ELEMENTAL ANALYSIS OF COW'S MILK APPLYING SEM-EDX SPECTROSCOPY TECHNIQUE

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Abstract

The present study has been conducted to investigate the microstructure and elemental analysis of cow's milk by applying scanning electron microscopy (SEM) using energy-dispersive X-ray spectroscopy (EDX) analytical technique. For this task cow's milk collected from the dairy farm situated in Landhi cattle colony, Karachi. After drying (above 85 ^{o}C), sample was exposed under the scanning electron microscope and EDX observations were recorded in four particulates. Results showed that this technique is adequate for qualitative measurement of elements in the milk sample. In contrast, quantitative measurement through EDX was satisfactory for measurement in comparison to other quantitative techniques such as Atomic Absorption Spectroscopy.

Introduction

Microscopy has been used for several years in controlling the adulteration of food commodity. These techniques based on particle size distributions can be obtained from micrographs of powders and particulate material by measuring the sizes of hundreds of particles in a micrograph field. A computer based image analysis systems having sophisticated statistical analysis software are commercially available and detailed analysis of micrographs are now more routinely on food products (Brennan and Grandison, 2012; Hui, 1992). A variety of devices have been developed in scanning electron microscopy (SEM) like wavelength-dispersive (WDS), energy-dispersive (EDS) spectrometers and proton induced x-ray emission (PIXE) are based on the detection of X-rays. Chemical characterization of a sample through a scanning electron microscope (SEM) is carried out non-destructively with energy dispersive X-ray (EDX) analysis. The electron beam strikes the atoms in the sample with uniform energy and they instantaneously emit characteristic X-rays of specific energies for every element. These X-rays provide information about the elemental composition of the sample. Elements starting with the atomic number 6 (carbon) can be determined with this analytic technique. The detection limit with SEM–EDS is ~0.1 wt%. The unique resolution of the SEM depends on the size of the electron spot which in turn depends on the magnetic electro-optical system which produces the scanning beam. The resolution is also limited by the size of the interaction volume, or the extent of material which interacts with the electron beam. The merits of SEM–EDX technique include the small sample size required (<1 mm^3). In contrast, there are limitations to this method that includes its poor sensitivity to trace elements and elements lighter than Na. Also, the loss of volatile elements (Na) during excitation is a well-recognized phenomenon (Kursula, 2000). In accordance with Reed (1996), the theoretical detection limits in SEM–EDX measurements are about 0.08 wt%.
Milk of any mammalian species has a wide range of organic and inorganic ingredients that are all interlinked in a specific manner. For example, Ca and P are bound with casein micelle, Fe is related to lactoferrin, transferring, xanthine oxidase and slightly to casein, Zn is associated with lactoferrin, Mn is bound to the milk fat membrane and Co is a key mineral in the large vitamin B12 molecule etc (Fennema, 1996; Whitney, 2005).

A successful study was reported to investigate the effect on solubility of NSAID in combination to fat free milk by applying SEM technique (Kumar and Mishra, 2006). Another study was conducted using SEM to examine the relationship between skimmed milk powder and physical properties of chocolate mass (Attaie *et al*., 2003).

In SEM analysis, sample protocol, such as drying, stabilizing through a fixative, mounting the sample on metallic holder and coating a thin layer by means of an electrical conductor are the fundamental steps (Bozzola and Russell, 1992).

The aim of this study was to focus on the chemical analytical aspects of elements in the cow's whole milk with and without damaging the organic portion through exploration at microstructure level.

Materials and Method

Sample collection: Sample of cow's whole milk just after milking was collected from Landhi cattle colony in the summer season in the month of June. Sample was taken in a polythene bag of 500 mL. and stored below 4 ^{o} C.

SEM analytical protocol: In SEM-EDX analysis two types of pretreatment were adopted (1) analysis through mineral oxides or ash and (2) analysis through coagulated whole milk. For the preparation of ash, an aliquot of 10ml was dried on hot plate at 105^{o} C and ignited in the furnace at 550^{o} C to constant weight (AOAC, 2000). For the coagulation an aliquot of the milk sample heated on hot plate over 85^{o} C (Twyman, 2005). After the removal of moisture a lumpy mass was obtained.

