Synchrotron soft x-ray and infrared microspectroscopy contributions to advances in feed chemistry and feed science technology

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Since synchrotron radiation was discovered, advanced synchrotron radiation based bioanalytical techniques have been rapidly developed such as synchrotron radiation based soft x-ray (SR-XMS) and synchrotron-based Fourier transform infrared microspectroscopy (SR-IMS). In this article, recently obtained information on potential applications of these bioanalytical techniques (SR-XMS & SR-IMS) was reviewed in feed chemistry and feed science. The emphasis of this review focused on basic concepts and theories of SR-XMS and SR-IMS techniques to feeds research and using SR-XMS and SR-IMS technique for feed chemistry, chemical speciation, feed molecular structure, feed mineral oxidation status, and feed science technology studies. The information described in this article may provide an insight how these two techniques (SR-XMS and SR-IMS) are contributing to advances in feed chemistry, feed molecular structure, and feed science technology.

Keywords Soft-x ray microspectroscopy; Infrared microspectroscopy; Synchrotron radiation; Feed chemistry; Feed molecular structure; Chemical speciation; Element oxidation

1. Introduction

1.1 Chemical speciation, feed chemistry, element oxidation, and feed structure

Mineral and elemental feeds are key ingredients in diets [1]. The toxicity and bioavailability of these mineral and elemental feeds are related to not only elemental concentration but also elemental oxidation status and chemical speciation in digestive tract. When feed minerals or elements move from rumen to small intestine, large intestine, blood and final organ tissues, the oxidation status of elements may also be changed in the digestive tract. These changes my be related to absorption and metabolization.

Nutrient availability and utilization of plant-based feeds are associated with various biomolecular or biopolymer conformations [2-6] and highly related to feed inherent structure on cellular and molecular levels [7-9]. Different feed structure layers have different biomolecular and biopolymer profiles, which affect the attachment of microorganism in rumen and attachment of enzymes in small intestine.

1.2 Conventional methods for feed structure, feed mineral and feed chemistry

Conventional methods for feeds, feed structure, feed mineral and feed chemistry studies include traditional wet chemical analysis [10], NIR analysis [11], MIR analysis [12,13], FTIR microspectroscopy, in vitro [14], in situ [15] and in vivo techniques [16] etc. However, these methods have limitation in revealing feed intrinsic structure, feed minerals and feed chemistry.

1.3 Limitation of conventional methods for feeds research

Traditional wet chemical analysis is unable to reveal feed intrinsic structures in their natural states because it destroys the feed inherent structure during the processing and lab analysis, and this technique heavily uses various chemicals which may alter original feed inherent structure [10]. Wet chemical analysis can only provide total chemical composition. No structural and spatial information can be obtained by this method.

The NIR [11] and MIR spectroscopy methods [12, 13] have been used for feed composition evaluation. However, these two methods are unable to analyze feeds on its origin and cannot reveal feed chemistry within intact tissue. Because before apply for NIR and MIR, feeds need to be ground to fine particle size or powder which destroys feed intrinsic structures.

The FTIR microspectroscopy can be used to analyze the feed chemical and structural information, but not to the cellular dimension [17,18]. It produces noised spectra when an aperture size reduces to cellular dimensions.

In vitro and in situ methods for feed evaluation combine model animal and lab technique together. Again, these methods cannot directly measure feed intrinsic structure, mineral oxidation status and chemical speciation. These two methods usually measure feed digestibility and degradation and it is also time consuming by in situ method [15,19].

In vivo method [16] is the method that animals are used for performance study after feeding certain feeds. This method evaluates feeds and mineral based on animal performance such as average daily gain, feed to gain ratio and carcass quality. It is time consuming and in practice it is also not suitable for lots of treatment evaluation.

1.4 Objective of this review article

The objective of this review article was to focus on introduction of two advanced synchrotron based bioanalytical techniques (a) synchrotron radiation-based soft x-ray (SR-XMS) and (b) synchrotron-based Fourier transform IR microspectroscopy (SR-IMS) as research tools for feed and animal studies: feed inherent structure, feeds chemical speciation, feed chemistry, and feed mineral oxidation status studies. The information described in this article may provide an insight in how feed structure and feed chemistry revealed by the SR-XMS and SR-IMS techniques on a molecular basis are related to nutrient utilization and availability and how these two techniques are contributing to advances in feed chemistry, feed molecular structure, and feed science technology.

