# Effects of pH, stirring rate, reaction time, and sequential ultrafiltration of whey protein solution on recovery and purification of glycomacropeptides

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SUPPLEMENTARY FILE

Figure S1. Flow process used for whey protein solution (WPS) and different mixing-pretreatments of feed solutions before their running in two sequential UF-1 and UF-2 for the four experiments of TRT-CON, CON-TRT, TRT-TRT and CON-CON.
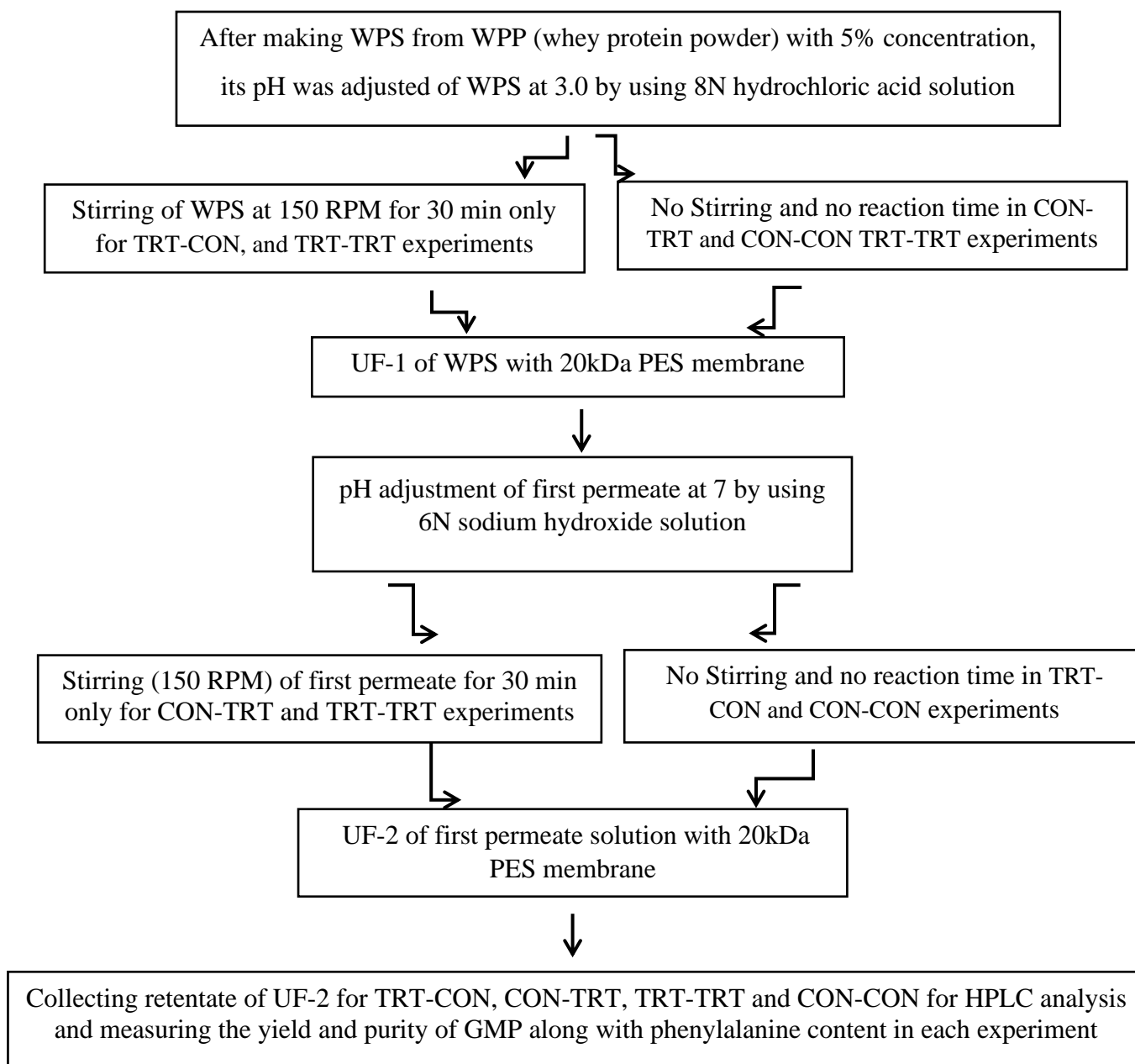
Description I.

