APPENDIX A

Microbiological analysis

1. Plate Count Agar (PCA) per liter (AOAC, 2000)

Tryptone	5.0	g
Yeast Extract	2.5	g
Dextrose (Glucose)	1.0	g
Agar	15.0	g
pH	7.0 ± 0.2	

Preparation

Suspend 22.5 g/l, autoclave 15 min at 121°C. If desired, add 1.0 g skim milk powder/liter prior to sterilization. The plates are clear and yellowish.

2. Potato Dextrose Agar (PDA) per liter (AOAC, 2000)

Potato infusion (infusion from 200 g potatoes)	4.0	g
Dextrose	20.0	g
Agar	15.0	g
pH	5.6 ± 0.2	

Preparation

Suspend 39 g/l, autoclave at 15 min for 121 °C. If the pH has to be adjusted to 3.5, add approx. 14 ml of a sterile 10 % tartaric acid solution/liter at a temperature of 45-50°C. The plates are clear and yellowish-brown.

3. Mcconkey Agar per liter (AOAC, 2000)

Peptone	20.0	g
Bile Salt	5.0	g
Lactose	10.0	g
Sodium Chloride	5.0	g
Neutral Red	0.05	g
Agar	12.0	g
pH	7.1 ± 0.2.	

Preparation of Mcconkey agar

Suspend 54 g in 1 l H_2O and mix thoroughly until homogeneous. Heat with occasional agitation. Boil 1-2 min until ingredients dissolve and autoclave 15 min at 121°C.

4. De Man Rogasa Sharp (MRS) per liter (AOAC, 2000)

Peptone	10.0	g
Meat Extract	8.0	g
Yeast Extract	4.0	g
Lactose	10.0	g
Sodium Acetate	5.0	g
Diammonium Citrate	2.0	g
Dipotassium Phosphase	2.0	g
Magnesium Sulfate	0.2	g
Manganese Sulfate	0.05	g
Tween 80 (Polysorbate)	1.0	g
2% Bromcresol Purple	2.0	g
Agar	14.0	g
pH	5.7 ± 0.2	

Preparation

Suspend 66.2 g in 1 l of purified water and heat to boiling to dissolve completely, autoclave for 15 min at 121°C. Autoclaving for 15 minutes at 118°C. The plates are clear and brown.

5. Tryptic Soy Agar (TSA) per liter (Busta, 1984)

Tryptone	15.0	g
Soytone (Soy Bean Peptone)	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
pH	7.4 ± 0.2	

Preparation

Suspend 40 g/l in 1 l H_2O, autoclave 15 min at 121°C. After preparation both media are clear and yellowish-brown.

6. Tryptose-Sulfite-Cycloserine (TSC) Agar per litre (AOAC, 2000)

Tryptose	15.0	g
Soytone	5.0	g
Yeast Extract	5.0	g
Sodium Metabisulfite	1.0	g
Ferric Ammonium Citrate	1.0	g
0.5% D-Cycloserine Solution	20.0	ml
50% Egg yolk Emulsion	20.0	ml
Agar	20.0	g
pH	7.6	

D-Cyclosrine Solution: Dissolve 1 g D-Cycloserine (Sigma, Germany) without heating in 200 ml 0.05 M phosphate buffer (pH 8.0 ± 0.1).

Egg yolk Emulsion: Wash fresh eggs with stiff brush and drain. Soak 1 h in 70% alcohol. Aseptically remove yolk and mix with equal volume sterile 0.85% NaCL aqueous solution (w/v). Store at 4°C.

Preparation

Diluted to 1 l H_2O. Dispense 250 ml portions into 500 ml flasks, autoclave 15 min at 121°C. Before plating, add 20.0 ml 0.5% D-Cycloserine to each 250 ml sterile melted medium at 50°C. To make egg yolk containing plates, add 20.0 ml 50% egg yolk emulsion to 250 ml sterile medium containing D-cycloserine.

7. Mannitol Egg Yolk Polymyxin (MYP) Agar per litre (AOAC, 2000)

Beef Extract	1.0	g
Peptone	10.0	g
D-Mannitol	10.0	g
Sodium Chloride	10.0	g
Phenol Red	0.025	g
50% Egg yolk Emulsion	12.5	ml
Polymyxin B Solution	2.5	ml
Agar	15.0	g
pH	7.2± 0.1	

Polymyxin B solution: Dissolve 500,000 units sterile polymyxin B sulfate in 50 ml sterile H_2O

Preparation

Suspend 21.5 g in 450 ml purified water, autoclave 15 min at 121°C. Cool to about 45 to 50°C. Add 50 ml (this volume can be varied depending on the degree of turbidity desired) of sterile egg-yolk emulsion. Pour plates. The plates (include egg yolk) are evenly turbid and slightly orange (red without egg-yolk).