2. Advanced synchrotron radiation based soft x-ray microspectroscopy (SR-XMS)

2.1 Bright light - electromagnetic radiation

A simple definition for synchrotron is that "a synchrotron light source is a source of electromagnetic radiation produced by a synchrotron facility, which is artificially produced for scientific and technical purposes by specialized particle accelerators, typically accelerating electrons." [20]. This particle accelerator is usually called a linear accelerator. The synchrotron light has the properties of high brightness, high intensity and high level of polarization. The synchrotron light contains IR and X-ray. The synchrotron-based IR source is thousands time brighter than conventional thermal IR sources. The synchrotron-based X-ray has many orders of magnitude more than with X-rays produced in conventional X-ray tubes [20]. The advantages of synchrotron based IR and X-ray make us enable to reveal various materials on a molecular basis with a ultra-spatial resolution.

2.2 Brief theory of synchrotron based soft x-ray microspectroscopy

As we know, electromagnetic radiation contains not only hard X-ray but also soft X-ray and IR bands. The function of an X-ray microscope is to make contrast images of very small objects by using soft X-ray electromagnetic radiation band to expose film or use a charge-coupled device detector to detect X-rays that pass through the sample objects [21]. Synchrotron radiation based soft X-ray microspectroscopy is a microspectroscopy that use synchrotron radiation as a light source and focus on soft X-ray band region in synchrotron electromagnetic radiation. Scanning transmission soft X-ray microscopy is an alternative approach to format X-ray image, because synchrotron radiation soft X-ray source has relatively low brightness of the required wavelengths [21, 22].

2.3 Strong point of synchrotron radiation based soft x-ray microspectroscopy

Synchrotron radiation based soft X-ray microspectrsocpy can be used for various applications not only in nanoscience, biological and biomedical research, nanomagnetic materials, environmental and materials sciences and biology but also in feed science and feed chemistry with highly spatial resolution (could reach to ~15 nm). Compared with conventional electron microscopy (EM), the SR-XMS has advantage to view biological sample in their natural state [21,22]. In other word, it is able to reveal chemistry within intact tissues.

Soft X-rays have shorter wavelengths in the nanometre range (100 eV to 2500 eV, or 12 nm to 0.5 nm) and therefore have a potential to provide much higher spatial resolution [22] for chemical speciation, fine structure identification and chemical mapping of heterogeneous materials. Karunakaran et al. [22] indicated that "when a monochromatic X-ray beam is incident on a sample, it is absorbed and excites a core electron localized at a specific atom in a molecule to unoccupied molecular orbitals giving rise to near edge X-ray absorption fine structure (NEXAFS) around the elemental absorption edges [23]. The NEXAFS structures are closely related to chemical bonding and can be used to determine and quantify the presence of functional groups" [24].

3. Advanced synchrotron based infrared microspectroscopy (SR-IMS)

3.1 Theory of synchrotron radiation based infrared microspectroscopy

Synchrotron radiation based IR microspectroscopy is a microspectroscopy that use synchrotron radiation as a light source and focus on IR band region in synchrotron electromagnetic radiation. So it uses synchrotron IR source which is different from thermal IR sources. The basic theory of synchrotron radiation based IR microspectroscopy to measure

plant-based feeds has been reported before [17,25-29]. The stretching vibration includes symmetrical stretching vibration (ν_s) and asymmetrical stretching vibration (ν_{as}). The bending vibration includes in-plane bending vibration (δ) [scissoring vibration (δ) and rocking vibration (ρ)] and out-of-plane bending vibration (γ) [wagging vibration (ϖ) and twisting vibration (τ)] [3,9,30-32]. Different atoms vibrate at different frequencies with different modes, which results in complicated matrix structural information. Different compounds exhibit their own characteristic IR absorption pattern [30-33]. Therefore an IR absorption profile is unique to a specific molecular vibration frequency. Resulting spectrum creates a molecular fingerprint of the sample. Identification of molecular functional groups is the major application of IR spectrometry [17,25-28].

3.2 Features of synchrotron radiation based infrared microspectroscopy

Bright synchrotron radiation based infrared microspectroscopy can reach diffraction limited [2,34,35]. The technique can link structural information to chemical information within cellular and subcellular levels. This technique can also provide feed composition, feed chemistry, and feed environment information at the same time [10]. It is capable of reveal the molecular chemistry of biological samples with high signal to noise ratio at ultraspatial resolutions as fine as 3~10 μm [17, 34-37].