Specification of different parameters used in 6 equations of ultrafiltration

Δm collected permeate weight (kg) or volume (L),

t   time (s)

A   membrane active surface (m^2)

μ_w viscosity of water (Pa. s)

L_p^0 membrane hydraulic permeability ($ms^{-1}Pa^{-1}$).

J_w water permeate flux through the membrane (ms^{-1})

ΔP transmembrane pressure (Pa)

L_p^4 membrane hydraulic permeability (after washing with acid)

J_w^4 water permeate flux after every experiment.

L_p^2 membrane hydraulic permeability (after washing with water)

J_w^2 Water permeate flux after this treatment

Different fouling including complete blocking, standard blocking, intermediate blocking, and cake formation happens during membrane filtration of different compounds. To identify the mechanism for the formation of predominant fouling researchers theorized Eq. 8 between permeate volume and filtration time during ultrafiltration (Mah et al. 2012).

$$\frac{d^2t}{dv^2} = k\left(\frac{dt}{dv}\right)^i \tag{8}$$

Where k and i are the fouling factor and blocking index, respectively. If $i = 0$, and the curve of t/v versus v is linear, then cake formation is the dominant mechanism. If $i = 1.0$ and the curve of $\ln(t)$ versus v is linear, intermediate blocking is the dominant mechanism. If $i = 1.5$ and the curve of t/v versus t is linear, standard blocking is the dominant mechanism. If $i = 2$, complete blocking is the dominant mechanism. These rules show the dominant fouling mechanism at any time of a specific UF process.
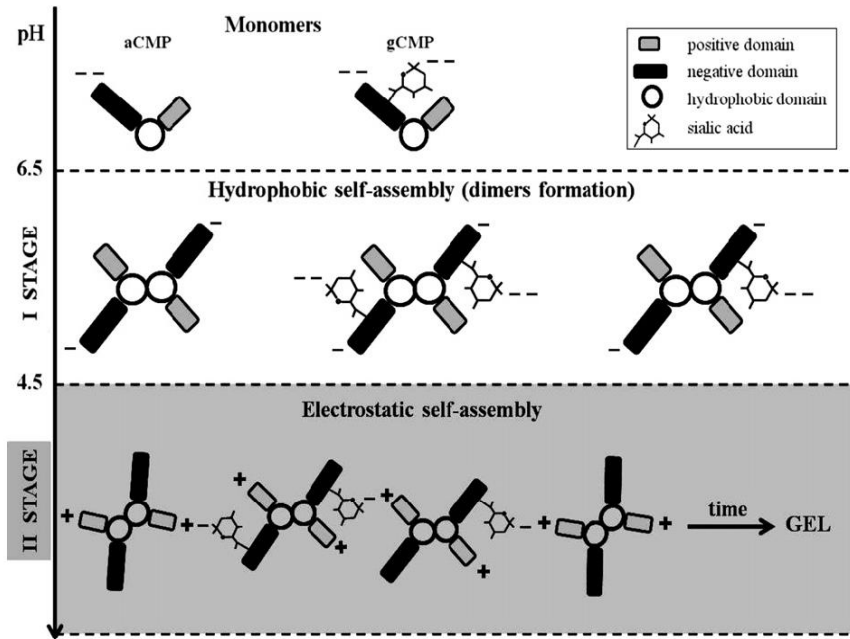
Figure S2. According to Kreub et al. (2008 and 2009), the self-assembling of GMP-A (or g CMP) and GMP-B (or a CMP) available in casein-derived peptides or CDP take places in two stages. In the first stage, the pH reduces from 6.5-7.0 to 4.5, and the dimer forms of two GMPs are made (as irreversible hydrophobic reactions) due to the absorption of positive and negative sides of each compound. In the second stage, the pH reduces from 4.5 to < 3.0, and tetramer compounds are generated by the attachment of two forms of GMPs dimers form (one with the negative charges of sialic acid, and the other one with positive peptide charges) because of electrostatic generation. Polymerization of GMP-A and GMP-B happens when the generated tetramers have enough time for the continuation of this process.

Description II.

