Indian Journal of Natural Products and Resources
Vol. 13(4), December 2022, pp. 543-551
DOI: 10.56042/ijnpr.v13i4.39616

Development of plant based nanoemulsion and its application as natural preservative having antioxidant and antimicrobial properties to deliver active compounds in apple juice

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Received 20 August 2020; revised received 12 October 2022; accepted 26 October 2022

In this study, active compound loaded stable nanoemulsion (surface charge -18 mV) was developed having small diameter (<100 nm) from the plant extract of raw *Syzygium aromaticum*, *Ocimum tenuiflorum* and rhizome of *Zingiber officinale*. The antimicrobial and antioxidant properties of formulated nanoemulsion were analyzed. The nanoemulsion showed good antimicrobial activity against various food poisoning organisms *viz.* bacteria (*Staphylococcus aureus* and *Escherichia coli*) and fungi (*Candida albicans*). For the antioxidant study, two different methods 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic 167 acid) diammonium salt (ABTS) were used, and a satisfactory result achieved (% inhibition for DPPH: 63.64±1.85 and ABTS: 48.36±1.12). The nanoemulsion in combination with a low dose of benzoic acid (200 ppm) is used as a preservative in formulated apple juice. The formulated juice quality was analyzed compared with standard fruit juice during a storage period of 30 days and quality of fruit juice remained good. This method thus allows reducing the use of synthetic preservatives with added benefit of natural beneficial compound in the formulated juice for human consumption.

Keywords: Antimicrobial, Antioxidant, Fruit juice, Nanoemulsion, Natural preservative.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 125/00, A61K 127/00, A61K 133/00, A61P 31/00, A61K 39/00

Introduction

Healthy foods production and their preservation are the biggest challenges in this era for sustainable development and reducing spoilage. Huge quantity of food is produced daily and sent to the market and some companies for their economic benefit use non-certified chemicals or overdose of them for long-time preservation[1]. Besides this, adulteration, cheap, and low-quality ingredient in processed food is a fatal problem that can have detrimental health effects to the consumer[2]. On considering consumer health, recently researchers are putting more attention towards the use of green method using natural products that can improve food quality and can be used to preserve food produced. Thus, plant bioactive compounds, which have antimicrobial properties, can play an important role to preserve the foods against the microbes increasing their self-life[3]. The bioactive compounds present in the essential oil of plants like benzaldehyde, carvacrol, carvone, cineole, cinnamaldehyde, citral, cymene, estragole, eugenol, geraniol, limonene, menthol, pinene, terpinene, terpineol, and thymol have antibacterial, antifungal, antigiardia, antioxidant, antiulcer, anti-yeast, anti-atherosclerotic, insecticidal and insect-repellent properties[4]. Formulation of oil-in-water nanoemulsion (NE) is an important route for the delivery of these antimicrobial compounds) into the food matrices[5]. The NEs with their nano-droplets size (less than 200 nm) are promisingly used for the formulation of food products with bioactive compounds and it has high solubility properties[6]. Recently many researchers have reported that use of NE is a useful technique for the incorporation of bioactive compounds into food matrices[7]. Numerous spices have been used as food, food ingredients, flavouring agent, and colouring agent from ancient times in India. Some of them are used as a medicine against specific diseases, and as food preservatives due to their antimicrobial properties. Detailed studies later on by many researchers have now proved their efficacy and active compounds present in these plants for a particular

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activity[8]. Herein the objective of this work is to utilize the extract of some important plants in food processing to preserve fruit juice reducing the use of a chemical preservative. The objective of this research is to deliver plant-based active compounds with antimicrobial activity in the food matrix to function as a food preservative. In this study, an active compound loaded NE was formulated using the extract of three plants parts mixture namely *Syzygium aromaticum*, *Ocimum tenuiflorum* and *Zingiber officinale* because they are known to have promising antimicrobial properties[3]. The formulated NE is characterized and incorporated into fruit juice to act as a preservative.

