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Microscopy—A Powerful Tool for Food Scientists

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Light microscopy is a valuable tool that can be used as an aid in meeting the demands of today's fast-paced product development process, food safety, and many other challenges encountered in food and industrial applications. Having spent many years in the cereal processing industry, Kenneth Yahl (KRY Microscopy & Consulting Service) and I have become very familiar with this powerful technique. As consultants to the food and other industries, we rely heavily on a wide variety of techniques, including light microscopy, to investigate ingredients and find solutions to diverse problems. I was first introduced to light microscopy by Majel McMasters at Kansas State University, while Kenneth spent considerable time honing his skills with the well-known microscopist Walter McCrone. Although there are numerous applications, our focus here is on the common uses of light microscopy in cereal processes and products. Our goal is to provide a few practical thoughts and examples.

Microscopy Techniques

Microscopy techniques can be used to study the quantitative microstructures of materials, ingredients, and in-process and finished products. Images can created for a broad range of food items, including macrostructures of meats and finished products, such as pizza, and microstructures of products, such as cheeses, doughs and baked goods, ice creams, fruits and vegetables, emulsions, foams, and gels. Microstructures characterized using high-quality imaging techniques illustrate chemical composition and thermomechanical processing, genetic, and structural properties.

Different types of imaging, used to measure various structural aspects, are obtained using light, scanning electron (SEM), transmission electron (TEM), and confocal and atomic force microscopy, as well as computed tomography. Microscopy at higher resolutions can aid in understanding what happens to the original biological materials, such as grain, muscle, or milk, when they are processed into products, such as bread or pasta, ham or steak, or yogurt or cheese.

Its versatility makes microscopy a useful tool for understanding what causes a product to fail or what an ingredient looks like in an acceptable product at the microstructural level. Light microscopy provides a rapid and effective means of examining the microstructure of products and obtaining information that can complement both chemical and physical analyses. In addition to revealing the structural details of globules and fibers, specific staining procedures make it possible to distinguish proteins, starches, gums, and minerals. The influence of ingredients and processing conditions on product structure, especially in the area of new product development, can be determined, which can help explain why foods with similar chemical compositions have markedly different textures.

Microscopes, Tools, and Methodology

In food and industrial laboratories, the optics most often used for compound microscopes with transmitted light illumination are bright field, simple polarization, and phase contrast. For most microscopy techniques, compound microscopes need not have research polarization optics (a rotating stage). Phase-contrast optics are ideal for viewing specimens with a refractive index (RI) close to that of the liquid phase, as in the case of gelatinized starch or hydrated gum particulates. Stereo microscopes, which are often enhanced with fiber optic light, can be used as a screening tool.

Specialized tools are needed for sample manipulation and preparation, including microspatulas, needles, single-edged stainless-steel razor blades, small brushes, disposable plastic eye droppers, bottles for stains, spot plates, and microsieves. It is important to obtain a representative sample for study. If the product is a liquid or gel, a good stirring should be enough, whereas dry products should be quartered down to a workable lab sample size. Ideally, before using a compound microscope a stereo microscope should be used to view powders and other particulate food products, such as dry soup and cake mixes. This aids in identifying minor constituents that could be missed when viewing the small amount of material used to prepare a slide for the compound microscope. When a known ingredient is observable, staining is not necessary. To investigate ingredients, it is helpful to establish reference samples of ingredients to compare with the sample being evaluated. This approach also allows you to become familiar with the optical properties of an ingredient.

Before using any microscope, check to see that it is in good optical alignment for Kohler illumination. Initial examinations should be done using bright-field optics at several magnifications. The specimens should be mounted in light mineral oil with a minimal depth of field. Make notes on specimen shape, size, color, and polarization properties. Photomicrographs should be taken for future reference. When working with powders, one should also become familiar with the appearance of the ingredient in an aqueous phase. Some ingredients when mixed with water may have a RI close to that of the liquid phase and may need to be stained to be detectable. Phase-contrast optics can be used to enhance the appearance of dry specimens with RIs near those of their liquid phase.

