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(54) Title: HIGH-PROTEIN CULTURED BEVERAGE PREPARED FROM REDUCED-MINERAL CASEIN

(57) Abstract: A method to make free-flowing beverages of relatively high protein concentration using mineral-depleted casein and a stabilizer and the resulting beverage.

HIGH-PROTEIN CULTURED BEVERAGE PREPARED FROM REDUCED-MINERAL CASEIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Priority is hereby claimed to U.S. Provisional Application Ser. No. 63/624,501, filed January 24, 2024, which is incorporated herein by reference.

BACKGROUND

[0002] Milk-derived, free-flowing fermented beverages such as kefir have become very popular. Kefir and other types of drinkable yogurt have a flavor that many find pleasing. As a result, they tend to find favor with athletes such as body builders, endurance runners, cyclists, and the like. Neutral pH milk protein shakes also tend to be popular with athletes for the reasons stated here, but they are not acidic or tart.

[0003] A well-known problem, though, with fermented milk beverages is that they are not as storage stable nor as temperature stable as compared to, say, electrolyte beverages, such as Gatorade, that do not contain protein. This is especially the case when the protein concentration of the fermented milk beverage approaches or exceeds about 5 wt%. At protein concentrations at or above about 5 wt%, it is difficult/impossible to keep the cultured milk beverage a free flowing, uniform liquid – even with added stabilizing compounds. At higher concentrations, the proteins react with each other, either agglomerating and causing phase separation of the liquid serum from the agglomerated proteins, or gelling and rendering the beverage no longer free-flowing (and thus not drinkable).

[0004] In the patent literature, see, for example, Rombouts et al. US 2022/0079186, published March 17, 2022. Rombouts describes a liquid composition comprised of a micellar casein concentrate (MCC) of non-bovine origin, along with food products made from the liquid. Roa et al., U.S. Patent No. 11,382,334, issued July 12, 2022, describes starch-based texturizers for use in low-protein yogurts.

[0005] Christiansen et al., US 2023/0014051, published January 19, 2023, describes a method for making shelf-stable yogurt products having viscosities from about 50 centipoise to about 20,000 centipoise and a total protein content of at least about 12 percent. About 75% of the whey protein in the product is undenatured. These products are free-flowing and drinkable. The product is made from a fermentable yogurt milk comprising the base milk to which has been added at least one casein-containing component and/or at least one whey protein-containing component, to give a whey/casein ratio of from about 20:80 to about 90:10 in the fermentable yogurt milk. The yogurt milk is then fermented in

conventional fashion and aseptically packaged. The product is heat-treated so that at least about 75% of the whey protein in the product is in an undenatured state. The Christiansen patent produces a high-protein cultured milk beverage by manipulating the ratio of proteins in milk. (In bovine' milk, the natural ratio of casein:whey is roughly 80:20. In contrast, Christiansen's beverage uses a 20:80 ratio to produce a thin, low-viscosity beverage.

[0006] WO 2017/109466, published June 29, 2017, to Arla Foods Limited, describes an extended shelf-life, protein-enriched milk product having a fat content of less than 0.5% v/v and a protein content of 4.2-4.5% v/v. The product is made by heat treating the centrifugate of fermented skim milk, cooling it to form a milk protein concentrate (MPC) and mixing the MPC with microfiltered and pasteurized skimmed milk to form the enriched milk protein product.

[0007] But there yet remains a long-felt and unmet need for a milk-derived, fermented beverage that will remain a homogenous, free-flowing liquid at elevated concentrations of protein.

SUMMARY OF THE INVENTION

[0008] Disclosed herein is a method to make a free-flowing beverage. The beverage may be derived from milk. Thus, disclosed herein are:

[0009] 1. A method to make a free-flowing beverage, the method comprising

(a) fermenting an aqueous solution comprising colloidal mineral-reduced casein for a time and at a temperature to yield a liquid yogurt having at least about 5 wt% protein concentration; and

(b) adding a stabilizer to the liquid yogurt.

[0010] 2. The method of claim 1, wherein step (a) comprises fermenting an aqueous solution comprising colloidal calcium phosphate-reduced casein.

[0011] 3. The method of claim 1, wherein step (b) comprises adding a stabilizer that comprises an anionic polysaccharide.

[0012] 4. The method of claim 1, wherein step (b) comprises adding a stabilizer selected from the group consisting of pectin, high-methoxy pectin, carboxymethylcellulose, alginate, soybean polysaccharide, carrageenan, and combinations thereof.

[0013] 5. The method of claim 1, wherein step (b) comprises adding a stabilizer comprising pectin.

[0014] 6. The method of claim 1, wherein step (a) further comprises, prior to fermenting, subjecting the aqueous solution to filtration, ultrafiltration, and/or diafiltration.

[0015] 7. The method of claim 1, wherein the liquid yogurt comprises from about 5 wt% protein to about 9 wt% protein.

[0016] 8. The method of claim 1, wherein the liquid yogurt comprises from about 6 wt% protein to about 8 wt% protein.

[0017] 9. The method of claim 1, wherein the stabilizer is added at a concentration of from about 0.5 wt% to about 3 wt%.

[0018] 10. A free-flowing beverage made by a process comprising:

(a) fermenting an aqueous solution comprising colloidal mineral-reduced casein for a time and at a temperature to yield a liquid yogurt having at least about 5 wt% protein concentration; and

(b) adding a stabilizer to the liquid yogurt.

[0019] 11. The free-flowing beverage of claim 10, wherein step (a) comprises fermenting an aqueous solution comprising colloidal calcium phosphate-reduced casein.

[0020] 12. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer that comprises an anionic polysaccharide.

