

**RESEARCH ARTICLE**

# Accelerated light protection performance measurement technology validated for dairy milk packaging design

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We report an accelerated measurement technology for prediction of light protection performance of milk packaging by quantifying the ability of a package to preserve a light-sensitive nutrient present in milk. This measurement technology consists of a light exposure instrument and a detection approach to track changes to the light-sensitive nutrient, riboflavin (RF), in a light-exposed solution sample. The light exposure instrument provided an intense, controlled light exposure and a consistent sample environment for an aqueous RF marker solution. Coupled with the light exposure instrument, two alternative detection approaches were used to determine the RF concentration of light-exposed marker solutions: an accelerated ex situ (AES) approach by high-performance liquid chromatography or an accelerated in situ (AIS) approach by ultraviolet-visible spectrometry. The capability of each RF determination method was confirmed using measurement systems analysis, and their statistical equivalency was demonstrated. To explore application of the measurement technology for use in package design, a set of high-density polyethylene packages incorporating surface-treated TiO<sub>2</sub> pigments was evaluated for light protection performance by AES and AIS. For the same package set, experiments monitoring RF degradation in extended-shelf-life milk products under retail dairy storage conditions showed strong correlation with the AIS method ( $R^2 > 0.97$ ). Riboflavin retention increased under both retail and accelerated light exposure conditions for package designs containing greater loadings of surface-treated TiO<sub>2</sub> confirming its light protection efficacy. This research illustrates the utility of the accelerated methods to quantitatively evaluate package designs for light protection performance for nutrient preservation.

**KEYWORDS**

accelerated light exposure, light protection, measurement systems analysis, measurement technology, package design, photo-oxidation

## 1 | INTRODUCTION

Food packaging protects food contents from potentially adverse conditions during distribution, retail display, and consumer use.<sup>1</sup> One protective function a food package can provide is light protection to minimize light-induced changes to food. The impact of food packaging

on protection of nutritional and sensory quality of packaged food is an ongoing topic of investigation.<sup>1-8</sup>

### 1.1 | Light sensitivity of food and riboflavin

Light induces various photochemical processes in foods by activating photosensitizers that can initiate chemical reactions, resulting in changes to the food components. For example, riboflavin (RF) is a micronutrient (vitamin B<sub>2</sub>) required to maintain human and animal cellular metabolic processes<sup>9</sup>; in foods, RF acts as a photosensitizer, initiating photochemical reactions.<sup>10</sup> In such processes, light energy at certain wavelengths leads to the photochemical excitation of RF. The resulting excited species can further react with other molecular

**Abbreviations used:** AES, accelerated ex situ; AIS, accelerated in situ; ESL, extended shelf life; GRR, gauge reproducibility and repeatability; HDPE, high-density polyethylene; HPLC, high-performance liquid chromatography; LDPE, low-density polyethylene; LPD, light power density; MSA, measurement system analysis; RF, riboflavin; TiO<sub>2</sub>, titanium dioxide; TGRR, total gauge repeatability and reproducibility; UV-vis, UV-visible

species present (eg, oxygen, proteins, and lipids) to induce chemical changes in the food.<sup>10,11</sup> These changes may include the degradation of nutritive food components, such as vitamins, lipids, and proteins, as well as the evolution of degradation products that negatively impact the sensory quality of the food.<sup>12,13</sup>

Milk contains RF and has undergone extensive study as a prototypical photosensitive food.<sup>14-16</sup> Aurand et al reported that raw milk in a clear glass flask lost 38% of its RF content in 1 hour of direct sunlight exposure,<sup>17</sup> and Allen and Parks reported similar loss of RF content after milk was exposed to fluorescent light for 8 hours.<sup>18</sup> Other components of milk are also impacted by light exposure, such as vitamins, amino acids, proteins, and lipids.<sup>10,14,19</sup> After 6 days of storage under typical fluorescent retail lighting, Cladman et al found a 58% reduction in vitamin A content for milk in unpigmented high-density polyethylene (HDPE) packaging.<sup>20</sup> Light exposure also changes the sensory character of milk.<sup>21-23</sup> The degradation of RF has been linked to a sensory quality decline in light-exposed milk products.<sup>12-14,24</sup> Riboflavin serves as the photoinitiator for chemical changes to milk components that have been identified as the source of sunlight flavour.<sup>14</sup> Specifically, photoactivated RF initiates the cascade of chemical reactions involving oxygen, lipids, and sulfur-containing amino acids that are attributed to sunlight flavour in milk.<sup>14,21</sup> Thus, monitoring the degradation of RF upon light exposure may serve as an indirect but leading indicator of sensory quality decline of light-exposed milk products.

## 1.2 | Performance evaluation of light protection in food packaging

Food packages can be designed to provide light protection to food contents, and titanium dioxide (TiO<sub>2</sub>) is one material that has been explored as a light protection treatment in plastic food packaging.<sup>6-8,20,24-27</sup> Titanium dioxide is already used broadly in plastic packaging applications to impart colour and opacity. In order to maximize the effectiveness of its light protection qualities, TiO<sub>2</sub> must be well dispersed in the plastic matrix. Surface treatments such as inorganic oxides and organic substituents can be applied to TiO<sub>2</sub> pigments to improve incorporation and processing.<sup>28</sup> Such surface-treated TiO<sub>2</sub> pigments allow for good dispersion in plastic matrices and permit processing using standard methods for plastic packaging. Here we demonstrate the utility of surface-treated TiO<sub>2</sub> pigments for use as light protection treatments in plastic packaging using a novel light exposure instrument and determination methods for light protection performance assessment.

