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Applications of fluorescence spectroscopy in dairy processing: a review

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The present review gives an overview of recent research on the application of fluorescence spectroscopy in dairy processing. The combination of fluorescence spectroscopy and chemometric analysis has been demonstrated to have significant potential to measure physical and chemical properties of dairy products. Furthermore, studies have shown that this technology can also be applied to efficiently quantify added nutrients and adulterants. This review outlines the potential of the fluorescence spectroscopy as a rapid, non-destructive tool for assessing the quality and safety characteristics of dairy products and identifies areas where further research is required.

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Introduction

There is increasing consumer demand for higher quality food products with improved safety [1]. Traditional methods to assess these parameters are time consuming and labour intensive. There is a need for process analytical technologies to measure the quality and safety of food during manufacture. Recent advancements in spectroscopic instrumentation and chemometric methods have facilitated the adaption of a 'quality by design' concept in food manufacturing. Over the last decade, fluorescence spectroscopy has been demonstrated to be a potential process analytical technology (PAT) tool suitable for the monitoring properties of a wide range of food products [2,3]. Fluorescence spectroscopy offers numerous advantages for the characterisations of molecular interactions and chemical reactions. It is much more sensitive than other spectroscopic methods, as fluorescent compounds are highly sensitive to their environment. Thus fluorescence may be used to characterise conformational changes that occur during different manufacturing and storage conditions [3].

Dairy products contain compounds that naturally or after chemical modification emit fluorescence, such compounds include Maillard reaction products, amino acid groups, riboflavin, vitamin A, chlorophylls, porphyrins, nicotinamide adenine dinucleotide (NADH) and lipid oxidation products. Each fluorophore has a unique excitation and emission spectrum, which can be used to identify compositional or structural changes of the particular molecule. An overview of fluorescence theory, instrumentation and data analysis methods along with applications in food systems can be found elsewhere [2–4].

This review outlines recent research on the use of fluorescence spectroscopy to assess the quality and safety of dairy products. This paper also discusses both emerging and established fluorescence techniques which have been investigated to quantify physiochemical properties of dairy products. These applications are discussed under three categories (i) assessment of quality, (ii) assessment of safety and (iii) monitoring of milk coagulation and syneresis. The current state-of-the-art and future research priorities for fluorescence spectroscopy are discussed in the last section.

Current status and applications of fluorescence spectroscopy for dairy products

Assessment of quality

Effects of heat treatment

Thermal treatment is an essential step in the manufacturing of dairy products which ensures microbiological safety and increased shelf life. However, heat treatment induces undesirable changes in dairy products including impaired sensory and nutritional properties. Chemical reactions resulting from heat treatment include protein denaturation, Maillard reaction and alteration of heat-labile vitamins. Numerous fluorescent markers have been shown to provide information about the degree of heat treatment such as tryptophan, advanced Maillard reaction products and riboflavin.

Timely determination of the protein composition and degree of protein denaturation is important for quality control in dairy processing. The amino acids present in these proteins contain at least one tryptophan residue [5]. Many studies propose tryptophan fluorescence as a good

indicator of the protein concentration in milk products [6]. Some studies found a decrease in the fluorescence intensity due to heat induced protein denaturation, thus, tryptophan fluorescence is reliable only for assessing low heat treatments [7]. Curcumin is a polyphenolic compound reported to have beneficial effects on human health. It can be added to dairy products as curcumin binds to casein micelles. Tryptophan quenching and direct fluorescence spectroscopy can be used to study the molecular interactions between curcumin and milk proteins during heating [8].

