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Article

Comparison of Antioxidant Compounds between *Glycine max* and *Bos taurus* milk

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Abstract— **Milks are dairy products that consists of high nutritional value which is good for health and provides energy to the body. Besides, milk also is believed to have antioxidant properties which helps to reduce the oxidative stress that may cause many diseases. Generally, cow milks are the most popular amongst all the dairy products as it is affordable and easy to find. However, dairy products that are originated from cow contains protein that can induce allergen reactions to some people. Thus, there is milk produced from the plant-based which is soybean that can be used to replace cow milk as it is believed to have similar nutritional value to cow milk. Therefore, to prove its similarity, an identification of antioxidant compounds was performed on soybean milk and cow milk extract using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. The extraction was done by using liquid-liquid extraction method and the excess solvent was removed by using rotary evaporator machine. As a result, soybean milk has been identified to have the similar antioxidant compounds to cow milk. It can be proven with the presence of functional group that are similar to tryptophan and tyrosine which are both existed in cow milk. Hence, this research is essential for community as it contribute maximum benefit for better healthcare.**

Keywords— ***Cow milk; soybean milk; antioxidant compound***

I. INTRODUCTION

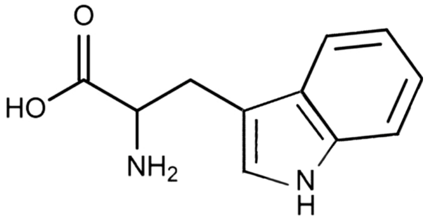
Milk is an essential source of nutrients for human consumption such as amino acid, vitamins and calcium and thus made it considered as a well-balanced food and regarded as a valuable part of a full diet by many. The presence of bioactive peptides, protein, conjugated linoleic acid, fatty acid, antioxidants, Vitamin D, selenium and calcium in milk play important roles in human body. These compounds act as anti-inflammatory, anti-tumor, antimicrobial activities, anti-oral squamous cancer and immune boosting (Khan[a] et. al. 2019; Ghafar et al., 2019). Moreover, α-lactalbumin, β-lactoglobulin, αs-casein, β-casein, and κ-casein are the most abundant milk proteins that contributes in enhancing the nutritional value in milk.

For example, tryptophan is a central amino acid residue for human nourishment as appeared by its various physiological capacities in the body (Nongonierma and FitzGerald, 2015). Few amounts of tryptophan have been distinguished for their potential in exhibiting antihypertensive, antidiabetic and antioxidant properties. Moreover, there is difference of Tryptophan concentration between several types of milk protein. Tryptophan concentration in human milk protein, cow milk protein and whey protein are 2.2-2.4%, 1.3% and 1.7% respectively. This difference is due to the α-lactalbumin portion of whey-casein protein (Tsopmo, 2009).

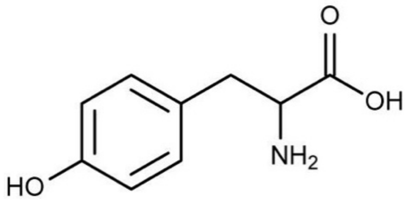
Also, there is tyrosine which is a phenylalanine-sparing amino acid that attributed to antioxidant properties and has an active hydroxyl group (Mauron et al., 1955). Tyrosine is an amine compounds which has the reducing power and a scavenging impact on responsive oxygen species (Yen, 1997). It is also categorized as a non-essential amino acid which also synthesized by the body rather than being only ingested from diet. Moreover, all foods that contains protein attributed to tyrosine compound. Tyrosine rich protein comes with soybean milk, cow milk and eggs which are 55mg/g, 48mg/g and 41mg/g protein respectively (Nongonierma and FitzGerald, 2012).

Indeed, milks are dairy products that consists of high nutritional value which is good for health and provides energy to the body. Thus, there is milk produced from the plant-based which is soybean that can be used to replace cow milk as it is believed to have similar nutritional value to cow milk.

Nonetheless, it is vital to know the difference between the two.