8. Lauryl Sulfate Tryptose (LST) Broth per litre (AOAC, 2000)

Tryptose	20.0	g
Lactose	5.0	g
Sodium Chloride	5.0	g
Dipotassium Hydrogen Phosphate	2.75	g
Potassium Dihydrogen Phosphate	2.75	g
Sodium Lauryl Sulfate	0.1	g
pH	6.8±0.1	

Preparation

Dissolved 35.6 g in 1 l H_2O with gentle heated, if necessary. Dispense 10 ml portions into 20×150 mm test tubes containing inverted Durham tubes, autoclave 15 min at 121°C.

9. Brillant Green Lactose Bile (BGLB) Broth per litre (AOAC, 2000)

Peptone	10.0	g
Lactose	10.0	g
Dehydrated oxgall or oxbile	20.0	g
0.1% Brilliant Green Solution	13.3	ml
pH	7.2± 0.1	

Preparation

Single strength: Dissolved 400 g in 1 l H_2O. Filter through cotton. Dispense 10 ml portions into 20×150 min test tubes containing inverted Durham tubes, autoclave 15 min at 121°C.

Double strength: Dissolved 80.0 g in 1 l H_2O. Filter through cotton. Dispense 10 ml portions into 20×150 min test tubes containing inverted Durham tubes, autoclave 15 min at 121°C.

Pour plate technique for bacterial enumeration (AOAC, 2000)

The pour plate technique was used in this research to determine the number of specific microorganisms in 1ml of noni juice samples. The technique was chosen because it did not require previously prepared plates and was often used to assay bacterial contamination in food products. The technique was conducted by first labeling the bottom of an empty sterile petri dish. After that 1 ml of each dilution of homogenate samples was aseptically transferred into the petri dish. An amount of 10 ml agar was poured into the dish and mixed well with the sample by gently rotating the dish. When agar inside the petri dish has solidified, the petri dish was placed in an upright position in an incubator at 37°C for 24 h. At the end of the incubation period, the petri dish was examined by considering each colony represents a Colony Forming Unit (CFU). The dilution that produced a microorganism number between 30-300 CFU/ml was taken into a calculation to determine the number of microorganism in the noni juice sample.

Plate count technique for *Clostridium* spp. (AOAC, 2000)

The number of *Clostridium* spp. in noni juice samples was examined using Tryptose Sulfite Cycloserine (TSC) agar. The examination was done by pouring approximately 5 ml TSC agar into a sterile petri dishes and spreading the agar evenly by rapidly rotating dish. When agar had solidified, the plate was labeled and 1 ml of each dilution of homogenate solution was aseptically transferred onto the agar surface in center of dish. An additional 5 ml TSC agar was poured into the dish and mix well with the sample solution by gently rotating dish. After the second agar addition had solidified, the petri dish was placed in an upright position in an anaerobic jar. The jar was incubated anaerobically for 20 h at 35°C. After the incubation time, the petri dish was removed from the jar and observed macroscopically for colony growth and black colony production. Petri dish that showed approximately 20-200 black colonies were selected to be counted. Using a piece of white tissue paper over counting area, count black colonies and calculate the number of *Clostridium* spp. in 1 ml of noni sample. *Clostridium perfringens* colonies in medium containing egg yolk are black and usually surrounded by 2-4 mm zone of white precipitate due to lecithinase activity. However, because a few strains are weak or negative for lecithinase, any black colonies suspected to be *Cl. perringens* was counted. The plate count technique for *Clostridium* spp. was done in duplicate for each dilution of noni juice samples.