Several methods to evaluate the light protective performance of food packages and packaging materials are described in the literature. Most of these consist of exposure of a packaged product to a light source and analysis of the changes to the food or beverage.<sup>5,6,26,27,29</sup> Two examples used a model mixture in a photoreactor to predict package performance with the real food product.<sup>30,31</sup> While these methods allow for the evaluation of a single light-exposed package and validate the photochemistry occurring within the system, they suffer from long experiment times and limited reproducibility. Variation in the intensity and spectral distribution of the light sources over time and batch variability in the food products tested have a large impact on these

methods, such that packages may only be accurately compared with one another if they were tested concurrently.<sup>31,32</sup> Further, the few examples of accelerated light exposure methods demonstrate only modest reductions in exposure time, and some experiments still require weeks to complete.<sup>7,32</sup> Robust light protection measurements are required to enable performance-based light protection food packaging design on a rapid time scale.

Accelerated methodologies can save a tremendous amount of time, product, and money and have been applied in food packaging design applications to estimate performance of key design parameters, such as gas barrier properties. Gas and small molecule permeation through packaging materials is generally a rapid laboratory evaluation method used to screen food packaging candidate materials for barrier performance. This approach has been useful in the design of packaging materials, such as multilayer films, to predict when the appropriate barrier properties are achieved that will allow for the protection of the food quality.<sup>33</sup> Similarly, development of laboratory assessments to quickly evaluate packaging material performance would improve speed and success of materials design for light protection packaging.

One strategy to accelerate assessment of package light protection performance is the use of model systems and markers to simplify analysis. Closely simulating the environment of a photosensitive compound of interest permits useful comparisons between the real food or beverage product and a more tractable model system. For example, Kline et al have previously explored the light sensitivity of lutein using a simplified model colloidal beverage,<sup>30</sup> and Manzocco et al used a standardized saffron extract to model the photobleaching of real beverages coloured with saffron.<sup>34</sup> King and Min used a model system in organic solvent to study the interaction between RF and vitamin D<sub>2</sub> when exposed to light.<sup>12</sup> Cardoso et al have also used model systems to examine light-induced reactions between RF and other milk components.<sup>35,36</sup> As RF is the key light-sensitive component of milk, we identified a buffer solution of RF as a model system to simulate the light sensitivity of the aqueous phase of milk. Riboflavin is a preferred marker as it is directly degraded by light exposure and its degradation is linked to sensory quality decline in milk products.<sup>12</sup> Thus, RF has the potential to serve as an indicator of both nutrient and sensory quality for light-exposed samples.

## 1.3 | Research goals

We propose that by monitoring changes to the concentration of RF in a model solution under accelerated light exposure, we can quantify the light protection performance of packaging materials rapidly and reproducibly. We hypothesize that once a correlation is established between the behaviours of the marker and the complete food product matrix, additional packaging materials can be evaluated with the marker alone to obtain indication of the light protection performance for the packaged food.

To assess the light protection performance of packaging materials, an accelerated light exposure instrument and two methods were designed for quantitative determination of RF in a marker solution as a function of the package light protection and exposure environment (eg, light intensity, pH, and temperature). This report describes the light exposure instrument, the high-performance liquid chromatography

(HPLC) and ultraviolet-visible (UV-vis) spectroscopy methods used to determine the RF concentration of light-exposed solution, and statistical evaluations of the capabilities of the accelerated methods. The two methods described are denoted accelerated ex situ (AES) and accelerated in situ (AIS) based on the method for RF concentration determination. The light protection performance of a set of packaging materials was assessed by the accelerated light exposure methods, and these data were compared with RF concentration data collected on 2 extended-shelf-life (ESL) milk products packaged in the same materials under retail storage conditions using further analysis of data reported by Johnson et al.<sup>24,37</sup> Extended-shelf-life milk products were chosen for safety for human consumption in the concurrent sensory evaluation during the month-long study. The sensory data have been published elsewhere.<sup>24,37</sup>

## 2 | MATERIAL AND METHODS

### 2.1 | Milk exposure study

A study light exposing 2% reduced fat ESL milks with several packaging conditions in a retail dairy case was conducted, and key details of the study are reproduced below as previously reported.<sup>24,37</sup>

#### 2.1.1 | Milk packages

The milk package bottles were produced for the study by extrusion blow moulding and consisted primarily of HDPE. Bottles were produced from unpigmented HDPE and from HDPE pigmented with surface-treated TiO<sub>2</sub> pigment incorporated into the polymer matrix using standard processing techniques. Three concentrations of surface-treated TiO<sub>2</sub> pigment were used as light protection treatments to yield four bottle designs that varied in TiO<sub>2</sub> content as shown in Table 1. All bottle designs were of the same shape and dimensions with a capacity of 16 fl oz (473 mL).<sup>24</sup> The unpigmented HDPE bottle design was used to generate packages for both control conditions. The light-exposed control (N) was simply the unpigmented bottle design. The light-blocked control (F) was obtained by wrapping unpigmented bottles in aluminium kitchen foil to completely block any light exposure. The surface-treated TiO<sub>2</sub> light protection treatment levels within the HDPE were confirmed by microwave ash analysis. Each package sample of known weight was combusted in two steps (2 h

**TABLE 1** Summary of HDPE bottle properties by light protection treatment

Treatment	Bottle Design	Level of TiO <sub>2</sub> , wt% <sup>a</sup>	
		Average	Standard Deviation
Unpigmented (none)	N	0.0	0.1
Low	L	0.6	0.2
Medium	M	1.3	0.3
High	H	4.3	0.4
Foil-wrapped	F	0.0	0.1

Abbreviations: HDPE, high-density polyethylene; TiO<sub>2</sub>, titanium dioxide.

