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Formulation of fortified burfi by using shredded bottle gourd (*Lageneria siceraria*), carrot (*Daucus carota*) and beetroot (Beta vulgaris) for its antioxidant potential

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Abstract

This investigation examined the influence of carrot or beetroot incorporation on the antioxidant properties of traditional bottle burfi. The objective of research is to investigate the antioxidant attributes of burfi prepared by fortifying bottle gourd burfi in different proportions (15, 25, 30 and 35) with carrot (10,15.20,25) and beetroot (10, 15, 20,25). Considering, the nutritional and therapeutic properties a nutrient rich burfi was developed by combining varied proportion of bottle gourd, carrot, beetroot and sugar leveraging the nutritional and therapeutic benefits of carrot and beetroot. 70% khoa and 30% sugar were formulated to prepare control sample T₀. The experimentally developed Burfi was prepared with 14 different proportions of bottle gourd, carrot, beetroot, sugar and khoa i.e. 25:10:00:30:35 (T₁), 25:00:10:30:35 (T₂), 30:10:00:25:35 (T₃), 30:00:10:25:35 (T₄), 35:10:00:20:35 (T₅), 35:00:10:20:35 (T_6) , 15:20:00:30:35 (T_7) , 15:00:20:30:35 (T_8) , 15:25:00:25:35 (T_9) , 15:00:25:25:35 (T_{10}) , 25:20:00:20:35 (T₁₁), 25:00:20:20:35 (T₁₂), 25:15:00:25:35 (T₁₃), 25:00:15:25:35 (T₁₄). Antioxidant properties in food is determined by its type and amount of bioactive compounds such as polyphenols, flavonoids and carotenoids. The experimentally developed burfi are an excellent source of beta carotene and vitamin C. Among the different treatments the Treatment T₅ (35:10:00:20:35) ratio of bottle gourd, beetroot sugar and khoa analysis showed the highest vitamin C and Treatment T9 (15:25:00:25:35) showed highest beta carotene. It was concluded that Treatment T₅ and Treatment T₉ resulted in highest antioxidant activity due to highest beta carotene and Vitamin C content. Result was also verified by t-test at a significance level of p<0.05.

 $\textbf{Keywords:} \ \text{Burfi, antioxidants, nutritional properties, the rapeutic, for tification}$

Introduction

Antioxidants play a crucial role in protecting biological systems by stabilizing free radicals through electron donation, thereby preventing oxidative stress and damage [12]. Oxidative stress is often linked to the presence of free radicals, which are unstable molecules formed through chemical reactions involving oxygen. These reactive molecules can pose a threat to cellular health by interacting with vital cell components, including genetic materials, protein and membrane structures [1]. Bottle gourd (Lagenaria siceraria) belongs to Cucurbitaceae family consisting of 825 species and 118 genera. Studies have shown that Bottle gourd seed extracts exhibit high antioxidant activity [1]. Research also showed that on comparing antioxidant properties of Bottle gourd pulp powder and Whole Bottle gourd powder later showed highest amount of antioxidants concluding that Bottle gourd and its processed products are beneficial for healthy life [2]. Carrots (Daucus carota) are a nutrient dense root vegetable that exhibit diverse array of colours, each within its unique characteristics. They are an excellent source of phytochemicals including flavonoids, carotenoids and polyacetylenes, which contribute to their nutritional value. The carotenoids, polyphenols and vitamins present in carrots exhibit a range of beneficial properties, including antioxidant, anticancer and immune boosting side effects. The biological and therapeutic benefits of carrots can be attributed to their high levels of antioxidants, particularly carotenoids with

beta carotene being a key contributor ^[15]. Beetroot (Beta vulgaris) is a nutrient dense vegetable that offers a wealth of inorganic micronutrients, phytoproteins, dietary fibre and bioactive compounds. The unique combination of these nutrients in beetroot makes it an excellent food for addressing numerous health concerns and micronutrient inadequacies ^[1]. Beetroot vibrant coloration is due to unique combination of bioactive compounds, including carotenoids, betalains (Betacyanin and betaxanthin). These compounds have been shown to possess a range of beneficial properties including antiviral, antioxidant, and antibacterial effects, making beetroot a valuable addition to a healthy diet ^[14]. Burfi is a cherished sweet delicacy made from khoa, a

concentrated milk product. Khoa is prepared by slowly evaporating milk in a wide pan, resulting in a semi solid consistency. This khoa is then used to create the delicious and popular dessert known as burfi [11]. The market offers a wide range of burfi varieties incorporating ingredients like fruits, cashews, almonds and many more. Due to its widespread popularity and appeal across India, innovative research has led to the development of numerous burfi types with various ingredients and flavors. Researchers continue to experiment with new ingredients and flavors, pushing the boundaries of this beloved Indian dessert. This study was aimed to focus on developing a nutrient dense Burfi with enhanced antioxidant properties, leveraging the functional benefits of Bottle gourd, Carrot and Beetroot. The main objective was to assess the impact of above mentioned ingredients on Burfi quality characteristics, including its physiochemical and antioxidant profiles.

