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Optical rotation measurements in process control and engineering research in foods

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Abstract

A system to measure optical rotation (OR) and transmitted light (TL) was built and tested by the Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada (CICATA, México). A similar system was built and tested by the Food Process Engineering Group (FPEG) at Oregon State University. OR and TL values, as a function of time and sample temperature, were recorded by a data acquisition system. Sucrose (1% w/v) and distilled water were used to calibrate both instruments while sucrose solutions were used to determine sensitivity, linearity and stability. The OR of sucrose solutions was measured with less than 3% (0.03 to 20 g/100 mL) and 2% error (0.01 to 20 g/100 mL) for the FPEG and CICATA system, respectively. The instrument setup was tested at CICATA by monitoring sucrose hydrolysis and by comparing initial and final OR with published reference values. Application examples tested by FPEG were the characterization of hard candies and the fermentation monitoring of *tepache*, a prehispanic alcoholic beverage consumed in Mexico and some Latin American countries. These tests confirmed the instrument versatility and the advantages of building modular units with light sources and electronic components available today at low cost. This work showed, using specific applications, that modular units for well-established optical measurements such as OR and TL could have additional applications in food process control and engineering research.

Additional key words: in-line/real-time control, sugar inversion, *tepache* fermentation.

Resumen

Aplicaciones de las mediciones de rotación óptica en el control de procesos y la investigación en ingeniería para el caso de los alimentos

Dos centros de investigación, el Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada (CICATA, México) y el Food Process Engineering Group (FPEG) de la Oregon State University, construyeron y evaluaron unidades similares de un sistema de medición de rotación óptica (OR, por sus siglas en inglés) y luz transmitida (TL, por sus siglas en inglés). Se utilizó un sistema de adquisición de datos para almacenar valores de OR y TL en función del tiempo y la temperatura de la muestra. Para calibrar ambos instrumentos, se utilizó una solución de sacarosa (1% p/v) y agua destilada, mientras que la sensibilidad, linealidad y estabilidad se determinaron utilizando soluciones de sacarosa. Las OR de las soluciones de sacarosa se pudieron medir con menos del 3% (0,03 a 20 g/100 mL) y 2% de error (0,01 a 20 g/100 mL) en los sistemas FPEG y CICATA, respectivamente. El sistema CICATA fue evaluado monitoreando la hidrólisis de azúcar y comparando el valor inicial y final de OR con valores de referencia. Para evaluar el instrumento del FPEG, se realizó la caracterización óptica de dulces comerciales y el seguimiento de la fermentación para la producción de *tepache*, una bebida alcohólica prehispánica consumida en México y otros países de América Latina. Estos estudios confirmaron la versatilidad del instrumento y las ventajas de una construcción modular empleando fuentes de señal óptica y componentes electrónicos disponibles a bajo costo. Con aplicaciones específicas se demostró que los sistemas modulares para mediciones ópticas bien establecidas, como la OR y la TL, podrían tener aplicaciones adicionales en el control de procesos e investigación en ingeniería de alimentos

Palabras clave adicionales: inversión de sacarosa, mediciones en línea y tiempo real, procesos de fermentación de *tepache*, rotación óptica.

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Abbreviations used: ASBC (American Society of Brewing Chemists), EBC (European Brewery Convention), FPEG (Food Process Engineering Group), IPN (Instituto Politécnico Nacional, México), NIST (US National Institute of Standards and Technology), OR (optical rotation), OSU (Oregon State University), PEM (photoelastic modulator), RTD (resistance temperature detector), TL (transmitted light), USP (United States Pharmacopeia).