[0021] 13. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer selected from the group consisting of pectin, high-methoxy pectin, carboxymethylcellulose, alginate, soybean polysaccharide, carrageenan, and combinations thereof.

[0022] 14. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer comprising pectin.

[0023] 15. The free-flowing beverage of claim 10, wherein step (a) further comprises, prior to fermenting, subjecting the aqueous solution to filtration, ultrafiltration, and/or diafiltration.

[0024] 16. The free-flowing beverage of claim 10, wherein the liquid yogurt comprises from about 5 wt% protein to about 9 wt% protein.

[0025] 17. The free-flowing beverage of claim 10, wherein the liquid yogurt comprises from about 6 wt% protein to about 8 wt% protein.

[0026] 18. The free-flowing beverage of claim 10, wherein the stabilizer is added at a concentration of from about 0.5 wt% to about 3 wt%.

[0027] 19. The free-flowing beverage of claim 10, which does not form a gel and does not phase separate after being heated to 72°C for 60 s.

[0028] 20. A free-flowing beverage comprising colloidal calcium phosphate-reduced casein and a stabilizer.

ABBREVIATIONS AND DEFINITIONS

[0029] CCP = colloidal calcium phosphate. CDR = Center for Dairy Research, Madison, Wisconsin US. HM = high-methoxy. LM = low-methoxy. MCI = micellar casein isolate. NaCas = sodium caseinate. NFDM = non-fat dry milk. SCC = soluble casein concentrate.

[0030] As used herein, the term "free flowing" as applied to a beverage is defined as the beverage being easily pourable with minimal shaking of its container. "Free flowing" may be quantitatively defined by use of a controlled-shear rheometer and application of a constant shear rate of 0.01 s^{-1} for several minutes as described by Luyten, Kloek, & van Vliet, "Yielding behaviour of mixtures of xanthan and enzyme-modified galactomannans, " *Food Hydrocoll.* 1994; **8**:431-440. Prior to analysis, the geometry (concentric cylinder) is inserted into the sample and allowed to rest, while maintained at 4°C, for 1 hour prior to the application of shear. A yield stress $\leq$ 5 Pa at 4°C is defined herein as "free flowing," and a yield stress > 5 Pa is a gel and not "free flowing."

[0031] As used herein, the terms "pectin" and "pectins" are defined broadly to include any heteropolysaccharide from any source whose principal monomeric unit is galacturonic acid, including high- and low-methoxy pectins, pectinates, pectates, acetylated forms of the foregoing, amidated forms of the foregoing, thiolated forms of the foregoing, and the like. "HM pectin" (high-methoxy pectin) is pectin in which 50% or more of the galacturonic acid residues are esterified. "LM pectin" (low-methoxy pectin) is pectin in which less than 50% of the galacturonic acid residues are esterified.

[0032] As used herein, the term yogurt is used colloquially to describe a cultured product, regardless of the method of acidification (e.g., fermentation, direct acidification, addition of glucono-delta-lactose, or other means of incorporating acid). It is not restrictive to legal definitions of yogurt as defined by the FDA, EU, or other entity which define certain allowable ingredients, starter cultures, and processing steps for yogurt.

[0033] Numerical ranges as used herein are intended to include every number and subset of numbers contained within that range, whether specifically disclosed or not. Further, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 2 to 8, from 3 to 7, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

[0034] All references to singular characteristics or limitations of the disclosed method shall include the corresponding plural characteristic or limitation, and vice-versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made. The indefinite articles "a" and "an" mean "one or more."

[0035] All combinations of method steps disclosed herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[0036] The method disclosed herein can comprise, consist of, or consist essentially of the essential elements and steps described herein, as well as any additional or optional ingredients, components, or limitations described herein or otherwise useful in synthetic / enzymatic organic chemistry.

BRIEF DESCRIPTION OF DRAWINGS

[0037] Fig. 1 is a graph showing yield stress of yogurt samples stored for 6 months at ambient temperature. The yogurt was prepared from micellar casein isolate dispersed in ultrafiltered milk permeate. Samples were thermally processed at 72°C for 60 s prior to storage. From Wilbanks et al. (2022) "Comparison of micellar casein isolate and nonfat dry milk for use in the production of high-protein cultured milk products," *J. Dairy Science* 106(1):61-74.

[0038] Fig. 2 is a histogram presenting storage modulus of yogurt samples prepared from micellar casein isolate dispersed in ultrafiltered milk permeate at 4-8% protein. After acidification, yogurts were mixed with high methoxy pectin (1.0%) and homogenized prior to analysis.

[0039] Fig. 3 is a histogram showing serum pectin content (non-adsorbed) of yogurt samples prepared at 4-8% protein from micellar casein isolate dispersed in ultrafiltered milk permeate.

[0040] Fig. 4 is a histogram showing the storage modulus (Pa) of 8% protein yogurt samples prepared with 1.0% high-methoxy pectin. High-protein yogurts were prepared from nonfat dry milk ("NFDM") in water, micellar casein isolate ("MCI") in ultrafiltered milk permeate, soluble casein concentrate ("SCC") in water, and sodium caseinate ("NaCas") in water. Samples were heated at 72°C for 60 s, cooled to ambient temperature, and measured by small amplitude oscillatory rheology.

[0041] Fig. 5 is a histogram showing non-adsorbed (serum) pectin content of 8% protein-cultured milks prepared with 1.0% HM pectin.

[0042] Fig. 6 is a graph showing the viscosity of 8% protein yogurt samples prepared with 1.0% high-methoxy pectin. High-protein yogurts were prepared from nonfat dry milk (NFDM) in water, micellar casein isolate (MCI) in ultrafiltered milk permeate, soluble casein concentrate (SCC) in water, and sodium caseinate (NaCas) in water. Samples were heated from 22-72°C over the course of 450 s, then held at 72°C for 60 s while continuously measuring viscosity at a constant shear rate of 50 s-1.

