MINERALS AND TRACE ELEMENTS COMPOSITION OF RAW COW'S MILK IN PENINSULAR MALAYSIA

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ABSTRACT. This study was conducted on raw cow's milk collected in seven states of Malaysia to investigate the composition of minerals and trace elements in the milk. Raw cow's milk samples were collected from Kedah, Perak, Johor, Melaka, Pahang, Selangor and Negeri Sembilan from January to November 2015. ICP-MS technique combined with microwave digestion were applied to determine the concentration of five major minerals (Na, Mg, K, Ca and P) and eleven trace elements (Cr, Mn, Fe, Ni, Cu, Zn, Se, Sr, Mo,Ba and Pb) in the milk samples. The result of analysis showed relatively constant composition of minerals and trace elements in the samples but some variations were observed in certain states. Application of principal component analysis (PCA) in the composition data showed different cluster exists between the states of Selangor and Negeri Sembilan as well as Kedah and Negeri Sembilan. There is no significant difference observed in the compositions of minerals and trace elements between other states. The compositions of minerals and trace elements in raw cow's milk in Malaysia are comparable with reports from other research. Further studies can be done to investigate the source of nutrient, food or environmental condition that produced the amounts of minerals and trace elements in the milk.

Keywords: raw cow's milk, minerals, trace elements, ICP-MS

INTRODUCTION

Milk is an important source of nutritious food which can provide essential fat, protein, carbohydrate, amino acids, vitamins and minerals to humans. In many countries around the world including Malaysia, milk is commonly given to children and people of all ages to enhance health and well-being. Being a healthy choice of beverage, it is also known as one of the main sources for major minerals and some important trace elements such as iron and zinc (Terrés *et al.*, 2001) as part of our healthy diet. The composition of minerals and trace elements in milk can be linked to the breed of the animal from which it is sourced (Linn, 1988), as well as forage, feed, water and the drugs administered (Angela *et al.*, 2013). Aside from that, research has shown that the concentration of minerals also varies according to the lactation period and can be modified during mastitis (Frédéric, 2005).

The geographical origin of food is fast becoming a very important issue worldwide especially when involving the authentication of expensive food. Aside from that, the geographical origin of food can be useful for other purposes such as investigating the factors directly or indirectly responsible for the quality and content of food. In elemental analysis, the PCA is one of the common techniques used to statistically evaluate and view grouping patterns in a large data and correlate between geographical origin and elements. In this study, preliminary analysis of the samples were by PCA to evaluate whether different clusters exist between data in different states.

Inductively coupled-plasma mass spectrometry (ICP-MS) has been widely known as a rapid and highly sensitive multielement technique in measuring minerals and low level trace elements. This technique offers a wider linear dynamic range which allows the determination of major and trace elements in the same sample injection (Ammann, 2007; Parson and Barbosa, 2007). In this research, ICP-MS was used to measure the concentration of minerals and trace elements in parts per billion (ppb). In addition to its capability in rapid multielement measurement, with very low detection limits, ICP-MS is able to provide simpler spectral interpretation and isotopic information (Nardi *et al.*, 2009) compared to other elemental analysis instruments. Its procedure for sample preparation is also easy by using a close-system microwave digestion which is a robust, quick and efficient tool in handling different types of sample matrix.

In Malaysia, there is scarcity of information on the current status of the composition of minerals and trace elements in raw cow's milk from local smallholder and commercial farms. This information is important to assess the quality of raw milk which will be a regulatory requirement for evaluating the quality of food for consumption. The aim of this study is to determine the composition of minerals and trace elements in raw cow's milk from farms in Peninsular Malaysia and evaluate statistically significant differences of samples from different states, as well as comparing the composition values with reports from other research.

MATERIALS AND METHODS

A total of 422 samples of raw cow's milk were collected from seven states – Kedah, Perak, Johor, Melaka, Pahang, Selangor and Negeri Sembilan, from January to November 2015.

Chemicals and reagents

Multi-element standards 1, 3, 4 and 5 consisting of major minerals Na, K, Ca, Mg and P, as well as trace elements Cr, Mn, Fe, Ni, Cu, Zn, Se, Sr, Mo, Ba and Pb were obtained from Perkin-Elmer (USA).

Ultrapure nitric acid (HNO_3), 65% and Ultrapure hydrogen peroxide (H_2O_2), 30% were from Merck (Darmstadt, Germany). Milli-Q Integral 5 Ultrapure water used in preparing standards and sample dilutions was from Merck Millipore (Darmstadt, Germany). Purified argon (99.999%) and purified helium (KED reaction gas) (99.999%) used in ICP-MS analysis was from MOX-Linde Malaysia Sdn Bhd.