<sup>a</sup>Number of samples analysed:  $n = 3$  for N;  $n = 4$  for L, M, H. Design F uses bottle N with externally applied foil; ash values from bottle N are repeated for design F.

at 300°C, 1 h at 900°C) in a laboratory microwave muffle furnace (MAS 7000, CEM Corporation, Matthews, NC). As treated TiO<sub>2</sub> was the only inorganic material present in substantial quantities and the TiO<sub>2</sub> surface treatment was of relatively negligible mass, the mass remaining after combustion was used to calculate the amount of TiO<sub>2</sub> in the original sample on a weight fraction basis. Reported values are the average weight fraction for each bottle design.

#### 2.1.2 | Milk bottle filling and storage

The RF levels of two 2% reduced fat milk products were tracked as a function of the bottle design and light exposure duration in a retail refrigerated dairy case with fluorescent lighting, as described in Johnson et al.<sup>24,37</sup> In brief, sets of sanitized bottles of each design were clean-filled under a positive laminar flow hood with ESL milk<sup>24</sup> or an ESL milk product fortified with omega-3 fatty acids, referred to hereafter as  $\omega$ -3-fortified ESL milk.<sup>37</sup> Efforts were made to avoid light exposure during processing and filling. Packages were stored at  $2.7 \pm 0.8^\circ\text{C}$  with an average light exposure of  $1122 \pm 439$  lux for a 5 week simulated retail storage exposure study. The exposure conditions and bottle sampling method are fully described in Johnson et al.<sup>24</sup>

#### 2.1.3 | Analysis of RF in milk products

At intervals of retail light exposure (day 0, 1, 3, 8, 15, 22, 29, and 36), two bottles of each design for each milk product were removed from the dairy case from different positions in the case to account for the known differences in light intensity at different positions in the case.<sup>24</sup> To look at the effect of the light exposure at a given time interval, the milk products from the set of two bottles for each package design were mixed together to form one sample and analysed for RF concentration in triplicate in a spectrofluorometer (Shimadzu Scientific Instruments, Columbia, MD).<sup>5</sup> This approach allowed for determination of the average RF concentration for each bottle design and milk product at each light exposure interval.

## 2.2 | Accelerated light exposure methodology

The sections below detail the accelerated exposure instrument, test solution conditions, production of samples to assess instrument capability. Two gauge repeatability and reproducibility (GRR) studies were conducted to determine (1) whether similar samples were differentiable and (2) the expected precision of results obtained from a wide range of potential samples.

### 2.2.1 | RF marker solutions

All reagents were obtained from Sigma (St. Louis, MO) with purity  $\geq 98\%$ . The pH 6.4 aqueous phosphate buffer was prepared by combining 4.94 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 3.81 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, and 0.09 g NaCl in a 1 L volumetric flask with water of an appropriate purity for the analytical technique. All solutions for HPLC analysis were made with water purified by a polishing system capable of producing water with resistivity  $\geq 18.2$  M $\Omega$ . Solutions for UV-vis spectral analysis were made with unpolished deionized water. This buffer solution was used to prepare aqueous solutions with initial RF concentrations ( $[\text{RF}]_0$ ) of either 15 or 30 mg/L. After preparation, buffer and RF solutions were stored in the dark at 4°C.

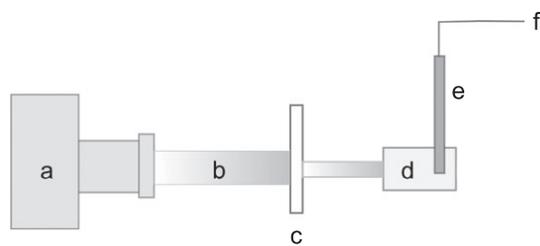
## 2.2.2 | Accelerated light exposure instrument and sample cell

The accelerated light exposure instrument provided a controlled light beam from a conditioned Xe arc lamp source (67005, 67015, and 61945; Newport, Irvine, CA) directed to the flat quartz face of the cylindrical sample cell (34-Q-50, Starna Cells, Atascadero, CA). The sample cell was filled with the aqueous RF marker solution and held under controlled temperature and agitation.<sup>38</sup> The light intensity was set using an input power controller (69911, Newport) and confirmed with a light power density (LPD) metre (1916-C, Newport) at a defined position in the light beam path close to the package sample position. The temperature of the sample cell was maintained at  $4 \pm 1^\circ\text{C}$ . The sample cell atmosphere was in exchange with the ambient atmosphere through agitation to allow for steady state concentration of oxygen in the solution. Light protection performance of a packaging material sample was evaluated by exposing a  $4\text{ cm} \times 4\text{ cm}$  area of the material secured in a sample holder placed between the light source and the sample cell normal to the arc lamp beam (Figure 1). Additional specifications of the light exposure instrument and sample cell are reported.<sup>38,39</sup>

## 2.2.3 | RF determination methods for the RF marker solutions

The RF concentration of the sample cell solution was measured over the duration of the accelerated light exposure period using one of two detection methods: ex situ HPLC or in situ UV-vis spectroscopy. The data from each detection method were used to determine the RF concentration in the sample solution as a function of light exposure time. Each of the two RF determination methods required different optimal experimental conditions for light exposure as documented in Table 2.

