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Microbial production of dicarboxylic acids from edible plants and milk using GC-MS

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Abstract

Objective: The present experiment was designed to assess the contents of organic acids such as citric acid, succinic acid, fumaric acid, and malic acid in edible food plants and milk under the influence of *Eschericia coli*.

Methods: Gas chromatography-mass spectrometry (GC-MS) was used to estimate the contents of organic acids in edible plants and milk. Two microliters of samples was injected into the GC-MS, and the contents of organic acids were computed using standard curves.

Results: Maximum citric content (204 mg/g DW, 24 h *E. coli* treatment) was observed in tomato followed by papaya (175 mg/g DW). Papaya and grapes (715 and 504 mg/g DW, 24 h *E. coli* treatment respectively) can be good sources of succinic acid. Malic acid content was highest in *E. coli*-treated milk (168 mg/g DW). In general, there was a decrease in average citric acid and increase in succinic acid contents in the food sources tested on treatment with *E. coli*.

Conclusion: It was found that among the tested raw food items and milk, with or without *E. coli* inoculation, tomato and papaya hold a good potential for citric acid production, grapes and papaya for succinic acid, and milk for malic acid production. The study can be a basis for utilization of vegetables, fruits, and milk for the production of dicarboxylic acids to boost the agrarian economy.

Keywords: *E. coli*, Food plants, Milk, Organic acids, Multivariate statistical techniques

Introduction

Organic acid production using microbial processes for industrial use is finding increasing attention worldwide (Sauer et al. 2008). Tricarboxylic acid cycle (TCA) operative in living organisms produces citric acid, succinic acid, fumaric acid, and malic acid as intermediates during the process of respiration. All these acids are extensively used in industry. Out of these, only citric acid is largely produced using microbial technology. Attempts are underway for the microbial production of other acids. The annual production of citric acid is 1.6 million tons, and it is mainly produced from the fermentation of glucose, sucrose or beet, and cane molasses using the fungi *Aspergillus niger* or *Yarrowia lipolytica* (Berovic and Legisa 2007; Gonçalves et al. 2014). The annual production of succinic acid is 1600 tons. Presently, succinic acid is largely produced by the catalytic hydrogenation of maleic anhydride, a fossil-based chemical. It has the potential to replace maleic anhydride as a raw material for many chemical industries, and its projected market is 270,000 tons (Sauer et al. 2008). The annual production of fumaric acid is 240,000 tons. The projected annual market of fumaric acid is 350,000 tons by 2020 (www.grandviewresearch.com). Malic acid is widely used as a food additive and also used in pharmaceutical and polymer industries. Malic acid is chemically produced by the hydration of maleic or fumaric acids (Moon et al. 2008). The demand for malic acid is expected to increase from 10,000 tons in 2008 to more than 200,000 tons (Sauer et al. 2008).

Escherichia coli is one of the preferred bacteria for studies on the energetics and regulation of respiration (Unden and Bongaerts 1997). Due to multitude of primary dehydrogenases, quinones, and terminal reductases, a large

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variability in the composition of the respiratory chains is observed. Since *E. coli* is a natural habitant of human intestines, the present study was designed to assess the effects of *E. coli* at 0 h, 24 h, and 72 h treatments on food plants such as *Triticumaestivum*, *Zea mays*, *Vigna mungo*, *Lens culinaris*, *Pisumsativum*, *Phaseolus vulgaris*, *Cicer arietinum* (black gram), *Cicer arietinum* (white gram), *Solanum tuberosum*, *Solanum lycopersicum*, *Brassica oleracea*, *Malus pumila*, *Musa paradisiaca*, *Vitisvinifera*, *Carica papaya*, and milk. The organic acids such as citric acid, succinic acid, fumaric acid, and malic acid were quantified in the food items and milk by using GC-MS. The results were analyzed by using various multivariate techniques such as cluster analysis (CA), factor analysis (CABFAC), and non-metric multidimensional scaling (NMDS).

Methods

E. coli culture

The bacterial strain of *E. coli* K-12 was procured from Microbial Type Culture Collection (MTCC) facility, Chandigarh, India. The culture was revived using Luria Bertani broth (composition used for the broth: casein enzymic hydrolysate 10 g/L, yeast extract 5 g/L, sodium chloride 10 g/L, and pH was adjusted to 7.3) and incubated in orbital shaker for 24 h at 37 °C. The chemicals and biochemicals were purchased from Himedia (India). The culture was diluted with the fresh medium in order to obtain the cell density of 10^7 cfu/ml. 0.1 ml of the inoculum was taken from the diluted culture to inoculate the experimental media.

Sample preparation

Two grams of each of the edible food source was taken in 100 ml of distilled water, autoclaved at 121 °C, inoculated with 100 μl of *E. coli* culture for 24 and 72 h, and kept in a BOD incubator at 37 °C. One hundred milliliters of milk was taken in a flask, autoclaved at 121 °C, inoculated with 100 μl of *E. coli* culture for 24 and 72 h, and kept in BOD incubator at 37 °C. All the samples were filtered with Whatman filterpaper#1 after 24 and 72 h and kept in a refrigerator for GC-MS analysis.

Organic acid determination by GC-MS

The determination of organic acids was done by following the protocol described by Sharma et al. (2016). The organic acids were extracted from 1 ml of the oven-dried samples by the addition of 0.5 N HCl (0.5 ml) and 0.5 ml of methanol. After that, the samples were shaken for 3 h proceeded by centrifugation (12,000 rpm; 10 min). The supernatant, methanol (300 μl), and 50% sulfuric acid (100 μl) were added and incubated for overnight in water bath at 60 °C. After cooling to 25 °C, 800 μl of chloroform and 400 μl of distilled water were added to the supernatant followed by vortexing for 1 min. The lower layer of chloroform was used to determine the organic acid contents.

