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Research article

The impact of raw and differently dried pineapple (*Ananas comosus*) fortification on the vitamins, organic acid and carotene profile of dairy rasgulla (sweetened cheese ball)

Tanmay Sarkar [a,b], Molla Salauddin [a], Sudipta Kumar Hazra [a], Runu Chakraborty [a,*]

[a] Department of Food Technology and Biochemical Engineering, Faculty of Engineering and Technology, Jadavpur University, Jadavpur, Kolkata 700032, India
[b] Malda Polytechnic, West Bengal State Council of Technical Education, Govt. of West Bengal, West Bengal 732102, India

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ABSTRACT

Pineapple, a tropical fruit that is well known for its excellent nutritional characteristics. Rasgulla (sweetened cheese ball) is a dairy based popular sweet found all over India. To enrich the nutritional profile, vitamin content, organic acid and carotene content profile of normal dairy rasgulla (sweetened casein ball) and to make it functionally more active for human health, fortification of rasgulla has been done by using normal pineapple pulp along with hot air, freeze, microwave and microwave convective dried pineapple pulp for overall comparative analysis and they are coded as PP, PH, PF, PMW and PMWC respectively. By using RP-HPLC method quantification of vitamin A, vitamin B group (B_1, B_2, B_3, B_5, B_6, B_7 and B_{12}), tocopherols (α, β, γ and δ form), vitamin C, carotene (α, β, γ and δ form) profile and organic acid profile (oxalic acid, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, propionic acid, succinic acid [SA], and fumaric acid) of all the rasgulla samples are estimated. A significant increase is found for differently fortified rasgulla samples in terms of vitamins content, organic acid and carotene content. B_1, B_3, B_7 and B_{12} are found maximum in PF whereas B_5, B_6 and B_2 are found maximum for PMWC, PP and NR. The maximum amount of vitamin C and tocopherol are found in PP and PF. PMWC is reported to have maximum carotene content compared to all other samples.

1. Introduction

Since ancient times in the context of vast Indian food culture milk has played a very significant role. Milk and milk products like cheese, butter, yogurt and different milk-based sweets are essential parts of the daily Indian diet. Milk products are well known for their rich nutritional characteristics [1]. India is the world's one of the largest milk producing country and in the year 2018–2019, India has produced 187.7 million tons of milk of which 55–60 % milk is used in the production of Indian traditional dairy products [2]. From the socio-cultural point of view, different dairy-based sweets have immense importance in Indian society and it predominates over all other types of sweets.

Among several types of dairy-derived sweets, rasgulla/rosogolla/rasogolla/sweetened cheese ball is one of the most popular sweets all over India which is originated in the eastern part of India. Though originated in India, it becomes popularized across the South Asian countries like Bangladesh, Nepal, Pakistan and Myanmar [3]; it is now exported to the countries like Togo, Ghana, Kenya, Finland, Turkey, Denmark, Netherlands, St Maarten, United States of America, New Zealand and Australia (HS code 21069099). India has exported rasgulla worth USD of 12,39,058 in the year 2016 [4]. It is a round-shaped small white-colored cheese ball with a spongy texture that has possessed excellent quality in terms of taste, flavor and mouthfeel. From the nutritional point of view, it is found that like other milk-derived products rasgulla lacks in the amount of vitamins, anti-oxidant, and organic acids. Under the new product development approach, there is a wider scope to enhance the vitamin, organic acids profile of rasgulla through fortification by natural products. Several authors have reported different types of newly developed rasgulla such as - use of carrot paste in normal dairy rasgulla by Bandyopadhyay et al., 2008; Sarkar *et al.* 2020 developed pineapple rasgulla; Kaur et al., 2019 reported use of stevia in dairy rasgulla; Pramanick *et al.* 2017 reported use of tulsi leaf extract in normal dairy rasgulla [3, 5, 6, 7].

Pineapple is well known for its excellent nutritional characteristics and can be used as a promising fortifying agent. Use of pineapple in different dairy products has been reported by several authors such as -

* Corresponding author.
E-mail address: crunu@hotmail.com (R. Chakraborty).

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Sawant *et al.* 2015 reported fortification of yogurt drink with pineapple pulp; Sarkar *et al.* 2020 reported fortification of rasgulla with pineapple juice; Gangwar *et al.* 2016 reported fortification of yogurt with pineapple juice [8, 9, 10].

Being a tropical seasonal fruit pineapple is available for a particular time of a year and therefore in order to enhance the ease of availability of pineapple throughout the year, drying of pineapple pulp can be an alternative. Better extraction of nutritional compounds such as carotenes and vitamins were reported in different dried products compared to fresh. To evaluate all these concerns here we have used four types of dried pineapple - hot air-dried, freeze-dried, microwave dried and microwave convective dried along with fresh pineapple pulp for the fortification of the normal dairy rasgulla.

Though rasgulla continuously increasing its influence in the segment of sweetmeat/dessert market in South Asian countries and gradually gaining popularity in African and European countries; the thorough nutritional, vitamin, carotene and organic acid profile are still unidentified. Southern North American (Mexico), Central American (Costa Rica), Latin American (Brazil, Colombia) and South East Asian (Philippines, Thailand, Indonesia, India and China) countries hold the major share in pineapple production and processing [8], though rasgulla is yet to be unveiled in a large portion of the world, pineapple fortification may assimilate with the perception of palatability of consumers from a vast section of the world. This may accelerate the transformation of this traditional Indian sweetmeat delicacy from the localized form to a more globalized form evolved through multicultural amalgamation. The objective of this study is to find out the efficacy of fortification through identification and quantification of vitamins (A, C, E, B_1, B_2, B_3, B_5, B_6, B_7, and B_{12}), organic acid and carotene profile of all the newly developed pineapple fortified rasgulla samples and normal dairy rasgulla sample by using RP-HPLC method.

2. Materials and methods

Fresh quality whole pineapple fruits and sugars were collected from the local market of Jadavpur, Kolkata, India. Fresh quality bovine milk was bought from a local outlet (HACCP and ISO 9622:2013 certified) of Jadavpur, Kolkata, India.

2.1. Preparation of pineapple

Raw pineapples (*Ananas comosus*) of Kew variety were cleaned, peeled and cut into small pieces of slab shape (length: 2 cm × breadth: 0.5 cm × thickness: 0.3 cm) and divided into five small parts. Among those one part was marked as PP and rest four parts were dried through four different drying methods – Convective drying/hot air oven drying (60 °C, 2.5 m/s air flow rate, 16 h) of pineapples was done by using a hot air oven (Concepts International, Kolkata, India) and marked as PH (moisture content 12%); After primary freezing at -50 °C for 22 h in a deep freezer (New Brunswick Scientific, England; Model no: C340-86), freeze-drying (-40 °C, 0.1 m-bar pressure, 20 h) of pineapple was done by using a freeze dryer (FDU 1200, EYELA, Japan) and marked as PF (moisture content 9%); Microwave drying (100 W, 108 min) of pineapples was done by using a microwave oven (Samsung, Combi CE1031LAT, Mumbai, India) and marked as PMW (moisture content 10%); Microwave convective drying (40 °C, 600W, 95 min) of pineapple was done by using a microwave oven (Samsung, Combi CE1031LAT, Mumbai, India) and marked as PMWC (moisture content 10%). A single layer of samples was there for each drying process [11]. All the four dried pineapple samples were ground by a mixer grinder.