Tryptophan



Tyrosine

Fig 1. Molecular structure of Tryptophan and Tyrosine

Soybean or *Glycine max* is one of the most consumed plant-based resource especially in China with 1.8 metric ton/hectare in 2017 (Li et al., 2020). It has been highly consumed and produced as soybean powder, soybean milk and soybean oil. Soybean milk are an aqueous extract of whole soybeans or *Glycine max* which are cholesterol and lactose free. Moreover, soybean milk believes to have almost similar nutritional values as cow milk as it has small amount of saturated fatty acids and different phytochemicals with beneficial properties which contributes to the presence of antioxidant properties (Durazzo, 2015).

Meanwhile, cow or *Bos taurus* is a domestic mammalian which known as the main sources of milk and dairy products. Cows were first domesticated as “all-purpose” animals as it can be consumed for its milk and meat. However, cows are consumed by humans in many other ways as well, such as its skin as source of leather for clothing and other products. Cows are usually milked twice per day and produces on average of 30 litres or 8 gallons of milk daily. Moreover, cow milk contains many bioactive components that boost one’s physiological processes (Khan[b], 2019).

This is because milks have a few of antioxidant factors such as enzymes and vitamins. The antioxidative activities are derived from the peptides that generated from the milk proteins digestion. Furthermore, many studies have focused on tryptophan and tyrosine as an antioxidant compound as its antioxidative peptides are composed of 5-11 amino acids (Pihlanto, 2006). It is due to the hydrolysates from milk proteins that could be utilized as natural antioxidants in upgrading the properties of antioxidant compound of useful nourishments and in forestalling oxidation reaction in food handling. Antioxidant is a bioactive compound that neutralize and inhibits the oxidation of other molecules caused by the harmful free radicals in human bodies. Antioxidant agent in dairy food plays an important role to protect one from oxidative damage in their body such as vitamins E and C and carotenoids derived from milk have attributed to the antioxidant properties in preventing illness counteraction.

However, the consumption of cow milk must be avoided in case of allergen reaction or intolerance of lactose. Therefore, the formulation of milk produced by using plant-based which is soybean is a valid alternative as the nutritional values are almost similar to cow milk. This formulation also benefits human with allergy and the vegetarians. Therefore, this research is a preliminary study on characterisation of soybean milk and cow milk and the comparison between both milks are made. In addition, this study also particularly focused on the evaluation of the functional groups of antioxidant properties in these milk samples.

II. MATERIALS AND METHOD

2.1 Material

Material used in order to carry out this study were two types of milk which are *Drinho* soybean milk and *Farm Fresh* cow milk. Ethyl acetate was used as solvent to extract both of cow and soybean milk.

2.2 Preparation of sample

20 ml of *Farm Fresh* cow milk and *Drinho* soybean milk were measured by measuring cylinder and poured into different conical flask. Then, 20 ml of Ethyl acetate solution which was used to extract the solvent were mixed into each conical flask and the mouth of the conical flask were covered with parafilm. The samples prepared were then left for 24 h in a refrigerator.

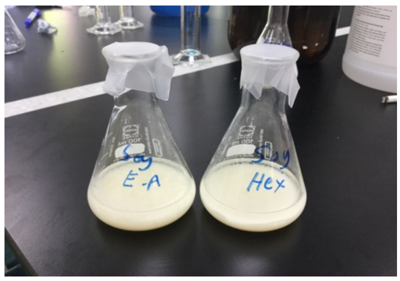


Fig 2. *Farm Fresh Cow milk* and *Drinho Soybean milk* with Ethyl acetate solvent

2.3 Extraction of sample

The samples were extracted by using liquid - liquid extraction. Ethyl acetate was poured into a filter funnel and followed by the sample solution. The filter funnel was then shaken lightly, and the gas was released through the tip of the filter funnel. The extraction was repeated three times with adding 20 ml of Ethyl acetate for each repetition. The extracted solution collected was then filtered using filter paper to maximize the extraction process.

2.4 Characterization of functional group

The excess solvent was then removed by using rotary evaporator to obtain samples' extract. The extract obtained are tested with ATR-FTIR to collect the data of extract's frequency and spectrum. Thus, with that, functional group of the milk can be identified to determine the antioxidant properties present in both milk samples.

