



“Optimization of fermentation Time for Enhancing Sensory Attributes and Functional Properties of Probiotics Rich Black Carrot Based Spread”

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ABSTRACT

This study aimed to assess the impact of different fermentation periods on the sensory attributes, physicochemical properties, nutritional composition, and antioxidant activity of a black carrot-based fermented spread. Different formulations were created using consistent proportions of black carrot, tomatoes, garlic, ginger, green chili, lemon juice, inoculum, and butter, subjected to fermentation for 6, 12, and 18 hours. Sensory evaluation, conducted using a 9-point hedonic scale, revealed that the spread fermented for 12 hours (T₂) received the highest overall acceptability score of 8.6, than other samples. Based on this sensory data and physico-chemical properties including pH, Total Acidity, and TSS further analyses were conducted to investigate the nutritional composition i.e moisture, ash, crude protein, crude fat, and crude fiber, Vitamin A and antioxidant activity like Total phenolic content, DPPH. Subsequent analyses indicated that the fermentation process enhanced nutritional analysis and antioxidant activity, potentially offering protection against oxidative stress and reducing the risk of chronic diseases. Additionally, the proliferation of beneficial microorganisms, particularly lactic acid bacteria with probiotic properties, was observed, contributing to improved gut health and overall well-being.

Keywords-Nutrition rich, Black Carrot, fermented Tomatoes, Inoculum, Sensory, Nutritional Composition, Antioxidant Activity, Probiotics.

INTRODUCTION

The Indian Black Carrot (*Daucus carota* ssp. *sativus* var. *atrorubens*), a biennial herbaceous species of the Apiaceae family, is primarily grown in regions of Asia and the eastern Mediterranean, including India. In India, it's notably cultivated in Haryana, Andhra Pradesh, Punjab, Karnataka, and Uttar Pradesh, with the "Punjab Black Beauty" being a popular variety. Black carrots are distinguished by their high content of bioactive compounds, particularly anthocyanins, which are responsible for their dark color and potent antioxidant properties. These bioactive compounds include dietary fiber, carotenoids, flavonoids, phenolic acids, and others, contributing to the carrot's multifaceted health benefits. The anthocyanins in black carrots are acylated, enhancing their stability and making them valuable as natural colorants in the food industry. Beyond their nutritional value, black carrots have been recognized for their potential in preventing various diseases, including cancer, cardiovascular conditions, obesity, and diabetes, due to their antioxidant and anti-inflammatory properties. The presence of

flavonoids, which are higher in black carrots compared to orange or red carrots, further enhances their pharmacological value. Including Fermentation for development of nutrition rich food products is the best way to increase their nutrients. Fermentation is a traditional method used to preserve black carrots, enhancing their shelf life, and the potential of black carrot, transforming it into nutritious, flavorful, with a wide range of culinary and functional applications. which protects the food against the growth of pathogenic bacteria with probiotics properties and increase the shelf life. Fermentation can enhance the stability and bioavailability of these compounds also, potentially increase their health benefits. Therefore, the aim of the study is to develop Black Carrot based fermented food products and to identify the optimal fermentation conditions and their effects on sensory and functional qualities. Creating spreads using fermented from black carrot puree provides a unique, nutrient-rich alternative to traditional spreads and dips, appealing to health-conscious consumers

Materials and Methods

Procurements of Raw Materials : The raw materials, includes black carrot, tomatoes, butter, green chilli, garlic, ginger, black pepper and lemon juice were purchased from local market of Naini in Prayagraj Uttar Pradesh. All the chemical, used for the study includes sodium carbonate, sodium hydroxide, phenolphthalein, petroleum ether, foline ciocalteu reagent, methanol, ethanol, acetone, gallic acid, sodium nitrite, copper sulphate, lithium sulphate, ferric chloride anhydrous, ascorbic acid, sulphuric acid, potassium phosphate, sodium carbonate anhydrous, 2,2- diphenyl-1-picrylhydrazyl (DPPH), and aluminum chloride anhydrous, were purchased from Sigma-Aldrich (St. Louis, MO, USA)

Formulation of Black Carrot based Fermented Spread:-

The black carrot based fermented spread was prepared by grating of the black carrots and tomato and putting into a pan, boiled with water for 5 minutes, once the vegetables have cooled to room temperature, put the black carrot and tomato into grinder jar with garlic cloves, green chilli and black pepper and grind it to make fine paste, after this add 2-3 drops of lemon juice and salt as per taste, Now add inoculum, leave it for 6, 12, and 18 hours at dark place. Once fermentation done, add butter and mix it well to form black carrot based fermented spread.