2.2. Preparation of pineapple rasgulla

Fresh milk (fat content of 3.6 % and solid not fat content of 8.7 %) was boiled (in gas oven) and after 30–60 s of boiling, the milk was cooled to 30 ± 5 °C. 1% (w/v) solution of lactic acid (70 °C) was mixed gradually into it, till the total amount of milk was coagulated with an appearance of greenish-yellow hue. The total coagulated mass was left for 10–15 min to stabilize completely. Then the total coagulated channa (casein portion) was transformed into a mesh made of stainless steel and thoroughly washed. Muslin cloth was used for the straining of the channa. The pressed channa was kneaded manually to obtain dough with a smooth texture.

PP, PH, PF, PMW and PMWC along with channa in 1:4 ratios were used to form round shaped balls each weighing about 10g [5]. Also, normal rasgulla was prepared by using only channa and marked as NR. Cooking sugar syrup having a soluble solid content in the range of 50–60 °Brix along with soaking sugar syrup having a soluble solid content of 30–40 °Brix were prepared. Channa balls were poured in the boiling cooking syrup first. To balance the amount of evaporated moisture during boiling, 80–100 ml/L of water were mixed in an interval of every 5 min and the process was continued for 20–25 min. After completion of the boiling period all the different types of rasgullas were dipped into warm (60–70 °C) soaking sugar syrup (35–40 °Brix) kept in a covered pot separately [9]. This soaking process was continued for a period of 6 h [Figure 1] and thereafter the rasgulla samples were taken for experimental results.

2.3. Sample extract preparation

1 gm. of each type of rasgulla samples (PP, PH, PF, PMW, PMWC, and NR) were mixed with a solution (15 ml of distilled water + 15 ml of isopropyl alcohol) and ultra-sonicated (Trans-O-Sonic, Mumbai) for 30 min followed by filtration using 0.45 μm millipore filter. Filtrates (PP, PH, PF, PMW, PMWC, and NR) were stored in a refrigerator for HPLC analysis of vitamin C and organic acids.

2.4. Nutritional analysis

Moisture content (MC) and crude protein of samples were estimated as per AOAC 2000 [12]. Carbohydrate, fat and ash content were measured according to Sarkar et al., 2020 [13].

2.5. HPLC analysis of organic acids and ascorbic acid (vitamin C)

By using an RP-HPLC system HPLC identification and quantification of organic acid profile and vitamin C were carried out. At first 20 μL of sample volume was injected into the system and the elution was carried out by using 0.01 M KH_2PO_4 solution (pH 2.5). The separation was performed by using a C_{18} column (4.6 × 150 mm^2 internal diameter, 3.5μm pore size) with a flow rate of 0.5 $mL.min^{-1}$. The final chromatogram was achieved at 214 nm and all the organic acids (oxalic acid, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, propionic acid, succinic acid, and fumaric acid), vitamin C were quantified by comparison with standard curve respectively [14].

2.6. HPLC analysis of vitamin B group

25 ml of (0.1 N) H_2SO_4 solution was mixed with 2 g of sample and the resulting solution was incubated at 121 °C for 30 min. After 30 min the mixture was cooled and the pH was set to 4.5 with 2.5 M sodium acetate solution. Then 50 mg α amylase enzyme was mixed with the mixture. Then again this mixture was incubated at 35 °C for an overnight period. Then the mixture was filtered (Whatmman No. 4 filter) and diluted by using 50 mL of distilled water and finally, the mixture was filtered by using a 0.45 μm millipore filter. In an RP-HPLC system, 10 μL filtrate was injected and separation was performed by using a C_{18} column having a 4.6 × 150 mm^2 internal diameter with a pore size of 3.5 μm. The H_3PO_4-Acetonitrile solution in a ratio of 1:60 was used as a mobile phase and the flow rate was maintained at 0.5 $mL.min^{-1}$. The final chromatogram was observed at 254 nm [14].

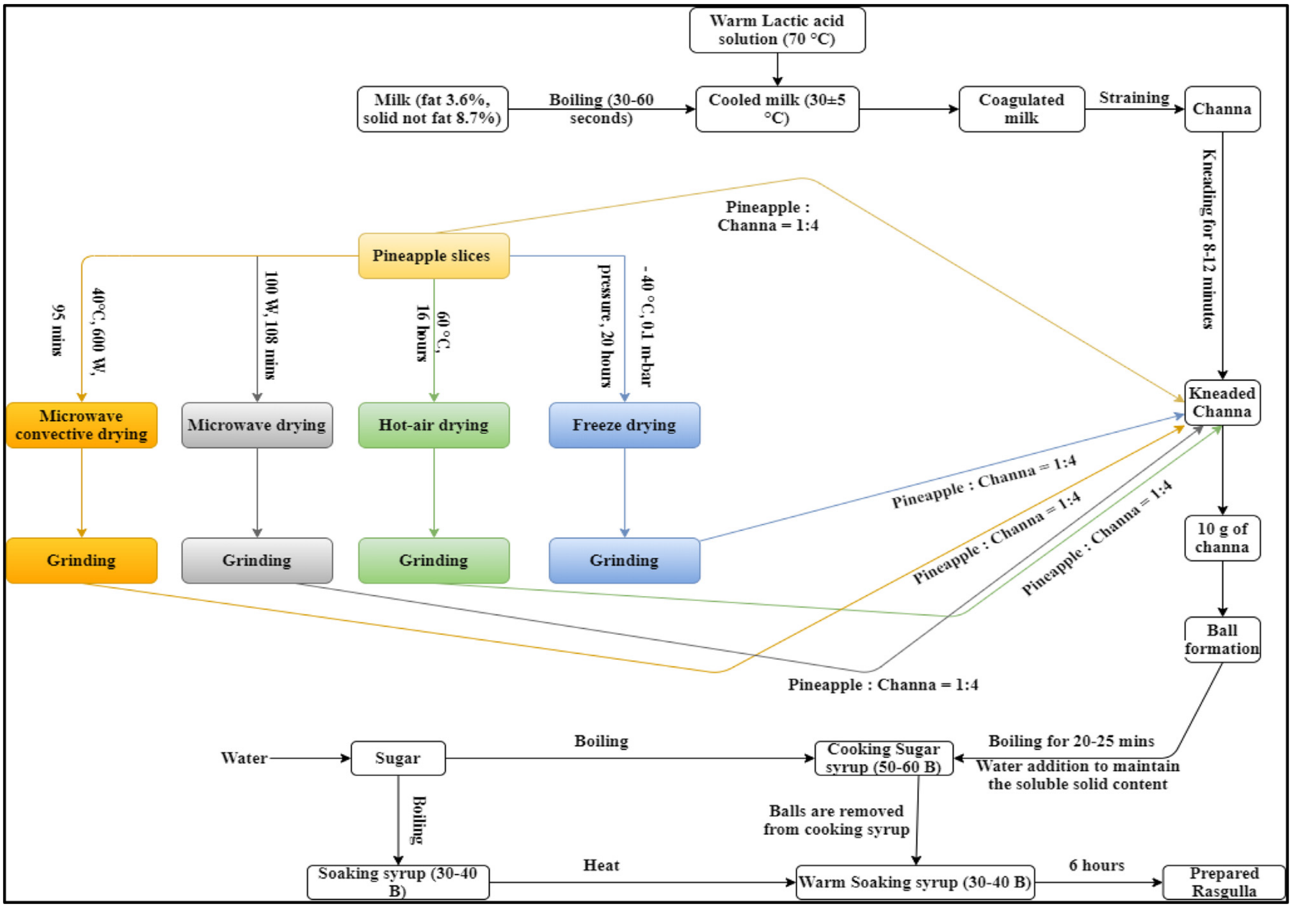

Figure 1. Flow chart for rasgulla preparation. Critical steps were: coagulating condition; kneading; soluble solid content in cooking syrup.

