Spectral Fluorescence Method for Protein Extraction Process Control

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Abstract: In this work we propose novel technology for the on-line monitoring of animal by-product (ABP) rendering using a spectral fluorescence signature (SFS) analyzer integrated into the recirculation line of the extracted liquid. First, we investigated various methods that could be used for on-line monitoring and control of protein extraction processes and determined that measuring the tryptophan fluorescence intensity is an effective measure of the protein content in the liquid phase. We measured the change in fluorescence intensity while performing liquid extraction on various raw materials. A series of samples was taken during each extraction and the dry matter content was determined using a standard technique. We found a good correlation between the tryptophan fluorescence intensity and the dry matter content (R^2>0.90). This spectral monitoring method could also be used to carry out express-analyses to determine the chemical composition of extracts from other processes, such as the heat-treatment of organic materials, and moreover can be used to control these treatment processes.

Keywords: Animal By-Products, rendering process, on-line control, Spectral Fluorescence Signature, fluorescence spectroscopy.

1. INTRODUCTION

One of the shortcomings of traditional ABP rendering technologies is an inability to control the chemical composition and properties of the materials that recirculate within the rendering system. This complicates the process of adjusting and adapting the process in real time. An on-line measurement and control system could be used to provide reliable information about the semi-processed materials and other processing parameters, yet not all measuring technologies are applicable for food process monitoring. However, it turns out that the control of ABP processing could be based on measurement techniques that have already been approved for meat processing, such as low and high frequency impedance measurement, microwaves, NMR, IR and UV light, and X-ray interactions. These techniques can describe a wide range of physical and chemical properties during processing [1]. One widely used technique based on impedance measurements is being used to characterize the properties of the solid materials being processed for the purpose of optimizing various unit operations during processing [2, 3], however, this non-destructive method is not suitable for liquid phase measurements.

Biological tissues consist of components that contain relatively strong fluorophores, including tryptophan residues in proteins, vitamin A, riboflavin, NADH, pyridinoline in collagen, protoporphyrin IX, and lipid oxidation products. Compared with a number of other methods, fluorescence spectroscopy offers several inherent advantages for the characterization of molecular interactions and reactions, such as the ability to use this method on-line and in real time. Using a front-face fluorescence spectrometer, one is able to determine both the chemical and rheological properties of the meat, including dry matter, fat, collagen, protein, peak load, the energy required to rupture, and the losses during cooking [4, 5]. Moreover, tryptophan fluorescence has been used to correlate the texture of meat emulsions and sausages and meat tenderness [6]. Combining fluorescence spectroscopy with chemo-metric methods improves the selectivity and accuracy of meat property assessment in complex intact samples [7].

Fluorescence spectrometry may be employed to characterize either solid or liquid samples. For opaque samples, such as meat and meat products, front-face fluorescence spectroscopy has proven to be particularly successful. Because these products contain the natural fluorophore tryptophan, which possessing high fluorescence, this technique has been used in meat science to investigate sample structure without resorting to the use of an extrinsic fluorophore probe

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[1]. To analyze liquid samples, one can cross-flow the sample through the detecting device. In this way, express optical analysis can be performed, which combines fluorescence and absorption measurements of the liquid fraction that circulates in a high-temperature rendering processes [8].

The fluorescence of folded proteins can be approximated by a mixture of fluorescence signals from individual aromatic substituents. For tryptophan measurements, the fluorescence excitation wavelength is often 280 nm or longer and commonly 295 nm. Most of the emissions from this excitation band originate from tryptophan residues, however, lower intensity emissions also originate from tyrosine and phenylalanine. These three amino acid residues display distinct absorption and emission wavelengths that differ greatly in their quantum yields and lifetimes. Because of these differences and also because of the resonance energy transfer from proximal phenylalanine to tyrosine and from tyrosine to tryptophan, the fluorescence spectrum of a protein containing these three residues usually resembles that of pure tryptophan [9].

The goals of this work is to develop a reliable, on-line system to monitor an ABP rendering process that makes use of an optical detector able to rapidly analyze liquid samples, such as a Skalar Fluo-Imager M53. This approach enables one to continuously measure and thereby control the chemical composition of the circulating liquid fraction in a rendering process in real time [10].

2. METHODS AND MATERIALS

An experimental setup was assembled to study the effectiveness of monitoring the protein content of the liquid stream during protein extraction and using this measurement in real-time as a process control variable. The fluorescence analyzer utilized in this study, a Fluo-Imager M53, is able to determine the tryptophan content of the protein rich liquid phase by measuring the intensity of the natural fluorescence signal (Figure **1**). This measurement system was equipped with sampling valves to extract and control the liquid level during processing.

This flexible measurement system allows one to manage the process by changing the processing parameters, when required. In contrast with many traditional laboratory analyses, this on-line method does not affect the physical and chemical properties of the material being analyzed.

