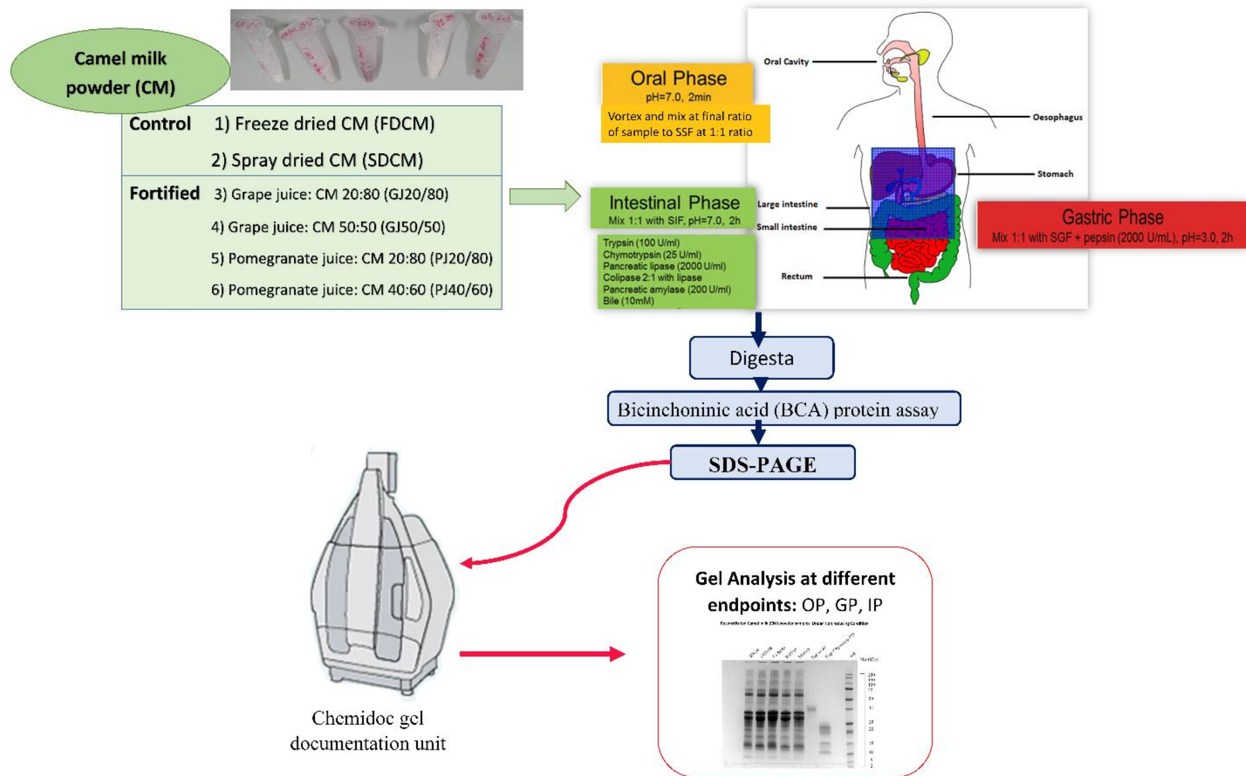
Supplementary material for the article:

Khulal, U.; Ghnimi, S.; Stevanovic, N.; Rajkovic, A.; Cirkovic Velickovic, T. Aggregability and Digestibility Study of Fruit Juice Fortified Camel Milk Powder Proteins. *LWT* **2021**, *152*, 112250. https://doi.org/10.1016/j.lwt.2021.112250.



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2 **Fig. S1**. Schematic diagram of optimized INFOGEST 2.0 simulated *in vitro* digestion protocol

3 *2.1. Camel milk (CM) samples preparation*

4 Fresh raw CM was purchased from a local farm in Al-Ain (UAE) and stored at 4°C. Fresh milk
5 was concentrated using a pilot unit vacuum evaporator (Model FT22, Armfield Ltd, UK). The
6 concentrated CM was dried using a pilot model spray dryer (Model FT80, Armfield Ltd, UK). The
7 spray dryer had a co- or counter-current flow configuration with a maximum water evaporation
8 rate of 3 l/h, the twin fluid pressure nozzle atomizer was used. The inlet temperature was set at
9 190°C. The flow rate of the feed stock was tested at 5 l/h. This spray dried CM (SDCM) was used
10 as control for the simulated *in vitro* digestion protocol.

