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Article

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. Therefore, this study focuses on the identification of antioxidant compounds in milk sample of Dutch Lady Fresh Milk and Goodday Fresh Milk using Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR). 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. This is the first research that studied on the antioxidant compounds in milk sample of *Dutch Lady* Fresh Milk and *Goodday* Fresh Milk. Further, the characterization of antioxidant compounds will be perform using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.**

Keywords— **Cow's milk, antioxidants, functional groups, ATR-FTIR**

I. INTRODUCTION

Dairy product is one of the biggest contribution to the economy which is also a part of industrial growth. In Malaysia, dairy product such as milk has been widely commercialised globally. Milk and dairy products play 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]. Sulphur containing the vitamins A, E, amino acids cysteine, carotenoids, superoxide dismutase, enzyme systems, catalase, and glutathione peroxidase are responsible for the antioxidant ability of milk and dairy products. [3].

Cow's milk has the most attention in dairy production as it is easily accessible by consumer. Thus, a lot of dairy manufacture industries produce various type of milk to fulfil the high demand from the consumers. The fatty acid content is one of milk's most significant contributions in terms of milk quality. Fatty acids are believed to act as antioxidants in cancer and other cells of the disease. [4]. Thus, the aim of this study was to compare the fatty acid contents in commercialised cow's milk using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) for the analysis of the functional group of the compounds in the milk while the Gas Chromatography-Mass Spectrometry (GC-MS) was used for the identification of fatty acids in milk compounds.

II. MATERIAL AND METHOD

Material

The milk samples which are Dutch Lady Fresh Milk and Goodday Fresh Milk were purchased from supermarket around Nilai. Hexane and ethyl acetate were used as the extractable solvents.

Preparation of sample

Two milk samples which were 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 as it is low on cost, reduce the time of analysis and easier. [5]. 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 was 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 as shown in **Figure 2.1**.

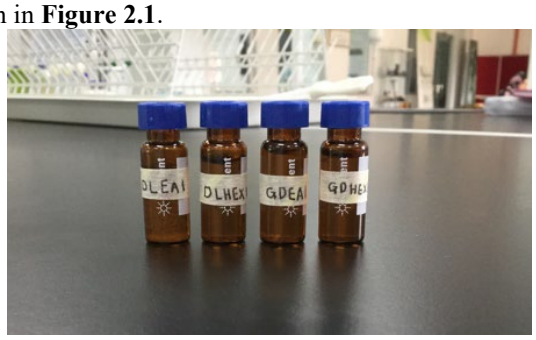


Figure 2.1

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. A drop of each extractable compounds 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 to remove the traces of the sample and ensure there is no residues. [6].

Gas Chromatography (GC) Instrument Parameter

The GC-MS study was carried out by Agilent on a GC-MS 5977B fitted with a pulsed split injector. 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 was 3 minutes. Both determinations have been performed in triplicates. The analysis was done based on method by [7].

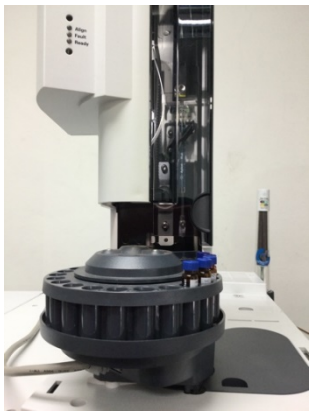


Figure 2.2 The samples were being injected into GC instrument

III. RESULT AND DISCUSSION

The functional group of antioxidant compound in the extractable compounds were detected using ATR-FTIR from the frequency of the spectrum obtained by using MicroLab software (Agilent Technologies, USA). Representative FTIR spectra of extracted compounds in the region 4000 – 600 cm^{-1} are shown in **Figure 3.1**, **Figure 3.2**, **Figure 3.3** and **Figure 3.4** and the results were tabulated in **Table 3.1.1**, **Table 3.2.1**, **Table 3.3.1** and **Table 3.4.1**. the extractable compounds of different brand with same solvents are been compared in **Figure 3.5** and **Figure 3.6**. The functional group were determined by the frequency of the peak in each spectrum.