Colony count (AOAC, 2000)

$$N = \Sigma C / [(1x\ n_1) + (0.1x\ n_2)]\ d$$

Where N = number of colonies per ml

ΣC = sum of all colonies on all plates counted

n_1 = number of plates in lower dilution counted

n_2 = number of plates in next highest dilution counted

d = dilution from which the first counts were obtained

Enumeration of coliforms and faecal coliforms (AOAC, 2000)

The presence of coliforms in a food usually indicates that it has been manufactured under unsanitary conditions. The presence of faecal coliforms usually indicates potential (post-processing) contamination of the product with faecal matter. This test involves a multiple tube fermentation technique, which estimates the "Most Probable Number" (MPN) of total coliforms and faecal coliforms

Presumptive Tests

1. Use Lauryl Sulfate Tryptose broth (LST). Dispense in 10 ml volumes into tubes containing gas vials (inverted Durham tubes).
2. Arrange LST broth tubes in rows of three and mark them identifying the sample unit and the dilution to be inoculated.
3. Inoculate each of separate sets of five tubes of LST broth with each dilution of food homogenate.

4. In order to verify growth conditions in the elevated temperature water baths, inoculate a culture of *E. coli* known to ferment lactose and produce gas at 45°C and a culture of *Salmonella berta* into tubes of LST broth as a positive and negative control, respectively, for each bath used. Transfer into all media used at different stages of the procedure. Set up an uninoculated tube of medium corresponding to each step in the procedure as a media control.

5. Mix inoculum and medium by gently shaking or rotating the tubes, but avoid entrapping air in the gas vials.

6. Incubate the inoculated LST broth tubes at 35°C for 24 ± 2 h. Examine for gas formation (gas formation may be either a gas bubble or effervescence), record the results and if required, begin on the same day the confirmed and presumptive *E. coli* (faecal coliform) tests for all gas positive tubes.

7. Incubate gas negative tubes for an additional 24 ± 2 h, examine, record the number of additional gas positive tubes, add to the result and begin the confirmed and presumptive *E. coli* (faecal coliform) tests for the additional gas positive tubes.

8. The absence of gas in all of the tubes at the end of 48 ± 4 h of incubation constitutes a negative presumptive test.

9. Compute the "MPN" of presumptive coliforms/ml of noni juice samples by following the table to convert the number of gas positive tubes to MPN values (Table A). Record the results.

Confirmed Test

Use Brilliant Green Lactose Bile (BGLB) broth dispensed in 10 ml volumes in tubes containing Durham tube.

1. Shake or rotate the positive LST broth tubes to mix the contents and transfer one loopful from each tube to a tube of BGLB broth (avoid transferring pellicle). Sterile wood applicator sticks may be used for making the transfers. Do not discard the LST broth tubes at this time.

2. Mix inoculum and medium by gently shaking or rotating the tubes, but avoid entrapping air in the gas vials.

3. Incubate the inoculated BGLB broth tubes at 35°C for 24 ± 2 h. Examine for gas formation (gas bubble or effervescence) and record results.

4. Incubate gas negative tubes for an additional 24 ± 2 h, re-examine, record the numbers of additional gas positive tubes and add to the result.

5. Formation of gas 48 ± 4 h incubation constitutes a positive confirmed test.

Table A. Value of the MPN inoculated from each of three successive decimal dilutions (AOAC, 2000).

Positive tubes			
0.1	**0.01**	**0.001**	**MPN/g**
0	0	0	<3
0	0	1	3
0	0	2	6
0	0	3	9
0	1	0	3
0	1	1	6.1
0	1	2	9.2
0	1	3	12
0	2	0	6.2
0	2	1	9.3
0	2	2	12
0	2	3	16
0	3	0	9.4
0	3	1	13
0	3	2	16
1	0	0	3.6
1	0	1	7.2
1	0	2	11
1	0	3	15
1	1	0	7.3
1	1	1	11
1	1	2	15
1	1	3	19
1	2	0	11
1	2	1	15
1	2	2	20
1	2	3	24
1	3	0	16
1	3	1	20
1	3	2	24
1	3	3	29
2	0	0	9.1
2	0	1	14
2	0	0	20
2	0	3	26
0	1	0	15
2	1	1	20
2	1	2	27
2	1	3	34
2	2	0	21
2	2	1	28
2	2	2	35
2	2	3	42
2	3	0	29
2	3	1	36
2	3	2	44
3	0	0	23
3	0	1	39
3	0	2	23
3	0	3	95
3	1	0	43
3	1	1	75
3	1	2	120
3	1	3	160
3	2	0	93
3	2	1	150
3	2	2	210
3	2	3	290
3	3	0	240
3	3	1	460
3	3	2	1100
3	3	3	>1100

Gram staining (Anonymous, 2000d)

Gram staining (or Gram's method) is an empirical method of differentiating bacterial species into two large groups based on the chemical and physical properties of their cell walls. The method is named after the inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 to discriminate between pneumococci and *Klebsiella pneumoniae* bacteria.