Many researchers have reported the antimicrobial and other biological properties of these selected plants. *O. sanctum* extract is known to contain the active compounds linalool (17.08%) and methyl chavicol (60.19%). These compounds possess antifungal properties and prevent fungal growth[9]. *S. aromaticum* is one of the important sources of phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propels. Among the other different compounds present the concentration of eugenol is high (9381.70 to 14650.00 mg/100g) in this plant extract[10]. The biological and pharmaceutical activity of *Z. officinale* rhizome mainly depends on active phytocompounds such as 6-gingerol, 6-shogaol, zingerone beside other phenolics and flavonoids[10]. The other compounds are shown in Table 1. Considering the active and beneficial compounds present, the plants were utilized for the extraction of active compounds. The active compounds were successfully delivered in fruit juice using a NE to inhibit microbial spoilage and to preserve the fruit juice with proper physiochemical quality.

Materials and Methods

Sample collection and identification

For apple juice preparation, mature apples *(Malus domestica)* were purchased (May 2020) from a local market in Kokrajhar, Assam, India. The selected essential plants (*S. aromaticum, O. tenuiflorum,* rhizome of *Z. officinale*) for extraction of bioactive compounds were also purchased from the same local market.

The plants were identified with the help of Mr. Sourav Borah, Curator, Herbarium, Gauhati University. All fresh plant samples were collected (May 2020) from the local market of Kokrajhar, Assam, India.

Extraction of fruit juice from *Malus domestica*

The fruits were washed carefully under fresh water, peeled, cut into pieces, deseeded, and then juiced with a mechanical extractor and then kept in a sterile glass container at 4ºC for further analysis.

Extraction of bioactive compounds

The leaves of *O. tenuiflorum,* flower buds of *S. aromaticum,* and rhizome of *Z. officinale* were dried using an oven at 45°C for 8 h. After grinding, the dried powders were mixed into 1:1:1 ratio. Exactly 50 mL of absolute ethanol was added to the 5 g of this sample and kept for 6 h with occasional stirring to extract the bioactive compounds. After the centrifugation at 8000 rpm for 15 minutes, the supernatant was separated and concentrated using a vacuum evaporator at 35°C and stored in refrigerated condition for further analysis (4°C)[11].

Formulation of active compound loaded NE

NE was developed according to the method adopted by Ghosh *et al*.[4] with some minor modifications. To formulate a stable NE using plant extract, a non-ionic surfactant tween 80 (HBL-15)

Table 1 — Active compounds in *Ocimum sanctum*, *Syzygium aromaticum* and rhizome of *Zingiber officinale*

Source	Method	Nano of active mazor compounds	Potential application	Ref.
Ocimum sanctum	GC-MS	Methyl chavicol, Linalool, Eugenol, Apigenin, catechins, quercetin, rutin, kaempferol, anthocyanins, eugenol, limonene, terpinene, carvacrol, geraniol, menthol, safrole, tannins, ursolic, *p*-coumaric, rosmarinic acid	Antioxidant, antimicrobial and Antinflammatory. Use as flavoring agent in food products. Applied in pharmaceutical, cosmetic, etc.	10
Syzygium romaticum	GC-MS	Eugenol, isoeugenol, acetyleugenol, sesquiterpene, pinene, vanillin, gallic acid,flavonoids, phenolic acids	Antioxidant, antimicrobial against all food-born pathogen, antifungal agent, Antinociceptive, etc.	20
Zingiber officinale	GC-MS	Eugenol, isoeugenol, acetyleugenol, sesquiterpene, pinene, vanillin, gallic acid,flavonoids, phenolic acids	Antioxidant and antimicrobial agent. application in therapeutic, aromatherapy as well as flavoring food industries.	22

was used since it possesses high hydrophilic as well as lipophilic properties. The mixture of plant extract (extracted from the mixture of *S. aromaticum, O. tenuiflorum,* and rhizome of *Z. officinale*) and the surfactant are mixed in a specific volume of water to obtain the desired concentration. The concentration of extract and surfactant are represented in Table 2. To obtain requisite morphological characteristics, the mixture was subjected to a sonication process using a sonicator (Q500; Qsonica, New York.) at 40°C for 30 minutes[4].