11 Freeze dried (lyophilized) CM (FDCM) was prepared by using a pilot-scale freeze dryer. The
12 concentrated CM was lyophilized at −15 °C for 24 h then freeze dried at −80 °C for four weeks
13 under 0.05 mbar. The FDCM powder was then stored at + 4 °C until further analysis.

14 2.1.1. Fruit juice fortified CM powder preparation

15 The whole CM and fresh fruit juice (grape and pomegranate, commercial pasteurized juices
16 purchased from local supermarket, free of added sugar, artificial colors and preservatives) were
17 concentrated using a vacuum rising film evaporator (40° C to 50° C) to obtain a concentrate with
18 a concentration of 28% and 45 % total solids respectively. The concentrated camel's milk and the
19 concentrated fruit juice were heated and then blended to uniformity in an agitated vessel (at a
20 temperature between 40° C and 50° C). The blended mixture was dried using a pilot spray dryer
21 to provide a homogenous, powdered fortified dairy product. The inlet temperature of the spray
22 dryer was in the range of 130° C to 150° C (for few seconds) to preserve the vitamins and heat
23 sensitive substances (US Patent number 20190239527A1). These fortified CM powder were tested
24 for pH so as to maintain pH between 4.50 to 7.00 (refer to Table 1 and Table 2 in US Patent number
25 20190239527A1) along with various physico-chemical properties such as water activity, bulk
26 density, angle of repose, moisture content, protein and ash content (refer to Table 3, 4 and 5 in US
27 Patent number 20190239527A1).

28 2.2.1. Fortified CM sample treatment at different pH

29 The CM protein aggregation and their stability in presence of fruit juice were further monitored at
30 different pH values 2.0, 4.0 and 7.5. The fortified milk powders were dissolved in appropriate
31 buffer (pH 2.0 buffer (Gly-HCl 50 mM), pH 4.0 buffer (Acetate buffer 50 mM), pH 7.5 buffer
32 PBS) to final concentration 50 mg/ml. The pH was checked and adjusted accordingly if necessary.

33 The latter steps were repeated: the samples were gently rocked at room temperature for 1h,
34 centrifuged at 13500 rpm for 10 min then both supernatants and pellets were analyzed on SDS
35 gels. Supernatants were defatted by dichloromethane extraction (1:1 v/v) prior BPA protein assay
36 and treatment with the electrophoresis buffer.

37 *2.4. Standardized static in-vitro simulation of gastrointestinal digestion*

38 The enzymes: pepsin for gastric digestion and individual intestinal enzymes trypsin and
39 chymotrypsin activity, bile concentration was determined following the protocol by Brodkorb et
40 al, 2019.

41 Oral phase (OP): CM powder and fruit juice fortified CM powder samples were reconstituted to
42 240 ul in milliQ water then mixed with 240 ul SSF solution. Human salivary α-amylase was not
43 added to the sample. $CaCl_2$ (2.4 μL, 150 mmol/L) was added separately to avoid precipitation in
44 stock solution to achieve final concentration of 0.75 mmol/L. The reaction mixture was incubated
45 for 2 minutes at 37 °C with agitation. All reagents were previously pre-warmed at 37 °C for 5
46 minutes. Salivary amylase enzyme was not used for oral phase.

47 Gastric phase (GP): Complete oral phase digesta was then mixed with 480 μL of SGF stock
48 solution including 3μL of CaCl2 (25 mM) to achieve a final concentration of 75 μM in the
49 digestion mixture. Porcine pepsin in 10 mM HCl (2500 U/mg, cat#P7012, Sigma-Aldrich) was
50 added, to achieve a final concentration of 2000 U/mL in the digestion mixture. Gastric lipase
51 enzyme was not added in gastric phase. The mixture was adjusted to pH 3 with 1 M HCl, then
52 water was added, such that the final volume of reaction mixture was 960 μL. The reaction mixture
53 was incubated for 120 minutes at 37 °C with continuous agitation (400 rpm) in thermoshaker.
54 Control samples were run in parallel: Pepsin enzyme controls at 0' and 120' without CM sample
55 (sample replaced by sand) were also included. Digestion in one replicate tube of each sample was

56 stopped by addition of 20 µL 48 uM protease inhibitor pepstatin A (Cat #P5318, Sigma Aldrich)
57 to achieve the final concentration of 1 uM in the final reaction mixture (the other replicate tube
58 was further continued with the intestinal phase as described below). The samples were centrifuged
59 at 10,000 g for 20 minutes; the supernatant was separated from pellet (insoluble solids) and both
60 were immediately frozen at −20 °C. Protein concentration of supernatant was determined using
61 BCA assay (Thermo Fisher Scientific Inc., Bremen, Germany) after necessary dilutions.

