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ORIGINAL RESEARCH PAPER

Effect of Different Treatments on Reduction of Oxalates in Starfruit (*Averrhoa carambola*) Juice

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Abstract

This study was aimed to evaluate the effect of EDTA blanching, NaCl blanching, and barley sprouts addition on the reduction of oxalates in starfruits juice. The effect of EDTA (0-0.6%), NaCl (0-10%), and sprouts (0-3%) treatments was evaluated by response surface methodology. EDTA, NaCl, and sprouts treatments had significant effects (p<0.05) on oxalate reduction. EDTA and NaCl treatments during blanching had a quadratic effect on the reduction of oxalate content of starfruit juice, while the addition of sprouts had a linear effect. Juice treated with all three factors had the maximum reduction, which was significantly higher (p<0.05) than individual treatments. The oxalates content was reduced to 6 mg/100 ml which is comparable to carrot and grapes juices and is considered as a normal oxalates level. Amongst the two factors treatment, the combination of EDTA and NaCl treatment had a significantly high reduction (p<0.05) as compared to other combinations. No significant difference (p>0.05) was obtained between the samples treated with EDTA (0.495%) or NaCl (10%) treatment in combination with sprouts addition. The regression expression for the oxalates reduction in response to these treatments was derived and validated.

Keywords:
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Introduction

Starfruit (*Averrhoa carambola*), variably recognized in Nepal as "*Kantaraa*", "*Chaarpaate*" or "*Madaane*" is valued for its distinct refreshing sweet and sour flavor. The ripe star fruits are usually consumed fresh as desserts or processed into fermented or unfermented drinks, confectionaries, and jam, and/or jelly (Minh, 2014; Patil et al., 2010). Starfruits are also valued for their nutritional and medicinal properties. They have high vitamin levels, crude fibre, and minerals, especially potassium (Othman et al., 2004). The bioactive compounds like pigments, vitamins, and polyphenols in starfruits promote the health of humans as anti-oxidative, anti-inflammatory, anti-microbial, anti-septic, anti-pyretic, anti-ulcer, anti-diabetic, anti-carcinogenic, and anti-aging agents (Kumar and Arora, 2016; Muthu et al., 2016). Star fruits are traditionally used for treating eye and throat infection, diarrhea, mouth ulcer, food poisoning, cough, and asthma (Barman et al., 2016) and also for diabetes mellitus because of their hypoglycemic effects (Wijayaratne et al., 2018).

Besides the sensory, nutritional, and medicinal benefits of starfruits, their high oxalic acid content has aroused serious concerns (Hönow and Hesse, 2002; Massey, 2007; Sorensen, 2014; Sá et al., 2019). The consumption of oxalic acid increases the formation of insoluble calcium oxalate salts which get deposited in the small blood vessels or to the units in the kidneys that clean blood (Chen et al., 2001; Wijayaratne et al., 2018). Excessive consumption of star fruit has been associated with the development of oxalate nephropathy in patients with both normal and abnormal baseline renal function (Barman et al., 2016; Wijayaratne et al., 2018). Consumption of high oxalic acid-containing food also decreases calcium absorption in the body (Von Unruh et al., 2004). The high amount of oxalic acid in starfruits (69-102 mg/100 g) (Gheewala et al., 2012; Patil et al., 2010) should therefore be reduced.

Various strategies have been used for the reduction of oxalates in foods. An attempt to reduce the oxalates content by thermal treatment was reported to follow first-order kinetics with a half-life of 2500-2800 years at 80°C and pH 5-7, which is practically not feasible (Crossey, 1991). Therefore, most of the strategies used to reduce oxalates like washing, soaking (with $CaCl_2$, NaCl), and blanching (normal or with EDTA, NaCl, $CaCl_2$, etc.) are primarily associated with the leaching out of oxalates present in the products (Dahal and Swamylingappa, 2006; Hefter et al., 2018; Rofi'ana et al., 2018; Savage and Dubois, 2006; Thapa et al., 2017). The effect of these treatments usually increases with increasing temperature and time (Dash and Gurumoorthi, 2011; Patel et al., 2018; Thapa et al., 2017). In recent years, the introduction of oxalate degrading enzymes such as oxalate-oxidase, oxalate decarboxylase, oxalyl-CoA decarboxylase, and formyl-CoA transferase is becoming popular for the reduction of oxalates content in foods (Conter et al., 2019; Federici et al., 2004; Kanauchi et al., 2009; Zhao et al., 2018). Barley sprouts are rich the oxalate degrading enzymes that can potentially be incorporated for the reduction of oxalates content in food products (Brudzyński and Salamon, 2011; Kanauchi et al., 2009).