Characterization of NE

Particle size and zeta potential analysis

The analysis of particle size and zeta potential of NE was done by dynamic light scattering method using zetasizer Nano ZS (Malvern Instruments, UK) at ambient temperature, and the average of three independent samples were taken[12].

Antimicrobial activity study

For the antimicrobial screening of NE, the agar well diffusion method was adopted[13]. Here three microbial species were selected, one fungus *Candida albicans* (ATCC 10231) and the other two include one Gram-positive *Staphylococcus* aureus (ATCC 6538), and one Gram-negative *Escherichia coli (*ATCC 11105) bacteria (Thermo Fisher Scientific Waltham, USA). Here 1 mL microbial culture was poured in the centre of the petri dish, and then cooled molten agar was added with proper mixing (for fungi Mueller Hinton Agar and for bacteria Nutrient Agar media were used). When the media became solidified, then 6 mm diameter wells were made using sterile borer into the media. The wells were filled with 40, 20, 10 µL NE, and for control, DMSO (10% in sterile water) was used. After 30 minutes of refrigeration at 4°C for proper diffusion, the plates were incubated for 24 h at 37°C and the zone of inhibition was calculated. Using the same procedure antimicrobial activity of NE was tested against unpasteurized *M. domestica* juice.

Stability study

Stability study was done during storage time of 30 days by virtual observation at room temperature. The physical changes were noticed and recorded for any changes like colour change, phase separation, flocculation or sedimentation[4].

Determination of antioxidant activity

The antioxidant activity of formulated NE was studied using two different methods 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic 167 acid) diammonium salt (ABTS). For more precise results, these two methods were adopted based on two different principles[14].

Antioxidant activity test with 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The methanolic DPPH solution was prepared at a concentration of 0.24 mg/mL, and 0.1 mL of NE was added in a 3.9 mL DPPH solution. After incubation at room temperature in dark place for 30 minutes, the optical density was recorded using a spectrophotometer at 515 nm (λ-35; PerkinElmer, USA)[14]. The percentage of inhibition was calculated using equation (I).

$$\%\text{Inhibition} = \frac{\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Blank}}} \text{ X } 100 \quad \text{(I)}$$

Antioxidant activity test with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS)

The ABTS assay was performed according to the method adopted by Costa *et al.*[14]. The 3 mL ABTS

Table 2 — Characteristics of formulated NE and stability during storage

Code	Extract (%)	Surfactant (%)	Water (%)	pH	Viscosity (mP)	Time (30 days)					
						5	10	15	20	25	30
A1	5	5	90	5.7=0.2^{a}	36.28±1.2^{b}	Us	Us	Us	Us	Us	Us
A2	10	5	85	5.5=0.32^{ab}	38.43±1.8^{c}	Us	Us	Us	Us	Us	Us
A3	5	12	83	5.6±0.54^{ab}	35.28±1.3^{f}	S	S	Us	Us	Us	Us
A4	5	18	77	5.8 ±0.21^{b}	34.81±1.4^{g}	S	S	S	S	S	S
A5	8	10	82	5.5±0.92^{ab}	35.43±1.7^{f}	Us	Us	Us	Us	Us	Us
A6	8	12	80	5.7±0.53^{a}	34.71±1.9^{g}	Us	Us	Us	Us	Us	Us
A7	8	16	76	5.6±0.37^{ab}	34.63±1.5^{e}	S	S	S	S	Us	Us
A8	8	20	72	5.7±0.52^{a}	34.52±1.6^{ef}	S	S	S	S	S	S
A9	8	24	68	5.7±0.91^{ab}	34.52±1.3^{ef}	S	S	S	S	S	S

Data are expressed as the means±SD (n = 3). Means with different lowercase letters within the same column are significantly different (P <0.05). S=Stable, Us = Unstable /Phase separated.

solution was mixed with 30 µL NE, and after the incubation for 12 minutes in a dark place, the optical density was recorded using the spectrophotometer at 734 nm (λ-35; PerkinElmer, USA). The percentage of inhibition was calculated using equation (I).