SEM-EDX analysis: The dual observations were recorded by applying scanning electron microscopy (SEM) in combination of energy-dispersive X-ray (EDX) spectrometer. For this task, the samples were first loaded on sample holder using double site tape then the samples were coated with auto coater from Joel Japan model no. JFC 1500. The coater has target of gold. The samples were coated up to 300^{o} A. Coated samples were then loaded on SEM from Joel Japan model JSM 6380 A. The EDX analyses were performed using EDX detector from Joel Japan model No. EX 54175 JMU.

Results and Discussion

In some studies, characterization with the confirmation of elemental composition through spectrum of silver nanoparticles synthesized by various sources by applying SEM-EDX technique has been reported (Guangquan *et al*., 2012 and Jegadeeswaran *et al*., 2012).

In present study, SEM-EDX was first performed on metal oxides (ash) on a trial basis that did not produce any identifiable particles. From this trial it appears that this technique may not be applicable without the presence of organic matter such as bovine milk. In the second trial, SEM was applied on the four different particulates of the dried cow's milk and a different scanning resolution of dehydrated milk that produced images and EDX mapping provides the conventional SEM image and a meaningful picture of the element distribution of a surface.

Fig.1 represents the qualitative inner structure of the different sites of the sample. Elemental composition of the surface cow milk particulates have been shown as spectra in Fig.2. The straight up axis shows the numeral of x-ray counts whereas the x-axis shows energy in KeV. Detection lines express the energies giving out by the elements has been exhibited and matches up with peaks in the spectrum. The percentages of several elements including metals and nonmetal (macro, micro and ultra-trace metals) were observed at different sites of the sample have been mentioned in the Table 1 applying Standard less Quantitative Analysis with ZAF correction Method (Trincavelli *et al*., 2014). All the estimated elements showed variation in their amounts in the different scan. Some of the elements were found in all four scan whereas others were recorded in only one or two scans. Scan1 and scan4 gave quantitative information about 12 elements; scan2 estimated 8 elements and scan3 showed percentages of 13 elements. The ranges of percentages of major, trace and toxic elements were depicted in Table 2.

Ca, which is rich in milk was estimated in two scans (3rd and 4th scans); but fairly high percentage among all elements. P was measured in all scans and highest in the 4th scan, but its percentage was noted as the second highest after Ca. Percentages of electrolytes such as Na and K differed in all trials and their ranges were: Na 0.31 - 0.56% and K 0.58 - 2.08%. Trace elements were found in the range of 0.01 - 0.08% (Table 2). Nevertheless, the quantities of essential trace elements (V, Cr, Mn, Fe, Co, Cu and Zn) were much lesser than that of the major elements. Fe and Cu were found in three trials, but percentage of Fe was uniform and comparatively lower than Cu. The percentages of Zn varied considerably but the maximum was measured as 0.08%. The percentages of Al were observed in all scan in the range of 0.01-0.18. However, other non-essential trace elements (Si, Sr and As) were observed in only a single scan. Si was measured in scan4 whereas Sr and As were found in scan1 and scan2, respectively as indicated in Table 1, but their levels were less than those of the major elements and higher than those of essential trace elements. In the light of the results, it is concluded that the concentration of elements in the sample was not homogeneous in all particles.

