 1 before HPLC Analysis

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3324.92	3350-3310	N-H stretch	Secondary Amine	Medium
2925.80	3000-2800	N-H stretch	Amine Salt	Strong
2854.44	3000-2800	N-H stretch	Amine Salt	Strong
2164.03	2175-2140	S-C≡N stretch	Thiocyanate	Strong
1744.33	1750-1735	C=O stretch	Esters	Strong
1637.11	1662-1626	C=C stretch	Alkene	Medium
1160.48	1160-1120	S=O stretch	Sulfone	Strong

Table 4.11: Functional Group of Okara Batch 2 before HPLC Analysis

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3319.06	3350-3310	N-H stretch	Secondary Amine	Medium
2138.60	2140-2100	C≡C stretch	Alkyne	Weak
1636.58	1662-1626	C=C stretch	Alkene	Medium

Samples after Conducting HPLC Analysis

After conducting HPLC analysis, elution wastes were collected for all four compounds according to their elution times. Thus each batches have 4 compounds (e.g. glutamate, aspartame, serine and asparagine) to be tested by the FTIR. From all the data we collected, the comparison from the data can be observed. Below is the graphs that have been collected from samples tested.

Samples Batch 1

Based on the result below, it can be observed each of the compounds contained same functional group which were alcohol, alkyne, alkene and alkane. The wave number of each compound also observed to be almost the same. However from detail observation, we can said that O-H bond represent alcohol in compound 4 have the most high wave number which is 3379.91 cm-1 with high strong intensity compared to compound 3, 2 and 1. While alkyne in compound 2 have the strongest bond with wave number of 2258.72 cm-1 and weak intensity compared to compound 1 followed by compound 4 and 3. Other than that, compound 1 also contained the highest alkene C=C bond with wave number 1638.75 cm-1 and compound 2 have wave number of 1374.70 cm-1 where it was the strongest C-H bend represents alkane.

After comparing before and after conducting HPLC analysis, the result showed that there were two new functional group in samples after conducting HPLC. From Figure 4.6, two functional group were highlighted in blue boxes that expressed alkyne and alkane. There were no alkyne and alkane in samples before conducting HPLC. Besides having two new functional group after performing HPLC, samples after HPLC still have three known functional group which were alkene, alcohol and secondary amine same as samples before HPLC. We also found that those alkene, alcohol and secondary amine were the same functional group in acetonitrile buffer that have been tested, thus we assumed there might be mixture of acetonitrile in samples that as for HPLC we used acetonitrile as buffer. We assumed in there were acetonitrile in the elution waste.

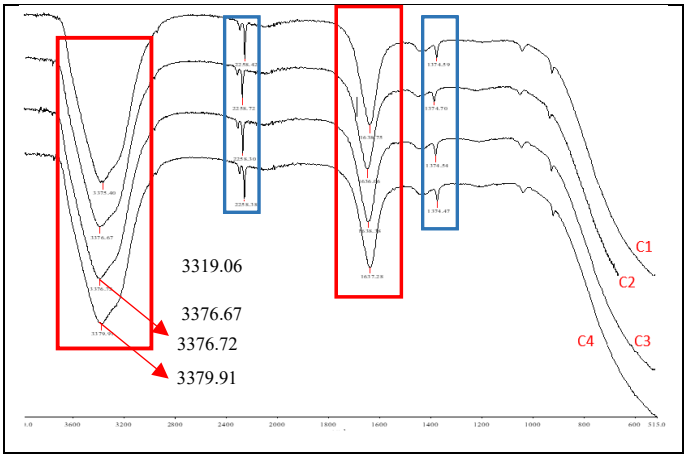


Figure 4.7: Compounds C1, C2, C3 and C4 for Batch 1 after conducting HPLC

Table 4.12: Functional Group of Compound 1 for Batch 1

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3375.40	3550-3200	O-H stretch	Alcohol	Strong
2258.42	2260-2190	C≡C stretch	Alkyne	Weak
1638.75	1662-1626	C=C stretch	Alkene	Medium
1374.59	1450-1375	C-H bend	Alkane	Medium

Table 4.13: Functional Group of Compound 2 for Batch 1

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3376.67	3550-3200	O-H stretch	Alcohol	Strong
2258.72	2260-2190	C≡C stretch	Alkyne	Weak
1636.06	1662-1626	C=C stretch	Alkene	Medium
1374.70	1450-1375	C-H bend	Alkane	Medium

Table 4.14: Functional Group of Compound 3 for Batch 1

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3376.72	3550-3200	O-H stretch	Alcohol	Strong
2258.30	2260-2190	C≡C stretch	Alkyne	Weak
1638.38	1662-1626	C=C stretch	Alkene	Medium
1374.54	1450-1375	C-H bend	Alkane	Medium

Table 4.15: Functional Group of Compound 4 for Batch 1

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3379.91	3550-3200	O-H stretch	Alcohol	Strong
2258.38	2260-2190	C≡C stretch	Alkyne	Weak
1637.28	1662-1626	C=C stretch	Alkene	Medium
1374.47	1450-1375	C-H bend	Alkane	Medium

Sample Batch 2

Based on the interpreted results from Batch 2 below by looking at yellow box in Figure 4.7, it can be observed that there was one new functional group compared to sample after HPLC for Batch 1 and acetonitrile. Nitro compound or known as N-O stretch was found at group band of 1550-1500 cm-1. While others functional group such as alcohol, alkyne, alkene and alkane were still in the same group band like Batch 1.