Gram stains are performed on body fluid or biopsy when infection is suspected. It yields results much quicker than culture, and is especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis. It necessitates the 24 hr staffing of microbiological laboratories in hospitals.

description of Gram staining

1. First, an inoculum is taken from a culture using an inoculation loop and put on a slide and then allowed to air dry. If the culture is solid, it is diluted by adding a drop of water or sterile saline on the slide and mixing with the loop. It is important here to take a very small inoculum so that the end result is a sparse single layer of bacteria. It is a common mistake for beginners to put far too much inoculum at this step.

2. The specimen is heat-fixed by passing the slide, inoculum side up, through a bunsen flame 1-2 times, without allowing the slide to become hot to the touch.

3. A basic dye, crystal violet or gentian violet, is used to stain the slide. This dye is taken up by both Gram positive and Gram negative bacteria. Allow to stain for 1 minute. The slide should look purple to the unaided eye, and if examined microscopically at this point both Gram positive and Gram negative bacteria are purple. Lugol can also be used instead of crystal violet.

4. Rinse off with water for a maximum of 5 s.

5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 min. This acts as a mordant and fixes the dye.

6. Rinse with water.

7. Apply 95% ethanol or a mixture of acetone and alcohol several times until no more colour appears to come from the sample. This washes away all the unbound basic dye, (usually crystal violet) and leaves Gram positive organisms stained purple and Gram negative organisms unstained (colourless).

8. Rinse with water immediately to prevent over-decolourisation.

9. Apply a suitable counterstain. Opinions vary as to the best choice but suitable stains include safranin. This stain is taken up by both Gram positive and Gram negative organisms, but does not alter the colour of Gram positive organism much, as they are already purple. It does, however, make the Gram negative organisms pinkish-red.

10. Blot gently and allow dried. Do not smear.

Interpretation

Inspect the slide under a microscope. Gram positive organisms will appear blue-black or purple. Gram negative organisms will appear red or pink. Organisms that cannot reliably be differentiated by this staining technique are said to be Gram variable.

Mechanism

Gram positive bacteria have a thick mesh-like cell wall made of peptidoglycan which is capable of retaining the violet dye/iodine complex. Gram-negative bacteria have a thin cell wall made of a layer of peptidoglycan. In addition to an inner membrane, they also have an outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space.

The decolorizing mixture causes dehydration of the multilayered peptidoglycan in the Gram positive cell wall, thus decreasing the space between the molecules and causing the cell wall to trap the crystal violet-iodine complex within the cell. But in Gram negative bacteria, the decolorizing mixture acts as a lipid solvent and dissolves the outer membrane of the Gram negative cell wall. The thin layer of peptidoglycan is unable to retain the crystal violet-iodine complex and the Gram negative cell is decolorized. The decolorization step is the crucial one, and requires some degree of skill, as Gram positivity is not an all-or-none phenomenon.

As a rule of thumb (which has exceptions), Gram negative bacteria are more dangerous as disease organisms, because their outer membrane is often hidden by a capsule or slime layer which hides the antigens of the cell and so acts as "camouflage" the human body recognises a foreign body by its antigens; if they are hidden, it becomes harder for the body to detect the invader. Often the presence of a capsule will increase the virulence of a pathogen. Additionally, Gram negative bacteria have lipopolysaccharide in their outer membrane. Lipopolysaccharide is an endotoxin which increases the severity of inflammation. This inflammation may be so severe that septic shock may occur. Gram positive infections are generally less severe because the human body does not contain peptidoglycan, and in fact the human body produces an enzyme called lysozyme which attacks the open peptidoglycan layer of Gram positive bacteria. Gram positive bacteria are also much more susceptible to beta-lactam antibiotics, such as penicillin.

APPENDIX B

Chemical analysis

Total titratable acids (AOAC, 2000)

Preparation of food homogenate

Using aseptic technique, weight 100 g food test portion into stomacher and remove undissolved soluble solid by Juicer (National: Model MJ-68M, Thailand). Measuring juice volume and juice weight for analized.

