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)

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ARTICLE INFO

Keywords:
Food analysis
Food technology
Fortification
Functional dairy products
Nutritional profile
Dairy rasgulla
Vitamins
Organic acid profile
Pineapple

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 (B1, B2, B3, B5, B6, B7 and B12), 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 fortifed rasgulla samples in terms of vitamins content, organic acid and carotene content. B1, B2, B7 and B12 are found maximum in PP whereas B6, B5 and B2 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/rosgolla/ 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 -

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https://doi.org/10.1016/j.heliyon.2020.e05233
Received 18 June 2020; Received in revised form 12 July 2020; Accepted 8 October 2020

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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, 600W, 95 min) of pineapple was done by using a microwave oven (Sungam, Combi CEI031LAT, 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 (Sungam, Combi CEI031LAT, 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.

### 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.

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.

### HPLC analysis of vitamin B group

25 ml of (0.1 N) H2SO4 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 a 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 (Whatman 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 C18 column having a 4.6 × 150 mm2 internal diameter with a pore size of 3.5 μm. The H2PO4- 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].
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 (Na2SO4) 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 C18 column having a 4.6 × 150 mm² 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. 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
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]; García-Amezquita et al., 2018 reported a higher amount of protein content in freeze dried pear-pear 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 differentially 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 B1, riboflavin/vitamin B2, niacin/nicotinic acid/vitamin B3, pantothenic acid/vitamin B5, pyridoxine/vitamin B6, biotin/vitamin B7, folate/vitamin B9 and cobalamin/vitamin B12. All of these vitamins are well known for their different functional characteristics to human health such as vitamin B6 and vitamin B12 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. Nicacin helps to lower the cholesterol level and improve blood circulation, pantothenic acid provides better functionality of lipids, vitamin B1 function as co-enzyme in different carboxylation process, riboflavin functioning as an electron carrier and important part of several coenzymes 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 B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B9 and vitamin B12.

In case of pineapple several studies reported that vitamin B1 (0.048–0.138 mg/100 g), vitamin B2 (0.011–0.04 mg/100 g), vitamin B3 (0.13–0.267 mg/100 g) and vitamin B6 (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 B1 (0.02–0.27 mg/100g), vitamin B2 (0.18–1.4 mg/100 g), vitamin B3 (0.09–0.07 mg/100 g), vitamin B6 (0.35–2.7 mg/100 g), vitamin B9 (0.036–0.2 mg/100 g), vitamin B12 (0.004–0.24 mg/100 g), vitamin B12 (0.0001–0.00043 ml/100 ml) [27, 28]. Though milk is a good source of vitamin B1, B2 and B12 but it possesses vitamin B3, B5, B9 and B6 in inadequate amounts [28]. Whereas pineapple is a rich source of vitamin B3 and B5, 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 B1 was found in all the rasgulla samples in the following order - PP > 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]; Noura et al. 2018 reported a decrease in vitamin B1 in oven drying, microwave drying of Spirulina platensis [30]; Ibrahim et al. 2014 reported a significant increase in vitamin B1 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 B2 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 > PH > PMW. Fortification of pineapple in rasgulla resulted insignificant (p > 0.05) changes in riboflavin amount for PP, PP 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, cyclohydroriboflavin and lumi flavin as a major by-product [35].

Significant amount of vitamin B3 or niacin was observed (Table 1) in all rasgulla samples in following order - PP > PMWC > PMW > PP > NR > PH. Ibrahim et al., 2014 reported a significant increase in vitamin B3 for freeze-drying of camel milk [31]; Kadakal et al. 2007 reported a decrease in niacin amount in hot air drying of bulgur [34]; Noura et al. 2018 reported a similar trend in vitamin B3 in oven drying, microwave drying and freeze-drying of Spirulina platensis [30].

