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Development of synbiotic added sucrose-free mango nectar as a potential substrate for *Lactobacillus casei*: Physicochemical characterisation and consumer acceptability during storage

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ABSTRACT

The study was carried out to evaluate the viability of probiotic bacteria as well as physicochemical and sensory characteristics of synbiotic added sucrose-free mango nectar. The mango nectar samples were prepared incorporating different concentrations of inulin and stevia (0, 2 and 4% w/w) and stored for 45 days at 4 °C. The results indicated that the growth of *Lactobacillus casei* was strongly influenced by the beverage composition. Therefore, the addition of inulin and stevia had a positive effect on the viability of *L. casei*, especially with 2% w/w inulin or inulin in combination with stevia. However, bacterial viability reduced during storage. According to the results, pH and total soluble solid content of the samples decreased during storage; however, the viscosity values of mango nectars were elevated by the addition of inulin and stevia. Additionally, all beverages were considered a suitable medium to keep the *L. casei* survival above the minimum recommended value (10^6–10^7 CFU mL^{-1}) during storage. In general, it can be concluded that the sample containing 2% inulin and 2% stevia in combination provided the best viability of *L. casei* with acceptable physicochemical properties and sensory characteristics that could be introduced as synbiotic and low-calorie mango nectar.

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KEYWORDS

added sucrose-free, mango nectar, prebiotic, probiotic, symbiotic

1. INTRODUCTION

In recent years, due to an increased consciousness towards health and nutrition, demand for the use of functional foods has increased among consumers (Gamage et al., 2016). *Lactobacillus casei is* one of the most favoured probiotic bacteria that provides health benefits to the host when administered in adequate amounts (10^6–10^7 CFU mL^{-1} or g^{-1}) (Ding and Shah, 2008). Inulin is a hydrocolloid classified as water-soluble dietary fibre that can be used in a variety of foods as a stabilising and thickening agent. In the literature, the effect of inulin as a thickener on the physical and prebiotic properties of sugar-free food products has been investigated (Aidoo et al., 2017; Kazemalilou and Alizadeh, 2017). Mango (*Mangifera indica* L.) nectar is a popular fruit juice among consumers. It is known as a rich source of carotenoids, with high contents of ascorbic acid and phenolic compounds. However, the high sucrose content of mango nectar limits its use for diabetics, which urges production of sugar-free nectar by using sucrose substitutes (Cadena et al., 2013). Stevia is a natural sweetener (the sweetness of which is 350–450 times higher than sucrose) used as a substitute for sugar in low-calorie food products (Gao et al., 2016; Rodrigues et al., 2017). Therefore, the objective of the current study was investigating physicochemical characteristics of synbiotic mango nectar sweetened with stevia as a natural low-calorie sweetener and their effects on consumer acceptance, throughout the 45 days of storage.

2. MATERIALS AND METHODS

2.1. Materials

Mango puree was obtained from Shadlee Co., Iran. Inulin (medium-chain) was purchased from Sensus Co., Netherlands. Stevia (purity of 95%) was purchased from Stevia Pack Co., Singapore. *L. casei* subsp.*casei* (PTCC 1608) was obtained from the IBRC. De Man, Rogosa, and Sharpe (MRS) agar for bacterial tests and all the applied reagents of analytical grade were purchased from Merck Chemicals Co., Germany.

2.2. Preparation of mango nectar

Mango nectars were prepared by blending 50% (v/v) of mango puree and 50% (v/v) boiled water. Inulin and stevia in certain proportions (Table 1) were added to the mango nectar, and the samples were pasteurised at 80 °C for 15 min in a water bath. After pasteurisation and cooling, 2 mL of previously prepared inoculum (Costa et al., 2013), containing 9.0 log CFU mL^{-1} of *L. casei*, were added to 200 mL of mango nectar under sterile conditions. Thus, the initial cell count in the samples was 7 log CFU mL^{-1}. Consequently, the samples were incubated for 24 h at 30 °C (Figure 1). After incubation, they were stored at 4 °C for 45 days in the refrigerator (Liu et al., 2014).

