One way to capture consumer interest in a probiotic health supplement is to offer a product loaded with multiple microbial strains in large concentrations per dose. Here we consider the special challenges of formulating, manufacturing, and handling these ultra-potent probiotics and offer advice on overcoming them.

The growing popularity of probiotics—beneficial live bacterial cultures, such as lactobacilli, taken orally to maintain good health—has motivated dietary supplement makers to offer products that stand out from their competitors. But this isn’t easy in the USA, where the structure-function claims allowed for any particular probiotic strain or clinically studied dose are fundamentally the same from brand to brand. Some companies promote their unique strain-specificity while others opt for a “more-is-better” appeal—both in terms of the variety of species contained in their product as well as its overall potency (live-cell count). This article focuses on the limitations of ultra-potent, high-count probiotics and the formulation considerations not seen in lower-count products: specifically, culture concentration, density, overage, processibility thresholds, and packaging.

**Culture concentration**

The maximum number of probiotic cells that can fit into a single dose depends largely on the concentration of the culture involved, in addition to the space needed for excipients in the formulation. Culture concentration—or cell density—may range from fewer than 50 billion colony-
forming units (CFUs) per gram to upwards of 1 trillion CFUs per gram. Some cultures are supplied in standardized concentrations, while the concentrations of others vary depending on how they were fermented. The physical density of a specific culture may also vary by strain and by manufacturer. When restricted by volume limitations, as in the case of a capsule shell, it may be helpful to think of cell density in terms of cells per unit of volume. When volume limitations are not a major factor, as with sachets, cell density may be calculated simply as cells per unit of weight.

Overage

In the language of probiotic manufacturing, “overage” is the number of live cells required in excess of the stated label claim to ensure the claim remains valid for the duration of the product’s shelf-life. Factors that contribute to cell death include culture stability, water activity, package permeability, heat exposure, and expected/desired length of shelf-life. Failure to account for these factors will force you to increase overage, which drives up the amount of cell culture required in the formulation. The inherent stability of the live cells themselves is fundamentally strain-specific. The more stable the strain, the less overage required to obtain a minimum label claim. Less stable strains on the other hand require greater overage, which crowds the already limited volume within the dose. Because every microliter of space is valuable with ultra-potent probiotics, only the most stable strains are good candidates for use.

Other ingredients

Probiotic products generally require carriers and processing aids to facilitate powder processibility. Microcrystalline cellulose dried to very low water content is commonly used as a carrier for probiotic capsules. Among processing aids, silicon dioxide and magnesium stearate are favored for their effectiveness as powder-flow agents at relatively small usage levels. Any secondary actives (plant extracts, vitamins, minerals) must be tested to ensure that they have no antimicrobial properties or other deleterious effects on the living probiotic cultures. It is important that all added ingredients be kept as dry as possible—re-drying them if necessary to prevent them from diminishing the stability of the probiotics.

Processibility

The fixed volume of the capsule shell is a key limitation for probiotic capsules. The density of the powder blend and its ability to be compressed into a slug determine the total fill weight of the capsule. In general, any product that exceeds 60 percent culture by weight should be tested in pilot studies using commercial machinery to determine feasibility. That said, there are products containing more than 80 percent culture by weight that succeed, while others with much lower culture percentages fail, often due to extreme hygroscopicity or stickiness.

Environmental control

Many of the factors affecting the success of ultra-probiotics involve control over process conditions, including maintaining humidity within a narrow range, usually less than 30 percent relative humidity (RH). Because the cultures are typically dried to water activity levels (A_w) of less than 0.100—and even as low as A_w 0.003—they are hygroscopic and tend to absorb water readily. [Editor's note: For more information on water activity, see “Measuring water activity as a means to reduce microbial limit testing” in the October 2008 issue.] Exposure to RH exceeding 40 percent will quickly cause the powder to become sticky. The biggest pitfall to manufacturing probiotic capsules is the buildup of sticky powder on the carrier segments, which leads to improper seating and closing of the capsule shells (photos). Therefore it's important to minimize bowl spillage and segment spillage during slug transfer from the bowl to the capsule body. Some manufacturers use a dosing disc one size smaller than the capsule (e.g., a size 1 disc for a size 0 capsule) to avoid depositing powder on the rim of the lower carrier segments.

Packaging concerns

The moisture (or water) vapor transmission rate (MVTR or WVTR) is the single most important criterion for
selecting packaging material. MVTR is usually calculated in grams of water per mil thickness per surface area per unit time. The size of a package significantly affects its surface area, so MVTR values must be scaled accordingly. For example, a bottle made of the same plastic and similar wall thickness as a smaller one will have greater surface area, leading to more total moisture ingress into the bottle. Similarly with films, the sachet or stick dimensions determine overall surface area. In thermoformed blisters, the major factors that determine moisture ingress are cavity size and material stretching, which reduces film thickness. In cold-formed blisters, the MVTR should remain near zero as long as the aluminum covering is sealed and has no holes.

Protecting the probiotic product from moisture during all blending, processing, and packaging steps is crucial to product quality—specifically, to meeting label claim throughout shelf-life. Remember: Moisture gain during production is cumulative. It’s not enough to maintain scrupulous environmental controls during one stage of production and then relax them in downstream steps. Any moisture gained will remain trapped inside the packaging for the product’s shelf-life, jeopardizing the probiotic’s viability. From the moment they’re produced onward, capsules containing ultra-probiotics must be carefully packaged and stored to prevent moisture uptake.

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