Fig. 1 Flow chart showing the preparation of Black Carrot Based Fermented Spread

Control and Treatment Combination for Preparation of Fermented Spread

T₀- Non-Fermented (83 g black carrot, 4g garlic, 3g ginger, 1 green chilli, 5g butter, 2g black pepper, 2g salt)

T₁-Fermentation for 6hrs(65g black carrot, 10 tomatoes, 8g inoculum 4g garlic, 3g ginger, 1green chilli, 5g butter, 2g black pepper, 2g salt)

T₂- Fermentation for 12hrs (65g black carrot, 10 tomatoes, 8g inoculum 4g garlic, 3g ginger, 1green chilli, 5g butter, 2g black pepper, 2g salt)

T₃- Fermentation for 18hrs (65g black carrot, 10 tomatoes, 8g inoculum 4g garlic, 3g ginger, 1green chilli, 5g butter, 2g black pepper, 2g salt)

Physico-Chemical Analysis

Determination of pH - The pH of each sample was measured after the fermentation process using a pH meter (Hand-Held EcoSense pH10An pen Tester, Beijing, China).

Determination of Acidity-The titratable acidity (TTA) analysis was carried out by titrating 2 g of sample mixed in 20 mL distilled water against 0.1 N NaOH, with the use of phenolphthalein as an indicator, the result obtained was expressed in percentage (% lactic acid).

Determination of Total Soluble Solids- The total soluble solids (TSS) determination was carried out using a refractometer (Hanna H 196801, Woonsocket, RI, USA), and the results were expressed as °Brix

Chemical Analysis

Proximate analysis was conducted following the methods described by **AOAC (2007)** to determine the moisture, ash, crude protein, crude fat, and crude fiber content of the sample. Each component was quantified using standard procedures, and results were expressed as percentages of the sample's total weight and vitamin A content was determined by quantifying beta-carotene using the method described by **Rodriguez-Amaya (2010)** were carried out according spectrophotometric by using 452nm.

Determination of Total Phenolic Content

Total phenols were estimated by the Folin-Ciocalteu method of Elfalleh et al., (2009),The Folin-Ciocalteu method is commonly used for TPC determination, where the phenolic compounds react with the reagent to produce a blue color, measurable by spectrophotometry.

where the absorbance of the sample was measured at 760 nm after reaction with the reagent. TPC was quantified as gallic acid equivalents (GAE) per gram of the sample, providing an estimate of the antioxidant capacity. which are key contributors to antioxidant activity and overall health benefits.

Radical Scavenging Activity

The radical scavenging activity was determined using the DPPH assay, as described by Brand-Williams et al. (1995), this test measures the ability of a substance to donate hydrogen atoms or electrons, effectively neutralizing free radicals and indicating its antioxidant potential. where the reduction of the DPPH radical by antioxidants in the sample was measured spectrophotometrically at 517 nm. The results were expressed as IC₅₀ values, indicating the concentration required to scavenge 50% of the DPPH radicals.(**Food science and technology , 1995**)

Statistical Analysis

The analysis was carried out in triplicate and values were expressed as mean. The data was statistically analysed by using Analysis of variance (Two-way ANOVA or Two-way classification) technique. A significant difference between the treatments was determine by using CD (Critical Difference) test (Appendix). 'T' Test was performed for comparing the difference in the nutritional content (moisture, ash, far, protein, fiber, and vitamin A) and antnioxidant activity(TPC, DPPH) between control and best treatment of the developed food products (**Chandel, 2006**).

