Application of Multiple Internal Reflection Spectroscopy to the Study of Food Surfaces

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Multiple internal reflection spectroscopy techniques were used to record the optical spectrum at the surface of some food materials. Relationships between refractive index of the sample, wavelength, and depth of penetration of the electromagnetic field into the surface of the sample were discussed with starch and fat-encapsulated food acids as examples. The type of sample holder was found to affect the intensities of the multiple internal reflection spectra. The stainless steel sample holder provided more intimate contact between the sample and reflection plate than did the Teflon holder and, therefore, increased the intensities of the observed reflections.

Techniques in internal reflection spectroscopy were developed simultaneously by Fahrenfort (1961) and Harrick (1960). In that form of spectroscopy, the sample is placed in contact with an optically denser but transparent medium (a prism), light is introduced into the prism, and a plot of the reflected energy, as a function of wavelength, is recorded. Internal reflection spectroscopy techniques complement conventional optical absorption techniques and are particularly useful for studying solids when harsh sample preparation procedures are to be avoided. For example, Harrick used multiple internal reflection infrared to study surfaces and thin films associated with semiconductors (Harrick, 1967).

Although multiple internal reflection offers advantages in ease of sample preparation, no information has been published on its utility in studying food materials. Reported here are examples where multiple internal reflection can be used to provide information on the characteristic functional groups on surfaces of food components.

EXPERIMENTAL SECTION

Samples. Fat-encapsulated fumaric acid and glucono-δ-lactone (GDL) were obtained from Durkee Chemical Co. (Cleveland, OH). The lipid coatings were removed from some samples by extracting overnight with petroleum ether (bp 35–60 °C) in a Goldfish apparatus. Fatty acid composition was determined by GC according to AOAC (1975) methods.

Layer cakes were prepared by a one-stage method. The formula consisted of 100 g of flour, 110 g of sugar, 155 mL of water, 6 g of monoglyceride, 0.8 g of lecithin, 3 g of $NaHCO_3$, 3.3 g of sodium aluminum phosphate, 1.5 g of salt, and 3.5 g of egg albumin. After the cakes were baked, the starch was washed from the cakes and freeze-dried as previously described (Varriano-Marston et al., 1980).

Infrared Spectroscopy. Conventional mulls were prepared for transmission IR by finely grinding the samples with an agate mortar and pestle, adding Fluorolube, and placing the mull between two KCl salt plates. The KCl plates were placed on the sample holder; spectra were recorded between 4000 and 500 cm^{-1}.

A KRS-5 reflection crystal (50 × 20 × 2 mm), with a 45° angle, was used in the multiple internal reflection studies. The crystal consists of 42 mol % TlBr and 58 mol % TlI with a refractive of 2.63. Samples to be analyzed were placed directly in contact with the KRS-5 crystal that was positioned in an MIR-2 stainless steel sample holder or a Teflon internal reflection plate holder. The holder was then placed in a Wilks Model 12 double-bean internal reflection attachment (Foxboro Analytical, North Haven, CT) that was situated in a Perkin-Elmer Model 457 grating instrument. Spectra were recorded between 4000 and 250 cm^{-1}.

Refractive Indexes. Refractive indexes of solid samples were determined by the Becke Line method as described by Allen (1962).

RESULTS AND DISCUSSION

Surface Interaction between Starch and Surfactants. Surface active agents (surfactants) are added to bakery foods to improve quality, but it is unknown whether the agent's main effect stems from colloidal adsorption or chemical absorption by flour components. Surfactants have been implicated in affecting starch gelatinization and solubilization (Collison, 1968), but the mechanism(s) by which they affect starch physicochemical characteristics is not understood. Multiple internal reflection can be used to determine the extent of surface adsorption by surfactants and fats on starch granules.

As an example, starch washed from cakes made with monoglyceride shows increased intensity of the CH_2 stretching frequencies at 3.42 and 3.51 μm (Figure 1a) and the appearance of a carbonyl stretching frequency at 5.88 μm compared with starch washed from cakes containing no monoglyceride (Figure 1b). To determine if the observed bands were reflections from the surface rather than from the interior, we placed a small portion (400 mg) of monoglyceride-treated starch in a 5.3-cm (i.d.) sintered glass filter funnel, and 10 mL of diethyl ether was rapidly suction-filtered through the sample. When the spectrum of that starch was determined, it was identical with that of the starch washed from cakes containing no monoglyceride (Figure 1b). Our mild fat extraction procedure removed the fat from the surface of the granules; a more exhaustive extraction of that starch with diethyl ether in a Goldfish apparatus for 12 h removed significantly more lipid, which suggests that multiple internal reflection detected only the lipids adsorbed on the surface of the granules.

