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 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 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 the 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.

***Keyword**: Cow milk; soybean milk; antioxidant compound*

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, Vitamin D, selenium and calcium in milk plays an important role in human body. They act as an anti-inflammatory, anti-tumor, antimicrobial activities, antioxidants and immune boosting (Khan 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.

Soybean or Glycine max is one of the most consumed plant-based resource with 200 million metric tons produced per year (FAO, 2006). The usage of soybean has been widen with the production of various products such 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 contribute to the presence of antioxidant properties (Durazzo, 2015).

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 used as draft animals and also for the production of milk and meat products. However, cows are used by humans in many other ways, such as the source of leather for clothing and other products. Cows which are specialised as dairy cows usually milked twice per day and produces on average 30 litres or 8 gallons of milk daily. Moreover, cow milk contains many bioactive components that boost the physiological processes in the body (Khan, 2019).

Milks are believed to 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.

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.

Material and Method

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.

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 hours in a chiller.

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 3 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.

*Characterization of functional grou*p

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.

Result and Discussion

Fourier Transform Infrared (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 an antioxidant properties. Referring to Khan 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 method are used in both cow milk and soybean milk extraction to ensure the accuracy in the experiment.

All the spectrum of ATR-FTIR were characterised by several peaks that could be related to functional group of the antioxidant compound.

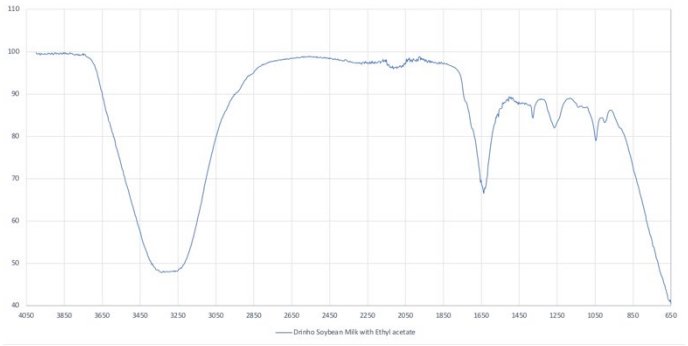

Fig 1. A spectrum graph of *Drinho Soybean Milk* with Ethyl acetate sample obtained from ATR-FTIR

Table 1 shows 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

The transmission measurements observed at 1638 cm^{-1} are attributed to amide I band due to N-H bending and at 1261 cm^{-1} are attributed to amide II band due to C-O stretching.

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 carboxylic acids compound due to O-H bending.

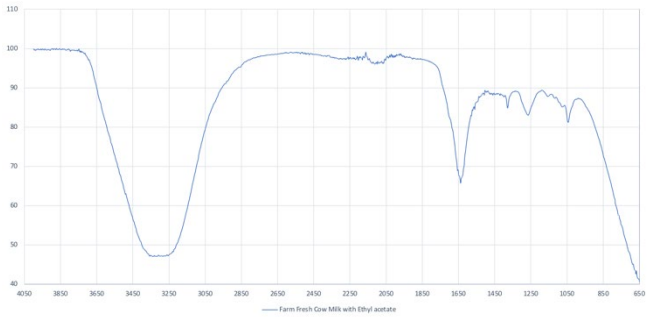

Fig 2. A spectrum graph of *Farm Fresh Cow Milk* with Ethyl acetate sample obtained from ATR-FTIR

Table 2 shows 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} are attributed to amide I band due to N-H bending and at 1259 cm^{-1} are attributed to amide II band due to C-O stretching.

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, aromatic amines and aliphatic amines are attributed by the transmission measurement at 1334 cm^{-1} and 1036 cm^{-1} due to the presence of C-N stretching bond. The transmission measurement at 652 cm^{-1} is then attributed to alkenes compound due to =C-H stretching bond.

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.

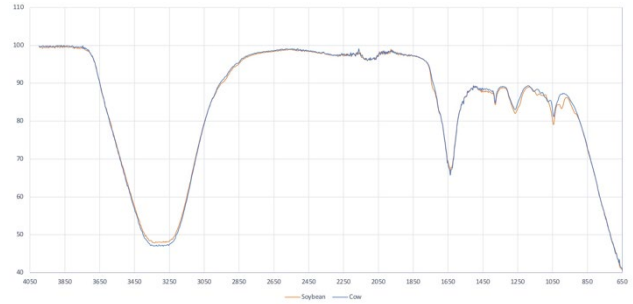

Fig 3. A spectrum graph of *Drinho Soybean Milk* and *Farm Fresh Cow Milk* with Ethyl acetate samples obtained from ATR-FTIR

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 the first peak which is at 3297 cm^{-1} attributed to phenols due to its O-H stretching bond. Then, the transmission measurement at 1638 cm^{-1} was then attributed to alkenes as due to -C=C- stretching bond. At 1044 cm^{-1}, the transmission measurement attributed to aliphatic amines with the presence of C-N stretching. 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 1260 cm^{-1} is then attributed to amide II band due to C-O stretching bond. Meanwhile, 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 are characterised by 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.

From the functional group recorded, functional group of tryptophan and tyrosine was identified. It can be proven with the presence of the functional groups in soybean and cow milk derived from the peak positions of the spectrums. Functional groups that present 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 these antioxidant compounds in both milk samples which are soybean milk and cow milk has been proven.

Conclusions

In this study, cow milk and soybean milk were studied to investigate the antioxidant properties of their major functional groups. Moreover, this study provided spectrum from ATR-FTIR of most representative functional groups and determined the similarity between antioxidant compound present in cow milk and soybean milk.

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