2.7. HPLC analysis of vitamin E, vitamin A and carotene profile

At first 1 g of the sample was mixed with 50 % 3 mL KOH, 7mL ethanol and 0.1 g of pyrogallic acid. Then the mixture was refluxed at 50 °C for 40 min. Double-distilled water and anhydrous sodium sulfate (Na_2SO_4) were used for neutralization and de-hydration of the solution respectively. By using a water bath maintained at 50 °C, the solution was concentrated to 5 mL and then by using methanol, the solution was diluted to 10 mL and finally with the aid of a 0.45 μm Millipore filter the mixture was filtered. 20 μL of the filtrate was injected in an RP-HPLC system for further analysis by using a C_{18} column having a 4.6 × 150 mm^2 internal diameter with a pore size of 3.5 μm. For *β*-Carotene a solution of acetonitrile-methyl alcohol-ethyl acetate in a ratio of 88:10:2 was used as eluent while for vitamin A and D, a 100 % solution of methyl alcohol was used as eluent with a flow rate of 0.5 $mL.min^{-1}$. The final chromatogram of carotene, vitamin E, and vitamin A were observed at 453 nm, 325 nm, and 290 nm respectively. All the quantifications were done by comparison with respective standard graphs [14].

2.8. Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and all the data were taken as the average of the independent experiments performed in triplicate. Student's t-test was carried out considering the significance level at $p < 0.05$. PCA (principal component analysis), HCA (hierarchical cluster analysis) and correlation analysis were carried out with R Studio software version 3.4.4 (2018-03-15) software.

3. Results

3.1. Nutritional composition

Table 1 represents the proximate composition of all the rasgulla samples. Sengupta et al., 2017 and Prodhan et al., 2007 also reported the proximate composition of normal dairy rasgulla [15, 16]. Table 1 clearly showed that the moisture content of NR was significantly lower than the moisture content of all the pineapple fortified rasgulla samples. Whereas, all the pineapple fortified samples were statistically insignificant ($p > 0.05$). This might be explained as, because of the incorporation of higher moisture from pineapple fruit pulp (80.78 ± 3.2 %) and differently dried pineapple pulp as well. According to different literature, the ash content of normal rasgulla was varied in the range of (0.75 ± 0.005–1.04 ± 0.03) % [12, 13] and in this study, the ash content of NR was found 1.19 ± 0.03 %.

The pineapple was reported to have an ash content of 0.45 ± 0.002 %. This value might alter from one cultivar to another [17]. As drying treatment showed no impact on mineral content, hence insignificant changes ($p > 0.05$) were observed in the ash content of all the pineapple fortified rasgulla samples [18]. Similarly, in the case of fat content, insignificant changes were observed between all the pineapple fortified rasgulla samples though NR was found to have a significantly higher amount of fat content due to the high amount of channa in it. NR and pineapple fruit were reported to have fat content (2.72 ± 0.10–14.41 ± 6.43) % and 0.956 % respectively [15, 16, 17]. The protein content of all the pineapple fortified rasgulla samples were significantly lower with respect to NR and this was due to different drying process which caused

Table 1. Nutritional composition.

	MC	Ash	Protein	Fat	Carbohydrate	Total solids
NR	45.31 ± 1.39	1.19 ± 0.07	9.03 ± 0.94	8.23 ± 0.49	37.02 ± 1.15	52.91 ± 2.39
PP	47.85 ± 1.88	1.09 ± 0.02	7.22 ± 0.69	6.58 ± 0.55	29.62 ± 1.01	42.33 ± 1.99
PF	46.31 ± 1.62	1.13 ± 0.05	7.31 ± 0.58	6.14 ± 0.35	45.22 ± 1.68	54.29 ± 2.59
PH	45.01 ± 1.03	1.17 ± 0.03	7.23 ± 0.91	5.90 ± 0.56	49.63 ± 1.72	52.26 ± 2.56
PMW	45.75 ± 1.69	1.11 ± 0.03	7.30 ± 0.80	6.08 ± 0.45	46.02 ± 1.65	52.92 ± 2.13
PMWC	45.96 ± 1.35	1.08 ± 0.05	7.30 ± 0.89	6.10 ± 0.39	48.03 ± 1.89	53.88 ± 2.03

All the results were reported in the above table as mean value ± SD (standard deviation).
MC = Moisture content.
[PP = Pineapple pulp rasgulla, PH = Hot air dried pineapple rasgulla, PF = Freeze-dried pineapple rasgulla, PMW = Microwave dried pineapple rasgulla, PMWC = Microwave convective dried pineapple rasgulla, NR = Normal rasgulla].

severe damage to the hydrogen bond and different hydrophobic interactions in the protein structure resulted in denaturation of the protein. Hassan et al., 2007 reported a decrease in protein content of hot air dried Capparaceae compared to fresh [19]; Garcia-Amezquita et al., 2018 reported a higher amount of protein content in freeze dried pear-peel compared to hot air drying [20]. NR and pineapple fruit were reported to have protein content (3.91 ± 0.12–8.24 ± 0.05) % and (1.19 ± 0.03) % respectively [12, 13, 14]. The carbohydrate content of NR (37.02 ± 1.03 %) was found higher than PP (29.616 ± 1.12 %) due to the higher carbohydrate content of NR than raw pineapple fruit pulp. But in the case of differently dried pineapple fortified rasgulla samples carbohydrate content was found significantly higher than PP and NR as well. This phenomenon occurred due to the different drying treatment, which caused an increase in the dry matter content of pineapple thus caused a higher carbohydrate amount in all dried pineapple pulp. Ajayi et al., 2017 reported an increase in carbohydrate content of microwave and oven dried ginger than raw ginger sample [21]. NR and pineapple fruit were reported to have protein content (36.57 ± 0.51–50.74 ± 0.49) % and (13.04 ± 2.03) % respectively [15, 16, 17]. The total solid content of all the dried pineapple fortified rasgulla samples were significantly ($p < 0.05$) increased compared to PP due to the removal of moisture from pineapple fruit pulp.