In the AES method, RF was monitored discretely by removing aliquots of solution from the sample cell for ex situ concentration measurement by HPLC. Samples of the RF marker solution were collected immediately before exposure and at 6 intervals during the light exposure period. High-performance liquid chromatography analysis used the following conditions: column: Kinetex C18  $100\text{ mm} \times 2.1\text{ mm}$ ; column particle size:  $2.6\text{ }\mu\text{m}$ ; column temperature:  $40^\circ\text{C}$ ; detector: Agilent 1100 DAD UV 268 nm Agilent 1100; mobile phase A: water with  $0.5\text{ mL}$  formic acid/L; mobile phase B: acetonitrile;



**FIGURE 1** Conceptual diagram of accelerated in situ instrument: (a) conditioned light source, (b) exposure light beam, (c) sample, (d) test solution, (e) ultraviolet-visible probe, and (f) spectrometry system and analysis software. For accelerated ex situ method, aliquots were removed for high-performance liquid chromatography analysis instead of using the in situ probe (e)

**TABLE 2** Accelerated light exposure method conditions

	AES	AIS
RF determination approach	ex situ	in situ
RF determination technique	HPLC	UV-vis
Light power density, $\text{mW}/\text{cm}^2$	375	400
Light exposure period, min	125	40
$[\text{RF}]_0$ , $\text{mg}/\text{L}$	30	15

Abbreviations: AES, accelerated ex situ; AIS, accelerated in situ; HPLC, high-performance liquid chromatography; RF, riboflavin; UV-vis, ultraviolet-visible.

isocratic: volume% A = 90, volume% B = 10; flow rate:  $0.5\text{ mL}/\text{min}$ ; injection volume:  $6\text{ }\mu\text{L}$ ; stop time: 15 minutes.

In the AIS method, the RF concentration was determined semicontinuously at 1 minute intervals by in situ UV-vis spectroscopy. The spectroscopic detection method consisted of a fibre-optic transreflective dip probe (Falcata 661.622-UVS, Hellma, Müllheim, Germany) placed inside the sample cell equipped with a deuterium-tungsten halogen dual light source (DT Mini 2 GS, Ocean Optics, Dunedin, FL) and spectrometer (USB 2000+, Ocean Optics). Ultraviolet-visible spectroscopic data were collected in absorbance mode with a 200 to 800 nm detection range.

## 2.2.4 | Packaging material prototype preparation

In order to rapidly assess the light protection performance of packaging materials independent of a final package size and shape, prototype parts were prepared for light protection performance evaluation. Several packaging material prototype samples containing surface-treated  $\text{TiO}_2$  were manufactured for the GRR study. As prototypes for flexible packaging applications, thin films ( $50\text{ }\mu\text{m}$ ) were manufactured by laboratory-scale cast film extrusion (Chemours Technical Service Center, Kuan Yin, Taiwan). As prototypes for rigid packaging applications, rigid plaques were manufactured by compounding low-density polyethylene (LDPE) (DuPont 20, DuPont, Wilmington, DE) and  $\text{TiO}_2$  pigment on a two-roll mill and compression moulding. Low-density polyethylene and  $\text{TiO}_2$  pigment concentrate pellets were preweighed in amounts to yield the final ratios desired in batches of 190 g. Pigment concentrate and resin mixtures were compounded to a stock on a two-roll mill (Stewart Bolling & Co., Cleveland, OH) at  $220^\circ\text{F}$  to  $240^\circ\text{F}$  with a nip gap of  $0.035\text{ in}$ . Standard techniques were used to compression mould rigid plaques from compounded stock using two hydraulic presses (Carver, Wabash, IN) in sequence, the first heated to  $350^\circ\text{F}$  to melt and mould the stock and the second water-cooled to freeze the plaque shape to yield sample plaques with average thickness of approximately 30 mil ( $0.76\text{ mm}$ ).

## 2.2.5 | Measurement systems analysis

Gauge repeatability and reproducibility (GRR) studies, a type of measurement systems analysis (MSA), were applied to assess the repeatability and reproducibility of the two accelerated methods, AES and AIS. Both studies were designed and analysed using MINITAB statistical software.<sup>40</sup>

The GRR studies were crossed designs and involved replicate evaluation of several packaging material samples, or parts, by multiple operators. Different sets of parts were used to assess the performance of the AES and AIS methods based on the goals of each study. The number of parts, operators, and replicate evaluations for each study design is shown in Table 3. The package prototype samples used for the AES GRR study consisted of TiO<sub>2</sub> pigmented LDPE cast films. Samples used for the AIS GRR study consisted of commercially available neutral density filters at specific optical densities (FS-3, Newport), TiO<sub>2</sub> pigmented LDPE cast films, and TiO<sub>2</sub> pigmented plaques.