An experimental version of this on-line measurement system was integrated with a laboratory vessel LR 2000.3 (IKA) that had both a stirrer (Velp) and circulator F-25-MC (Julabo). A Fluo-Imager M53, together with its respective control software, was used to carry out fluorescence analyses. To circulate the extracted liquid through the detector housing, we used a peristaltic pump manufactured by Skalar B.V. (The Netherlands), together with valves and piping.

Each test was carried out as follows: the raw materials (ground meat or meat-and-bone) were placed into the vessel for heat-treatment. The temperature

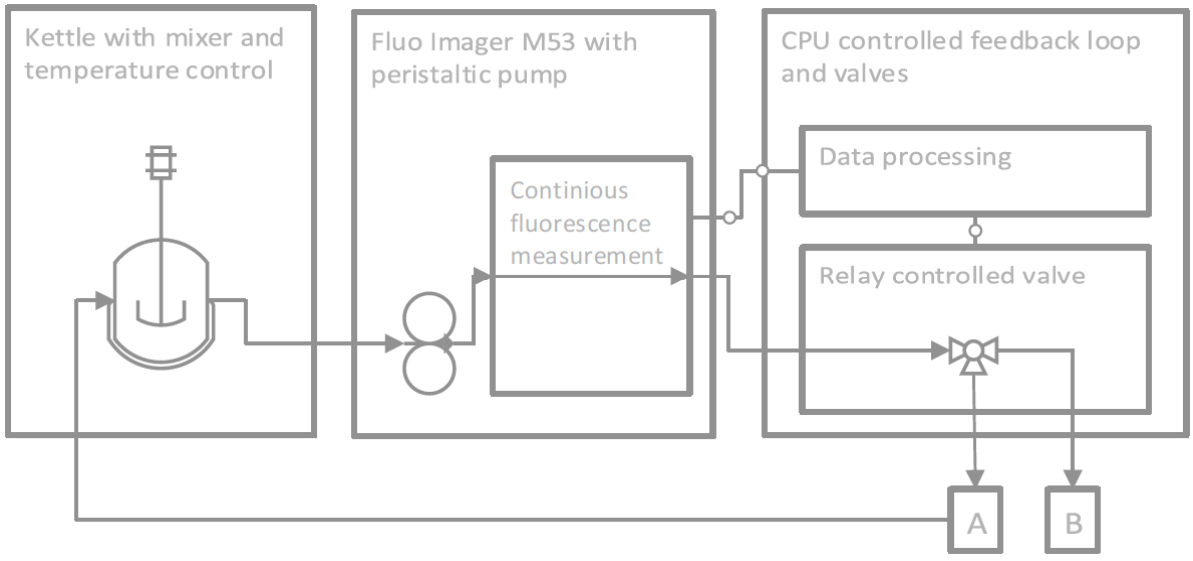

Figure 1: Test-equipment for the fluorometric determination of the tryptophan content during protein extraction. The control valve either recirculates the semiproduct (**A**) or remove the ready product (**B**) from the recirculation loop.

Table 1: Chemical Composition of the Raw Materials Under Test

Material	Water content % (MettlerToledo HR83 Moisture Halogen Analyser)	Protein content % (Kjeldhal method) Velp Scientifica UDK 142	Fat content % (Soxhlet method Velp Scientifica SER 148 Solvent Extractor)	Aches content % (lyophilized powder, +300 C - 2 hours, + 500 C - 6 hours)
Pork minced meat (Company 1)	44.3±5.4	15.5±0.3	26.7±5.8	1.84±0.02
Briquette from chicken MDM (Company 2)	53.4±0.39	19.8±3.3	11.5±0.74	5.15±0.02%
Ragout (Company 3)	31.03±3.6	25.03±0.4	22.3±4.4	22.32±0.01

was held constant in these experiments at 99+/-1°C because the main effect under investigation was the dependence of fluorescent intensity over time. A portion of the liquid fraction (water/fat/protein solution) exiting the reactor vessel was diverted through a fluorescence detector/analyzer that continuously determined the content of tryptophan. One sample was taken out of the system every minute and the dry matter for each of these was determined using an HR83 moisture analyzer (Mettler-Toledo). Based on data from the reference literature, a wavelength of 280 nm is required to excite tryptophan fluorescence (thereby characterizing 95% of the fluorescent protein compounds in the substance). The intensity of fluorescence emission was analyzed at a wavelength of 348 nm [2].

Using this experimental setup, it was possible to follow changes in the fluorescence intensity of the extract and take samples for traditional laboratory analyses without disturbing the treatment processes.

Some consumer meat products are analogous to the most common types of ABPs and thus were chosen as raw materials for these experiments. The general chemical composition of the raw materials tested is presented in Table **1**.