Results and Discussion

Sensory Analysis of Black Carrot based Fermented Spread

The result of the present study reported that there are significant differences ($p < 0.05$) in all sensory parameters among treatments. The mean sensory score in term of colour and appearance were studied and found that T₂ has

the highest score followed by T₀, T₁ and T₃. It indicates that the treatment T₂ was liked very much by the responders, Whereas T₀, T₁ and T₃ were liked moderately by the responders. Our finding reported that fermentation duration increases to 18 hrs in T₃, and the color acceptability decreases. Further, the mean of texture scores of fermented spread, shows that T₂ has the highest score (8.6), followed by T₀ (7.0), T₁ (7.66), and T₃ (6.8). This might be because the tanginess increases with increasing the fermentation duration in the spread, resulting in a tangy spread flavor. The result of the taste and flavor score shows that T₂ has the highest score (7.8), followed by T₁ (6.1), and T₃ (6.8). It indicates that all treatments were significantly liked by consumers while treatment T₂ was liked very much by the responders, whereas T₀, T₁ and T₃ were liked slightly. This might be attributed to the fermentation and combination of tomatoes, Further, the mean of sensory scores obtained and analyzed for the fermented spread concerning Overall Acceptability, shows that T₂ has the highest score (8.6), followed by T₁ (7.66) and T₃ (7.26). It indicates that the sensory acceptability of the product increases with increasing the fermentation period. This shows that treatment T₂ is best overall due to 12 hrs of fermentation for 37^o C.

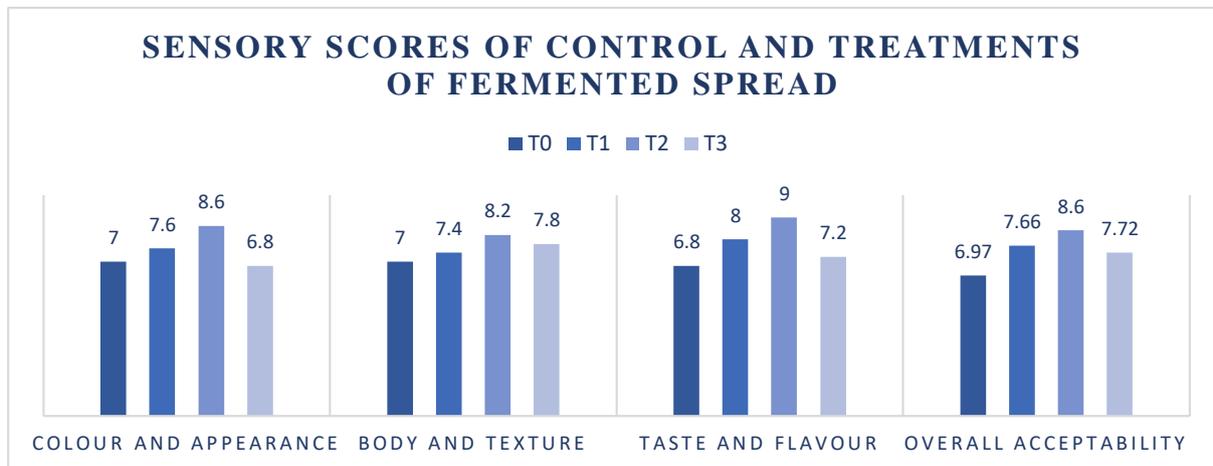


Fig. 1: Sensory Scores of Non-Fermented and Fermented Spread

Physico-Chemical Analysis of Non-Fermented Spread and Fermented Spread

Table 1:- Physico-Chemical of Fermented spread

	T ₀	T ₁	T ₂	T ₃	F.cal(5%)	F.tab(5%)	S
pH	6.3	4.3	4.0	3.6	22.31	4.75	S*
TOTAL ACIDITY (%)	5.3	1.75	2.2	2.35	2.85	4.75	S*
TOTAL SOLUBLE SOLIDS (BRIX%)	14	10	7.8	6	57.65	4.75	S*