For both GRR studies, the evaluation of parts was conducted in predetermined random order using blind codes to the extent possible to avoid bias in the resultant data. The measured response of each evaluation for the GRR analysis was the pseudo first order rate constant ( $k'$ ) for both studies. Variability in the study from sources including parts, operators, and inherent measurement variation was determined by a random effects analysis of variance (ANOVA) method with the part-operator interaction term excluded ( $\alpha = 0.05$ ).<sup>41</sup>

### 3 | RESULTS AND DISCUSSION

The utility of our methods was investigated for accelerated, quantitative light protection performance evaluation of plastic milk packaging materials. Using RF as a marker, light protection performance of dairy packaging materials was evaluated by measuring the degradation of a RF marker solution under accelerated light exposure. The changes to the concentration of RF under accelerated light exposure were further related to the retention of RF in fluid milk products with the same package designs under retail light exposure.

#### 3.1 | Rate constant determination for milk products and for accelerated exposure methods

The decomposition of RF in dilute aqueous solution under ambient atmosphere has been shown to follow pseudo first order reaction kinetics when exposed to UV or visible light.<sup>42</sup> More specifically, under conditions during which the intensity and energy distribution of the incident light is held constant, the decomposition of RF in solution can be described by the following integrated rate expression:

$$\ln[\text{RF}]_t = -k' t + \ln[\text{RF}]_0 \quad (1)$$

**TABLE 3** Summary of gauge R&R study design for AES and AIS methods

	AES	AIS
No. parts	5	8
No. operators	2	3
No. replicates	2	3
Total measurements	20	72

Abbreviations: AES, accelerated ex situ; AIS, accelerated in situ; R&R, repeatability and reproducibility.

where  $t$  is the light exposure time,  $[\text{RF}]_t$  is the RF concentration at time  $t$ ,  $[\text{RF}]_0$  is the initial RF concentration prior to light exposure, and  $k'$  is the pseudo first order rate constant.

Johnson et al reported the influence of these packaging systems on RF and sensory quality preservation for plain<sup>24</sup> and  $\omega$ -3-fortified<sup>37</sup> 2% reduced fat ESL milks under retail storage conditions. Here, further analysis of the RF concentration data for the milk products was performed to determine the rate constants of the RF degradation. These  $k'$  data were then applied to predict RF retention at a storage time of 1 month and to compare the light protective performance across the package designs and milk products.

Pseudo first order kinetics were observed for all RF degradations in the milk products under retail storage conditions and in the RF marker solutions under accelerated light exposure. The associated rate constants were determined using linear regression with Equation 1 and are shown in Table 4. The coefficients of determination ( $R^2$ ) observed for these linear correlations were above 0.95 in all analyses for the RF marker solutions. In the milk products, the coefficients of determination were above 0.8 with the exception of one milk trial. Because each study condition was performed in duplicate, the data from the trial with low  $R^2$  were excluded from further analysis.

#### 3.2 | Influence of bottle design on RF degradation in milk products in retail storage

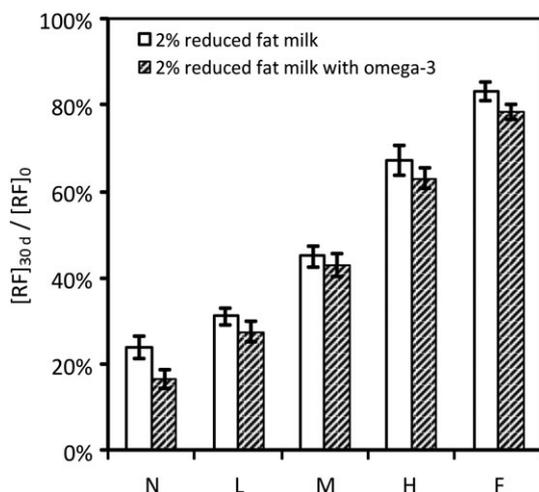
Food packagers must balance competing factors in package design, including cost, aesthetics, and performance metrics. Packagers may prioritize these factors differently or have different thresholds of acceptable performance. In this study, several light protection performance levels were evaluated to address a range of options relevant for food packagers and packaging converters.

Rate constants for RF degradation were determined for each bottle design for the two milk products under retail light exposure as well as under accelerated light exposure with both AIS and AES RF determination (Table 4). Equation 1 was algebraically transformed to determine the RF retention fraction:  $[\text{RF}]_t/[\text{RF}]_0 = \exp(-k' t)$ . For the milk studies, the RF retention fraction at 1 month ( $[\text{RF}]_{30d}/[\text{RF}]_0$ ) was calculated to determine the light protection efficacy of the packaging treatments in retail conditions (Figure 2). The time interval of 30 days was chosen as a uniform reference point for the analysis to be within but towards the end of the study interval. This analytical

**TABLE 4** Pseudo first order rate constants for RF under varied light exposure conditions

Bottle Design	RF $k'$ , day <sup>-1</sup>			
	Accelerated		Retail	
	AES	AIS	2% Milk	2% Milk with $\omega$ -3 Fatty Acids
N	14.72	16.86	0.048	0.060
L	3.19	2.74	0.039	0.043
M	1.70	0.88	0.027	0.028
H	0.60	0.34	0.013	0.015
F	0.27	0.14	0.006	0.008

Abbreviations: AES, accelerated ex situ; AIS, accelerated in situ; RF, riboflavin.



