by blending in an electric blender. The bentonite fraction, which does not pellet in 3 min at 600*g* (first centrifugation), but which does pellet in 15 min at 5500*g* (second centrifugation), is resuspended in 100 mL of buffer and kept overnight at 4°C. The following day, the same procedure is repeated, but the last pellet obtained is finally resuspended in 50 mL of buffer A using a blender, resulting in a suspension ready to use, and which contains about 40–50 mg/mL of bentonite. It is important to correctly estimate the bentonite titer by weighing 1 mL of the bentonite suspension after water evaporation.

3.2. Virus Purification

3.2.1. Grinding

ACLSV is propagated in the herbaceous host *Chenopodium quinoa* (*see* **Note 1**). Symptoms vary depending on the ACLSV strain: Sunken or necrotic lesions can develop after 4–6 d on inoculated leaves, followed by systemic yellow spotting or mottling 2 d later on noninoculated apical leaves.

ACLSV is purified from systemically infected leaves harvested 7–10 d after inoculation. Leaves can be kept frozen before purification, but should not be kept longer than 2 mo at –20°C. Leaves (100 g) are homogenized in 3 vol of buffer B in a blender. Both Mg^{2+} ions and polyamines tend to limit viral particle degradation during the purification process.

3.2.2. Clarification

The homogenate is strained through cheesecloth and then clarified by adding bentonite suspension in steps, starting with an initial amount of 10 mg of bentonite per gram of leaves. The homogenate is mixed, kept at 4°C for 10 min, and then centrifuged 5 min at 1400*g*. The supernatant is recovered, and bentonite is added again to a final concentration of 5 mg/g of leaves. The homogenate is mixed and kept at 4°C for 10 min, followed by another centrifugation at 1400*g* for 5 min. This step is repeated until the supernatant becomes straw yellow in color and the pellet grayish, each time using decreasing amounts of bentonite (2.5 mg/g, 1.25 mg/g, and so on).

Bentonite is used to adsorb plant material (organelles, membranes, and so on), which is then pelleted and eliminated after centrifugation. It is important to keep in mind that an excessive use of bentonite will lead to the adsorption of virus particles, and loss of virus. Therefore, the bentonite clarification procedure must be performed very carefully and should generally not exceed four successive steps.

3.2.3. Polyethylene Glycol (PEG) Precipitation

The virus is precipitated from the bentonite-clarified extract by adding PEG (mol wt 6000, Merck) to 8% (w/v) of the volume of the clarified extract (*see*