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Analyzing a Complex System

An important principle in microscopy is that the less done to the specimen the better. It is important to preserve what is often a fragile structure, especially when it is the precise nature of the structure that is of interest, as in the study of emulsions. Histological methods have evolved to suit intact tissues that can withstand fixation, wax embedding, and lengthy staining procedures. Many of these techniques are unsuitable for food and industrial applications, because any one of the stages may so alter a food product that the information that is subsequently obtained can be misleading.

Before looking at a food product, key components of the ingredients used in the system should be considered first. For example, microscopy can be used to characterize the lipids, starches, proteins, and gums that are present.

Lipids

Lipids play an important part in the microstructure of many processed foods. Their presence and the way they are dispersed affect the taste and mouthfeel of the food that contains them. Fat that is solid at room temperature can be seen using polarized light, which reveals the crystalline nature of the solid glycerides. Fat can be identified further by gently warming the slide and observing whether the crystalline material melts and loses its birefringence. Upon recrystallization, the newly formed crystalline material often has a different form than the original; it is usually larger and sometimes forms spherulites.

Starches

Starches are widely distributed in nature and in many processed foods. Important commercial starches include those extracted from cereals (e.g., corn, rice, wheat) and roots and tubers (e.g., potato, tapioca). The size and shape of starch granules are characteristic of the plant source and can be identified microscopically. The birefringent pattern of the granules seen under polarized light assists in identification. The position of the hilum (the point of intersection of lines known as the Maltese cross), central or eccentric, and any observable striations are important as well. Microscopy can also be used to determine the starch type and amount of the two main components of starch—amylose and amylopectin. Iodine staining of unmodified starch granules can be used to identify the type of starch present (1,2). Waxy starches consist almost entirely of amylopectin molecules. Common starches have amylose concentrations of ≈17–28%. High-amylose starches have amylose concentrations of ≈50–90%. The amount of amylose in a starch and the type of starch modification affect the gelatinization temperature range of starch-containing products. In addition, the presence of fats, proteins, gums, and soluble solids in a formulation also affect product properties.

The degree of cook of a starch granule can be determined using microscopy. There are many technical articles regarding the gelatinization of heated starch granules. In general, there is a noticeable, gradual swelling of the starch granule and increase in the diameter of the hilum. At this point some of the soluble components are leached from the granule. Mechanically damaged starch granules usually show a disturbance in their crystalline regions and sometimes swell when mounted in water. Starch modification by cross-linking can prevent granules from fragmenting, creating swollen, intact granules. The paste or gel formed by cooking also has specific functional properties that are associated with the type of starch and affect its applications.

Proteins

Protein concentrates and isolates, commonly from soybean, wheat, and milk, are added to a wide variety of products, from baked goods to meat products. They can contribute functional properties as well as nutritional benefits and can be added to aid in dough formation or as meat extenders. Protein isolates (soluble protein) provide a challenge in microscopy because a different method of sample preparation is sometimes needed. Iodine, which develops a yellow color, is one of the stains that can be used to identify protein particulates. The best approach is to obtain a sample from the supplier that may be used in the formulation and determine its microscopic characteristics.

Gums

Gums are used as thickeners, stabilizers, and emulsifiers. They are high molecular weight, water-soluble carbohydrates (hydrocolloids) derived from natural sources such as fruits, cellulose fibers, seeds, plant exudates, and microorganisms. No one specific stain can be used to identify these ingredients. In general, dry gum particulates exhibit their own birefringent patterns under polarized light. However, gums are often fully hydrated (no longer birefringent) in food systems and dispersed with high shear to provide optimal functional properties. When gum particulates are completely hydrated and dispersed, they are difficult to detect. When all birefringence is gone, specific stains can be used to identify the gum type.

