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(54) Titre : MATRICE SECHE AMELIOREE POUR L'INCORPORATION D'ESCHERICHIA COLI VIABLE, PROCEDE DE FABRICATION ET UTILISATION ASSOCIES
(54) Title: IMPROVED DRY MATRIX FOR EMBEDDING VIABLE ESCHERICHIA COLI, METHOD OF MAKING SAME AND USE THEREOF

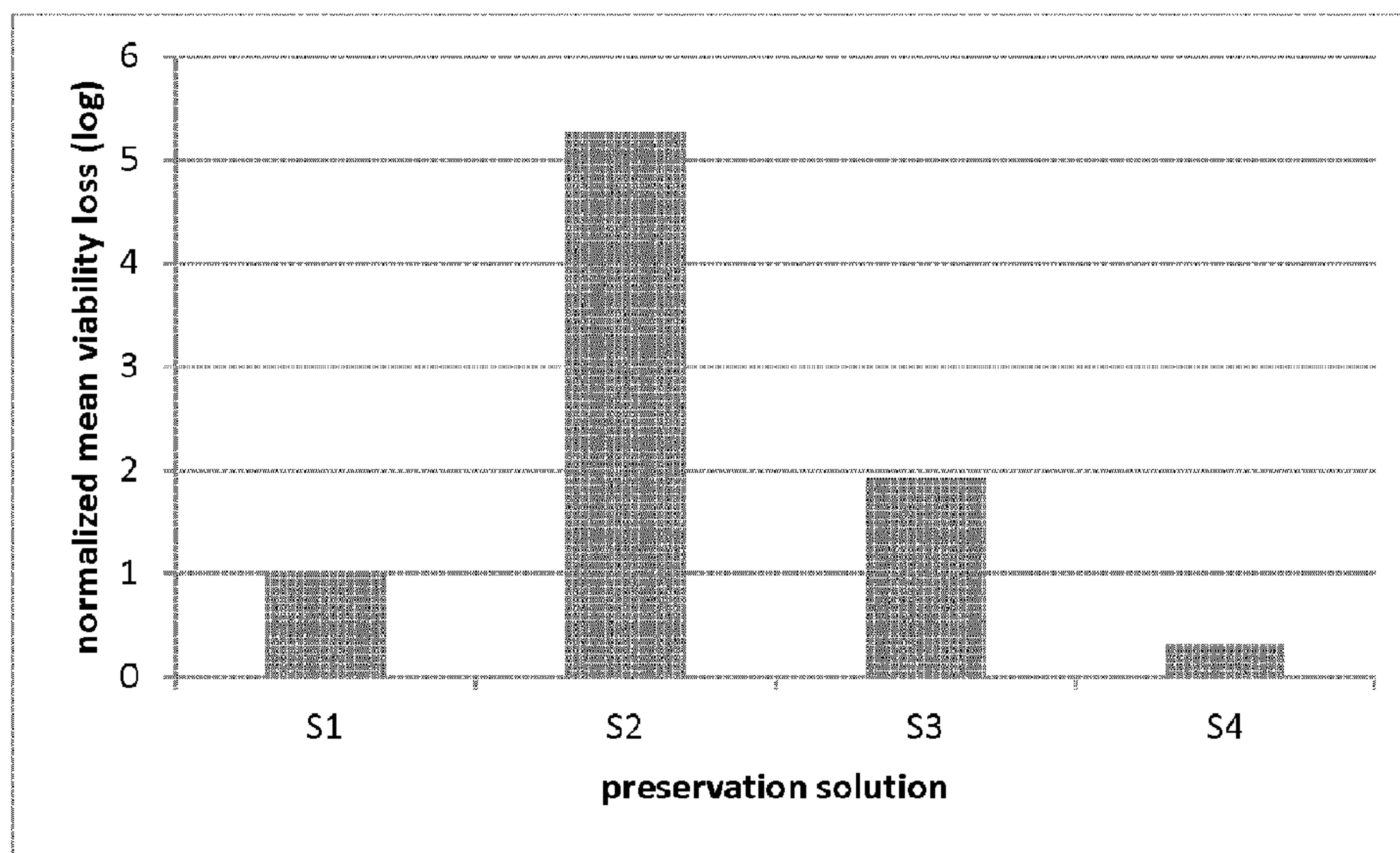


Figure 3

(57) **Abrégé/Abstract:**

There is provided viable Escherichia coli (E. coli) embedded in a matrix, wherein said matrix has a water activity (a_w) ≤ 0.3 , and wherein said matrix comprises a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof. There is also provided methods of making same and use thereof.



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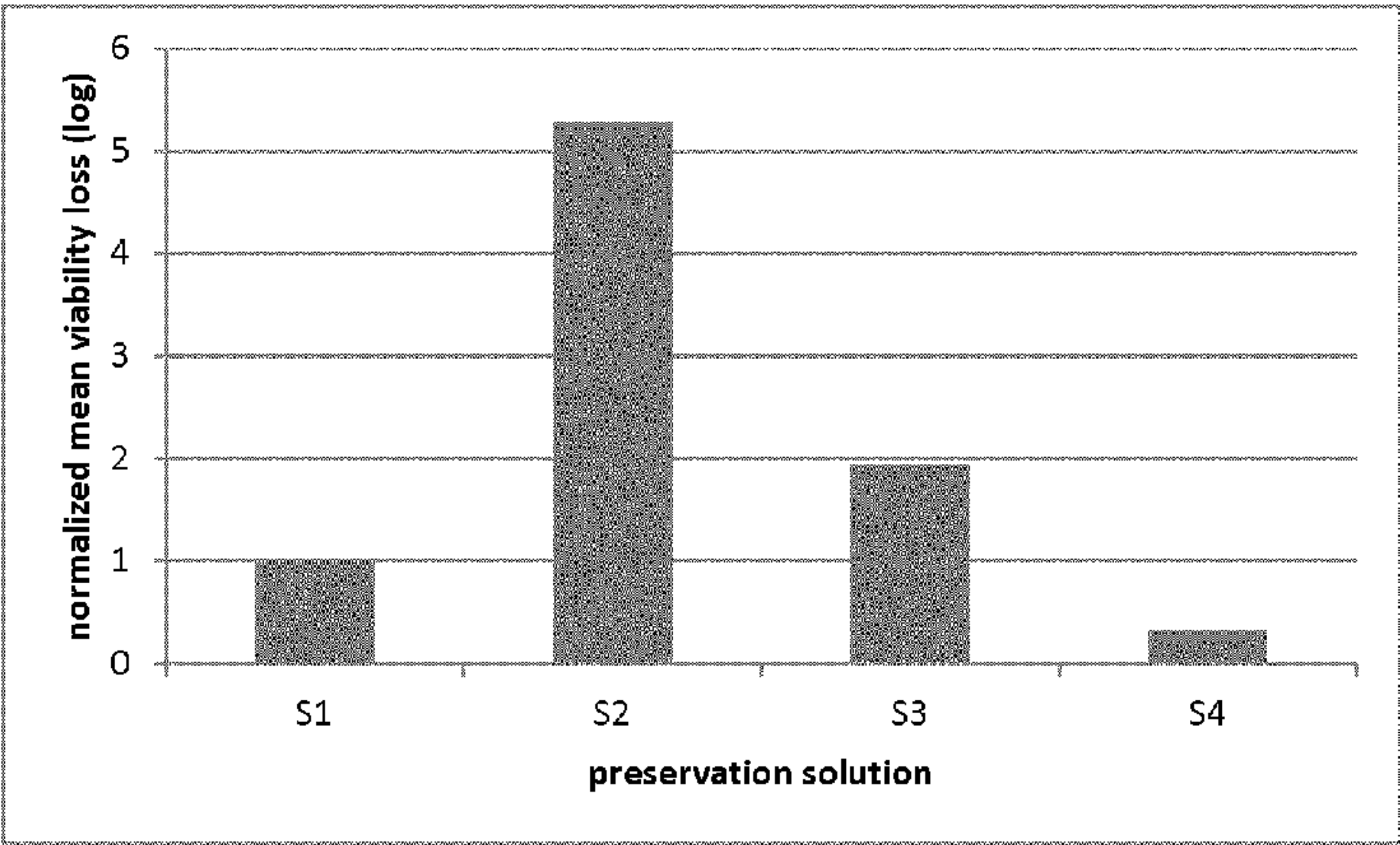


Figure 3

(57) Abstract: There is provided viable *Escherichia coli* (*E. coli*) embedded in a matrix, wherein said matrix has a water activity (a_w) ≤ 0.3 , and wherein said matrix comprises a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof. There is also provided methods of making same and use thereof.

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TITLE: IMPROVED DRY MATRIX FOR EMBEDDING VIABLE ESCHERICHIA COLI, METHOD OF MAKING SAME AND USE THEREOF

RELATED APPLICATIONS

[01] The present application claims the benefit of U.S. provisional patent application serial number 62/114,829 filed on February 11, 2015 by Eric Nadeau. The contents of the above-referenced document are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[02] This application generally relates to the field of improved dry matrices for embedding viable *E. coli*, method of making same and use thereof.

BACKGROUND

[03] Bacterial spores are dormant life forms which can exist in a desiccated and dehydrated state indefinitely. For humans, bacterial spores are available either as over-the-counter prophylactics for mild gastrointestinal disorders, such as diarrhea, or as health foods or nutritional supplements. In the agricultural industry, bacterial spores are also receiving increasing attention as potential alternatives to antibiotics as growth promoters (Hong et al., *FEMS Microbiology Reviews*, 2005,29: 813-835). *Escherichia coli* (*E. coli*) are, however non-spore-forming, and as such, are less resistant to desiccation and/or dehydration conditions than spore-forming bacteria. In many applications, it is nevertheless necessary to preserve and store *E. coli* bacteria in a form that affords sufficient viability and/or sufficient bacterial bioactivity for a given purpose.

[04] In this regard, various practical preservation and storage conditions for bacteria have been previously suggested.

[05] Freeze-drying (also named lyophilisation) is often used for preservation and storage of bacteria because of the low temperature exposure during drying (Rhodes, *Exploitation of microorganisms* ed. Jones, DG, 1993, p. 411-439, London: Chapman & Hall). However, it has the undesirable characteristics of significantly reducing viability as well as being time and energy-intensive. Protective agents have been proposed, but the protection afforded by a given additive during freeze-drying varies with the species of micro-organism (Font de Valdez et al., *Cryobiology*, 1983, 20: 560-566).

[06] Air drying such as with desiccation has also been used for preservation and storage of bacteria. While vacuum drying is a similar process as freeze-drying, it takes place at 0° - 40° C. for 30 min to a few hours. The advantages of this process are that the product is not frozen, so the energy consumption and the related economic impact are reduced. In the product point of view, the freezing damage is avoided. However, desiccation at low or ambient temperature is slow, requires extra precautions to avoid contamination, and often yields unsatisfactory viability (Lieveense et al., *Adv Biochem Eng Biotechnol.*, 1994, 51:71-89).

[07] Encapsulating bacteria in hydrocolloid-forming polysaccharide matrix, such as Calcium-alginate (Ca-alginate) beads, has also been used for preservation and storage of bacteria in a broad and increasing range of different applications (Islam et al., *J. Microbiol. Biotechnol.*, 2010, 20:1367–1377). To maintain the bacteria in a metabolically and physiologically competent state and thus obtain the desired benefit, it has been suggested to add to such matrices a suitable preservative formulation. Preservative formulations typically contain active ingredients in a suitable carrier and additives that aid in the stabilization and protection of the microbial cells during storage, transport and at the target zone.

[08] Mannitol has been described as an effective preservative formulation component for Ca-alginate encapsulated bacteria during freeze-drying as it affords high bacterial viability up to 10 weeks under room temperature and water activity (a_w) of less than 0.2 (Efiuvwevwere et al., *Appl. Microbiol. Biotechnol.*, 1999, 51:100–104). A synergistic mixture of trehalose and a sugar alcohol has also been described as an effective preservative formulation component for air-dried Ca-alginate encapsulated bacteria, where trehalose is used instead of sucrose for its significantly higher glass transition temperature, *i.e.*, 110° C. vs. only 65° C., respectively (U.S. 8,097,245). A synergistic mixture of carboxylic acid salts and hydrolyzed proteins has also been described as an effective preservative formulation component for freeze-dried Ca-alginate encapsulated bacteria (U.S. 2013/0,296,165). In both cases, the synergistic mixture affords an enhanced glassy structure without the need for foaming or boiling under vacuum to facilitate effective drying.