3.2. Vitamin B group

Vitamin B group refers to a group of water-soluble vitamins. Vitamin B group comprises a total of eight vitamins which are thiamine/vitamin B_1, riboflavin/vitamin B_2, niacin/nicotinic acid/vitamin B_3, pantothenic acid/vitamin B_5, pyridoxine/vitamin B_6, biotin/vitamin B_7, folate/vitamin B_9 and cobalamins/vitamin B_{12}. All of these vitamins are well known for their different functional characteristics to human health such as vitamin B_6 and vitamin B_{12} are essential in the conversion process of homocysteine to methionine which lowers the homocysteine level in the body otherwise it will cause several detrimental effects on health, Niacin helps to lower the cholesterol level and improve blood circulation, pantothenic acid provides better functionality of lipids, vitamin B_1 function as co-enzyme in different carboxylation process, riboflavin functioning as an electron carrier and important part of several co-enzymes such as flavin adenine dinucleotide (FAD) [22, 23, 24]. Here we had identified and quantified a total of seven vitamin B group members namely vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_7 and vitamin B_{12}.

In case of pineapple several studies reported that vitamin B_1 (0.048–0.138 mg/100 g), vitamin B_2 (0.011–0.04 mg/100 g), vitamin B_3 (0.13–0.267 mg/100 g) and vitamin B_6 (0.08–0.110 mg/100 g) are the most predominant among all [25, 26]. In different milk and milk products several studies found the presence of vitamin B_1 (0.02–0.27 mg/100g), vitamin B_2 (0.18–1.4 mg/100 g), vitamin B_3 (0.09–0.07 mg/100 g), vitamin B_5 (0.35–2.7 mg/100 g), vitamin B_6 (0.036–0.2 mg/100 g), vitamin B_7 (0.004–0.024 mg/100 g), vitamin B_{12} (0.0001–0.00043 ml/100 ml) [27, 28]. Though milk is a good source of vitamin B_1, B_2 and B_{12} but it possesses vitamin B_3, B_5, B_6 and B_9 in inadequate amounts [28]. Whereas pineapple is a rich source of vitamin B_3 and B_5, thus pineapple fortification is a potential way to enhance these vitamins (Table 2) for better nutrition [26].

In our observation (Table 2) different amount of thiamine/vitamin B_1 was found in all the rasgulla samples in the following order - PF > PMWC > NR > PP > PH > PMW. Among all the samples NR and PP were found statistically insignificant ($p > 0.05$). Rodríguez *et al.* 2016 reported a decrease in thiamine content for convective drying of pineapple [29]; Alajaji *et al.* 2006 reported a similar kind of result for microwave dried chickpea [24]; Nouri *et al.* 2018 reported a decrease in vitamin B_1 in oven drying, microwave drying of *Spirulina platensis* [30]; Ibrahim *et al.* 2014 reported a significant increase in vitamin B_1 for freeze-drying of camel milk [31]. In different types of thermal treatment, the thiamine degradation process took place by following first-order reaction kinetics and several parameters like temperature, oxygen concentration, pH and oxidation-reduction potential promoted the degradation process, where –CH bridge in between thiazole and pyrimidine was destructed resulting hydrogen sulfide as a reaction product [32].

Riboflavin or vitamin B_2 is a relatively stable vitamin that is less affected by temperature, oxygen concentration, and very much sensitive to light [33]. Significant amount of riboflavin was found (Table 1) in all rasgulla samples in a following order - NR > PMWC > PP > PF > PH > PMW. Fortification of pineapple in rasgulla resulted insignificant ($p > 0.05$) changes in riboflavin amount for PP, PF and PMWC. Bawa *et al.* 2020 reported a decrease in riboflavin for oven drying and microwave drying of house crickets (*Acheta domesticus*) [33]; Alajaji *et al.* 2006 reported a decrease in riboflavin in microwave cooking of chickpea [24]; Kadakal *et al.* 2007 reported a decrease in riboflavin amount in hot air drying of bulgur [34]; Rodríguez *et al.* 2017 reported a decrease in riboflavin content for convective drying of pineapple [29]. The stability of riboflavin is affected mostly by light rather than types of heating treatment or temperature. But if the pH varies in the median range of the acidic-alkaline zone, then with an increase in temperature, the stability of riboflavin hampered to a great extent. Under the presence of light riboflavin transferred to its singlet excited state and triplet excited state by absorbing the light, energy and undergone several photochemical degradations resulted in carboxymethyl flavin, cyclodehydroribo flavin and lumi flavin as a major by-product [35].

Significant amount of vitamin B_3 or niacin was observed (Table 1) in all rasgulla samples in following order - PF > PMWC > PMW > PP > NR > PH. Ibrahim *et al.*, 2014 reported a significant increase in vitamin B_3 for freeze-drying of camel milk [31]; Kadakal *et al.* 2007 reported a decrease in niacin amount in hot air drying of bulgur [34]; Nouri *et al.* 2018 reported a similar trend in vitamin B_3 in oven drying, microwave drying and freeze-drying of *Spirulina platensis* [30].

Vitamin B_5 or pantothenic acid was found least affected by drying temperature. In all the samples of rasgulla pantothenic acid was found (Table 1) in following order - PMWC > PF > PMW > PH > NR > PP. Ibrahim et al., 2014 reported a significant increase in vitamin B_5 for freeze-drying of camel milk [31]. Higher thermal stability of aliphatic

Table 2. HPLC analysis of vitamin profile and carotene profile.

Vitamin	PP	PH	PF	PMW	PMWC	NR
Vitamin B (mg/100 g DB)						
B_1	0.101 ± 0.005	0.093 ± 0.0008	0.221 ± 0.001	0.075 ± 0.0005	0.181 ± 0.0009	0.104 ± 0.002
B_3	0.412 ± 0.009	0.305 ± 0.005	0.806 ± 0.003	0.047 ± 0.0006	0.624 ± 0.005	0.393 ± 0.008
B_6	0.097 ± 0.0009	0.018 ± 0.0001	0.071 ± 0.0006	0.085 ± 0.0003	0.044 ± 0.006	0.096 ± 0.0008
B_5	0.415 ± 0.006	0.547 ± 0.004	0.728 ± 0.0004	0.615 ± 0.002	0.984 ± 0.007	0.472 ± 0.005
B_7	0.025 ± 0.003	0.052 ± 0.0003	0.106 ± 0.0005	0.023 ± 0.0001	0.037 ± 0.0002	0.022 ± 0.0003
B_{12}	0.034 ± 0.004	0.032 ± 0.0001	0.066 ± 0.0007	0.046 ± 0.0008	0.050 ± 0.0001	0.039 ± 0.0001
B_2	0.570 ± 0.002	0.290 ± 0.003	0.571 ± 0.003	0.180 ± 0.006	0.594 ± 0.008	0.737 ± 0.004
Ascorbic acid/Vitamin C (mg/100 g DB)						
C	17.71 ± 1.895	4.69 ± 0.0085	8.6 ± 1.009	5.86 ± 0.0095	11.038 ± 1.013	5.14 ± 0.098
Tocopherol/Vitamin E (mg/100 g DB)						
α	ND	ND	ND	ND	ND	ND
β	ND	ND	0.029 ± 0.002	0.024 ± 0.001	ND	ND
σ	0.225 ± 0.003	0.205 ± 0.004	0.055 ± 0.0008	0.135 ± 0.009	0.153 ± 0.004	0.237 ± 0.001
γ	0.384 ± 0.008	0.409 ± 0.009	0.649 ± 0.005	0.469 ± 0.005	0.549 ± 0.009	0.401 ± 0.006
Vitamin A (mg/100 g)						
A	0.0292 ± 0.0005	0.0275 ± 0.0003	ND	0.0223 ± 0.0004	0.0211 ± 0.0005	0.0363 ± 0.0007
Carotene (mg/100 g DB)						
α	0.004 ± 0.0001	0.006 ± 0.00001	0.00773 ± 0.008	0.00180 ± 0.0005	0.00165 ± 0.0003	0.00101 ± 0.0008
β	0.039 ± 0.0009	0.016 ± 0.0006	0.0310 ± 0.0005	0.044 ± 0.003	0.056 ± 0.002	0.015 ± 0.003
σ	0.00026 ± 0.00002	0.00012 ± 0.00002	0.00087 ± 0.00003	0.00031 ± 0.00007	0.00078 ± 0.00002	0.00033 ± 0.00005
γ	0.00194 ± 0.0002	0.00157 ± 0.0004	0.00683 ± 0.0003	0.00472 ± 0.0002	0.00850 ± 0.0003	0.00076 ± 0.00002