[0043] Fig. 7 is a photograph showing cultured milk samples prepared at 7% protein and 1.0% HM-pectin before (left) and after (right) heating at 72°C for 60 s.

[0044] Fig. 8 is a graph showing viscosity of yogurt beverages stabilized with 1.0% pectin prepared at 8% protein using various milk protein sources. Shown are nonfat dry milk (NFDM), micellar casein isolate (MCI), sodium caseinate (NaCas), soluble casein concentrate (SCC), and reduced calcium MPC (MPC low Ca).

DETAILED DESCRIPTION OF THE INVENTION

[0045] Cultured milk beverages include a wide range of milk-derived products that can be generally described as acidic (pH $\leq$ 4.6). Acidification is typically achieved by bacterial fermentation, although direct acidification is also be used. Examples of cultured milk beverages include yogurt beverages, yogurt smoothies, kefir, ayran, doogh, lassi, koumiss, and the like.

[0046] Acidifying milk causes the proteins to coagulate, thereby yielding a semi-solid gel. The resulting gel can be broken by stirring. However, the acidic environment promotes re-aggregation of the milk protein particles (given sufficient time). Because milk proteins are responsible for gel formation in acidic conditions, many traditional cultured milk beverages are prepared by diluting the cultured milk product in water, aqueous sugar solutions, or aqueous salt solutions to maintain a low protein concentration. The lowered protein concentration prevents coagulation and thus the beverage remains a flowable liquid (rather than a gel).

[0047] In addition to gel reformation (which is a defect in a product designed to be a flowing liquid), another major defect in cultured milk beverages is phase separation. The mechanism for phase separation in cultured milk beverages is similar to gel reformation. Protein particles aggregate under the acidic conditions to a point where the density of the aggregated protein particles cause them to separate from the liquid portion of the beverage (the serum) by gravity. The primary defect in cultured milk beverages having a protein concentration less than about 3.4% protein is phase separation. Concentrated cultured milk beverages having protein levels greater than about 3.4% protein are more likely to experience gel reformation. Gel formation largely prevents (or greatly inhibits) phase separation but renders the product undrinkable – it's no longer a free-flowing beverage. It now requires intense shaking or a spoon or some other implement to consume. To prevent both defects (gelling and phase separation), stabilizers are used by commercial manufacturers.

[0048] Stabilizers for cultured milk beverages inhibit or prevent the aggregation of protein particles either by adsorption onto the surface of protein particles or by forming a much

weaker gel in the serum phase which prevents/limits the Brownian motion that leads to further protein-protein interactions. Given the net-positive charge of protein particles below their isoelectric point, anionic polysaccharides are common stabilizers for cultured milk beverages. High-methoxy (HM) pectin, carboxymethylcellulose (CMC), alginate, soybean polysaccharide, and carrageenan are common anionic stabilizers used in cultured milk beverages. At pH values below the isoelectric point of milk proteins (roughly pH 4.6 to 5.3), protein molecules become more positively-charged, which improves adsorption of anionic stabilizers to the protein surface. For stabilizers that limit protein aggregation by adsorption to the protein surface, adequate adsorption of stabilizer is critical. The adsorption process itself depends on several factors, including protein concentration, protein particle size, pH, processing steps (*e.g.*, homogenization, heating), and ionic strength.

[0049] Initial efforts by the present inventors to develop a high-protein cultured milk product failed, even with high levels of added HM pectin. In the initial effort, yogurt samples were fermented to pH 4.1, mixed with HM pectin, and homogenized. Yogurt samples were fluid momentarily during homogenization, but quickly reformed gels. These gels were very unstable to heat (72°C for 60 s), and large yield stress values were observed for yogurt samples prepared at 5-9% protein and 0-1% HM pectin. See Fig. 1. In Fig. 1, protein concentration (wt%) is on the X-axis; yield stress is on the Y-axis, and pectin concentration (wt%) is on the Z axis. Gels prepared with less than 5% protein were fluid (storage modulus, G', < 1 Pa) and remained stable with high levels of added pectin. See Fig. 2. In Fig. 2, yogurts were prepared having 4, 5, 6, 7, and 8 wt% protein. Each type of yogurt was stabilized with 1 wt% high-methoxy pectin. Only the 4 wt% yogurt had a storage modulus below 1 Pa. This yogurt was a free-flowing liquid. The others all formed gels.

[0050] The pectin adsorption efficiency of the yogurts from Fig. 2 were analyzed. Unexpectedly, higher protein concentration correlated strongly with lower pectin adsorption. That is, the higher-protein yogurts had higher serum pectin content (*i.e.*, pectin not adsorbed to the milk protein). See Fig. 3. In Fig. 3, the serum pectin concentration (g pectin/100 g serum) for the 4, 5, 6, 7, and 8 wt% protein yogurts was measured. As is clearly shown in Fig. 3, the serum pectin concentration increased with increasing concentration of milk protein, even though each type of yogurt had the same concentration of pectin added (1 wt%).

[0051] Milk is the primary food source for mammalian infants and designed in part to deliver very high concentrations of calcium and phosphate minerals to the young. Calcium and phosphate concentrations in milk exceed typical solubility limits because of their unique packaging within nanoclusters inside the structure of milk proteins (casein). This

type of calcium and phosphate in milk is known as colloidal calcium phosphate (CCP). During the acidification of milk, however, calcium and phosphate are solubilized, released into the serum, and dramatically increase the ionic strength of the serum. High-protein cultured milk beverages thus tend to exhibit a higher overall ionic strength compared to lower-protein variants, due to the solubilization of milk minerals normally found within the casein structure.