How deep did the electromagnetic field penetrate into the starch granule surface? Harrick (1967) has defined the penetration depth into a sample as

$$d_p = \frac{\lambda_1}{2\pi(\sin^2\theta - n_{21}^2)^{1/2}}$$

where $\lambda_1 = \lambda/n_1$ is the wavelength in the KRS-5 plate and $n_{21} = n_2/n_1$ is the ratio of the refractive index of the sample

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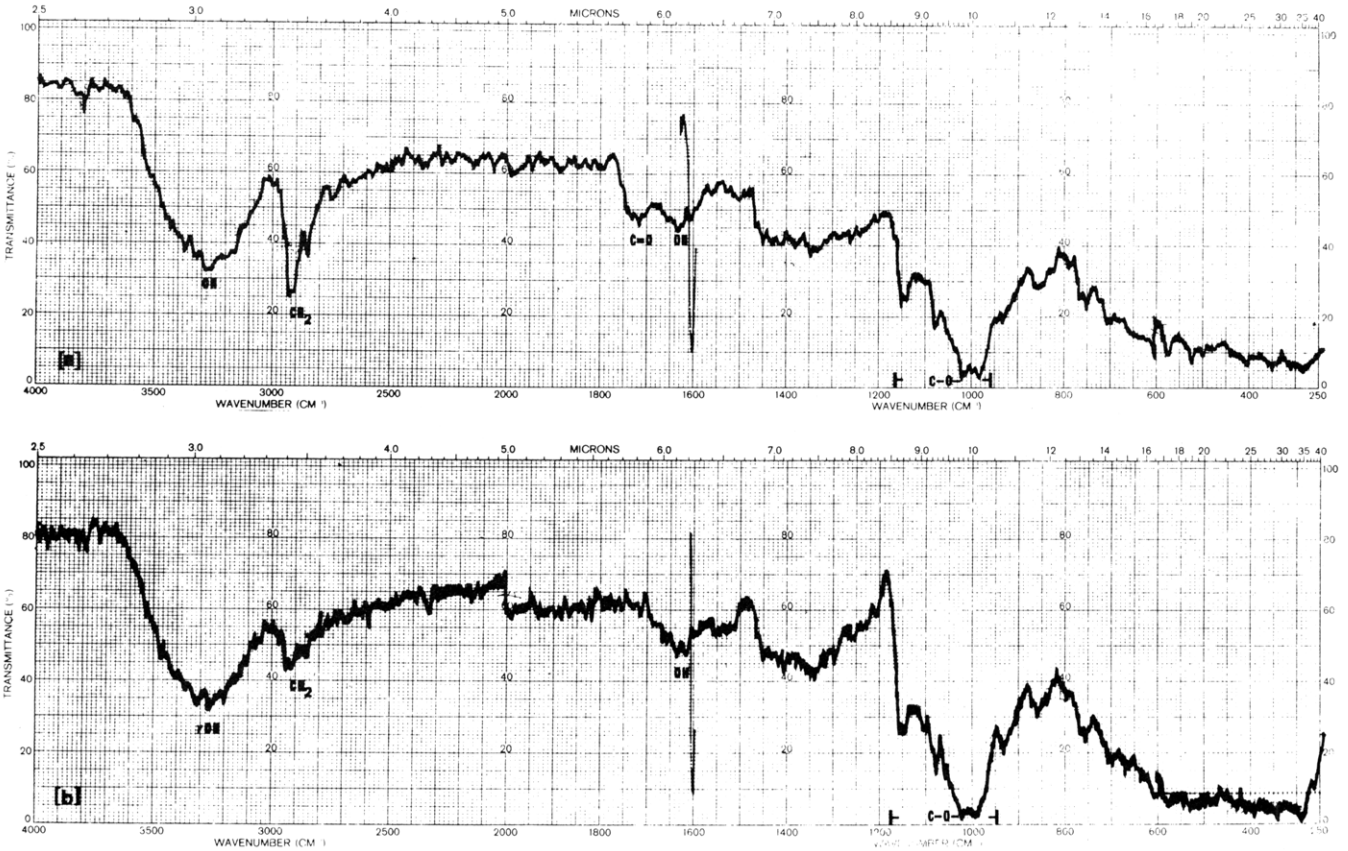

Figure 1. Multiple internal reflection spectra of starch washed from cakes with (a) and without (b) monoglyceride.