[09] The development of novel formulations is, however, a challenging task and not all formulation are effective for a given bacteria (Young et al., *Biotechnol Bioeng.*, 2006 Sep 5;95(1):76-83).

[10] In light of the above, there is a need to provide improved preservation and storage conditions for *E. coli* bacteria.

SUMMARY

[11] The present disclosure relates broadly to a viable *Escherichia coli* (*E. coli*) embedded in a matrix, wherein said matrix has a water activity (a_w) of ≤ 0.3 , and wherein said matrix comprises a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof.

[12] The present disclosure also relates broadly to a composition for forming a matrix, said composition comprising a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof, and an *Escherichia coli* (*E. coli*).

[13] The present disclosure also relates broadly to a method for providing a particulate comprising viable *Escherichia coli* (*E. coli*).

[14] The present disclosure also relates broadly to a matrix comprising viable *Escherichia coli* (*E. coli*), wherein said matrix has a water activity (a_w) of ≤ 0.3 , and wherein said matrix comprises a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof.

[15] All features of embodiments which are described in this disclosure and are not mutually exclusive can be combined with one another. Elements of one embodiment can be utilized in the other embodiments without further mention. Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments in conjunction with the accompanying Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[16] A detailed description of specific embodiments is provided herein below with reference to the accompanying drawings in which:

[17] Figure 1 shows a non-limiting flow diagram for preparing a bacteria culture in accordance with an embodiment of the present disclosure.

[18] Figure 2 shows a non-limiting flow diagram for drying beads with embedded *E. coli* in accordance with an embodiment of the present disclosure.

[19] Figure 3 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S2, S3 and S4 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[20] Figure 4 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S5, S6 and S7 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[21] Figure 5 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S0, S8 and S9 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[22] Figure 6 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S10, S11 and S12 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[23] Figure 7 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S13, S14 and S15 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[24] Figure 8 shows a non-limiting bar graph that depicts the effect of preservation solutions S1, S16, S17 and S18 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[25] Figure 9 shows a non-limiting bar graph that depicts the effect of preservation solutions S1 and S19 on bacterial viability following air-drying in accordance with an embodiment of the present disclosure.

[26] Figure 10 shows the raw data regarding Figures 3 to 9.

[27] In the drawings, embodiments are illustrated by way of example. It is to be expressly understood that the description and drawings are only for the purpose of illustrating certain embodiments and are an aid for understanding. The scope of the claims should not be limited by the embodiments set forth in the present disclosure, but should be given the broadest interpretation consistent with the description as a whole.

DETAILED DESCRIPTION OF EMBODIMENTS

[28] Specific examples will now be described to illustrate the manner in which the principles of the present disclosure may be put into practice.

[29] The herein described *E. coli* bacteria are viable bacteria, in other words, while the bacteria embedded in a dry matrix can be considered as being in a non-active state, these bacteria can be restored to an active state upon exposing the matrix to moisture.

[30] The herein described *E. coli* bacteria comprise any recombinant or wild *E. coli* strain, or any mixtures thereof. In one embodiment, the *E. coli* is a non-pathogenic strain. In one embodiment, the non-pathogenic *E. coli* strain is the strain deposited at the International Depository Authority of Canada (IDAC) on January 21, 2005 under accession number IDAC 210105-01, or the strain deposited at the International Depository Authority of Canada (IDAC) on June 20th 2013 and attributed accession number 200613-01, or a combination thereof.

[31] The herein described matrix comprises a hydrocolloid-forming polysaccharide. Several polysaccharides are suitable for use as described herein, alone or in any combination thereof.

[32] High amylose starch is a polysaccharide capable of forming firm gel after hydrating the starch granules in boiling water, dispersing the granules with the aid of high shear mixer and then cooling the solution to about 0-10° C. The firmness and strength of the gel depend on the concentration of the starch in the solution, with a maximal workable concentration of up to 10% w/v. The sliced starch gel matrix is also capable of retaining the live bacteria in the preservation mixture, and since it is mostly non-digestible by intestinal or gastric juices, the bacteria are protected from gastric destruction while within the starch matrix. The controlled release mechanism is provided by the fact that high amylose starch is readily digestible by the gut microflora at which time the delivered live bacteria are then released in their intact form.

[33] Pectin is another suitable polysaccharide that performs very similar to high amylose starch. Pectin has an additional advantage since the strength of the pectin gel matrix can be further increased by the addition of divalent cations such as Ca^{2+} that forms bridges between carboxyl groups of the sugar polymers.

[34] Alginate is another suitable polysaccharide that can form a firm gel matrix by cross-linking with divalent cations. The alginate can be hardened into a firm gel matrix by internally cross-linking the alginate polysaccharides with a dication, e.g. Ca^{2+} , for example by extruding the alginate in the form of thin threads, strings, or substantially spherical beads into a Ca^{2+} bath. The alginate hardens upon interaction with Ca^{2+} . An alternative method of preparation of the matrix is to spray atomize the mixture into a bath containing Ca^{2+} .

[35] In one embodiment, the hydrocolloid-forming polysaccharide is present in the matrix in percent by weight of total dry matter at a value of from 0.1% to 20%. In one embodiment, the hydrocolloid-forming polysaccharide is present in the matrix in percent by weight of total dry matter at a value of from 0.1% to 19%, or from 0.1% to 18%, or from 0.1% to 17%, or from 0.1% to 16%, or from 0.1% to 15%, or from 0.1% to 14%, or from 0.1% to 13%, or from 0.1% to 12%, or from 1% to 12%, including any value therein.

[36] The herein described matrix further comprises a disaccharide and a polysaccharide. The present disclosure discloses several concentrations and proportions suitable for inclusion in the matrix. In one embodiment, a suitable ratio of disaccharide / polysaccharide in wt.%/wt.% is of less than 10 or more preferably of less than 5. In one embodiment, the ratio of disaccharide / polysaccharide in wt.%/wt.% is of about 1.

[37] In one embodiment, the disaccharide is present in the matrix in percent by weight of total dry matter at a value of from 0.1% to 90%, or from 0.1% to 75%, or from 0.1% to 50%, or from 0.1% to 35%, or from 0.1% to 20%, or from 0.1% to 15%, or from 0.1% to 10%, including any value therein.

[38] In one non-limiting embodiment, the disaccharide includes sucrose.

[39] In a further non-limiting embodiment, the disaccharide includes trehalose.

[40] In one non-limiting embodiment, the polysaccharide includes maltodextrine.

[41] In a further non-limiting embodiment, the polysaccharide includes dextran.

[42] In a further non-limiting embodiment, the dextran has a molecular weight between 20 and 70 kDa.

[43] In one embodiment, the matrix further includes a salt of L-glutamic acid. In one non-limiting embodiment, the salt is a sodium salt of L-glutamic acid.

[44] The herein described matrix has a water activity ("a_w") which is of $0.04 \leq a_w \leq 0.3$, for example $0.04 \leq a_w \leq 2.5$, $0.04 \leq a_w \leq 2.0$, $0.04 \leq a_w \leq 1.5$, and the like. "Water activity" or "a_w" in the context of the present disclosure, refers to the availability of water and represents the energy status of the water in a system. Water activity may be measured according to materials and procedures known in the art, for example, using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.).

[45] There is also provided a composition for forming a matrix, the composition comprising a first hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof and an *Escherichia coli* (*E. coli*).

[46] There is also provided a method for providing a particulate comprising viable *Escherichia coli* (*E. coli*), the method comprising providing particles comprising a first hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof and *E.coli* and drying said particles to water water activity (a_w) ≤ 0.3 .

[47] In one non-limiting embodiment, the viable *E. coli* sustains an a_w fold reduction in the particles of at least 0.4, or at least 0.5, or at least 0.6, or at least 0.7.

EXAMPLES

[48] In each of the following examples, three preservation solutions were tested along with preservation solution S1. The tests were performed in triplicates and one standard deviation was calculated according to the following formula:

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n}}$$

with n : number of samples and $\bar{x}$: mean of sample population.

[49] In each of the following examples, bacterial viability was assessed by measuring the number of colony-forming units (CFU) according to protocols known in the art.

[50] The preservation solutions used in the following examples are shown in Table 1.

Table 1

preservation solution	Polysaccharide	Disaccharide	salt of L-glutamic acid	Ratio polysaccharide / disaccharide / salt of organic acid
S0	x ¹	x	x	N/A ²
S1	dextran 40	sucrose	yes	5:7:1
S2	dextran 40 (5 wt%)	x	x	N/A
S3	x	Sucrose (7 wt%)	x	N/A
S4	dextran 40	trehalose	yes	5:7:1
S5	dextran 20	sucrose	yes	5:7:1
S6	dextran 70	sucrose	yes	5:7:1
S7	maltodextrine	sucrose	yes	5:7:1
S8	dextran 40	sucrose	yes	10:1:1
S9	dextran 40	sucrose	yes	1:10:1
S10	dextran 40 (different manufacturer)	sucrose	yes	5:7:1
S11	x	sucrose	yes	7:1
S12	dextran 70	trehalose	yes	5:7:1
S13	dextran 40	sucrose	x	5:7
S14	dextran 40	sucrose	yes	5:3:1
S15	dextran 40	sucrose	yes	5:5:1
S16	maltodextrin	trehalose	yes	5:7:1
S17	maltodextrin	trehalose	yes	10:1:1
S18	maltodextrin	trehalose	yes	1:10:1
S19	dextran 40	maltose	yes	5:7:1

¹ x means absent

² N/A means not applicable

1. Example 1

a. *E. coli* culture

[51] With reference to Figure 1, an *E. coli* strain was cultivated in a first step 100 on Tryptic Soy Agar of non-animal origin. Six (6) isolated colonies were then used to cultivate the *E. coli* strain in a second step 200 for 2 hours at 37° C and agitation at 200 rpm in 30 mL of Tryptic Soy Broth (TSB) of non-animal origin (for 1L of TSB: 20 g of Soy Peptone A3 SC – (Organotechnie), 2.5 g anhydrous dextrose USP –(J.T. Baker), 5 g sodium chloride USP – (J.T. Baker), and 2.5 g dibasic potassium phosphate USP –(Fisher Chemical)). The resulting Culture 1 was diluted by a factor of 10 in TSB and was then used to cultivate the *E. coli* strain in a third step 300 for 2 hours at 37° C and agitation at 200 rpm in 100 mL of TSB of non-animal origin. The resulting Culture 2 was diluted by a factor of 10 in TSB and was then used to cultivate the *E. coli* strain in a fourth step 400 for 5 hours at 37° C and agitation at 200 rpm in 1 L of TSB of non-animal origin. The resulting Culture 3 was then used to embed *E. coli* in matrix. Variations and refinements to the culture protocol herein described are possible and will become apparent to persons skilled in the art in light of the present teachings. For example, the non-pathogenic *E. coli* may also be cultivated in anaerobic conditions according to protocols known in the art (Son & Taylor, *Curr. Protoc. Microbiol.*, 2012, 27:5A.4.1 – 5A.4.9). In preparing the beads of the subsequent examples, the non-pathogenic *E. coli* strain deposited at the International Depository Authority of Canada (IDAC) on January 21, 2005 under accession number IDAC 210105-01 may be selected.

b. Matrix preparation

[52] BactoTM peptone (1.5 g, BD, Mississauga, Canada) was mixed with 1.5 L of heated water to obtain a mixture. Alginate (30 g Grindsted[®], DuPontTM Danisco[®], Mississauga, Canada) was slowly added to the mixture while mixing with a magnetic bar at 360 rpm. Complete solubilisation of alginate was obtained in about 3 h to obtain a 2% alginate (m/v) solution. The solution including the magnetic bar was then autoclaved under standard

conditions. Variations and refinements to the matrix preparation protocol herein described are possible and will become apparent to persons skilled in the art in light of the present teachings.

c. Embedding *E. coli* in matrix

[53] The following was added, in order and while mixing with the magnetic bar, to the autoclaved matrix solution to obtain a slurry: 1 L of TSB of non-animal origin and, with reference to Figure 1, 0.5 L of the resulting Culture 3 of *E. coli* in 1 L of TSB of non-animal origin. The slurry was extruded into a polymerization bath (300 mM CaCl₂, 0.1 wt./v. % Bacto™ tryptone, 0.1 wt./v. % Bacto™ peptone, and 0.05 wt./v. % g Bacto™ yeast extract in water) to form beads using a 9 exit syringe system adapted from the Thermo Scientific™ Reacti-Vap™ Evaporators. The bath was gently stirred while injecting the slurry. The matrix beads were allowed to cross-link for about 30 minutes, and the resulting hardened beads were then harvested. Variations and refinements to the embedding protocol herein described are possible and will become apparent to persons skilled in the art in light of the present teachings.

d. Drying and testing of embedded *E. coli*

[54] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 the beads with embedded *E. coli* in the matrix were placed in a preservation solution S1, a preservation solution S2, a preservation solution S3 or a preservation solution S4 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[55] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[56] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[57] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[58] The results are shown in Figure 3. Preservation solution S4 showed a normalized average viability loss of 0.32 while sustaining a water activity of 0.142 ± 0.004 .