This study was aimed to evaluate if the treatments, namely EDTA blanching, NaCl blanching, and sprouts addition can reduce the high oxalates content in star fruit juice to a normal level. The individual and combined effects of these treatments were studied to derive a prediction expression for the reduction of oxalates content in starfruit juice.

Materials and Methods

Materials

Fully mature starfruit was collected from Gaindakot-1, Nawalpur. Barley seeds (*Hordeum vulgare* var. Bonus) grown at Rampur, Chitwan was collected from *Saajha Agrovet*, Bharatpur, Chitwan. Ethylene-diamine-tetra-acetate (EDTA) disodium salt (Qualikem's Fine Chemical Pvt. Ltd.) and NaCl (Salt Trading Corporation Ltd.) available at Nagarik College were used.

Preparation of starfruit juice

Starfruits were washed with clean water and were cut vertically crease-wise after removing the side ridges. The core and seeds were discarded as they impart tartness. The fruit pieces were blanched at 85±2°C for 3 min. Constant blanching water to fruit ratio of four was used (Behera et al., 2017). Blanching was carried out in batch, with different concentrations of EDTA (0-0.6%) and NaCl (0-10%). The blanched samples were rinsed with sterile water and crushed to obtain juice. Fresh barley sprouts (Day-7, 25°C) coleoptile (0-3%) were plucked, crushed, and added to the juice for 4 h at 37°C. Then, the juice was filtered and clarified through Whatman No. 4 filter paper, then filled in a sterile bottle and pasteurized at 72±2°C for 30 s. The sample was immediately transferred to a deep-freezer (-18±2°C) until final analysis.

Proximate and physiochemical evaluation

The raw starfruit sample was taken to determine the proximate components and the starfruit juice sample was taken for determining physiochemical components. The contents of moisture, crude protein, crude fat, total ash, crude fibre, calcium, potassium, sodium and iron, pH, and acidity (as % citric acid), were analyzed as per Ranganna (1986). Total carbohydrate was evaluated by the difference method. Total soluble solids (TSS) were evaluated by using a refractometer and the Brix-acid ratio was calculated.

Oxalic acid determination

Estimation of oxalic acid content was carried out as described by Naik et al. (2014) with minor modifications. Briefly, 0.5 ml of the sample was transferred to 30 ml of 0.25 N HCl and was boiled in a water bath for 15 min. Then the total sample volume was made 50 ml by adding 0.25 N HCl. In a culture tube, 1 ml of sample extract was taken, then 5 ml of 2N H_2SO_4 and 2 ml of 0.003M $KMnO_4$ were added and incubated at 37°C for 10 min. Absorbance was read at 308 nm in UV-Vis Spectrophotometer (Shimadzu, USA). Oxalates contents were determined by comparing the absorbance with the calibration curve obtained for the standard oxalic acid solution (0-1 mg/ml).

Experimental design of treatments

Response surface methodology (RSM) was used to understand the effect of EDTA (0-0.6%) blanching, NaCl (0-10%) blanching, and sprout (0-3%) addition on the reduction of oxalates in star fruit juice. The juice sample obtained with normal blanching i.e., EDTA 0%, NaCl 0%, and sprouts 0% were considered as control with 100% oxalates, while the response for different combinations of these treatments was expressed as the oxalates reduction (%) as presented in Table 1. Regression evaluation was carried out to predict oxalates reduction (%) and was validated for different combinations.

Statistical evaluation

The results are the mean ± standard deviation (SD) of triplicate evaluations. Analysis of data was carried by one-way analysis of variance (ANOVA) and the mean comparisons were done by the Tukey-HSD test where necessary. The main, interaction and quadratic effect of each of the treatments on the reduction of oxalates were evaluated. All statistical evaluations were analyzed at a 5% level of significance. All statistical evaluations and figures were obtained using JMP pro 15 (SAS Inc., US).

Results and Discussion

Proximate and physiochemical evaluation

The proximate compositions of the starfruit sample and various physicochemical parameters of starfruit juice are presented in Table 2. The results are in accordance with that reported previously (Bhaskar and Shantaram, 2013; Narain et al., 2001; Patil et al., 2010).

