
1
Co-express kappa-casein and beta/alpha casein in our "serum media" using gram-positive bacteria.
2
Caseins form protein micelles in our "serum media"
3
After removing expression bacteria and adding regular cheese starter culture, curd and later cheese is formed

FIG. 1

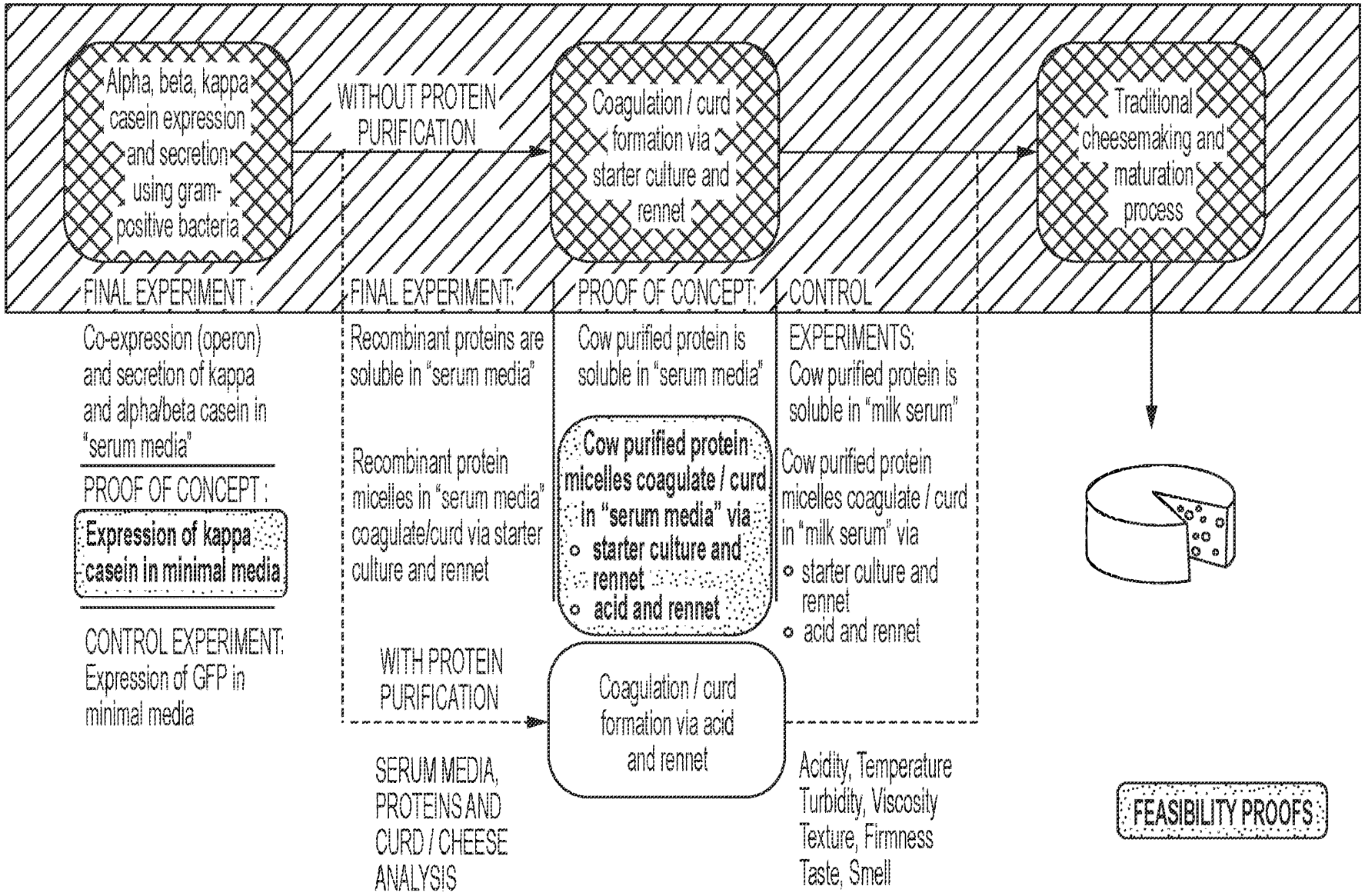
Alpha, beta, kappa casein expression and secretion using gram-positive bacteria
WITHOUT PROTEIN PURIFICATION
Coagulation / curd formation via starter culture and rennet
Traditional cheesemaking and maturation process
FINAL EXPERIMENT :
Co-expression (operon) and secretion of kappa and alpha/beta casein in "serum media"
PROOF OF CONCEPT :
Expression of kappa casein in minimal media
CONTROL EXPERIMENT:
Expression of GFP in minimal media
FINAL EXPERIMENT:
Recombinant proteins are soluble in "serum media"
Recombinant protein micelles in "serum media" coagulate/curd via starter culture and rennet
WITH PROTEIN PURIFICATION
SERUM MEDIA, PROTEINS AND CURD / CHEESE ANALYSIS
PROOF OF CONCEPT:
Cow purified protein is soluble in "serum media"
Cow purified protein micelles coagulate / curd in "serum media" via
starter culture and rennet
acid and rennet
Coagulation / curd formation via acid and rennet
CONTROL EXPERIMENTS:
Cow purified protein is soluble in "milk serum"
Cow purified protein micelles coagulate / curd in "milk serum" via
starter culture and rennet
acid and rennet
Acidity, Temperature
Turbidity, Viscosity
Texture, Firmness
Taste, Smell
FEASIBILITY PROOFS

FIG. 2

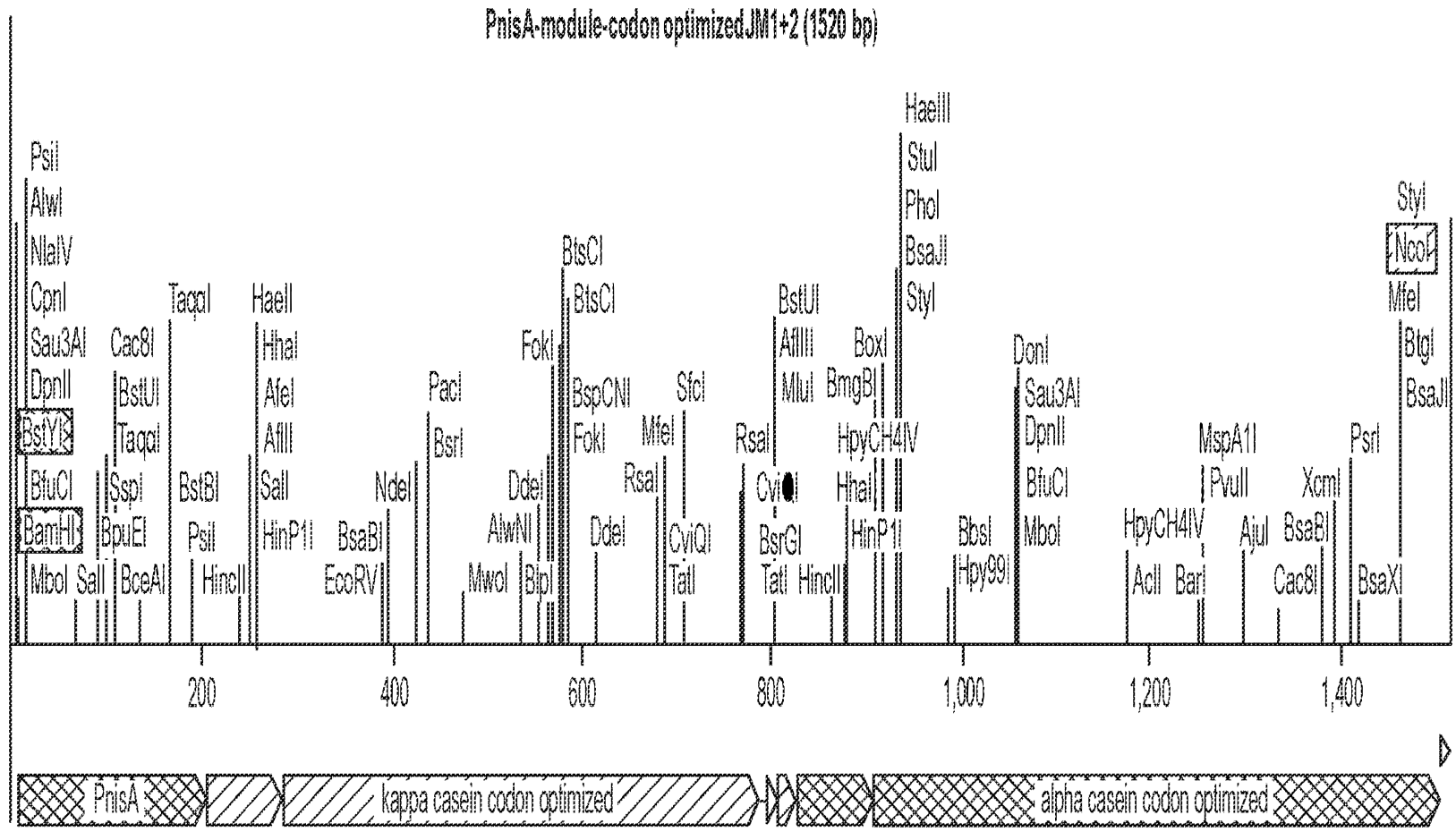
PnisA-module-codon optimizedJM1+2 (1520 bp)
PsiI
AlwI
NlaIV
CpnI
Sau3AI
DpnII
BstYI
BfuCI
BamHI
MboI
SalI
Cac8I
BstUI
TaqαI
SspI
BpuEI
BceAI
TaqαI
BstBI
PsiI
HincII
HaeII
HhaI
AfeI
AflII
SalI
HinP1I
BsaBI
EcoRV
NdeI
PacI
BsrI
MwoI
AlwNI
DdeI
BlpI
FokI
BtsCI
BtsCI
BspCNI
FokI
DdeI
RsaI
MfeI
SfcI
CviQI
TatI
RsaI
CviQI
BsrGI
TatI
HincII
BstUI
AflIII
MluI
BmgBI
BoxI
HpyCH4IV
HhaI
HinP1I
HaeIII
StuI
PhoI
BsaJI
StyI
BbsI
Hpy99I
DonI
Sau3AI
DpnII
BfuCI
MboI
HpyCH4IV
AclI
BarI
MspA1I
PvuII
AjuI
XcmI
BsaBI
Cac8I
PsrI
BsaXI
StyI
NcoI
MfeI
BtgI
BsaJI
200
400
600
800
1,000
1,200
1,400
PnisA
kappa casein codon optimized
alpha casein codon optimized