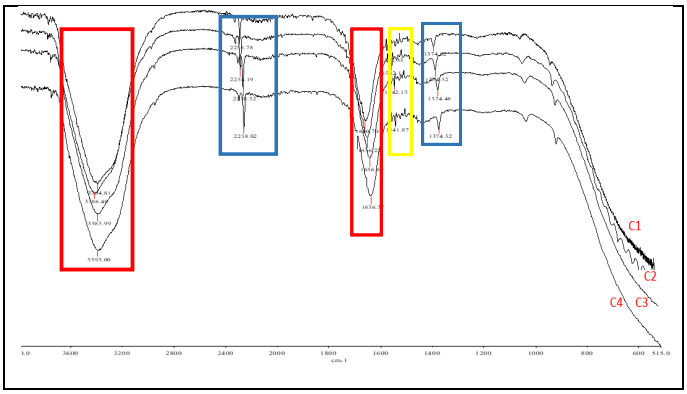


Figure 4.8: Compounds C1, C2, C3 and C4 for Batch 2 after conducting HPLC

Table 4.16: Functional Group of Compound 1 for Batch 2

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3364.81	3550-3200	O-H stretch	Alcohol	Strong
2258.78	2260-2190	C≡C stretch	Alkyne	Weak

1640.70	1662-1626	C=C stretch	Alkene	Medium
1542.33	1550-1500	N-O stretch	Nitro Compound	Strong
1374.02	1450-1375	C-H bend	Alkane	Medium

Table 4.17: Functional Group of Compound 2 for Batch 2

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3386.49	3550-3200	O-H stretch	Alcohol	Strong
2258.19	2260-2190	C≡C stretch	Alkyne	Weak
1636.22	1662-1626	C=C stretch	Alkene	Medium
1542.15	1550-1500	N-O stretch	Nitro Compound	Strong
1374.52	1450-1375	C-H bend	Alkane	Medium

Table 4.18: Functional group of Compound 3 for Batch 2

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3385.99	3550-3200	O-H stretch	Alcohol	Strong
2258.32	2260-2190	C≡C stretch	Alkyne	Weak
1636.69	1662-1626	C=C stretch	Alkene	Medium
1542.13	1550-1500	N-O stretch	Nitro Compound	Strong
1374.46	1450-1375	C-H bend	Alkane	Medium

Table 4.19: Functional group of Compound 4 for Batch 3

Wavenumber (cm-1)	Group band (cm-1)	Bond	Functional Group	Intensity
3393.00	3550-3200	O-H stretch	Alcohol	Strong
2258.02	2260-2190	C≡C stretch	Alkyne	Weak
1636.37	1662-1626	C=C stretch	Alkene	Medium
1541.87	1550-1500	N-O stretch	Nitro Compound	Strong
1374.39	1450-1375	C-H bend	Alkane	Medium

IV. CONCLUSION

Isolation and characterization of okara have been studied in order to know their antimicrobial and antioxidant properties. The analysis of okara using FTIR and HPLC have been discussed. Okara was abundant in many parts of the world, and could serve as a cheap and easily available source for new antibiotics that have slightly alkali properties. . Elemental analyzer revealed amount of protein about 69% in okara after become residue of soybean. HPLC analysis indicated that okara have compounds (e.g. aspartame, glutamate, asparagine, serine) that detected within 1.0 to 4.5 minutes and the enzymatic hydrolysis process might affected the type of compounds in it. While FTIR analysis revealed the functional group (e.g. alkane, alkene, alkyne and alcohol) was found after HPLC analysis which alkyne and alcohol also were found in elution buffer (e.g. acetonitrile).

As HPLC required very fine particles to do the elution. We analyzed that maybe the non-filtered particles were actually contained the desired compounds or functional group for our studies. It is recommended to use membrane separation techniques to characterize okara samples. This is because membrane separation processes can separate at the molecular scale up to a scale at which the particles can be seen, which means this implied that a very large number of separation needs might actually be met by membrane processes. Furthermore, membrane processes are increasingly used for removal of bacteria, microorganisms, particulates, and natural organic material, which can impart color, tastes, and odors to water thus the it can give resulting in better characterizing process

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