[59] A compilation of the results of Example 1 is set forth in Tables 2 and 3. These results demonstrate that the elements of preservation solution S4 provided a significant effect to the viability of the *E. coli* embedded in the dried matrix and its resistance to the drying process 700.

Table 2

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss ($\log_{10}$)	Normalized CFU loss ($\log_{10}$)
S1	$3 \times 10^{11} \pm 9 \times 10^{10}$	$1.4 \times 10^{11} \pm 4.3 \times 10^9$	0.32 ± 0.14	1
S2	$2.3 \times 10^{11} \pm 5.5 \times 10^{10}$	$5.4 \times 10^9 \pm 2.7 \times 10^9$	1.66 ± 0.3	5.18
S3	$2.6 \times 10^{11} \pm 4.9 \times 10^{10}$	$7.4 \times 10^{10} \pm 4.3 \times 10^{10}$	0.61 ± 0.32	1.90
S4	$3.1 \times 10^{11} \pm 7 \times 10^{10}$	$2.5 \times 10^{11} \pm 9.7 \times 10^{10}$	0.11 ± 0.08	0.34

Table 3

Sample	step 650	step 760	
	average a_w	average a_w	a_w fold reduction
S1	0.473 ± 0.020	0.165 ± 0.010	0.65
S2	0.278 ± 0.021	0.054 ± 0.009	0.81
S3	0.423 ± 0.022	0.150 ± 0.026	0.64
S4	0.488 ± 0.022	0.142 ± 0.004	0.71

e. Incorporating dried embedded *E. coli* into a feed (“pelleting”)

Protocol for incorporating dried matrix into a feed, for example in the form of a feed additive are known in the art. An illustrative example of doing such can be done, e.g., by incorporating 500 g to 1000 g of dried matrix beads into a ton of feed. If desired, the feed can also include inactivated yeast product in suitable amounts. For instance, the dried matrix beads comprising the embedded *E. coli* are mixed in a homogenization tank with all other ingredients. Preferably, the mixture is continuously mixed during the pelleting process. The mixed material is then pumped towards an extruder. Steam is then applied on the mixed material that is about to enter the extruder (i.e., hence, the temperature of the mixture increases at this stage). Suitable pressure is then applied on the mixture during its passage inside the extruder (pressure and temperature increase, point where highest temperature reached, around 75°C). The formed pellets are then expelled out of the extruder into a cooling tank (rapid temperature drops to 30-40°C followed by another cool down, to reach ambient temperature). Pelleted feed including the feed additive (matrix comprising embedded *E. coli*) can then be stored, for example in bags / containers. Variations and refinements to the pelleting protocol herein described are possible and will become apparent to persons skilled in the art in light of the present teachings.

2. Example 2

[60] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in a preservation solution S1, a preservation solution S5, a preservation solution S6 or a preservation solution S7 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water

a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[61] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[62] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[63] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[64] The results are shown in Figure 4. Preservation solution S7 showed a normalized average viability loss of 0.38 while sustaining a water activity of 0.298 ± 0.013 .

[65] A compilation of the results of Example 2 is set forth in Tables 4 and 5. These results demonstrate that the elements of preservation solution S7 provided a significant protective effect to the viability of the *E. coli* embedded in the dried matrix and its resistance to the drying process 700.

Table 4

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss ($\log_{10}$)	Normalized CFU loss ($\log_{10}$)
S1	$3.3 \times 10^{11} \pm 9.2 \times 10^{10}$	$1.8 \times 10^{11} \pm 2.7 \times 10^{10}$	0.26 ± 0.07	1
S5	$3.5 \times 10^{11} \pm 7.7 \times 10^{10}$	$2.6 \times 10^{11} \pm 6 \times 10^{10}$	0.13 ± 0.17	0.5
S6	$3.1 \times 10^{11} \pm 5.8 \times 10^{10}$	$2.8 \times 10^{11} \pm 1.7 \times 10^{11}$	0.10 ± 0.29	0.38
S7	$3.4 \times 10^{11} \pm 3.7 \times 10^{10}$	$2.7 \times 10^{11} \pm 1 \times 10^{10}$	0.10 ± 0.06	0.38

Table 5

Sample	step 650	step 760	
	average a_w	average a_w	a_w fold reduction
S1	0.535 ± 0.020	0.230 ± 0.012	0.57
S5	0.530 ± 0.049	0.249 ± 0.009	0.53
S6	0.586 ± 0.143	0.260 ± 0.013	0.56
S7	0.541 ± 0.045	0.298 ± 0.013	0.45

3. Example 3

[66] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S0, a preservation solution S8 or a preservation solution S9 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[67] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[68] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU\ loss = \log_{10}(550) - \log_{10}(750)$$

[69] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[70] The results are shown in Figure 5.

[71] A compilation of the results of Example 3 is set forth in Tables 6 and 7.

Table 6

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss (log ₁₀)	Normalized CFU loss (log ₁₀)
S1	$2.2 \times 10^{11} \pm 2.9 \times 10^{10}$	$1.7 \times 10^{11} \pm 9.3 \times 10^9$	0.11 ± 0.05	1
S0	$1.9 \times 10^{11} \pm 2 \times 10^{10}$	$1.5 \times 10^6 \pm 1.5 \times 10^6$	5.28 ± 0.53	47.7
S8	$2.8 \times 10^{11} \pm 4.6 \times 10^{10}$	$5.9 \times 10^{10} \pm 2.3 \times 10^{10}$	0.70 ± 0.15	6.28
S9	$2.4 \times 10^{11} \pm 5.4 \times 10^{10}$	$1.5 \times 10^{11} \pm 1.5 \times 10^{10}$	0.18 ± 0.04	1.64

Table 7

Sample	step 650	step 760	
	average a _w	average a _w	a _w fold reduction
S1	0.453 ± 0.010	0.241 ± 0.005	0.47
S0	0.331 ± 0.022	0.037 ± 0.002	0.89
S8	0.366 ± 0.010	0.062 ± 0.006	0.83
S9	0.451 ± 0.010	0.275 ± 0.032	0.39

4. Example 4

[72] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S10, a preservation solution S11 or a preservation solution S12 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator

for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[73] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[74] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[75] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[76] The results are shown in Figure 6. Preservation solution S7 showed a normalized average viability loss of 0.58.

[77] A compilation of the results of Example 4 is set forth in Tables 8 and 9.

Table 8

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss ($\log_{10}$)	Normalized CFU loss ($\log_{10}$)
S1	$3.7 \times 10^{11} \pm 5.2 \times 10^{10}$	$2.8 \times 10^{11} \pm 4.46 \times 10^{10}$	0.12 ± 0.01	1
S10	$4 \times 10^{11} \pm 1 \times 10^{11}$	$3.3 \times 10^{11} \pm 3.11 \times 10^{10}$	0.07 ± 0.12	0.58
S11	$3.3 \times 10^{11} \pm 3.9 \times 10^{10}$	$1.9 \times 10^{11} \pm 1.77 \times 10^{10}$	0.24 ± 0.03	1.91
S12	$4 \times 10^{11} \pm 2.9 \times 10^{10}$	$5.3 \times 10^{11} \pm 9.27 \times 10^{10}$	-0.12 ± 0.08	-0.94

Table 9

Sample	step 650	step 760	
	average a_w	average a_w	a_w fold reduction
S1	0.475 ± 0.023	0.123 ± 0.007	0.74
S10	0.490 ± 0.026	0.135 ± 0.007	0.72
S11	0.419 ± 0.016	0.201 ± 0.038	0.52
S12	0.494 ± 0.026	0.165 ± 0.006	0.66

5. Example 5

[78] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S13, a preservation solution S14 or a preservation solution S15 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[79] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[80] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[81] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[82] The results are shown in Figure 7. Preservation solution S7 showed a normalized average viability loss of 0.35.

[83] A compilation of the results of Example 5 is set forth in Tables 10 and 11.

Table 10

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss ($\log_{10}$)	Normalized CFU loss ($\log_{10}$)
S1	$4.1 \times 10^{11} \pm 3.8 \times 10^{10}$	$2.7 \times 10^{11} \pm 3.44 \times 10^{10}$	0.18 ± 0.10	1
S13	$3.8 \times 10^{11} \pm 4.2 \times 10^{10}$	$2.6 \times 10^{11} \pm 2.7 \times 10^{10}$	0.15 ± 0.02	0.85
S14	$3.8 \times 10^{11} \pm 6.1 \times 10^{10}$	$2.2 \times 10^{11} \pm 3.55 \times 10^{10}$	0.24 ± 0.08	1.35
S15	$4.4 \times 10^{11} \pm 1.5 \times 10^{10}$	$3.8 \times 10^{11} \pm 6.37 \times 10^{10}$	0.06 ± 0.07	0.35

Table 11

Sample	step 650	step 760	
	average a_w	average a_w	a_w fold reduction
S1	0.501 ± 0.041	0.177 ± 0.008	0.65
S13	0.562 ± 0.101	0.247 ± 0.012	0.56
S14	0.465 ± 0.031	0.133 ± 0.013	0.71
S15	0.502 ± 0.037	0.198 ± 0.016	0.60

6. Example 6

[84] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S16, a preservation solution S17 or a preservation solution S18 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon

Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[85] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[86] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[87] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[88] The results are shown in Figure 8.

[89] A compilation of the results of Example 6 is set forth in Tables 12 and 13.

Table 12

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss (log ₁₀)	Normalized CFU loss (log ₁₀)
S1	$3.3 \times 10^{11} \pm 3.4 \times 10^{10}$	$3.1 \times 10^{11} \pm 4.92 \times 10^{10}$	0.03 ± 0.07	1
S16	$3.6 \times 10^{11} \pm 4.1 \times 10^{10}$	$4.4 \times 10^{11} \pm 9.91 \times 10^{10}$	-0.07 ± 0.11	-2.68
S17	$3.2 \times 10^{11} \pm 4.5 \times 10^{10}$	$2.3 \times 10^{11} \pm 5.05 \times 10^{10}$	0.15 ± 0.05	5.57
S18	$2.7 \times 10^{11} \pm 3.9 \times 10^{10}$	$4.2 \times 10^{11} \pm 4.76 \times 10^{10}$	-0.19 ± 0.06	-6.98

Table 13

Sample	step 650	step 760	
	average a _w	average a _w	a _w fold reduction
S1	0.734 ± 0.164	0.155 ± 0.003	0.79
S16	0.575 ± 0.862	0.136 ± 0.015	0.76
S17	0.742 ± 0.167	0.039 ± 0.004	0.95
S18	0.536 ± 0.003	0.176 ± 0.029	0.67

7. Example 7

[90] For each preservation solution the drying and testing was performed at least in triplicates. With reference to Figure 2, in a first step 500 beads prepared as in Example 1 were placed in either a preservation solution S1 or a preservation solution S19 with gentle stirring for about 20 minutes. In each case, a determination of total CFU 550 was performed after soaking in the preservation solution. In a second step 600 the beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. In each case, a measurement of water activity a_w 650 was performed on the semi-dry beads using an Aqualab Water Activity Meter 4TE (Decagon Devices, Inc., U.S.A.). In a third step 700 the semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. In accordance with an embodiment of the present disclosure, the drying process 800 includes at least two steps: a step 600 which includes placing beads in an air dryer for 24 hours at room temperature and to obtain semi-dry beads and a step 700 which includes placing the semi-dry beads in a desiccator for 64 hours to obtain dry beads. In each case, a determination of total CFU 750 and a measurement of water a_w 760 were performed on the dry beads. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[91] In each case, and with reference to Figure 2, the a_w fold reduction was calculated according to the following:

$$a_w \text{ fold reduction} = \frac{650 - 760}{650}$$

[92] In each case, and with reference to Figure 2, viability loss was calculated according to the following:

$$CFU \text{ loss} = \log_{10}(550) - \log_{10}(750)$$

[93] In each case, an average viability loss and normalized average viability loss relative to the results obtained with preservation solution S1 was calculated.