Fourier Transform Infrared Spectroscopy is a commendable and outstanding tool for quantitative and qualitative studies of a compound. The primary advantages of FTIR spectroscopy over non-conventional and other analytical methods are accuracy, fast identification, simplicity, low financial cost of acquisition, non-destructive, non-burdening and minimal to low sample preparation. [8]. It also allows the structure of compounds to be evaluated for a comprehensive detection of milk quality. [9]. 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 antioxidant compounds in milk samples may contain the functional group found from the analysis of the spectra.

3.1 Milk sample A with ethyl acetate

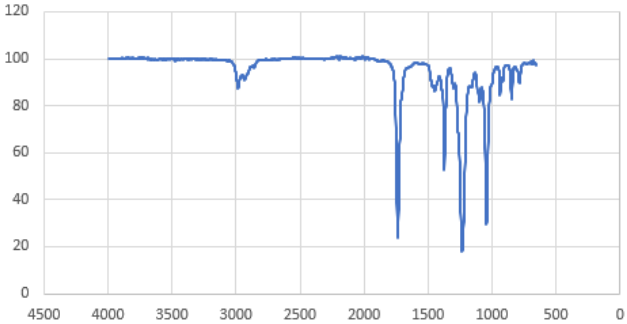


Figure. 3.1 Transmitted spectrum of milk sample A with ethyl acetate.

Frequency (cm^{-1})	Transmittance (%)	Functional group
2988.08	87.30	O-H carboxylic acid and C-H alkane
2938.81	90.89	=C-H aromatic
1741.05	24.40	-C=O carboxylic acid
1449.64	86.15	-C=C aromatic
1235.37	17.86	-C-O carboxylic acid
1046.82	30.80	-C-N aliphatic amine
939.68	84.48	=C-H aromatic and O-H carboxylic acid
849.69	82.95	-N-H 1°,2° amines and C-Cl alkyl halides

Table 3.1.1

3.2 Milk sample B with ethyl acetate

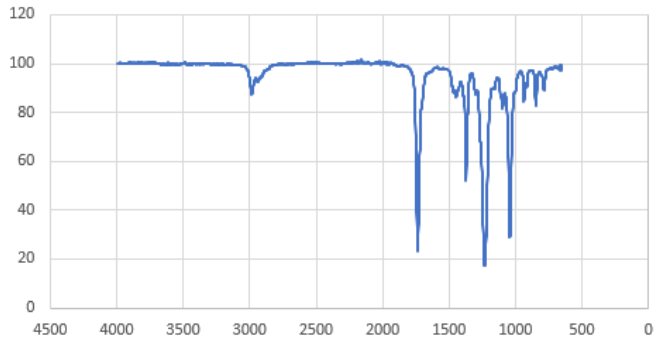


Figure. 3.2 Transmitted spectrum of milk sample B with ethyl acetate.

Frequency (cm^{-1})	Transmittance (%)	Functional group
2988.09	87.15	O-H carboxylic acid and C-H alkane
1741.05	23.93	-C=O carboxylic acid
1449.64	86.38	-C=C aromatic
1235.37	17.18	C-N aliphatic amines and -C-O carboxylic acid
1100.38	81.96	C-N aliphatic amines and -C-O carboxylic acid
939.68	84.17	O-H carboxylic acid
849.69	82.48	-N-H 1°,2° amines and -C-Cl alkyl halides