Table 1 depicted the influence of fermentation duration on acidity, titratable acidity (TA), and Total Soluble Solids (TSS) of a black carrot-based fermented spread. pH provides the free acidity (the immediate sourness perceived) but does not account for the buffering capacity of the food. Initially, the pH of the non-fermented spread is 6.3, maintaining its natural sweetness. As fermentation progressed, the pH dropped, to 4.3, 4.0, and 3.6 after 6 hours, 12 hours, and 18 hours respectively. Total acidity is contributed by all total acids present in the food, not even all the acids are dissociated. High TA often results in a tangy taste, typical of fermented products. It also helps in preserving the spread by inhibiting the growth of spoilage microorganisms. In the non-fermented spread, Titratable Acidity (TA) was around 5.3% with increasing fermentation duration, titratable acidity decreased to 1.75%, 2.2%, and 2.35% respectively. It reflects mild tanginess due to initial lactic acid production. Inconsistent to present finding, previous studies indicated a rise in acidity shifts the flavor of the product from sweet to tangy and acidic flavors.

Total Soluble Solids (TSS) refers to the total concentration of dissolved substances in a liquid, often measured in degrees Brix. These dissolved substances include sugars, acids, vitamins, minerals, and other soluble compounds. TSS is commonly used as an indicator of the sweetness or maturity of fruits and vegetables, and it reflects the overall quality, flavor, and ripeness of fresh produce or processed products like juices, jams, and syrups. The total soluble solids of non-fermented spread were 14 %, indicating high sugar content, while the total soluble solids of fermented spread varied from 10%, 7.8%, and 6% after 6 hours, 12 hours, and 18 hours respectively. The result is attributed to sugars being converted into acids during fermentation, leading to a progressively tangier and sour flavor

Nutritional Composition of Non-Fermented and Optimized Fermented Spread.

Table 2 Nutritional composition of black carrot non-Fermented and Optimized Fermented Spread.

Parameters	Control (T0)	(T2) 12hrs	t - test
Moisture (%)	70±1.8	20±1.2	28.57%
Ash (%)	1.5±0.5	6.25±0.8	316.6%
Fat (%)	2 ±0.01	2.2 ±0.06	10%
Protein (%)	0.75±0.04	1.56±0.02	0.81*
Fiber (%)	2.2±0.3	3±0.2	40%
Vitamin A (%)	42.58±0.5	54.31±2.3	27.54%

The nutritional composition of Fermented optimized spread was based on the sensory and physico-chemical analysis, control and best treatment was conducted for comparison which are presented in Table 2. The study reported the significant increment of protein, fat, ash and fiber content of Fermented optimized spread were observed; however moisture content were decreased from &70g/100g to 20 g/100g after fermentation.. The increment in protein content of the Fermented optimized spread was 0.75g/100g to 1.56g/100g. it attributed to quantitative reduction in antinutritional factors (tannin, and phytic acid) and production of some additional amino acids in the samples as a result of fermentation (Hassan et al., 2006). The ash content was increased from 2g/100g to 2.2g/100g in Fermented optimized spread, this reflects the high increment in mineral content in optimized spread due to butter its inclusion in the spread directly raises the fat content. Similarly several studies reported the increase in mineral content during fermentation (Rodríguez-Sánchez et al., 2023) Adebisi et al., (2016) mentioned that crude fat content of the control spread was 0.2% where as after the fermentation fat has increase to 2 g/100gm. Further, Vitamin A content in Fermented optimized spread found increase from 42.58µg/100g to 54.31µg/100g .due to microbial activity can synthesize vitamin A precursors like β-carotene and enhance their bioavailability by breaking down cell walls, releasing more nutrients. These results confirmed with the finding reported by Hassan et al., (2006).

Antioxiant Activity in Non- Fermented Spread and Optimized Fermented spread.

TPC is used to measure the concentration of phenolic compounds in a sample. They can scavenge free radicals, which are linked to oxidative stress and various health issues. The DPPH assay measures the antioxidant capacity of a substance by assessing its ability to reduce the DPPH radical, It is a simple, rapid, and widely used method to evaluate the free radical scavenging ability of antioxidants present in foods.