Oxalate content in raw and control juice

The oxalates contents were significantly reduced ($p<0.05$) in response to different treatments. The oxalates content of the starfruit juice was reduced from 93.21 ± 1.27 mg/100 ml to 80.01 ± 1.53 mg/100 ml after normal water blanching. This was a 14.16 ± 0.54% reduction of initial oxalates content. This reduction was possibly associated with leaching loss in aqueous solution which increases with increasing temperature and treatment time (Dash and Gurumoorthi, 2011; Patel et al., 2018; Thapa et al., 2017). The reduction of oxalates could also be partly attributed to the hydrolysis of soluble oxalates into carbonates with a slight increase in pH due to water (Blum, 1912).

Table 1

Treatment combinations for the response surface evaluation and percentage reduction in oxalates content in star fruit juice

Treatments	*EDTA (%)*	*NaCl (%)*	*Sprouts (%)*	*Oxalates reduction (%)*
S1	0.33	0	1.53	43.48 ± 1.29^{fg}
S2	0.6	10	3	92.18 ± 2.75^{a}
S3	0	10	3	54.98 ± 1.59^{e}
S4	0.3	5	1.5	71.34 ± 2.07^{c}
S5	0.6	10	0	81.02 ± 2.42^{b}
S6	0.6	0	0	41.07 ± 1.22^{fg}
S7	0	0	3	15.03 ± 0.47^{h}
S8	0.3	5	0	65.76 ± 1.95^{d}
S9	0	5	1.5	38.47 ± 1.11^{g}
S10	0.3	5	1.5	71.34 ± 2.12^{c}
S11	0	10	0	43.83 ± 1.27^{f}
S12	0.6	2.3	3	67.82 ± 2.02^{cd}
Control	0	0	0	0^{i}

Note. The values in the table are arithmetic mean ± SD value of triplicate samples. Different superscript letters in the column indicate a statistical difference among the sample means ($p<0.05$).

Effect of EDTA blanching

The reduction in oxalates content with increasing concentrations of EDTA in the absence of NaCl and sprout treatment is presented in Figure 1a. EDTA (0-0.6%) blanching had a quadratic effect ($p<0.05$) on the oxalates content in starfruit juice. With increasing concentration of EDTA during blanching, there was a higher reduction in oxalates but flattened above 0.3% EDTA. At EDTA concentration of 0.4-0.6%, the oxalates reductions were comparable and ranged about 40-42%. The reduction in oxalic acid content with an increasing concentration of EDTA treatments was also reported in previous studies (Dahal and Swamylingappa, 2006; Thapa et al., 2017). Thapa et al. (2017) reported that blanching the leaves of *Spinacia oleracea* with EDTA solution at boiling temperature for 2 min with different concentrations of EDTA (0.1-3 mM) reduced the oxalates content of spinach by 37-67%. They further reported that the increase in EDTA concentration from 2 mM to 3 mM had no significant difference in the oxalate reduction. In another study by Dahal and Swamylingappa (2006), the best method for the reduction of oxalates in Colocasia tuber was concluded to be 0.1 mM EDTA.

Table 2
Proximate and physicochemical composition of sample starfruit juice

Parameters	*Starfruit*
Moisture (%)	92.24±1.34
Crude protein (%)	0.42±0.03
Crude fat (%)	0.29±0.03
Ash (%)	0.34±0.03
Crude fibre (%)	1.07±0.05
Carbohydrate (%)	5.88±2.06
Calcium (mg/100 g)	4.34±0.48
Potassium (mg/100 g)	125±0.35
Sodium (mg/100 g)	3.8±0.05
Iron (mg/100 g)	0.52±0.34
pH	3.13±0.06
Acidity (as % citric acid)	0.42±0.04
Total soluble solids (°Bx)	7.2±0.06
Brix-acid ratio	16.14±1.53
Oxalic acid content (mg/100ml)	93.21±1.27

Note. The values in the table are arithmetic mean ± SD value of triplicate samples.

The enhanced reduction of oxalates during EDTA blanching could be associated with the chelating activity of EDTA, which has a very high affinity for calcium, resulting in the formation of calcium edetate (calcium-disodium-EDTA). In the presence of EDTA, the calcium in solution phase complexes with EDTA, shifting the reaction equilibrium and lower the calcium oxalate salts, and releasing oxalic acid (Verplaetse et al., 1986). EDTA concentration 0.03M reportedly dissolved two mm calculus within 48 h (Burns and Cargill, 1987). It is well recognized that oxalic acid is five to seven times more soluble than sodium oxalates, and therefore has a higher potential of leaching in an aqueous solution.