**FIGURE 2** Calculated riboflavin (RF) retention fraction in milk products for each bottle design remaining after 1 month of retail storage conditions: 2% reduced fat milk (open bars) and  $\omega$ -3-fortified 2% reduced fat milk (shaded)

approach used data from all time intervals to make the performance prediction and increases the predictive power over using the data from only one time interval.

Riboflavin degradation through the study period was measurable for both milk products (Figure 2). This analysis can be used to compare the light protection performance across the bottle designs and milk products. The content of surface-treated  $\text{TiO}_2$  in each treatment is shown in Table 1. In the case of bottle design N, which had no light protection treatment, the modelled 30-day RF retention fractions were 24% and 16% for plain and  $\omega$ -3-fortified 2% reduced fat ESL milk, respectively (Figure 2). This further supports the conclusions reported by Johnson et al<sup>24,37</sup> showing the lack of light protection provided by bottle design N for RF and milk sensory quality preservation. The surface-treated  $\text{TiO}_2$  treatments in bottle designs L, M, and H showed improved light protection performance with enhanced RF retention fraction versus bottle design N.

The improvement of RF retention trended with the level of surface-treated  $\text{TiO}_2$  in the bottle design for both milk products over the 1-month retail storage study. This confirms that the surface-treated  $\text{TiO}_2$  is an effective light protection treatment. Light protection treatment H enhanced the RF retention substantially, with over twice the RF retention as compared with N for both milk products. While the light protection treatments L, M, and H showed enhanced RF protection as compared with N, they did not provide the same degree of light protection for RF as the F control. The milk product composition appeared to have a slight influence on the light protection efficacy of the packages; the  $\omega$ -3-fortified milk was more susceptible to the impact of light exposure. While recent light exposure studies on  $\omega$ -3-fortified milk products do not necessarily show a change in RF concentration during the exposure period, they do report higher levels of lipid oxidation in samples with high  $\omega$ -3 fatty acid content.<sup>43,44</sup> This observation is consistent with the accelerated RF-mediated oxidation reactions of polyunsaturated fatty acids demonstrated in model systems.<sup>14,45</sup>

### 3.3 | Accelerated light exposure methodology

The sections below discuss the complementary utility of the HPLC and UV-vis detection methods used in the accelerated exposure studies and the results of the GRR studies using the AIS and AES methods. Further, an orthogonal regression analysis is shown which demonstrates the equivalence of the two detection methodologies.

#### 3.3.1 | Determination of RF in AES and AIS methods

In the accelerated light exposure methods, RF was determined by either ex situ HPLC or in situ UV-vis spectroscopy. For both methods, development work was performed to allow for the determination of exposure conditions (Table 2) to minimize data collection time while ensuring a robust methodology as confirmed by GRR studies. A Xe arc lamp was chosen for its intense light output, which is stable and uniform in intensity and in spectral distribution over time. High-performance liquid chromatography was chosen for the AES method because it is a well-established method for RF determination.<sup>46-48</sup> With AES using HPLC detection, the evaluation of the light protection performance is accelerated 200-fold on a light exposure intensity basis over retail light exposure methods for milk products. To provide further acceleration in light protection performance evaluation, additional detection and data collection approaches were considered. Integration of real-time in situ RF determination was explored to further accelerate measurement and data processing in development of the AIS method. The RF marker solution was well suited for concentration analysis by UV-vis spectroscopy because RF is the only spectroscopically active component in the detection region.<sup>9</sup> A distinct advantage of using a UV-vis spectroscopic approach in the AIS method is the potential for automation of real-time data acquisition and analysis using an in situ probe. With this approach, an increased number of RF concentration analyses per light exposure experiment can be collected, permitting calculation of robust  $k'$  data with a substantially shorter light exposure period.

The AIS method provides enhanced acceleration and integrates light exposure and determination of  $k'$  from RF concentration; however, the AES method remains a valuable alternative. As the light exposure technology is applied to additional test solution analytes, the properties of the specific light-sensitive species will determine the optimal detection approach for each. For example, HPLC is particularly beneficial for analysis of multicomponent samples that require separation for accurate species determination, systems with complex spectral behaviour, or species that cannot be monitored by UV-vis spectroscopy. Thus, the AIS and AES methods are complementary, each potentially providing benefits depending upon the system studied.

#### 3.3.2 | GRR studies of AES and AIS RF determination methods

Measurement systems analysis is the set of methods used to design and execute experiments to determine the impact of variance factors in measurement systems. The capabilities of the AES and AIS methods for light protection performance evaluation were assessed using GRR studies, a form of MSA used to determine the capability of a measurement system to discriminate samples.<sup>49,50</sup> Generally speaking, the precision and accuracy of a gauge (eg, AIS and AES) are defined by the

sources of error or variability because of the gauge (repeatability) and error due to the operator, or person conducting the measurement with the gauge (reproducibility). For AES and AIS, the measured response of interest,  $k'$ , was tested with defined sets of samples, or parts, in a randomized order with replicate measurements performed by at least two operators. The data were then analysed using an ANOVA random effects model to assess the amount of variability observed in the  $k'$  that originates in the measurement system as compared with the total variability observed, which also includes the impacts of the operators and parts.