Formulation of apple juice with active compound loaded NE

A standard apple juice was developed with NE, according to the method described by Syed *et al.*[15]. In this study, two different batches of fruit juice were prepared. One batch was prepared with preservative benzoic acid (600 ppm) as a control (T1). Another batch (T2) was formulated by adding benzoic acid (200 ppm) to fruit juice with NE (NE and fruit juice ratio- 0.1:10) to reduce the dose of benzoic acid in fruit juice. The formulated fruit juice was kept for analysis during the storage period 30 days.

Physicochemical study of fruit juice during storage

Physiochemical studies of fruit juice were done according to the method described by Nonga *et al.*[16] during the storage time of 30 days at room temperature. The physiochemical parameters colour, pH, titratable acidity (TTA) and total soluble solids (°Brix) were analyzed. Colour changes of fruit juice were studied during the storage period using hunter colourimeter (Hunterlab D25LT, Reston, VA). The pH of formulated fruit juice was analyzed using pH meter (Oakton® pH 700, Cole-Parmer, USA), and buffer solution of pH 4.2 and 7 were used to calibrate the pH meter. According to AOAC (1999) method, the TTA of fruit juice was measured. The TTA was calculated by the formula (II). For the total soluble solid analysis, the TSS meter (MASTER-53Pα, Atago co. Ltd.) was calibrated using distilled water at 0°Brix and sucrose solution of 25°Brix then the sample was analyzed.

$$\%\text{Acid (w/w)} = \frac{(\text{Net mL Titrant})\ (\text{N Titrant})\ (0.064)\ \text{X}\ 100}{\text{Sample weight}} \quad \text{(II)}$$

where 0.064 = the acid factor for citric acid, Net mL Titrant = titre value of NaOH and N Titrant = Normality of titrant (NaOH).

Statistical analysis

The statistical analysis was done using statistical SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). The three determinants were taken of every analysis as the mean±standard deviation (SD). For significance of difference ANOVA was used and $P < 0.05$ was considered statistically significant.

Results and Discussion

Extraction of bioactive compounds

It is known that the plants *S. aromaticum*, *O. tenuiflorum*, and rhizome of *Z. officinale* discretely possess antimicrobial potential[17]. Here in this work, all the plants were mixed and extracted using the method described above for getting highly efficient mixed-antimicrobial compounds. To prevent the decomposition of heat-sensitive compounds, extraction was done at low temperatures for a long time. The extract was used in microbial analysis and NE formulation. The solvent has a significant impact on the extraction yield of active compounds. Ngo *et al.*[18] reported that extraction yield of absolute methanol is high (15.6%) fallowed by 50% ethanol (14.3%), 50% methanol (12.3), and 50% acetone (12.2%) when experiment was carried out using *Schisandra chinensis* root. It was reported that the extraction yield of dry leaves of *O. sanctum* leaves is 7%,[19] and *S. aromaticum* is 12.9%,[20] using ethanol as solvent. Although methanol has high extraction efficiency, ethanol was used for our extraction purpose because the extract was used to formulate NE, which was used for food preservation. In this study, the extraction yield of 6.25% was achieved. Gahlot *et al.*[21] reported almost similar result using ethanol as solvent for extraction.

Formulation of active compound loaded NE

A total of nine different non-identical emulsions were prepared using diverse percentages of water as aqueous phase, plant extract as oil phase, and tween 80 as a surfactant. The ratio of extract, tween 80, and water are represented in Table 2.

Stability study NE during storage

Here for physical changes study, a virtual observation was done on formulated NEs during 30 days of storage. In this study, nine different samples of NE were prepared based on different compositions. We observed that sample with low extract/oil concentration with high surfactant concentration is more stable than higher extract/oil contained NE. The characteristic changes of NEs during storage are represented in Table 2. Phase separation, flocculation, sedimentation, and colour change not found only in two samples code A8 and A9 during storage. These characteristics properties confirmed its stability. In this study, A8 formulation was accepted because in A8 sample surfactant concentration was lesser than A9 which is more economical.