62 Intestinal phase (IP): The resulting 960 ul of complete gastric digesta was mixed with 960µL of
63 SIF stock solution including 23µL of CaCl2 (25 mM) to achieve a final concentration of 300 µM
64 in the digestion mixture. Individual enzymes porcine trypsin (Measured activity was 194.44
65 TAME U/mg, cat #T0303, Sigma-Aldrich) and α-chymotrypsin from bovine pancreas (Measured
66 activity was 27 BTEE U/mg, Cat #C7762, Sigma-Aldrich) (to obtain final activity ratio of 4:1 in
67 the digesta) prepared in SIF was added. The bile extract porcine (measured concentration was 256
68 mM, cat # B8631, Sigma-Aldrich) was prepared in SIF and added to achieve final concentration
69 of 10mM in the final digesta. The amylase, pancreatic lipase and the colipase enzymes were not
70 added. The mixture was adjusted to pH 7 with 1 M NaOH, then milliQ water was added, such that
71 the final volume of reaction mixture was 1920 µL. The reaction mixture was incubated for 120
72 minutes at 37 °C with continuous agitation (400 rpm) in thermoshaker. Control samples were run
73 in parallel: trypsin and chymotrypsin enzyme controls at 0' and 120' without CM sample (sample
74 replaced by sand) were also included. The enzyme activity in the final digestion mixture was
75 stopped by addition of 20 µL of 480 mM AEBSF/Pefabloc SC (Cat #76307, Sigma Aldrich) to
76 obtain 5 mM final concentration in the reaction mixture. The samples were centrifuged at 10,000
77 g for 20 minutes; the supernatant was separated from pellets (insoluble solids) and immediately

78 frozen at −20 °C until further assessment. Protein concentration of supernatant was determined
79 using BCA assay (Thermo Fisher Scientific Inc., Bremen, Germany) after necessary dilutions.

80 *2.5. Protein profiling by gel electrophoresis*

81 *2.5.1. Native electrophoresis and Sodium dodecyl sulphate polyacrylamide gel electrophoresis*
82 *(SDS-PAGE)*

83 FDCM and fortified samples: GJ20/80, GJ50/50, PJ20/80, and PJ40/60 prepared with
84 sodium phosphate buffer for their solubility tests were characterized by native electrophoresis. Just
85 the supernatants were dissolved in the native electrophoresis buffer (62.5 mM Tris-HCl, pH 6.8,
86 40% glycerol, 0.01% Bromophenol Blue) prepared according to the Biorad specifications and run
87 on Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories) with the native electrophoresis
88 running buffer (10x Tris/Glycine: 25mM Tris, 192 mM glycine, pH 8.3) according to the Biorad
89 protocol based on modified Ornstein and Davis 1964 method.

90 They were also comparatively characterized via SDS-PAGE under both reducing
91 conditions and non-reducing conditions using 4-20% gradient precast gels (Mini-PROTEAN
92 TGX, Bio-Rad Laboratories) as explained below.

93 *2.5.2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)*

94 In addition to solubility test, FDCM and fortified samples: GJ20/80, GJ50/50, PJ20/80, and
95 PJ40/60 prepared with different buffers for their pH stability tests were characterized via SDS-
96 PAGE under both reducing conditions and non-reducing conditions using 4-20% gradient precast
97 gels (Mini-PROTEAN TGX, Bio-Rad Laboratories). The supernatant and the pellets protein
98 profile of the samples were acquired for pH test as well as the digestibility studies. SDCM instead
99 of FDCM was used as the control sample in case of digestibility study. The detailed steps of sample
100 preparation for the SDS-PAGE are explained below.