Example of Problem Solving in a Complex System

A midsized manufacturer in the baking industry wants to determine why a competitor's cakes are more moist than its own. In taste tests, more favorable ratings for the competitor's cakes were attributed to the moistness of the cake. To determine the differences between the products, the two dry cake mixes are purchased, and the ingredients listed on the cake mixes are studied. Next, some of the ingredients are placed in groups according to their characteristics (insoluble, soluble, or immiscible in water):

Insoluble	Soluble	Immiscible
Wheat flour	Sugar	Partially hydrogenated oils
Wheat starch	Sodium bicarbonate	Soy lecithin
Cellulose gum	Dicalcium phosphate	Polyglycerol esters of fatty acids
Xanthan gum	Sodium aluminum phosphate	Mono- and diglycerides
Cocoa	Monocalcium phosphate	
Modified wheat starch	Dextrose	
Cornstarch	Salt	
Whey protein concentrate	Polysorbate 60	
	Propylene glycol	

Wheat flour, wheat starch, cocoa, cornstarch, and whey protein concentrate are easily detected in oil or water. Modified wheat starch may appear as unmodified wheat starch granules, depending on the type of modification or processing used. Although textured wheat protein and wheat protein isolates may be present in bakery formulations, one should not assume that all proteins used in bakery applications are from wheat. In the current example, we can see from the ingredient list that whey protein concentrate has been added to the formulation. As previously mentioned, protein concentrates and isolates require specific sample preparations for microscopic examination.

Cellulose and xanthan gum have unique shapes and specific birefringent patterns when they are not hydrated. Although they lose all of their birefringence when completely hydrated, as in a cake, they can still be observed in the cake mix, which is a low-water system. Partially hydrogenated oils consist of two phases—solid and liquid. The solid phase is seen in fully hydrogenated flakes, which have a specific birefringent pattern. These ingredients are easily detected in a dry blend or in batter prior to processing. In the final batter for a cake system, however, the oil droplets should be as small as possible, indicating that the best emulsion has been formed. You should also expect to find smaller pieces of hydrated gum particulates in the final batter.

Microscopic examination of the two cake products reveals that the competitor's product contains higher amounts of cellulose and xanthan gum, which produce a more moist product. As a result, the recommendation would be to reformulate the midsize manufacturer's cake mix with higher levels of cellulose and xanthan gum.

(Note, when using microscopy techniques you should observe the sample in both nonaqueous and aqueous preparations to prevent missing those components that are soluble in water.)

Solution of Different Types of Product Challenges Using Microscopy

As described earlier, microscopy can be used in a wide range of applications to help solve problems in food and industrial applications. Several examples of potential challenges and their solutions are provided below.

Sauces

Problem: Unexpected thinning of sauce after retorting.

Microscopic examination: Cooked starch granules are fragmented, and starch granules are overcooked.

Recommendation: Check temperature control of starch cooker; reduce temperature of starch cooker; use a starch product that has a higher degree of cross-linking.

Problem: Tapioca-like clumps and brown globules are present in a retorted cheese sauce.

Microscopic examination: The tapioca-like clumps consist of strong birefringent crystalline particulates typical of certain gums. The brown globules consist of clusters of hydrated gum particulates.

Recommendation: Blend all of the dry ingredients to separate gum particulates and then add to liquid ingredients. If clumps are still present, increase the holding time after the mixture containing gum is added to the liquid ingredients and use a higher level of shear.

Formed Meats

Problem: Even though starches were used in a hot dog formulation, the packaged hot dogs are swimming in liquid.

Microscopic examination: Waxy and common starch granules are not optimally swollen; they are disrupted and, therefore, not able to retain liquid.

Recommendation: Change the type of starch used to ensure an optimally swollen, fully functional starch.

Paper Sizing

Problem: Streaks on the surface of sized paper.

Microscopic examination: Residue from the edge of the trailing blade coater reveals it to be a high concentration of small and large retrograded amylose spherulites.

Recommendation: A starch product containing amylose was used to prepare the paper sizing formulation. The cooked formulation needs to be kept at a temperature >190°F at all points in the process to prevent retrograded amylose from forming.

Summary

If you need to determine the best ingredients and processing methods for producing an acceptable product, microscopy is one of the tools you'll need for your forensic tool kit. Light microscopy techniques are useful in new product development, enabling quick identification of ingredients and processing problems.

References

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