Introduction

High sensitivity measurements based on the optical activity of specific food and beverage components can be used as feedback information to control processes and improve product quality. In the food industry, sugar inversion and alcoholic fermentations are important commercial processes that could be monitored by following changes in optical rotation (OR). For example, sugar inversion must be monitored and controlled in hard candy manufacturing to obtain products with the desired sweetness, texture and stickiness (Hess, 1995; Woo and Symanski, 2001). Off-line measurements, even after process and product formulation standardization, are often insufficient to control sugar inversion. This leads to an undesirable variability in candy production with deleterious effects on product shelf-life and quality. An in-line/real-time system based on OR measurements could allow timely adjustments of temperature, processing time and acid addition to improve the control of sugar inversion. OR measurements are used in Greece, Italy and the UK to classify honey types. For example, average OR values reported by Dinkov (2003) for *Robinia sp.* and multifloral honeys were -17.0 ± 1.2 and -4.8 ± 4.9, respectively. However, the correct botanical classification of a honey type may require additional information such as electrical conductivity measurements and microscopic analysis of pollen (Pridal and Vorlova, 2002). In another application, the relationship between carbohydrate composition and specific OR values has been used to develop a classification procedure for gum acacia. Application of this approach to 50 gum samples allowed their identification and clustering (Biswas *et al.*, 2000; Biswas and Phillips, 2003). Finally, OR measurements can be used also to study dynamic aspects of gel formation. For example, Schafer and Stevens (1996) showed that the OR for carrageenan decreased when gels became disordered as a result of the formation of more extended chains. In previous work, the same authors developed an OR approach to reexamine the double-helix model for agarose gels. OR determinations were consistent with a double helix model of agarose gelation with wide diameter helices capable of intertwining (Schafer and Stevens, 1995).

Another potential application of OR measurements is in-line/real-time monitoring of alcoholic fermentations including regional beverages such as *tepache* (Moreno-Terrazas *et al.*, 2000, 2001a,b). Tepache is a drink known since prehispanic times in Mexico just like pulque and other alcoholic beverages. Tepache has a low alcohol content and is a drink better served cold. When consumed in Mexico, the alcohol comes mostly from the addition of a small amount of beer. Determinations of ethanol content have been standardized by industry associations but they rely mostly on off-line measurements (e.g., ASBC, 1992; EBC, 2003). These measurements are often insufficient to control fermentations by timely adjustments to process variables such as temperature and nutrient supplementation (Torija *et al.*, 2003). Alternative *in-situ* techniques based on indirect measurements have been proposed to monitor industrial fermentations including methods based on ultrasonic velocity (Resa *et al.*, 2004a, 2005). Ultrasonic velocity and density values were measured for 0-50% aqueous solutions of glucose, fructose, sucrose and water, and as a function of time (0-13 d) during the fermentation of these solutions by *Saccharomyces cerevisiae* (Resa *et al.*, 2004b). Also promising are enzyme-covered platinum probes proposed as a means to monitor wine production and prevent unwanted metabolic changes during fermentation (Esti *et al.*, 2003).

The objective of this work was to build and evaluate a modular OR measurement system to monitor changes in optically active solutions and determine their potential for food applications in process control and food process engineering research. Intended applications include the control of processes such as sugar inversion in hard candy manufacturing and fermentations for the production of alcoholic beverages. In-line/real-time monitoring facilitates the identification of process completion and the optimization of process conditions for best product quality and cost control.

Material and methods

Two systems measuring optical rotation (OR) and transmitted light (TL) were built, one by the Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada (CICATA, Campus Querétaro) from the Instituto Politécnico Nacional (IPN, México) and a similar unit by the Food Process Engineering Group (FPEG) at Oregon State University (OSU, USA). The same procedures were followed to calibrate both instruments and to determine and compare their sensitivity, linearity and stability. The CICATA instrument performance was tested by monitoring sucrose hydrolysis and comparing initial and final OR values with reference values reported in the literature. Characterization of hard candies and fermentation monitoring of *tepache* were used to demonstrate the performance of the FPEG unit.

Optical system description

The CICATA and FPEG systems shared the same optical measurement design and used lasers or lamps as light sources (Fig. 1). Two light sources were utilized for the CICATA system. One was a tungsten lamp with a SPEX-270 Monochromator to produce 350 to 850 nm monochrome light. A 643 nm semiconductor laser beam (Lasermate Group, Inc., Pomona, CA, USA) was also used. The light from both sources passed through a Glan Taylor polarizer (Model MGTYB20, Karl Lambrecht Corp., Chicago, IL, USA). The polarization state of the light beam was then modulated at 50 KHz using a photoelastic modulator (PEM) (Model PEM-90, Hinds Instruments, Inc., Hillsboro, OR, USA) (Kemp, 1969). The modulated light beam was then passed through the sample solution contained in a custom-built cell and then through a second Rochon polarizer acting as analyzer (Model RPPC-0912, Lambda Research Optics, Inc., Costa Mesa, CA, USA).