The study highlights significant increases in bioactive components and antioxidant activity in optimized spread compared to non-fermented spread (controls). The TPC in the fermented optimized spread was 345.8 mg GAE/g, a significant increase from the control spread, which had 104 mg GAE/g. This rise ($P \leq 0.05$) during the 12-hour bacterial fermentation is attributed to enzymes produced by microbes that release bound phenolic compounds into free phenols.

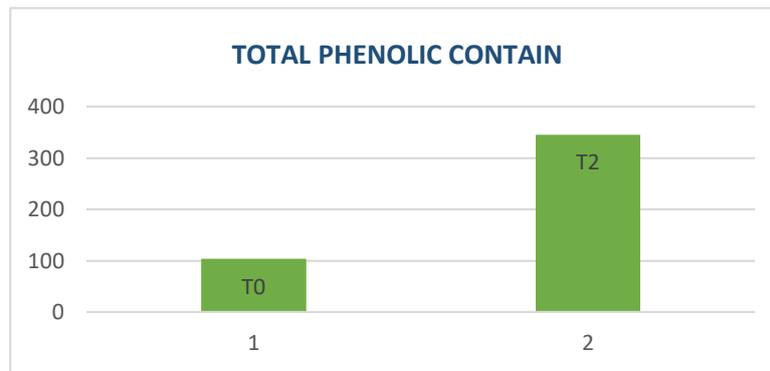


Fig .- 2 Total Phenolic Content of Non-Fermented and Optimized Fermented Spread

The DPPH radical scavenging activity of the fermented optimized spread was 76.16%, compared to 40% in the control. The higher antioxidant activity in the fermented Optimized spread is likely due to the increased levels of total phenolic as supported by **Shin et al. (2021)** and **Yen et al. (2022)**

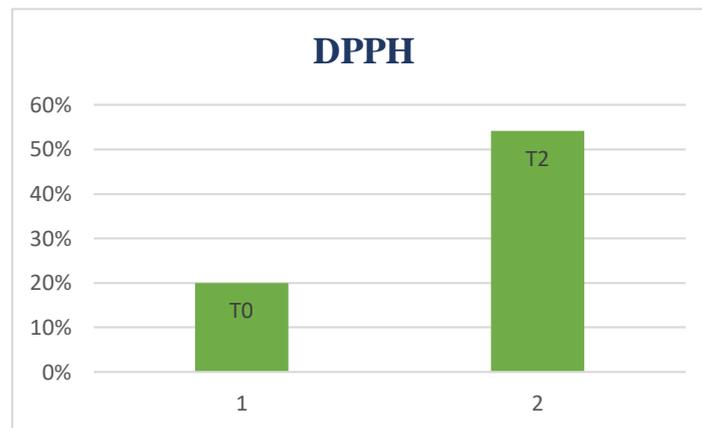


Fig .- 3 DPPH Value of Non-Fermented and Optimized Fermented Spread

CONCLUSION

On the basis of findings it is concluded that Black Carrot, Tomatoes, Garlic, Ginger, Green Chilli, and Butter were successfully incorporated in the preparation of Development of Nutritionally Food Products namely **Fermented Spread** from Black Carrot (65g Black Carrot, 10 g tomatoes, 7 g inoculum 2 g garlic , 2 g ginger 1g green chilli and 2-3 drops of lemon juice 5 g butter) for **12hrs of fermentation** was liked very much. Which is further optimized (on the basis of sensory score) nutritive values of the fermented spread products was calculated and observed that the moisture content was 20% which is quite less than control spread (70%) , Ash was calculated and observed that ash was 6.25% for fermented spread from black carrot whereas protein was 1.56, fat was 2 in bacterial fermentation food products. and crude fiber was 3% which was more than control spread increase 5.67 with bacterial fermentation. The Physico-chemical value of best treatment of Fermented Spread from Black Carrot products was calculated and observed that the pH content was 4.0, Total Acidity was 2.2% and TSS was 7.8%. The Total phenolic content obtained from the optimized fermented spread from Black Carrot products was 345.8mg and DPPH for Optimized fermented spread from black carrot was calculated and observed was 76.16% during the fermentation process.

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