Table 3.2.1

3.3 Milk sample A with hexane

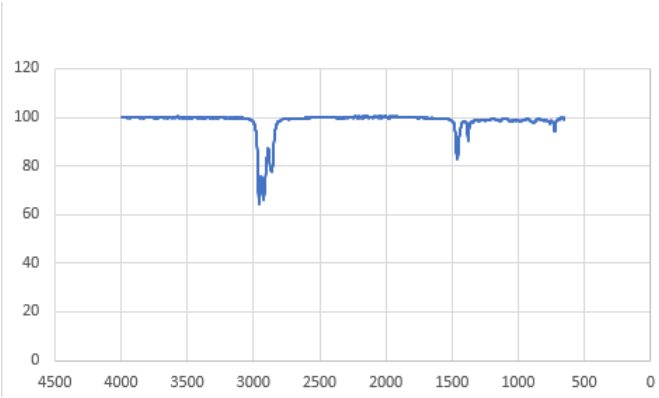


Figure. 3.3 Transmitted spectrum of milk sample A with hexane.

Frequency (cm^{-1})	Transmittance (%)	Functional group
2956.91	64.36	O-H carboxylic acid and C-H alkane
2860.35	77.56	-C-H alkane
1459.28	82.42	-C=C aromatic

Table 3.3.1

3.4 Milk sample B with hexane

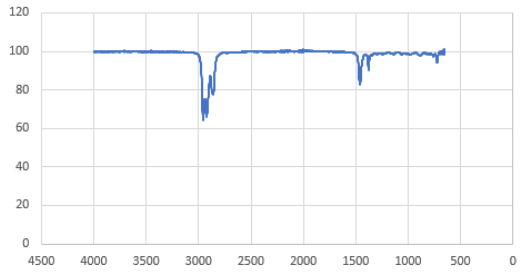


Figure. 3.4 Transmitted spectrum of milk sample B with hexane.

Frequency (cm^{-1})	Transmittance (%)	Functional group
2955.95	64.56	O-H carboxylic acid and C-H alkane
1462.50	83.08	-C=C aromatic
727.56	94.39	C-Cl alkyl halides

Table 3.4.1

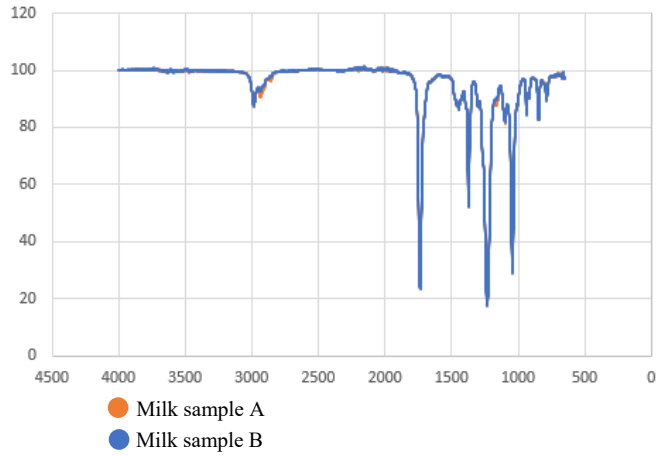


Figure. 3.5 Transmitted spectrum of milk sample A and milk sample B with ethyl acetate.