FIG. 3A

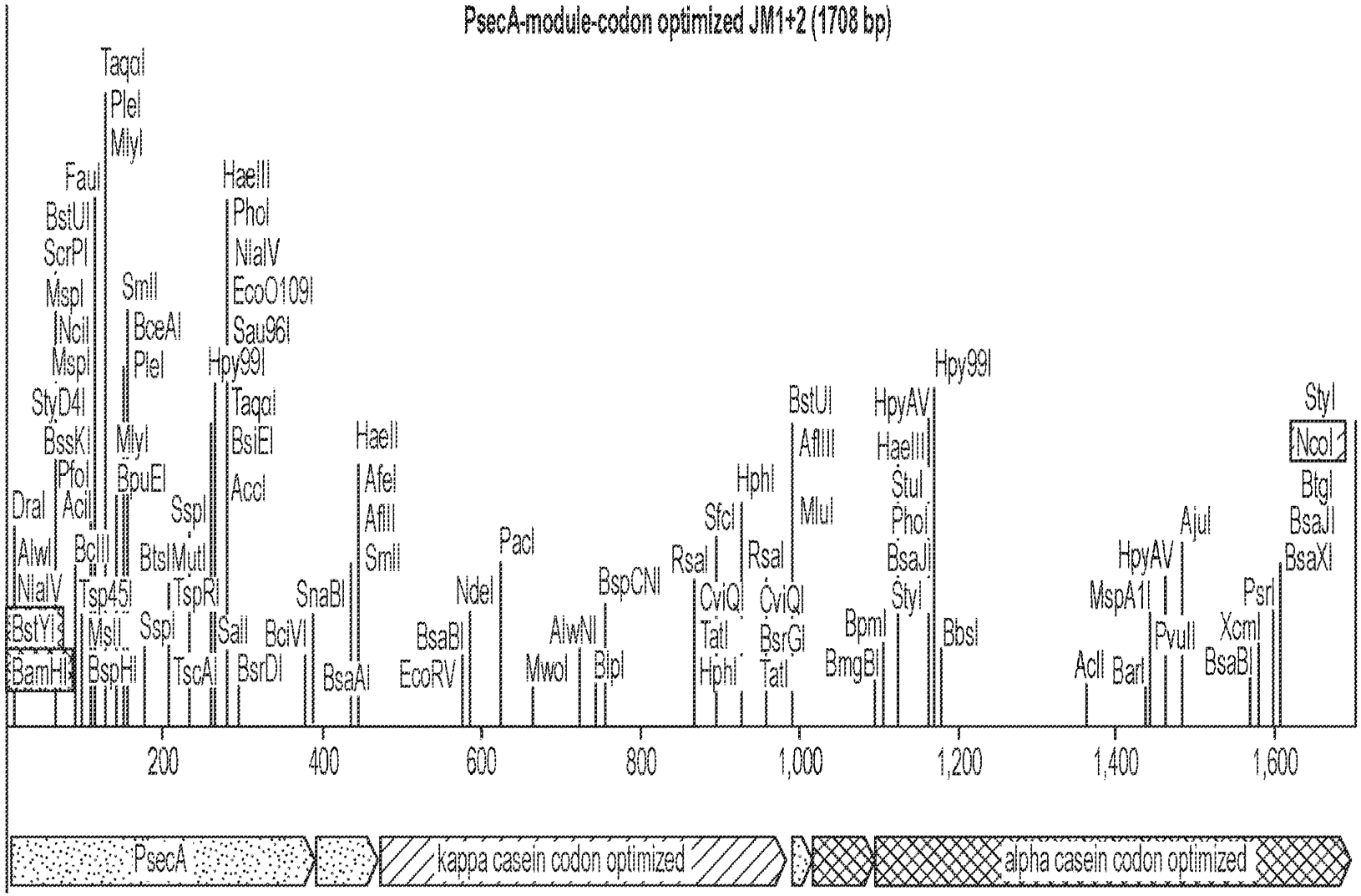
PsecA-module-codon optimized JM1+2 (1708 bp)
TaqαI
PleI
MlyI
FauI
BstUI
ScrPI
MspI
NciI
MspI
StyD4I
BssKI
PfoI
AciI
DraI
AlwI
NlaIV
BstYI
BamHI
BclI
Tsp45I
MslI
BspHI
SmlI
BceAI
PleI
MlyI
BpuEI
SspI
BtsI
MutI
TspRI
SspI
TscAI
HaeIII
PhoI
NlaIV
EcoO109I
Sau96I
Hpy99I
TaqαI
BsiEI
AccI
SalI
BclVI
BsrDI
SnaBI
BsaAI
HaeII
AfeI
AflII
SmlI
BsaBI
EcoRV
NdeI
PacI
MwoI
AlwNI
BspCNI
BipI
RsaI
SfcI
CviQI
TatI
HphI
HphI
RsaI
CviQI
BsrGI
TatI
BstUI
AflIII
MluI
BpmI
BmgBI
HpyAV
HaeIII
StuI
PhoI
BsaJI
StyI
Hpy99I
BbsI
AclI
BarI
HpyAV
MspA1I
AjuI
PvuII
BsaBI
XcmI
PsrI
StyI
NcoI
BtgI
BsaJI
BsaXI
200
400
600
800
1,000
1,200
1,400
1,600
PsecA
kappa casein codon optimized
alpha casein codon optimized