[0052] Given the presence of CCP within casein, we hypothesized that the higher protein levels were producing a cultured milk product with a high ionic strength that was preventing electrostatic interaction of pectin and protein. We hypothesized that reducing the CCP content in milk would produce a high-protein cultured milk product with a lower ionic strength. The lower ionic strength would promote more adsorption of pectin onto the protein surface, thereby providing stability by inhibiting protein aggregation, gel reformation, and phase separation.

[0053] Approximately 1/3 of the calcium content in milk is soluble and can be removed by simple filtration (*e.g.*, ultrafiltration). The remaining calcium is bound within milk proteins and can be partially removed by gentle acidification (pH ~5.6), ion-exchange, or extensive diafiltration with water. Extensive removal of CCP results in the disintegration of the casein micelle. The Center for Dairy Research (CDR) and co-inventor Dr. John Lucey have studied the impact of partial demineralization by acidification for several decades. See, for example, Lucey, Dick, Singh, and Munro (1997) "Dissociation of colloidal calcium phosphate-depleted casein particles as influenced by pH and concentration of calcium and phosphate," *Milchwissenschaft* 52:603–606. The CDR manufactures a demineralized micellar casein product known as soluble casein concentrate, SCC. (See also Silva et al. (2013) "pH-induced demineralization of casein micelles modifies their physico-chemical and foaming properties," *Food Hydrocolloids* 32(2):322-300.)

[0054] SCC was used to prepared high protein milks with about 50% CCP removed, acidified to pH 4.0, combined with 1.0% HM pectin and homogenized to produce fluid cultured milk beverages with G' values < 1 Pa. See Fig. 4. Fig. 4 shows the G' values for 8% protein yogurts made from non-fat dry milk (NFDM), from micellar casein without demineralization (MCI), from yogurt made from demineralized SCC, and from yogurt made from sodium caseinate (NaCas). The NFDM, MCI, and NaCas yogurts formed gels even after after stabilizer was incorporated. In contrast, the high-protein (8%) yogurt made from SCC (*i.e.*, demineralized milk proteins) exhibited higher adsorption rates for pectin. See Fig. 5. Fig. 5 presents that non-adsorbed (serum) pectin content for the yogurts made as per Fig. 4. (In Fig. 5, n = 2 batches.) As shown in Fig. 5, the amount of non-adsorbed pectin in the SCC yogurt was than in all of the other types of yogurts.

[0055] The demineralized SCC beverages were so stable, in fact, that high heat (72°C for 60 s) could be applied to them without protein aggregation. (Heat of that nature typically leads to high viscosity, gel reformation, and/or phase separation.) A simple test was used to demonstrate the heat stability of the SCC liquid yogurt. The homogenized beverages were heated to 72°C over the course of 7 min, and held at 72°C for 60 s to simulate a batch pasteurization process. The viscosity of milk ***decreases*** with higher temperatures, so an ***increase*** in viscosity at any point during the temperature ramp and hold period would indicate particle aggregation. The results are shown in Fig. 6. The viscosity the SCC beverage started low and decreased continuously throughout the test. In contrast, the MCI beverage had increased viscosity after about 500 s. The NaCas beverage also had a small increase in viscosity at around 450 s. A photograph is shown in Fig. 7 demonstrating particle aggregation that occurs during heat treatment without proper stabilization of protein particles. These were yogurt samples prepared at 7% protein and 1.0% HM pectin. The panel on the left shows the product before heating at 72°C for 60 s; the panel on the right shows the same product after the heat treatment. Even with the added pectin, the product clearly suffered coagulation and phase separation.

[0056] Fig. 8 depicts the viscosity of several working examples of beverages made according to the presently disclosed method. A reduced calcium MPC was obtained commercially from Milk Specialties Global (now Actus Nutrition, Eden Prairie, Minnesota, US) (20.9 mg Ca per gram protein) that is reduced in calcium content by approximately 50%. The calcium reduction in the MPC powder is approximately equivalent to the levels achieved in the Soluble Casein Concentrate powder produced by CDR. The primary difference between the low Ca MPC and SCC is the presence of whey proteins in the low Ca MPC. Yogurts prepared with 1% pectin at 8% protein using reduced calcium MPC exhibited heat stability after heating to 72°C (1°C/min) and holding at 72°C for 60 seconds. The viscosity of yogurt beverages prepared at 8% protein using various milk protein sources was measured during the heating process and is shown in Fig. 8. An increase in viscosity at any point during the heating process or during the hold time (from 470-530 seconds) is indicative of protein aggregation (instability).

Claims

1. A method to make a free-flowing beverage, the method comprising

(a) fermenting an aqueous solution comprising colloidal mineral-reduced casein for a time and at a temperature to yield a liquid yogurt having at least about 5 wt% protein concentration; and

(b) adding a stabilizer to the liquid yogurt.

2. The method of claim 1, wherein step (a) comprises fermenting an aqueous solution comprising colloidal calcium phosphate-reduced casein.

3. The method of claim 1, wherein step (b) comprises adding a stabilizer that comprises an anionic polysaccharide.

4. The method of claim 1, wherein step (b) comprises adding a stabilizer selected from the group consisting of pectin, high-methoxy pectin, carboxymethylcellulose, alginate, soybean polysaccharide, carrageenan, and combinations thereof.

5. The method of claim 1, wherein step (b) comprises adding a stabilizer comprising pectin.

6. The method of claim 1, wherein step (a) further comprises, prior to fermenting, subjecting the aqueous solution to filtration, ultrafiltration, and/or diafiltration.