4. Current applications of synchrotron soft x-ray and infrared microspectroscopy

4.1 Recent applications of synchrotron infrared microspectroscopy

The review of applications of synchrotron radiation infrared microspectroscopy were reported by Yu [17,38]. With this technique, we can find the pure protein body in plant-based feeds and then analyze its protein molecular structure to avoid other functional groups effect [2,39,40]. For example, in the studies by Yu et al. [41] and Jonker et al [13], both studied the effect of gene-transformation on protein structure conformation of transgenic alfalfa feeds. The comparison of two spectra in amide I and II region in these two studies shows differences in the spectra of transgenic Lc-alfalfa from normal FT/IR-ATR spectroscopy and from synchrotron radiation infrared microspectroscopy. The published figures show the difference between these two methods. The SR-IMS spectra clear shows amide I and II band which make protein conformation study much easier, but the FT/IR-ATR spectra from normal IR source does not show clear amide I and II bands, which make it hard to study protein structure on a molecular basis. With synchrotron radiation IR microspectroscopy, we could detect processing or treatment induced-structural changes [38] such as effect of gene-transformation [41], bioethanol processing [4], heat processing [7,8], dry heating and wet heating [6,42]. The structural changes could be determined by measuring functional group band intensity changes and molecular spectral pattern changes using univariate molecular spectral analysis. To detect the treatment effect, we can also use multivariate molecular spectral analysis to classify the differences by molecular clutering and analysis of intermolecular relationship [1,33]. We also can detect the interrelationship between different feed or seed varieties using cluster analysis and principal component analysis. Using synchrotron radiation IR microspectroscopy, we can also relate the inherent feed structure to nutrient availability and utilizations of feeds in animals and we can also image molecular chemistry of feeds [43.44] and do structural biology study and plant physiology study [45].

4.2 Recent applications of synchrotron soft x-ray microspectroscopy

Recent application 1: Synchrotron soft x-ray microspectroscopy as an advanced technique for plant polysaccharides research

Karunakaran et al. [22] studied plant polysaccharides with different parts of the cell using synchrotron based soft X-ray spectromicroscopy in comparison with IR microspectroscopy. The authors found that soft X-ray NEXAFS has similar chemical speciation capabilities to IR but have much higher spatial resolution. The technique is better than IR technique to be able to differentiate different polysaccharides and cell components and to be able to clearly show linkage of different polysaccharides to different parts of the cell in plant-based seed tissue. In order words, chemical information and visible information and structural information can be linked together. The authors also found that it is easier to interpretate the spectra obtained from synchrotron based soft X-ray spectromicroscopy because it produces the fewer peaks than IR technique. The authors also found that the carbon 1s spectra are better suited to differentiate plant polysaccharides than the oxygen 1s spectra. The authors concluded that the soft X-ray technique is able to provide real space quantitative maps of plant polysaccharides and other biochemical components at a sub-cellular level, with ~30 nm spatial resolution for non-destructive characterization of polysaccharides for bio-products research. Synchrotron based soft X-ray spectromicroscopy is a powerful technique for characterizing bio-products with higher spatial resolution and similar chemical sensitivity compared to IR technique [22]. From this study, we can image that all different types of

biopolymers and polysaccharides in various grains and plants can be tested using this methods in combination with synchrotron based IR microspectroscopy.

Recent application 2: Synchrotron based x-ray spectromicroscopy to study the effect of chemical treatment on flax fibres

In this study, Oraji et al. [24] reported the effect of chemical treatment on the structure and composition of flax fibres using Scanning Electron Microscopy (SEM) and Scanning Transmission X-ray Microscopy techniques (STXM). In this study, Oraji et al. (2008) treated flax fibres with 5% NaOH for 1, 2, 3, and 4 h and then analyzed differences in morphological changes and composition (focused on lignin and cellulose) between the untreated and treated flax fibres using SEM and STXM technique. The STXM carbon (C) 1s NEXAFS data were collected. For STXM, untreated or treated single flax fibres were embedded in an amine epoxy resin. 90 nm thick longitudinal sections were cut using an ultra-microtome. These authors found that the chemical treatments affected flax fibres structure and composition and caused dramacal changes and thus affected the quality of bio-composites. Authors concluded that the SEM and STXM techniques are a powerful combination for studying the composition and structural interfacial properties of agricultural fibres and biocomposites that will help optimize and refine the preparation of improved bio-composites. These two techniques are helping to systematically optimize the chemical treatment of agricultural fibres [24].

In the previous study [7,8] used synchrotron based IR microspectroscopy to study protein molecular structure of flaxseeds in relation to protein digestion and degradation in dairy cattle. The results clear showed that synchrotron IR can be used to do protein conformation study and synchrotron X-ray can be used to detect treatment effect. Combination of the two techniques will enable use to do a more powerful study for plant-based feeds

Recent application 3: Synchrotron soft x-ray microspectroscopy as a tool to characterize wheat grain tissues