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FIG. 3B

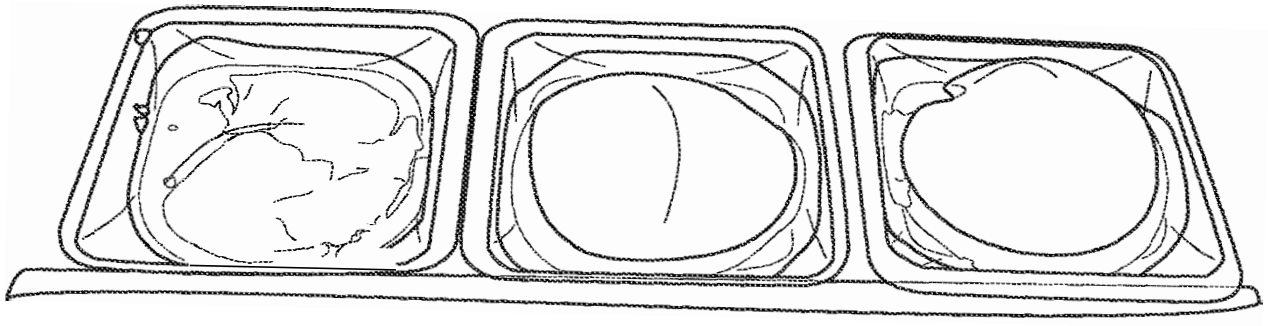

FIG. 4A

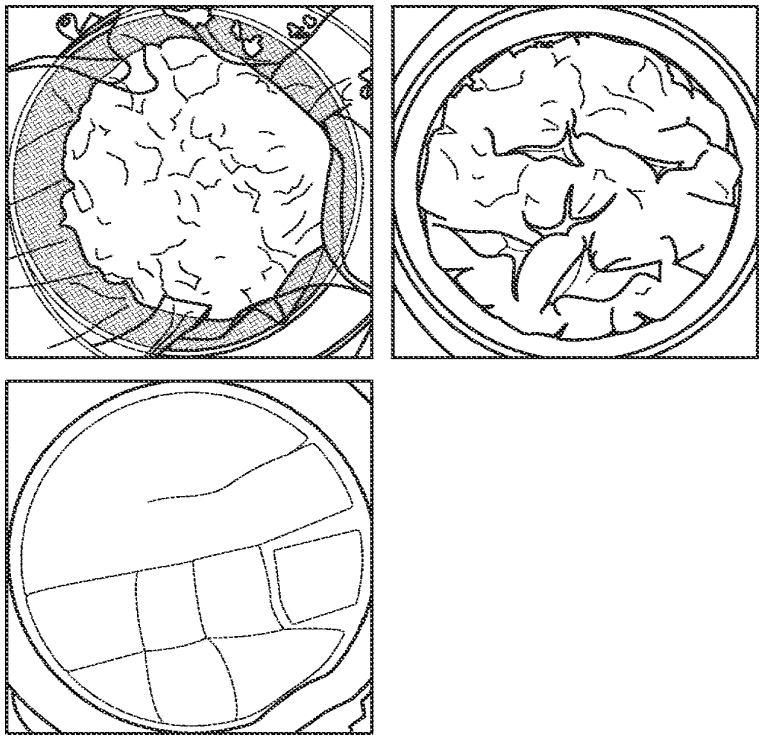

FIG. 4B

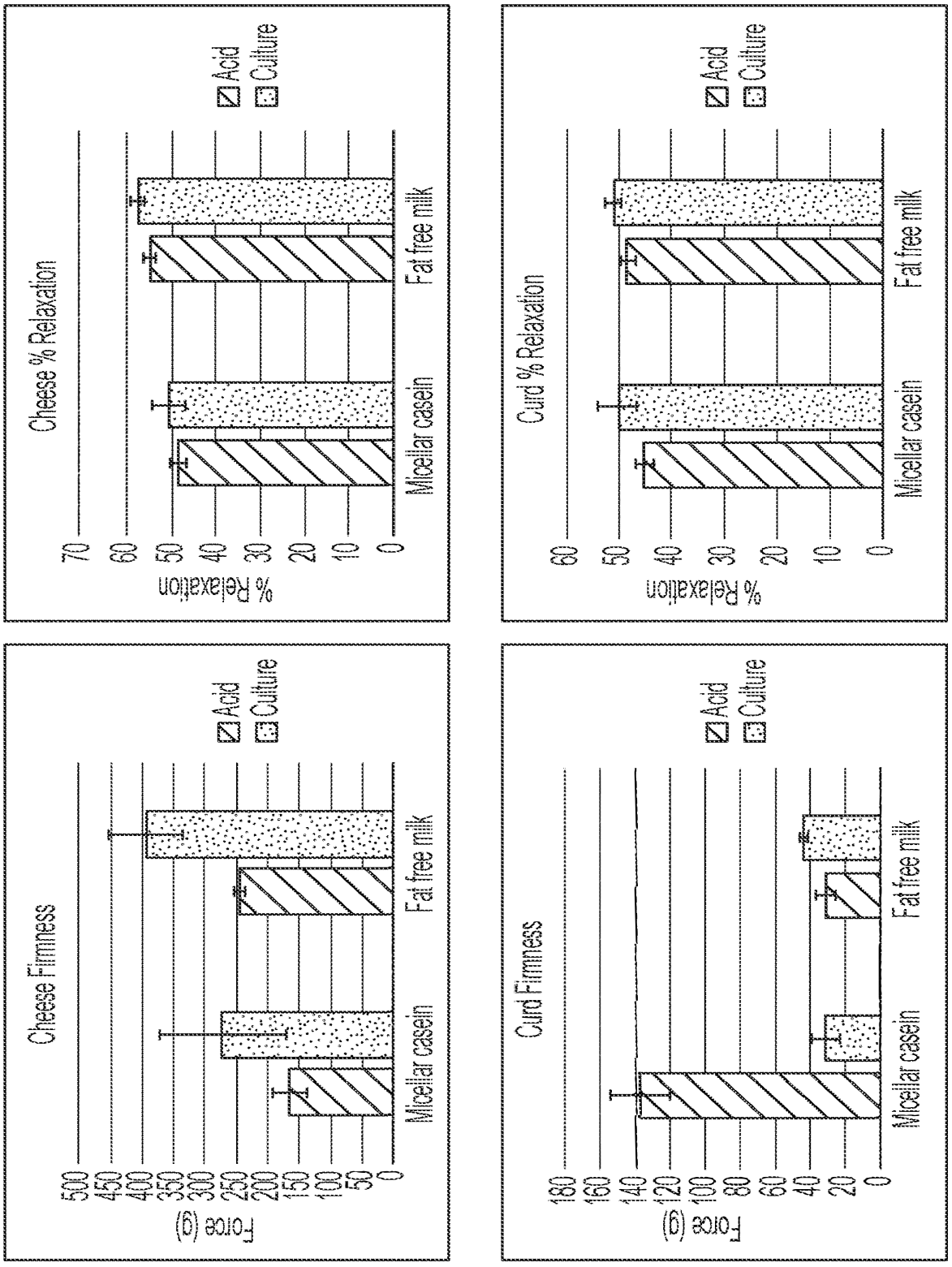
Cheese % Relaxation
% Relaxation
70
60
50
40
30
20
10
0
Acid
Culture
Micellar casein
Fat free milk
Curd % Relaxation
% Relaxation
60
50
40
30
20
10
0
Acid
Culture
Micellar casein
Fat free milk
Cheese Firmness
Force (g)
500
450
400
350
300
250
200
150
100
50
0
Acid
Culture
Micellar casein
Fat free milk
Curd Firmness
Force (g)
180
160
140
120
100
80
60
40
20
0
Acid
Culture
Micellar casein
Fat free milk

FIG. 4C

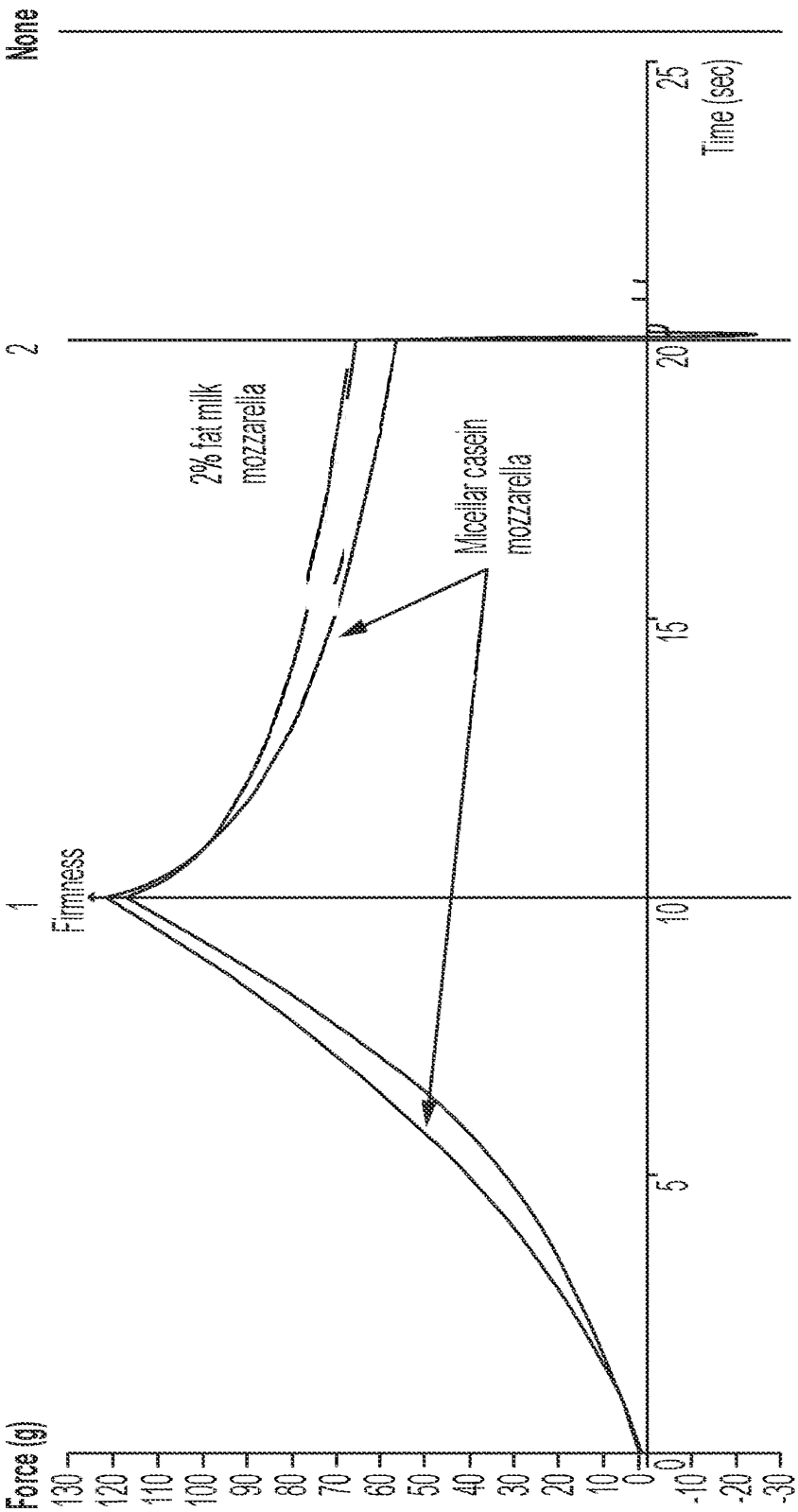
None
Force (g)
Time (sec)
Firmness
2% fat milk mozzarella
Micellar casein mozzarella
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
5
10
15
20
25
1
2