Conditions of GC-MS

Helium was used as the carrier gas, and the starting column temperature was set at 50 °C, stopped for 1 min which was increased to 125 °C at 25 °C/min, followed by additional increment to 300 °C at 10 °C/min, and detained for 15 min. Injection temperature was 250 °C, injection mode was split, gas flow in the column was 1.7 ml/min, and analytical column DB-5ms was used. MS conditions are as follows: Ion source temperature was fixed at 200 °C and interface temperature was 280 °C, solvent cut time was 3 min, and detector gain mode was relative. The sample preparation procedure resulted in the derivatization of organic acids, and the concentrations of citric acid trimethylester, succinic acid dimethyester, fumaric acid dimethyester, and malic acid dimethyester were determined by using standard curves.

Statistical analysis

All the experiments were performed in triplicate, and the data were presented as mean ± SD. The data was also analyzed by using multivariate techniques. Two-way analysis of variance (ANOVA) and post hoc Tukey's honestly significant difference (HSD) test were applied to the contents of different organic acids with respect to the duration of *E. coli* treatment. Similarities among the species in n-dimensional space were computed using Ward's method of cluster analysis with Euclidean distance as distance measure. Factor analysis (CABFAC) was done by regressing the variables (organic acids) on environmental variable (hours of *E. coli* treatment) and varimax rotation. Factor analysis brings out common variables governed by the same factor. A loading of a factor of magnitude (0.7 or − 0.7) or more was treated to be significant.

Non-metric multidimensional scaling (NMDS) is a multivariate technique of data reduction. In NMDS, ranked differences among the points in multidimensional space are maintained in a two- or three-dimensional space using a similarity measure, correlation in the present study. The environmental variable taken was *E. coli* treatment. In the present analysis, NMDS software developed by Taguchi and Oono (2005) was used. The other software used were PAST3 (Imbrie 1971; Klovan and Imbrie 1971; Sieger et al. 1999), MINITAB-14, and self-coded software in Microsoft Excel.

Results and discussion

Table 1 shows mean ± SD, *F*-ratios of two-way analysis of variance, and Tukey's HSD values of organic acids with respect to different durations of *E. coli* treatment.

Table 1 Organic acid analysis of edible food plants and milk under *E. coli* treatments (0 h, 24 h, and 72 h) using GC-MS

Species and family	Common name and edible part	*E. coli* treatment (h)	Citric acid (mg/g DW)	Succinic acid (mg/g DW)	Fumaric acid (mg/g DW)	Malic acid (mg/g DW)	Source of variation	*F*-ratios	HSD
Triticum aestivum L.	Wheat grain	0	1.97 ± 0.24	0.46 ± 0.08	0.29 ± 0.03	0.93 ± 0.27	Acids	183	7.09
Fam. Poaceae		24	1.39 ± 0.10	32.48 ± 5.35	0.27 ± 0.05	0.81 ± 0.20	Time	49	
		72	1.28 ± 0.09	45.12 ± 8.05	0.27 ± 0.005	0.58 ± 0.10	Interaction	52	
Zea mays L.	Maize/corn grain	0	4.56 ± 0.36	0.47 ± 0.10	0.22 ± 0.07	0.59 ± 0.10	Acids	157	3.28
Fam. Poaceae		24	1.29 ± 0.02	4.21 ± 0.79	0.23 ± 0.05	0.59 ± 0.08	Time	107	
		72	1.40 ± 0.29	30.77 ± 4.50	0.26 ± 0.04	0.61 ± 0.07	Interaction	126	
Vigna mungo (L.)	Urad bean/Maah seeds	0	19.71 ± 3.68	0.72 ± 0.03	0.24 ± 0.05	0.86 ± 0.14	Acids	237	5.64
Hepper		24	ND	30.21 ± 5.82	0.35 ± 0.05	0.87 ± 0.14	Time	68	
Fam. Fabaceae		72	1.42 ± 0.23	34.10 ± 5.21	2.88 ± 0.96	0.83 ± 0.23	Interaction	59	
Lens culinaris Medicus	Lentil/Masoor seeds	0	10.44 ± 2.59	5.40 ± 0.88	0.24 ± 0.08	0.84 ± 0.19	Acids	253	7.08
Fam. Fabaceae		24	1.80 ± 0.30	48.24 ± 8.24	1.56 ± 0.24	0.72 ± 0.08	Time	33	
		72	1.36 ± 0.31	43.93 ± 5.48	0.24 ± 0.07	1.30 ± 0.19	Interaction	59	
Pisum sativum L.	Peas seeds	0	82.43 ± 17.34	1.81 ± 0.04	0.76 ± 0.004	5.39 ± 0.63	Acids	59	3.69
Fam. Fabaceae		24	5.14 ± 1.05	3.87 ± 0.04	1.35 ± 0.042	2.32 ± 0.50	Time	41	
		72	4.67 ± 1.13	35.28 ± 5.93	0.80 ± 0.004	3.07 ± 0.67	Interaction	70	
Phaseolus vulgaris L.	Kidney beans/Rajmaah seeds	0	6.82 ± 1.80	1.75 ± 0.28	0.22 ± 0.069	1.45 ± 0.10	Acids	121	5.23
Fam. Fabaceae		24	11.41 ± 1.94	2.02 ± 0.16	0.37 ± 0.092	0.60 ± 0.11	Time	78	
		72	1.50 ± 0.26	45.74 ± 6.72	0.29 ± 0.058	0.72 ± 0.08	Interaction	133	
Cicer arietinum L.	Chickpea/black gram seeds	0	4.90 ± 0.65	23.22 ± 3.71	0.22 ± 0.02	Nd	Acids	291	3.71
var. black gram		24	1.31 ± 0.19	25.67 ± 5.48	0.23 ± 0.05	Nd	Time	255	
Fam. Fabaceae var.		72	1.32 ± 0.25	81.81 ± 7.93	0.22 ± 0.03	Nd	Interaction	274	
Cicer arietinum L.	Chickpea/white gram/Kabuli gram seeds	0	21.72 ± 4.47	42.31 ± 5.21	0.22 ± 0.03	0.60 ± 0.09	Acids	362	5.37
var. White gram		24	1.50 ± 0.35	24.24 ± 2.64	0.54 ± 0.05	0.59 ± 0.04	Time	93	
Fam. Fabaceae		72	1.47 ± 0.24	19.44 ± 3.09	0.44 ± 0.06	0.60 ± 0.10	Interaction	33	
Solanum tuberosum L.	Potato tubers	0	6.06 ± 1.16	3.01 ± 0.19	0.87 ± 0.19	2.43 ± 0.53	Acids	2844	0.110
Fam. Solanaceae		24	5.67 ± 0.77	18.82 ± 2.91	0.97 ± 0.24	2.47 ± 0.005	Time	1985	
		72	5.38 ± 0.97	2.43 ± 0.48	0.92 ± 0.29	2.43 ± 0.44	Interaction	1969	
Solanum lycopersicum L.	Tomato fruits	0	77.72 ± 11.7	52.73 ± 11.04	2.50 ± 0.92	6.71 ± 0.92	Acids	144	3.50
Fam. Solanaceae		24	204.09 ± 29.1	275.36 ± 44.7	2.50 ± 0.65	6.58 ± 1.18	Time	153	
		72	27.88 ± 4.20	35.37 ± 6.57	2.50 ± 0.65	6.58 ± 1.05	Interaction	56	
Brassica oleracea.	White cauliflower inflorescence	0	16.22 ± 1.31	2.17 ± 0.05	2.11 ± 0.006	9.99 ± 1.78	Acids	920	0.358
var. *botrytis* L.		24	6.79 ± 1.08	35.80 ± 0.45	1.08 ± 0.011	2.97 ± 0.05	Time	199	