7. The method of claim 1, wherein the liquid yogurt comprises from about 5 wt% protein to about 9 wt% protein.

8. The method of claim 1, wherein the liquid yogurt comprises from about 6 wt% protein to about 8 wt% protein.

9. The method of claim 1, wherein the stabilizer is added at a concentration of from about 0.5 wt% to about 3 wt%.

10. A free-flowing beverage made by a process comprising:

(a) fermenting an aqueous solution comprising colloidal mineral-reduced casein for a time and at a temperature to yield a liquid yogurt having at least about 5 wt% protein concentration; and

(b) adding a stabilizer to the liquid yogurt.

11. The free-flowing beverage of claim 10, wherein step (a) comprises fermenting an aqueous solution comprising colloidal calcium phosphate-reduced casein.

12. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer that comprises an anionic polysaccharide.

13. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer selected from the group consisting of pectin, high-methoxy pectin, carboxymethylcellulose, alginate, soybean polysaccharide, carrageenan, and combinations thereof.

14. The free-flowing beverage of claim 10, wherein step (b) comprises adding a stabilizer comprising pectin.

15. The free-flowing beverage of claim 10, wherein step (a) further comprises, prior to fermenting, subjecting the aqueous solution to filtration, ultrafiltration, and/or diafiltration.

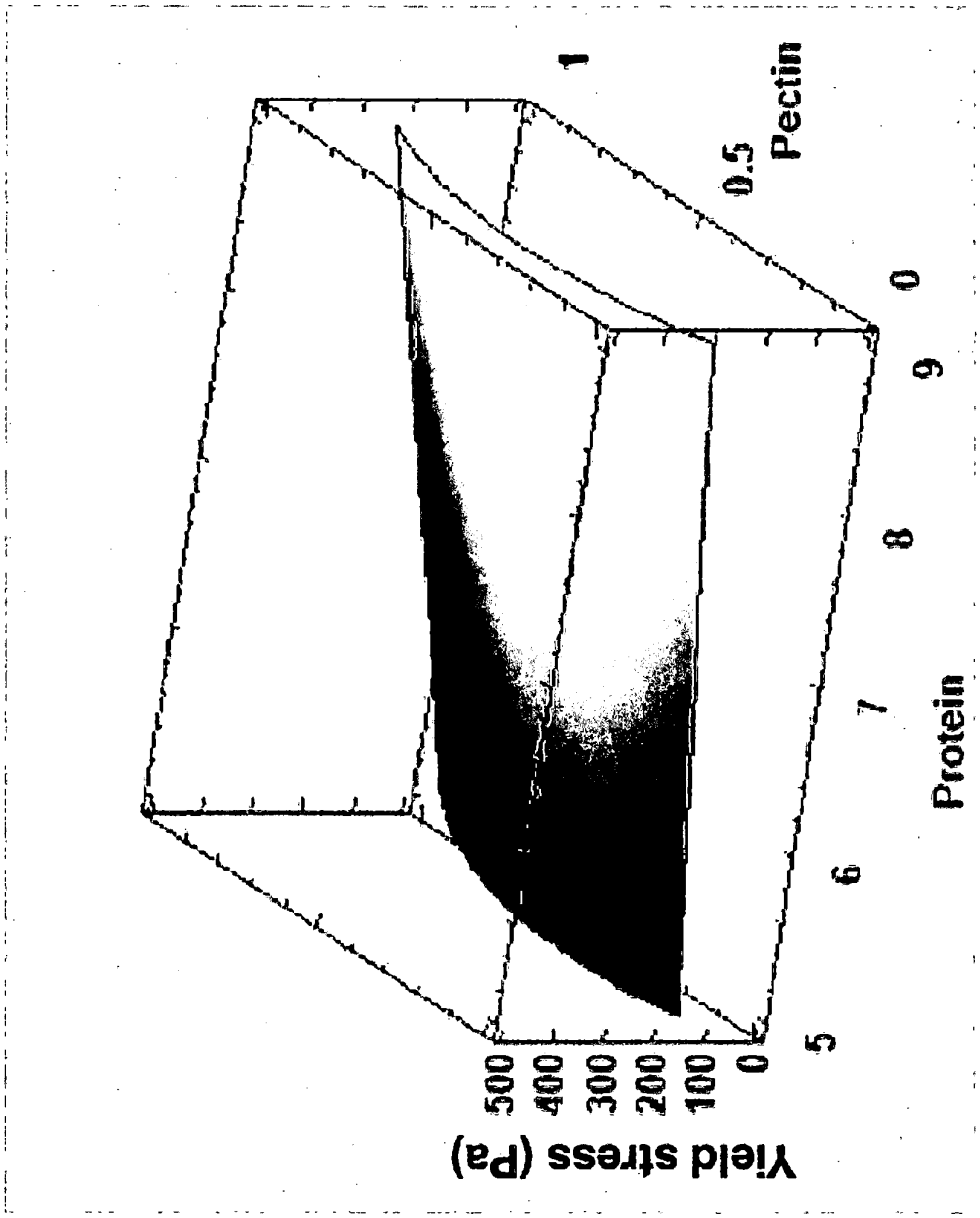
16. The free-flowing beverage of claim 10, wherein the liquid yogurt comprises from about 5 wt% protein to about 9 wt% protein.

17. The free-flowing beverage of claim 10, wherein the liquid yogurt comprises from about 6 wt% protein to about 8 wt% protein.

18. The free-flowing beverage of claim 10, wherein the stabilizer is added at a concentration of from about 0.5 wt% to about 3 wt%.

19. The free-flowing beverage of claim 10, which does not form a gel and does not phase separate after being heated to 72°C for 60 s.