FIG. 5A

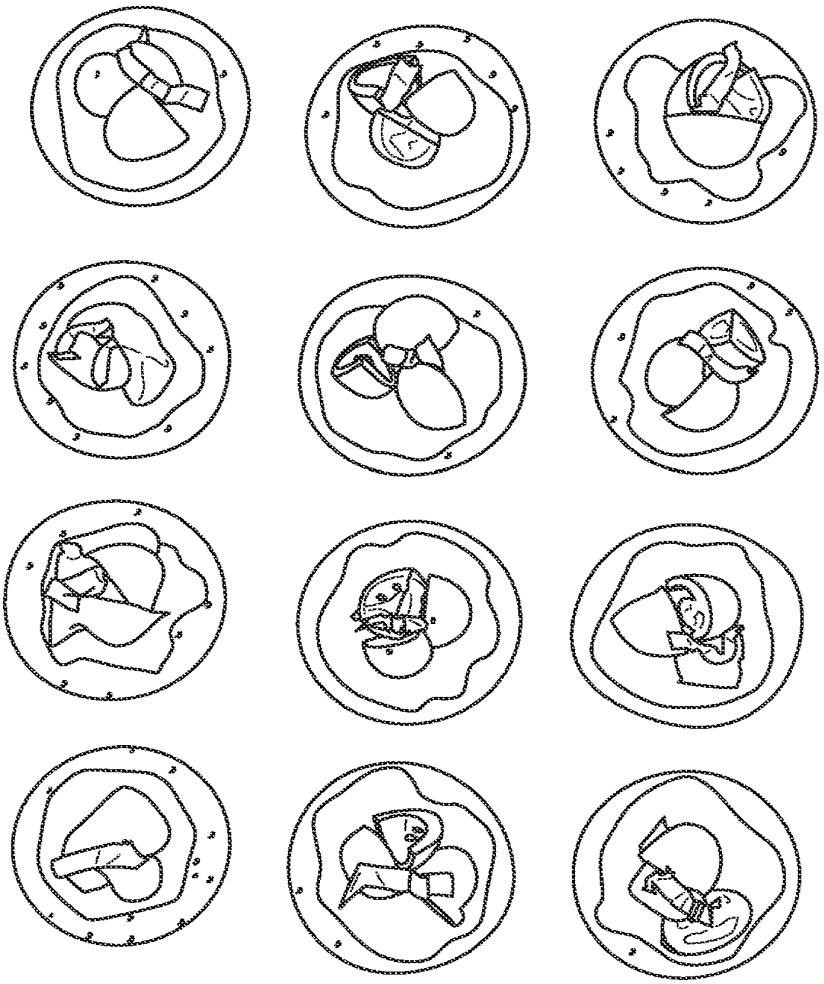

FIG. 5B

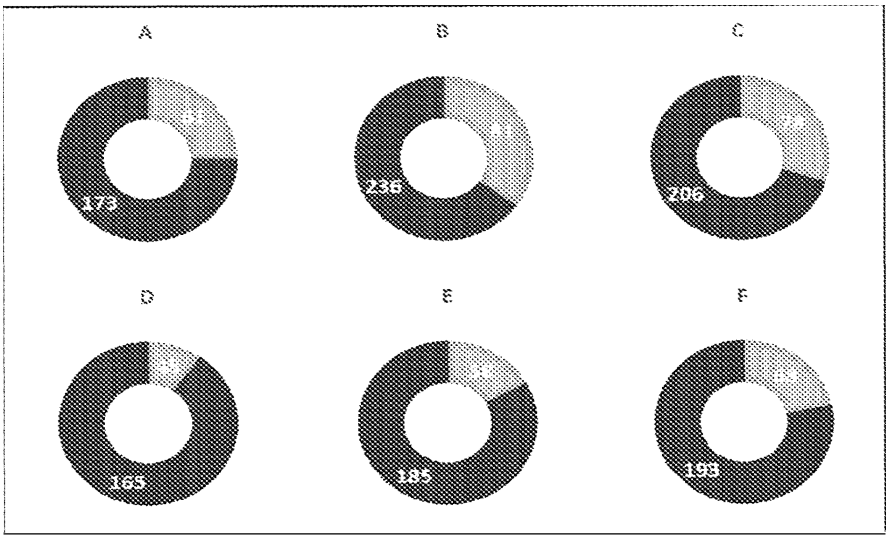

FIG. 6

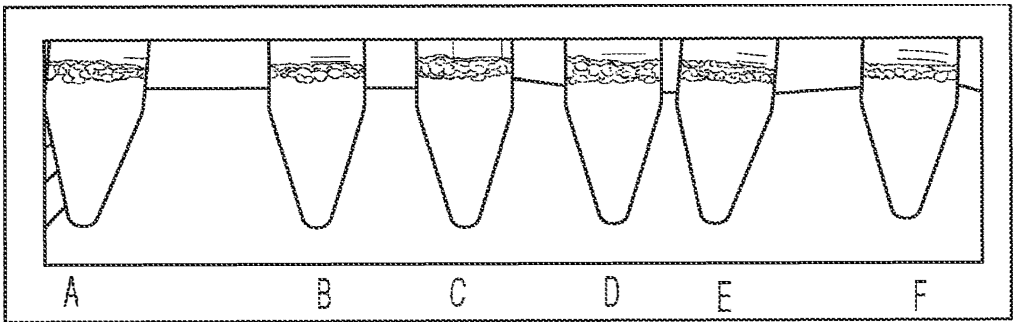

FIG. 7A

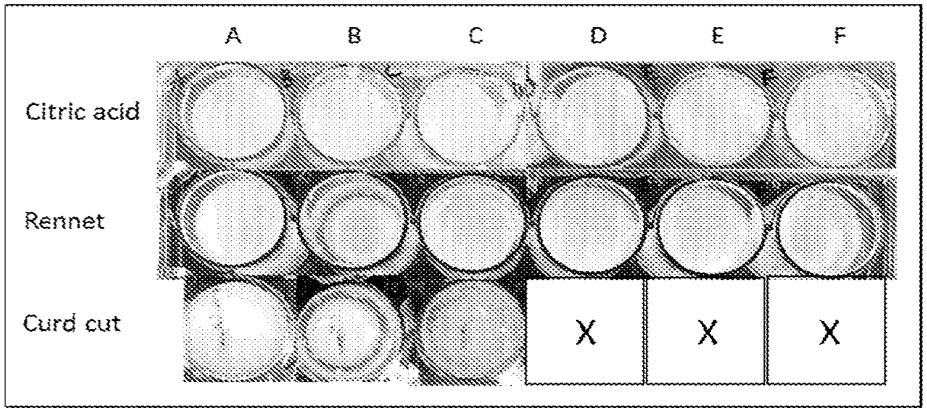

FIG. 7B

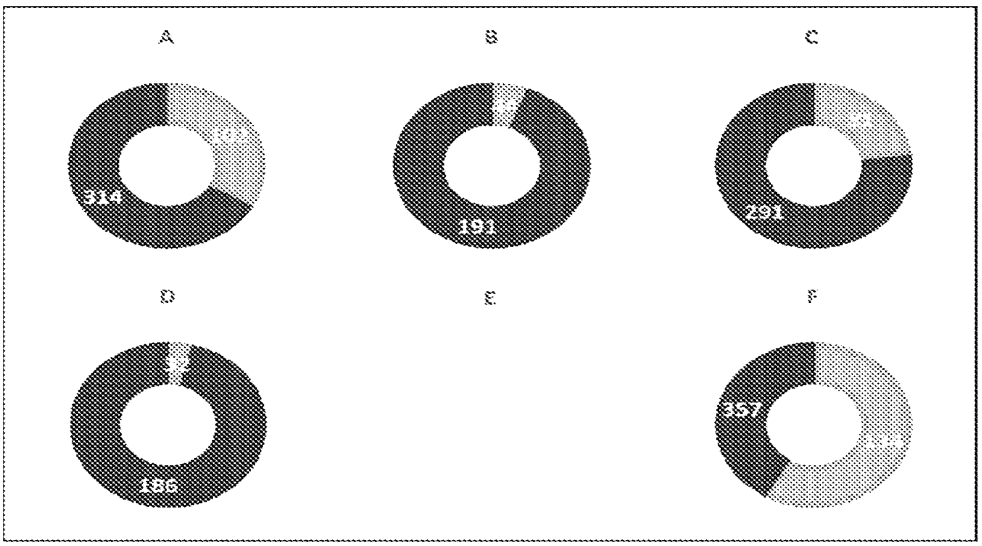
A
B
C
314
191
291
D
E
F
186
357

FIG. 8

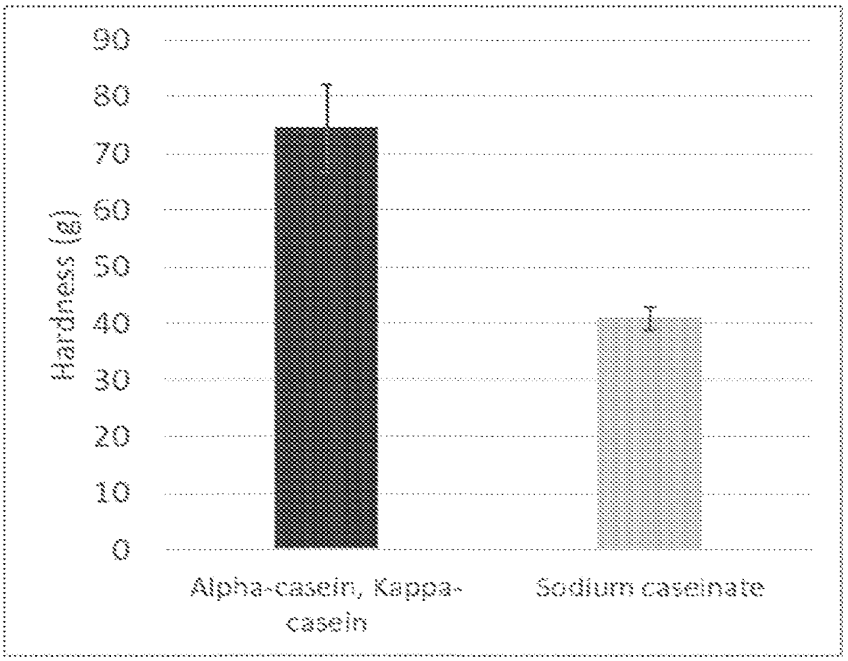
90
80
70
60
50
40
30
20
10
0
Hardness (g)
Alpha-casein, Kappa-casein
Sodium caseinate