[94] The results are shown in Figure 9.

[95] A compilation of the results of Example 7 is set forth in Tables 14 and 15.

Table 14

Sample	step 550	step 750		
	average CFU	average CFU	average CFU loss ($\log_{10}$)	Normalized CFU loss ($\log_{10}$)
S1	$3.5 \times 10^{11} \pm 4.4 \times 10^8$	$3.2 \times 10^{11} \pm 4.13 \times 10^{10}$	0.03 ± 0.05	1
S19	$3.3 \times 10^{11} \pm 2.6 \times 10^{10}$	$2.4 \times 10^{11} \pm 2.06 \times 10^{10}$	0.13 ± 0.06	3.83

Table 15

Sample	step 650	step 760	
	average a_w	average a_w	a_w fold reduction
S1	0.660 ± 0.200	0.158 ± 0.026	0.76
S19	0.561 ± 0.085	0.129 ± 0.010	0.77

8. Example 8

[96] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in a preservation solution S1, a preservation solution S2, a preservation solution S3 and a preservation solution S4 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at

room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[97] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[98] The results of Example 8 are shown in Table 16 where all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 16

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0.2
S2	0.1
S3	0.1
S4	0

9. Example 9

[99] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in a preservation solution S1, a preservation solution S5, a preservation solution S6 and a preservation solution S7 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[100] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[101] The results of Example 9 are shown in Table 17 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 17

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0.2
S5	0.1
S6	0
S7	0.1

10. Example 10

[102] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S0, a preservation solution S8 and a preservation solution S9 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[103] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[104] The results of Example 10 are shown in Table 18 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 18

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0
S0	2.4
S8	0.1
S9	0

11. Example 11

[105] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S10, a preservation solution S11 and a preservation solution S12 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[106] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[107] The results of Example 11 are shown in Table 19 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 19

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0.1
S10	0.1
S11	0.4
S12	0.2

12. Example 12

[108] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S13, a preservation solution S14 and a preservation solution S15 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[109] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[110] The results of Example 12 are shown in Table 20 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 20

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0
S13	0
S14	0.1
S15	0.1

[111]

13. Example 13

[112] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in either a preservation solution S1, a preservation solution S16, a preservation solution S17 and a preservation solution S18 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[113] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[114] The results of Example 13 are shown in Table 21 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 21

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0
S16	0
S17	0
S18	0.1

[115]

14. Example 14

[116] For each preservation solution the drying and testing was performed at least in triplicates. Beads prepared as in Example 1 were placed in either a preservation solution S1 and a preservation solution S19 with gentle stirring for about 20 minutes. The beads were then placed on a tray dryer in an air dryer at room temperature for about 24 h to obtain semi-dry beads. The semi-dry beads were then placed in a desiccator for about 64 h, in which dry and filtered air was blown. Dry beads having a water activity a_w of ≤ 0.3 were obtained.

[117] In each case, the strain viability was tested over a period of four (4) weeks under storage conditions at 4°C by measuring the CFU/g of the dried beads. The tests were performed at least in triplicates and one standard deviation was calculated.

[118] The results are shown in Table 22 and all the preservation solutions tested afforded feed additive strain stability during 4 weeks when stored at 4°C.

Table 22

Preservation solution	Difference CFU/g after 4 weeks (log)
S1	0.1
S19	0.1

[119] In brief, the present inventor has surprisingly and unexpectedly observed that a matrix comprising embedded viable *E. coli* as described herein was capable of preserving viability of sufficient bacteria CFU over a given period of time, e.g. 4 weeks, for a commercial use thereof. For example, the matrix was successfully incorporated into a pelleted animal feed such that the animal feed could be stored / transported / handled and eventually administered to an animal

while retaining sufficient viable CFU / g of animal feed to provide the beneficial effect normally associated with the bacteria.

[120] Note that titles or subtitles may be used throughout the present disclosure for convenience of a reader, but in no way these should limit the scope of the invention. Moreover, certain theories may be proposed and disclosed herein; however, in no way they, whether they are right or wrong, should limit the scope of the invention so long as the invention is practiced according to the present disclosure without regard for any particular theory or scheme of action.

[121] All references cited throughout the specification are hereby incorporated by reference in their entirety for all purposes.

[122] It will be understood by those of skill in the art that throughout the present specification, the term “a” used before a term encompasses embodiments containing one or more to what the term refers. It will also be understood by those of skill in the art that throughout the present specification, the term “comprising”, which is synonymous with “including,” “containing,” or “characterized by,” is inclusive or open-ended and does not exclude additional, un-recited elements or method steps.

[123] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In the case of conflict, the present document, including definitions will control.

[124] As used in the present disclosure, the terms “around”, “about” or “approximately” shall generally mean within the error margin generally accepted in the art. Hence, numerical quantities given herein generally include such error margin such that the terms “around”, “about” or “approximately” can be inferred if not expressly stated.

[125] Although the present disclosure has described in considerable detail certain embodiments, variations and refinements are possible and will become apparent to persons skilled in the art in light of the present teachings.

CLAIMS:

1. Viable *Escherichia coli* (*E. coli*) embedded in a matrix, comprising a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof, wherein said matrix has a water activity (a_w) ≤ 0.3 .
2. The *E. coli* embedded in the matrix of claim 1, wherein $0.04 \leq a_w \leq 0.3$
3. The *E. coli* embedded in the matrix of claim 1 or 2, wherein said matrix comprises a ratio disaccharide / second polysaccharide of less than 10, wherein the ratio is wt.%/wt.%.
4. The *E. coli* embedded in the matrix of claim 3, wherein the ratio is of less than 5.
5. The *E. coli* embedded in the matrix of claim 3, wherein the ratio is of about 1.
6. The *E. coli* embedded in the matrix of any one claims 1 to 4, wherein said hydrocolloid-forming polysaccharide is alginate.
7. The *E. coli* embedded in the matrix of any one of claims 1 to 6, wherein said matrix further comprises a salt of L-glutamic acid.
8. The *E. coli* embedded in the matrix of claim 7, wherein said salt is sodium salt of L-glutamic acid.
9. The *E. coli* embedded in the matrix of any one of claims 1 to 8, wherein the second polysaccharide is maltodextrine.
10. The *E. coli* embedded in the matrix of any one of claims 1 to 8, wherein the second polysaccharide is dextran.
11. The *E. coli* embedded in the matrix of any one of claims 1 to 10, which is a non-pathogenic *E. coli*.
12. A composition for forming a matrix, said composition comprising a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof, and an *Escherichia coli* (*E. coli*).

13. The composition of claim 12, wherein said matrix comprises a ratio disaccharide / second polysaccharide of less than 10, wherein the ratio is wt.%/wt.%.
14. The composition of claim 13, wherein the ratio is of less than 5.
15. The composition of claim 13, wherein the ratio is of about 1.
16. The composition of any one claims 12 to 15, wherein said hydrocolloid-forming polysaccharide is alginate.
17. The composition of any one of claims 12 to 16, wherein said matrix further comprises a salt of L-glutamic acid.
18. The composition of claim 17, wherein said salt is sodium salt of L-glutamic acid.
19. The composition of any one of claims 12 to 18, wherein the second polysaccharide is maltodextrine.
20. The composition of any one of claims 12 to 18, wherein the second polysaccharide is dextran.
21. The composition of any one of claims 12 to 20, wherein said *E. coli* is a non-pathogenic *E. coli*.
22. A method for providing a particle comprising viable *Escherichia coli* (*E. coli*), the method comprising:
 - providing particles comprising a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof, and the *E. coli*; and
 - drying said particles to obtain a water activity (a_w) of ≤ 0.3 .
23. The method of claim 22, wherein said drying is performed to obtain $0.04 \leq a_w \leq 0.3$.
24. The method of claim 22 or 23, wherein providing said particles comprises:
 - mixing said *E. coli* with the first polysaccharide to form a mixture;

- forming particles from said mixture; and
- contacting said particles with a preservation solution comprising the sucrose or trehalose, and the second polysaccharide.

25. The method of any one of claims 22 to 24, wherein said preservation solution comprises a ratio disaccharide / second polysaccharide of less than 10, wherein the ratio is wt.%/wt.%.

26. The method of claim 25, wherein the ratio is of less than 5.

27. The method of claim 25, wherein the ratio is of about 1.

28. The method of any one claims 22 to 27, wherein said hydrocolloid-forming polysaccharide is alginate.

29. The method of any one of claims 22 to 28, wherein said matrix further comprises a salt of L-glutamic acid.

30. The method of claim 29, wherein said salt is sodium salt of L-glutamic acid.

31. The method of any one of claims 22 to 30, wherein the second polysaccharide is maltodextrine.

32. The method of any one of claims 22 to 30, wherein the second polysaccharide is dextran.

33. The method of any one of claims 22 to 32, which is a non-pathogenic *E. coli*.

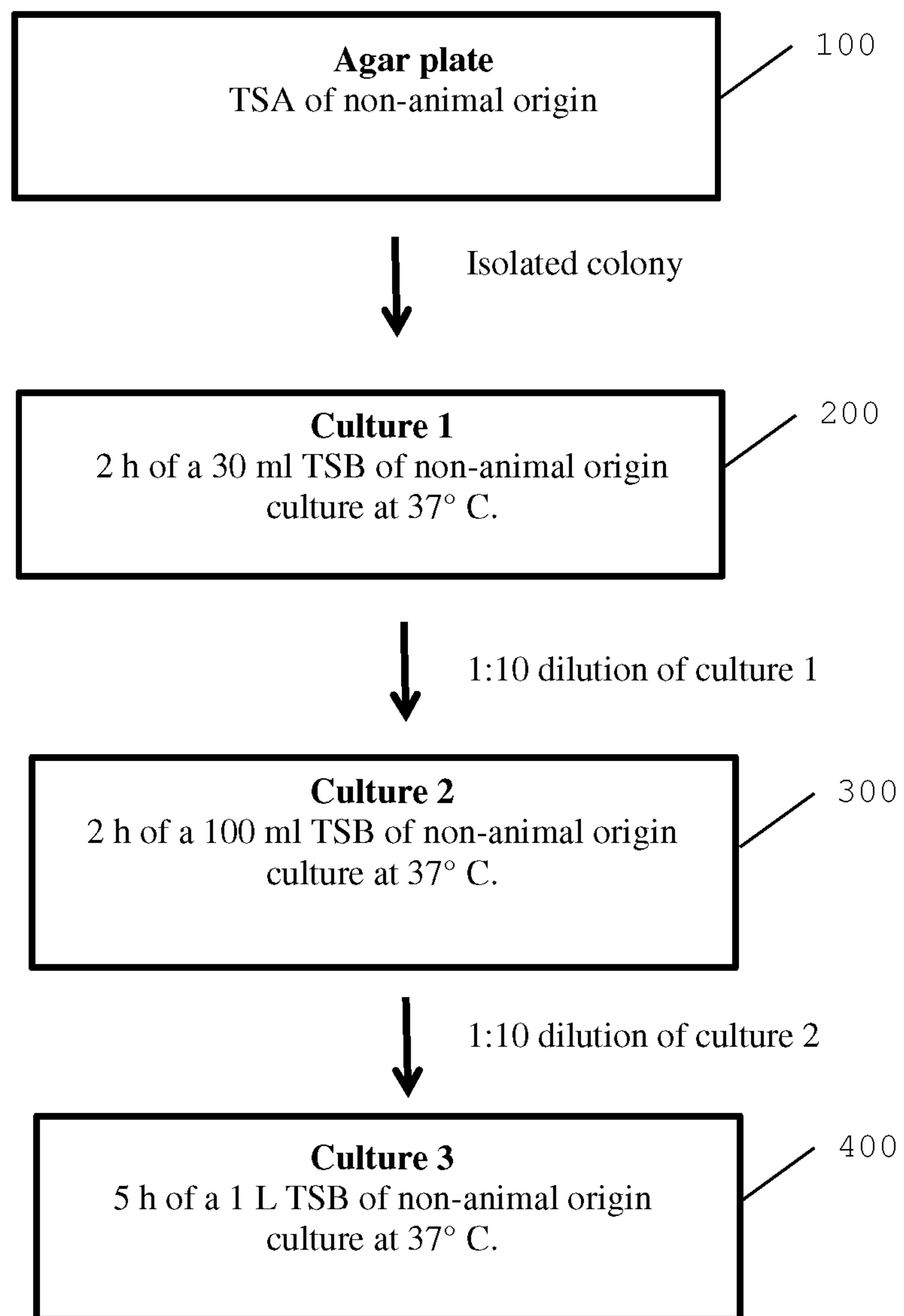
34. The method of any one of claims 22 to 33, wherein said drying includes air drying.