Table 1 Organic acid analysis of edible food plants and milk under *E. coli* treatments (0 h, 24 h, and 72 h) using GC-MS *(Continued)*

Species and family	Common name and edible part	*E. coli* treatment (h)	Citric acid (mg/g DW)	Succinic acid (mg/g DW)	Fumaric acid (mg/g DW)	Malic acid (mg/g DW)	Source of variation	*F*-ratios	HSD
Fam. Brassicaceae		72	6.74 ± 1.14	14.62 ± 0.17	1.08 ± 0.40	2.91 ± 0.22	Interaction	626	
Malus pumila Miller	Apple mesocarp of the fruit	0	5.81 ± 0.45	1.89 ± 0.05	0.97 ± 0.25	2.70 ± 1.73	Acids	121	2.34
Fam. Rosaceae		24	Nd	44.83 ± 7.85	0.97 ± 0.35	2.96 ± 0.51	Time	32	
		72	Nd	36.11 ± 9.28	0.97 ± 0.005	3.01 ± 0.51	Interaction	32	
Musa paradisiaca	Banana mesocarp of the fruit	0	15.16 ± 2.64	8.55 ± 1.17	0.70 ± 0.18	5.87 ± 0.44	Acids	451	7.93
Colla		24	87.71 ± 18.05	207.58 ± 17.5	0.66 ± 0.11	2.06 ± 0.36	Time	152	
Fam. Musaceae		72	4.07 ± 0.40	233.01 ± 25.6	0.70 ± 0.22	2.75 ± 0.22	Interaction	125	
Vitis vinifera L.	Grapes Fruits	0	24.31 ± 5.19	5.66 ± 0.94	2.60 ± 0.82	34.46 ± 6.01	Acids	345	5.88
Fam. Vitaceae		24	12.86 ± 2.12	504.57 ± 57.46	2.24 ± 0.59	7.20 ± 1.41	Time	83	
		72	21.71 ± 2.24	409.58 ± 58.17	2.24 ± 0.11	26.08 ± 1.18	Interaction	99	
Carica papaya L.	Papaya fruit	0	175.68 ± 48.02	3.70 ± 0.81	1.33 ± 0.37	8.58 ± 1.48	Acids	259	11.31
Fam. Caricaceae		24	54.32 ± 12.21	715.8 ± 78.21	1.41 ± 0.44	5.62 ± 0.07	Time	86	
		72	73.70 ± 17.24	262.5 ± 30.63	1.33 ± 0.37	7.33 ± 0.88	Interaction	137	
Milk		0	0.19 ± 0.009	0.09 ± 1.01	0.04 ± 0.009	1.20 ± 0.09	Acids	642	9.72
		24	0.19 ± 0.009	4.72 ± 0.55	0.04 ± 0.009	2.41 ± 0.18	Time	685	
		72	0.19 ± 0.009	8.98 ± 1.48	0.04 ± 0.009	168.2 ± 11.29	Interaction	618	