Fig 3. *Farm Fresh Cow milk* and *Drinho Soybean milk* extraction sample

Table 1: The functional groups and compounds identified from *Drinho Soybean Milk* sample

Drinho Soybean Milk with Ethyl acetate		
Frequency (cm^{-1})	Transmittance (%)	Functional group
3305.2043	46.41	O-H stretching
1638.1973	65.74	N-H bending
1334. 3869	88.83	C-N stretching
1261.7039	82.18	C-O stretching
999.6778	83.39	=C-H bending
918.2558	82.08	O-H bending
654.7058	39.31	=C-H bending

III. RESULT AND DISCUSSION

3.1 ATR-FTIR Spectra

Fourier Transform Infrared (FTIR) spectroscopy is a procedure used to acquire an infrared spectrum of retention or discharge of a solid, fluid or gas. Infrared spectrum is a plot of estimated infrared light force versus a property of light. Analysis of infrared spectra can be utilized to recognize the presence of specific compound in a sample. Thus, it can be distinguished by the peak positions of known molecules derived from the spectra in an obscure sample (Smith, 2011).

Moreover, the infrared measurements can be used for the qualitative and quantitative analysis of a specific compound, rapid determination and prediction of a category of bioactive compound (i.e., polyphenols) and rapid determination and prediction of antioxidant activity (Lu and Rasco 2012). Hence, in this study, FTIR spectroscopy is a rapid technique used for the determination of the functional group that exhibit characteristic vibrational absorption frequencies in specific infrared regions.

Thus, the presence of the functional group obtained from the spectrum and frequency can be used to identify any antioxidant properties. Referring to Khan[b] et.al. (2019), antioxidant capacity of milk and milk products is mainly due to sulphur-rich amino acids, such as tyrosine and cysteine. However, the different method of processing milk could help to enhance the bioactive compound that are found naturally in the milk. Therefore, same methods were used in both cow milk and soybean milk extraction to ensure the accuracy in the experiment.

According to Durazzo (2015), all samples contains water with the percentage over 83%. Thus, the spectra obtained were corrected with the spectrum of water as typical absorption bands for water are located between 3650-3000 cm-1 and 1680-1600 cm-1. With that, the absorption of the studied samples is able to be secured. All the spectrum of ATR-FTIR were characterised by several peaks that could be related to functional group of the antioxidant compounds.

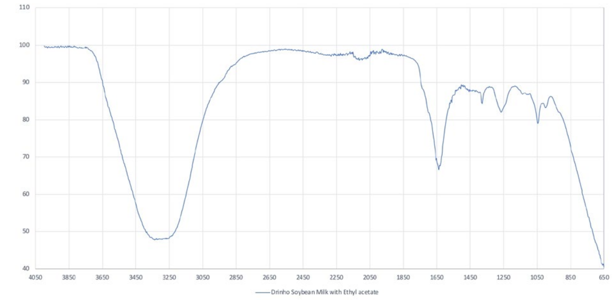


Fig 4. ATR-FTIR spectrum graph of *Drinho Soybean Milk* that was extracted with Ethyl acetate

The transmission measurements observed at 1638 cm^{-1} are attributed to N-H bending of amide I, and peak at 1261 cm^{-1} is attributed to C-O stretching of amide II compound. The transmission measurement at 3305 cm^{-1} is attributed to phenols as due to the O-H stretching bond. At 1334 cm^{-1}, the transmission measurement is corresponded to aromatic amines with the presence of C-N stretching bond. Besides, alkenes are attributed by the transmission measurement at 999 cm^{-1} and 654 cm^{-1} due to the presence of =C-H bending bond. The transmission measurement at 918 cm^{-1} is then attributed to O-H bending of carboxylic acids compound

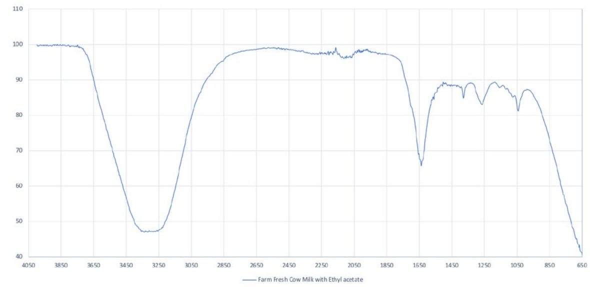


Fig 5. ATR-FTIR spectrum graph of *Farm Fresh Cow Milk* that was extracted with Ethyl acetate.