FIG. 9A

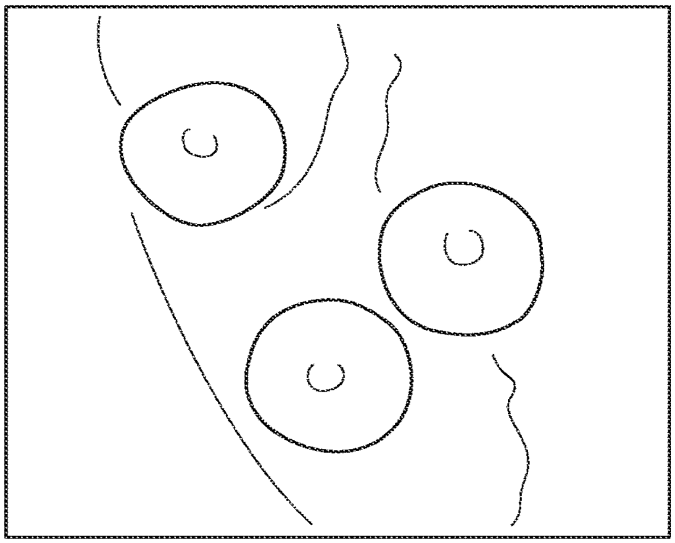

FIG. 9B

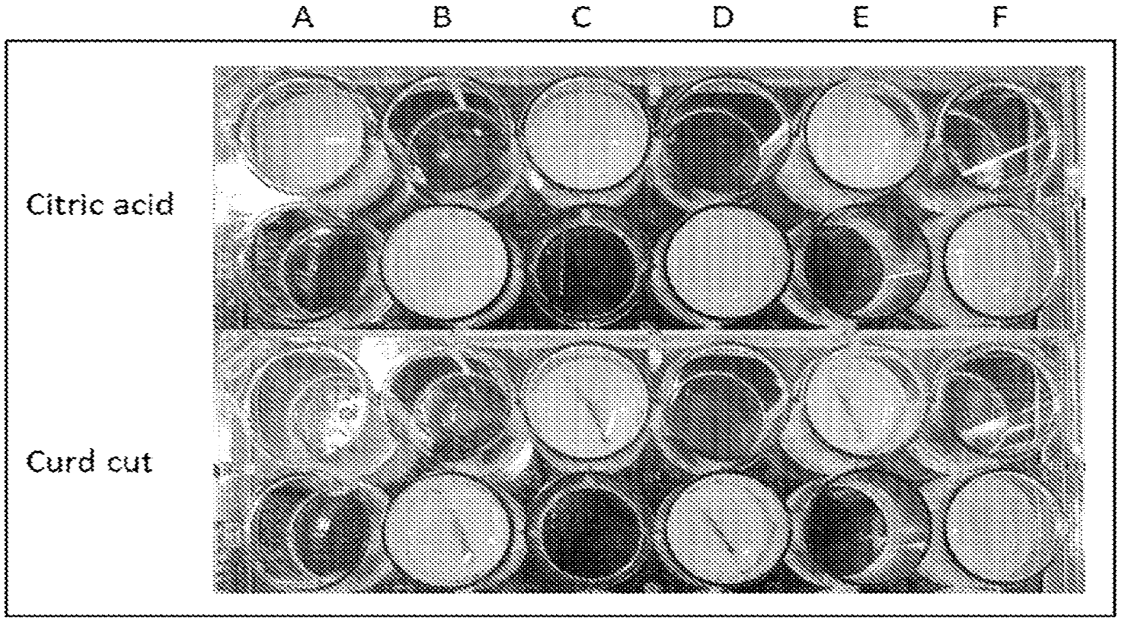

FIG. 10

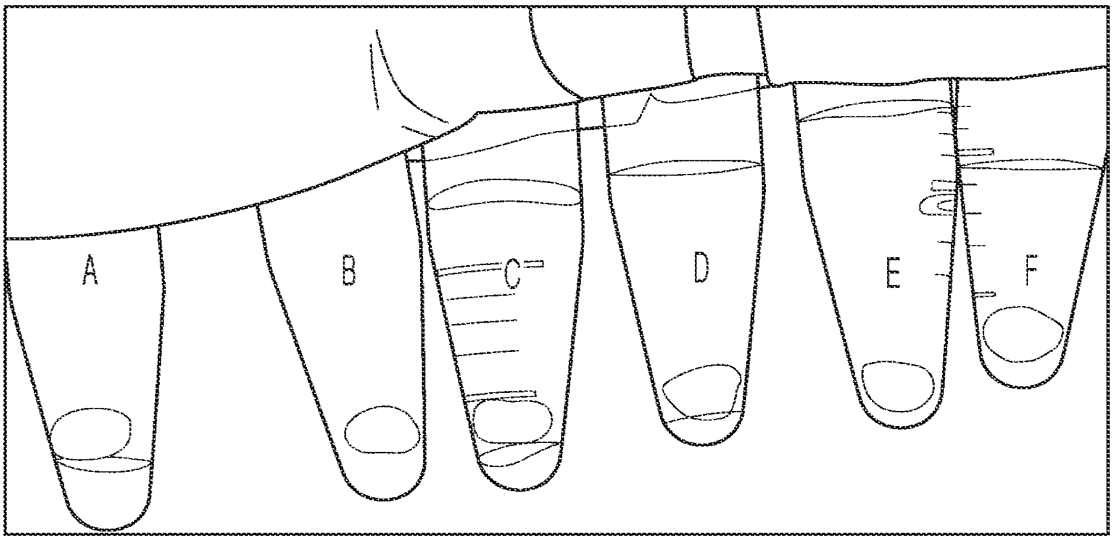
A
B
C
D
E
F

FIG. 11

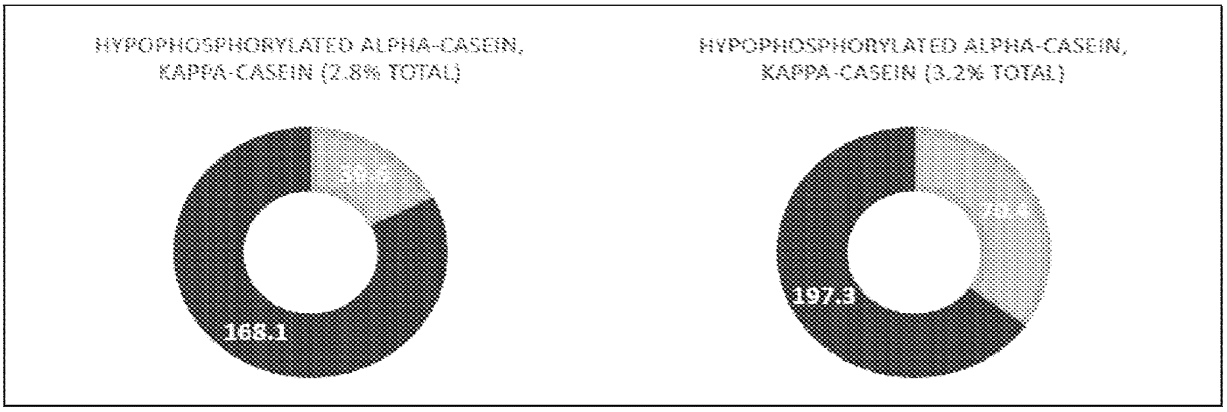
HYPOPHOSPHORYLATED ALPHA-CASEIN, KAPPA-CASEIN (2.8% TOTAL)
163.1
HYPOPHOSPHORYLATED ALPHA-CASEIN, KAPPA-CASEIN (3.2% TOTAL)
197.3

FIG. 12

