# Identification of Antioxidants and Active Compounds in Cow's Milk

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**Abstract.** This paper aims to analyze and identify the antioxidant compounds in cow's milk. Milk contains high biological molecules protein, essential fatty acids, calcium, fat, amino acid, vitamins that are soluble in water and several bioactive compounds. In Al-Quran and Hadith, milk has been mentioned several times especially for breast feeding which is essential for infant immunity. Antioxidant can suppress free radicals and unstable molecules produced by the body as an environmental and other pressure reaction. In this research, the commercialized milks were successfully extracted by liquid-liquid extraction method. Therefore, this study focuses on the identification of antioxidant compounds in commercialized milk samples using Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR) and Gas Chromatography-Mass Spectroscopy (GC-MS). Based on the ATR-FTIR spectrum, we are able to identify the functional groups of antioxidant compounds which are C–H stretch of alkanes, C=O of aldehydes, C–N stretch of aliphatic amine, C–Cl stretch of alkyl halide, O–H bend of carboxylic acids and N–H wag of 1 , 2 amines. Further, the spectrum of the chromatogram from the GC-MS can determine the antioxidants in the milk samples. This study enables the dairy product factory to improve their quality of the milk to enhance the consumers' health.
***Keyword:*** Cow's milk, antioxidants, functional groups, ATR-FTIR

## Introduction

*Bos Taurus* (cow) Milk
*Bos taurus*'s milk or Cow's milk has the most attention in dairy production as it is easily accessible by consumers. Thus, a lot of dairy manufacture industries produce various type of milk to fulfil the high demand from the consumers. Milk plays an important role in human daily life food intake as it contain proteins, lactose, fat and minerals but also have countless active substances such as vitamins, antioxidants and bioactive peptides which are good for the welfare of human beings. [1]. In recent years, due to high level of milk consumption, many milk or dairy products have been modified to enhance the human diet according to the health agency guidelines. [2]. The fatty acid in the milk have various benefits such as lowering blood pressure, promotes healthy functioning of brain, nervous system and sleep wake cycles.

## Material and methods

*Materials*
The milk samples (milk sample A and milk sample B) were purchased from supermarket around Nilai. Hexane and ethyl acetate were used as the extractable solvents.Following paragraphs should be indented.

*Preparation of sample*

Two milk samples, milk sample A and milk sample B were measured at 20 ml and were poured into four conical flasks. 20 ml of hexane is added into two different milk samples and 20 ml of ethyl acetate is also added into the remaining milk samples. The conical flasks were covered using parafilm and the samples were left and kept in chiller for 24 hours.

*Liquid-liquid extraction*

The antioxidants compounds and active compounds were extracted using liquid-liquid extraction to reduce cost, simpler and easier [3]. Then, the mixture of the milk samples and the solvents formed two layers which are, a clear layer containing extractable compounds and a white layer which contain the water molecules and remaining chemical compound of the milk. The lower layer is needed to be separated from the sample. The technique was repeated for two times to maximize extraction of compounds. After that, the remaining solvent is removed using rotary evaporator in which the bath temperature was set at 76°C for ethyl acetate solvent while 68°C for hexane solvent. The extractable compounds were collected and placed in sample bottles.

*Characterization of functional group of milk samples using ATR-FTIR analysis*

The absorption of samples using FTIR spectrometer with diamond crystal cell ATR is used to scan the extractable compounds. [4]. A drop of each extractable compound were placed on the diamond. The functional group for each milk samples were determined by the frequencies obtained from the analysis. The ATR crystal was wiped with methanol and soft tissue paper after the scanning of each sample.

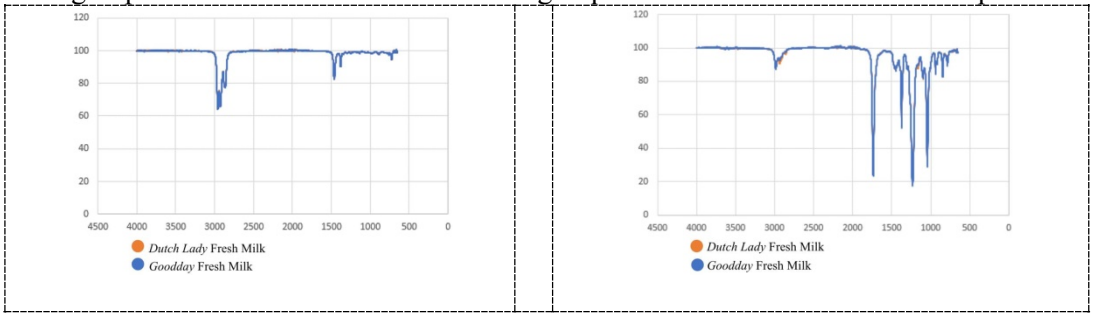
*Gas Chromatography-Mass Spectrometry*

The GC-MS study was carried out by Agilent on a GC-MS 5977B fitted with a pulsed split injector. [5]. Using the DB-WAX Ultra Inert column (30 m × 0.18 mm × 0.18 µm film thickness), separation was achieved. At flow rates of 2.1 mL / min and a 5:1 split ratio, helium gas was used as the carrier gas. The temperature of the injector was 250 °C. The temperature of the oven was programmed to be 50 ° C at a holding time of 1 minute and at a rate of 25 ° C / min, then increased to 200 ° C, then increased to 230 ° C at a rate of 3 ° C / min and kept at the final temperature for 23 minutes. The activity of GC-MS was managed using the Intuvo MS programme. Mass spectrometry (MS) spectra were obtained at range width m / z 46-500 u, transfer line temperature 250 ° C, source temperature 230 ° C, quadrupole temperature 150 ° C and solvent cut time is 3 minutes. Both determinations have been performed in triplicates.

