Collagen extraction for isotopic analysis

1. To ensure sufficient protein after extraction, use aprox. 200mg of bone.
2. If the simple is not powered, clean bones by ultrasound or scraping.
3. Record weights of bone used.
4. Crush bone using agate mortar or ball mil.
5. Add 1M HCl.
6. Shake by ultrasound for 15min.Centrifugar a 12.000rpm, 5 min.
7. Centrifuge at 12.000rpm for 5min.
8. Decant off the supernant.
9. Rinse with water MilliQ, centrifuge and decant off the supernant (X3).
10. Add 0.1M NaOH for 20 hours.
11. Rinse in water MilliQ, centrifuge and decant supernant (X3).
12. Gelatinize the remaining collagen pellet by heating in pH2 HCl (0.01M) at 57ºC for 17 hours. (The test tubes must be closed).
13. Centrifuge at 12.000rpm for 5min.
14. Cover the test tubes with parafilm.
15. Freeze the samples overnight at -20ºC at a sharp angle.
16. Then put into the freeze-dryer the samples for 24 hours until dry.
17. The freeze-dried material contains collagen and possibly some aacid salts. Weight tube + sample after drying and this will give you the yields of collagen.
18. Weight and encapsulate.