Preparation of standard solutions

Three sets of standard solutions were prepared. For the first set, a combination of multi-element standards 3, 4 and 5 consisting of trace elements Cr, Mn, Pb, Se, Ni, Mo and Ba was prepared as intermediate of 100 ppb from stock solution and consequently prepared in four concentrations of 1, 2, 5 and 10 ppb thereafter. The second set of standard consisting of trace elements and minerals Cu, Fe, Zn, Sr and P was prepared directly using multi-element standards 3 and 5 into three points of working standard concentrations of 50, 100 and 500 ppb. In the third set of standard, an intermediate of 20 ppm was prepared from multi-element standard single stock solution consisting of minerals Na, Mg, Ca and K. The intermediate was then diluted to five concentrations of working standard of 0.1, 0.5, 1, 2 and 5 ppm. Internal standard solution (1000 ml) was prepared directly from stock solution of 10 ppm in the concentration of 2 ppb. All standard solutions and internal standards were prepared using 1% nitric acid (HNO_3) in Ultrapure water.

Analysis Procedure

Microwave digestion of samples

The microwave digestion of samples was performed according to Milestone Pro 16 Rotor Application Book. The raw cow's milk was thawed and shaken well before 500 mg of samples were weighed using an analytical balance (A&D, Oxfordshire, UK) and pre-mixed with nitric acid, HNO_3 and hydrogen peroxide, H_2O_2 as accelerator in a Teflon vessel. The vessels were assembled and inserted into the Milestone (Sorisole, (BG) Italy) microwave digestion system. The temperature was set to 180 °C for complete digestion in 45 minutes. Samples digested were diluted to a final volume of 50 ml before subjected to ICP-MS analysis.

Elemental analysis using ICP-MS and data analysis

Nexion 300X ICP-MS (Perkin-Elmer, USA) system was employed for sample analysis using 2 modes, kinetic energy discrimination (KED) and standard mode. KED mode was employed to cater for interferences in analysing low mass elements. Online internal standard solution was employed to correct physical and matrix interferences. Corrections by equation were applied automatically by the software during data analysis and reported. Data were analysed statistically and applied with principal component analysis using Minitab 17.

RESULTS

The highest number of samples were from Kedah (n=101) followed by Johor (n=100), Selangor (n=66), Perak (n=53), Negeri Sembilan (n=49), Melaka (n=30) and Pahang (n=23) (see Table 1). The states with high number of samples have progressive dairy farms which supply milk for the nearby high density population areas. The overall result of analysis showed relatively constant composition of minerals and trace elements in the samples but some variations can be observed in certain states. The result obtained (see Table 2) was also compared with research values from other countries and values reported by the FAO (Wijesinha-Bettoni and Burlingame, 2013).

Table 1. The compositions of minerals and trace elements (reported in range) according to states.

	State						
	Johor n = 100	Kedah n = 101	Melaka n = 30	N. S. n = 49	Pahang n = 23	Perak n = 53	Selangor n = 66
Minerals (µg/g)							
Ca	340-1673	423-1546	412-1387	563-1716	575-1709	425-2254	532-2400
Mg	56-142	93-192	57-151	82-229	72-248	91-192	71-199
Na	229-709	236-742	224-610	282-1033	283-816	265-799	222-799
K	857-2354	1156-2431	821-2059	842-3079	1304-3136	1148-2555	943-2519
P	219-882	338-1236	309-753	363-1061	405-1004	279-1375	312-1496
Trace Elements (µg/g)							
Mn	0.01-1.23	0.01-4.15	0.01-0.32	0.02-5.02	0.06-1.44	0.02-4.69	0.04-7.32
Fe	0.44-4.84	0.14-5.94	0.54-4.45	0.46-6.13	0.52-5.69	0.38-4.89	0.51-5.04
Cu	Tr-1.17	0.02-0.70	0.03-0.57	0.03-0.44	0.07-0.74	0.03-1.27	0.05-0.54
Zn	0.42-3.87	0.45-3.54	0.60-4.68	0.52-3.48	0.90-2.54	0.60-5.25	0.87-5.74
Se	Tr-0.03	Tr-0.06	Tr-0.03	Tr-0.03	Tr-0.04	Tr-0.04	Tr-0.04
Cr	Tr-0.70	Tr-1.43	Tr-0.70	0.01-0.30	0.01-0.35	0.01-0.24	0.01-0.35
Ni	0.01-6.23	0.01-6.24	0.04-2.86	0.05-1.25	0.12-1.75	0.04-0.34	0.05-2.27
Sr	Tr-2.34	0.12-5.73	0.22-1.20	Tr-9.75	Tr-5.54	0.18-9.57	0.25-2.17
Mo	0.02-0.30	Tr-0.27	0.02-0.12	Tr-0.26	0.03-0.09	0.01-0.29	0.02-0.31
Ba	Tr-0.59	0.01-0.86	0.02-0.91	0.01-0.17	0.03-0.92	Tr-0.87	0.01-0.21
Pb	Tr-0.22	Tr-0.42	0.02-0.15	Tr-0.41	0.02-0.17	Tr-0.20	Tr-0.24