In this study, Karunakaran et al. [45, 47] used STXM at the C 1s edge to measure the distribution of biopolymers in different parts of the outer layers of wheat grain from pericarp, aleurone to starchy endosperm. The detailed molecular chemistry of wheat tissue imaged by synchrotron based IR microspectroscopy in wheat tissue was also reported by Yu et al. [18]. In this study, Yu et al. [18] prepared the thin tissue and mounted onto BaF_2 windows. The authors showed various functional group maps from outside to inside of wheat from pericarp, seed coat, aleurone layer and endosperm. The intensity and distribution of functional groups are clearly to be seen in the chemical maps related to spatial structure. In the study [47], the author prepared the wheat tissue specimens for STXM according to two different methods. The authors freezed small pieces of specimen zones corresponding to the aleurone and most outer layers at high pressure and embedded them in LR-white resin by freeze-substitution. Then authors cut ultrathin slices from the resin-embedded specimens and placed on Si3Ni4 windows. In order to check an alternative sample preparation route avoiding the use of resin, the authors used ultramicrotomy and cut ultrathin sections from native wheat grains without using any fixation and resin-embedding steps and prepared 70 nm thick sections of the wheat starchy endosperm zone for analysis. The slices were placed on holey carbon coated copper grids. The authors concluded that the STXM enable us to study the fine assemblies of biopolymers in grain tissues. Combination of synchrotron based x-ray and infrared techniques to study feed structures could be a new tool for feed and nutrition scientists.

5. Current study on feed chemistry using synchrotron soft x-ray microspectroscopy and synchrotron x-ray absorption near edge structure (XANES) spectroscopy

5.1 Study on feed chemistry using synchrotron soft x-ray microspectroscopy in our team

Currently, we are using synchrotron soft x-ray spectromicoscopy to study grain seeds and trying to characterize the difference between seeds in nutrient fractions and nutrient availability in animals [48]. The more detailed information as follow:

Project: Feed Inherent Polysaccharides and Proteins Research Program through Newly Advanced Technology (Synchrotron x-ray and IR spectroscopy). The objectives of this study are: To study feed inherent structure in relation to nutrient availability through SR-IMS and Soft x-ray techniques. A series of experiments will be conducted:. PART 1: chemistry Analyses to Determine Chemical Characterization and Nutrient Profiles of feeds and processing feeds Using standard lab and animla work. PART 2: Modeling Nutrient Supply: Structural effect and Processing effect. PART 3: Study processing-induced structural changes in relation to nutrient availability using synchrotron-based soft x-ray Microspectroscopy. PART 4: Study processing-induced structural changes in relation to nutrient availability using synchrotron-based IMS Microspectroscopy.

5.2 Study on feed chemistry using synchrotron x-ray absorption near edge structure (XANES) Spectroscopy

Another project is to study metal element oxidation changes in ruminant animal. We try to detect whether the oxidation status in metals from mineral feeds can be changed by ruminant digestive track and trying to understand oxidation status changes in relations to bioavailability in ruminants. The more detailed information as follow:

Project: Use of Synchrotron X-ray to Study Toxicity, Bioavailability, Chemical Forms and Oxidation State of Metal Elements in Plant, Feeds, Food and Mineral Supplements" Background and Motivation: Synchrotron X-ray absorption near edge structure (XANES) spectroscopy is a cutting-edge synchrotron-based technique which is able to provide chemical information on spectral characteristics of mineral elements such as Cu, Fe, P and Se. The information includes oxidation state and chemical forms of mineral elements in biological samples in term of spatially resolved x-ray absorption spectra. This information is invaluable in unraveling the element chemistry, bioavailability and toxicity of biological samples. The objective of this study is to use synchrotron-based x-ray technique (XANES) to study oxidation states and chemical forms in feeds and mineral supplements (such as organic vs. inorganic trace element supplements) and relate this information (such as oxidation state) to bioavailability, toxicity and nutrient utilization of plants, feeds, food and commercial minerals fed to animals. The effect of technological treatment on changes of oxidation state and chemical form and the changes of oxidation states of elements in animal digestive tract (such as rumen) will be reported. It is expected that the result from this spectroscopic analysis with synchrotron source will provide us information for understanding oxidation state in relation to bioavailability and toxicity of mineral elements to animals.

6. Conclusions and future research

Advanced synchrotron radiation-based soft x-ray and IR microspectroscopy are able to chemically and structurally characterize feeds at the cellular and subcellular level and can be used as advanced powerful tools for feed chemistry, elemental oxidation status and feed fine structure studies. After comparison between synchrotron radiation-based soft x-ray and synchrotron based IR microspectroscopy, it was found that combination of these two techniques may be the best way to systematically characterize feeds in chemical and structural senses, that affected by various treatments.

Future study is needed to further quantify the relationship between feed chemistry and structure and nutrient availability of feeds in animals and further study the relationship between elemental oxidation status and bioavailability and toxicity of mineral feeds in animals using synchrotron IR and X-ray technique simultaneously. Advanced synchrotron based X-ray and IR techniques have a high potential to make contributions to advances on feed science technology and feed chemistry:

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