Preparation of reagents

0.1 N NaOH solution: weight 4 g NaOH, dissolve in distilled water and adjust to a volume flask markea at 1 l. NaOH solution standardization by titrate with 0.1 N H_2So_4 standard solution and use phenolphthalein for indicator.

Analysis

1. Pipette 10 ml sample into 100 ml beaker. Add 50 ml boiled and cooled down distilled water.
2. Titrate with 0.1 N NaOH solution by Magnetic stirrer and Magnetic bar.
Measuring pH by pH-meter during titration.
3. Titrate until pH 7 endpoint for tartaric acid and pH 8.1 for citric and malic acid. Recorded the titrated NaOH solution volume for calculate the total titratable. Analyzed 3 times for each sample.

Calculation

Formula to calculate TA of noni juices in % lactic acid

1 ml of 0.1 N NaOH = 0.009 g lactic acid

APPENDIX C

Nutraceuticals identified in noni

1-butanol

1-hexenol

1-methoxy-2-formyl-3-hydroxy anthraquinone

2, 5-undecadien-1-ol

2-heptan one

2-methyl-2-butanoyl decanoate

2-methyl-2-butanoyl hexanoate

2-methyl-3, 5, 6-trihydroxyanthraquinone-6-δ-primeveroside

2-methyl-3, 5, 6-trihydroxyanthraquinones

2-methyl butanoic acid

2-methylpropanoic acid

24-methylcycloartanol

24-methylene cholesterol

24-methyenecycloartanyl linoleate

3-hydroxyl-2-Butazone

3-hydroxymorindone

3-hydroxymorindone-6-δ-primeveroside

3-methyl-2-buten-1-ol

3-methyl-3-buten-1-ol

3-methylthiopropanoic acid

5, 6-dihydroxylucidin

5, 6-dihydroxylucidin-3-δ-primeveroside

5, 7-acacetin7-O-δ-D- (+)-gluco pyranoside

5, 7-dimethyl apigenin-4Õ-O-δ-D-D (+)-galactopyranoside

6, 8-di methoxy-3-methyl anthraquinone-1, -O-δ-rhamnosyl

Gluco pyranoside

6-dodecanoic-y-lactone

7-hydroxy-8-methoxy-2-methyl anthraquinone
8, 11, 14-eicosatrienoic acid
acetic acid
alizarin
alkaloids
anthragallol 1,2-dimethyl ether
anthraquinones
anthragallol 2,3-dimethyl ether
asperuloside
benzoic acid
benzyl alcohol
butanoic acid
calcium
campesteryl glycoside
campesteryl linoleic glycoside
campesteryl palmitate
campesteryl palmityl glycoside
campesterol
carbonate
carotene
cycloartenol
cycloartenol linoleate
cycloartenol palmitate
damnacanthal
decanoic acid
elaidic acid
ethyl decanoate
ethyl hexanoate
ethyl octanoate
ethyl palmitate
eugenol
ferric iron

gampesteryl linoleate

glucose

glycosides

heptanoic acid

hexadecane

hexa-amide

hexanedioic acid

hexanoic acid

hexose

hexyl hexanoate

iron

isobutyric acid

iso caproic acid

iso fucosterol

isofucosteryl linoleate

isovaleric acid

lauric acid

limonene

linoleic acid

lucidum

lucidum-3-δ-primeveroside

magnesium

methyl 3-methylthio-propanoate

methyl decanoate

methyl elaidate

methyl hexanoate

methyl octanoate

methyl oleate

methyl palmitate

morenone-1

morenone-2

morindadiol

morindanigrine

morindin

morindone

morindone-6-δ-primeveroside

mucilaginous matter

myristic acid

n-butyric acid

n-valeric acid

nonanoic acid

nordamnacanthal

octadecanoic acid

octanoic acid

oleic acid

palmitic acid

paraffin

pectins

pentose

phenolic body

phosphate

physcion

physcion-8-O [{L-arabinopyranosyl} (1-3) {δ-D-g-D-galactopyranosyl (1-6) {δ-D-galactopyranoside}]

potassium

protein

proxeronine

proxeroninease

resins

rhamnose

ricinoleic acid

rubiadin

rubiadin-1-methyl ether

scopoletin

sitosterol
sitosteryl glycoside
sitosteryl linoleate
sitosteryl linoleyl glycoside
sitosteryl palmitate
sitosteryl palmityl glycoside
sodium
sorandjidiol
δ-sitosterol
stearic acid
sterols
stigmasterol
stigmasteryl glycoside
stigmasteryl linoleate
stigmasteryl linoleyl glycoside
stigmasteryl palmitate
stigmasteryl palmityl glycoside
terpenoids
trixymethylantraquinone
undecenoic acid
ursolic acid
xeronine