Maillard reaction is a key physiochemical modification triggered by the thermal treatment of dairy products. Intermediate advanced Maillard reaction products formed during heating such as hydroxymethylfurfural (HMF), pyrrole and imidazole are fluorescent and fluorometric analysis of these products can be exploited to characterise the thermal treatments given to dairy products [9–11]. Birlouez-Aragon *et al.* proposed a new univariate fluorometric method to estimate the different heat treatments given to the liquid milk samples, which quantifies the protein denaturation by using fluorescence of advanced Maillard reaction products and soluble tryptophan (FAST) [12]. The FAST index is a ratio of fluorescence acquired from tryptophan and advanced Maillard reaction products in the milk fraction soluble at pH 4.6. Studies have shown that fluorescence of Maillard reaction products is directly proportional to the heat treatment received. Increase in heating time and temperature intensifies fluorescence of the thermally treated products [13]. The impact of thermal treatment on the chemical composition of infant formula is more complex compared to standard dairy products. The addition of different vitamins increases the rate of Maillard reactions in infant formulas. Numerous studies have shown that the FAST index is a highly sensitive indicator of the thermal impact on infant formula caused by direct or microwave heating [14,15].

Infant formulas are supplemented with macro and micro nutrients including lactose, whey proteins and vitamins to meet the infant nutritional requirements [16]. The Amadori products formed during the early stage of Maillard reaction in infant formulas initiate the glycation process. Numerous studies have found higher rates of glycation processes in infant formulas than any other dairy products [17,18]. Consumption of advanced glycation end (AGE) products are reported to cause serious health problems in adults as well as infants [19,20]. After assessment of different protein glycation markers in infant formulas, it was found that the FAST index is a rapid and sensitive method to determine AGE products in infant formulas [17]. In other studies good correlations were found between the FAST index and AGE, and also N epsilon-carboxymethyllysine and oxalic acid monoalkylamide [14].

Although the FAST method has been demonstrated as an improved and rapid method to analyse the effects of heat treatment of dairy products, further research is required before its adoption in industry. Fluorescence spectra are very sensitive to the environment of the sample, thus parameters like pH, temperature and presence of retardant or accelerating salts regulate the fluorescence yield of Maillard products [21]. Feinberg *et al.* suggested that no fluorescent tracer can be selected to universally analyse properties of heated milk samples unless it is coupled with other chemically determined tracers [22].

Lactulose and furosine can be used as markers of the degree of heat treatment in dairy products. Kulmyrzaev and Dufour applied front face fluorescence spectroscopy to determine lactulose and furosine in milk treated with different heat treatments. Lactulose was found only in ultra heat treated (UHT) milk but no traces were detected in milk samples treated below 110 °C [23]. Tryptophan fluorescence intensity was associated with lactulose and furosine content when measured at a wavelength range of 305–450 nm with an excitation at 290 nm. However, some studies suggest that lactulose and furosine are not universal indicators of milk heat treatments [24]. A recent study predicted lactulose concentration in heat treated reconstituted skim milk powder using fluorescence spectroscopy [25]. This study was conducted using samples with different heat treatments and a large number of fluorescent markers were analysed to obtain correlation to the lactulose content. The best model to predict lactulose content used tryptophan fluorescence emitted at 307 nm and a combination of two markers, that is, ratio of fluorescence of tryptophan and dityrosine emitted at 327 nm and 377 nm respectively. This study demonstrated that combinations of different fluorescent indicators are an effective method to determine the lactulose content in thermally treated dairy products.

Kulmyrzaev *et al.* proposed that intrinsic fluorophores of alkaline phosphatase, that is, coenzymes NADH and flavin adenine dinucleotide (FADH), could be used to determine β-lactoglobulin in milk samples which had been heat treated [26]. However, this method can only be applied for mild heat treatments. Development of Maillard reaction cannot be monitored using fluorescence of the alkaline phosphatase [27]. The β-lactoglobulin and alkaline phosphatase are protein derivatives with good correlation with tryptophan fluorescence at different emission wavelengths [28]. In a review of fluorescence spectroscopy it was suggested that the deviation in the emitted wavelength of tryptophan fluorescence may be due to an indirect relationship between fluorophores, phosphatase activity, and β-lactoglobulin content induced by heat [2].

Fat is another vital constituent in the dairy products, different studies have successfully detected change in

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