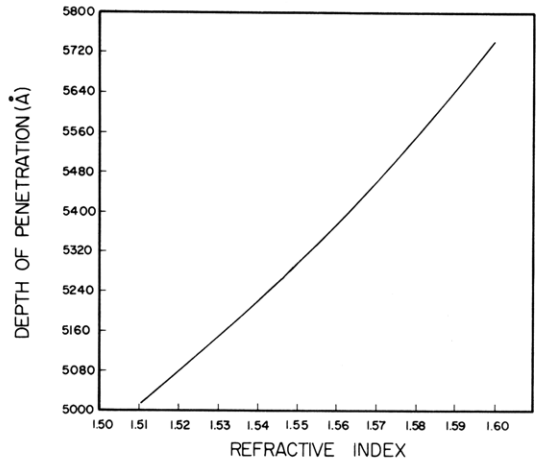

Figure 2. Effect of refractive index on the penetration depth of the electromagnetic field into the sample at a constant wavelength of 3.42 μm.

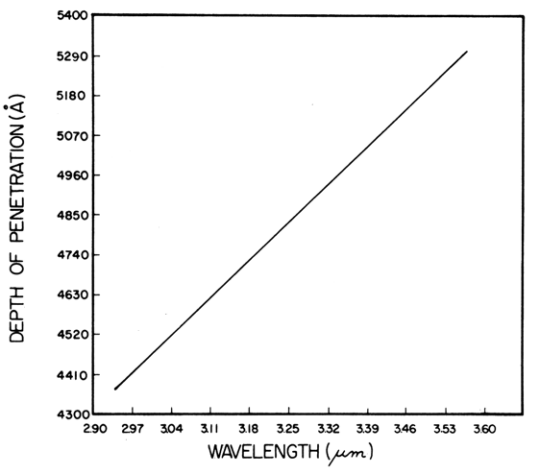

Figure 3. Penetration depth of the electromagnetic field as a function of wavelength at a constant refractive index of 1.52.

divided by the refractive index of the KRS-5 plate. The formula indicates that depth of penetration depends on the refractive index of the sample. For starch where n = 1.52, the depth of penetration of a constant wavelength of 3.42 μm (CH_2 stretch) is 5091 Å (Figure 2). The depth of penetration is also proportional to the wavelength; penetration is greater at higher wavelengths. For starch (n = 1.52), Figure 3 shows that the penetration depth of the electromagnetic field increases from 4546 Å at 3.06 μm (OH stretch) to 5091 Å at 3.42 μm (CH_2 stretch). The wavelengths between 3.06 and 5.88 μm are of greatest interest to us since that area provides information on absorption bands characteristic of the monoglyceride component (ν_{CH_2} at 3.42 and 3.50 μm; $\nu_{C=O}$ at 5.81 μm). Within that range, the maximum penetration of the electromagnetic field into the starch would be 8745 Å. Since the mean diameter of intact (unheated) starch is 25 μm, multiple internal reflection should prove to be useful in studying surface interactions between starch and fats or surfactants.

As expected, the intensity of the OH stretching frequency increases in the presence of high-moisture contents. Therefore, hydrated (not freeze-dried) gelatinized starch exhibits a more intense OH stretching frequency than dry intact starch. However, identical spectra were obtained for dry intact starch and freeze-dried gelatinized starch. We found that starch samples placed on the KRS-5 plate could not contain excess water because after prolonged periods of contact, the plate rapidly deteriorated, causing drastic reductions in reflection intensities.

Encapsulated Acids. For further illustration of the potential for studying food surfaces using multiple internal reflection, fat-encapsulated food acids were studied. Since