Nd no data

Among the analyzed samples, citric acid content on dry weight basis was found to be maximum in tomato (204 mg/g in 24 h treatment), papaya (175 mg/g), banana (87 mg/g), pea (82 mg/g), and potato (77 mg/g). *E. coli* treatment decreased average citric acid content of cereals and pulses. *E. coli* fermentation increased the succinic acid content in cereals, pulses, vegetables, and fruits tested except for white gram. Papaya, grapes, tomato, and banana yielded 175, 504, 275, and 233 mg/g DW of succinic acid on inoculation with *E. coli*. In *S. lycopersicum* and *M. paradisiaca*, the citric acid content initially increased followed by a decrease on *E. coli* treatment. There was no significant difference in fumaric acid contents in the samples inoculated with *E. coli*. Malic acid content was increased in milk with *E. coli* inoculation. However, no specific trend was found for the other analyzed food plants. In 11 out of 15 cereals, pulses, vegetables, and fruits, the average citric acid content was reduced on inoculation with *E. coli.* The average values of fumaric and malic acid contents were recorded to be very low in the cereals, pulses, vegetables, and fruits (Figs. 1 and 2). Milk yielded the highest malic acid content on *E. coli* treatment (168 mg/g).

In vegetables, the average citric acid and succinic acid contents were found to be maximum for 24 h duration on *E. coli* treatment and decreased after 72 h with *E. coli* treatment. The average values of fumaric acid and malic acid contents were found to be very low in the vegetables (Fig. 3). In fruits, the average citric acid content was reduced with inoculation of *E. coli*, whereas the average succinic acid content was found to be maximum for 24 h duration of *E. coli* treatment. The average fumaric acid and malic acid contents were found low in the fruits (Fig. 4). In milk, malic acid content was enhanced under *E. coli* treatment with respect to the control (Fig. 5). Citric acid and fumaric acid contents were recorded very low in milk. Analysis of data by using two-way ANOVA and Tukey's HSD test showed significant differences for citric acid (F_{acids}, F_{time}, and $F_{acids \times time}$ $p < 0.05$), succinic acid (F_{acids}, F_{time}, and $F_{acids \times time}$ $p < 0.05$), fumaric acid (F_{acids}, F_{time}, and $F_{acids \times time}$ $p < 0.05$), and malic acid (F_{acids}, F_{time}, and $F_{acids \times time}$ $p < 0.05$) in different edible plants.

Cluster analysis (CA) was applied to the contents of different organic acids (Fig. 6). *C. papaya* and *V. vinifera* are included in the same cluster, and both are fruits. The