All the results were reported in the above table as mean value ± SD (standard deviation).
ND = Non-detectable.
[PP = Pineapple pulp rasgulla, PH = Hot air dried pineapple rasgulla, PF = Freeze-dried pineapple rasgulla, PMW = Microwave dried pineapple rasgulla, PMWC = Microwave convective dried pineapple rasgulla, NR = Normal rasgulla].

hydrocarbon and the presence of the amide group along with two methyl groups in the aliphatic chain of pantothenic acid resulted in more stability of pantothenic acid under different drying condition. Vitamin B_6, vitamin B_7, and vitamin B_{12} were also found in significant amounts in all the pineapple rasgulla samples. Bawa *et al.* 2020 reported a decrease in vitamin B_{12} for oven drying of house crickets (*Acheta domesticus*) [33]; Alajaji *et al.* 2006 reported a decrease in vitamin B_7 for microwave dried chickpea [21]; Nouri *et al.* 2018 reported a similar trend in vitamin B6 in oven drying, microwave drying and freeze-drying of *Spirulina platensis* [30].

3.3. Vitamin A

Vitamin A refers to a group of the compound that possesses the activity of retinol or any compound that has a similar structure of retinol [36]. Carotenoids also have a significant contribution to vitamin A as pro-vitamin-A activity in the case of plant food, as a plant does not contain any retinoic substances. In animal body after in vivo ingestion, carotenoids and similar types of compounds from different plant sources that have pro-vitamin A activity converted into vitamin A in the intestinal mucosa through a complex pathway [37].

In this study, we observed different content of vitamin A in all samples except PF where vitamin A activity was not detected (Table 2). Pineapple fortification in normal rasgulla insignificantly ($p > 0.05$) affected vitamin A content of different pineapple rasgulla samples. Plant food such as pineapple was not reported to have any vitamin A activity, though it possesses pro-vitamin A activity derived through different carotenoids, which after consumption converted to vitamin A inside the animal body system. In all the rasgulla samples a significant amount of vitamin A was contributed through channa only as cow milk and milk products were reported to have a significant amount of vitamin A activity in a different study [38]. Vitamin A in cow milk was found in the form of retinol, retinyl esters and vitamin A amount ranging from (0.01–0.1) mg/100 g [39]. Hulshof *et al.* 2006 reported a different amount of retinol in raw milk, cheese and butter [40].

3.4. Vitamin C

Vitamin C or L-ascorbic acid has immense importance in human nutrition for its crucial role in human physiology and body metabolism. Vitamin C has possessed several beneficial roles in the human body such as it can act as a strong anti-oxidant agent that can prevent lipid peroxidation, it can act as a strong free radical scavenging agent that can remove different types of the harmful free radical present in the human body, and it can also act as pro-oxidant agent.

Tropical fruit such as pineapple (*Ananas comosus*) is well known for its excellent nutritional qualities. Pineapple is a rich source of vitamin c along with other micro-nutrient as well. With respect to different cultivar and environmental conditions, vitamin c content of fresh pineapple fruit varies in a range of 5.08 mg/100 g to 33.57 mg/100 g [41]. In milk ascorbic acid or vitamin C is present as one of the main water-soluble anti-oxidant which ranging from 1.65 mg/100 g to 2.75 mg/100 g [42]. Therefore, pineapple possesses 3 to 12-fold higher vitamin C content than that of milk. Thus, pineapple fortification will increase the ascorbic acid content of rasgulla significantly.

In our study, we observed (Table 3) that fortification of pineapple in NR successfully caused a significant increase in vitamin C content in different pineapple rasgulla. The highest ascorbic acid or vitamin C content resulted in PP. Due to the use of original pineapple fruit pulp without any treatment, the highest retention of vitamin C activity in PP was observed. Vitamin C or ascorbic acid is the most thermo-sensitive bioactive compound in plants and very susceptible to degrade under the influence of different parameters among which temperature, moisture content, and oxygen concentration are considered as the most

Table 3. HPLC analysis of organic acid profile.

(mg/100 g DB)	PP	PH	PF	PMW	PMWC	NR
Oxalic acid (OA)	22.28 ± 1.89	11.32 ± 0.78	15.39 ± 0.96	8.12 ± 0.25	9.22 ± 0.36	11.94 ± 0.85
Tartaric acid (TA)	1.19 ± 0.005	0.68 ± 0.003	0.77 ± 0.03	1.05 ± 0.05	0.39 ± 0.003	0.47 ± 0.005
Mallic acid (MA)	9.78 ± 0.98	4.82 ± 0.03	6.95 ± 0.09	10.09 ± 0.68	6.37 ± 0.13	0.89 ± 0.008
Lactic acid (LA)	199.1 ± 10.36	33.12 ± 1.65	130.8 ± 11.32	117.8 ± 5.36	84.72 ± 3.96	105.3 ± 4.35
Acetic acid (AA)	105 ± 9.89	37.36 ± 2.36	120.9 ± 7.65	35.74 ± 2.39	87.47 ± 4.02	ND
Citric acid (CA)	160.2 ± 8.96	130.5 ± 7.98	ND	74.56 ± 3.15	114.6 ± 4.96	38.63 ± 1.85
Propionic acid (PA)	17.99 ± 1.03	32.25 ± 2.35	81.21 ± 3.36	53.66 ± 2.03	ND	20.38 ± 1.06
Sinapic acid (SA)	2.026 ± 0.58	0.38 ± 0.005	1.18 ± 0.03	0.33 ± 0.001	5.22 ± 0.05	0.213 ± 0.002
Fumaric acid (FA)	ND	28.17 ± 1.01	4.69 ± 0.65	33.3 ± 1.65	ND	3.37 ± 0.85