Source: Hirazumi and Furusawa (1999)

APPENDIX D

Results on microbiological quality of pasteurized noni juices

Table A The effect of storage temperature at 4°C on the microbiological quality of pasteurized noni juices at 64°C 15 min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.81 ± 0.01[a]	2.82 ± 0.18[a]	2.79 ± 0.02[a]	3.21 ± 0.01[ab]
Yeasts and moulds (log CFU/ml)	2.82 ± 0.18[ns]	2.82 ± 0.01[ns]	2.53 ± 0.01[ns]	2.75 ± 0.01[ns]
Gram negative bacteria (log CFU/ml)	2.58 ± 0.03[ns]	2.54± 0.02[ns]	2.71 ± 0.03[ns]	2.69 ± 0.00[ns]
Lactic acid bacteria (log CFU/ml)	2.63 ± 0.01[ns]	2.66 ± 0.01[ns]	2.53 ± 0.03[ns]	2.57 ± 0.01[ns]
Coliform (MPN/g)	<3[ns]	<3[ns]	<3[ns]	<3[ns]
Proteolytic bacteria (log CFU/ml)	2.80 ± 0.10[a]	3.26 ± 0.05[b]	2.81 ± 0.01[a]	3.01 ± 0.16[ab]
Bacillus spp. (log CFU/ml)	3.32 ± 0.02[ab]	3.59 ± 0.01[b]	2.66 ± 0.02[a]	3.27 ± 0.00[b]
Clostridium spp. (log CFU/ml)	3.60 ± 0.01[b]	3.46 ± 0.02[b]	2.53 ± 0.03[a]	2.74 ± 0.01[a]

Different letter within a row showed significantly different ($p \leq 0.05$)

Table B The effect of storage temperature at 4°C on the microbiological quality of pasteurized noni juices at 72°C 1 min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.68 ± 0.04[ns]	2.82 ± 0.01[ns]	2.83 ± 0.01[ns]	2.69 ± 0.04[ns]
Yeasts and moulds (log CFU/ml)	2.64 ± 0.04[ns]	2.65 ± 0.02[ns]	2.79 ± 0.04[ns]	2.79 ± 0.02[ns]
Gram negative bacteria (log CFU/ml)	2.87 ± 0.01[a]	3.05 ± 0.14[ab]	2.70 ± 0.01[a]	2.69 ± 0.00[b]
Lactic acid bacteria (log CFU/ml)	2.65 ± 0.00[ns]	2.64 ± 0.01[ns]	2.63 ± 0.01[ns]	2.64 ± 0.01[ns]
Coliform (MPN/g)	<3[ns]	<3[ns]	<3[ns]	<3[ns]
Proteolytic bacteria (log CFU/ml)	2.71 ± 0.02[a]	2.70 ± 0.00[a]	3.49 ± 0.02[b]	3.44 ± 0.02[b]
Bacillus spp. (log CFU/ml)	3.29 ± 0.04[ns]	3.26 ± 0.05[ns]	3.27 ± 0.06[ns]	3.60 ± 0.01[ns]
Clostridium spp. (log CFU/ml)	2.68 ± 0.01[a]	3.47 ± 0.02[b]	2.81 ± 0.01[a]	3.45 ± 0.02[b]

Different letter within a row showed significantly different ($p \leq 0.05$)

Table C The effect of storage temperature at 4°C on the microbiological quality of pasteurized noni juices at 80°C 15 sec