Particle size and zeta potential analysis

After the completion of 30 days of storage, the stable NE without phase separation the stable sample (A8) was analyzed, and the droplet size of NE was found to be less than 100 nm (Fig. 1a). The particle size of NE depends upon the plant extract/oil concentration when amount of oil in NE increases the particle size also increases[22]. The zeta potential analysis of the NE gave a value -18.6 mV (Fig. 1b) indicating that the NE was very stable.

Antimicrobial activity study of the NE

Investigation of antimicrobial activity of NE against bacteria Gram-positive (*S. aureus*), Gram-negative *E. coli*), and fungi (*C. albicans*) showed satisfactory results. The highest inhibition zone found against *C. albicans (Fig. 2a) and then* Gram-positive *S. aureus (Fig. 2b)*. The inhibition zone using NE (40 µL) recorded against *C. albicans* 14.0±0.61, *S. aureus* 12.0±0.82, and *E. coli 11.0±0.73 mm* (*Fig. 2c)*. The inhibition zone of other wells with a lower volume of NE found smaller inhibition zone showing that the inhibition zone is directly related to the volume of NE used. Since more quantity of NE has higher concentration of antimicrobial compounds[23]. No further improvement in inhibition zone was observed up to the increased volume of 50 µL NE.

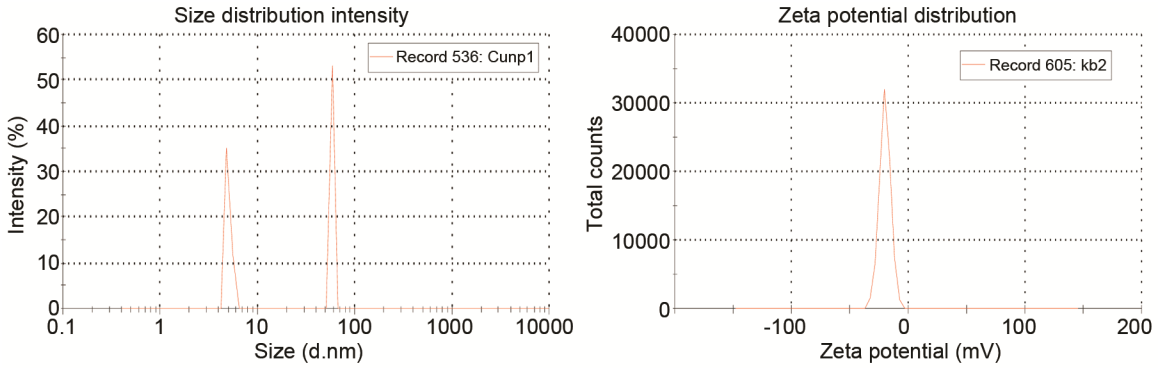

Fig. 1 — Particle size and zeta potential analysis.