All the results were reported in the above table as mean value ± SD (standard deviation).
ND = Non-detectable.
[PP = Pineapple pulp rasgulla, PH = Hot air dried pineapple rasgulla, PF = Freeze-dried pineapple rasgulla, PMW = Microwave dried pineapple rasgulla, PMWC = Microwave convective dried pineapple rasgulla, NR = Normal rasgulla].

important parameter [43]. Due to prolonged drying time and exposure to hot air caused the lowest vitamin C activity in PH (4.69 mg/100 g DB). Santos *et al.* 2008 also suggested the same reason for lower vitamin C activity in case of hot air drying of green bell peppers [44]. Ramallo *et al.* 2004 reported that degradation of vitamin C in pineapple followed first-order reaction kinetics where under aerobic condition first ascorbic acid converts into dehydro-ascorbic acid via a reversible reaction pathway and then further hydrolysis and oxidation took place in an irreversible way [45], followed by PH, PMW (5.86 mg/100 g DB) also resulted in low vitamin C activity compared to PP. Here in the case of PMW, rather than drying temperature, drying time along with microwave power is the main crucial parameter which caused severe damage to vitamin C activity in pineapple [46]. In the case of PF (8.6 mg/100 g DB) the vitamin C activity is comparatively higher than PH and PMW. Lower drying temperature and absence of liquid water caused lower degradation of vitamin C activity in PF [47]. Saini *et al.* 2014 also reported a similar trend of vitamin C or ascorbic acid activity in the case of hot air, freeze and microwave dried *Moringa oleifera* [48]. Pineapple fortification caused insignificant ($p > 0.05$) changes in ascorbic acid values in PH and PMW. In our study, we observed the highest retention of vitamin C activity in PMWC (11.04 mg/100 g DB) with respect to PP. The lowest drying time along with microwave- convective dual treatment resulted in the lowest degradation of vitamin C activity in PMWC. Pham *et al.* 2018 also reported a similar result for microwave convective dried kiwi fruit [49].

3.5. Vitamin E

Vitamin E is a fat-soluble vitamin and contains four types of tocopherols - α, β, σ and γ tocopherols [50]. All four tocopherols found in different types of plants in different concentrations contribute significantly to total vitamin E activity. Vitamin E is well known for its impressive antioxidant activity that can successfully inhibit the lipid peroxidation, can effectively remove the free radicals, and plays an important role in preventing several chronic diseases [42, 51]. Vitamin E content in pineapple is quite low and it was varied in a range of 0.019 mg/100 g to 0.09 mg/100 g with respect to different cultivar [50]. In milk, vitamin E content was reported in a range of 0.2 mg/lit to 0.7 mg/lit [52]. In this study vitamin E activity of different rasgulla was estimated.

From the result (Table 2) it was observed that the highest vitamin E activity was found in PF and for all the samples vitamin E activity was found as follows – PF > PMWC > PMW > PH > PP > NR. The following trend is a clear indication that pineapple fortification in normal rasgulla caused an increase in vitamin E activity in all types of pineapple rasgulla. In PF the vitamin E activity was increased by 20.23 % compared to NR and this may be due to the loss of effectiveness of tocopherol oxidase enzyme at low water activity which is the key player in the degradation process of vitamin E activity. Also in the case of freeze-drying decrease in the water content caused an increase in solid content thus increased the vitamin E activity. Knecht *et al.* 2015 reported an increase in vitamin E activity in freeze-dried broccoli [53]. PMWC, PMW and PH also caused an increase of vitamin E activity by 15.36 %, 4.72 % and 3.08 % respectively. The changes in vitamin E activity of PH and PMW, PF and PMWC were found statistically insignificant ($p > 0.05$). Saini et al., 2014 reported that under different drying environments stability of α-tocopherols was much higher compared to other vitamins [48]. Miranda et al. 2010 found that in the case of hot air drying vitamin E activity of quinoa seeds was rapidly increased at 80 °C compared to fresh samples [54]. Casal *et al.* 2006 found that microwave treatment of stingless bee (*Tetragonula biroi*) pollens caused a significant increase in vitamin E activity [55]. Tocopherols were reported to scavenge peroxide radicals by forming tocopheroxyl radical and at higher temperature through reacting with other antioxidants this tocopheroxyl radical may be added back to tocopherol form, thus caused an enhancement in tocopherol content for PH, PMWC and PMW [56]. In the case of PH at a higher temperature, Vitamin E that was conjugated to protein or phospholipid may be released due to structural breakdown at a higher temperature and caused enhancement of tocopherol content [55]. Insignificant ($p > 0.05$) changes in vitamin E activity was observed in the case of PP and NR. σ tocopherol is a potential free radical scavenger and has the ability to protect the living cell against damage due to oxidative degradation [55]. Pineapple fortification significantly increases ($p < 0.05$) the β and σ tocopherol content in rasgulla, both of this tocopherol were possessed by milk in much lower quantity.

3.6. Carotene profile

Carotenes are fat-soluble color pigments that are comprising of four sub-groups – α, β, σ and γ carotenes. Amongst all, β-carotene is predominant and both α and β-carotene possess pro-vitamin A activity [57]. Different carotenoids have shown different effective actions against several diseases like cardiovascular problems, cataract problems [22]. β -Carotene is also reported to have pro-oxidant and anti-oxidant activity against lipid peroxidation [58].

In our study, it was found that a significant amount of - α, β, γ, and δ carotenes were present in all types of rasgulla. Among all carotenes, though β-Carotene was found predominant, other carotenes also had a significant contribution to the carotene content of all types of rasgulla. In our study we have found carotene content for all types of rasgulla in the following order – PF > PMW > PMWC > PP > PH > NR (Table 2). Fortification of pineapple in all rasgulla samples significantly ($p < 0.05$) increased the β carotene content. In different types of drying processes, the solid content of the particular product was increased as a result of moisture loss. Due to heat treatment, the different bioactive compounds were also degraded but with respect to the increment in total solid content their amount increased up to a certain extent. In this study due to this phenomenon, though all the carotenes were degraded due to heat treatment, their amount was found increased with respect to PP. For the same reason Incedayi *et al.*, 2016 reported a 1.9–3.4-fold increase in β-carotene of dried apricots compared to fresh apricots [59]. Similar to our result Incedayi *et al.*, 2016 also reported higher β-carotene value for microwave -convective dried apricot compared to microwave and hot air-dried apricot. Gao et al., 2012 reported an increase in β-Carotene content of freeze-dried Jujubes compared to fresh samples [60]. β-carotene is mainly degraded through oxidation either by diradical or singlet oxygen molecule and also degradation occurred due to the isomerization process took place at high temperature [61]. In PH the carotene content came least due to the aerobic condition in prolonged hot-air drying treatment. Hiranvarachat *et al.* 2008 reported much less value of β-carotene in hot air drying compared to other drying treatments for carrots [61]. Though pineapple was reported to have carotene content in a range of 0.0252–0.497 mg/100g in a few studies [39, 62], other studies reported specifically the presence of α and β-carotene in pineapple. According to several studies, α and β-carotene of pineapple fruit were found in a range of 0–0.2 mg/100 g and 0.08–0.31 mg/100 g respectively [63, 64]. In different cow and cow milk products β -Carotene (0.007–0.43 mg/100 g) was found in a much higher amount than α-carotene (0–0.002 mg/100 g) [40].