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.87 ± 0.01^{a}	2.83 ± 0.02^{a}	3.22 ± 0.02^{b}	2.81 ± 0.02^{a}
Yeasts and moulds (log CFU/ml)	2.78 ± 0.01^{a}	2.54 ± 0.01^{a}	2.69 ± 0.02^{a}	3.26 ± 0.06^{b}
Gram negative bacteria (log CFU/ml)	2.88 ± 0.02^{a}	3.42 ± 0.03^{b}	2.81 ± 0.01^{a}	3.27 ± 0.06^{b}
Lactic acid bacteria (log CFU/ml)	3.27 ± 0.05^{b}	3.25 ± 0.02^{a}	2.78 ± 0.01^{a}	2.79 ± 0.02^{a}
Coliform (MPN/g)	$<3^{ns}$	$<3^{ns}$	$<3^{ns}$	$<3^{ns}$
Proteolytic bacteria (log CFU/ml)	2.73 ± 0.03^{ns}	2.72 ± 0.03^{ns}	2.88 ± 0.01^{ns}	2.73 ± 0.01^{ns}
Bacillus spp. (log CFU/ml)	3.03 ± 0.16^{ns}	3.46 ± 0.02^{ns}	3.30 ± 0.06^{ns}	3.28 ± 0.04^{ns}
Clostridium spp. (log CFU/ml)	3.54 ± 0.01^{ns}	3.30 ± 0.05^{ns}	3.47 ± 0.02^{ns}	3.59 ± 0.05^{ns}

Different letter within a row showed significantly different ($p \leq 0.05$)

Table D The effect of storage temperature at 4°C on the microbiological quality of pasteurized noni juices at 100°C 10 min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.66 ± 0.01^{ns}	2.74 ± 0.01^{ns}	2.71 ± 0.03^{ns}	2.75 ± 0.02^{ns}
Yeasts and moulds (log CFU/ml)	3.22 ± 0.01^{ns}	3.33 ± 0.02^{ns}	3.20 ± 0.00^{ns}	3.20 ± 0.00^{ns}
Gram negative bacteria (log CFU/ml)	2.82 ± 0.03^{ab}	2.38 ± 0.02^{a}	2.29 ± 0.03^{a}	2.53 ± 0.05^{a}
Lactic acid bacteria (log CFU/ml)	ND	2.38 ± 0.02^{ns}	2.37 ± 0.02^{ns}	2.50 ± 0.03^{ns}
Coliform (MPN/g)	$<3^{ns}$	$<3^{ns}$	$<3^{ns}$	$<3^{ns}$
Proteolytic bacteria (log CFU/ml)	ND	ND	2.56 ± 0.02^{ns}	2.59 ± 0.03^{ns}
Bacillus spp. (log CFU/ml)	2.65 ± 0.01^{a}	2.67 ± 0.01^{a}	2.66 ± 0.02^{a}	3.29 ± 0.05^{b}
Clostridium spp. (log CFU/ml)	2.55 ± 0.01^{a}	2.55 ± 0.02^{a}	3.53 ± 0.02^{b}	3.31 ± 0.01^{b}

Different letter within a row showed significantly different ($p \leq 0.05$)

Table E The effect of storage temperature at room temperature on the microbiological quality of pasteurized noni juices at 64°C 15 min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.82 ± 0.01[a]	4.35 ± 0.01[b]	6.30 ± 0.02[c]	8.01 ± 0.01[d]
Yeasts and moulds (log CFU/ml)	2.52 ± 0.01[a]	5.32 ± 0.01[b]	7.21 ± 0.01[c]	7.95 ± 0.01[d]
Gram negative bacteria (log CFU/ml)	2.52 ± 0.01[a]	4.26 ± 0.01[b]	6.87 ± 0.00[d]	5.21 ± 0.01[c]
Lactic acid bacteria (log CFU/ml)	2.54 ± 0.01[a]	3.21 ± 0.02[c]	2.71 ± 0.00[a]	3.41 ± 0.02[b]
Coliform (MPN/g)	<3[a]	14[b]	75[c]	150[d]
Proteolytic bacteria (log CFU/ml)	2.80 ± 0.02[a]	4.46 ± 0.01[b]	4.36 ± 0.01[b]	4.40 ± 0.02[b]
Bacillus spp. (log CFU/ml)	3.26 ± 0.07[a]	4.85 ± 0.00[b]	7.38 ± 0.01[c]	7.85 ± 0.04[c]
Clostridium spp. (log CFU/ml)	3.09 ± 0.20[a]	4.86 ± 0.01[b]	7.47 ± 0.36[c]	7.40 ± 0.01[c]

Different letter within a row showed significantly different ($p \leq 0.05$)