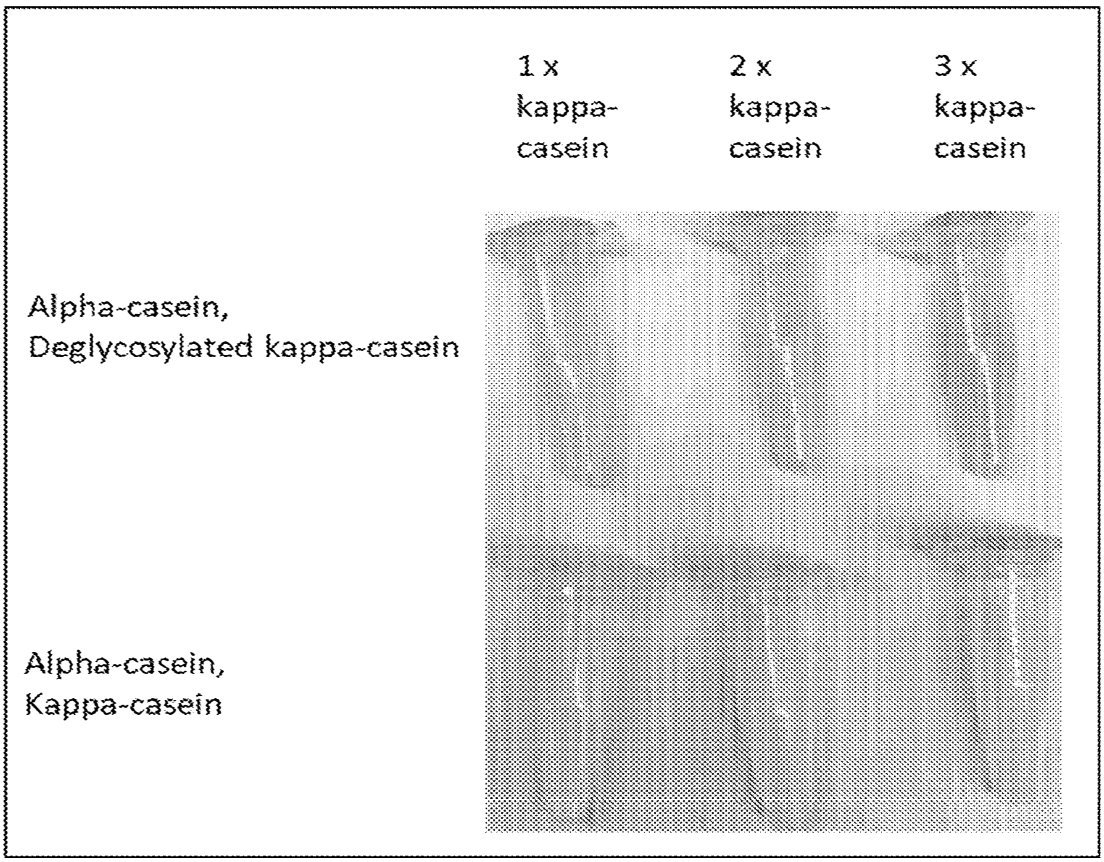
1 x kappa-casein
2 x kappa-casein
3 x kappa-casein
Alpha-casein, Deglycosylated kappa-casein
Alpha-casein, Kappa-casein

FIG. 13

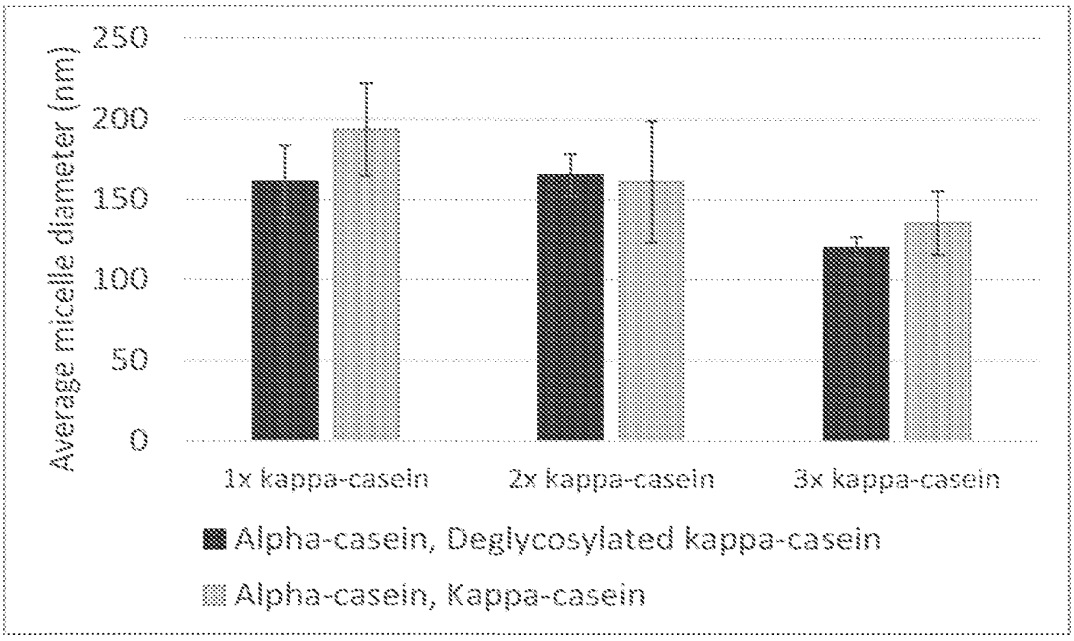
Average micelle diameter (nm)
250
200
150
100
50
0
1x kappa-casein
2x kappa-casein
3x kappa-casein
Alpha-casein, Deglycosylated kappa-casein
Alpha-casein, Kappa-casein