Table 2: The functional groups and compounds identified from *Farm Fresh Cow Milk* sample

Farm Fresh Cow Milk with Ethyl acetate		
Frequency (cm^{-1})	Transmittance (%)	Functional group
3290.2056	44.59	O-H stretching
1638.1973	65.97	N-H bending
1411.0729	87.35	C-C stretching
1334.3869	89.15	C-N stretching
1259.8402	83.36	C-O stretching
1036.1999	82.94	C-N stretching
652.5632	38.84	=C-H bending

The transmission measurements observed at 1638 cm^{-1} and 1259 cm^{-1} are attributed to N-H stretching of amide I band and C-O stretching of amide II, respectively.

The transmission measurement at 3290 cm^{-1} is attributed to phenols as due to the O-H stretching bond. At 1411 cm^{-1}, the transmission measurement attributed to aromatics compound with the presence of C=C stretching bond. Besides, C-N stretching of aromatic amines and aliphatic amines are attributed by the transmission measurement at 1334 cm^{-1} and 1036 cm^{-1}, respectively. The transmission measurement at 652 cm^{-1} is then attributed to =C-H stretching of alkenes compound.

Referring to both table, amide groups of soybean milk showed higher transmittance measurements that cow milk. Based on previous research, pure soybean proteins exhibit specific absorption bands in the region of amide I, II and III.

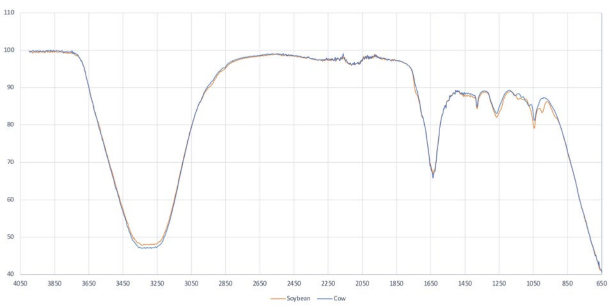


Fig 6. ATR-FTIR spectra of *Drinho Soybean Milk* and *Farm Fresh Cow Milk* that were extracted with Ethyl acetate.

Durazzo, A., Gabrielli, P., & Manzi, P. (2015). Qualitative study of functional groups and antioxidant properties of soy-based beverages compared to cow milk. Antioxidants, 4(3), 523-532.

As for the overlay graph above, the similar transmission measurements are recorded to find the similarities of antioxidant properties between both milk samples. The transmission measurement at the first peak which is at 3297 cm^{-1}. The transmission measurement at 1636 cm^{-1} attributed to alkenes as due to -C=C- stretching bond. At 1559 cm^{-1}, the transmission measurement attributed to 1° amines with the presence of N-H bending. Besides, aromatics compounds are attributed by the transmission measurement at 1379 cm^{-1} due to the presence of C-C stretching bond. The transmission measurement at 1265 cm^{-1} is then attributed to aromatic amines due to C-N stretching bond. While, the transmission measurement of 652 cm^{-1} attributed to alkenes compounds with the presence of =C-H bending.

Regarding the evaluation of the overlay spectrums, it is found that there were differences of functional group between the region of 900-1400 cm^{-1}. However, both milk sample showed almost similar functional group that characterised several antioxidant compounds like tryptophan and tyrosine. Thus, conclude that soybean milk has obtained higher absorption frequency of amides which made it richer in antioxidant capacities.

The presence of functional groups of tryptophan and tyrosine in soybean and cow milk can be proven from the peak positions of the ATR-FTIR spectra. Functional groups that are presence in tryptophan are an α-amino group, an α-carboxylic acid group, and a side chain indole. Thus, it makes tryptophan as a non-polar aromatic amino acid. Besides, tyrosine has the functional groups of isopropyl side chain, aromatic group and hydroxyl group which make it as an aromatic amino acid. Therefore, the presence of antioxidant compound in both milk samples which are soybean milk and cow milk has been proven.

IV. CONCLUSIONS

In this study, cow milk and soybean milk were studied to investigate the functional groups of antioxidants. Moreover, this study determined the similarity between antioxidant compound present in cow milk and soybean milk.

V. ACKNOWLEDGEMENT

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