Changes in the polarization state of the light beam were monitored using a silicon photodiode (Model UDT UV-001, UDT Sensors Inc., Hawthorne, CA, USA) with a quartz window and a gain setting yielding a TL signal in the best linear response range (between 2-7 V output for a TL-071 operational amplifier powered with 9V batteries). The photodiode electrical signal was filtered by a lock-in amplifier (Model SR830, Stanford Research Systems, Inc., Sunnyvale, CA, USA). The sample solution temperature, measured using a resistance temperature detector (RTD), and the OR and TL optical signals were collected and recorded in real-time. The FPEG system used a semiconductor laser with the same wavelength as the CICATA system, a Model PEM-80 photoelastic modulator (Hinds Instruments, Inc.), a lock-in amplifier Model 5207 (Princeton Applied Research, Princeton, NJ, USA) and a silicon photodiode Model UDT UV-005 (UDT Sensors Inc.). The optical signals and sample temperature recording system was built using a data acquisition card (DAQ 6036E, National Instruments Inc., Austin, TX, USA) and operated using LabView (Version 7 Express, National Instrument Inc.).

Optical system measurements

The following equation was used to obtain the OR value α in degrees (Wang, 1999):

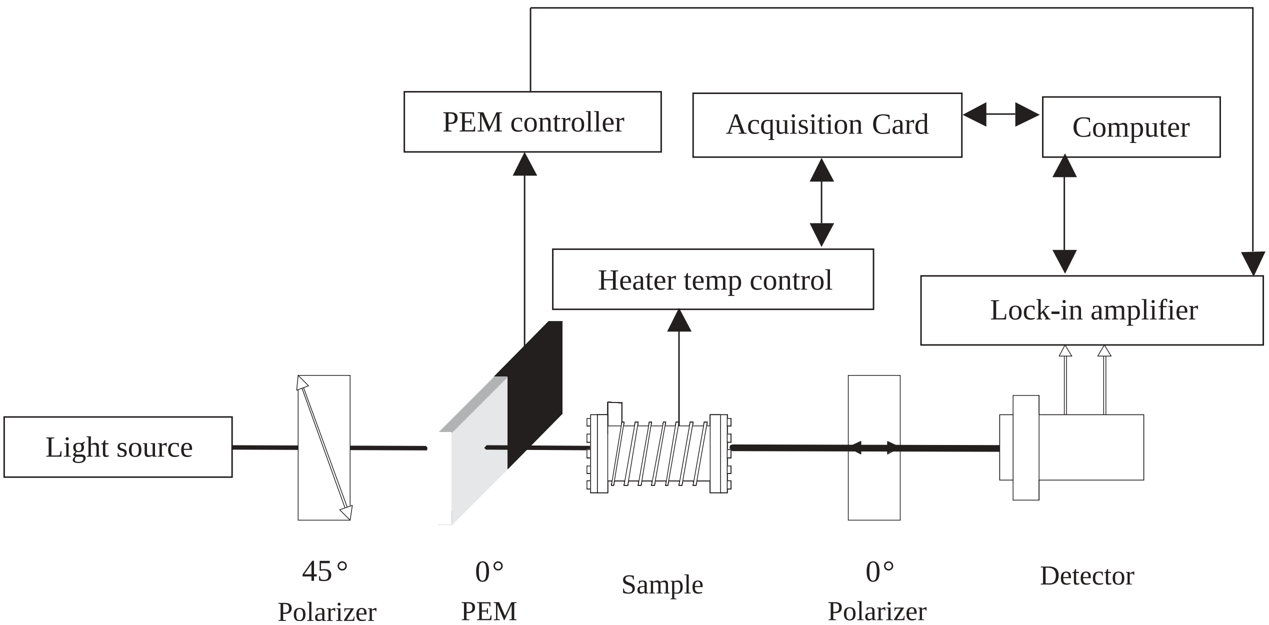

Figure 1. Schematic description of the CICATA and FPEG optical rotation systems (PEM = photoelastic modulator)

$$\alpha = \left(\alpha^{ref} \cdot \frac{I_{dc}^{ref}}{I_{2f}^{ref}}\right)\left(\frac{\pm I_{dc}}{I_{2f}}\right)^{-1} \quad [1]$$

where:

$\alpha^{ref}(\lambda)$ = OR for the reference standard as a function of the wavelength λ.