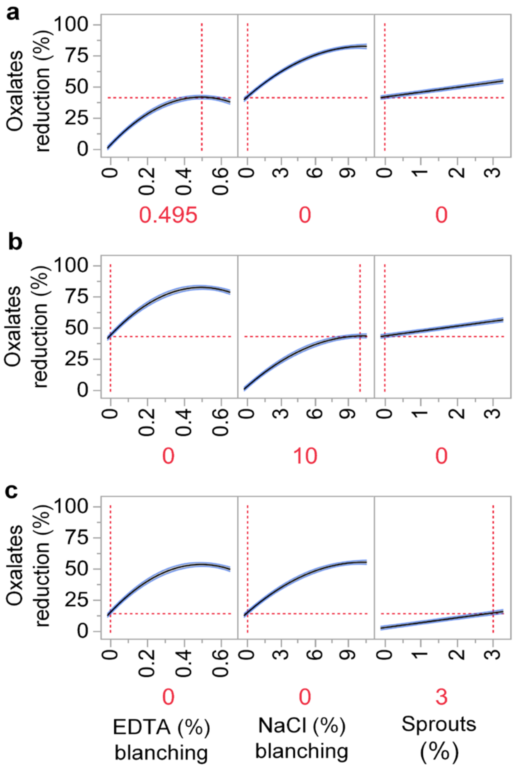

Figure 1
The effect of different treatments on the reduction of oxalates in starfruit juice. a) EDTA blanching; b) NaCl blanching; c) Sprouts addition

Although EDTA treatment reportedly reduced the total oxalates in different foods, the majority of reductions occur in bound fractions, while the overall reduction of soluble fraction needs further elaboration. Insoluble oxalates in starfruit could range from one-tenth to more than a half of the total oxalates content (Hönow and Hesse, 2002; Sá et al., 2019). Oxalates in a bound state have limited bioavailability and very low toxicity (Sorensen, 2014). Some evidence has revealed a proportional increase in oxalate absorption with an increase in soluble oxalate content (Massey, 2007). However, reduction of total oxalates could reduce the risk of associated toxicities, if the soluble oxalates content is not increased.

Effect of salt treatment

The reduction in oxalates content with increasing concentrations of NaCl in the absence of EDTA and sprout treatment is presented in Figure 1b. NaCl (0-10%) blanching had a quadratic effect ($p<0.05$) on oxalates reduction in starfruit juice. With an increasing concentration of NaCl during blanching, there was a higher reduction in oxalates with the maximum reduction of 43.83±1.27% at 10% NaCl. Thapa et al. (2017) also reported a higher reduction of oxalates in leaves of *Spinacia oleracea* with increasing concentration of NaCl during blanching. In their study, boiling spinach leaves for 2 mins in 1% NaCl solution reduced oxalate content by 10.45%; and when NaCl was 5%, the reduction was 23.79%.

The reduction in oxalates during NaCl treatment could be associated with the conversion of insoluble calcium oxalates into soluble derivatives and subsequent leaching into the aqueous solution (Rofi'ana et al., 2018). In this study, the reduction in oxalic acid content was highly effective with increasing concentration of NaCl in the initial stage, while the effectiveness was gradually reduced at higher NaCl concentrations. This could be associated with the reduced solubility of sodium oxalates with increasing concentration of NaCl (Hefter et al., 2018). Hefter et al. (2018) reported that the calcium oxalates in purple yam were also reported to reduce more with increasing NaCl concentration up to 10%, whilst there was less reduction when NaCl was further increased to 50%.

Table 3
Optimized treatment for maximum oxalates reduction with desirability function

S. No.	*EDTA blanching (%)*	*NaCl blanching (%)*	*Sprouts addition (%)*	*Desirability*	*Predicted oxalates reduction (%)*
T1	0.495	10	3	0.92	93.88±1.65
T2	0.495	10	0	0.81	82.08±1.51
T3	0.495	0	3	0.53	53.05±1.56
T4	0	10	3	0.54	54.77±1.44

Note. The values in the table were obtained by using the statistical tool: JMP Pro 15 (SAS Inc.).