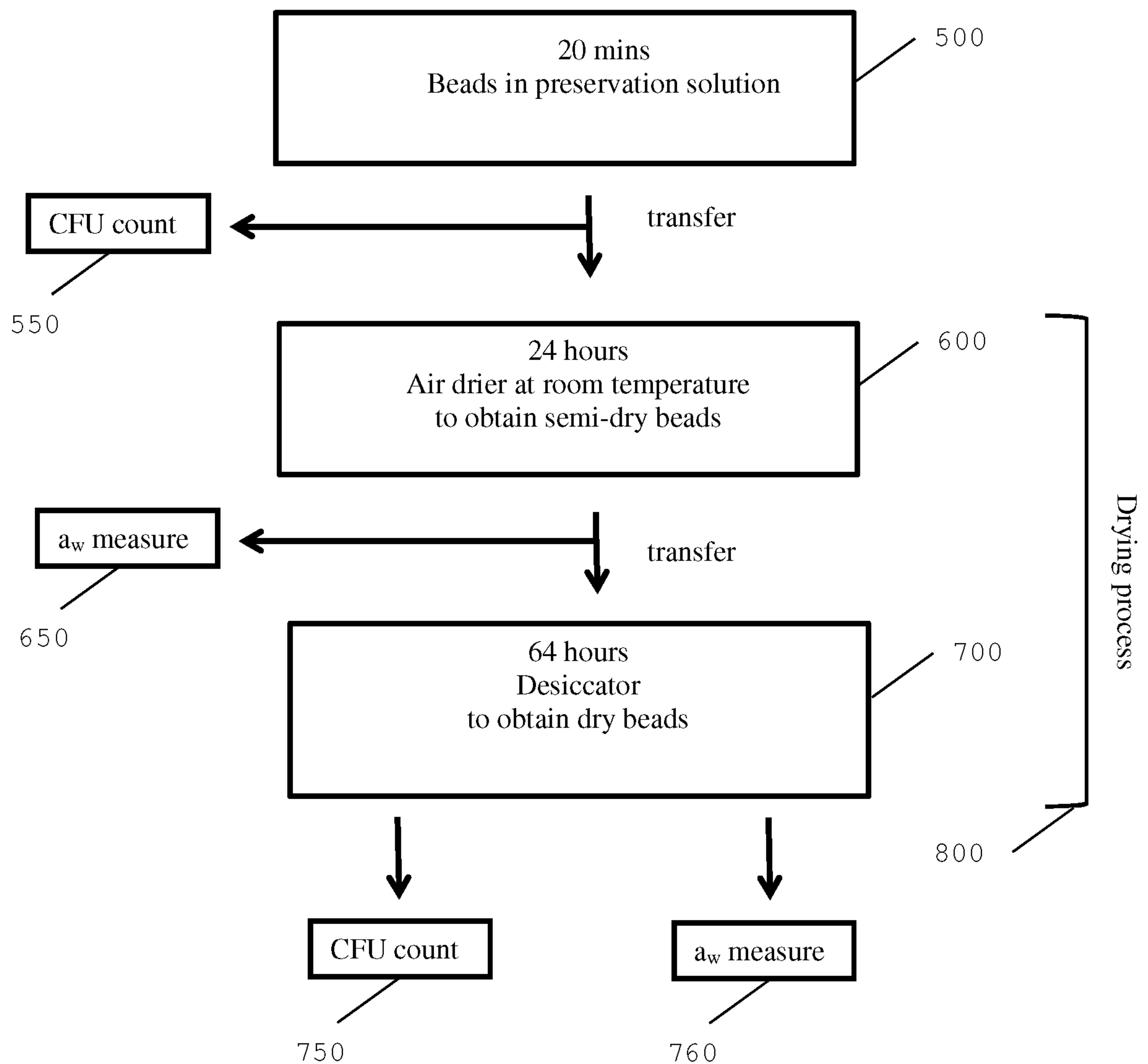
35. The method of claim 34, wherein said drying comprises drying said particles in an air dryer.

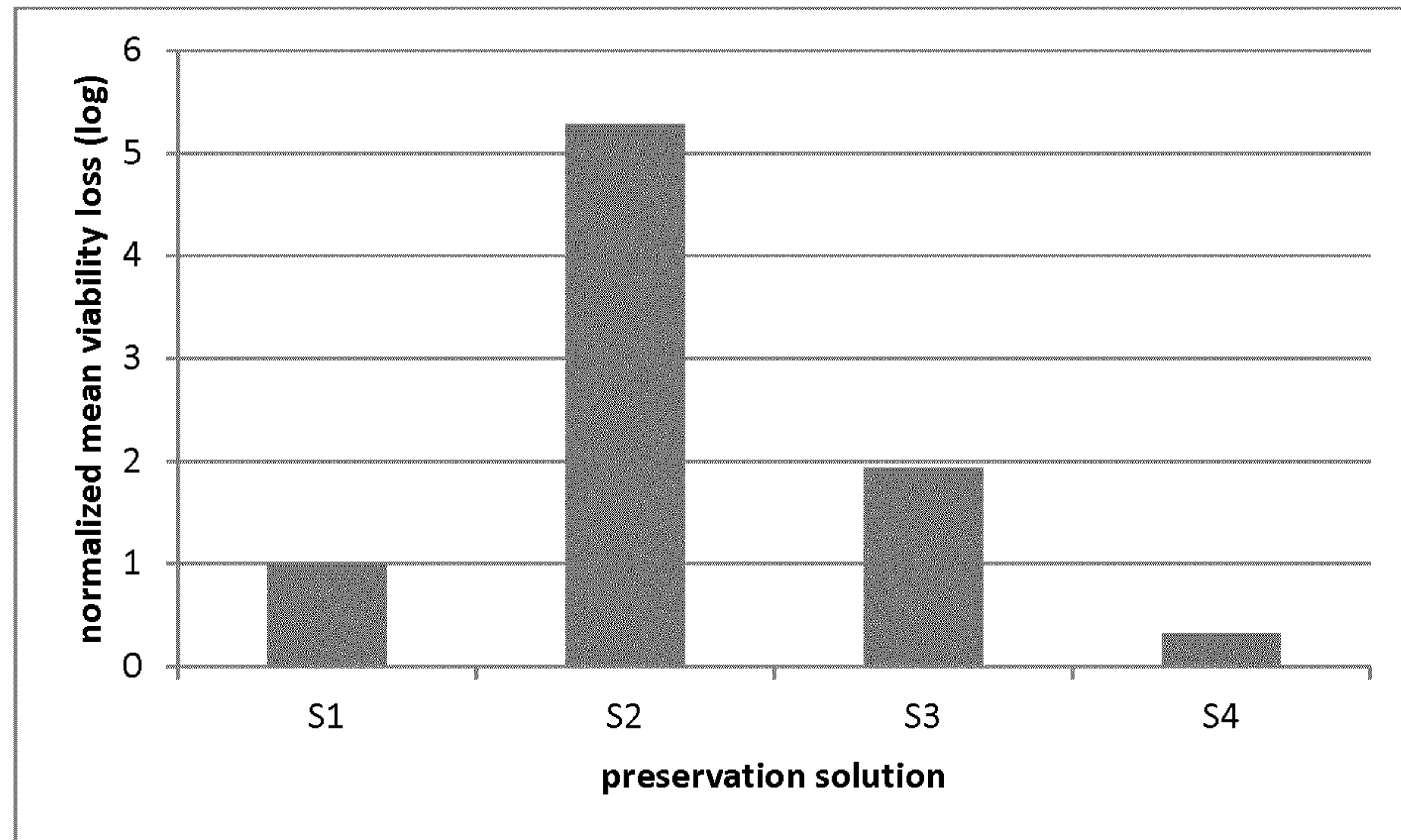
36. The method of claim 34, wherein said drying comprises drying said particles in a desiccator.

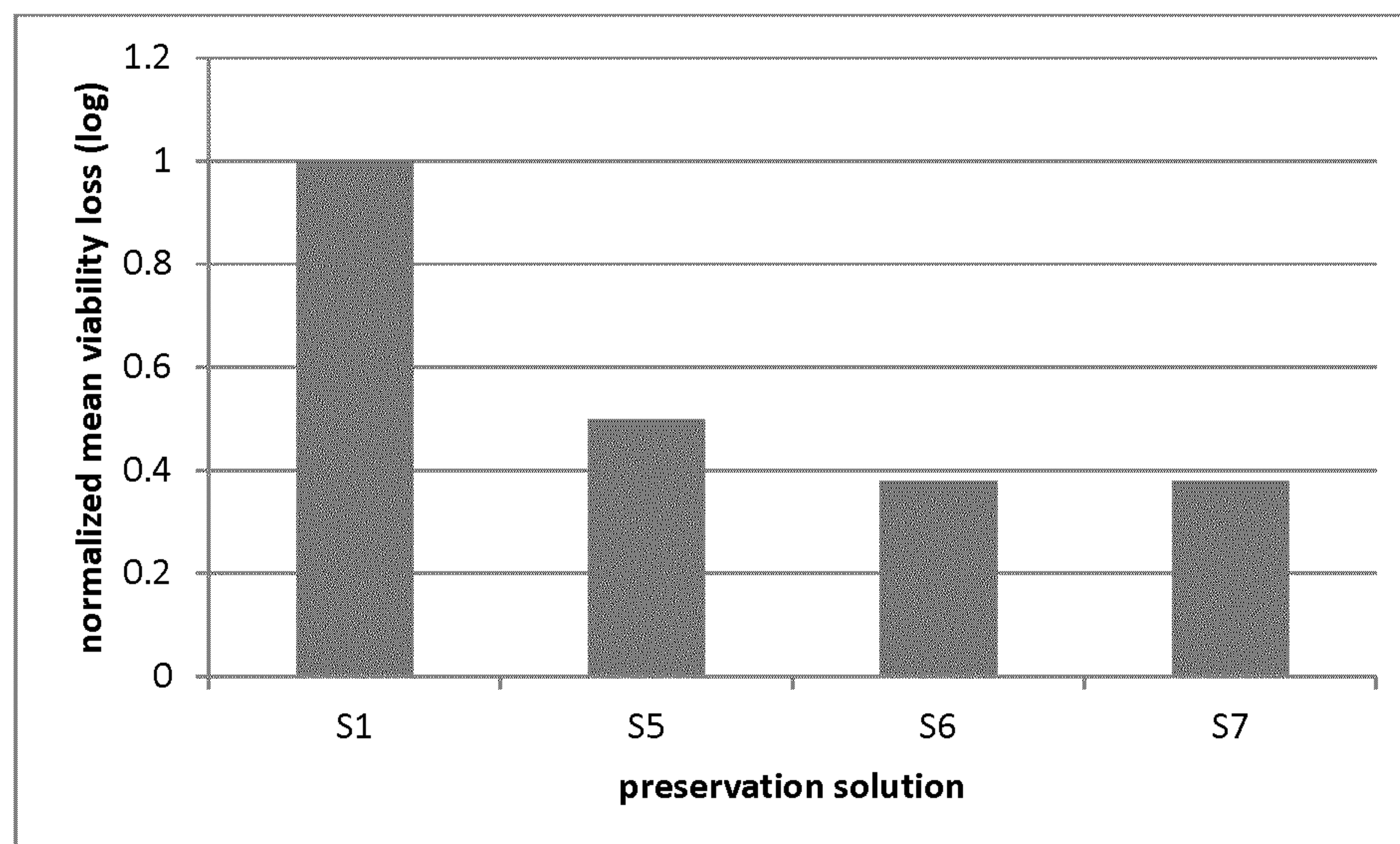
37. The method of claim 36, wherein said drying comprises a first drying in an air drier and a second drying in a desiccator.