I_{dc} = detector signal generated by the average intensity of the light beam.

I_{2f} = signal from the second harmonic of the PEM's working frequency.

$I_{dc}^{ref} / I_{2f}^{ref}$ = reference standard normalized OR signal obtained from the lock-in amplifier.

$\pm I_{dc} / I_{2f}$ = normalized OR signal for unknown sample with the ± indicating OR direction.

The two parameters in the first bracket in Eq. [1] are the instrumental function of the system defined as the instrumental response inherent to the system itself. Therefore, for a light beam at a given wavelength λ the instrumental function F(λ) can be defined as:

$$F(\lambda) = \alpha^{ref}(\lambda) \cdot \left(\frac{I_{2f}^{red}}{I_{dc}^{ref}}\right)^{-1} \quad [2]$$

where:

$$\alpha^{ref}(\lambda) = \frac{\alpha_{0.5462271}}{a_0 + a_1\lambda^2 + a_2\lambda^4 + a_3\lambda^6} \quad [3]$$

$\alpha^{ref}(\lambda)$ is the function that represents the calculated sucrose OR value at the specified wavelength λ, where a_i are coefficients to obtain best curve fitting for the standard reference material. These coefficients are published by the US National Institute of Standards and Technology (Standard Reference Material 17e; NIST, 2003). The OR value for an unknown sample at a given wavelength is then:

$$\alpha = F(\lambda) \cdot \frac{\pm I_{2F}}{I_{dc}} \quad [4]$$

Optical system calibration, sensitivity, linearity and stability

The CICATA and FPEG systems were tested using a 50-mm cell path length following the same methodology. Both instruments were calibrated using distilled water for baseline and sucrose (1% w/v solution) as reference standard (NIST, 2003). Sucrose solutions (0.01 to 20% w/v) were used to determine instrument sensitivity, linearity and stability. The evaluation of the OR signal stability as a function of temperature was performed and reported by Mendoza-Sánchez *et al.* (2005). The stability of the system when monitoring brewing can be found in Huerta *et al.* (2004). Instrument performance while monitoring thermal effects on protein solution was reported by Kongrasawech *et al.* (2007, 2008).

Application test using the CICATA system

OR measurements at 589 nm to allow comparisons with published values were used to analyze the hydrolysis of a 5% w/v sucrose solution (Vukov, 1965). A pH 2.1 buffer was prepared by dissolving first 970 mg KCl in 200 mL distilled water. After 5 min stirring, 1.62 mL HCl and water to complete 250 mL were added with stirring continuing for another 5 min. A batch sugar solution was prepared by dissolving 12.5 g sucrose in distilled water to complete 250 mL. To neutralize the hydrolysis reaction, 387 mg NaOH were dissolved in 96 mL water. Sixteen 15-mL sugar solution aliquots were heated to 60°C in test tubes placed in a water bath. At time 0, 2 mL pH 2.1 buffer were added to each tube. The reaction was stopped at different times by adding 1.6 mL NaOH solution and cooling the tube in an ice bath. After temperature equilibration to room temperature, OR measurements were made using a 5-mm path length cell. Experimental initial and final OR values were compared with standard values reported in the literature (USP, 2003).

Application tests using the FPEG system

The performance of the FPEG instrument was evaluated by characterizing solutions made from commercial hard candies (five flavors, Jolly Ranchers, Hershey Foods Inc., Hershey, PA, USA) and by monitoring the fermentation of *tepache*, an alcoholic beverage made from pineapple peel following the procedures described by Moreno-Terrazas *et al.* (2000, 2001a,b).

Characterization of commercial hard candy. The OR at 25°C of commercial hard candy solutions was determined using a 10-mm path length cell filled with a solution of 5 g samples in 10 mL distilled water. This concentration was chosen to obtain OR values with the best signal to noise ratio for the instrument. Solutions were prepared in triplicate, and to ensure stability in time, the OR of each solution was measured during 5 min.