One key output from the GRR analysis is the study variation for total GRR (TGRR), which includes contributions to the measured variation in  $k'$  from repeatability, reproducibility, and the potential interaction terms. In addition, an aggregate GRR standard deviation is calculated for the measurements in the sample set, which can be used to define confidence intervals for the response. The quality of a measurement system is assessed from the TGRR contribution to the study variation. Generally, when this value is 10% or less, the measurement system is highly capable of discriminating differences in the responses for unique samples.<sup>51</sup>

Two GRR studies were designed for the AES and AIS methods to confirm acceptable capability of each method in the measurement of light protection performance, represented by the  $k'$  of RF decay. The AES study evaluated whether thin film samples within a relatively narrow range of compositions resulted in differentiable  $k'$  measurements. An expanded sample set was used for the AIS study including thin films, rigid plaques, and neutral density optical filters. This sample set allowed characterization of the dynamic range for  $k'$  measurement, including performance near upper and lower detection limits. Table 3 shows key parameters of the GRR study design. This complete characterization of the precision and dynamic range of the accelerated exposure instrument with well-characterized samples permits robust analysis of packaging materials relevant to current commercial milk packaging designs and needs.

The GRR studies confirmed that both AES and AIS methods produced  $k'$  data that can be replicated with high statistical confidence. Table 5 provides the ranges of  $k'$  evaluated as well as the TGRR contributions to the standard deviation, standard error, and study variance. The TGRR study variations were both less than 10%, indicating AES and AIS demonstrate excellent capability for light protection performance assessment.

One concern in the AIS study results is that the TGRR standard deviation exceeded the smallest measured  $k'$  due to inclusion of the

**TABLE 5** Summary of gauge R&R study results for AES and AIS methods

	AES	AIS
Maximum $k'$ , day <sup>-1</sup>	28.64	24.36
Minimum $k'$ , day <sup>-1</sup>	2.28	0.17
TGRR standard deviation, day <sup>-1</sup>	0.78	0.35
TGRR standard error, day <sup>-1</sup>	0.17	0.04
TGRR study variation	7.6%	4.2%

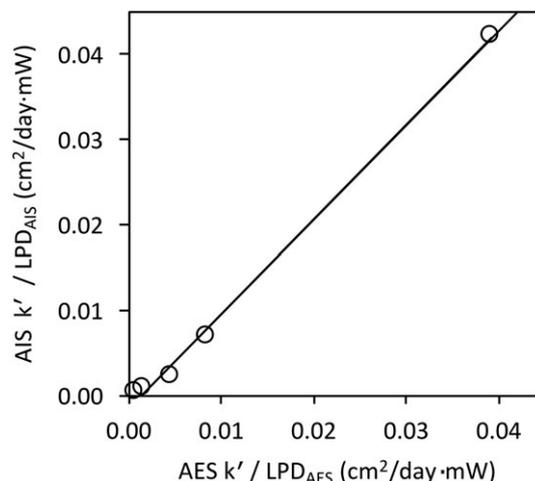
Abbreviations: AES, accelerated ex situ; AIS, accelerated in situ; R&R, repeatability and reproducibility; TGRR, total gauge repeatability and reproducibility.

larger variations of faster rate constants. However, for each part, the standard deviation of  $k'$  was at most 10% of the average value. The calculation of standard error includes the effects of sample size and replication, while the study standard deviation represents an unweighted average for samples, which span two orders of magnitude. The standard error for the AIS study is less than the minimum measured  $k'$  value and similar to the standard deviation of the lowest  $k'$  part. For this reason, the standard error is our preferred representation of the magnitude of measurement variation, particularly for samples with very low  $k'$  values.

### 3.3.3 | Demonstrating equivalency of the RF determination methods in RF marker solutions in accelerated light exposures

Both accelerated light exposure methods were used to evaluate samples of each bottle design. The effects of the different LPD of the AES and AIS methods were accounted for by normalizing the data. Specifically, the RF degradation rate constants for the two determination methods were normalized by the LPD of each method, 375 or 400 mW/cm<sup>2</sup> for AES and AIS, respectively. The resultant  $k'/\text{LPD}$  data for AES and AIS were compared for equivalency using orthogonal regression in MINITAB. Orthogonal regression is a modified least squares analysis considering two continuous variables, where both variables contain measurement error. This approach is often used in laboratories to determine if two instruments or methods are equivalent.<sup>52</sup> In this analysis, the normalized AES  $k'$  was the predictor (x) and the normalized AIS  $k'$  was the response (y). The error variance ratio (y/x) of 0.33 was applied to the analysis as determined by the variance component for repeatability from the GRR studies conducted for both methods.

The analysis revealed a strong linear correlation between the AES and AIS methods (Figure 3). The slope of the correlation was 1.1 with a 99% confidence interval of 1.0 to 1.2. The intercept was -0.002 with a 99% confidence interval of -0.003 to 0.000. For perfectly equivalent methods, the regression would show a slope of 1 and an intercept of 0. The correlation data show that the confidence interval for the slope contained 1 and the confidence interval of the intercept contained 0.