Notes : N.S. = Negeri Sembilan; Tr = traces

Table 2. The compositions and trace elements of cow milk reported in mean

	Peninsular Malaysia (2015)	Wijesinha-Bettoni and Burlingame – FAO (2013)	Gabryszuk *et al.* (2010)	Zamberlin *et al.* (2012)	Frédéric (2005)
Minerals (mg/kg)					
Ca	468-1812	910-1120	637.4	1070-1330	1043-1283
Mg	75-193	100-110	61.85	90-160	97-146
Na	249-787	380-450	421	400-580	391-644
K	1010-2590	1320-1550	894.6	1440-1780	1212-1681
P	318-1115	840-950	456.3	630-102	930-992
Trace Elements (mg/kg)					
Mn	0.02-3.45	0.04-0.10	0.02	0.013-0.04	-
Fe	1.82-5.28	Tr-2.0	0.79	0.3-0.7	-
Cu	0.03-0.76	Tr-Tr	0.16	0.02-0.3	-
Zn	0.62-4.16	3.0-4.0	1.59	0.74-1.45	-
Se	Tr-0.04	0.01-0.037	0.019	0.013-0.017	-
Cr	0.01-0.58	-	0.02	0.01-0.04	-
Ni	0.02-2.99	-	0.04	0.004-0.06	-
Sr	0.11-5.19	-	0.14	-	-
Mo	0.01-0.23	-	0.09	0.024-0.06	-
Ba	0.01-0.65	-	0.03	-	-
Pb	0.006-0.26	-	0.006	-	-

Tr = traces; - = no data

DISCUSSION

From the results, it can be observed that milk from the seven states contain high amount of calcium with Selangor giving the highest value (532-2400 mg/kg) while milk in Negeri Sembilan and Pahang were observed to be high in magnesium (Negeri Sembilan: 82-229 mg/kg), (Pahang: 72-248 mg/kg) and potassium (Negeri Sembilan: 842-3079 mg/kg) (Pahang: 1304-3136 mg/kg). Sodium was observed to be highest in Negeri Sembilan (282-1033 mg/kg) while phosphorus content was observed to be highest in Selangor (312-1496 mg/kg) followed by Perak (279-1375 mg/kg) and Kedah (338-1236 mg/kg). Meanwhile for trace elements, manganese showed high value in Selangor (0.04-7.32 mg/kg), Negeri Sembilan (0.02-5.02 mg/kg), Perak (0.02-4.69 mg/kg) and Kedah (0.01-4.15 mg/kg). Whereas for copper, high values were observed in Perak (0.03-1.27 mg/kg) and Johor (traces amount to 1.17 mg/kg).

Iron, zinc and selenium showed constant and similar value for all states.

The variation of the results in mineral composition can be due to several reasons. During the lactation period, the most important changes in composition occur at around parturition, this caused the calcium concentration in colostrum to be much higher than that of normal milk and near the end of lactation (Frédéric, 2005). Research has also proven that the yield of minerals increased with increasing milk production (Eppard *et al.*, 1985).

PCA of the data for all seven states shows that different clusters exists between the states of Selangor and Negeri Sembilan (Figure 1) as well as Kedah and Negeri Sembilan (Figure 2), while for other states, the results showed no significant difference. The difference in composition of milk between different states analyzed by PCA showed that geographical condition, type of feed and climate conditions may also play a role in determining the composition of the milk besides other factors mentioned. This information can be useful as a preliminary tool to predict the geographical origin of the milk, while knowing the characteristics of the milk, and thus, the factors that makes the milk higher in quality. Future research is recommended to further improve the quality of milk produced in Malaysia by investigating the effects of feeding, environment and disease treatment.

