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SYNERGISTIC ROLE OF PROBIOTICS AND NON-THERMAL PRESERVATION TECHNIQUES IN ENHANCING THE FUNCTIONAL VALUE OF INDIGENOUS FRUITS

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ABSTRACT

Native fruits provide key macro-nutrients, natural antioxidant, and bear a significant cultural value; but due to their high perishability limits its potential commercialization. It can be enhanced to make these fruit matrices functional foods by introducing probiotic cultures to them and providing them with added health benefits. Regrettably, the viability of most probiotics is negatively affected by the common preservation pressures which include heat, oxygen, and acidic condition making the traditional preservation method inappropriate. Recent developments in non-thermal preservation, especially, High Pressure Processing (HPP), Pulsed Electric Field (PEF), ultrasound, UVc irradiation, cold plasma, and preserve sensory and nutritional qualities and provide microbial safety. To that end, the current study evaluates the synergistic effect of probiotic inoculation in the combination with these non-thermal modalities (in indigenous fruit systems) on probiotic stability, shelf-life prolongation, and nutrient preservation.

The empirical results indicate that non-thermal processes significantly support the survival of the probiotics in comparison to thermal procedures and form the basis of functional food production. The non-thermal indigenous fruit matrices fortified with probiotics had better amounts of vitamins, phenolic compounds, and sensory properties during the storage period. Shelf-life increased to 28 days and quality parameters were not sacrificed. These results provide a scientific basis to the synergistic nature of probiotics and non-thermal preservation and provides a viable direction to value-added, non-processed fruit products. In general, the study can add new knowledge about sustainable fruit processing and functional food technology.

Keywords: Indigenous fruit, probiotics, non-thermal preservation, functional foods, high-pressure processing, pulsed electric field, bioactive, combination effects, shelf life.

INTRODUCTION

The background of Indigenous Fruits 1.1 Indigenous Fruits background.

Indigenous fruits, also known as underutilised, wild, or native variety, are plant species that have developed in particular geographical boundaries, and that are long traditions on the menus of local people. Some of them are jamun (Syzygium cumini), amla (Phyllanthus emblica), bael (Aegle marmelos), wood apple (Limonia acidissima), phalsa (Grewia asiatica), karonda (Carissa carandas) and many many others that are distributed in tropical and subtropical ecosystems. The fruits, despite outperforming most of the commercially available counterparts, in terms of nutritional content, are not exploited in the modern food systems. They have phytochemical portfolios that include polyphenols, flavonoids, anthocyanins, carotenoids and vitamins especially vitamin C, minerals and dietary fibres. As an example amla has ascorbic acid ranging between 400 and 900 mg / 100g, much more than the citrus fruits, whereas jamun provides higher anthocyanin levels with reported antidiabetic activity. The antioxidant activity of many native fruits is also greater than the antioxidant activity of blueberries and other popular so-called superfruits.

However these nutritional resources are faced with major challenges: high perishability (shelf life of 2-5 days at ambient conditions), high post-harvest losses (40-50 per cent), seasonal supply, lack of

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commercialised processing systems, inefficiency of cold-chain infrastructure and consumer ignorance outside the more traditional agricultural areas. This means that farmers are forced to live with significant financial losses and the society loses chances of nutritional security and development of nutritious food.

Overall, Probiotics are the constituents of functional foods, and their importance must not be undervalued.

Such change of paradigm whereby the capacity to think of food as sustenance is replaced by the ability to understand its effect on diseases and health has placed functional foods in frontline of nutritional science today. The so-called probiotics, namely distinctly defined by FAO/WHO as the live microorganisms that, when taken in the right amounts, provide a health benefit to the host, can be regarded as one of the most widely researched components of this area.

LAB, especially the species of Lactobacillus and Bifidobacterium, do have wide-ranging health-promotional properties: they make the gut microbiome efficient, strengthen intestinal barrier function, regulate immune responses, synthesise vitamins of the B-complex, produce antimicrobial compounds, reduce serum cholesterol, and possibly have anticancer properties. The world probiotic market is estimated to be worth about 58 billion dollars in 2023 and it reflects the demand towards the products which provide health benefits over basic nutrition.

Fruit-based probiotic products form a new category, which is going to meet various consumer needs, such as a dairy probiotic alternative (plants), a natural prebiotic source (fruit fibres and oligosaccharides), a high concentration of phytochemicals, and a pleasant taste. The fermentation of fruit matrices not only survives, but also actively modifies the substrate via an enzymatic reaction, synthesising bioactive metabolites - short-chain fatty acids, exopolysaccharides, bacteriocins - and biotransforming complex phytochemicals into more accessible forms when fermented by probiotics.

1.3 Gonzalez Requirement of Non-Thermal Preservation Technologies.

Conventional thermal (pasteurisation, sterilisation) will successfully inactivate spoilage and pathogenic microorganisms and lengthen shelf life, but causes serious destruction of the heat-sensitive bioactive constituents, vitamins, pigments and flavour compounds in fruits. Destruction of ascorbic acid, oxidisation of polyphenols, degradation of anthocyanin, loss of colour and volatile aromatic compounds corrode not only the nutritional value but the sensory quality as well, depleting the same properties which make indigenous fruits attractive as constituents of functional food.