Material and Methods Raw Materials

The study utilized fresh vegetables Bottle gourd, Carrot and Beetroot from local vendors in Prayagraj. Milk, ghee and sugar were source from local Departmental Store in Naini, Prayagraj. The entire investigation was conducted in Warner College of Dairy Technology (WCDT), Department of Food Science and Technology, SHUATS, Prayagraj. The selected vegetables subjected to rigorous quality control ensuring they were fresh, mature and disease free. Following cleaning, peeling and shredding the vegetables were uniformly cooked and incorporated into traditional koa burfi formulations. This investigation aimed to coparatively evaluate the physiochemical properties and potential health benefits of burfi variants enriched with beetroot and carrot.

Production of Experimental Burfi

The milk was preheated to 35-40 °C and filtered through a clean muslin cloth. It was then continuously heated on a flame until it reached semi thick consistency. For the control sample 70 g of khoa was measured and added to pan with ghee, scraped continuously over low heat until the colour slightly changed. Sugar (30 g) was added and cooked until uniformly mixed. The mixture was transferred to a tray, set and cut into bars (2*4 cm). The fresh and disease free vegetables were washed, peeled and shredded. The shredded vegetables were weighed according to the treatment composition T_1 , T_2 , T_3 , T_4 , T_5 , T_6 , T_7 , T_8 , T_9 , T_{10} , T_{11} , T_{12} , T_{13} and T_{14} . Sugar was added to each treatment as per the formulation, and the mixture was further heated until solidification. The mixture was then transferred to trays,

cooled and cut into rectangular bars (2*4 cm), which were stored at room temperature.

Table 1: Composition of Experimentally Developed Burfi by incorporating Bottlegourd (*Lagenaria siceraria*), Carrot (*Daucus carota*) and Beetroot (*Beta vulgaris*).

Treatment	Bottle gourd	Carrot	Beetroot	Sugar	Khoa
T_0				30	70
T_1	25	10	00	30	35
T ₂	25	00	10	30	35
T ₃	30	10	00	25	35
T ₄	30	00	10	25	35
T ₅	35	10	00	20	35
T_6	35	00	10	20	35
T ₇	15	20	00	30	35
T ₈	15	00	20	30	35
T ₉	15	25	00	25	35
T_{10}	15	00	25	25	35
T ₁₁	25	20	00	20	35
T ₁₂	25	00	20	20	35
T ₁₃	25	15	00	25	35
T ₁₄	25	00	15	25	35

Chemical Evaluation

The sample of finished product obtained from various treatment combinations were chemically analysed for free radical scavenging capacity and beta carotene.

Determination of beta carotene

The experimental procedure involved mixing approximately 5g of sample with 2.5 ml of DMSO in individual test tubes. The test tubes were subjected to vortexing for 30 seconds to ensure thorough mixing. Subsequently the tubes were incubated in water bath at 50 °C for a period of 30 minutes. During the incubation the tubes were removed after 10 minutes, vortexed for additional 10 seconds and then returned to water bath. Following centrifugation at 4200rpm for 3 minutes, the supernatant was carefully collected using a dropper and transferred to funnel. A glass piece was coated with methanol by pipetting a sufficient amount to cover it. The tube was sealed, vortexed for 30 seconds and after removing the top layer, 4ml of methanol was added. The tube was sealed and subjected to multiple 30 seconds vortexing cycle. The methanol solution developed a color due to the presence of beta carotene. The supernatant was collected and diluted to 25mL with methanol, followed by centrifugation as needed. An 8 ml aliquot of methanol extract was then transferred to funnel and subsequently poured into a 15ml centrifuge tube. Finally 1.5 ml of saturated sodium hydroxide and 5ml of heptane were added to the methanol mixture and the tube was sealed. The saponification reaction proceeded for 15 minutes in darkness, followed by gentle tube inversion. This process was repeated after 30 minutes. Subsequent to vortexing (15 seconds) and centrifugation (4200 RPM, 3 minutes) the heptane layer was carefully aspirated and transferred to a 10ml cylinder. The interface was then washed with 1 ml of fresh heptane, and the tube was sealed and gently agitated (8 times). After phase separation (2 minutes) the heptane layer was collected and added to cylinder. A 5 ml of aliquot of heptane extract was then mixed with water, vortexed briefly and centrifuged (4200 RPM, 3 minutes) [7].