Table 1. The prepared mango nectar samples

Samples	Inulin (% w/w)	Stevia (% w/w)
Control	–	–
MN/I2%	2	–
MN/I4%	4	–
MN/S2%	–	2
MN/S4%	–	4
MN/S2%/I2%	2	2
MN/S2%/I4%	4	2
MN/S4%/I2%	2	4
MN/S4%/I4%	4	4

MN: Mango nectar; I: inulin; S: stevia.

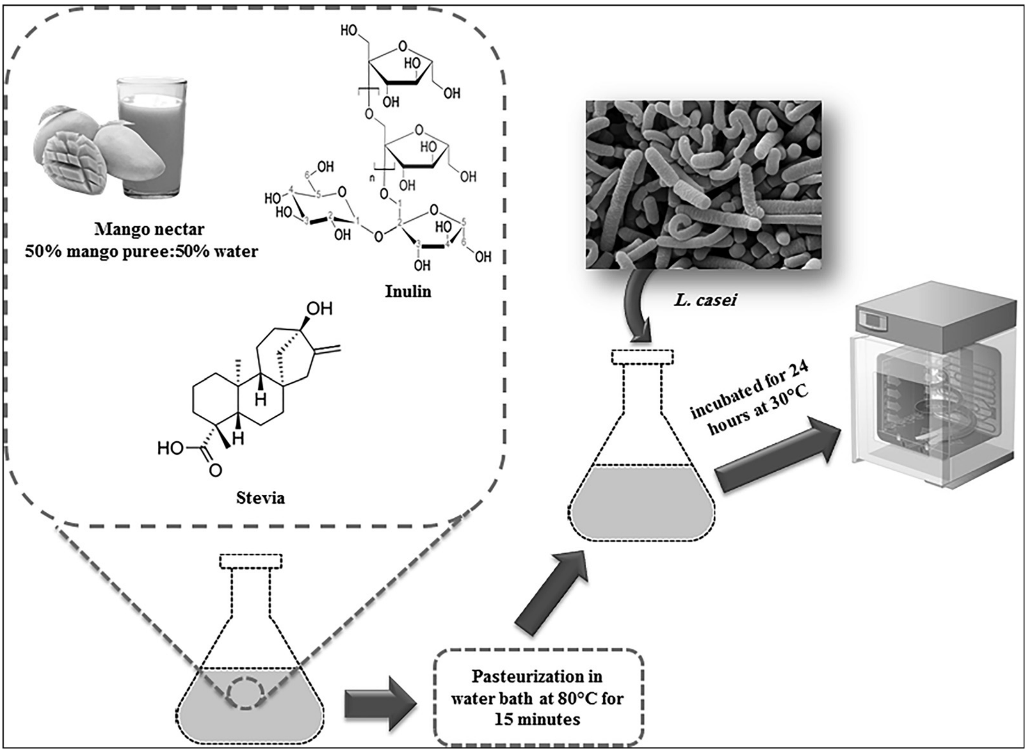

Fig. 1. The schematics of preparation of the synbiotic added sucrose-free mango nectar. Inulin: prebiotic texturiser, Stevia: natural low-calorie sweetener, *L. casei*: probiotic bacteria

2.3. Viability of probiotic bacteria

The viability of probiotic strain (*L. casei*) was determined after fermentation at the 1st, 15th, 30th and 45th days of storage. For this purpose, 10-fold serial dilutions of each sample were prepared in sterile peptone water, spread on MRS agar plates, and incubated at 37 °C for 48 h. The results were expressed as log CFU mL^{-1} of mango nectar (Valero-Cases and Frutos, 2017).