3.7. Organic acids

Quantification and identification of organic acids present in a particular food product are acknowledged as a very important parameter due to its crucial interference with the quality, stability, and nutritional profile of the final product [60]. Also, all these organic acids have several beneficial effects on human health and metabolism like - fumaric acid is found effective in the prevention of cardiovascular diseases, citric acid may be helpful in the protection against diabetics, malic acid may act as an anti-microbial agent against different harmful microorganism [65].

In accordance with our result, several studies also reported that the main predominant organic acid in pineapple fruit is citric acid [66] and in the case of milk products citric acid and lactic are found major compared to other organic acids [67]. In our study (Table 3) we found that the highest and lowest amount of citric acid was achieved in PP and NR respectively, while a significant decrease in citric acid value was observed in the rest of the samples. Fortification of pineapple in normal rasgulla caused a significant increase in the citric acid amount in all the pineapple fortified samples. Compared to PP 82.37 %, 76.85 %, 72.58 % and 47.23 % retention of the organic acid amount were found in PH, PF, PMWC and PMW respectively. Gao *et al.* 2012 also reported similar trends of citric acid in differently dried Jujubes [60]. Both in PP and NR the maximum amount of organic acid was found in terms of lactic acid compared to other organic acids present in it. Due to the channa making process with lactic acid or calcium lactate and the presence of lactic acid bacteria in milk may be th reason for the highest amount of lactic acid in NR. Similar to citric acid, significant ($p < 0.05$) changes in lactic acid amount were also observed in all the rasgulla samples. With respect to PP 16.35 %, 65.72 %, 61.17 % and 43.64 % retention of the lactic acid was observed in PH, PF, PMW and PMWC respectively.

The third predominant organic acid found in PP was acetic acid, while in NR it was not detectable. Barretto *et al.* 2013 also reported the presence of acetic acid in pineapple fruit [68]. Though compared to PP significant decrease of acetic acid was observed in PH, PMW and PMWC, in PF the acetic acid amount was increased by 11.6 %. Adamczak *et al.* 2009 reported that freeze-drying resulted in stabilizing the organic acid content compared to other thermal treatments in European cranberry [69].

Oxalic acid was the fourth predominant organic acid found in PP and unlike acetic acid, it was found in NR. Different thermal treatment also resulted in the decrease of oxalic acid content in the following order – PF > PH > PMWC > PMW and from this trend it is clear that the higher the thermal treatment higher the decrease in oxalic acid content.

In the case of propionic acid different thermal treatment caused a significant increase of the acid amount with respect to PP in the following order- PF > PMW > PH while in PMWC it was found non-detectable. The increase in organic acid content may be due to the reaction of the nitrogen-free carboxylic acid with sugar present in fruit [65].

With respect to PP, a decrease in the amount of sinapic acid, tartaric acid and malic acid were observed for all the samples. In the case of sinapic acid the changes in the amount of all the samples were found significant while for malic and tartaric acid insignificant ($p > 0.05$) changes were observed in PP and PMW. During different types of drying: dehydration, decarboxylation and Maillard reactions – all these three facts may be responsible for the decrease of most of the organic acids.

3.8. Multivariate analysis

For all the rasgulla samples, to find out the different interference in between all the studied parameters namely nutritional profile (carbohydrate, ash, protein, total solids and moisture content), vitamins (A, C, tocopherols, B_1, B_2, B_3, B_5, B_6, B_7, and B_{12}), organic acids and carotene profile principal component analysis (PCA) and hierarchical cluster analysis (HCA) analysis with heat map were done. In the PCA analysis (Figure 2) it was seen that the principal component (PC) 1 and principal component (PC) 2 were accounted for 87.73 % of total variance and if we add a principal component (PC) 3 then all total they were accounted for 94.34 % of the total variance. From the Figure 1, it was seen that a major and relatively bigger cluster was formed in between PMW-PH-NR-PMWC and all together they shared the maximum number of parameters such as - vitamins (A, B_1, B_2, B_3, B_5, B_6, B_7, and B_{12}), tocopherols (α, β, σ, and γ), carotene (α, β, σ, and γ), carbohydrate, fat, protein, moisture content, total solids, fat. To study the inter-correlation of the results obtained from

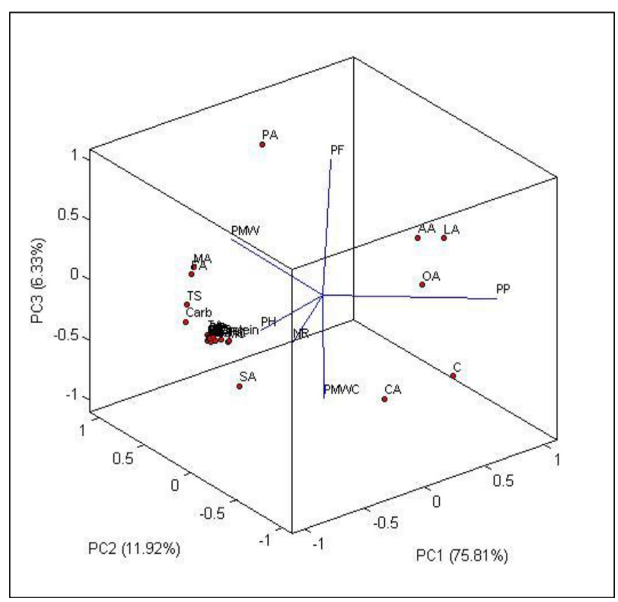

Figure 2. Tri-plot of different parameters** of all the rasgulla samples*. *[NR = Normal rasgulla, PP = normal pineapple pulp rasgulla, PH = hot air dried pineapple pulp rasgulla, PF = freeze dried pineapple pulp rasgulla, PMW = microwave dried pineapple pulp rasgulla, PMWC = microwave-convective dried pineapple pulp rasgulla.]. **[A = Vitamin A, TP = Tocopherol, CA = Citric acid, MA = Malic acid, TA = Tartaric acid, B6 = Vitamin B_6, LA = Lactic acid, OA = Oxalic acid, C = Vitamin C, AA = Ascorbic acid, BC = Beta carotene, B_7 = Vitamin B_7, PA = Propionic acid, FA = Fumaric acid, SA = Sinapic acid, B5 = Vitamin B_5, B12 = Vitamin B_{12}, B1 = Vitamin B_1, B3 = Vitamin = B_3, B2 = Vitamin B_2, Carbohydrate, Fat, Protein, Ash, MC = Moisture content, Total Solids].