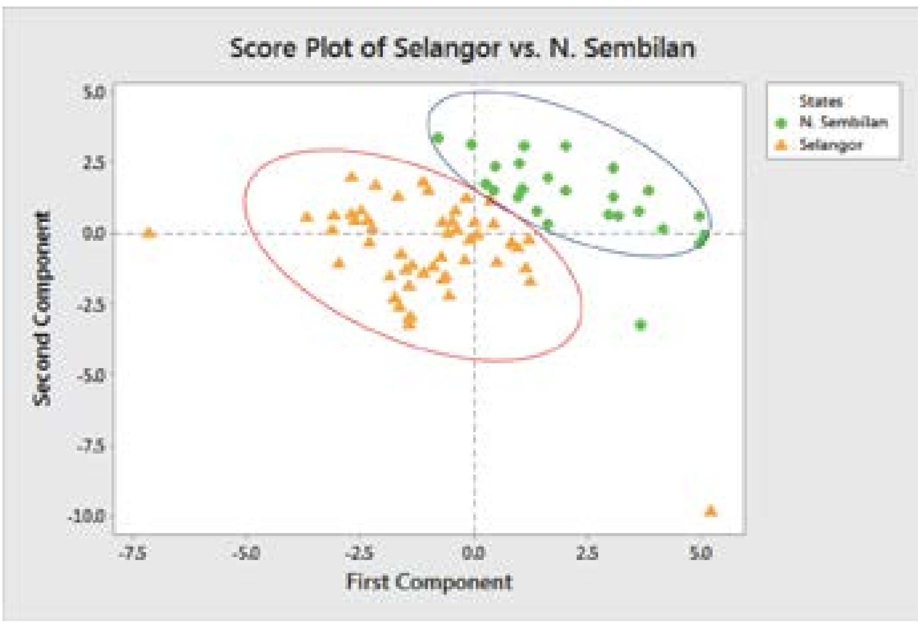

Figure 1. Principal component analysis of milk composition between Negeri Sembilan and Selangor states.

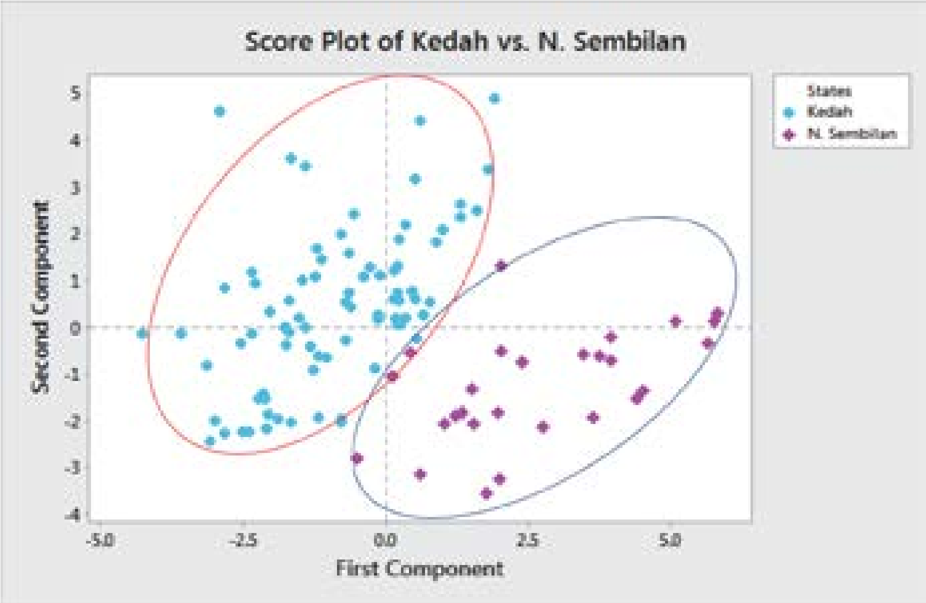

Figure 2. Principal component analysis of milk composition between Kedah and Negeri Sembilan states.

CONCLUSION

The composition of cow's milk produced in Peninsular Malaysia is comparable with values reported by research in other countries. Preliminary PCA showed that clustering exists between states based on composition of minerals and trace elements. This can be further investigated by including other parameters such as iodine, chloride and carbon content. The concentration of minerals and trace elements are dependent on the stage of lactation, breed, feed given to the animals as well as regional diversity and soil conditions affecting feed production or grasses among other factors. Knowledge on these will help to assess the reasons for the diversity in raw milk mineral composition. In evaluating the mineral composition, farmers and consumers will greatly benefit by being able to improve their management of animals as well as to be able to produce the best milk quality for human consumption. Regular monitoring of these elements is needed so as to evaluate milk quality and animal management.

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