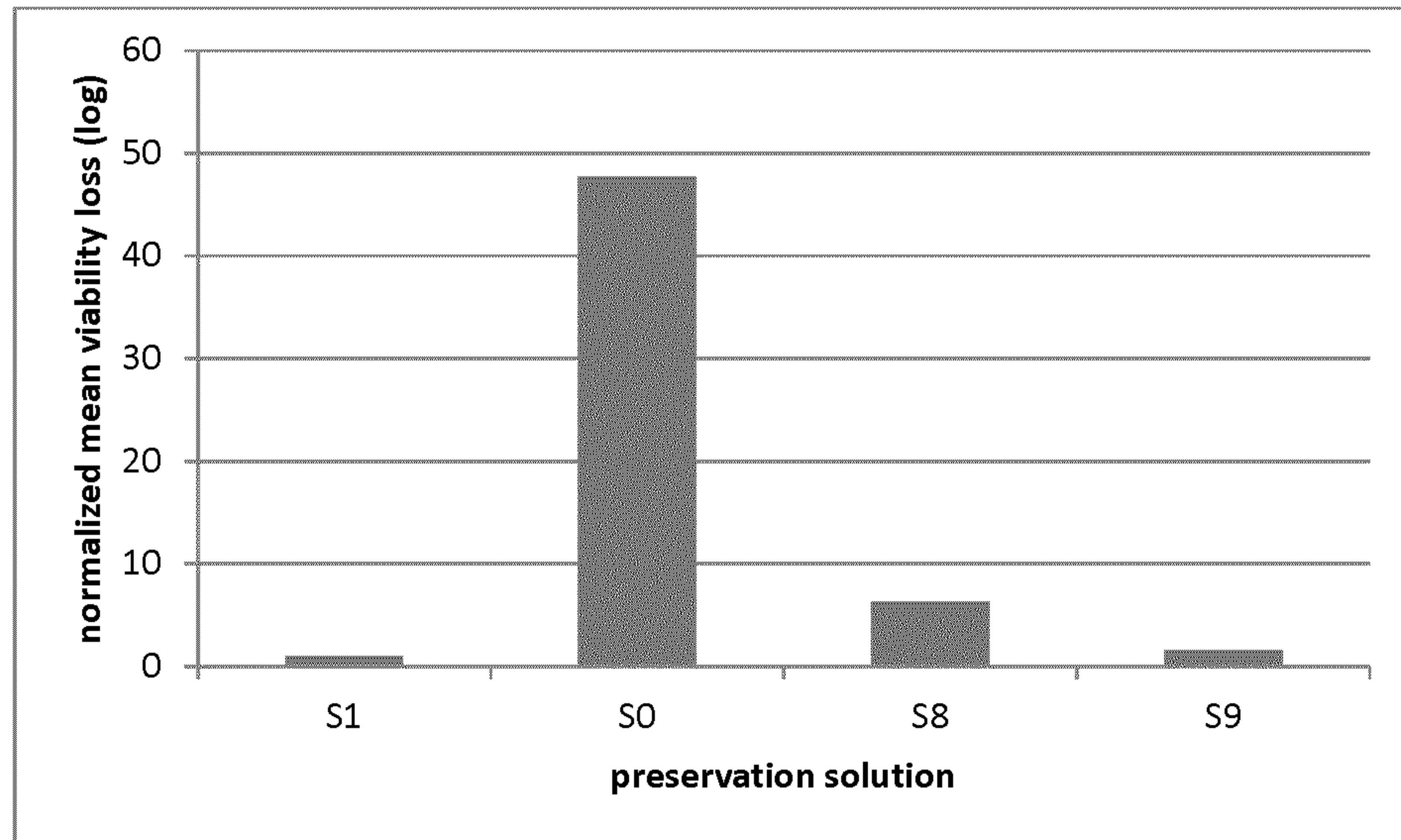
38. The method of any one of claims 22 to 33, wherein said drying includes freeze drying.
39. A matrix comprising viable *Escherichia coli* (*E. coli*), comprising a first polysaccharide which is a hydrocolloid-forming polysaccharide, a second polysaccharide which is different from the first polysaccharide, and a disaccharide which includes sucrose, trehalose, or a combination thereof, wherein said matrix has a water activity (a_w) ≤ 0.3 .
40. The matrix of claim 39, wherein $0.04 \leq a_w \leq 0.3$.
41. The matrix of claim 39, wherein said matrix comprises a ratio disaccharide / second polysaccharide of less than 10, wherein the ratio is wt.%/wt.%.
42. The matrix of claim 41, wherein the ratio is of less than 5.
43. The matrix of claim 41, wherein the ratio is of about 1.
44. The matrix of any one claims 39 to 43, wherein said hydrocolloid-forming polysaccharide is alginate.
45. The matrix of any one of claims 39 to 44, wherein said matrix further comprises a salt of L-glutamic acid.
46. The matrix of claim 45, wherein said salt is sodium salt of L-glutamic acid.
47. The matrix of any one of claims 39 to 46, wherein the second polysaccharide is maltodextrine.
48. The matrix of any one of claims 39 to 46, wherein the second polysaccharide is dextran.
49. The matrix of any one of claims 39 to 48, which is a non-pathogenic *E. coli*.