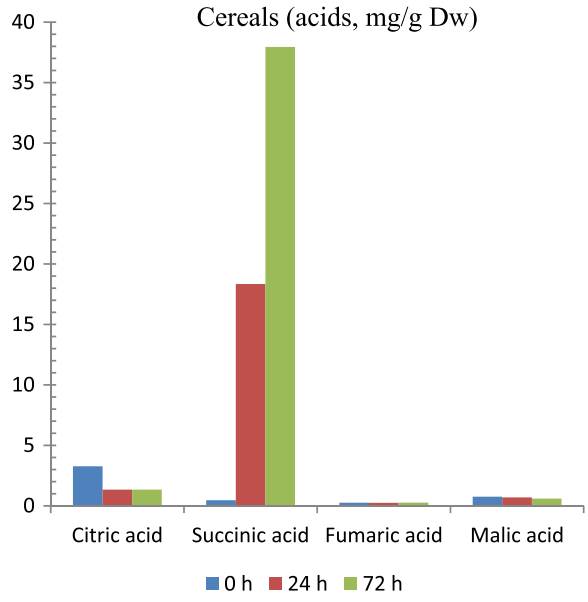

Fig. 1 Average values of organic acids of all the analyzed cereals

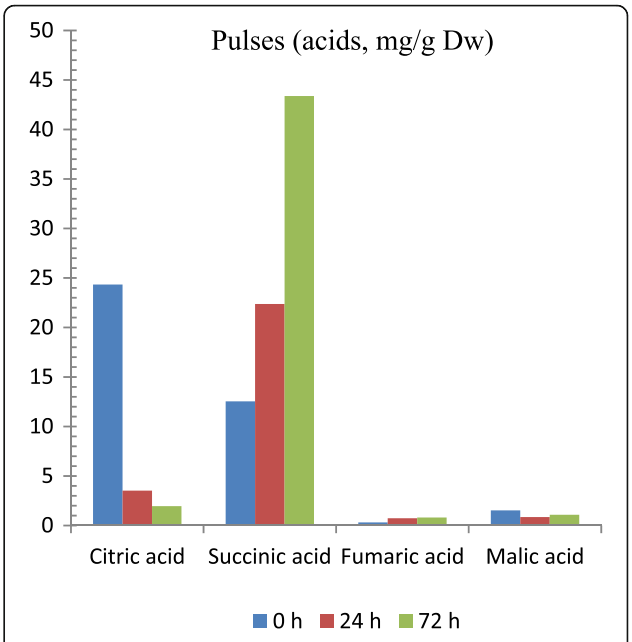

Fig. 2 Average values of organic acids of all the analyzed pulses

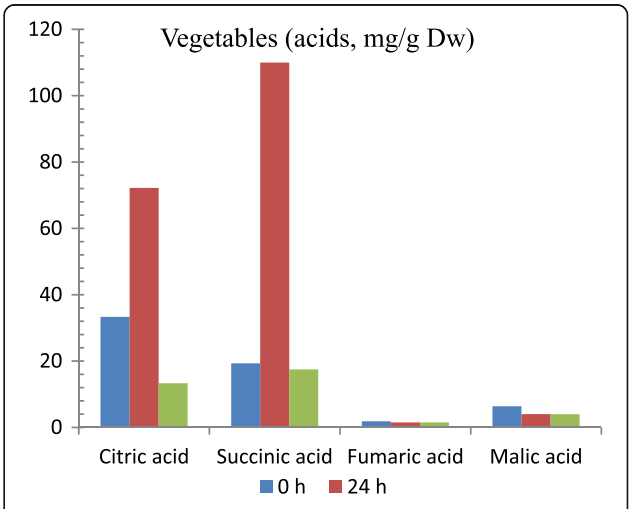

Fig. 3 Average values of organic acids for all the analyzed vegetable

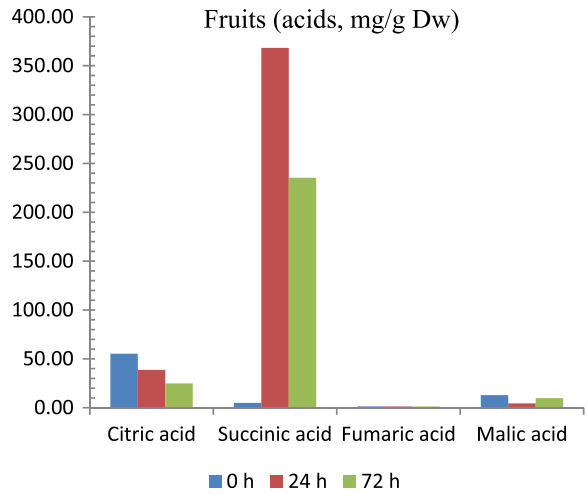

Fig. 4 Average values of organic acids in all the analyzed fruits

pulses, *C. arietinum* w.g, *C. arietinum* b.g, and *L. culinaris*, are included in the same cluster and had close proximities with each other. *B. oleracea* and *P. sativum* are included in the same cluster, and both are vegetables. CABFAC factor analysis yielded segregation of untreated and *E. coli*-treated organic acid variables in two different factors (Fig. 7). In *C. arietinum*, however, both the treatments are represented in the same first factor implying that there is another factor governing acid formation in these pulses. In grapes, sugar content may be the governing factor. In milk, the segregation of the factors was different, and lactose present in milk could be the deciding factor. First three factors explained 99.87% of the total variance, and the Eigen value was more than one for the first three factors (Table 2).This factor is contributed by *E. coli*. Both CABFAC scatter plot and NDMS

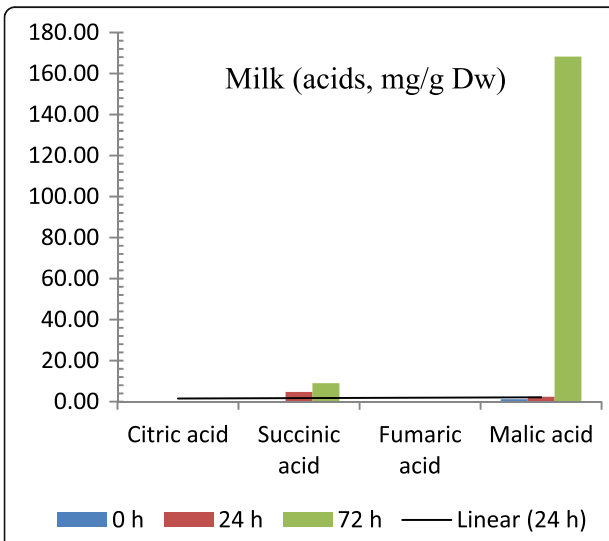

Fig. 5 Average values of organic acids in milk

scatter plot reveal three points (point no. 48 = milk 72 h, 46 = milk 0 h, and 40 = grapes 0 h) segregated from the main group (Figs. 8 and 9). Since the stress in NMDS Shepard curve (Fig. 9) is 0.03785, i.e., less than 0.05, the data shows a good fit to NMDS. As also seen in the factors, chemical composition of these items might be segregating them from the other items.

E. coli are Gram-negative bacteria mainly occupying the lower intestinal tract of humans and animals and are regularly excreted into the environment by feces or wastewater discharge. The existence of *E. coli* in the environmental waters has been measured as a marker of fecal pollution (Jang et al. 2017). The wild strain of *E. coli* under anaerobic conditions produces acetate, formate, ethanol, and succinate, whereas aerobically succinate is formed as an intermediate of TCA cycle. *E. coli* has been used for the production of succinic acid (Thakker et al. 2013; Skorokhodova et al. 2013) under aerobic conditions using raffinose, galactose, sucrose, and stachyose. Bioenergetics of *E. coli* involve aerobic and anaerobic respiration and fermentation, which require different carriers to transport different substrates and products across membranes. Succinic, fumaric, and malic acids play different roles in different respiratory pathways (Unden and Bongaerts 1997). In aerobic respiration, careers mediate the uptake of succinic acid. However, in anaerobic process, exchange of fumarate with succinate, uptake of fumarate, and efflux of succinate occurs.