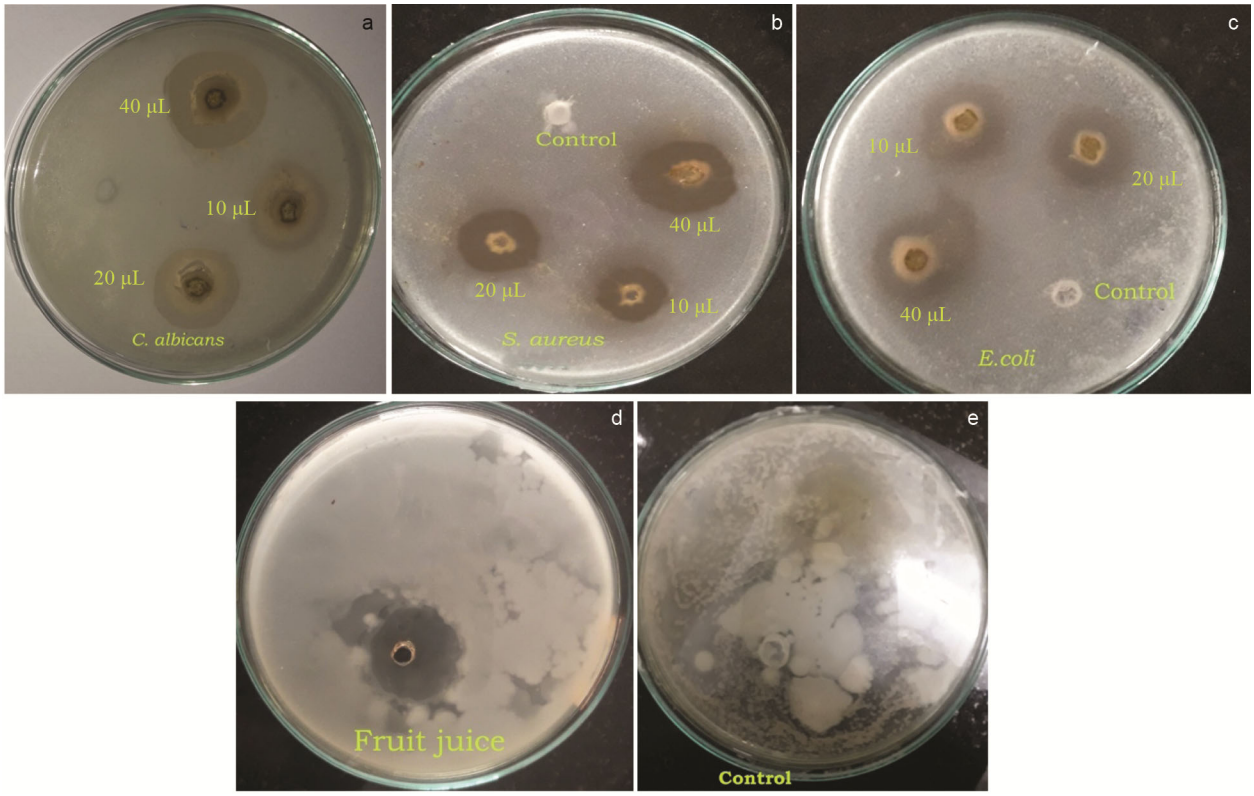

Fig. 2 — Antimicrobial activity tests.

To study the effectiveness of the NE against microbes present in unpasteurized juice, an investigation was carried out effectively using well diffusion method and the finding was very satisfactory. The inhibition zone 18±0.53 mm found against unpasteurized fruit juice (Fig. 2d) and in control perti dish (10% DMSO) a huge microbial colony was observed (Fig. 2e). The individual plant extracts are active against many pathogens[24] and thus a mixture of these plant extracts has all the benefits as it contain many bioactive compounds. As a result, the NE showed good antimicrobial activity against unpasteurized juice although it contains a jumble of microbes. The NE has comparatively increased bioactivity due to nano-droplet size formation and cell absorption mechanisms. From these results, it is confirmed that the emulsion has good potential against the food poisoning pathogens and is effective for food preservation

Antioxidant activity study

The NE has a high potential to be used in food and beverage as food antioxidants and nutraceuticals for the benefit of consumer health. From the antioxidant activity analysis of NE, the inhibition percentage recorded using ABTS and DPPH methods are 48.36±1.12, 63.64±1.85. These results are similar to Seibert *et al*.[25] and Noori *et al.*[26] The ABTS method is more effective because it determines the scavenging activity in both polar and non-polar medium. The working solution of ABTS is soluble in an aqueous and organic solvent at large pH ranges. Comparatively, ABTS method has short reaction time with acceptable result and reactivity of ABTS working solution is higher[27]. Components that are responsible for antioxidant properties in NE and other natural products are phenolic compounds. The most active phenolic compounds responsible for antioxidant activity might be eugenol, gingerols, and the source of these compounds was the extract used to formulate NE[28]. Therefore, this investigation exhibited that the formulated NE possessed an excellent antioxidant potential to scavenge free radicals.