## Results and discussions

*ATR-FTIR analysis*

Representative FTIR spectra of extractable compounds in the region 4000 – 600 cm-1 are shown in Figure 3.1.1 and Figure 3.1.2. The major spectral absorption was assigned to -OH stretching, -C-H stretching, -C=O stretching, -C-N stretching,-O-H bending and N-H wagging, respectively at 2988 cm-1, 2938 cm-1, 1741 cm-1, 1449 cm-1, 1235 cm-1, 1046 cm-1 and 939 cm-1 along with 849 cm-1. The functional group detected would be the functional group of antioxidants in the milk samples.



| | | |
|---|---|---|
| **Figure 3.1.1.** Transmitted spectrum of milk sample A and milk sample B with ethyl acetate | | **Figure 3.1.2.** Transmitted spectrum of milk sample A and milk sample B with hexane |

*GC-MS analysis*

The extractable compounds were injected in the GC-MS system. The gas chromatography spectrums for the milk sample are shown in **Figure 3.2.1** (a) and (b). Fatty acid detection was allocated from NIST mass spectral library in accordance with the artificial analysis diagram by contrasting their retention time and spectrum with corresponding data from the reference compounds data. [7].

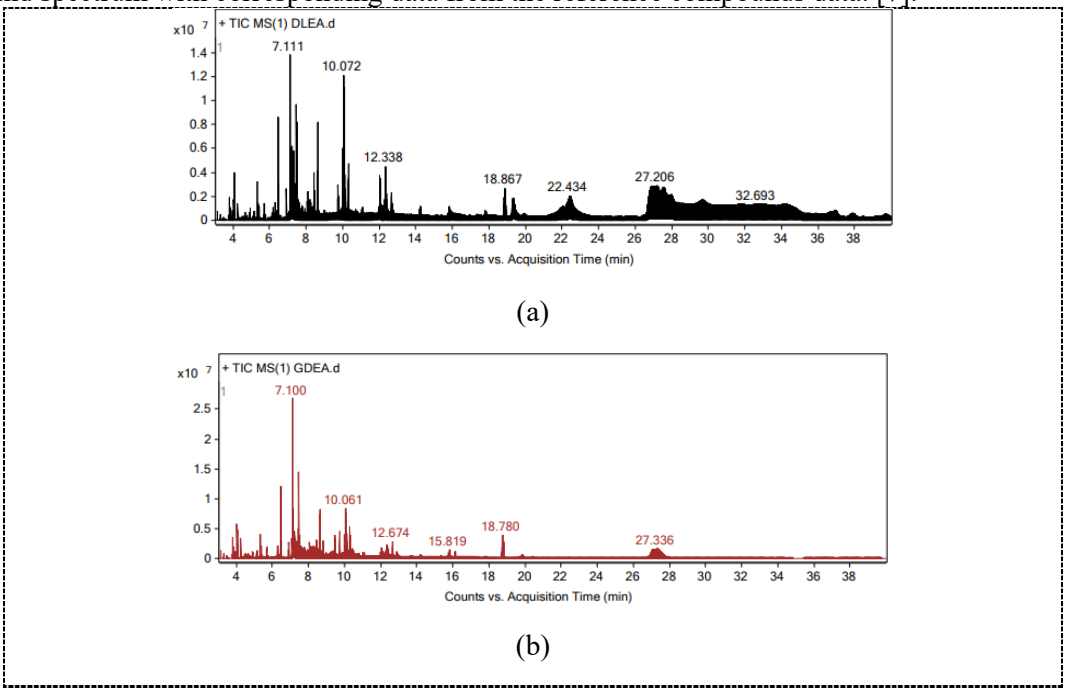



**Figure 3.2.1.** Gas chromatography spectrums for sample milk A and milk sample B

The fatty acid composition in milk are presented in **Table 3.2.3** with the real time for each fatty acid compounds. There are a few significant differences between milk in the form of fatty acid that results in various health effects. There are eight different fatty acids were detected in each milk. Milk sample B has the highest number of fatty acids detected different fatty acid. Palmitic acid, oleic acid, stearic acid and myristic acid were found in both milk while capric acid, decanoic acid, caprylic acid and lauric acid were only found in milk sample B.

| Fatty Acid | Molecular weight (MW) | Ethyl Acetate | | Hexane | |
|---|---|---|---|---|---|
| | | Milk Sample A | Milk Sample B | Milk Sample A | Milk Sample B |
| **Palmitic** | 256 | 10.072 | 10.061 | 10.007 | 10.039 |
| **Oleic** | 282 | 12.035 | nd | 12.035 | 12.002 |
| **Stearic** | 282 | 12.338 | 12.349 | nd | nd |
| **Capric** | 172 | nd | 6.298 | nd | nd |
| **Decanoic** | 172 | nd | nd | nd | 6.320 |
| **Myristic** | 228 | nd | nd | 8.445 | 8.456 |
| **Caprylic** | 144 | nd | nd | nd | 5.159 |
| **Lauric** | 200 | nd | nd | nd | 7.328 |

**Table 3.2.3**. Fatty acids compounds detected from the peak of gas chromatography spectra

## Conclusions

In this research, the commercialize cow's milk were studied in order to identify the antioxidant properties, active compounds and the functional group of the milk samples.

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