In the present study, fruits and vegetables, especially papaya, grapes, and tomato, were found to be good sources of succinic acid. *E. coli* is a preferred bacterium for study of succinate production technology due to its known genomics and proteomics (Thakker et al. 2012). Nghiem et al. (2017) suggested that there can be two metabolic pathways for the production of succinic acid from glucose. In the TCA pathway, succinic acid is an intermediate of oxidation of glucose via citrate. On the other hand, succinic acid is more reduced molecule than glucose, and a reduction pathway for its production is:

$$7\,C_6H_{12}O_6 + 6\,CO_2 \rightarrow 12\,C_4H_4O_4\,(\text{succinic acid}) + 6\,H_2O$$

Microbial production of succinic acid can be achieved using *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *E. coli*, *Actinobacillus succinogenes*, and *Anaerobiospirillum succiniciproducens* from glucose, molasses, and wheat (Sauer et al. 2008). Toker et al. (2004) analyzed organic acid contents in the fresh parts of different varieties of *C. arietinum*. They reported the highest organic acid content to be that of succinic acid followed by malic acid, the minimum being citric acid. The glycolysis of glucose produces pyruvate which is then metabolized to yield

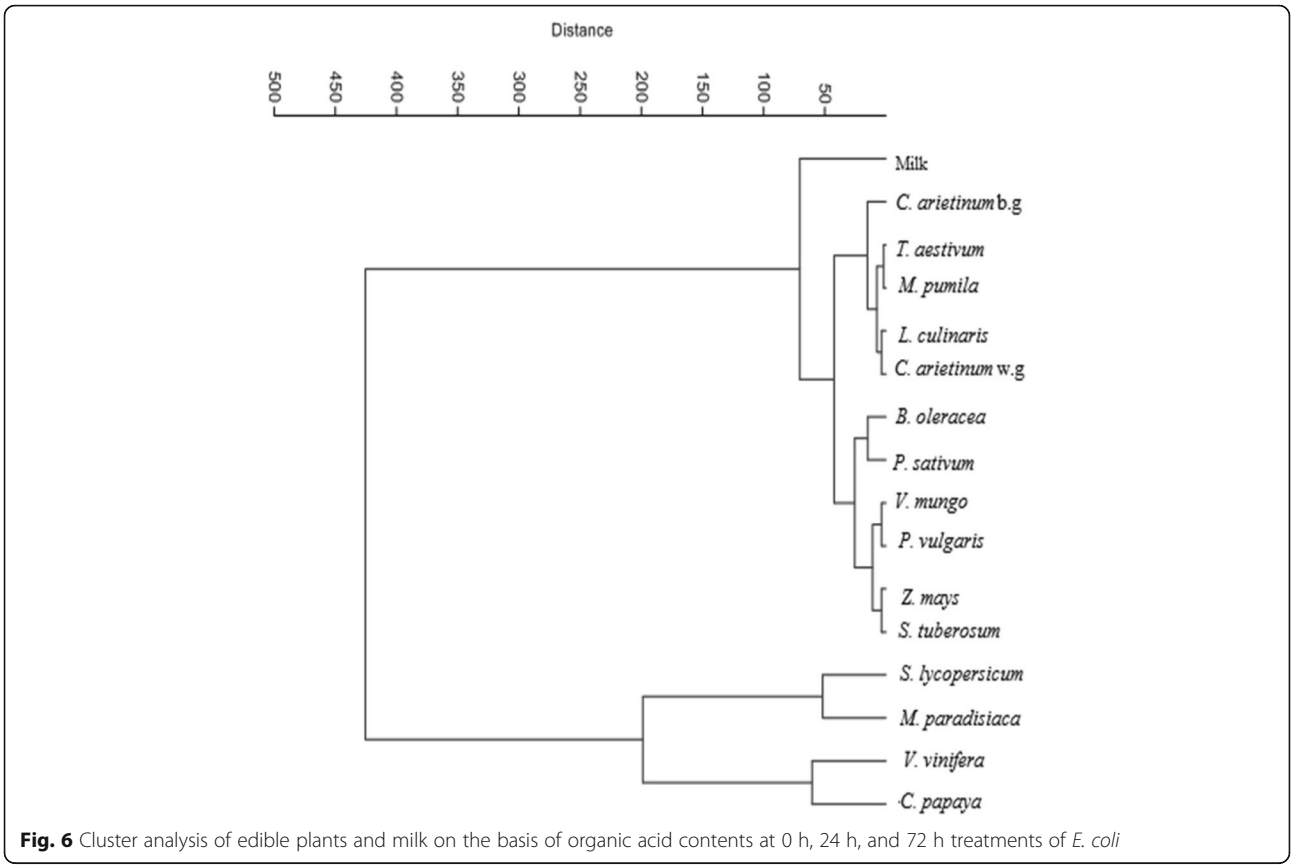

Fig. 6 Cluster analysis of edible plants and milk on the basis of organic acid contents at 0 h, 24 h, and 72 h treatments of *E. coli*