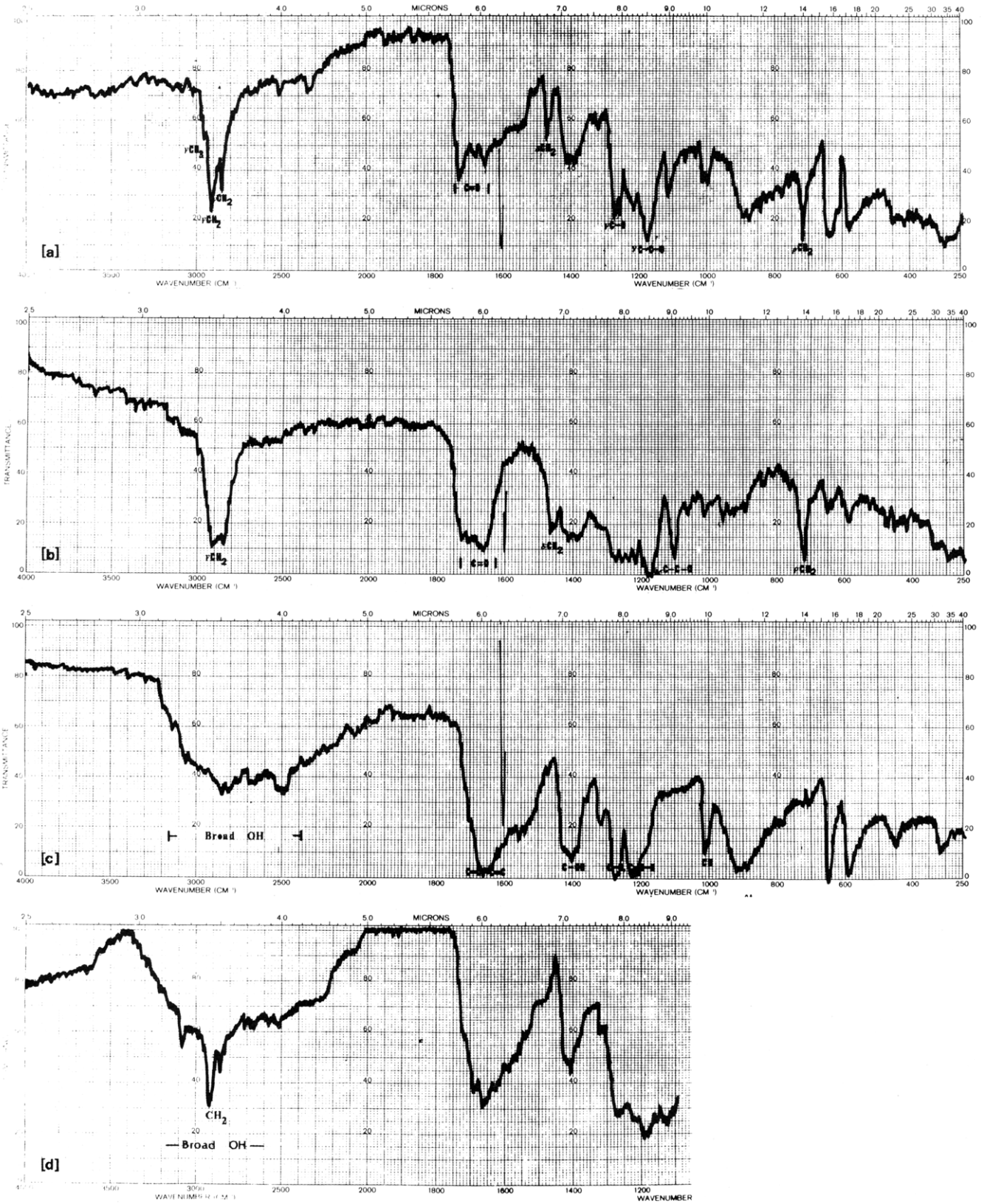

Figure 4. Multiple internal reflection spectra of fat-encapsulated fumaric acid (a), fat extracted from the encapsulated acid (b), and fumaric acid crystals (c). A Fluorolube mull of fat-encapsulated fumaric acid is shown in (d).

these samples consist of two distinct components, a crystalline food acid surrounded by a solid fat, they provide immediate visual evidence of the penetration depth of the electromagnetic field into the sample.

The multiple internal reflection spectrum of encapsulated fumaric acid is shown in Figure 4a. Comparing that spectrum with the multiple internal reflection spectrum of the extracted fat (Figure 4b) indicates that they have the same pattern, i.e., the same frequency vibrations for the various functional groups. Assignments of the functional groups in the spectrum of the fat were based on its GC pattern, which indicated that it consisted of $C_{18:0}$ and $C_{16:0}$ fatty acids in the ratio of 2.4:1 with no unsaturated acids. The results in Figure 4a,b clearly indicate that the