FIG. 14

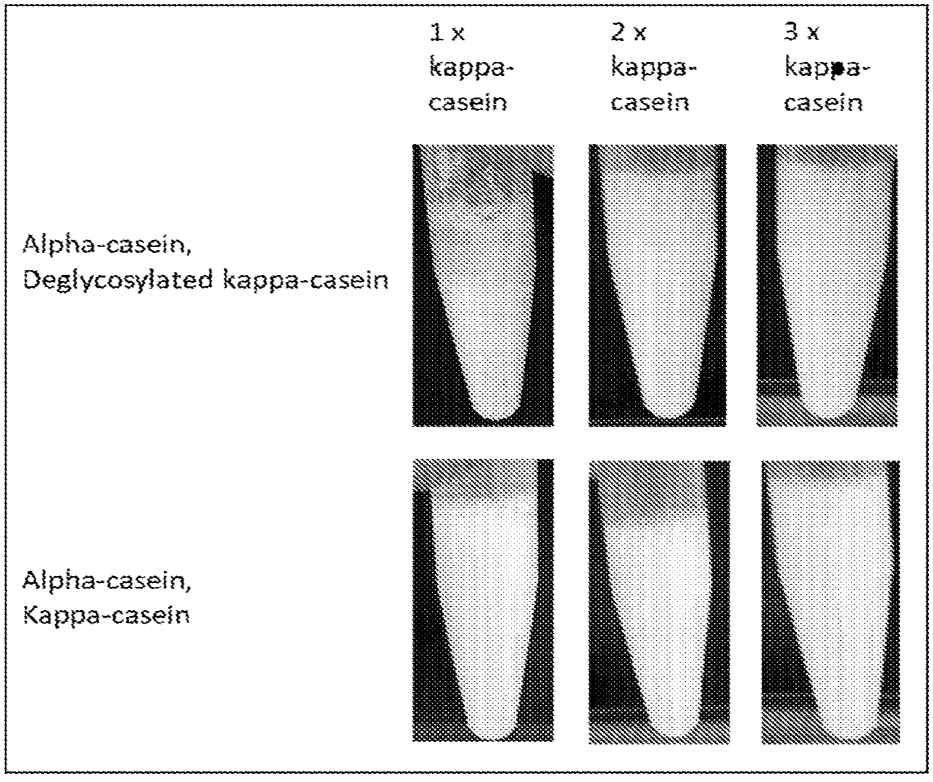
1 x kappa-casein
2 x kappa-casein
3 x kappa-casein
Alpha-casein, Deglycosylated kappa-casein
Alpha-casein, Kappa-casein

FIG. 15

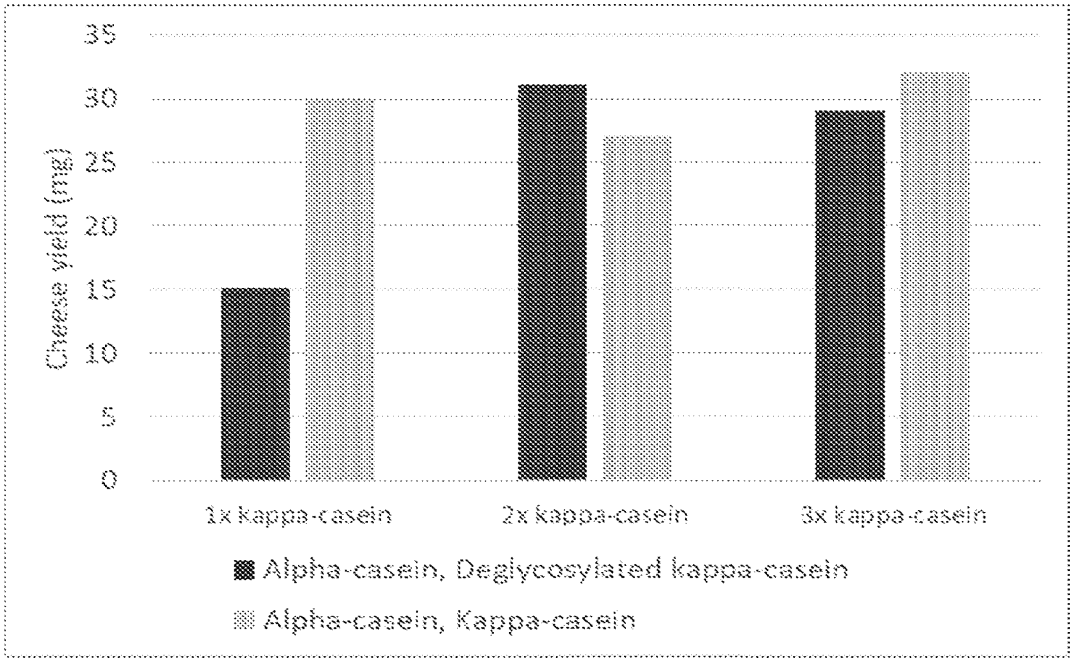
35
30
25
20
15
10
5
0
Cheese yield (mg)
1x kappa-casein
2x kappa-casein
3x kappa-casein
Alpha-casein, Deglycosylated kappa-casein
Alpha-casein, Kappa-casein

FIG. 16

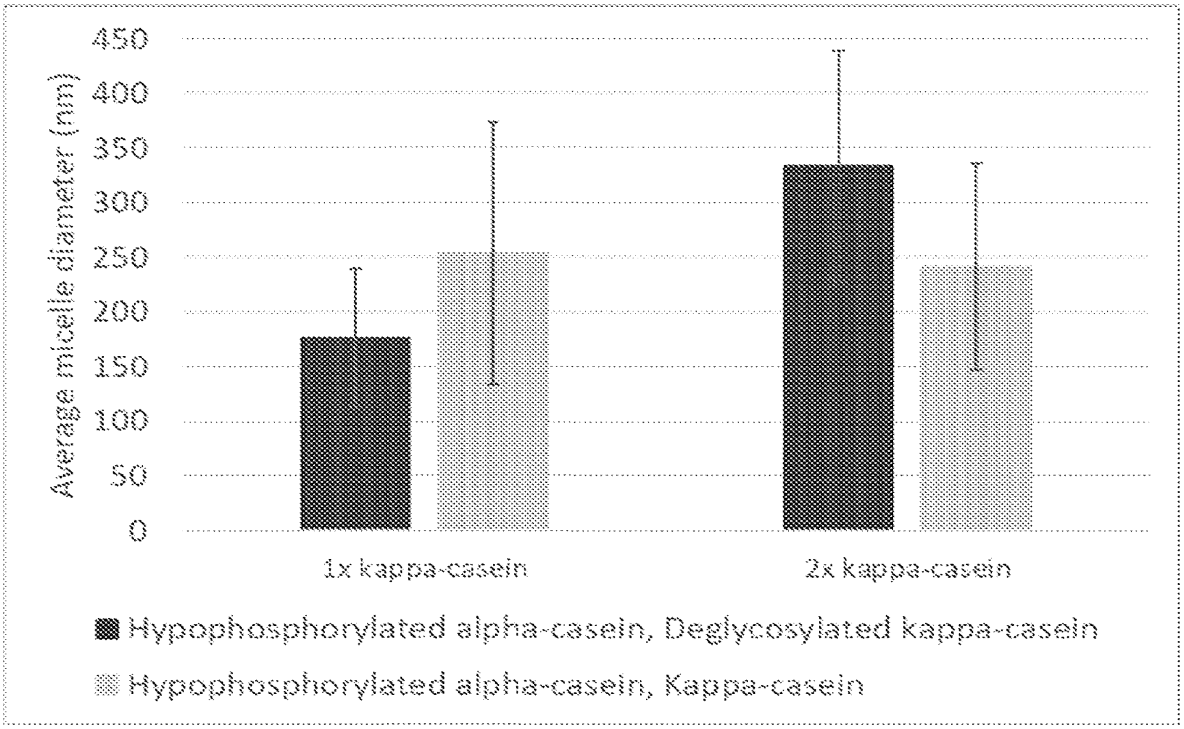
450
400
350
300
250
200
150
100
50
0
Average micelle diameter (nm)
1x kappa-casein
2x kappa-casein
Hypophosphorylated alpha-casein, Deglycosylated kappa-casein
Hypophosphorylated alpha-casein, Kappa-casein

FIG. 17

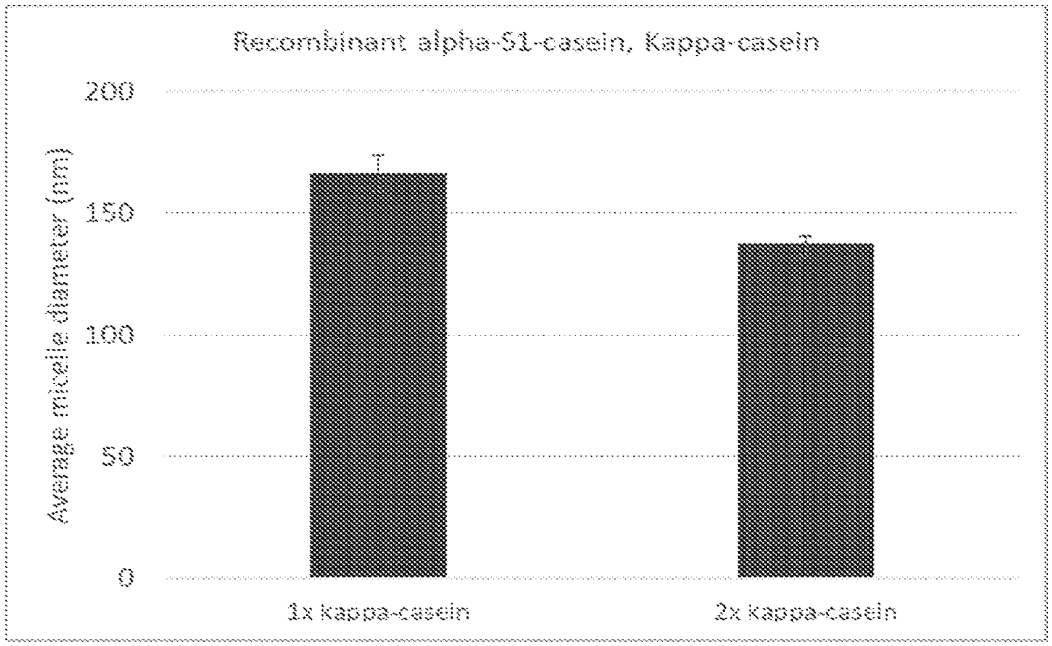
Recombinant alpha-S1-casein, Kappa-casein
Average micelle diameter (nm)
200
150
100
50
0
1x kappa-casein
2x kappa-casein