Monitoring the fermentation of an alcoholic beverage. A controlled-heating 100-mm path length cell (Fig. 2a) was used for in-line/real-time monitoring of *tepache* fermentation at 35ºC. The cell was filled with a 100 mL solution of distilled water containing 10 g sucrose and 8 g pineapple peel previously rinsed with tap water. For comparison purposes, off-line fermentation experiments were conducted using three flasks with 1 L solution of distilled water containing 100 g sucrose and 80 g pineapple peel previously rinsed with tap water (Fig. 2b). Two flasks were placed in 30°C and 37°C water baths, respectively, while a third flask was kept at room temperature (25 ± 0.5°C) for tests at temperatures similar and above that in the controlled-heating cell. The fermentation temperatures selected were chosen as typical for the production of this beverage (Moreno-Terrazas *et al.*, 2001a,b).

Results

All experimental data reported had a TL signal in the best linear response range (2 to 7 V output) to ensure correct determinations of OR changes of the light beam passing through the samples used in this study.

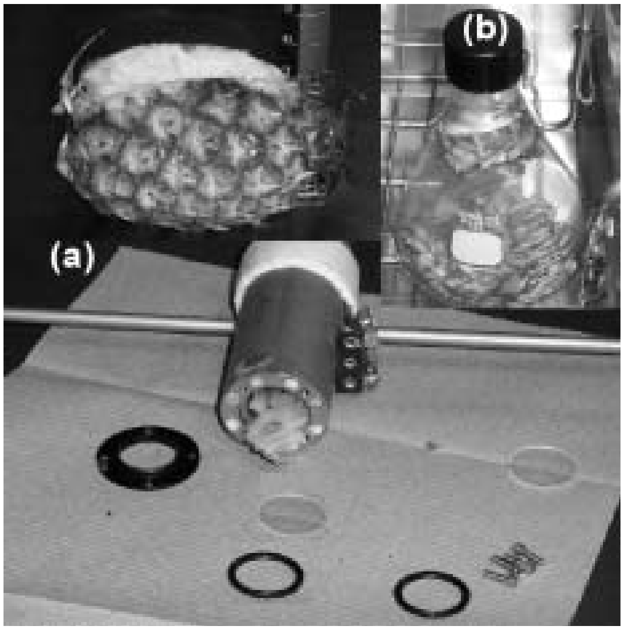

Figure 2. Monitoring fermentation for *tepache* production using an optical cell (a) and 1-L flasks (b) for in-line/real-time and off-line measurements, respectively.

Optical system characterization

The OR values measured using the FPEG and CICATA systems at the semiconductor laser central wavelength of 643 nm, and the calculated error expressed in degrees with respect to the sucrose solution used as reference, are shown in Table 1. For the FPEG system evaluated in the range from 0.03 to 20% w/v (0.01% w/v sucrose solution was below the detection limit), the average percentage error was 3%, whereas the CICATA system, evaluated in the range from 0.01 to 20% w/v, had an average percentage error estimated as 2%. System response linearity in terms of OR in degrees with respect to sucrose concentration gave for both systems a correlation factor of 0.999 for concentrations up to 20% w/v. Log scales were used to show concentration data differing by nearly three orders of magnitude (Fig. 3). Error bars are visible only for the FPEG unit when measuring low sucrose concentrations. As expected, a higher linear correlation factor (0.9999) was determined for both systems when the concentration was restricted to a maximum 1% w/v.

Sucrose concentrations as low as 0.01% w/v could be differentiated from the zero baseline corresponding to the distilled water signal by the CICATA unit (Fig. 4). The FPEG unit was slightly less sensitive and could differentiate from distilled water sucrose concentrations lower than 0.1 but higher than 0.03% w/v (data not shown). The instrument response stabi-

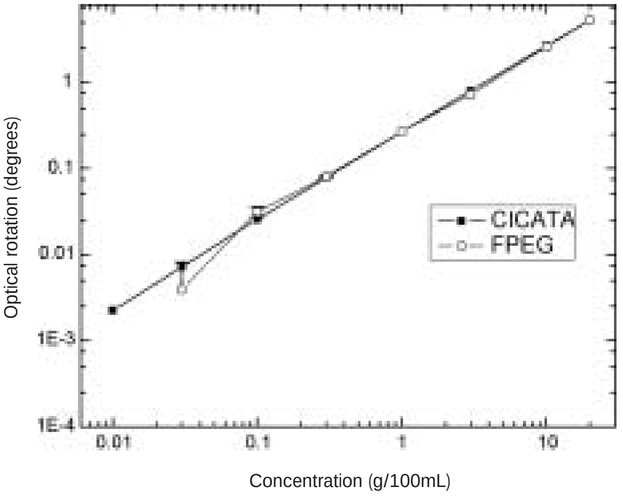

Figure 3. Optical system calibration for the CICATA and FPEG system.