2.4. Physico-chemical and sensory properties

The pH of mango nectar was determined using a pH meter (Mettler, Swiss), and the acidity of samples was expressed as a percentage (%) of citric acid (Valero-Cases and Frutos, 2017). The total soluble solids content (TSS) of mango nectars was determined at 25 °C using a hand refractometer (RE – 380, Atago, Japan) and expressed as Brix (Valero-Cases and Frutos, 2017). The viscosity of the samples was determined with a viscometer (RV-DV II, Brookfield, USA) after 2 min by using spindle No. 3 at a shear rate of 100 r.p.m. at 4 °C (Valero-Cases and Frutos, 2017). The image processing method was used to determine colour parameters (Amjadi et al., 2018). The sensory acceptance study was performed by 25 semi-trained panellists (Alizadeh et al., 2019).

2.5. Statistical analysis

All experiments and analyses were made in triplicate. Statistical analysis was performed based on one-way analysis of variance (ANOVA) and Tukey's mean comparison tests at a 5% significance level by using Minitab 20 software (Minitab Inc. State College, PA, USA). Chemical, sensorial and microbiological characteristics were tested on the 1st, 15th, 30th and 45th days of storage. However, the viscosity test was carried out on the 1st and 45th days and colour measurement was done on the 45th day.

3. RESULTS AND DISCUSSION

3.1. Viability of probiotic bacteria

Table 2 presents the viability of *L. casei* in mango nectar samples during 45 days of storage. As a result, the count of *L. casei* in the MN/S2%/I2% sample was higher than in the other samples, and the lowest value was attributed to the control sample on the first day. However, the highest viability *of L. casei* was observed in samples containing inulin (MN/I2%, MN/I4% and MN/S2%/I2%) at the 30th and 45th days of storage. Parameters such as the type of prebiotic and the storage time are the most important factors affecting the growth and survival of the bacteria (Talwalkar and Kailasapathy, 2003). Therefore, differences in the viability of probiotic bacteria of samples containing inulin could be affected by the buffering properties of inulin that prevented drastically changes caused by metabolites derived from *L. casei* in mango nectars (Ding and Shah, 2008). The results suggested that the incorporation of inulin and stevia improved the viability of *L. casei* in the synbiotic mango nectar. However, the effect of inulin was greater than stevia ($P < 0.05$). Konar et al. (2018) demonstrated that the stability of probiotic bacteria improved by using prebiotic agents like inulin. In the present study, during storage, the viability of *L. casei* decreased ($P < 0.05$), but all samples could be considered probiotic (>10^6 CFU mL^{-1}). Furthermore, Rodrigues et al. (2017) reported that accumulation of lactic acid, diacetyl and acetaldehyde produced by probiotic bacteria decreased their viability during storage.

Table 2. The viability of probiotic bacteria in the mango nectar samples during storage time

	L. casei viability (log CFU mL^{-1})			
	Storage time (days)			
Samples	1	15	30	45
Control	10.23 ± 0.05^{Ad}	9.58 ± 0.24^{Ba}	7.57 ± 0.20^{Cd}	7.19 ± 0.10^{Cd}
MN/I2%	10.65 ± 0.09^{Aab}	9.66 ± 0.29^{Ba}	8.72 ± 0.15^{Ca}	8.67 ± 0.07^{Ca}
MN/I4%	10.61 ± 0.03^{Aabc}	9.65 ± 0.30^{Ba}	8.70 ± 0.14^{Ca}	8.66 ± 0.23^{Ca}
MN/S2%	10.38 ± 0.11^{Abcd}	9.20 ± 0.11^{Ba}	8.28 ± 0.21^{Cc}	7.69 ± 0.07^{Dc}
MN/S4%	10.34 ± 0.14^{Acd}	9.66 ± 0.12^{Ba}	7.51 ± 0.20^{Cd}	7.47 ± 0.05^{Cc}
MN/S2%/I2%	10.79 ± 0.03^{Aa}	9.73 ± 0.28^{Ba}	8.76 ± 0.25^{Ca}	8.77 ± 0.04^{Ca}
MN/S2%/I4%	10.40 ± 0.07^{Abcd}	9.65 ± 0.14^{Ba}	8.26 ± 0.09^{Cc}	7.87 ± 0.07^{Db}
MN/S4%/I2%	10.59 ± 0.14^{Aabc}	9.58 ± 0.18^{Ba}	8.24 ± 0.04^{Cc}	7.92 ± 0.06^{Db}
MN/S4%/I4%	10.27 ± 0.14^{Ad}	9.65 ± 0.06^{Ba}	8.38 ± 0.04^{Cbc}	7.45 ± 0.05^{Dcd}