FIG. 18

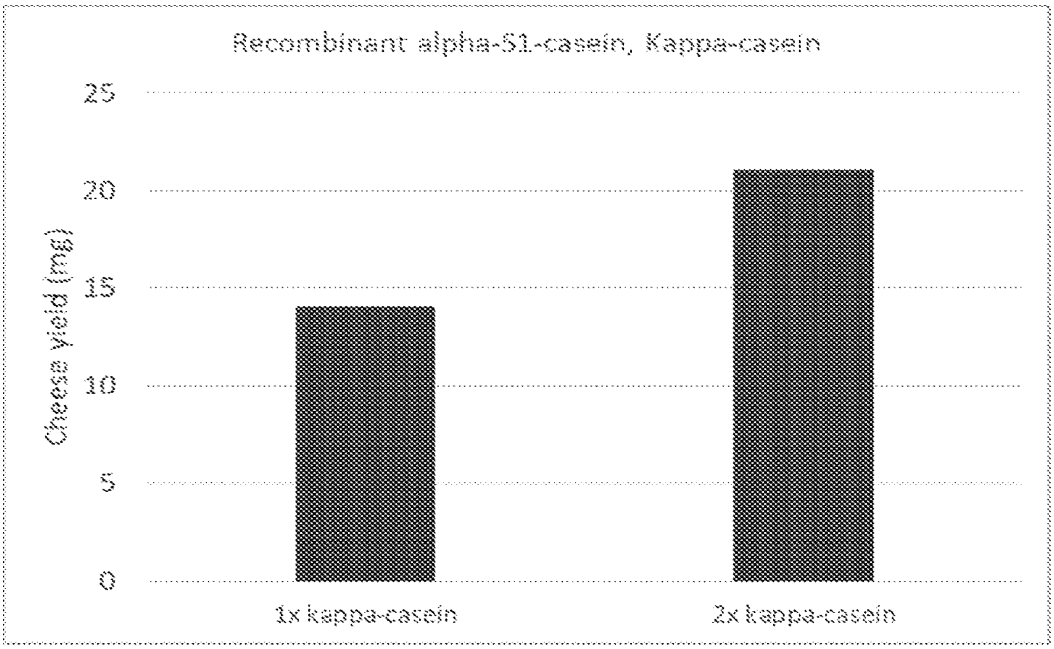
Recombinant alpha-S1-casein, Kappa-casein
Cheese yield (mg)
25
20
15
10
5
0
1x kappa-casein
2x kappa-casein

FIG. 19

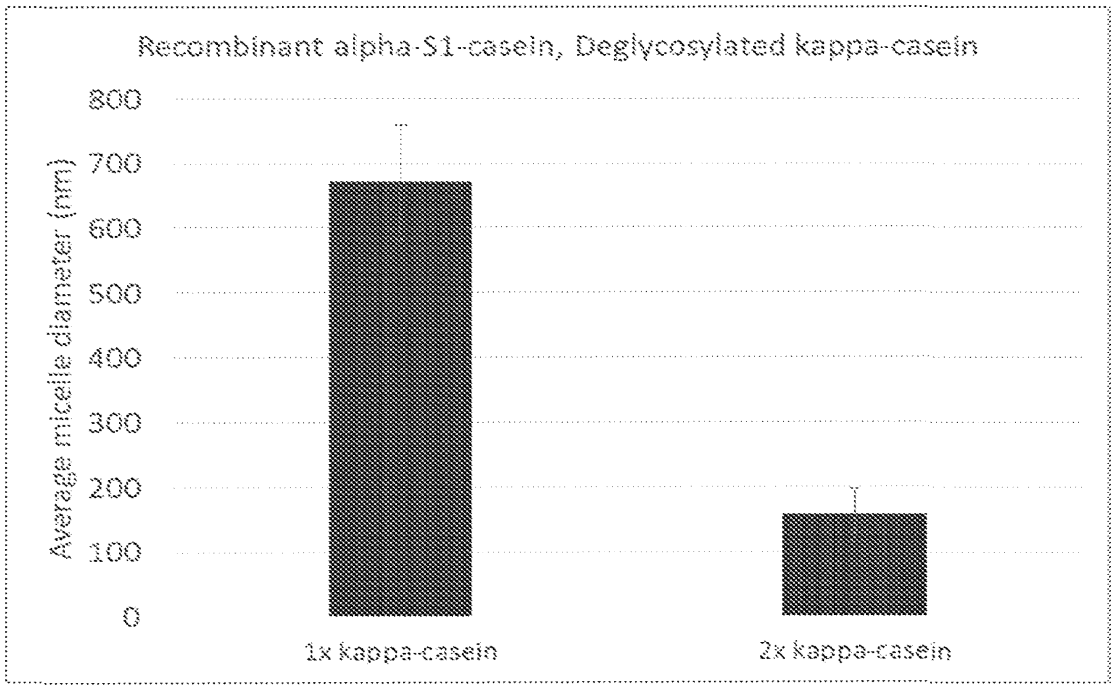
Recombinant alpha-S1-casein, Deglycosylated kappa-casein
Average micelle diameter (nm)
800
700
600
500
400
300
200
100
0
1x kappa-casein
2x kappa-casein

FIG. 20

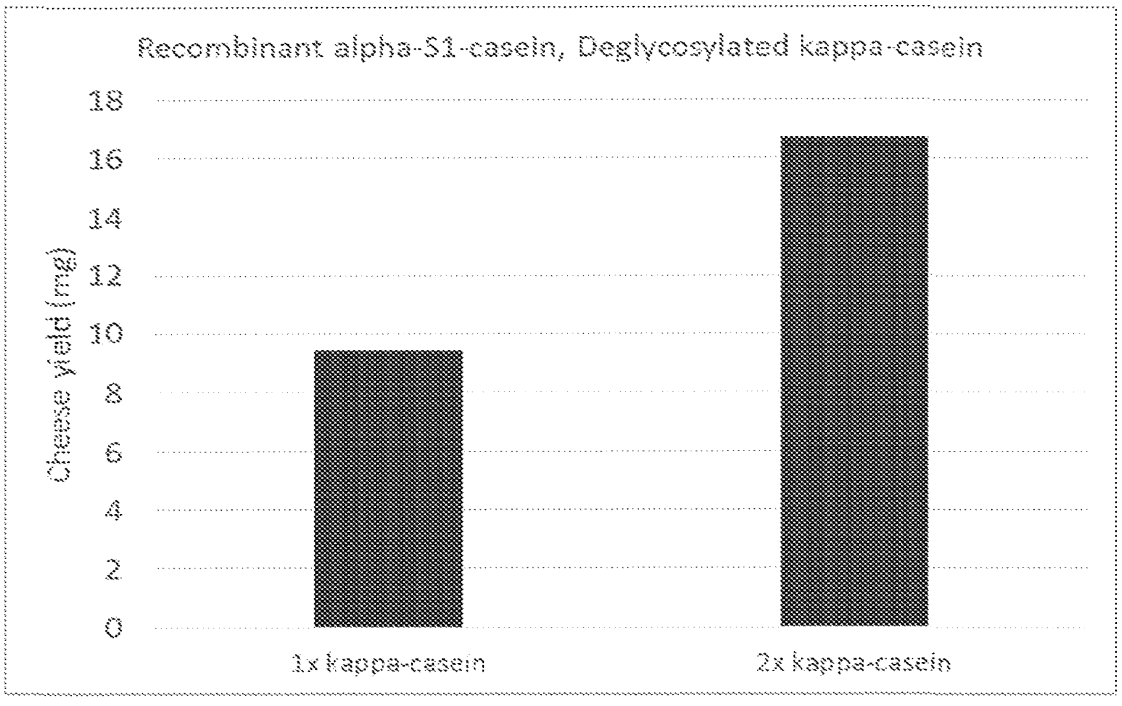
Recombinant alpha-S1-casein, Deglycosylated kappa-casein
Cheese yield (mg)
18
16
14
12
10
8
6
4
2
0
1x kappa-casein
2x kappa-casein

FIG. 21