Table 1. Calculated and experimental optical rotation values

Sucrose % w/v	A = Calculated[1] (degrees)	B = Experimental (degrees)		Error = A – B (degrees)	
		CICATA	FPEG	CICATA	FPEG
0.01	0.0027	0.0023	0.0074[2]	0.0004	-0.0047[2]
0.03	0.0081	0.0072	0.0106	0.0009	-0.0025
0.10	0.0270	0.0259	0.0310	0.0011	-0.0040
0.30	0.0811	0.0790	0.0828	0.0021	-0.0017
1.00	0.2702	0.2684	0.2702	0.0018	0.0000
3.00	0.8106	0.8190	0.7803	0.0084	0.0303
10.0	2.7018	2.6970	2.7165	0.0048	-0.0147
20.0	5.4037	5.3244	5.5133	0.0793	-0.1096

[1] Calculated using Equation [3] for λ= 643 nm. [2] Value below detection limit.

lity determined from a 24-h test on 1% w/v sucrose solution (data not shown) was under 2% for both systems.

CICATA system application example

Sucrose hydrolysis (Fig. 5) showed experimental OR values beginning at α = 1.65°, equivalent to a specific rotation [α] = -66° which compares well with the reported value of $[\alpha]_{589\ nm}$ = -66.5° (USP, 2003). The hydrolysis end point sample reached a value of α = -0.39° which was also similar to the literature value for inverted sucrose, α = -0.5°, equivalent to a specific rotation of $[\alpha]_{589\ nm}$ = -20° (USP, 2003).

FPEG system application examples

The OR values for solutions of five hard candy flavors of the same brand showed significant OR differences between flavors and low variability during 5 min OR experimental measurements on the same sam-

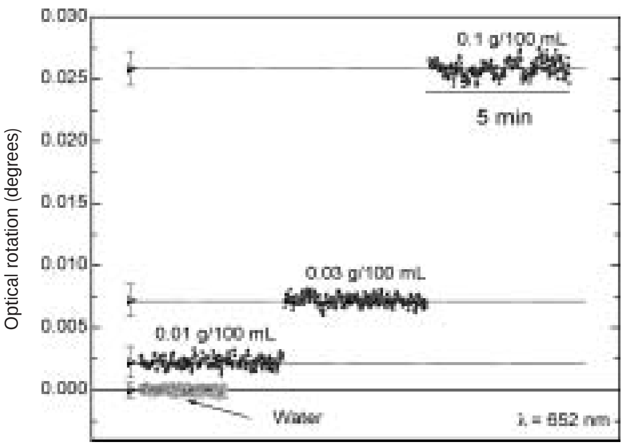

Figure 4. Optical rotation response at low sucrose concentration illustrating the sensitivity and stability (CICATA unit, error bars determined from 300 measurements obtained in 5 min).

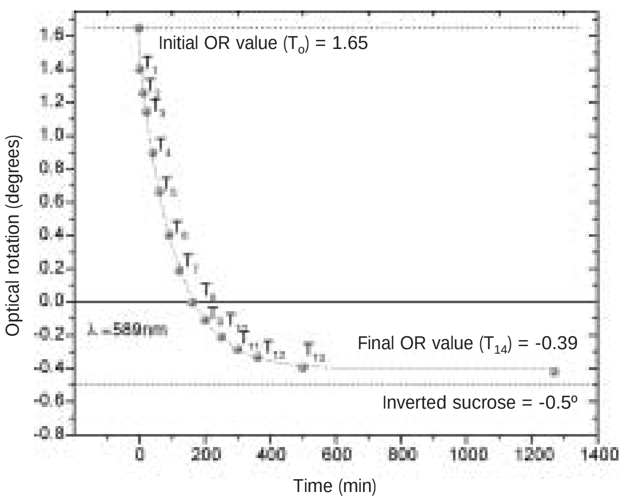

Figure 5. Acid hydrolysis of a 5% w/v sucrose solution at pH 2.09 and 60°C. Also shown is the OR value for inverted sucrose**.**