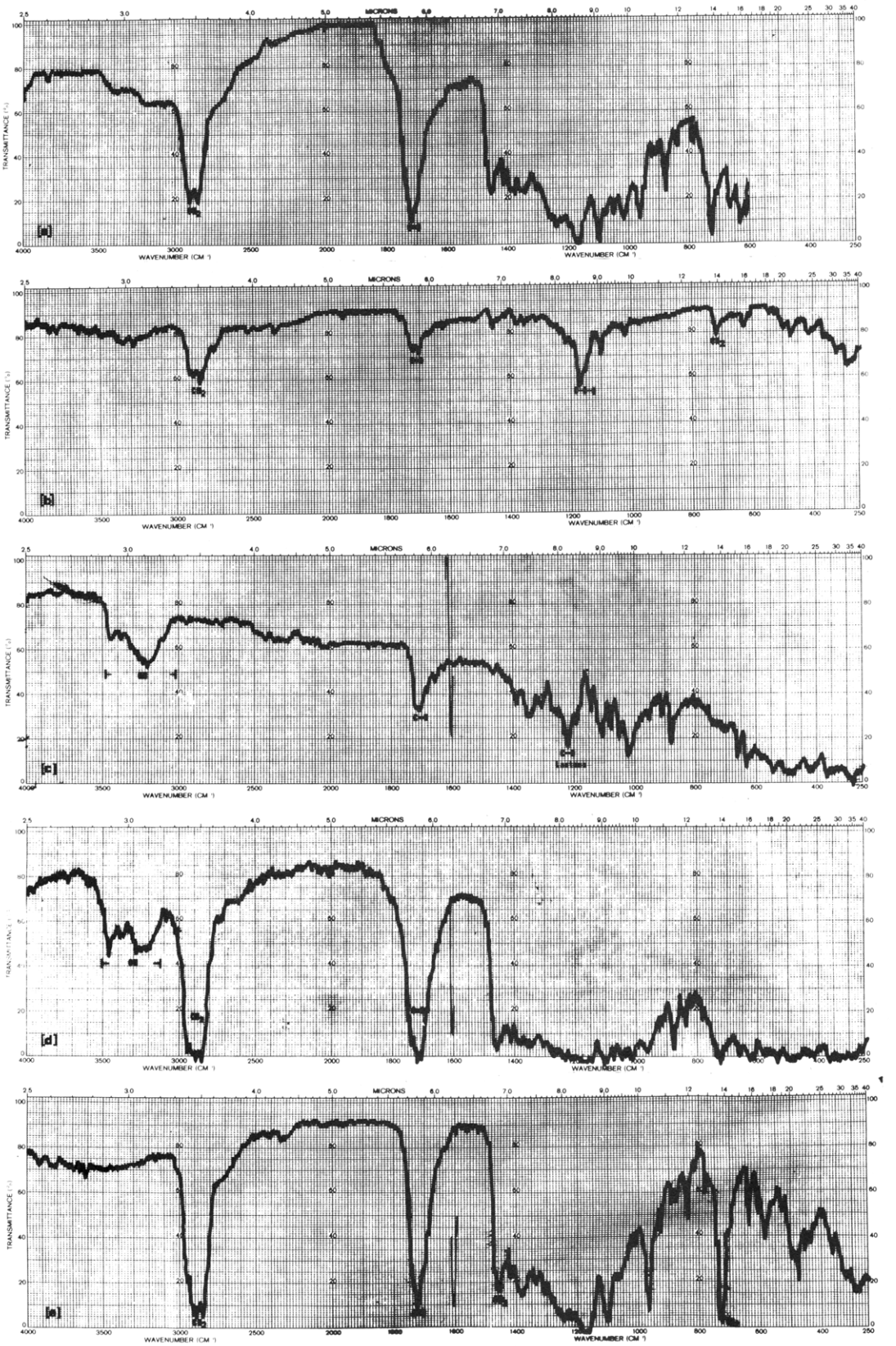

Figure 5. Multiple internal reflection spectra of fat-encapsulated GDL (a, b, and d), crystalline GDL (c), and fat extracted from the encapsulated acid (e). A Teflon holder was used with sample b; stainless steel was used with all others.

electromagnetic wave did not penetrate deep into the sample. In fact, a refractive index of 1.483 for the fat gives a calculated penetration depth of 4621 Å at a wavelength of 3.42 μm.