chemical analysis, singular value decomposition and eigen-decomposition were carried out, which in turn provide orthogonal variables or PC with dimension reduction but with the retention of maximum variance within the data set. Malic acid, vitamin A, carbohydrate content and total solid content clustered together and positioned in close proximity with PMW, implied that these properties were in coherence with the PMW sample; similarly, all carotenes, tocopherols, vitamin B group, fat, protein ash, moisture content and fumaric acid formed the largest cluster and situated along with PH and NR samples, which implicit the stronger association of these inherent attributes of rasgulla sample with PH and NR. Vitamin C, oxalic acid and lactic acid clustered together and placed them closer to PP, which describe the strong connotation of these features with the PP sample. In the HCA analysis with a heat map (Figure 3) it was observed that horizontally four main clusters were formed in between - NR, PP, PH, PF, PMW, and PMWC. Depending on the correlation values and association with the experimental parameters it was obvious that PF and PMWC clustered together; which substantiate the experimental results that PF and PMWC were close enough in terms of vitamin B_1, B_3, vitamin C, γ tocopherol, σ and γ carotene content (Table 2). PMW and PH were more correlated in terms of analyzed parameters like acetic acid, fumaric acid, propionic acid and sinapic acid (Table 3), thus clustered together. NR clustered alone which could be explained through the distinguishing features (nutritional, vitamin profile, organic acid profile and carotene profile) possessed by NR. Due to fortification with raw pulp only PP also possessed a separate cluster. According to the heat map, the darker coloured attributes stand for a higher correlation value (>0.5). The heat map analysis was in accordance with the cluster formation characteristics observed in the PCA. Vertically, all the studied properties (vitamins, organics acids, carotene profile) formed three major clusters. The linear co-relationship for the observed parameters was in agreement with the classification behavior obtained from HCA. Though it was the first kind of approach to study the interrelationship within the nutritional, vitamin, carotene and organic acid profile of fortified dairy based sweetmeat products through multivariate analysis; further investigations may explore the chemical associations and product characteristics in the field of food fortification.

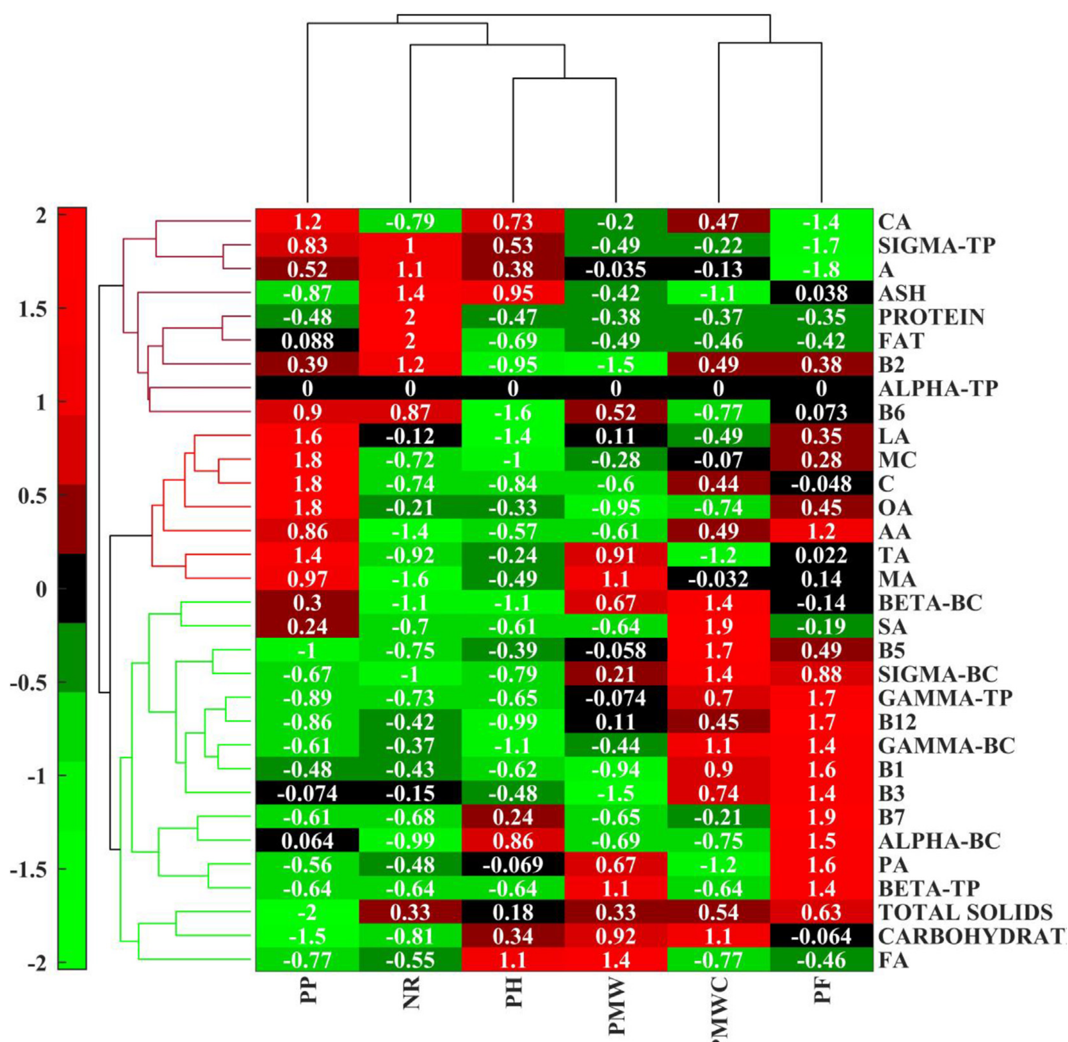

Figure 3. Hierarchical cluster analysis with heat map for different parameters** of all the rasgulla samples*. *[NR = Normal rasgulla, PP = normal pineapple pulp rasgulla, PH = hot air dried pineapple pulp rasgulla, PF = freeze dried pineapple pulp rasgulla, PMW = microwave dried pineapple pulp rasgulla, PMWC = microwave-convective dried pineapple pulp rasgulla.]. **[A = Vitamin A, TP = Tocopherol, CA = Citric acid, MA = Malic acid, TA = Tartaric acid, B6 = Vitamin B_6, LA = Lactic acid, OA = Oxalic acid, C = Vitamin C, AA = Ascorbic acid, BC = Beta carotene, B_7 = Vitamin B_7, PA = Propionic acid, FA = Fumaric acid, SA = Sinapic acid, B5 = Vitamin B_5, B12 = Vitamin B_{12}, B1 = Vitamin B_1, B3 = Vitamin = B_3, B2 = Vitamin B_2, Carbohydrate, Fat, Protein, Ash, MC = Moisture content, Total Solids].

4. Conclusions

From all the discussions it is concluded that the amount of vitamins, organic acid and carotene significantly increased in pineapple fortified different rasgulla samples. In the case of vitamin B groups, B_1, B_6, B_5, B_3 were found maximum in PF, PP, PMWC, PF respectively whereas B_2 was found maximum in NR. Vitamin C was found maximum in PP and the amount was 3 fold higher than NR. But in the case of Vitamin A, the maximum result was obtained for NR. In the case of tocopherol and carotene content, the highest amount was found in PF for both the cases. Therefore it can e concluded from the overall results, PF was found better compared to other types of pineapple fortified rasgulla samples.

Declarations

Author contribution statement

Tanmay Sarkar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Molla Salauddin: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sudipta Kumar Hazra: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Runu Chakraborty: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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