101 For CM protein digestibility study, the protein concentration was determined by
102 Bicinchoninic acid Assay (BCA) (Smith et al., 1985) in triplicates using Bovine serum albumin
103 (BSA) as standard then the dilutions were made to maintain uniform protein concentration of 1
104 ug/ul among all the samples. Prior to sample loading, each sample was mixed well with the
105 respective sample buffer, β-mercaptoethanol was used as a reducing agent with the Laemmli
106 sample buffer (#1610747, Bio-rad Laboratories) while preparing sample under reducing
107 conditions. The samples were prepared in the Laemmli sample buffer without β-mercaptoethanol
108 under non-reducing conditions. The samples were denatured by heating at 95°C for 5 min at 400
109 rpm on thermoshaker (Thermo Scientific), cooled to room temperature then 20 ug protein was
110 loaded unto the gel well. The gels were run on Mini-PROTEAN Tetra Cell system (Bio-Rad
111 Laboratories) following standard protocol: constant voltage of 200V for approximately 34 min.
112 Precision Plus Protein Dual Xtra standard, 2-250 KDa (#1610377, Bio-rad Laboratories) was used
113 for the molecular weight determination of the protein bands. Similarly, the pellets were also
114 solubilized with the Laemmli sample buffer with and without β-mercaptoethanol. Then, 20 ul of
115 this sample was loaded onto the gel well. After running the gel, they were stained with Coomassie
116 Blue staining solution followed by destaining to visualize the gel. Gel visualization, image export
117 and protein bands quantification were completed using Gel documentation unit: Chemidoc™
118 XRS+ and Image lab software version 6.0 (Bio-Rad Laboratories).

119 *3.2. Aggregability study of the soluble CM proteins by size exclusion chromatography (SE-*
120 *UHPLC)*

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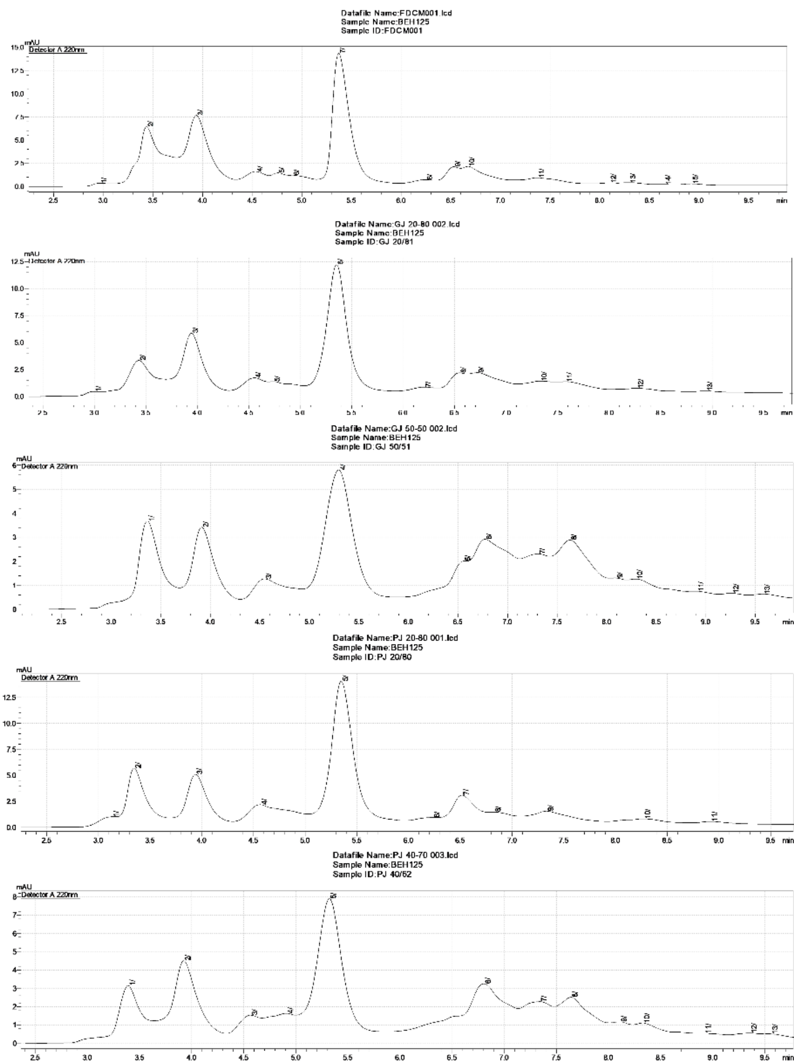