Table 2: The burfi prepared under various treatment were analysed for beta carotene of different level of incorporation of bottle gourd, carrot and beetroot presented in Table 1.

Treatments BG:C:BR: Sugar	B-carotene(mg)	Vitamin C (mg)
T0 (30:70)	98.3±0.03	0.76±0.01
$T_1(25:10:00:30)$	174±0.01	3.5±0.04
T ₂ (25:00:10:30)	93±0.04	3.4±0.01
T ₃ (30:10:00:25)	248±0.01	4.01±0.01
T ₄ (30:00:10:25)	124.2±0.02	3.91 ±0.03
T ₅ (35:10:00:20)	805.2±0.03	4.51±0.01
T ₆ (35:00:10:20)	169±0.02	4.41±0.02
T ₇ (15:20:00:30)	559±0.05	3.08±0.02
T ₈ (15:00:20:30)	70.35±0.01	2.88±0.04
T ₉ (15:25:00:25)	951.2±0.01	3.38±0.03
T ₁₀ (15:00:25:25)	98.3±0.02	3.23±0.01
T ₁₁ (25:20:00:20)	881.3±0.03	4.09±0.03
T ₁₂ (25:00:20:20)	154±0.02	3.89±0.01
T ₁₃ (25:15:00:25)	781.6±0.01	3.80±0.02
T ₁₄ (25:00:15:25)	101.35±0.02	3.65±0.03
Mean	353.92	3.55
Minimum	93	0.76
Maximum	951.2	4.22
F-test	S	S
S. Ed. (±)	3.457	0.032
C. D. $(P = 0.05)$	6.914	0.065

Values are the mean up to 3 replicates \pm standard deviation (SD)

Where T_0 (Khoa: Sugar) 70:30 [Control], T_1 (Bottle gourd: Carrot: Beetroot: Sugar) = 25:10:00:30, T_2 (Bottle gourd: Carrot: Beetroot: Sugar) = 25:00:10:30, T_3 (Bottle gourd: Carrot: Beetroot: Sugar) = 30:10:00:25, T_4 (Bottle gourd: Carrot: Beetroot: Sugar) = 30:00:10:25, T_5 (Bottle gourd: Carrot: Beetroot: Sugar) = 35:10:00:20, T_6 (Bottle gourd: Carrot: Beetroot: Sugar) = 35:00:10:20. T_7 (Bottle gourd: Carrot: Beetroot: Sugar) = 15:20:00:30, T_8 (Bottle gourd: Carrot: Beetroot: Sugar) = 15:00:20:30, T_9 (Bottle gourd: Carrot: Beetroot: Sugar) = 15:00:25; T_{10} (Bottle gourd: Carrot: Beetroot: Sugar) = 15:00:25:25, T_{11} (Bottle gourd: Carrot: Beetroot: Sugar) = 25:20:00:20, T_{12} (Bottle gourd: Carrot: Beetroot: Sugar) = 25:00:20:20, T_{13} (Bottle gourd: Carrot: Beetroot: Sugar) = 25:15:00:25) and T_{14} (Bottle gourd: Carrot: Beetroot: Sugar) = 25:00:15:25.

Statistical Analysis

The experimental data were presented as mean \pm standard deviation and subjected to one way ANOVA, followed by Tukey's post hoc test to determine significant differences (p<0.05) among the means. Statistical analysis was performed using Minitab software (version 17.0.1).

Determination of vitamin C

The spectrophotometric method for determining vitamin C content involves measuring the absorbance of ascorbic acid in a sample solution at a specific wavelength. This method is based on the principle that ascorbic ultraviolet (UV) light at a wavelength of around 265 nm [3].

The free radical scavenging activity of the sample extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A 3.9 ml of aliquot of 0.0634mM DPPH solution in 95% methanol was added to 0.1ml of methanolic sample extract. The mixture was shaken and incubated in the dark for 30 minutes. The absorbance was

then measured at 515nm. The percentage inhibition of DPPH was calculated using the formula:

% inhibition= $[(A_0 - A)/A_0]*100$

Where A_0 is the absorbance of the control sample (DPPH solution without sample extract) at 515nm. 95% methanol was used as a blank to caliberate the spectrophotometer. This assay measures the ability of the sample extract to scavenge free radicals, which is important indicator of its antioxidant activity $^{[10]}$.

Statistical Analysis

The experimental data were presented as mean \pm standard deviation and subjected to one way ANOVA, followed by Tukey's post hoc test to determine significant differences (p<0.05) among the means. Statistical analysis was performed using Minitab software (version 17.0.1).