Specifications of different reactions take place during (acidification & mixing), neutralization & mixing) respectively before UF-1 and UF-2

When pH of acidified liquid whey is reduced from its original (~ 6.7) to <3.0, it affects the polymer formation of GMP-A and GMP-B in WPS. Actuality, the negative charges of the glutamine and asparagine residues of GMP-A are attached to the C-terminus of the sialic acid. Consequently, this

new residue of GMP-A is masked with the hydrophobic domains of the peptide chain. The peptide domains cannot interact with the hydrophilic part of WPS because of its zeta potential and its generated charged particles (More detail in **Figure S2 of the SF**). In other words, the GMP-A and GMP-B compounds repeal each other when zeta potential is high at these conditions (Kreub, **2009**). Since GMP-A has a higher content of carbohydrate residues (mainly sialic acid) and phosphorus than GMP-B, they are heterogeneous compounds. Additionally, the sialic acid of GMP-A and peptide chain of GMP-B respectively have hydrophilic and hydrophobic properties. Consequently, the IP of GMP-A (pH=2.2) is lower than the one in GMP-B (pH=4.0). Therefore, the monomers of GMP-A and GMP-B require acceptable time for dimmer, tetramer, and polymer formation because of pH reduction (Kreub et al., **2009**). In other words, their complete glycosylation can convert them to amphiphilic (possessing both hydrophilic and lipophilic) form (Tolkach and Kulozik, **2005**).
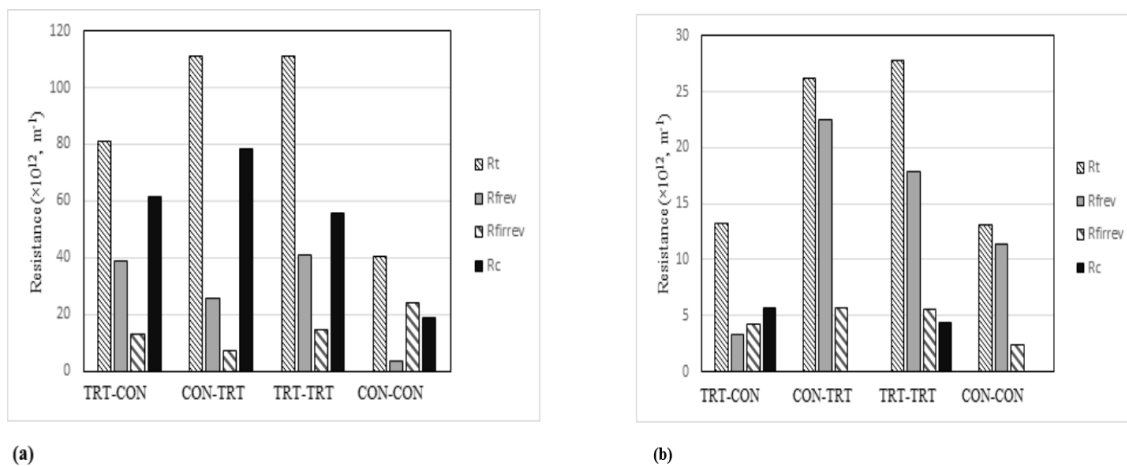

Figure S3. Values of fouling resistances obtained for running WPS (as a feed with 6.5% concentration) through two sequential UF-1 **(a)** and UF-2 **(b)** for the TRT-CON, CON-TRT, TRT-TRT and CON-CON experiments through the 20K pore-sizes of PES membrane.