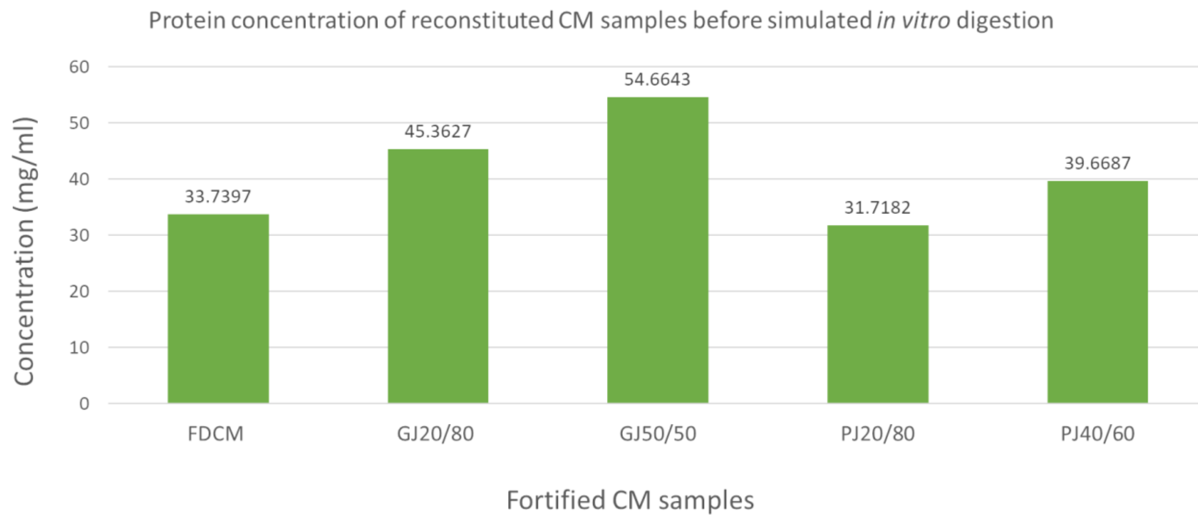
122 **Fig. S2.** Ultra-High Performance size exclusion chromatography(SE-UHPLC) chromatograms of

123 fortified camel milk powder samples in the order from FDCM as control (top), GJ20/80, GJ50/50,

124 PJ20/80, PJ40/60 (bottom).

125 **3.4.1.** 1-D Gel Electrophoresis for fortified CM protein digestibility study in supernatant (soluble

126 fraction)



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128 **Fig S3.** The total protein concentration in reconstituted fortified CM samples before simulated *in*
129 *vitro* digestion based on Bicinchoninic Acid Assay (BCA). [The protein concentrations were
130 determined in triplicates] FDCM: Freeze dried Camel milk as control, GJ20/80: Grape Juice
131 fortified CM at 20:80 ratio, GJ50/50: Grape Juice fortified CM at 50:50 ratio, PJ20/80:
132 Pomegranate Juice fortified CM at 20:80 ratio, PJ40/60: Pomegranate Juice fortified CM at 40:60
133 ratio.