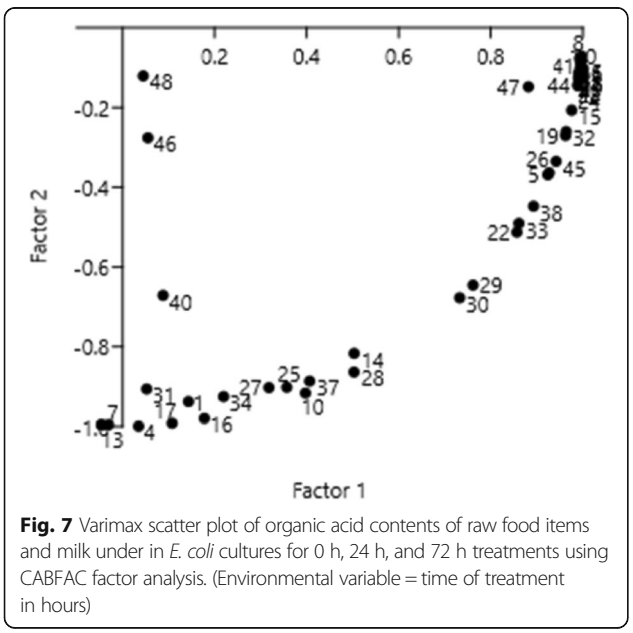

Fig. 7 Varimax scatter plot of organic acid contents of raw food items and milk under in *E. coli* cultures for 0 h, 24 h, and 72 h treatments using CABFAC factor analysis. (Environmental variable = time of treatment in hours)

one or more end products such as lactate, acetate, ethanol, formate, malate, succinate, hydrogen, and carbon dioxide (Förster and Gescher 2014).

In the present study, fumaric acid was not produced or enhanced to be exploited on an industrial scale with or without *E. coli* treatment. TCA cycle produces fumaric acid as an intermediate from glucose metabolism. Fumaric acid is used in the manufacture of polyesters, resins, inks, and as an animal feed and a food additive to tortilla, fruit juice, wines, etc. In view of the increasing cost of production, microbiological methods offer an economically feasible solution.

$$C_6H_{12}O_6 + 2CO_2\,(\text{from } CaCO_3) \rightarrow 2C_4H_4O_4\,(\text{fumaric acid}) + 2H_2O$$

The reductive pathway for fumaric acid from glucose occurs via carboxylation of pyruvate to oxaloacetate, then to malate and fumerate. The reductive pathway is catalyzed by pyruvate carboxylase under aerobic conditions (Das et al. 2017). Commercial production of fumaric acid is done from maleic anhydride using vanadyl pyrophosphate as a catalyst (Martin-Dominguez et al. 2018).

Table 2 CABFAC factor analysis of edible food plants and milk on the basis of organic acid contents for 0 h, 24 h, and 72 h under *E. coli* treatments. Values in italics represent significant factor

No.	Food source	Treatment (h)	Factor 1	Factor 2	Factor 3
1	*Triticum aestivum*	0	0.140	− 0.930	0.300
2		24	*0.993*	− 0.112	0.020
3		72	*0.995*	− 0.097	0.010
4	*Zea mays*	0	0.035	*− 0.999*	0.011
5		24	*0.924*	− 0.369	0.098
6		72	*0.993*	− 0.115	0.015
7	*Vigna mungo*	0	− 0.031	*− 0.996*	− 0.074
8		24	*0.997*	− 0.071	0.029
9		72	*0.991*	− 0.115	0.020
10	*Lens culinaris*	0	0.397	*− 0.916*	− 0.033
11		24	*0.994*	− 0.107	0.011
12		72	*0.994*	− 0.101	0.026
13	*Pisum sativum*	0	− 0.045	*− 0.996*	− 0.052
14		24	0.503	*− 0.817*	0.245
15		72	*0.976*	− 0.207	0.071
16	*Phaseolus vulgaris*	0	0.178	*− 0.980*	0.089
17		24	0.108	*− 0.992*	− 0.064
18		72	*0.995*	− 0.101	0.012
19	*Cicer arietinum* var. black gram	0	*0.962*	− 0.270	− 0.024
20		24	*0.993*	− 0.118	− 0.005
21		72	*0.997*	− 0.083	− 0.001
22	*Cicer arietinum* var. white gram	0	*0.857*	− 0.513	− 0.041
23		24	*0.991*	− 0.132	0.018
24		72	*0.989*	− 0.146	0.022
25	*Solanum tuberosum*	0	0.357	*− 0.902*	0.236
26		24	*0.927*	− 0.364	0.091
27		72	0.318	*− 0.903*	0.277
28	*Solanum lycopersicum*	0	0.503	*− 0.863*	− 0.026
29		24	*0.761*	− 0.645	− 0.050
30		72	*0.732*	− 0.677	0.072
31	*Brassica oleracea.* var. *botrytis*	0	0.052	*− 0.906*	0.416
32		24	*0.964*	− 0.260	0.060
33		72	*0.861*	− 0.491	0.129
34	*Malus pumila*	0	0.220	*− 0.925*	0.297
35		24	*0.995*	− 0.076	0.066
36		72	*0.993*	− 0.078	0.083
37	*Musa paradisiaca*	0	0.406	*− 0.886*	0.221
38		24	*0.893*	− 0.448	− 0.036
39		72	*0.996*	− 0.086	0.010
40	*Vitis vinifera*	0	0.088	− 0.671	*0.736*
41		24	*0.995*	− 0.094	0.012
42		72	*0.990*	− 0.127	0.057
43	*Carica papaya*	0	− 0.046	*− 0.995*	− 0.069

Table 2 CABFAC factor analysis of edible food plants and milk on the basis of organic acid contents for 0 h, 24 h, and 72 h under *E. coli* treatments. Values in italics represent significant factor *(Continued)*

44		24	*0.990*	− 0.143	− 0.001
45		72	*0.942*	− 0.335	− 0.005
46	Milk	0	0.055	− 0.276	*0.960*
47		24	*0.882*	− 0.148	0.448
48		72	0.045	− 0.121	*0.990*