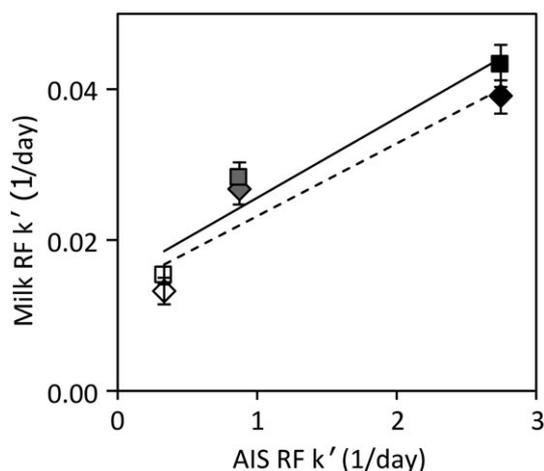
**FIGURE 3** Riboflavin degradation rate constants for bottle pieces by accelerated ex situ (AES) and accelerated in situ (AIS) normalized by light power density (LPD) plotted with regression line ( $R^2 = 0.998$ ). Errors for the data points fall within the symbols

Thus, the equivalency of the AES and AIS RF determination methods was demonstrated.

### 3.4 | Validating the accelerated light exposure of RF marker solution to predict light protection performance for milk products under retail light exposure

To confirm the validity of the accelerated light exposure approach and its relevance to a complete packaged food system, the RF  $k'$  for each milk product exposed to retail light was compared with the corresponding  $k'$  value of the RF marker solution under accelerated light exposure using the AIS determination method for bottle designs L, M, and H (Figure 4). A linear correlation was observed between the rate constants for the packages containing the light protection treatments, the slope of which was dependent upon the milk product. These linear correlations establish that evaluation of light protection performance of a package using accelerated light exposure of the simple aqueous buffered RF solution by the AIS method is sufficient to predict the effect of retail light exposure on RF in a packaged milk product. The differences in the linear regression coefficients for the relationship between the  $k'$  values in the RF marker solution and those of the milk products illustrate that RF degradation is a complex process involving other species present in the milk<sup>14</sup> and the  $\omega$ -3 fatty acids themselves have complex effects in milk products.<sup>53,54</sup> Through these studies, we confirmed that RF is an effective marker to monitor the impacts of retail lighting on a milk product.

While assessment of relative light protection performance of a packaging material is possible using only accelerated methods, the translation of those accelerated results to retail performance requires an established correlation with the behaviour of the milk product of interest under retail storage conditions. Once determined, the correlation for a given milk product could be applied to efficiently predict the light protection efficacy of a milk package design for RF protection



**FIGURE 4** Riboflavin (RF) rate constants for milk products plotted against accelerated in situ (AIS) RF rate constants for H (open), M (grey), and L (black) milk packages:  $\omega$ -3-fortified 2% reduced fat milk (squares); 2% reduced fat milk (diamonds). Least squares correlation lines are shown for  $\omega$ -3-fortified 2% reduced fat milk (solid,  $R^2 = 0.96$ ) and 2% reduced fat milk (dashed,  $R^2 = 0.93$ ). Errors for AIS RF  $k'$  values fall within the symbols

based on evaluation of the packaging material using our accelerated methods. While determination of  $k'$  for milk took approximately 30 days, the measurement of  $k'$  in the AIS method took less than 1 hour. This accelerated evaluation of light protection efficacy for milk packages could improve the efficiency of package design and allow for light protection performance optimization.

## 4 | CONCLUSIONS

We have demonstrated that accelerated light exposure of a simple aqueous RF solution under a controlled environment can be advantageously used to assess light protection performance of milk packaging in lieu of the much more involved light exposure and testing of a milk product. In as little as 40 minutes, these methods predict the light protection performance of a milk package for RF preservation. These accelerated results correlate to the light protection observed in a 1-month study of ESL milk products under retail storage conditions.

The change in concentration of RF solution under accelerated light exposure was monitored by AES and AIS determination methods, which were confirmed to be statistically equivalent. Each of these determination methods offers advantages and selection of the preferred method is based upon the experimental needs. The AES method employing HPLC allows for determination of species in more complex systems and could potentially track concentration profiles for multiple species during a single light exposure. The AIS method is advantageous for the speed of the data collection, offering light exposure, RF concentration profiles, and rate constants in only 40 minutes. The AIS method also provides ease of data collection through the integration of the exposure, detection, and data analysis operations. These accelerated methods allow optimization of the light protection performance of packaging materials using representative pieces that are independent of the size and shape of the package. Using these methods could allow for improved optimization for light protection package performance.

The AIS and AES methods were validated as accelerated and quantitative evaluation methodologies to assess for the light protection performance of milk packages. Using these methods, light protection treatments can be designed into milk packages to achieve improved protection of the milk within. With proper light protection treatment incorporation into milk package design, the nutrient content of the milk can be preserved, as demonstrated by the retail light exposure study of two ESL milk products. The light protection observed for RF in each of the two milk products was dependent upon the treatments employed. As compared with the control package (N) with no light protection treatment, the surface-treated TiO<sub>2</sub> (L, M, H) and the foil-wrapped control (F) treatments provided light protection benefits to the packages evidenced by improved retention of RF in the milk products.

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