134 *3.3. Gel Electrophoresis of fortified CM protein*

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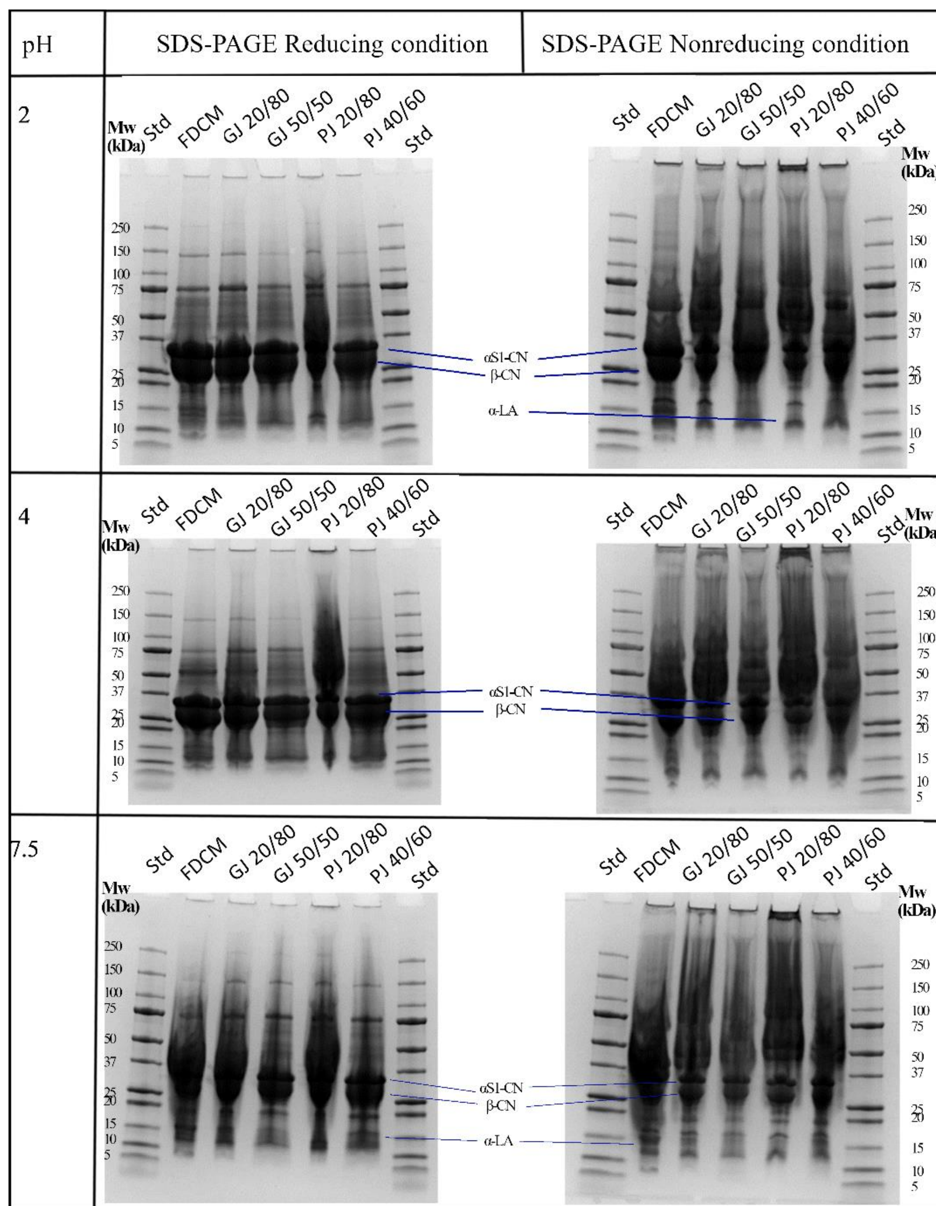

136 **Fig. S4**. SDS-PAGE electropherogram of pellets (insoluble protein) of fortified CM powder
137 samples under reducing and nonreducing conditions at varying pH. FDCM: Freeze dried Camel
138 milk as control, GJ20/80: Grape Juice fortified CM at 20:80 ratio, GJ50/50: Grape Juice fortified
139 CM at 50:50 ratio, PJ20/80: Pomegranate Juice fortified CM at 20:80 ratio, PJ40/60: Pomegranate
140 Juice fortified CM at 40:60 ratio, Std: Biorad precision plus protein standard.

141 **Supplementary Tables**

142 **Table S1**. Stock solutions preparation of simulated digestive fluids for 125 ml (4x concentration)
143 of SSF, Simulated Salivary Fluid; SGF, Simulated Gastric Fluid and 250 ml (2x concentration) of
144 SIF, Simulated Intestinal Fluid. OP, Oral phase; GP, Gastric phase; IP, Intestinal phase

Constituent	**SSF stock salt concentration (mM)**	**SGF stock salt Concentration (mM)**	**SIF stock salt Concentration (mM)**
KCl	15.1	6.9	6.8
KH_2PO_4	3.7	0.9	0.8
$NaHCO_3$	13.6	25	85
NaCl	-	47.2	38.4
$MgCl_2\ (H_2O)_6$	0.15	0.1	0.3
$(NH_4)_2CO_3$	0.06	0.5	-
HCl	1.1	15.6	8.4
$CaCl_2(H_20)_2$	1.5	0.15	0.6
pH	**7**	**3**	**7**

145 **Note**: $CaCl_2(H_20)_2$ is added immediately before use to avoid the precipitation in stock solutions
146 during storage.