Variance of first three factors using CABFAC factor analysis

Principal component	Eigenvalue	% variance
I	33.36	69.50
II	11.78	24.54
III	2.79	5.83
Total	47.93	99.87

$$C_4H_2O_3\,(\text{maleic anhydride}) \xrightarrow{\text{Hydrolysis}} C_4H_4O_4\,(\text{maleic acid}) \xrightarrow{\text{Isomerisation}} C_4H_4O_4\,(\text{fumaric acid})$$

In the present study, highest malic acid content was observed in milk inoculated with *E. coli* after 72 h. Dobrowolska-Iwanek et al. (2015) compared the juice composition of apple cultivars. It was opined by Martin et al. (2000) that carboxylic acid salts enhance the conversion of lactic acid into propionic acid by ruminant bacteria through succinate-propionate pathway. The concentration of malic acid was found to be maximum (6.58 g dm^{-3}) in *Reinette simirenk* cultivar. Wang et al. (2009) observed increase in milk yield in dairy cows fed on fodder supplemented with malic acid. Martínez-González et al. (2015) proved that when the diet of ewes was supplemented with 4 g malic acid/kg diet, their milk production and milk protein contents increased.

Conclusions

From the present study, it was concluded that tomato and papaya can be used as potential sources for citric acid production. Similarly, *E. coli* treatment of papaya, grapes, and tomato increases their succinic acid content. Milk on treatment with *E. coli* can be a potential source of malic acid. However, the samples analyzed did not prove to be good sources of fumaric acid. It is also concluded that *E. coli* treatment decreases the citric acid content of *T. aestivum*, *V. mungo*, *L. culinaris*, *P. sativum*, *C. arietinum*, *S. tuberosum*, and *B. oleracea*. The succinic acid content was increased with *E. coli* treatment in all samples except for white gram and milk.

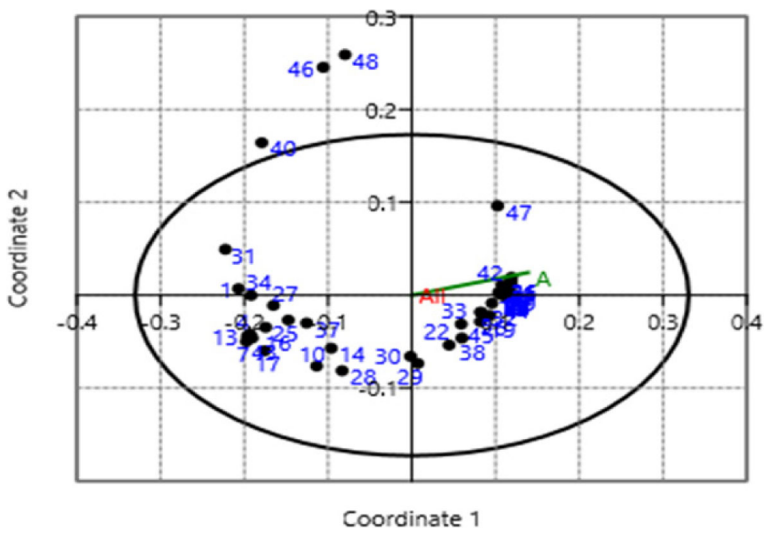

Fig. 8 NMDS scatter plot (95% eclipse) of organic acid formation in *E. coli* cultures for 0 h, 24 h, and 72 h treatments containing raw food items and milk using correlation as a similarity measure.(Environmental variable = time of treatment in hours)

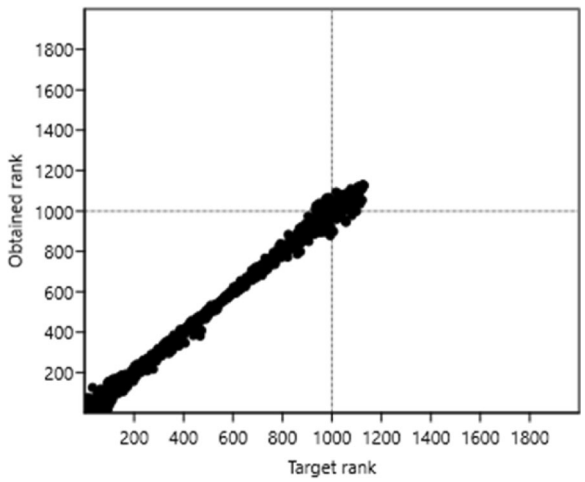

Fig. 9 NMDS Shepard 2-D plot of organic acid formation of raw food and milk for 0 h, 24 h, and 72 h treatments of *E. coli* cultures (Environmental variable = time of treatment in hours, stress = 0.03785, R^2 for axis 1 = 0.8091 and axis 2 = 0.2267)

Malic acid increased in milk on *E. coli* treatment. Microbial production of dicarboxylic acids, citric acid, succinic acid, and malic acid using microbial technology employing *E. coli* holds a good potential. The study will provide a baseline data for utilization of surplus vegetables, fruits, and milk for industrial production of dicarboxylic acids and improve the agrarian economy.

Abbreviations

ANOVA: Analysis of variance; CA: Cluster analysis; FA: Factor analysis; GC-MS: Gas chromatography-mass spectrometry; HSD: Honestly significant difference; NMDS: Non-metric multidimensional scaling

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Authors' contributions

This work was carried out in collaboration between all authors. The authors RK and AS collected and analyzed the samples. The authors VK, MS, and AKT carried out literature search and drafted the manuscript. The authors RK, RB, and AKT designed the experiment and statistically analyzed the data. All authors read and approved the final manuscript.

Competing interests

All authors declare that they have no competing interests.

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