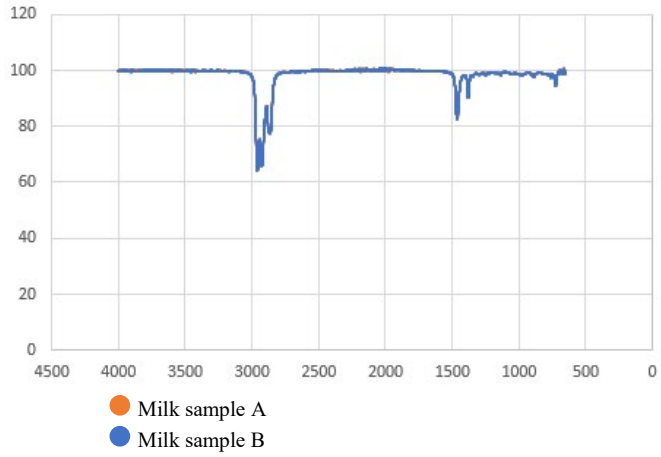


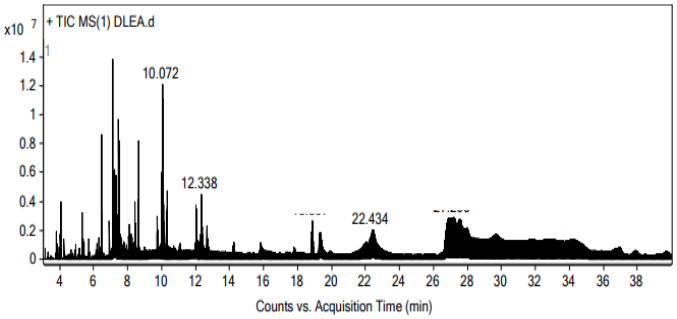
Figure. 3.6 Transmitted spectrum of milk sample A and milk sample B with hexane.

The extractable compounds were injected in the GC-MS system. Characteristics mass spectra of the compounds are shown in **Figure 3.7**. 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. [10].

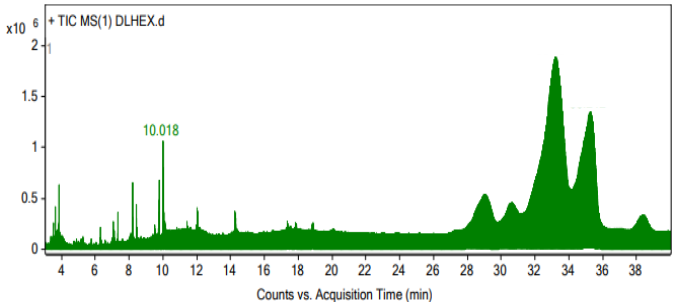
The fatty acid contents in milk different brand are almost similar. There are seven different fatty acids detected in all milk samples. Capric acid, lauric acid and caprylic acid are not detected in milk sample A.

Based on **Table 3.9.1** and **Table 3.9.2**, palmitic acid is one of the highest percentages of fatty acids detected in both milk samples. Palmitic acid is usually found in palm oil. Although this fatty acid often said to have harmful effects on chronic diseases but it is also an essential component of cell membranes. [11]. The least percentage of fatty acid contents is oleic acid. Oleic acid is important as it is the main fatty acid component of brain myelin phospholipid formed during after two years of birth. [12].

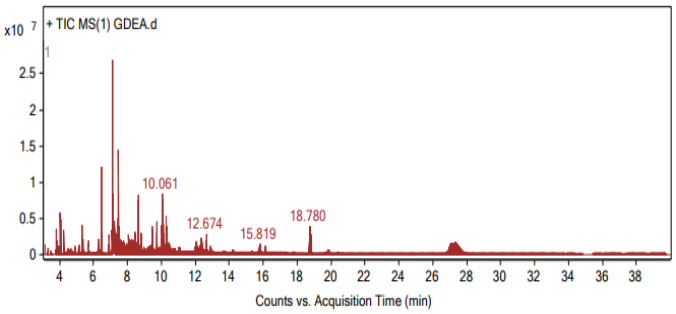
Consumption of myristic acid increases the amount of long-chain omega-3 fatty acids in plasma phospholipids, which may boost cardiovascular health parameters in human. [13]. In recent studies, there is an antiviral activity in both caprylic and capric acid while the lauric acid shown both antiviral and antibacterial activity. [14].