Data are presented as mean ± standard deviation ($n = 3$), and different letters indicate significant differences at the 5% level in Tukey's test ($P < 0.05$). Capital letters indicate storage time effect and small letters indicate treatment effect. MN: Mango nectar; I: inulin; S: stevia.

3.2. pH and acidity

The pH and the acidity of mango nectar samples during the storage are shown in Fig. 2A and B, respectively. In all samples, the pH value decreased significantly over time that may be due to the production of lactic acid by probiotic bacteria's activity during the storage (Ding and Shah, 2008). Moreover, the incorporation of inulin and stevia increased the pH of the nectars, and it was significant in the samples containing stevia and those containing a combination of inulin and stevia, which could be influenced by buffering properties of inulin (Rodrigues et al., 2017). Miranda et al. (2019) reported that probiotics may produce organic acids from the consumption of sugars or by the catabolism of amino acids (Miranda et al., 2019). The highest acidity of the samples was obtained for MN/S4%/I4% at the end of the storage. These findings could be due to the desirable conditions for bacterial activity which consequently resulted in higher fermentation rate and acid production (Ding and Shah, 2008). The same results were reported by Yoon et al. (2005) on fermented red beet juice containing *Lactobacillus plantarum* and *Lactobacillus delbrueckii* (Yoon et al., 2005).

3.3. Total soluble solids (TSS)

The TSS values of mango nectars during storage are shown in Fig. 3. The results revealed that the TSS values of mango nectars were increased by incorporating inulin and stevia separately and in combination. As a result, this phenomenon was due to the fibre nature and the water absorption properties of the inulin (Alizadeh et al., 2019). It was also concluded that the effect of inulin on increasing the brix of the mango nectar samples was greater than of stevia. Moreover, the TSS values of mango nectars containing inulin and inulin in combination with stevia significantly ($P < 0.05$) decreased during storage, due to metabolism of sugars by microorganisms (Ding and Shah, 2008). However, the