Table F The effect of storage temperature at room temperature on the microbiological quality of pasteurized noni juices at 72°C 1 min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.70 ± 0.02[a]	4.37 ± 0.01[b]	5.47 ± 0.02[c]	8.42 ± 0.02[d]
Yeasts and moulds (log CFU/ml)	2.63 ± 0.03[a]	4.20 ± 0.03[b]	5.22 ± 0.02[c]	7.37 ± 0.00[d]
Gram negative bacteria (log CFU/ml)	3.29 ± 0.05[a]	3.64 ± 0.01[b]	5.14 ± 0.02[c]	5.89 ± 0.01[d]
Lactic acid bacteria (log CFU/ml)	2.61 ± 0.03[a]	3.28 ± 0.07[b]	4.49 ± 0.03[c]	6.38 ± 0.01[d]
MPN Coliform (MPN/g)	<3[a]	15[b]	43[c]	150[d]
Proteolytic bacteria (log CFU/ml)	2.76 ± 0.03[a]	4.48 ± 0.02[b]	4.41 ± 0.01[b]	5.86 ± 0.01[c]
Bacillus spp. (log CFU/ml)	3.26 ± 0.06[a]	3.65 ± 0.01[b]	5.87 ± 0.00[c]	6.26 ± 0.02[d]
Clostridium spp. (log CFU/ml)	3.54 ± 0.02[a]	4.27 ± 0.00[b]	5.32 ± 0.02[c]	7.41 ± 0.00[d]

Different letter within a row showed significantly different ($p \leq 0.05$)

Table G The effect of storage temperature at room temperature on the microbiological quality of pasteurized noni juices at 80°C 15 sec

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	3.21 ± 0.01[a]	4.28 ± 0.04[b]	6.25 ± 0.02[c]	5.38 ± 0.02[d]
Yeasts and moulds (log CFU/ml)	2.77 ± 0.02[a]	4.50 ± 0.01[b]	6.95 ± 0.07[c]	8.42 ± 0.01[d]
Gram negative bacteria (log CFU/ml)	3.25 ± 0.00[a]	4.37 ± 0.02[b]	5.28 ± 0.03[c]	4.86 ± 0.02[b]
Lactic acid bacteria (log CFU/ml)	3.24 ± 0.06[a]	4.38 ± 0.02[b]	3.53 ± 0.01[a]	4.38 ± 0.03[b]
Coliform (MPN/g)	<3[a]	14[b]	75[c]	210[d]
Proteolytic bacteria (log CFU/ml)	2.72 ± 0.02[a]	4.49 ± 0.02[b]	4.85 ± 0.01[c]	6.33 ± 0.06[d]
Bacillus spp. (log CFU/ml)	2.88 ± 0.01[a]	3.65 ± 0.01[b]	5.50 ± 0.01[c]	5.98 ± 0.05[d]
Clostridium spp. (log CFU/ml)	2.77 ± 0.01[a]	3.68 ± 0.02[b]	4.52 ± 0.00[c]	6.26 ± 0.01[d]

Different letter within a row showed significantly different ($p \leq 0.05$)

Table H The effect of storage temperature at room temperature on the microbiological quality of pasteurized noni juices at 100°C 10min

No. of microorganisms	Storage time (day)			
	0	7	14	21
TPC (log CFU/ml)	2.66 ± 0.01^{a}	4.38 ± 0.00^{b}	6.14 ± 0.01^{c}	8.43 ± 0.01^{d}
Yeasts and moulds (log CFU/ml)	3.23 ± 0.01^{a}	4.24 ± 0.02^{b}	6.87 ± 0.01^{c}	7.45 ± 0.01^{d}
Gram negative bacteria (log CFU/ml)	2.38 ± 0.03^{a}	3.20 ± 0.04^{b}	4.87 ± 0.00^{c}	6.20 ± 0.02^{d}
Lactic acid bacteria (log CFU/ml)	ND	2.56 ± 0.00^{a}	2.69 ± 0.03^{b}	8.57 ± 0.02^{c}
Coliform (MPN/g)	$<3^{a}$	14^{b}	75^{c}	210^{d}
Proteolytic bacteria (log CFU/ml)	ND	3.28 ± 0.03^{a}	4.88 ± 0.02^{b}	8.32 ± 0.02^{c}
Bacillus spp. (log CFU/ml)	2.57 ± 0.04^{a}	3.68 ± 0.02^{b}	5.50 ± 0.00^{c}	6.28 ± 0.01^{d}
Clostridium spp. (log CFU/ml)	2.55 ± 0.01^{a}	4.27 ± 0.00^{b}	5.11 ± 0.02^{c}	6.47 ± 0.02^{d}

Different letter within a row showed significantly different ($p \leq 0.05$)

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