20. A free-flowing beverage comprising colloidal calcium phosphate-reduced casein and a stabilizer.


Yield stress (Pa)
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300
200
100
0
5
6
7
8
9
Protein
0
0.5
1
Pectin

FIG. 1

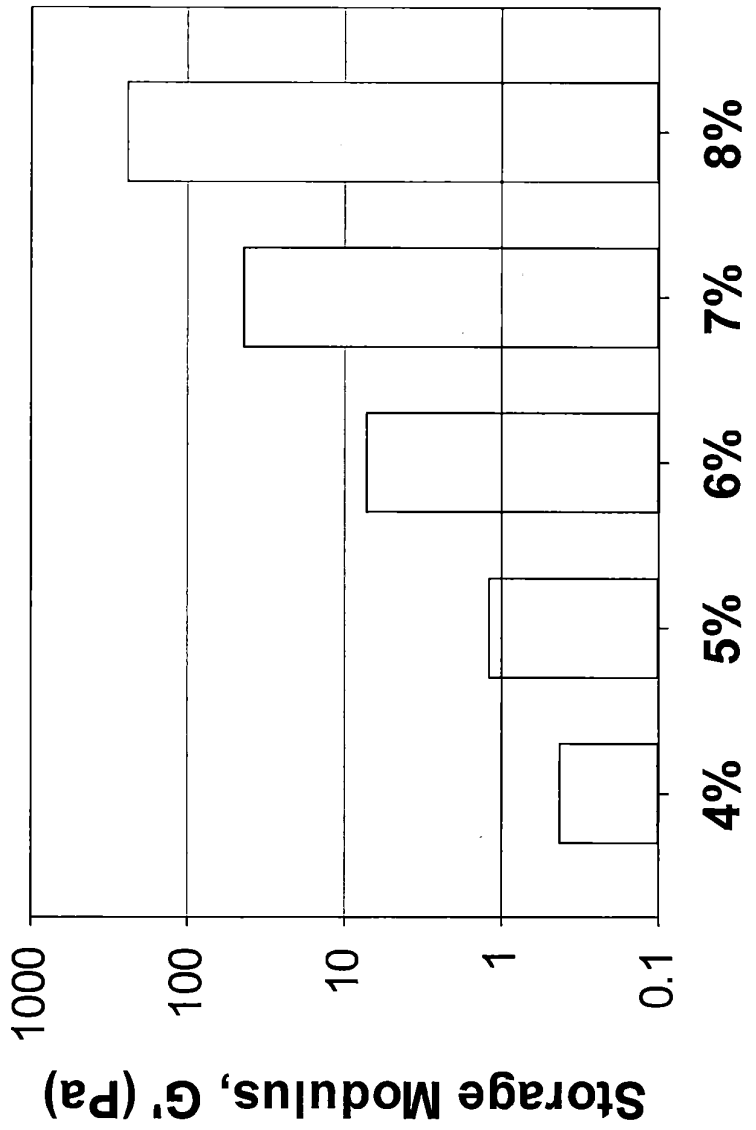

FIG. 2

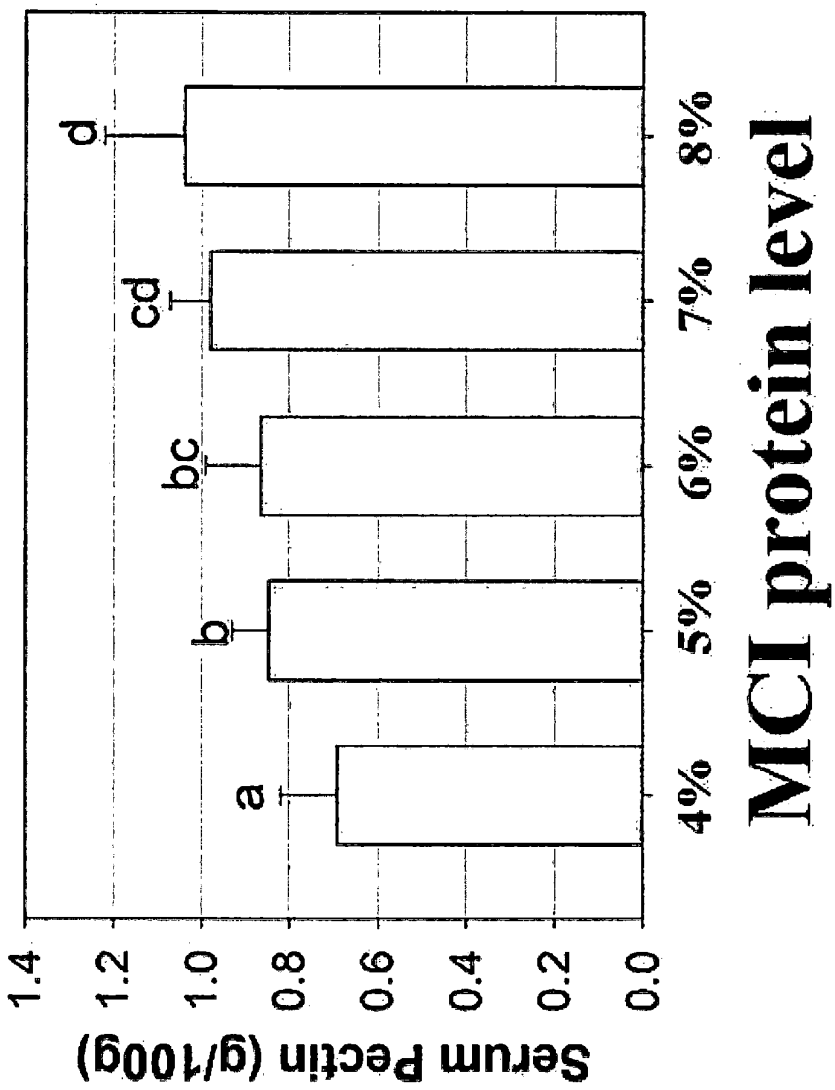
MCI protein level
Serum Pectin (g/100g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
bc
cd
d
4%
5%
6%
7%
8%