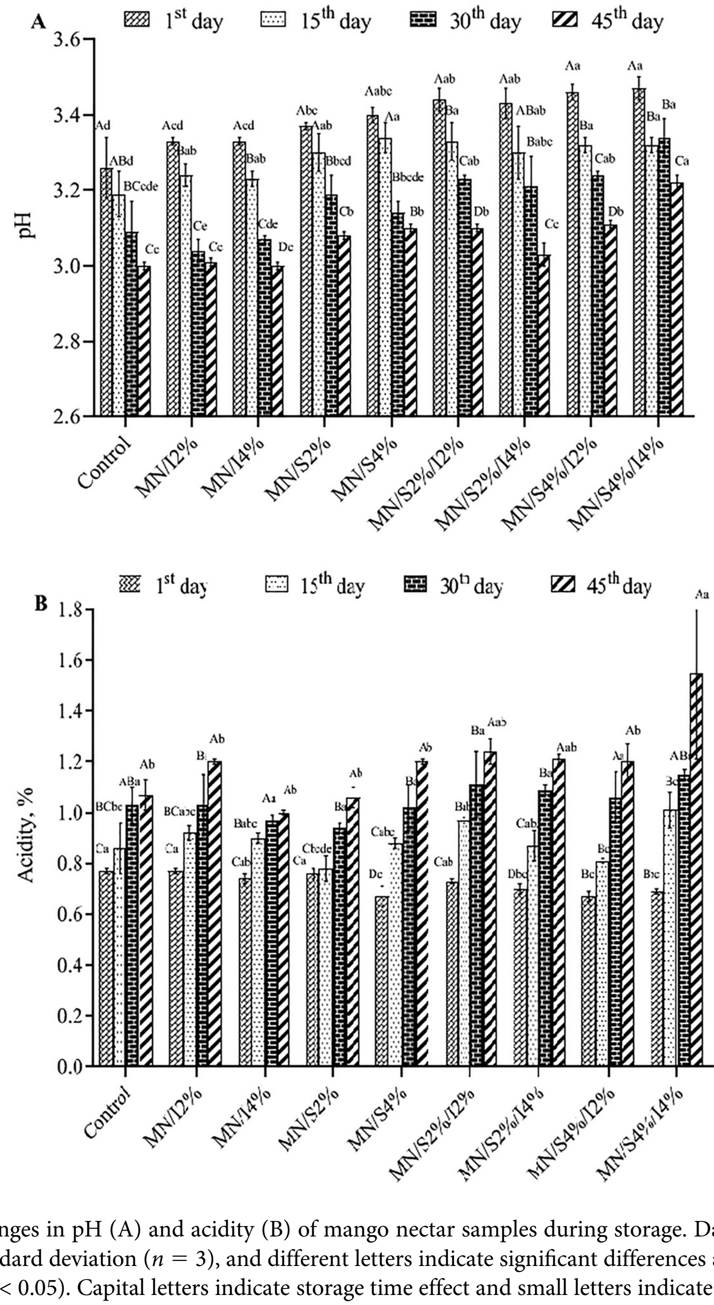

Fig. 2. The changes in pH (A) and acidity (B) of mango nectar samples during storage. Data are presented as mean ± standard deviation ($n = 3$), and different letters indicate significant differences at the 5% level in Tukey's test ($P < 0.05$). Capital letters indicate storage time effect and small letters indicate treatment effect. MN: mango nectar; I: inulin; S: stevia

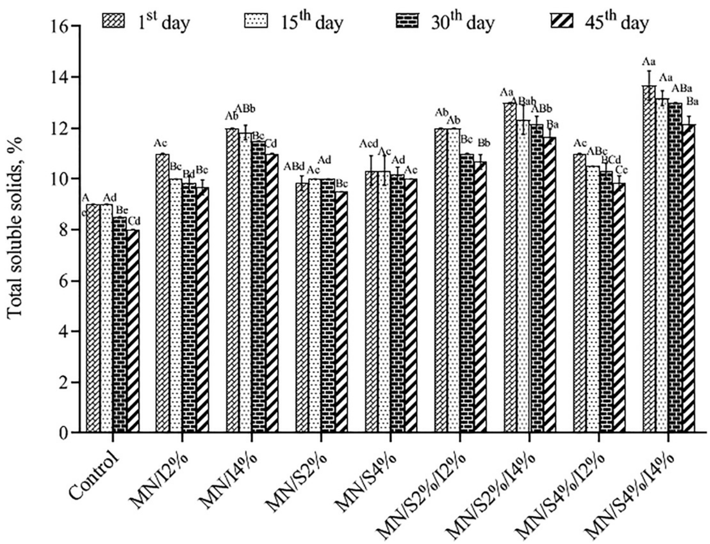

Fig. 3. The total soluble solid changes of mango nectar samples during storage. Data are presented as mean ± standard deviation ($n = 3$), and different letters indicate significant differences at the 5% level in Tukey's test ($P < 0.05$). Capital letters indicate storage time effect and small letters indicate treatment effect. MN: mango nectar; I: inulin; S: stevia

incorporation of inulin, which was not used by microorganisms in the early hours, provided a higher TSS value in samples containing inulin (Alizadeh et al., 2019). Additionally, the low TSS value of the samples containing stevia was compensated by using the combination of stevia with inulin.