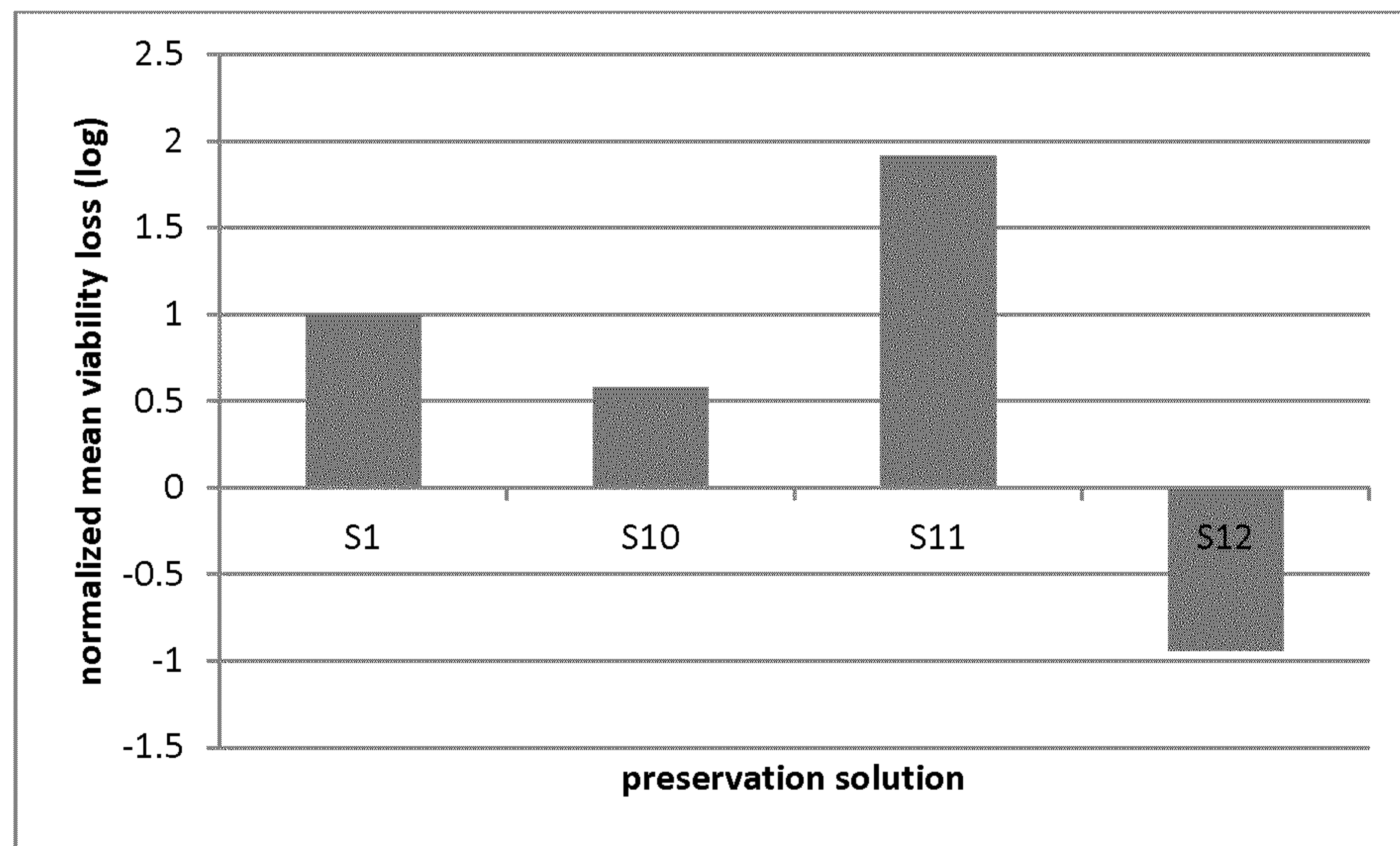
**Figure 1**

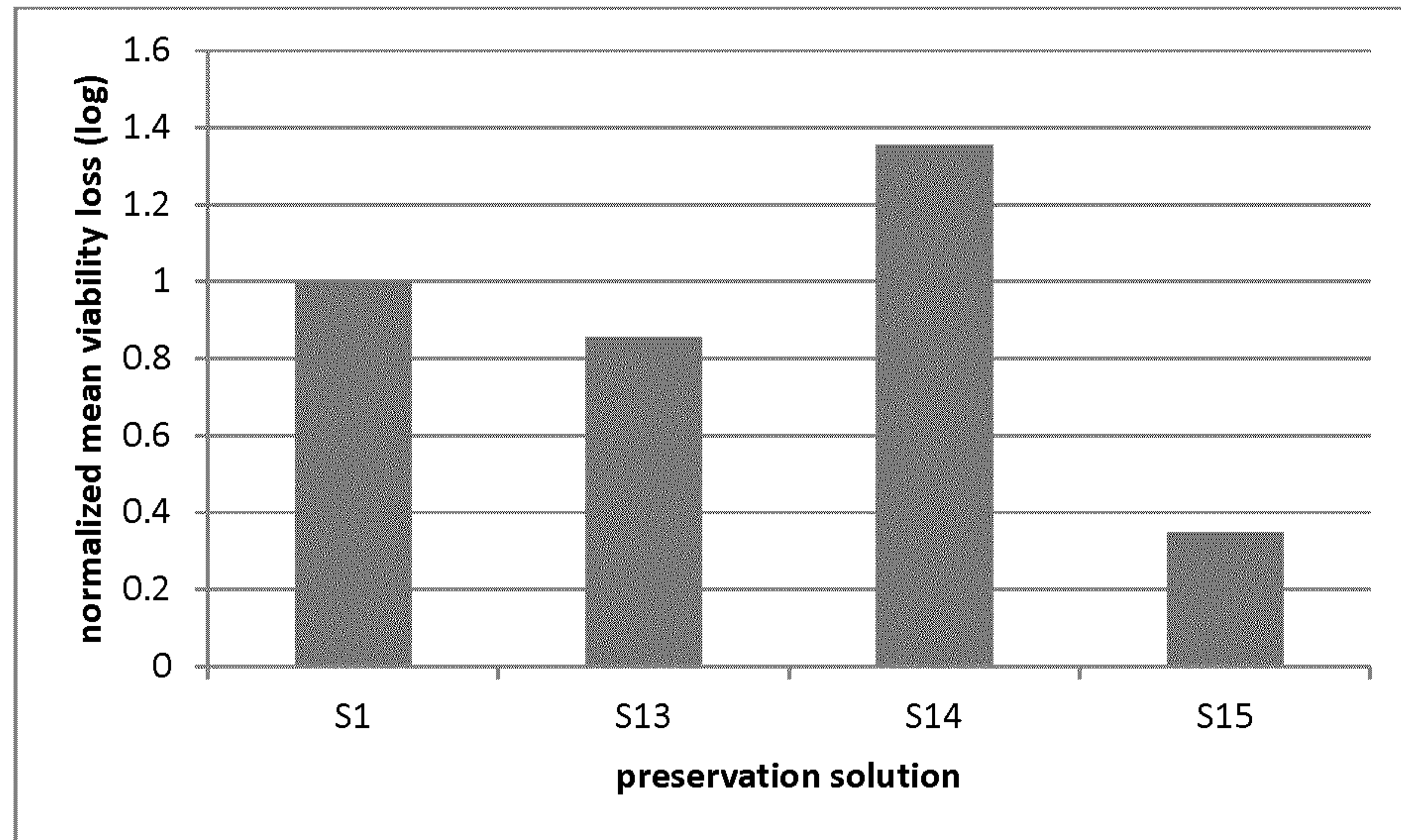
**Figure 2**

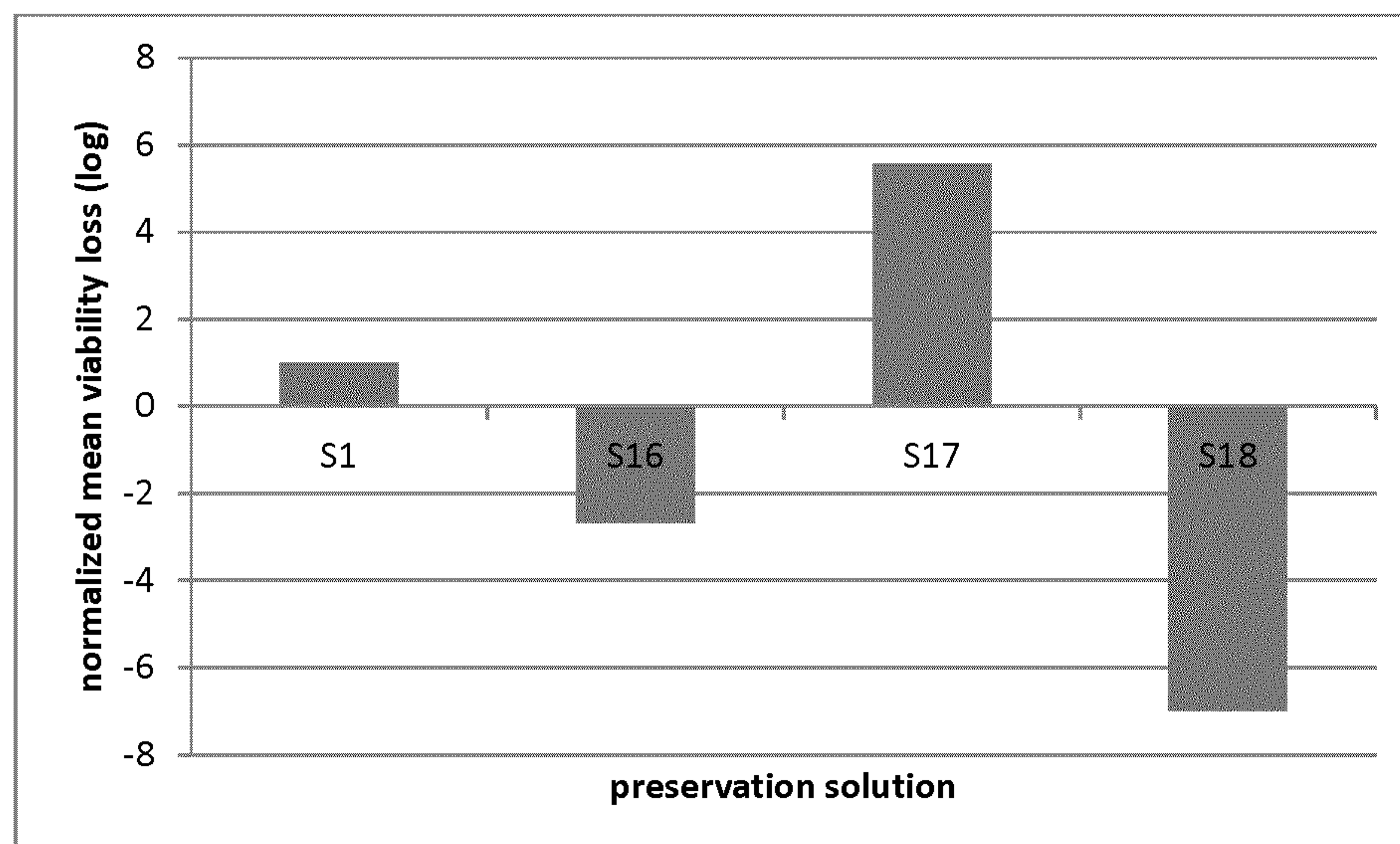
**Figure 3**

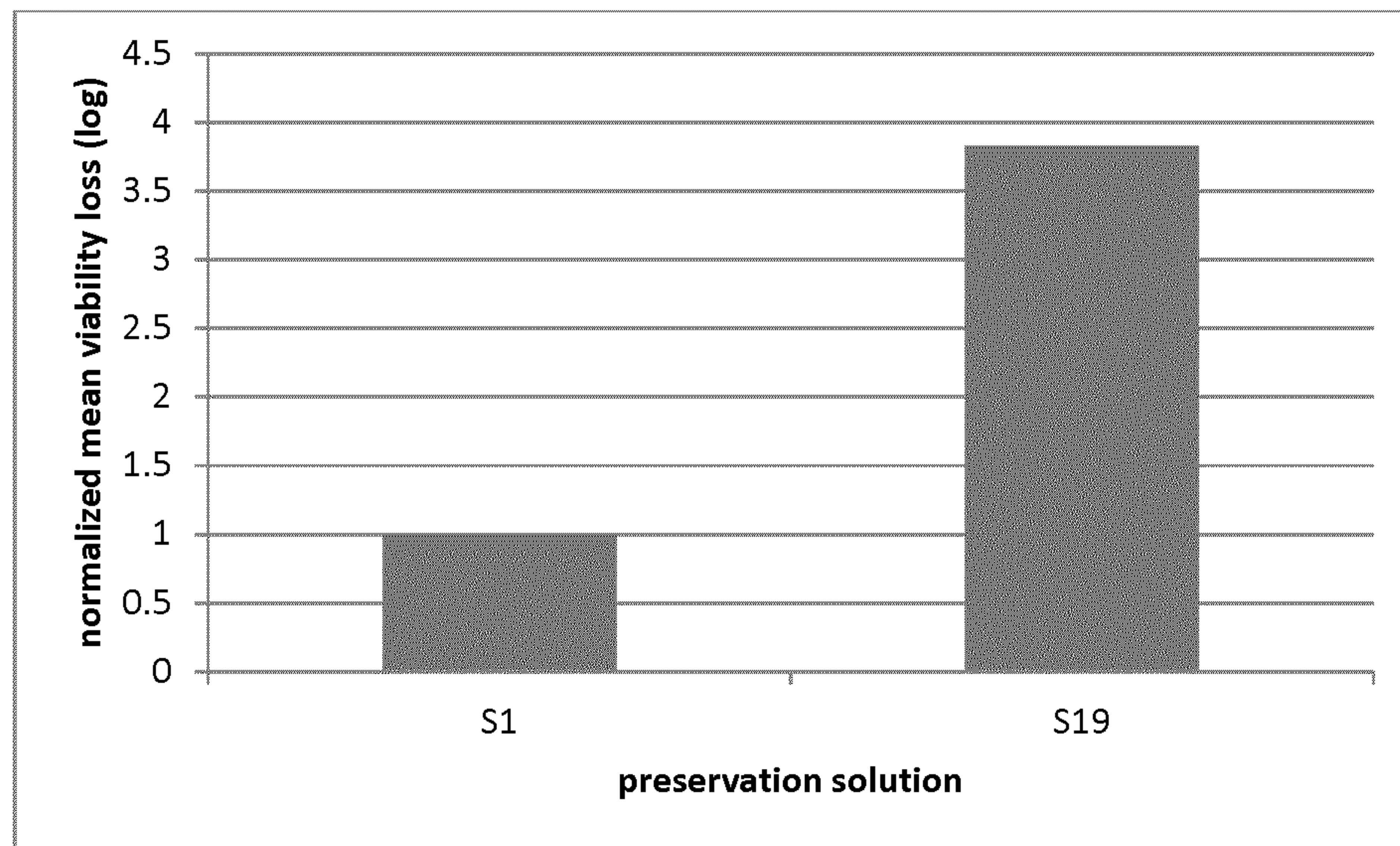
**Figure 4**

**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8**

**Figure 9**

Test	Solution code	Replicates	Drying			a _w		Stability per week (CFU/g)				
			Before (CFU total)	After (CFU total)	difference (log)	Before	After	0	1	2	3	4
1	S1	A	3.1E+11	1.4E+11	-0.36	0.4502	0.1536	3.4E+09	4.5E+09	4.1E+09	4.4E+09	8.6E+09
		B	2.1E+11	1.4E+11	-0.17	0.4831	0.1740	3.8E+09	5.4E+09	4.8E+09	3.2E+09	4.8E+09
		C	3.9E+11	1.4E+11	-0.43	0.4871	0.1671	3.8E+09	5.0E+09	4.3E+09	4.9E+09	5.5E+09
	S2	A	2.3E+11	2.8E+09	-1.92	0.2549	0.0616	1.9E+08	9.9E+07	1.3E+08	1.9E+08	3.5E+08
		B	1.8E+11	8.1E+09	-1.34	0.2836	0.0561	5.4E+08	6.2E+08	4.5E+08	8.2E+08	5.4E+08
		C	2.9E+11	5.3E+09	-1.74	0.2961	0.0434	3.4E+08	1.1E+08	1.5E+08	2.0E+08	4.6E+08
	S3	A	2.3E+11	1.0E+11	-0.34	0.4215	0.1274	4.3E+09	1.8E+09	1.5E+09	4.5E+09	4.4E+09
		B	2.3E+11	2.5E+10	-0.96	0.4011	0.1437	1.1E+09	1.2E+09	1.7E+09	1.5E+09	2.3E+09
		C	3.1E+11	9.1E+10	-0.53	0.4459	0.1791	3.9E+09	5.9E+09	6.1E+09	3.2E+09	6.1E+09
	S4	A	2.9E+11	2.4E+11	-0.08	0.4687	0.1376	6.6E+09	6.3E+09	4.9E+09	5.0E+09	6.3E+09
		B	2.6E+11	1.6E+11	-0.20	0.4848	0.1429	4.6E+09	4.0E+09	5.3E+09	5.6E+09	4.2E+09
		C	3.9E+11	3.5E+11	-0.04	0.5114	0.1463	9.7E+09	5.8E+09	6.6E+09	6.2E+09	6.2E+09
2	S1	A	2.5E+11	1.5E+11	-0.24	0.5219	0.2174	3.7E+09	7.4E+09	5.8E+09	6.6E+09	5.9E+09
		B	4.3E+11	2.0E+11	-0.34	0.5582	0.2299	5.0E+09	5.0E+09	4.8E+09	6.9E+09	7.7E+09
		C	3.0E+11	1.9E+11	-0.20	0.5255	0.2416	4.9E+09	5.1E+09	6.3E+09	4.9E+09	5.9E+09
	S5	A	3.6E+11	2.0E+11	-0.24	0.5016	0.2470	5.4E+09	1.0E+10	8.3E+09	4.7E+09	6.4E+09
		B	4.3E+11	2.6E+11	-0.22	0.5011	0.2413	6.5E+09	5.8E+09	6.6E+09	6.2E+09	8.0E+09
		C	2.8E+11	3.2E+11	0.07	0.5864	0.2592	8.1E+09	7.4E+09	5.6E+09	5.7E+09	9.4E+09
	S6	A	2.8E+11	4.8E+11	0.23	0.7511	0.2747	1.2E+10	9.4E+09	6.2E+09	8.1E+09	8.8E+09
		B	3.8E+11	2.5E+11	-0.19	0.5017	0.2539	6.2E+09	5.6E+09	7.1E+09	5.9E+09	7.5E+09
		C	2.8E+11	1.3E+11	-0.33	0.5065	0.2511	3.3E+09	4.6E+09	7.2E+09	4.7E+09	5.0E+09
	S7	A	3.3E+11	2.8E+11	-0.07	0.5929	0.2840	7.0E+09	1.0E+10	8.5E+09	6.9E+09	1.0E+10
		B	3.8E+11	2.6E+11	-0.16	0.5190	0.3027	6.5E+09	8.1E+09	4.8E+09	4.7E+09	8.6E+09
		C	3.1E+11	2.7E+11	-0.06	0.5104	0.3086	6.6E+09	4.9E+09	4.9E+09	4.4E+09	6.2E+09
3	S1	A	2.0E+11	1.6E+11	-0.10	0.4418	0.2399	4.5E+09	5.6E+09	2.9E+09	4.1E+09	3.2E+09
		B	2.5E+11	1.7E+11	-0.17	0.4611	0.2366	4.9E+09	5.4E+09	4.0E+09	5.3E+09	4.6E+09
		C	2.1E+11	1.8E+11	-0.07	0.4561	0.2468	5.1E+09	5.3E+09	3.1E+09	4.9E+09	4.8E+09
	S0	A	1.8E+11	2.9E+05	-5.79	0.3066	0.0391	3.8E+04	4.4E+05	3.6E+04	4.7E+05	4.7E+05
		B	2.1E+11	1.0E+06	-5.31	0.3343	0.0362	1.4E+05	7.1E+05	9.4E+05	2.8E+06	1.5E+08
		C	1.7E+11	3.2E+06	-4.73	0.3510	0.0356	4.2E+05	8.5E+05	7.2E+05	9.4E+05	3.1E+06
	S8	A	3.2E+11	4.5E+10	-0.85	0.3631	0.0680	1.5E+09	1.9E+09	3.4E+09	3.2E+09	2.5E+09
		B	2.3E+11	4.7E+10	-0.69	0.3585	0.0565	1.5E+09	1.1E+09	1.6E+09	2.2E+09	1.9E+09
		C	3.0E+11	8.6E+10	-0.54	0.3777	0.0614	2.8E+09	1.9E+09	3.0E+09	4.2E+09	3.3E+09
	S9	A	2.3E+11	1.5E+11	-0.17	0.4625	0.2392	4.5E+09	4.5E+09	3.6E+09	3.8E+09	4.0E+09
		B	3.0E+11	1.7E+11	-0.25	0.4433	0.2862	4.8E+09	3.9E+09	3.8E+09	4.4E+09	4.5E+09
		C	1.9E+11	1.4E+11	-0.14	0.4472	0.3011	4.0E+09	5.0E+09	4.3E+09	4.8E+09	2.9E+09