FIG. 3

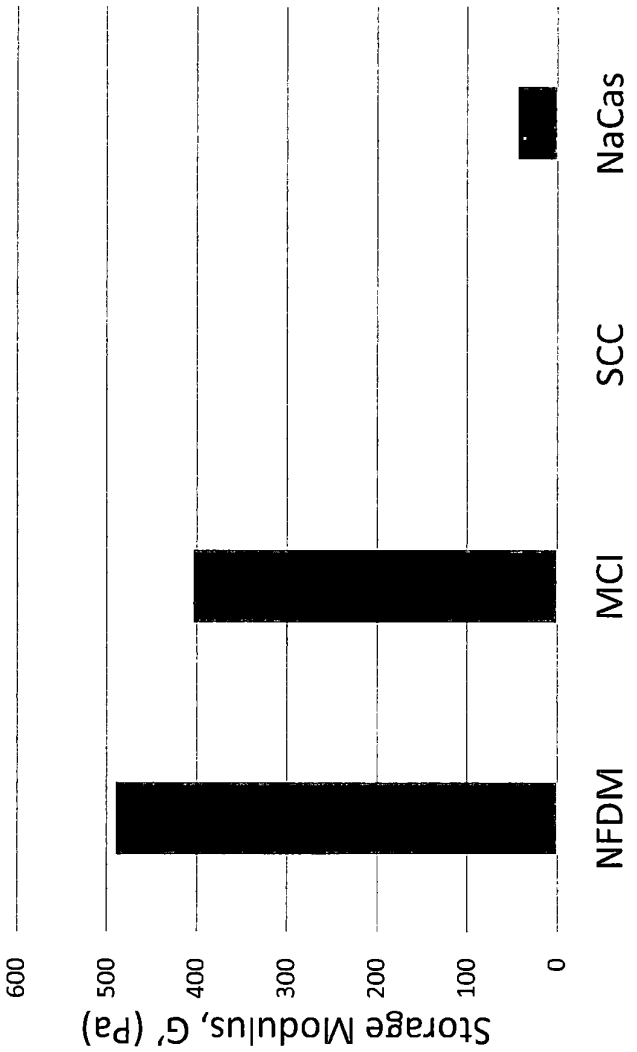
Storage Modulus, G' (Pa)
600
500
400
300
200
100
0
NFDM
MCI
SCC
NaCas