Evidence that the electromagnetic field did not penetrate through the entire sample is provided by the spectrum of fumaric acid (Figure 4c). The broad OH stretching frequency (3.09–4.76 μm) of fumaric acid was not observed in the multiple internal reflection spectrum of fat-encapsulated fumaric (Figure 4a) but was observed in its Fluorolube mull (Figure 4d).

Spectra of encapsulated GDL (Figure 5) demonstrate several points. First, the type of sample holder used with solids may affect intensities of observed spectra. With a

stainless steel sample holder, uniform pressure is applied that allows for maximum contact between the KRS-5 plate and the sample and produces the spectrum of encapsulated GDL presented in Figure 5a. On the other hand, with a Teflon holder, the sample contact with the plate depends on adhesive characteristics of the sample which, therefore, limits the usefulness of this type of holder. Figure 5b shows the spectrum of encapsulated GDL using the Teflon holder. Figure 5a,b illustrates that the stainless steel holder permits closer contact between the sample and the KRS-5 plate than does the Teflon holder since the intensities of the observed reflections were greater when the stainless steel holder was used.

Second, the amount of pressure applied to the sample in the stainless steel holder affects its usefulness in surface analysis. When excessive pressure is applied to the sample, vibrational frequencies characteristic of crystalline GDL (Figure 5c) are observed in the encapsulated sample (Figure 5d). In this instance, the excess pressure caused the fat to flow exposing the underlying GDL.

Third, in cases where pressure cannot be applied to the sample, a Teflon holder is more applicable to surface analysis. For example, the multiple internal reflection spectrum of encapsulated GDL using the Teflon holder (Figure 5b) has essentially the same spectrum as the extracted fat (Figure 5e) when one allows for expected differences in intensities. Functional groups characteristic of GDL (Figure 5c) are not observed in that spectrum as they are in Figure 5a, indicating that the electromagnetic field penetrated only the sample's surface.

From the above discussion, it is obvious that the characteristics of the food material will dictate the conditions to use for surface analysis. Optimum conditions can only be defined through experimentation.

Although only a few examples showing the possible application of multiple internal reflection to the study of food materials were given, numerous other possibilities exist. Solid or liquid food substances can be easily studied since the only sample preparation necessary is bringing the substance in contact with the reflecting surface. Large particles as well as very thin films can be investigated with ease in their natural state, whereas in conventional spectroscopy few samples can be studied without elaborate sample preparation.

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In Vitro Digestibility and Functional Properties of Chemically Modified Casein

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Casein amino groups were modified by reaction with aldehydes and ketones via reductive alkylation at pH 9.0 to give stable, non-cross-linked lysine-modified derivatives. The degree of alkylation was controlled by the amount of alkylating reagent. There was a pronounced decrease in the initial rates of α-chymotrypsin-catalyzed hydrolysis of alkylated caseins. The initial rates decreased as the size of the modifying group increased and as the degree of modification increased. This decreased rate of hydrolysis was not due to product inhibition. Extents of hydrolysis after 48 h of the alkylated caseins were essentially independent of the degree of alkylation and the nature of the alkyl group. The conformation of alkylated casein was different from the native casein. Solubilities of methylcasein and isopropylcasein were increased slightly over that of native casein; with bulkier alkyl groups, the solubilities were significantly lower than that of native casein. Emulsifying activities of alkylated caseins, except for butylcasein, were higher than that of native casein.

Increased attention has been directed toward the development of low-cost protein foods (Altschul, 1974; Forsythe and Briskey, 1977; Friedman, 1978). Although there are a multitude of alternative sources of proteins (e.g., trash fish, grain, microbial, and leaf), the feasibility of using them as food proteins has been limited due to their low biological value, undesirable organoleptic properties, toxic contamination, and poor functional properties. The above problems may be overcome by one or more of the following methods: physical or mechanical treatment (Huang and Rha, 1974), enzyme modification (Spinelli et al., 1972; Kuehler and Stine, 1974; Hermansson et al., 1974; Liener, 1977; Miller and Groninger, 1976; Phaff, 1977; Richardson, 1977; Whitaker, 1977; Fujimaki, 1978; Zakaria and McFeeters, 1978), microbial modification (Whitaker, 1978; Beuchat, 1978), chemical modification (Franzen and Kinsella, 1976; Barman et al., 1977; Feeney, 1977; Meyer and Williams, 1977; Puigserver et al., 1979a,b).

We have studied chemical procedures to overcome some of the above limitations. Reductive alkylation was used because it has been shown that under mildly alkaline conditions, the amino groups in protein can be reductively

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