4	S1	A	3.1E+11	2.3E+11	-0.14	0.4589	0.1150	5.7E+09	5.8E+09	4.5E+09	5.7E+09	4.9E+09
		B	3.9E+11	3.0E+11	-0.11	0.4655	0.1267	7.6E+09	6.0E+09	4.5E+09	6.6E+09	4.5E+09
		C	4.1E+11	3.1E+11	-0.12	0.5011	0.1272	7.8E+09	6.3E+09	4.7E+09	4.6E+09	6.1E+09
	S10	A	2.9E+11	3.2E+11	0.04	0.4617	0.1279	8.3E+09	6.6E+09	5.6E+09	5.9E+09	6.5E+09
		B	4.9E+11	3.1E+11	-0.20	0.4977	0.1363	7.6E+09	7.5E+09	6.9E+09	5.9E+09	6.2E+09
		C	4.2E+11	3.7E+11	-0.06	0.5117	0.1417	9.0E+09	7.8E+09	8.5E+09	6.6E+09	6.3E+09
	S11	A	2.8E+11	1.8E+11	-0.20	0.4289	0.1567	6.2E+09	3.9E+09	4.1E+09	5.5E+09	3.0E+09
		B	3.3E+11	1.8E+11	-0.27	0.4003	0.2199	6.2E+09	5.0E+09	4.0E+09	3.1E+09	2.6E+09
		C	3.6E+11	2.1E+11	-0.24	0.4287	0.2259	7.6E+09	8.1E+09	5.0E+09	6.3E+09	2.4E+09
	S12	A	3.8E+11	6.1E+11	0.20	0.5165	0.1639	1.6E+10	1.2E+10	1.1E+10	1.1E+10	9.0E+09
		B	4.4E+11	5.5E+11	0.10	0.4994	0.1719	1.5E+10	1.2E+10	1.1E+10	1.3E+10	9.7E+09
		C	3.9E+11	4.3E+11	0.04	0.4652	0.1596	1.1E+10	8.6E+09	1.1E+10	7.9E+09	5.2E+09
5	S1	A	4.5E+11	2.4E+11	-0.28	0.4816	0.1677	5.6E+09	4.5E+09	3.1E+09	5.5E+09	5.0E+09
		B	3.7E+11	3.1E+11	-0.09	0.5490	0.1824	7.3E+09	6.8E+09	7.0E+09	7.0E+09	7.6E+09
		C	4.0E+11	2.7E+11	-0.18	0.4739	0.1821	6.1E+09	5.9E+09	6.4E+09	5.5E+09	5.9E+09
	S13	A	3.7E+11	2.5E+11	-0.17	0.6726	0.2386	6.1E+09	5.6E+09	6.2E+09	6.0E+09	6.7E+09
		B	3.4E+11	2.5E+11	-0.14	0.4740	0.2422	6.1E+09	7.4E+09	5.0E+09	6.8E+09	5.9E+09
		C	4.2E+11	3.0E+11	-0.15	0.5405	0.2602	7.3E+09	6.3E+09	3.7E+09	7.5E+09	6.3E+09
	S14	A	3.1E+11	2.0E+11	-0.19	0.4778	0.1371	6.5E+09	4.9E+09	3.5E+09	5.3E+09	4.7E+09
		B	4.2E+11	2.6E+11	-0.20	0.4295	0.1184	8.4E+09	8.6E+09	6.2E+09	6.6E+09	5.0E+09
		C	4.2E+11	2.0E+11	-0.33	0.4864	0.1434	6.3E+09	8.4E+09	8.2E+09	9.0E+09	6.5E+09
	S15	A	4.2E+11	3.5E+11	-0.08	0.5324	0.1938	9.5E+09	8.5E+09	8.3E+09	1.1E+10	8.3E+09
		B	4.5E+11	3.4E+11	-0.12	0.4612	0.1854	9.5E+09	9.1E+09	8.3E+09	7.9E+09	7.6E+09
		C	4.5E+11	4.6E+11	0.01	0.5118	0.2164	1.2E+10	9.1E+09	8.9E+09	8.4E+09	7.3E+09
6	S1	A	2.9E+11	2.9E+11	0.00	0.7314	0.1529	7.1E+09	6.5E+09	7.3E+09	6.7E+09	6.7E+09
		B	3.5E+11	3.7E+11	0.02	0.8990	0.1590	9.0E+09	8.3E+09	8.9E+09	9.4E+09	8.2E+09
		C	3.5E+11	2.8E+11	-0.11	0.5714	0.1540	6.9E+09	5.3E+09	5.5E+09	6.8E+09	6.2E+09
	S16	A	3.5E+11	3.2E+11	-0.04	0.5313	0.1219	8.1E+09	8.9E+09	1.3E+10	1.2E+10	9.0E+09
		B	4.1E+11	4.9E+11	0.08	0.5198	0.1326	1.2E+10	1.1E+10	1.3E+10	1.7E+10	1.2E+10
		C	3.3E+11	4.9E+11	0.18	0.6745	0.1524	1.3E+10	1.1E+10	9.0E+09	1.3E+10	1.1E+10
	S17	A	3.0E+11	1.9E+11	-0.21	0.5994	0.0372	5.3E+09	6.5E+09	6.8E+09	5.5E+09	7.3E+09
		B	3.8E+11	2.9E+11	-0.12	0.9267	0.0439	8.2E+09	8.5E+09	9.8E+09	8.9E+09	1.1E+10
		C	3.0E+11	2.2E+11	-0.13	0.7010	0.0371	6.5E+09	7.0E+09	5.9E+09	5.8E+09	5.8E+09
	S18	A	2.5E+11	3.7E+11	0.17	0.5388	0.1469	9.6E+09	7.9E+09	1.2E+10	7.8E+09	6.3E+09
		B	3.2E+11	4.4E+11	0.14	0.5327	0.1771	1.1E+10	1.3E+10	8.7E+09	1.1E+10	1.1E+10
		C	2.5E+11	4.6E+11	0.26	0.5359	0.2048	1.2E+10	1.1E+10	9.4E+09	1.1E+10	9.4E+09
7	S1	A	3.5E+11	3.1E+11	-0.05	0.6498	0.1473	8.1E+09	8.0E+09	-	6.9E+09	5.9E+09
		B	3.5E+11	3.7E+11	0.03	0.4659	0.1387	9.7E+09	8.8E+09	-	7.3E+09	-
		C	3.5E+11	2.9E+11	-0.08	0.8656	0.1871	7.6E+09	9.9E+09	-	9.2E+09	7.9E+09
	S19	A	3.0E+11	2.5E+11	-0.08	0.6582	0.1401	6.8E+09	5.7E+09	-	4.3E+09	5.7E+09
		B	3.5E+11	2.6E+11	-0.13	0.5270	0.1194	7.0E+09	7.2E+09	-	5.8E+09	6.0E+09
		C	3.4E+11	2.2E+11	-0.19	0.4994	0.1280	6.0E+09	5.0E+09	-	4.6E+09	4.5E+09

Figure 10

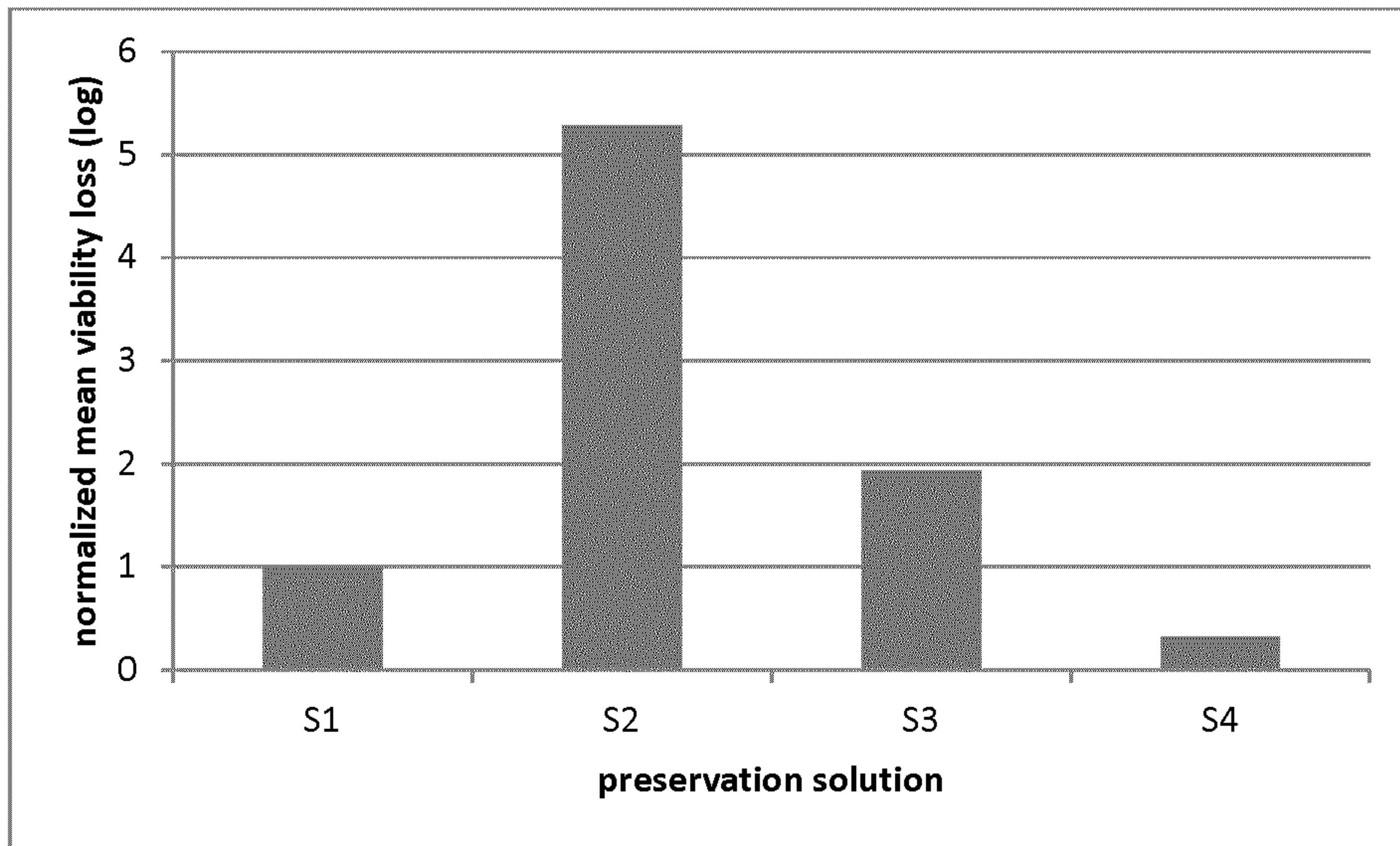


Figure 3