Time-Resolved $\mu$-XRF and Elemental Mapping of Biological Materials

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Abstract
We have developed a micro-XRF instrument using a polycapillary x-ray lens. In addition, a time-resolved MCA (multi channel analyzer) was installed for x-ray energy analysis. This micro-XRF system with the time-resolved MCA enables continuous x-ray analysis as a function of time at localized region on the sample. After the micro x-ray beam was fixed on the quinoa seed, x-ray fluorescence intensities of several elements emitted from the quinoa were monitored. Furthermore, elemental distributions in biological materials, such as wheat and rice grains were investigated by the micro-XRF method.

INTRODUCTION
In x-ray fluorescence analysis, a non-destructive elemental analysis is possible at ambient air pressure. This is a unique advantage because other instrumental analytical methods such as EPMA require special sample preparation and high vacuum condition. In addition, XRF analysis at small region, that is, micro-XRF ($\mu$-XRF), has been possible by applying recent technological advances. For example, a polycapillary x-ray lens [1-6] is useful for obtaining micro x-ray beam in laboratory. We have studied applications of $\mu$-XRF with polycapillary x-ray lens [7,8]. One of interesting samples is in vivo biological material such as seed and plants. It is difficult to measure them by other micro analytical methods, because they can not be usually put into a vacuum chamber, and the damage due to electron beam is a serious problem for the sample. XRF at ambient air pressure will be the best method for them.

Rice, wheat and quinoa seeds are important grains to as nutrient in the human body. Elemental distributions of minerals in grains give us useful information for processing of food materials besides basic research on nutrition and botany. The minerals in grains have been analyzed by atomic spectrometry such as ICP-AES (-MS) and AAS (atomic absorption spectroscopy) [9]. However, only averaged composition is obtained by these methods. In addition, sample preparation for these atomic spectroscopic methods is a time-consuming process and requires a skill for sample preparation. On the other hand, $\mu$-XRF directly gives us elemental distributions without any special sample preparation.
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EXPERIMENTAL DETAILS

Figure 1 shows our experimental setup for μ-XRF [7,8]. A polycapillary x-ray lens (made by Beijing Normal University) was attached to an x-ray tube (Mo target). The focus size of x-ray emission in the x-ray tube was 1 x 10 mm. Finally, a spot size of about 80 μm was obtained at a focal output distance of 15 mm from the end of the lens. A whole grain sample was put on an X-Y-Z sample stage, which was controlled by a motor controller and a computer. A CCD camera was used for observation and confirmation of the position of the sample. A silicon drift detector (SDD; X-Flash Detector Type 1201, RÖNTEC, Germany; Sensitive area: 10 mm², Energy resolution <150 eV, FWHM at 5.9 keV) was used for detection of x-ray fluorescence. The distance between the sample and the SDD was less than 30 mm.

A time resolved multi-channel analyzer (time-resolved MCA; NT-2400M, Laboratory Equipment Co., Japan) was used to obtain the time-dependent XRF intensity. By using this equipment, measuring life time, interval time and total time are controlled and x-ray fluorescence area-intensities (ROI: range of interest) are automatically saved by computer. The cotton and water shown in Fig.1 were used for observation of growth process of the quinoa seed. All measurements were performed at ambient air pressure.

Fig. 1 Experimental setup for μ-XRF.
RESULTS AND DISCUSSIONS

First, a black wheat grain was measured to know the distribution of minerals in the grain. Figure 2 shows element maps for K, Ca, Mn, Fe and Zn. X-ray generator was operated at a tube voltage of 30 kV and a tube current of 20 mA. One pixel was 100 x 100 μm, and preset time for each pixel was 10 s. Each element showed different distributions. For example, K and Ca distributed on the grain coat, while Ca, Mn and Zn dominantly existed at embryo. On the edge surface near the SDD (right side of each element map in Fig. 2), excited x-ray fluorescence was not absorbed by sample itself; therefore, high x-ray fluorescence intensity is usually observed compared with XRF intensity at other positions.

Fig. 2 Element maps obtained for black wheat.
Figure 3 shows the elemental distributions obtained for buckwheat (soba wheat). Similar result was obtained, although K, Ca and Fe distributed on the coat while Ca dominantly distributed on the embryo. Figs. 2 and 3 indicate that μ-XRF is useful for elemental mapping of biological samples such as grains.

Next, a quinoa sample was measured by using a time-resolved MCA. As shown in Fig.1, the quinoa seed was put on the cotton using double sides tape. The end of the cotton was put into the water (pure water; Milli-Q); therefore, the seed could be germinated. We measured the x-ray fluorescence from the seed in the growth process.

Figure 4 shows the growth of the quinoa seed. After germination of the seed, micro x-ray beam was fixed at the germinated region (stem). The time-dependence of XRF intensities was monitored.
Fig. 5 Time dependence of x-ray intensities of each element, measured at stem of the quinoa seed without nutrition solution.

Figure 5 shows the time dependence of x-ray intensities of K, Ca, Mn, Zn, P, S and Ca. A preset time of 100 s and an interval time of 200 s were applied. During the growth in 16 hours, no change in the time dependent intensities was observed in the stem region.

![Diagram showing time dependence of x-ray intensities](image)

Fig. 6 Time dependence of x-ray intensities of each element at root of the quinoa seed. A nutrition solution was put into the water at 2 hours after starting the measurement.

Figure 6 shows the time dependent XRF intensities (K lines) during the growth at the root of the quinoa. A nutrition solution is used to fasten the growth of the plant. The nutrition solution, including N, P, Mg, Fe, Cu, Zn and Mo, was added to the water at 2 hours after starting the measurement. Before adding the nutrition solution, K signal decreased. However, after adding the nutrition solution, the decease of K signal stopped and slightly increased. In contrast, Ca signal increase until 2 hours; however, it decreased after the nutrition solution was added. The reason of these trends of x-ray intensities is not clear; however, it was demonstrated that the time-resolved MCA is useful to acquire the time dependence of x-ray fluorescence.

CONCLUSIONS

A micro XRF instrument was developed using a polycapillary x-ray lens. In addition, a time-resolved MCA, which enables continuous x-ray analysis as a function of time, was developed. Using this analytical system, we studied the growth of a quinoa seed. After the micro x-ray beam was fixed on the Quinoa sample, x-ray fluorescence intensities of several elements emitted from the quinoa were monitored. It was demonstrated that the time-resolved MCA is useful to acquire the time dependence of x-ray fluorescence. It is expected that the
time-resolved MCA is useful for studying and monitoring the compositional changes during growth processes as well as environmental monitoring of harmful elements.

REFERENCES