3.4. Viscosity

The viscosities of mango nectars are shown in Fig. 4. As a result, measured viscosity of mango nectars revealed that the viscosity of the samples increased on the first day with the addition of inulin and stevia, which was significant in inulin-containing samples ($P < 0.05$). Additionally, in the samples containing inulin and stevia in combination, the viscosity was significantly higher than of the control sample ($P < 0.05$). Although the viscosity of the inulin containing samples increased during the storage, the change was not significant ($P > 0.05$). It can be explained that over time, the remaining parts of inulin increased viscosity of the sample by absorbing and encapsulating the water in the nectar (Alizadeh et al., 2019). Results also revealed that incorporating a combination of inulin and stevia increased the water uptake compared to the stevia containing samples due to the presence of higher amounts of water-absorbing compounds and increased viscosity of mango nectar samples (Alizadeh et al., 2019). In this regard, Aidoo et al. (2017) have reported that the changes in TSS value and a particular amount of sucrose in beverages could change the viscosity of the fruit juices. Also, it has been reported that inulin and sweeteners like stevia have synergistic effects on viscosity (Konar et al., 2018).

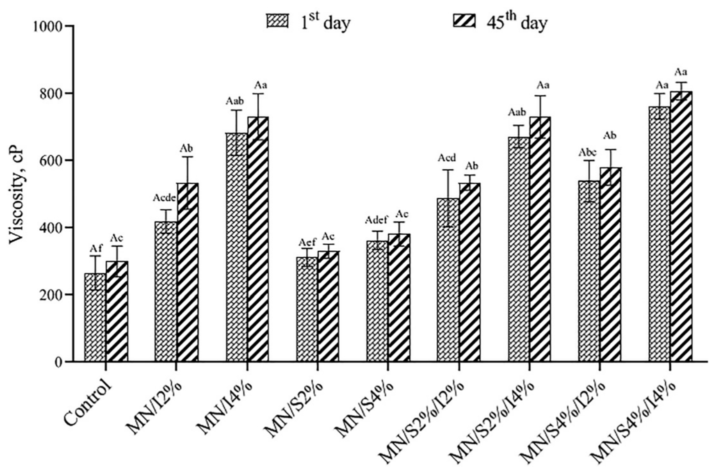

Fig. 4. The viscosity changes of mango nectar samples in the 1st and 45th days of storage. Data are presented as mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). Capital letters indicate storage time effect and small letters indicate treatment effect. MN: mango nectar; I: inulin; S: stevia

3.5. Colour

The colour properties of mango nectars are shown in Table 3. According to the results, the L^* and b^* values of samples increased significantly ($P < 0.05$) with the addition of inulin and stevia. Additionally, a^* values decreased by incorporating inulin and stevia or a combination of them. The findings could be explained by the original colour of stevia and inulin. In this regard, Barakat et al. (2017) have revealed that the L^* and b^* values of the low-calorie nectars increased due to the addition of stevia and inulin (Barakat et al., 2017).

Table 3. Colour parameters of mango nectar samples

	Colour parameters		
Samples	L^*	a^*	b^*
Control	54.98 ± 0.54^{e}	7.61 ± 0.84^{a}	33.28 ± 1.54^{e}
MN/I2%	56.89 ± 1.41^{de}	4.83 ± 0.14^{b}	40.96 ± 1.06^{de}
MN/I4%	60.70 ± 1.69^{cd}	3.89 ± 0.29^{b}	46.74 ± 5.34^{cde}
MN/S2%	62.44 ± 1.55^{bc}	4.44 ± 0.37^{b}	51.12 ± 3.55^{bcd}
MN/S4%	68.18 ± 2.32^{a}	4.67 ± 1.12^{b}	63.91 ± 4.40^{ab}
MN/S2%/I2%	67.55 ± 1.15^{ab}	4.51 ± 0.99^{b}	62.51 ± 4.12^{ab}
MN/S2%/I4%	66.75 ± 1.88^{ab}	4.62 ± 0.76^{b}	60.21 ± 8.16^{abc}
MN/S4%/I2%	70.23 ± 1.40^{a}	4.32 ± 1.38^{b}	66.47 ± 2.24^{a}
MN/S4%/I4%	70.25 ± 3.56^{a}	4.35 ± 1.32^{b}	69.97 ± 7.28^{a}

Data are presented as mean ± standard deviation ($n = 3$), and different letters indicate significant differences at the 5% level in Tukey's test ($P < 0.05$). Small letters indicate the treatment effect. MN: Mango nectar; I: inulin; S: stevia.