Vitamin B5 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 > PP > PMW > NR > PP. Ibrahim et al., 2014 reported a significant increase in vitamin B5 for freeze-drying of camel milk [31]. Higher thermal stability of aliphatic

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**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 ± 1.18 | 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 ± 0.29 | 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].**
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Table 2. HPLC analysis of vitamin profile and carotene profile.

| Vitamin | PP (mg/100 g DB) | PH (mg/100 g DB) | PF (mg/100 g DB) | PMW (mg/100 g DB) | PMWC (mg/100 g DB) | NR (mg/100 g DB) |
|---------|------------------|------------------|------------------|-------------------|------------------|------------------|
| A       | 0.0292 ± 0.0005  | 0.0275 ± 0.0003  | ND               | 0.0223 ± 0.0004  | 0.0211 ± 0.0005  | 0.0363 ± 0.0007  |
| Carotene | 0.004 ± 0.0001  | 0.006 ± 0.0001  | ND               | 0.00773 ± 0.0008 | 0.00190 ± 0.0005 | 0.00165 ± 0.0003 |
| β       | 0.029 ± 0.0009  | 0.016 ± 0.0006  | ND               | 0.0310 ± 0.0005  | 0.044 ± 0.0003   | 0.056 ± 0.0002   |
| δ       | 0.00026 ± 0.00002 | 0.00012 ± 0.00002 | ND               | 0.00087 ± 0.00003 | 0.00031 ± 0.00007 | 0.000076 ± 0.00002 |
| γ       | 0.00194 ± 0.0002 | 0.00157 ± 0.0004 | ND               | 0.00683 ± 0.0003 | 0.00472 ± 0.0002 | 0.00850 ± 0.0003 |

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 B6, vitamin B7, and vitamin B12 were also found in significant amounts in all the pineapple rasgulla samples. Bawa et al. 2020 reported a decrease in vitamin B3 for oven drying of house crickets (Acheta domestica) [33]; Alajaji et al. 2006 reported a decrease in vitamin B2 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 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 PP 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 2) 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
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 PP (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 – PP > 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-tocopherol 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 single bee (Tetragonula biori) pollens caused a significant increase in vitamin E activity [55]. Tocopherols were reported to scavenge peroxide radicals by forming tocopheroxy radical and at higher temperature through reacting with other antioxidants this tocopheroxy 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

Carotenoids are fat-soluble color pigments that are comprising of four sub-groups – α, β, σ and γ carotenes. Amongst all, β-carotene is predominate 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 – PP > PMW > PMWC > 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

### 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 |
| Malic acid (MA) | 9.78 ± 0.98  | 4.82 ± 0.03  | 6.95 ± 0.09  | 10.09 ± 0.68 | 3.67 ± 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 ± 0.89 | 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 | 53.63 ± 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]
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 influence 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 a main predominant organic acid in pineapple fruit is citric acid [66] and sinapic acid the changes in the amount of all the samples were found significant for all the thermal treatments in European cranberry [69].

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 lactate 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 increase of the acid amount with respect to PP in the following order - PF > PMW > PH while in PMW 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, B1, B2, B3, B6, B9, B12), organic acids and caroten 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, B1, B2, B3, B6, B9, B12), 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 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 PMW 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].

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 B9, LA = Lactic acid, OA = Oxalic acid, C = Vitamin C, AA = Ascorbic acid, BC = Beta carotene, B7 = Vitamin B7, PA = Propionic acid, FA = Fumaric acid, SA = Sinapic acid, BS = Vitamin B9, B12 = Vitamin B12, B1 = Vitamin B1, B3 = Vitamin B3, B2 = Vitamin B2, 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 carotenoids, 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 B1, B3, vitamin C, γ tocopherol, α 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 B6, LA = Lactic acid, OA = Oxalic acid, C = Vitamin C, AA = Ascorbic acid, BC = Beta carotene, B9 = Vitamin B9, PA = Propionic acid, FA = Fumaric acid, SA = Sinapic acid, B5 = Vitamin B5, B12 = Vitamin B12, B1 = Vitamin B1, B3 = Vitamin B3, B2 = Vitamin B2, 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, B1, B6, B9, B3, were found maximum in PP, PF, PMWC, PF respectively whereas B2 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; Contributed reagents, materials, analysis tools or data. 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.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We would like to thank Dr. Ramdhan Majhi (MDLC facility, Indian Institute of Chemical Biology) for his continuous support and guidance in HPLC analysis.

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