FIG. 4

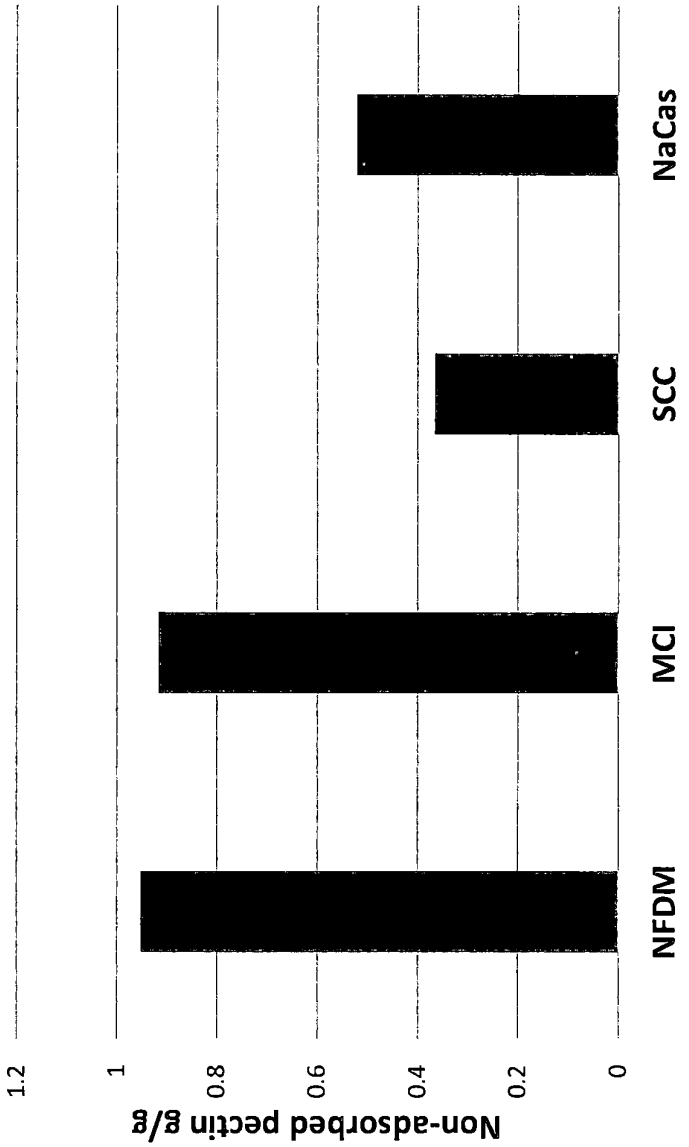

FIG. 5

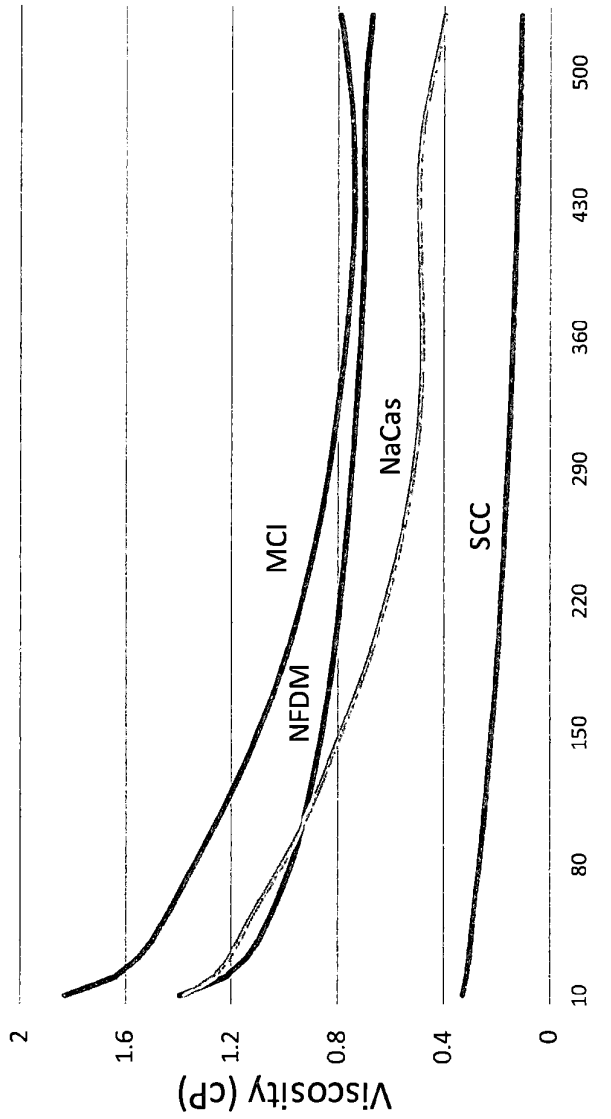
Viscosity (cP)
2
1.6
1.2
0.8
0.4
0
10
80
150
220
290
360
430
500
Time (s)
MCI
NFDM
NaCas
SCC

FIG. 6

FIG. 7

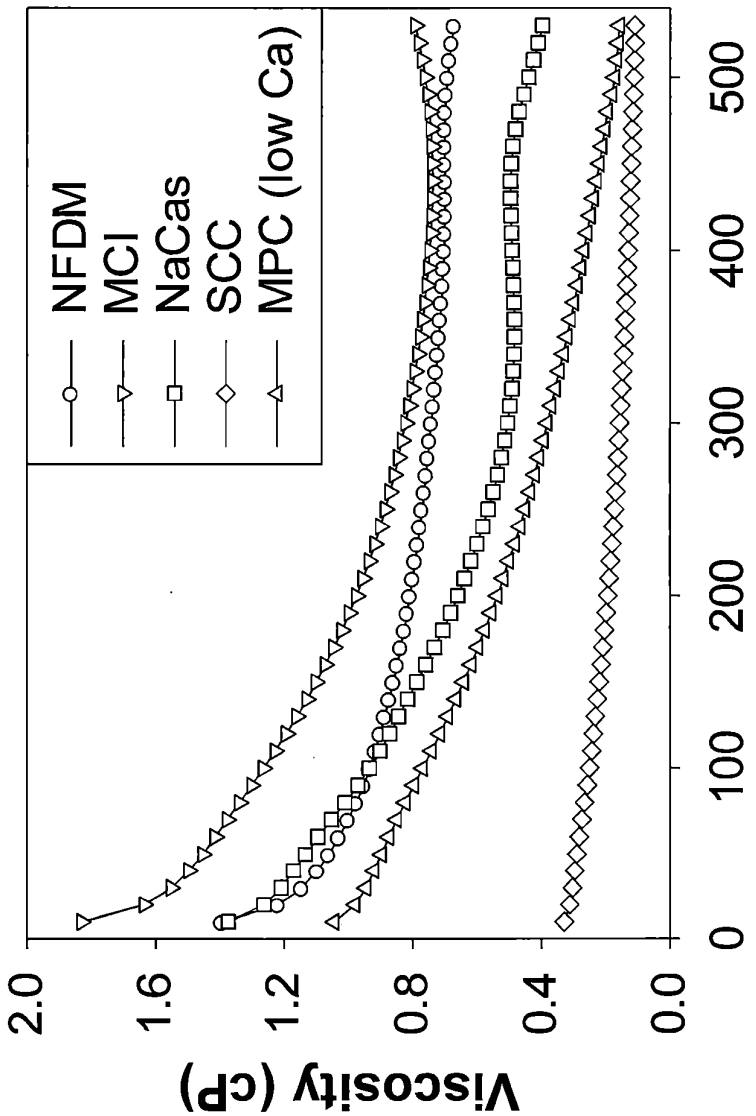
NFDM
MCI
NaCas
SCC
MPC (low Ca)
Viscosity (cP)
2.0
1.6
1.2
0.8
0.4
0.0
0
100
200
300
400
500
Time (s)

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/000003

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A23C 9/13*** (2025.01); ***A23C 9/142*** (2025.01); ***A23C 9/152*** (2025.01); ***A23L 2/38*** (2025.01); ***A23L 2/66*** (2025.01); ***A23C 1/00*** (2025.01); ***A23C 9/123*** (2025.01)

CPC: ***A23C 9/13; A23C 9/123; A23C 9/142; A23C 9/152; A23L 2/38; A23L 2/66; A23C 1/00***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2023/0014051 A1 (CHRISTIANSEN et al.) 19 January 2023 (19.01.2023) entire document	1-20
X	WILBANKS, Investigations into the use of micellar casein for the manufacture of high protein cultured milk products, A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph. D.), 30 August 2022 [retrieved on 08 May 2025]. Retrieved from the Internet: <URL:https://asset.library.wisc.edu/1711.dl/F2GDDFAWKS JPO8D/R/file-aec89.pdf>. Pgs. 167-214	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 May 2025 (10.05.2025)	**19 May 2025 (19.05.2025)**

Name and mailing address of the ISA/US	Authorized officer
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