3.6. Sensory acceptance

According to the results (Table 4), the addition of inulin had a significantly ($P < 0.05$) positive effect on the overall acceptance of the prepared samples, and this positive effect decreased by elevating the inulin concentration. The same trend was observed in samples containing stevia and the samples containing a combination of inulin and stevia. Additionally, due to the presence of rebaudioside A in stevia, incorporating stevia in high concentrations may cause a bitter taste that could affect the sensory acceptance of the samples (Alizadeh et al., 2019; Barakat et al., 2017). In this regard, Cardoso and Bolini (2008) reported that incorporating stevia in the peach nectar samples caused an exotic herbal flavour (Cardoso and Bolini, 2008).

4. CONCLUSION

In this study, mango nectar was demonstrated to be a good medium to maintain *L. casei* viability above the minimum recommended value during storage. The analysis of the viability of probiotic bacteria in the mango nectar samples revealed that the stability of *L. casei* during storage time was improved by the addition of inulin and stevia; however, the effect of adding inulin was significantly greater than stevia. The results of pH and acidity measurements were consistent with the results of microbial viability. Moreover, the TSS and viscosity values of mango nectars were elevated by the addition of inulin and stevia. Additionally, inulin improved the sensory properties without significantly changing the colour of the samples; however, stevia had a negative effect on the colour and organoleptic characteristics of prepared mango nectars. According to the results, it could be concluded that MN/S2%/I2% sample maintained the highest viability of probiotic bacteria for 45 days, with the least changes in colour and optimal physical properties, and higher sensory acceptability.

Table 4. Sensory analysis of mango nectar samples

	Overall acceptability			
	Storage time (days)			
Samples	1	15	30	45
Control	2.52 ± 0.65^{Cc}	2.56 ± 0.31^{Cc}	2.61 ± 0.26^{Cc}	2.50 ± 0.74^{Cc}
MN/I2%	4.16 ± 0.69^{Aa}	4.11 ± 0.54^{Aa}	4.24 ± 0.73^{Aa}	4.14 ± 0.29^{Aa}
MN/I4%	3.52 ± 0.59^{Bb}	3.48 ± 0.47^{Bb}	3.55 ± 0.89^{Bb}	3.52 ± 0.69^{Bb}
MN/S2%	3.32 ± 0.56^{Bb}	3.40 ± 0.62^{Bb}	3.41 ± 0.56^{Bb}	3.37 ± 0.11^{Bb}
MN/S4%	2.40 ± 0.50^{Ccd}	2.51 ± 0.48^{Ccd}	2.38 ± 0.44^{Ccd}	2.43 ± 0.33^{Ccd}
MN/S2%/I2%	4.12 ± 0.60^{Aa}	4.20 ± 0.11^{Aa}	4.15 ± 0.22^{Aa}	4.17 ± 0.58^{Aa}
MN/S2%/I4%	3.36 ± 0.49^{Bb}	3.44 ± 0.53^{Bb}	3.39 ± 0.38^{Bb}	3.34 ± 0.43^{Bb}
MN/S4%/I2%	2.32 ± 0.48^{Ccd}	2.36 ± 0.66^{Ccd}	2.33 ± 0.19^{Ccd}	2.33 ± 0.52^{Ccd}
MN/S4%/I4%	2.01 ± 0.41^{Dd}	2.14 ± 0.27^{Dd}	2.09 ± 0.47^{Dd}	2.08 ± 0.61^{Dd}

Data are presented as mean ± standard deviation ($n = 3$) for overall acceptance (1 – dislike very much; 5 – like very much). Different letters indicate significant differences at the 5% level in Tukey's test ($P < 0.05$). Capital letters indicate storage time effect and small letters indicate treatment effect. MN: Mango nectar; I: inulin; S: stevia.

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