**SOP – Bioaccessibility of Polyphenols 5.0**

*Adapted from Minekus et al., 2014*

**Release:** Take 0.5mL (x2) at 60, 90 and 120 min. Replace with 1mL SIF. Add 20μL of 0.1M HCl and store at -20 °C.

Add 4.5 mL SGF or SGF with 0.05% gelatine. Add 25 μL 0.3M CaCl2.

Then, transfer 0.4-1.6 of film pieces**\*** or 0.024 GTE in 50mL falcon tube. Add 3.5 mL SSF, 250 μL amylose (1,500 U/mL) and 1.3 mL distilled water. Incubate in a shaker water bath for 2 min (100 rpm, 37 °C)

Bring to pH 3 with 150 μL HCl (1M). Add 250 μL pepsin (39,000 U/mL) and incubate in a shaker water bath for 2h (100 rpm, 37 °C). Add 75 μL distilled water

Use Folin SOP 2.0 to analyse polyphenolic content of all aliquots.

**Release:** Take 0.5mL (x2) at 60, 90 and 120 min. Replace with 1mL SGF and store at -20 °C.

Add 5.5 mL SIF (pH = 7). Correct pH if required with NaHCO3 (0.1M). Add 20 μL 0.3M CaCl2. Add 250 μL pancreatin (4,350 U/mL) and 4 mL distilled water. Incubate in a shaker water bath for 2h (100 rpm, 37 °C)

Dissolve 0.2-0.8g film or 0.012g GTE in 50 mL distilled water under constant stirring and quantify phenolic content by Folin SOP 2.0.

**Release:** Take 0.5mL (x2) at t=1min, 2 min. Replace with 1mL SSF. Add 20μL of of 0.1M HCl and store at -20 °C.

**\***Take a small amount of film sample and place in a mortar. Disrupt the film structure to simulate mastication for 60s.

Material preparation:

* Enzyme stock solutions (can be stored up to 1 week at -20°C).
  + Salivary alpha-amylase (1,500 U/mL) = 0.007g amylase in 14mL of SSF
  + Pepsin (39,000 U/mL) = 0.325 g pepsin in 5mL of SGF.
  + Pancreatin (4,350 U/mL) = 0.1 g pancreatin in 5mL of SIF
* Stock solutions to prepare SDF and SIF:
* KCl (0.5M) = 9.4 g in 250mL
* KH2PO4 (0.5M) = 17 g in 250mL
* NaHCO3 (1M) = 42 g in 500mL
* NaCl (2M) = 29.5 g in 250mL
* CaCl2(H2O)2 (0.3M) = 11 g in 250mL
* 0.1M NaHCO3 = Dissolve 8.4 g of NaHCO3 in 1L of distilled water.
* SSF (1L): combine the following proportions of stock solution. Then adjust to pH 7 with. Split in 2 500mL Duran bottles and add 0.25g of gelatine to one of them (heat up gently under agitation to ensure full dissolution).
  + KCl (0.5M) = 15mL
  + KH2PO4 (0.5M) = 3.7mL
  + NaHCO3 (1M) = 6.8mL
  + Distilled water = bring to 1L
* SDF (1L): combine the following proportions of stock solution. Then adjust to pH 5.5 with 0.4mL of 1M HCl. Split in 2 500mL Duran bottles and add 0.25g of gelatine to one of them (heat up gently under agitation to ensure full dissolution).
* KCl (0.5M) = 17mL
* KH2PO4 (0.5M) = 2.2mL
* NaHCO3 (1M) = 32mL
* NaCl (2M) = 30mL
* Distilled water = bring to 1L
* SIF (1L): combine the following proportions of stock solution. Then adjust to pH 7.4 with HCl (1M).
* KCl (0.5M) = 17mL
* KH2PO4 (0.5M) = 2mL
* NaHCO3 (1M) = 107mL
* NaCl (2M) = 24mL
* Distilled water = bring to 1L