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### **An instant fly medium and a convenient method to dispense it.**



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### **Introduction**

Most small research and teaching laboratories use instant media to grow fruit flies, with Carolina Formula 4-24 being a popular choice, although simpler and less expensive media such as mashed bananas (Bennett, 1961) or instant mashed potatoes (Duenas *et al.*, 2002) can also be used. Other than the higher price relative to cooked versions and other types of media (Formula 4-24 is currently \$5.13 per lb from Carolina Biologicals in a 50 lb bag), the principal disadvantage of instant medium comes from how it is commonly mixed in the laboratory: the dry food powder and water are added separately to individual vials or bottles, a procedure that will result in variable ratios of dry media to water across the bottles or vials (unless each bottle or vial is individually weighed after the sequential additions of dry medium and water). This is not a trivial concern: dietary depletion – produced by varying the amounts of macronutrients relative to agar – is well known to increase the lifespan of fruit flies, as well as to affect the expression of behaviors, including locomotion. Although the mechanism underlying the lifespan extension produced by dietary depletion is controversial, the end result – longer lived and differently acting flies – is not.

On the practical side, filling even a relatively small number of vials (50-75) in this manner is tedious. To address this problem, Laverty (1986) fabricated a device that will concurrently fill multiple vials with approximately the same amount of dry media, to which water is then added with a repeater pipette, reducing the labor associated with filling vials. Since the amount of dry medium added to each vial will still vary with this apparatus, however, the food concentrations will vary across individual vials. Rather than adding the dry ingredients and water separately into individual vials, mixing the dry ingredients and water prior to dispensing ensures that all vials and bottles will contain media of the same concentration. Described here is a recipe for an inexpensive medium similar to that described by Duenas *et al.* (2002) with physical properties similar to Formula 4-24, as well as a simple and efficient method to dispense rapidly this or any other instant medium by using a vertical screw-type sausage stuffer.

**Recipe for potato/yeast/dextrose instant medium for fruit flies.**

The dry ingredients are (by weight) potato flakes (75%), yeast flakes (15%), glucose (9.3%), and methylparaben (0.7%). To prepare the media, the dry ingredients and water at 4 times the weight of the dry ingredients are mixed in a large bowl to form a thick paste. Depending on the hydration of the ingredients, more or less water might be needed. Once mixed, the media is dispensed into vials or bottles as described below. At the time of this writing, the total cost of the ingredients is \$2.35 per pound, which is substantially less than that of Formula 4-24 and compares very favorably with the cost of a typical cooked medium. An advantage of this instant medium relative to proprietary formulations is that the exact constituents are known, which allows the specific nutritional properties of the diet to be varied as needed, by changing the ratio of yeast and glucose to potato flakes, for example. If freezer space is available, the media could be mixed and frozen in containers, then thawed prior to use. Bulk storage of the mixed dry ingredients is not recommended unless individual use aliquots are made, as there will be separation of the ingredients with time.

**The use of a vertical screw-type sausage stuffer to dispense fly food.**

Dispensing requires a device with a large enough diameter to allow the dough-like medium to be easily scooped in, a capacity large enough to fill a reasonable number of vials and bottles, and, most importantly, sufficient power to dispense this or another viscous medium. Manual or electric caulking guns and similar devices have been used with simple media, such as mashed bananas (Bennett), but unless the media can be mixed directly inside the cylinder of the gun (difficult given the small diameter of the tubes) filling them with medium is problematic. Also because of their relatively small size, most caulking guns will require repeated refillings as the vials are filled, detracting from any added efficiency afforded by the gun. The *Drosophila* Species Center (<http://stockcenter.ucsd.edu>) mixes a saguaro potato media in a resealable plastic bag, cuts off a corner, and dispenses the media into bottles directly from the bag. A similar approach might be used to fill a caulking gun or other device with media, but would probably not work well for the direct filling small bottles or vials, since the media would not be forced to the bottom of the vials by the bag, which would result in food adhering to the sides of the container.

An alternative to these dispensing approaches is the use of a screw-type sausage stuffer, which is normally used to force chopped meat into sausage casings. To dispense with the stuffer, the medium is mixed in a bowl and then scooped into the stuffer cylinder. Turning the hand crank lowers a plunger into the cylinder, forcing the medium out of a dispensing tube into the bottoms of vials or bottles. Since this or any other instant media is very viscous, each turn of the crank results in a fairly continuous rate of extrusion, allowing a single operator to use the device while managing the empty and filled vials and bottles. The author can easily fill ~15 standard 30 mm vials/min with ~12 g media using this device. At the time of this writing, a 5-lb capacity, vertical sausage stuffer (Lem model #606) can be purchased new for about \$130. Other models of stuffers would also work, but screw-type stuffers, including the model used here, are easier for a single operator to use than other types.

References: Bennett, J., 1961, *The American Biology Teacher* 23(2): 79-82; Dueñas, I.E., M.E. Heres, P.L. Castañeda, and U. Graf 2002, *Dros. Inf. Serv.* 84: 166; Laverly, T.R., 1986, *Dros. Inf. Serv.* 63: 146.