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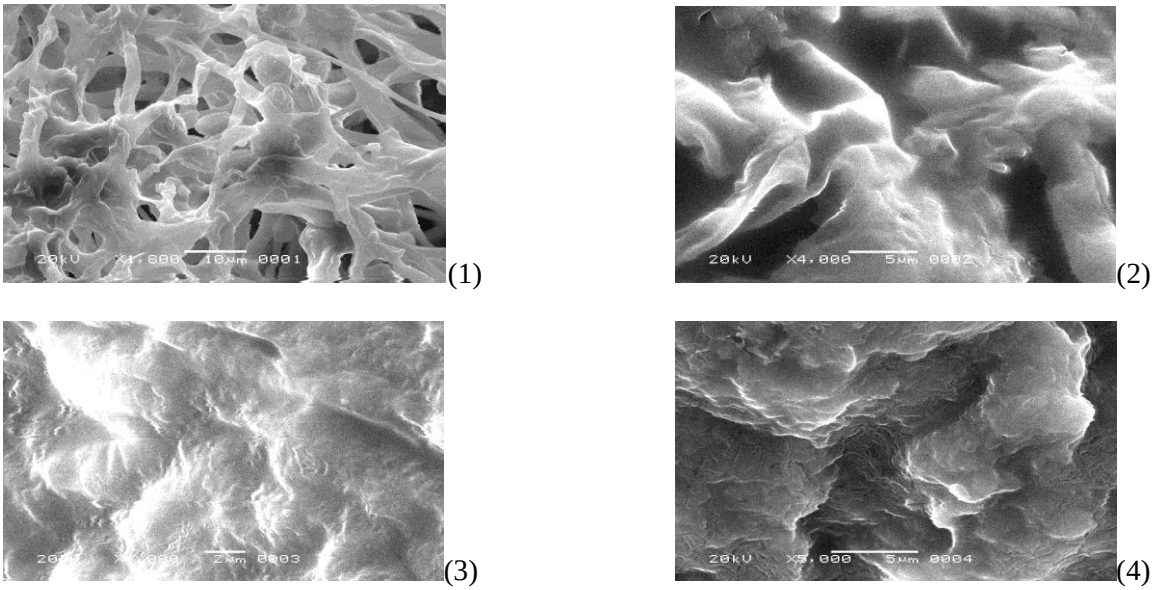

Fig.1. SEM Images of dried Cow Milk particulate, scan (1) at 20 µm, (2) and (4) at 10 µm and (3) at 5.0 µm.