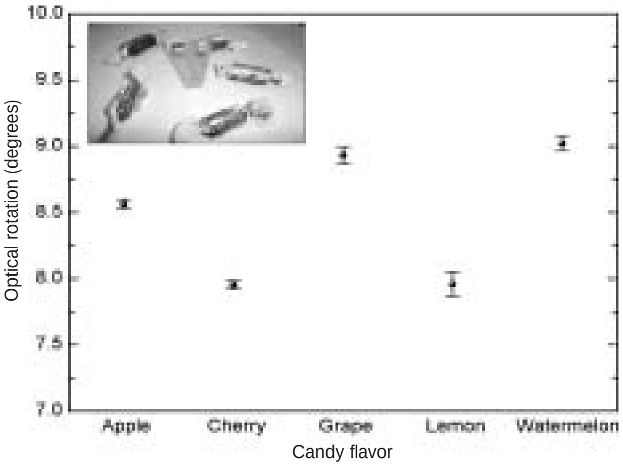

Figure 6. Optical rotation of commercial hard candy solutions**.**

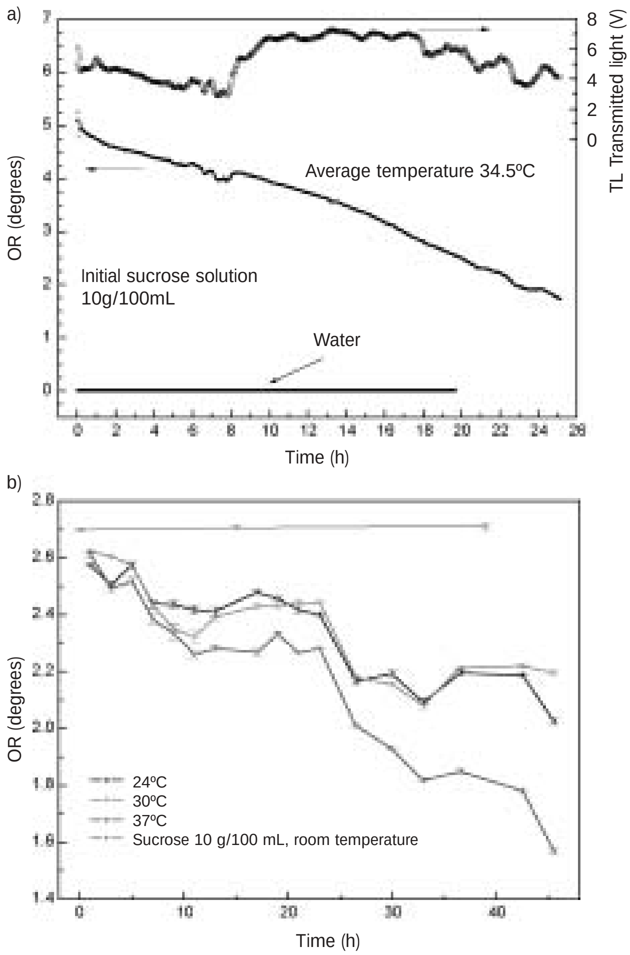

Figure 7. Tepache fermentation monitoring by OR (optical rotation) measurements. (a) In-line/real-time measurements including distilled water as control and TL (transmitted light) signal to ensure valid OR measurements (cell path length 100 mm). (b) Off-line measurements for flasks incubated at 25, 30 and 37ºC including 10% w/v sucrose solution kept at 25ºC as control (cell path length used to test aliquots removed from flasks, 50 mm).

ple (Fig. 6). Higher candy solution concentrations, as compared to the sucrose solution measurements above reported, were possible by using a shorter optical path length (1 versus 5 cm path length cell). Although hydrolysis variability can reduce life-shelf and lead to undesirable properties such as stickiness, commercial process variability was not an objective of this study. Instead, OR measurements were used to determine if different flavor products with different sugar inversion could be detected by OR measurements. The ability to differentiate products based on OR measurements suggest that an in-line/real time data acquisition system could be used to control sugar hydrolysis in hard candy manufacturing. However, OR differences between different flavor samples may reflect also compositional differences associated with each type of hard candy.