Formulation of apple juice with active compound loaded NE

The apple fruit juice was prepared and NE was used as an active agent against spoilage due to microbes. The total fruit content (45%) and acid content (1%) were maintained for both batches (T1, T2). To preserve the fruit juice mainly sulphur dioxide and benzoic acid are used with a limited dose (300 and 600 ppm)[29]. Benzoic acid is very active against the growth of yeasts and moulds in processed fruit products[30]. In the present study, minimum quantity of benzoic acid 200 ppm was used in combination with the NE. To prevent the other microbes, active compounds were delivered using the formulated NE. The NE was used to minimize the use of chemical preservatives that may cause health hazards, especially to infants. The active compounds present in NE improve nutritional quality[31] and it has antioxidant properties also. The findings of this study exhibited that the ratio of NE and fruit juice (0.1:10) was good enough to preserve the fruit juice against spoilage by microbes.

Physicochemical studies

Comparative physicochemical analysis of both formulations (T1, T2) was carried out. The physicochemical properties are directly associated with the quality of processed food[32]. Changes in these properties indicate lowering quality of food product. From this point of view, an investigation carried out on the physiochemical properties of both juices (T1, T2) during storage time. The physicochemical observations are discussed below.

Change in pH

The pH of fruit juice is inversely proportional to acid content. When the pH of the fruit juice suddenly increases at high rate, its means the degradation in quality due to microbial contamination or chemical reaction during the storage period[33]. In both formulations, a slight pH increase was recorded (Fig. 3a). Minor increase of pH may cause acid hydrolysis of poly-saccharides and formation of mono-saccharides and disaccharides resulting in increase in sweetness and decrease in sourness. The pH changes of fruit juice during storage may be due to the storage temperature. Alaka *et al*.[34] also reported similar results. The pH changes were observed in both formulated samples (T1 and T2). The pH changes rate was minor and similar changes were noticed in both samples (T1 and T2) which indicated that NE was acting as preservative.

Titratable acidity (TTA)

The TTA acidity of both fruit juice decreased significantly during storage of 30 days. The TTA decrease is observed in both cases (T1, T2) of fruit juice formulations (Fig. 3b). Similar result was

reported by Kaddumukasa *et al.*[35]. These chemical characteristic changes might be due to the acid hydrolysis of polysaccharides where acid is used to convert non-reducing sugar into reducing sugar[36]. Here no sudden chemical changes were observed that indicate stability without spoilage or quality degradation[37].

Total soluble solid (TSS)

The TSS of T1 and T2 were 10.2°Brix and 10.3°Brix at the starting (zero time) of storage. After completion of the storage time, significant difference of both formulations was not observed (10.26°Brix and 10.35°Brix). The changes of TSS of all formulation during storage time were pictorially represented in Fig. 3c. A slight increase in TSS during storage is acceptable for quality improvement[37]. The TSS increase may be due to the solubilization or hydrolysis by acid and formation of sugars. A similar result was shown by Deka and Sethi[38] in mixed fruit juice and by Singh and Mathur[39] in cashew apple juice.

Colour

From the virtual observation, the colour of both formulations (T1 and T2) was monitored. Both formulations were reddish-yellow but T2 was deeper in colour due to addition of NE. The values of colour change are represented in Table 3. The L* represents the whiteness/darkness, ranged from 0 to 100. The increasing rate of L*values control (T1) were comparatively slightly higher than new formulation (T2) and this phenomena confirm of colour stability new formulation (T2). The a* represents redness for positive value and greenness for the negative. The a* value in T2 is slightly higher than T1 due to the addition of NE. The a* value increase in both cases (T1 and T2), may be due to the non-enzymatic brow reaction resulting in slight increase of redness[40]. The b* represented the yellowness for positive and blueness for the negative value. In the case of b* values, in both cases, parallel changes were observed. The studies on colour changes of both formulation (T1 and T2) were similar, and it proves that NE is effective in preserving the juice compared to the juice using high dose of benzoic acid.

Discussion

In this study, a nanoemulsion with a very small diameter (<100 nm) and negative surface charge (-18 mV) was developed using beneficial plants extract.