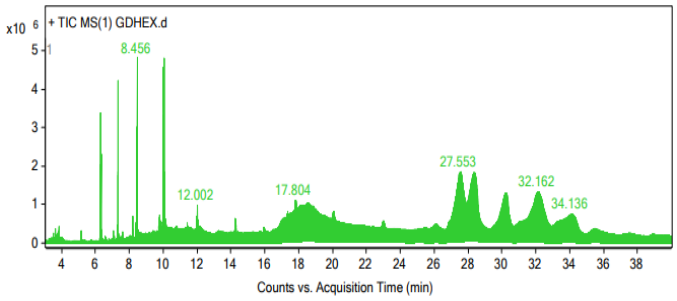
(a)



(b)

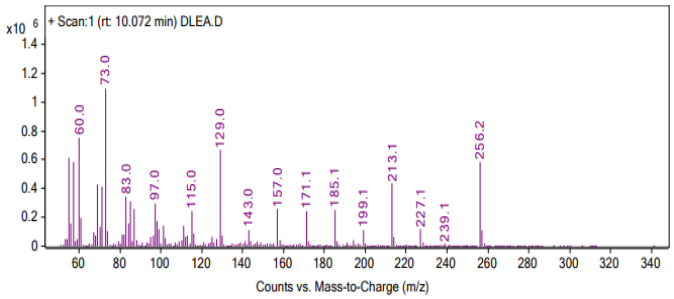


(c)

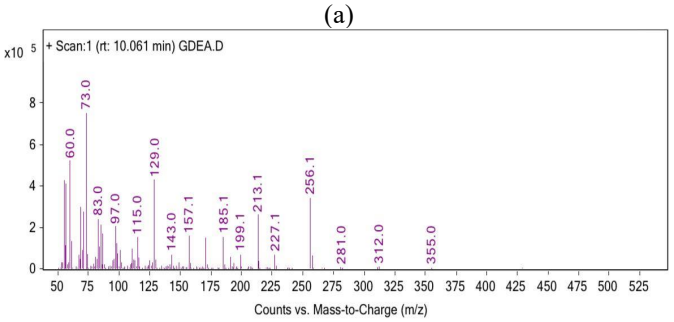


(d)

Figure 3.7 (a), (b), (c) and (d) are the gas chromatography spectra for extractable compounds in milk sample A with ethyl acetate, milk sample A with hexane, milk sample B with ethyl acetate and milk sample B with hexane respectively.



(a)



(b)

Figure 3.8. (a) and (b) are the mass spectroscopy spectra for palmitic acid in both milk sample A and milk sample B in ethyl acetate.

Table 3.9.1 shows fatty acid compounds detected *in* milk sample A and milk sample B that had been extracted with ethyl acetate. The data shows the real time of each compounds and the match percentage.

Fatty Acid	MW	Milk Sample A		Milk Sample B	
		Real time (min)	Match percentage (%)	Real time (min)	Match percentage (%)
Palmitic acid	256	10.072	90.2	10.061	87.1
Oleic acid	282	12.035	20.6	nd	nd
Stearic acid	284	12.338	57.8	12.349	17.8
Capric acid	172	nd	nd	6.298	84.9

*nd refer to not detected

Table 3.9.2 shows fatty acid compounds detected *in* milk sample A and milk sample B that had been extracted with ethyl acetate. The data shows the real time of each compounds and the match percentage.

Fatty Acid	MW	Milk Sample A		Milk Sample B	
		Real time (min)	Match percentage (%)	Real time (min)	Match percentage (%)
Palmitic acid	256	10.007	47.3	10.039	87.9
Oleic acid	282	12.035	12.1	12.002	24.3
Capric acid	172	nd	nd	6.320	91.1
Myristic acid	228	8.445	66.8	8.456	89.2
Caprylic acid	144	nd	nd	5.159	82.0
Lauric acid	200	nd	nd	7.328	89.7

*nd refer to not detected

IV. CONCLUSIONS

In summary, the commercialized cow's milk were studied in order to identify the fatty acid compounds that may serve as antioxidants. There was a slightly different amount of fatty acids in each milk sample A and milk sample B. This finding suggest that milk sample B showed the most detection of fatty acids. Therefore, this research can be utilized by the consumers in comparing the contents of the milk to ensure the body health.

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