Effect of sprouts treatment

The reduction in oxalates content with increasing concentrations of sprouts in the absence of NaCl and EDTA treatment is presented in Figure 1c. Barley sprouts (0-3%) addition linearly reduced ($p<0.05$) oxalates in starfruit juice. At 3% sprouts addition, oxalates reduction of 15.03±0.47% was obtained. The reduction of oxalates could be associated with the activity of the oxalate oxidase enzyme in the sprouts. Barley sprouts especially embryo and aleurone are rich in oxalate oxidase and the enzyme activity was reported to increase with an increasing number of days in its first week (Brudzyński and Salamon, 2011; Kanauchi et al., 2009). Oxalate oxidase also referred to as 'germin' is heat resistant and has the maximum activity at pH 4.0 with more than 80% relative activity in the pH range of 3.0 to 5.0 (Kanauchi et al., 2009). The oxidase enzyme catabolize oxalates into carbon and hydrogen derivatives (Kanauchi et al., 2009; Kotsira and Clonis, 1997). The insoluble calcium oxalates are usually not accessible to the enzymes (Chipps et al., 2005). Therefore, the reduced oxalates are primarily soluble oxalate derivatives. Considering the linear activity of sprouts addition in starfruit juice, higher reductions in oxalates can be further achieved by increasing the concentration of sprouts or by increasing the treatment time. Considering the high thermal resistance of oxalate oxidase (Kanauchi et al., 2009), further reduction in the soluble oxalates in starfruit juice during storage can be expected.

Predictions and validations

From response surface evaluation, a prediction expression for the reduction of oxalates in comparison to control was obtained as presented below:

$$\textbf{Oxalates reduction (\%)} = 23.35 + 68.90 \times [\text{EDTA (\%)}] + 4.24 \times [\text{NaCl (\%)}] + 3.94 \times [\text{Sprouts (\%)}] - 159.50 \times [\text{EDTA (\%)} - 0.28]^2 - 0.38 \times [\text{NaCl (\%)} - 4.79]^2$$

Using the above expression, optimum conditions for two and three factors were predicted for the validation of the expression. The desirability functions used for the prediction and the predicted values are presented in Table 3. The reduction of oxalates in response to optimized treatments when practically evaluated was statistically similar ($p>0.05$) to the predicted reduction of oxalates. The final result of optimized treatments is presented in Figure 2.

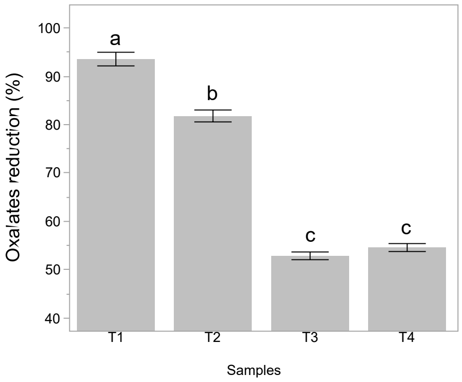

Figure 2
Oxalates reduction in starfruit juice in response to optimized two and three factor treatments (T1: 0.495% EDTA + 10% NaCl + 3% sprouts, T2: 0.495% EDTA + 10% NaCl, T3: 0.495% EDTA + 3% sprouts, T4: 10% NaCl + 3% sprouts)

The combination of all three treatments (0.495% EDTA + 10% NaCl + 3% sprouts) had the maximum percentage of oxalates reduction. The combined treatments reduced oxalate content to about 6 mg/100 ml from the initial content of 93.21 ± 1.27 mg/100 ml. This oxalates level is comparable to carrot and grapes juices and is considered as a normal oxalates level (Siener et al., 2016). Amongst the two factors treatment, the combination of EDTA and NaCl treatment had a significantly high reduction ($p<0.05$) as compared to other combinations. The maximum oxalates reduction that could be achieved by the combination of EDTA (0.495%) and sprouts addition (3%) was statistically similar to that achieved by the combination of NaCl (10%) and sprouts addition (3%).

Conclusions

The proximate and physicochemical composition of the starfruit was comparable with previous studies. EDTA and NaCl treatments during blanching had a quadratic effect on the reduction of oxalate content of starfruit juice, while the addition of sprouts had a linear effect. The overall oxalates reduction in response to these treatments could also be predicted effectively. Juice treated by the combination of all three parameters had the maximum reduction, which was significantly higher ($p<0.05$) than other samples. This was followed by a combination of EDTA and NaCl treatment. No significant difference ($p>0.05$) was obtained between the samples treated with EDTA and NaCl treatment in combination with sprouts addition. EDTA and NaCl treatments during blanching reduce both the soluble and insoluble oxalates. The insoluble oxalates are firstly solubilized and subsequently leached out to an aqueous solution during blanching. However, in the case of sprouts addition, the oxalate oxidase enzyme catabolizes the soluble oxalates into carbon and hydrogen derivatives. Considering the heat resistance of oxalate oxidase enzymes in sprouts, the oxalates in products are expected to further decrease during storage and are recommended for further exploration.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

The study did not involve any inhumane animal study.

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