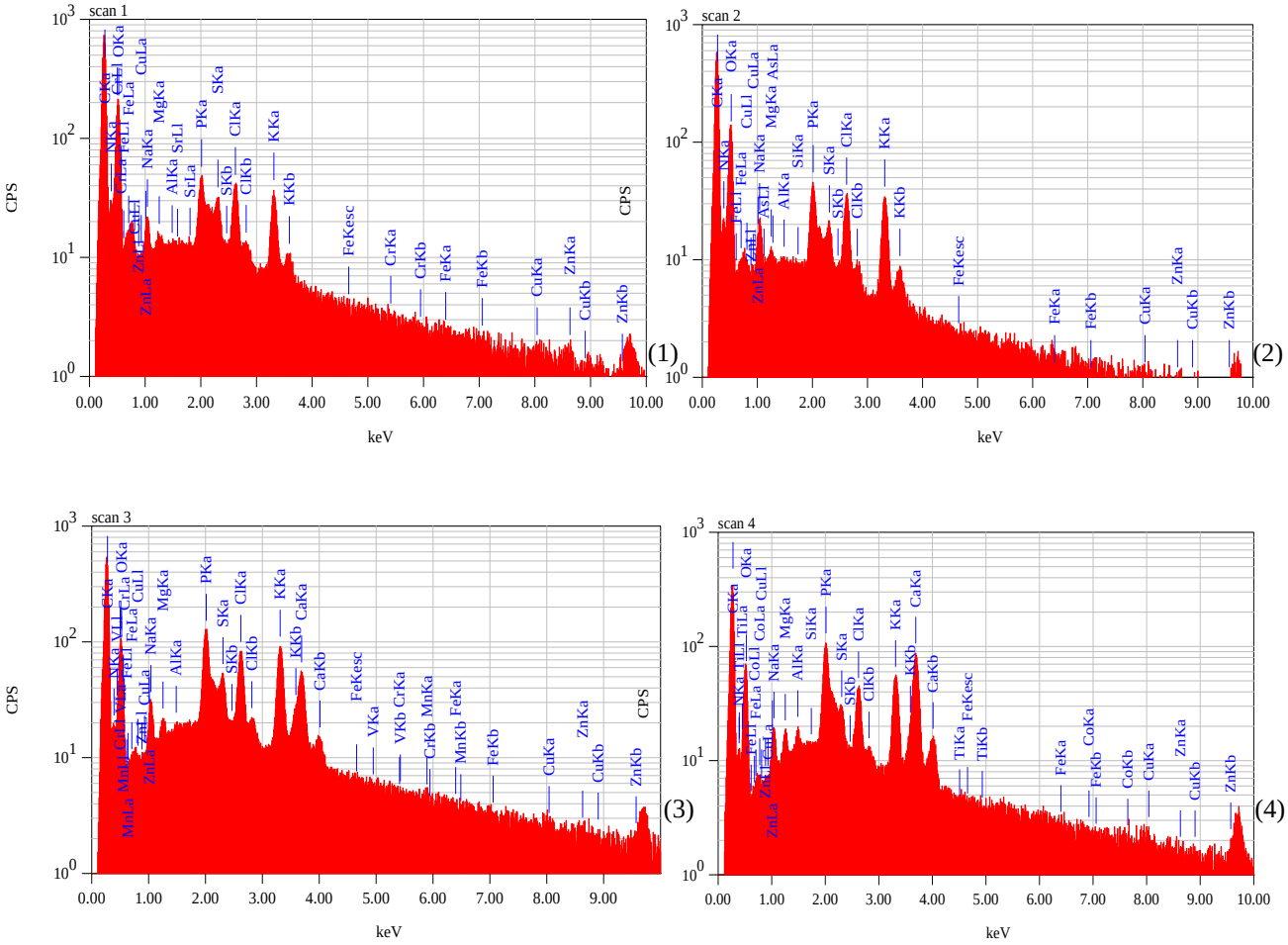

Fig.2. EDX spectra of the element distribution of a surface dried Cow Milk particulate, scan (1) at 20 µm, (2) and (4) at 10 µm and (3) at 5.0 µm.

Table 1. Precisions of major, essential trace elements and non-essential trace elements of the Cow's milk obtained using the EDX spectroscopy.

Element	Mass % of scan 1	Mass % of scan 2	Mass % of scan 3	Mass % of scan 4	Error %	Maximum %
Na	0.31	0.45	0.56	0.51	0.26	0.56
Mg	0.05	0.05	0.15	0.26	0.17	0.26
Al	0.01	0.01	0.04	0.18	0.15	0.18
Si	0.00	0.00	0.00	0.01	0.11	0.01
P	0.46	0.63	1.88	2.24	0.14	2.24
S	0.24	0.20	0.49	0.31	0.11	0.49
Cl	0.53	0.61	1.35	1.01	0.13	1.35
K	0.58	0.80	2.08	1.83	0.18	2.08
Ca	0.00	0.00	1.23	3.23	0.18	3.23
V	0.00	0.00	0.01	0.00	0.29	0.01
Cr	0.01	0.00	0.00	0.00	0.58	0.01
Mn	0.00	0.00	0.04	0.00	0.40	0.04
Fe	0.01	0.00	0.01	0.01	0.49	0.01
Cu	0.04	0.00	0.12	0.04	0.86	0.04
Zn	0.08	0.03	0.05	0.01	1.02	0.08
Sr	0.05	0.00	0.00	0.00	0.36	0.05
As	0.00	0.03	0.00	0.00	3.17	0.03

Table 2. Precision ranges of elements of the Cow's milk obtained using the EDX spectroscopy.

Type	Element	Range of mass %
Major elements	Ca, P, Na, K, Cl , Mg, S	0.26-3.23
Essential trace elements	V, Cr, Mn, Fe, Cu, Zn	0.01-0.08
Non-essential trace elements	Al, Si, Sr, As	0.01-0.18

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