The chemical compositions of the raw materials used in these tests are comparable with the mean chemical properties of the main types of ABPs. This allowed us to carry out the experiments with materials that have well defined properties and thus provide good reproducibility of the results obtained.

3. RESULTS AND DISCUSSION

During thermal extraction of the three materials presented in Table **1**, we measured both the changes in the intensity of the spectral fluorescence signature (SFS) of tryptophan and the dry matter content, as described in the previous section. The reliability and precision of SFS has been demonstrated in other investigations carried out in complex food matrixes, e.g., determination of the optimal cheese-milk coagulation time [11], assessment of the freshness of meat [7, 12], and monitoring the production process during cheese maturation [13]. We found that measurements of the dry matter content correlated well with measurements of the fluorescence intensity dynamics (measured every minute) (see Figure **2**).

The results of these tests indicate that the proposed automated monitoring of SFS enables one to simultaneously follow both the tryptophan (protein)

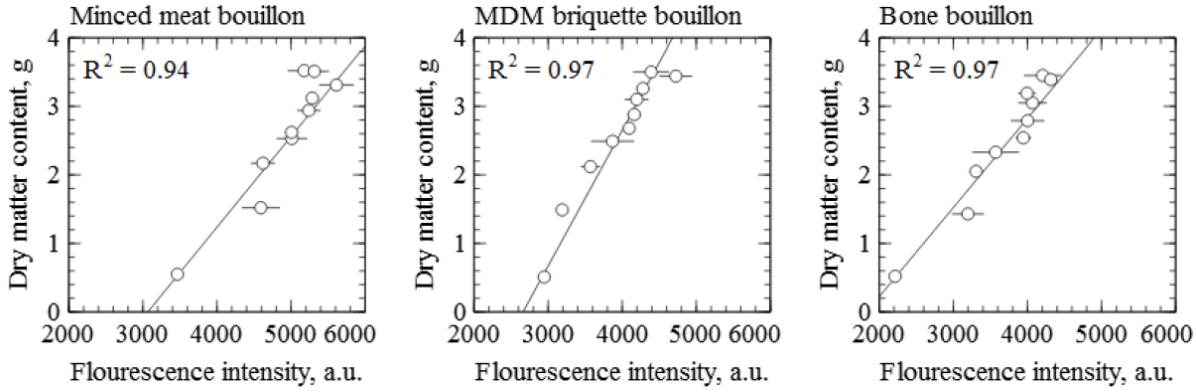

Figure 2: Changes of tryptophan fluorescence intensity and dry matter content in meat and meat-bone bouillons during continuous thermal extraction.

content and dry matter content during processing. Further elaboration of this method could also be used to detect and identify other material and chemical properties from various organic mixtures without prior processing. Furthermore, this technique can be conducting in real-time without chemical reagents and other ordinary expendable materials and labor.

A number of authors have indicated a need for efficient automation, control, and monitoring systems that can handle complex technological processes in real-time [14, 15]. The measurement system prototype we present here is suitable for monitoring various technological processes and can be used to control existing ABP thermal treatment systems.

The protein-rich stock separated from the solid fraction can be redirected from the output of the thermal treatment device back into the process until its tryptophan content reaches the desired level. This monitoring method could also be implemented to carry out express-analyses to determine the chemical composition of other extracts during the heat-treatment of organic materials and used to control these treatment processes.

The tryptophan content estimated using the measurement system described herein could also be used as a proxy measurement of the concentration of protein in the extract because the tryptophan/protein ratio in meats of different origin is quite stable.

4. CONCLUSIONS AND SUGGESTIONS

We present an on-line real-time fluorescence spectroscopy based method that can be used to measure and control the protein extracted during the thermal treatment of meat products. The technology makes use of a Fluo-Imager M53 (Skalar B.V.), which is able to track changes in the tryptophan content. This measurement is a proxy measurement of the protein content and therefore often the quality of the liquid fraction. We performed continuous thermal rendering experiments on well-defined meat products and determined that the tryptophan content determined from the tryptophan fluorescence dynamics also correlates well with the dry matter content of the extract (R^2>0.90). This laboratory scale apparatus allowed us to demonstrate that rapid measurement of the tryptophan fluorescence can be used to both control the thermal treatment process and stop the process when the tryptophan fluorescence intensity (and the corresponding increase in the dry matter content) ceases to increase. The measurement method is rapid (1-2 minutes), accurate (0.01%) and does not require any sample preparation.

Existing methods used to monitor changes within food matrixes are not suitable for the on-line control of rapid processes carried out for thermal treatment of ABP's. This study presents a novel biophysical process control method based on the SFS that provides an on-line estimation of the tryptophan content in the extract. Tryptophan estimation using this SFS method can be used as a variable to control ABP processing because it is fast, reliable, and precise.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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