Figure S4. Apparent views for separation of GMPs (GMP-A and GMP-B) from other proteins of WPS (as a feed). The right one is WPS before passing through the UF-1 and the left one is the UF-2 retentate obtained from TRT-TRT experiment with highest yield, purity, and lowest phenylalanine among the four different experiments.
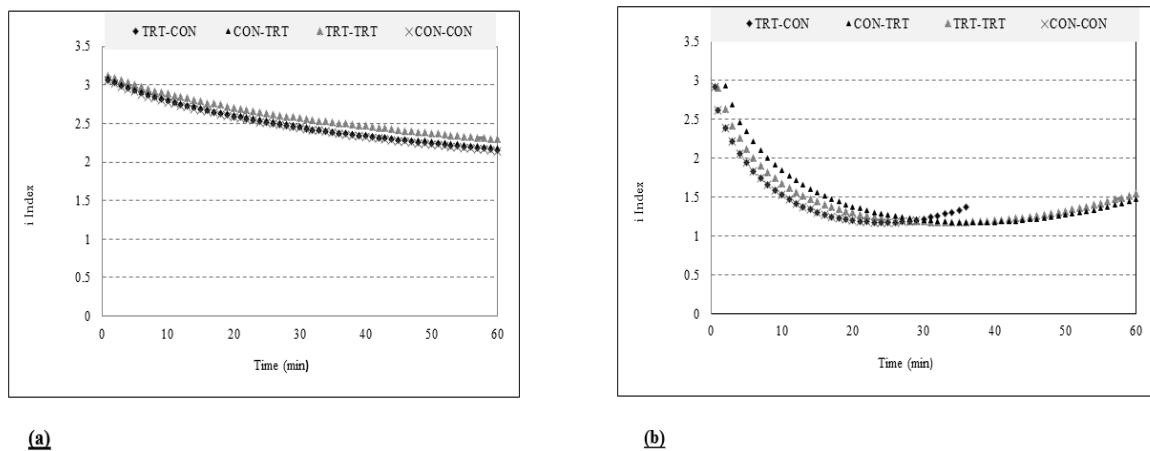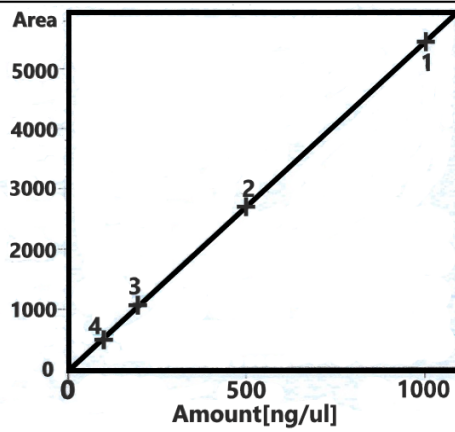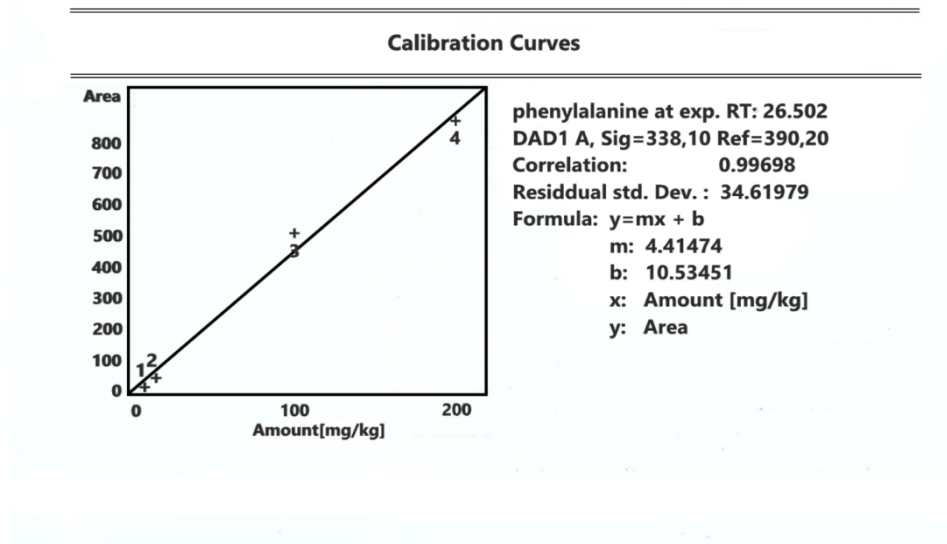

Figure S5. Effects of ultrafiltration time on the i (blocking) indexes of TRT-CON, CON-TRT, TRT-TRT and CON-CON after passing the WPS through the sequential UF-1 **(a)** and UF-2 **(b)** each one with 20K pore sizes of PES membrane.

Calibration curves

GMP at exp. RT: 2.023
MWD1 A, Sig= 226,10 Ref= 390,20
Correlation: 0.99995
Residental Std. Dev: 25.81531
Formula = y= mx + b
          m: 5.42508
          b: -13.38859
          x: Amount [ng/ul]
          y= Area

(a)- SPGMP

Calibration Curves

phenylalanine at exp. RT: 26.502
DAD1 A, Sig=338,10 Ref=390,20
Correlation: 0.99698
Residdual std. Dev. : 34.61979
Formula: y=mx + b
          m: 4.41474
          b: 10.53451
          x: Amount [mg/kg]
          y: Area

(b)-phe

Figure S6. Calibrations data for standard powder of glycomacropeptides or SPGMP **(a)** and phenylalanine or Phe **(b)**.