Results and Discussions

Beta carotene and Vitamin C were assessed in Burfi samples T0, T_1 , T_2 , T_3 , T_4 , T_5 , T_6 , T_7 , T_8 , T_9 , T_9 , T_{10} , T_{11} , T_{12} , T_{13} and T_{14} and compared. A two sample t-test (95% confidence level) was used to compare the optimised Burfi sample with the control, with superscripts denoting significant differences.

Beta carotene analysis

The $\beta\text{-carotene}$ content value added burfi made by incorporating Bottle gourd, Carrot and Beetroot with different compositions are shown in Table 2. For samples T_0 to T_{14} , the $\beta\text{-carotene}$ content ranged from 93 μg to 951.2 μg . The highest $\beta\text{-carotene}$ content was found in treatment T_9 which is 951.2 μg and the lowest content was found in T_2 which is 93 μg . The $\beta\text{-carotene}$ content in the control sample T_0 was observed to be 98.3 μg The observed results are graphically displayed in Figure 1.

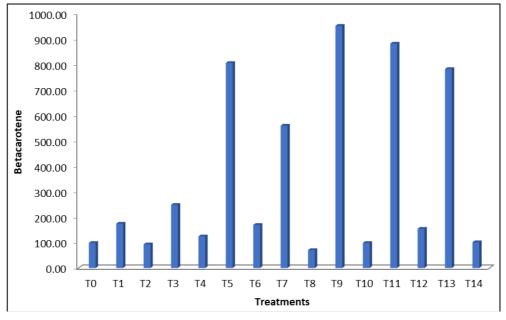


Fig 1: Graphical Representation of β-carotene Analysis (µg/100g) of Experimentally Developed Burfi

The result of the present study indicates that β -carotene content increases with the increase of Bottle gourd, Carrot and Beetroot. The beta carotene content range was affected by change in compositional parameter. Bottle gourd contains 4.6371 µg/100 g beta carotene ^[9] while Carrot contains 7.88 µg/100 g beta carotene ^[9] and Beetroot 1.9 µg ^[9]. In a study reported by ^[5] where ice cream were prepared by different proportion of Beet root juice. β -carotene content tends to increase with the increase of beetroot. The results are also in accordance with study on incorporation of carrot powder in different ratios on the quality of pasta ^[13] higher beta carotene in carrot candy and jam when compared to

carrot orange juice. Similar findings were also observed in the present research for Bottle gourd burfi.

Vitamin C analysis

The vitamin C content of value added burfi made by incorporating Bottle gourd, Carrot and Beetroot with different compositions are shown in Table 2. For samples T_0 to T_{14} , the vitamin C content ranged from 0.76mg to 4.51mg. The highest vitamin C content was found in treatment T_5 which is 4.51 mg/100g and the lowest content was found in control sample T_0 which is 0.77 mg. The observed results are graphically displayed in Figure 1.

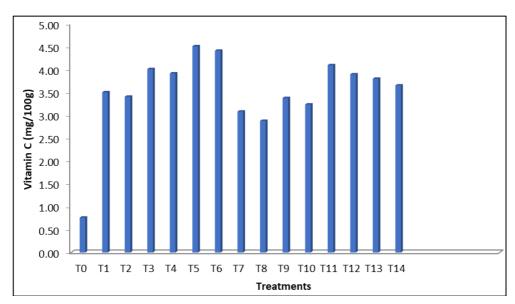


Fig 2: Effect of different treatments (T0-T14) on Vitamin C content (mg/100g).

The result of the present study indicates that Vitamin C content increases with the increase of Bottle gourd, Carrot and Beetroot. The Vitamin C content range was affected by change in compositional parameter. Bottle gourd contains 10.1 mg/100 g Vitamin C ^[9] while Carrot contains 5.9 mg/100 g Vitamin C ^[9] and Beetroot 4.9 mg/100 g ^[9]. Similar results were observed when tomato sauce samples were enriched by adding different proportion of Bottle

gourd ^[13]. Vitamin C content tends to increase with the increase of beetroot. The results are also in accordance with study on incorporation of carrot powder in different ratios on the quality of pasta ^[13] higher beta carotene in carrot candy and jam when compared to carrot orange juice. Similar findings were also observed in the present research for Bottle gourd burfi

Conclusion

Study leads to conclusion that treatment T_9 composed of 15 parts of Bottle gourd, 25 parts of Carrot, 25 parts of sugar and 35 parts of khoa showed highest amount of beta carotene thus resulting in high antioxidant activity. Also Treatment T_5 prepared by incorporating 35 parts of Bottle gourd, 10 parts of Carrot and 20 parts of sugar and 35% khoa analysis showed highest percentage of vitamin C. It may concluded that the Treatment T_9 and Treatment T_5 can be prepared to develop high antioxidant enriched burfi.

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