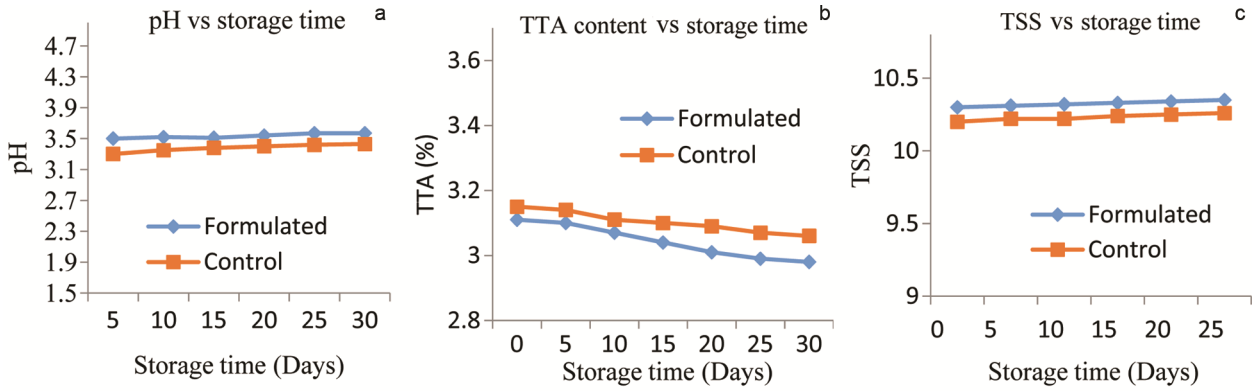

Fig. 3 — Physicochemical changes during storage period. Data are expressed as the means±SD (n = 3), (P <0.05).

Table 3 — Studies on colour change during storage time

Storage time (Days)	L		a*		b*	
	T1	T2	T1	T2	T1	T2
0	81.63±0.18^{a}	80.41±0.18^{b}	11.50±0.58^{a}	11.70±0.58^{b}	22.45±0.18^{a}	20.4±0.18^{b}
5	82.30±0.19^{a}	80.44±0.19^{b}	11.63±0.31^{a}	11.91±0.41^{b}	22.32±0.25^{a}	20.53±0.25^{b}
10	82.40±0.18^{ab}	80.47±0.09^{c}	11.82±0.43^{ab}	12.23±0.32^{c}	21.91±0.017^{ab}	20.22±0.17^{c}
15	82.65±0.19^{bc}	80.9±0.31^{cd}	12.18±0.57^{b}	12.45±0.27^{cd}	21.23±0.28^{ab}	20.10±0.28^{c}
20	82.71±0.18^{c}	81.2±0.20^{de}	12.20±0.15^{b}	12.59±0.25^{cd}	20.01±0.34^{c}	19.7±0.34^{cd}
25	82.95±0.19^{cd}	81.5±0.56^{de}	12.51±0.3^{bc}	12.73±0.37^{de}	19.80±0.45^{cd}	19.2±0.45^{d}
30	83.01±0.09^{d}	82.5±0.34^{e}	12.98±0.25^{c}	12.92±0.43^{de}	19.52±0.19^{d}	19.01±0.19^{d}

Data are expressed as the means ± *SD* (n = 3). Means with different lowercase letters within the same column are significantly different (P <0.05). The L* = whiteness/darkness, ranged from 0 to 100; a* = redness for positive value and greenness for the negative; b* = yellowness for positive and blueness for negative value. T1= control, T2= formulated.

Through this study, the use of synthetic preservative in formulated fruit juice is lowered. Here the dose of benzoic acid was reduced to 200 ppm and NE played an important role to preserve the fruit juice. The formulated fruit juice could be preserved for a long time using the nanoemulsion without affecting the quality due to the presence of natural antimicrobial compounds in the nanoemulsion. The fruit juice has further added benefit of having natural antioxidant compounds from the nanoemulsion. Thus it is expected that this method would allow reducing the use of chemical preservative and would be beneficial to consumer health.

Conflict of interest

The authors have no conflict of interest to declare.

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