A second application tested with the FPEG system was the monitoring of *tepache* fermentation. When comparing *in-situ*/real-time OR readings (Fig. 7a) with off-line OR measurements (Fig. 7b) made on 10 mL aliquots removed at various sampling times from 1-L fermentation vessels incubated at 25, 30 and 37°C, deviations from the expected monotonic decrease in the OR signal were observed in the latter. The off-line measurements have the disadvantage of removing solution from the vessel, changing the pineapple solids to solution ratio. The expected faster disappearance of sucrose with higher fermentation temperature was observed in the off-line OR measurements.

Discussion

Optical system characterization and performance

The importance of the selection of components for a system with identical design was highlighted when characterizing and comparing the performance of the two systems used in this study. For example, the average percentage errors of the CICATA and the FPEG instrument with respect to sucrose solution measurements used as reference were different (Table 1). Most likely, the lower error characterizing the CICATA system when compared to the FPEG unit, reflects the differences in electronic components, particularly in the PEM and lock-in amplifier models utilized. The level of undesirable signal or noise present in an instrumentation system determines the smallest quantity of the sample being analyzed that can be measured with accuracy and also determines the measurement precision at higher concentrations. System response linearity in terms of OR in degrees with respect to sucrose concentration showed also minor differences between both systems. For sucrose solutions with concentrations up to 20% w/v (Fig. 3), the maximum OR difference between both systems was 0.19° observed at the highest sucrose concentration tested. In addition to the differences in the electronic instrumentation, this instrument behavior difference could reflect also environmental conditions. A third difference in instrument response was in their ability to differentiate from distilled water, samples with a small OR signal. Again, the performance of the CICATA unit was slightly better as it could differentiate from distilled water sucrose solutions with concentrations as low as 0.01% w/v. The sensitivity of the FPEG system was slightly lower, i.e., between 0.01 and 0.03% w/v. The detection limit can be defined as $x - x_B = 3s_B$ with x defined as the signal for a solution with the minimum detectable analyte concentration, x_B is signal for the baseline and s_B is the standard deviation of the baseline (Fig. 4).

The only comparison with no differences between the CICATA and FPEG units was the 24-h stability test which showed less than 2% variability for both systems. Although both systems were tested under different laboratory conditions, this 2% variability might be attributed to variations in external ambient conditions including room temperature, effects of power supply on the electronic instrumentation response, and even changes in the test solution (e.g., evaporation). Although in future versions of this laboratory device, this low instrument response variability could be reduced even further, an industrial control device version could be less stable under the harsh conditions of a processing environment. In conclusion, the evaluation of the FPEG and CICATA instrument characteristics demonstrated that these two units can be utilized to obtain reliable and comparable data.

System application examples

The hydrolysis of a sucrose solution (5% w/v) was monitored at 589 nm using the CICATA system because this test required measuring OR values at the same wavelength (589 nm) reported in the literature (USP, 2003). The comparison of the instrument response with published OR values required using polychromatic light processed through a monochromator set at 589 nm. The ease in implementing this modification, i.e., the use of additional optical components such as a monochromator, showed well the advantages of a modular design for optical measurements.

The monitoring of *tepache* fermentation using the FPEG unit showed the expected monotonic decreases in the OR signal. This was not the case of off-line measurements requiring the removal of samples from the fermentation vessel. This suggested that opening the container to remove aliquots for off-line determinations disturbed the *tepache* fermentation conditions. The ability of the FPEG system to obtain consistent readings without the need for recalibration during long term operation was confirmed by measuring the OR of a 10% w/v sucrose solution after 0, ~15 and ~40 h of continuous operation giving values of 2.730, 2.745 and 2.748 degrees, respectively (Fig. 7b). To further demonstrate the stability of the system during these extended continuous OR measurement experiments, the OR of distilled water was monitored during a comparable length of time (Fig. 7a).

Conclusions

The performance characteristics of the optical system, its modular construction and the application demonstrations here reported confirmed that this design can be used for in-line/real-time process monitoring. Two systems with different instrumentation resources were developed and tested for several potential applications. Similar commercial units could be built to monitor industrial fermentations, thereby identifying fermentation deviations, facilitating the identification of the fermentation completion and the optimization of process conditions. Optical rotation measurements might assist in controlling sugar hydrolysis in commercial hard candy manufacturing. The proposed optical system could also be used as a research instrument, e.g., to study protein denaturation by heat, acidification, pressure and other factors important in food processing. The sensitivity of OR measurements combined with modular designs using components available now at low cost could facilitate the development of these and other in-line/real-time process control and research tool applications.

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