Alternative preservation methods provide the means of providing graceful solutions since most of them maintain microbial stability and prolong shelf life without thermal degradation. Advanced modalities including high-pressure processing (HPP), pulsed electric field (PEF), ultrasonics, cold plasma and ozone treatment are used to inactivate spoilage and pathogenic organisms without heating food and cannot degrade the structure, colour, flavour and bioactive compounds. These technologies resonate with what consumers are seeking which are minimal processing, fresh like products that have clean labels and natural properties.

Most importantly, a variety of non-thermal technologies can be tailored to reduce the thermal activity of unwanted microorganisms and still preserve the viability of probiotics, allowing people to develop safe, stable and probiotic-enhanced fruit products, something that is impossible to achieve using standard thermal processing systems that inevitably cause the killing of probiotic organisms.

The study justification and gap in research can be explained as follows:

Although proficient studies have been done on probiotic dairy products and to a minor degree on probiotic

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fortification of commercial fruit juices, there still remains a significant gap in knowledge with regards to the utilisation of probiotics on indigenous fruits in parallel with non-thermal preservation technologies. Current literature is available on either probiotic fermentation or non-thermal preservation as independent domains, but its application as synergistic entities has been least explored.

The specific gaps in the research are: (1) no systematic testing on the performance of probiotic strains in different indigenous fruit matrices with diverse pH, sugar content and phytochemical profiles; (2) no data on the most suitable parameters of specific non-thermal technologies to be applied to probiotic-enriched native fruits; (3) inadequate research on the synergistic effects through which combined processes/methodologies should improve functional properties over single treatments; (4) a gap in large-scale research in the area of shelf-life performance; and (5) inade

The research will improve these gaps by undertaking a systematic research that explores the synergistic impact of probiotic fortification and various non-thermal preservation techniques on selected native fruit products. The study will provide evidence-based guidelines of the manufacture of commercially viable and health-enhancing functional foods that may ensure that indigenous fruits that are produced as perishable and locally consumed products are converted to value added products that have potential markets both nationally and internationally. This would reduce at the same time, post-harvest losses, improve the incomes of farmers and benefit the overall national nutrition.

LITERATURE REVIEW

1. Tripathi & Giri (2019)

Tripathi and Giri (2019) clarified the reason why fruits are an ideal carrier of probiotics are the presence of natural sugars, fibers, and antioxidants in fruits. They pointed out that fruits can be kept in an acidic environment as probiotics more than dairy. Their research highlighted the increase of the non-dairy probiotic foods demand among the vegan and lactose- intolerant population. Another discovery concerning fruit environments by the authors was that L. plantarum and L. rhamnosus adjust to fruit environments. The problems found included pH sensitivity and stability of storage. The authors made a conclusion that fruit-based probiotics possess high marketing potential. Yet, they observed that processing procedures have to be optimized, so as not to lose viability. Indigenous fruits are useful as functional carriers as promoted in this study. It provides foundations on which probiotic-fruit interactions are to be explored.

2. Patras et al. (2010):

Patras et al. (2010) studied the preservation of fruits using High Pressure Processing (HPP) on both nutritional and sensory attributes. Their study revealed that HPP preserves color, flavor and bioactive compounds in a better manner than thermal processing. The experiment indicated that anthocyanins and ascorbic acid were highly retained in berries and juices. HPP was also able to create a complete microbial inactivation without any deterioration of nutrients. The authors observed that pressure beyond 500 MPa enhances the shelf-life significantly. They however warned of extreme pressure that can have an influence on texture. Their results endorse HPP as an apt technology in the enriched fruit with probiotics. The research demonstrates that the non-thermal processes do not destroy heat-sensitive elements. It also has solid reasons as to why HPP should be applied in indigenous fruit processing.

3. Barba et al. (2017):

Barba et al. (2017) determined the effect of Pulsed Electric Field (PEF) on fruit juices and fruit purees. The article stated that PEF is an efficient approach to the reduction of microbial load and preservation of vitamins and phenolic compounds. The membrane disruption is brought about by the electric pulses without heating up the membrane excessively. The authors discovered that PEF can be used to enhance

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extraction of antioxidants and juice homogeneity. They also observed that flavor is better retained than in case of pasteurization. Barba et al. found PEF as an appropriate process of fruit drinks with minimal processing. It was demonstrated that the technique can help preserve the life of probiotics in the process of storage. This paper identifies the possible synergy between probiotics and PEF-treated products. It reinforces the suggestion to study the use of PEF with indigenous fruits.

4. Pankaj et al. (2018):

Pankaj et al. (2018) examined coating cold plasma as a new non-thermal technology in decontaminating the surface of fruits. They documented that cold plasma has the capability of inactivating bacteria, yeast and molds by means of reactive species. The therapy maintained the color, hardness as well as phytochemicals of the fruits. Cold plasma did not result in significant nutrient degradation, as was the case in thermal methods. The authors made a point of the capacity of plasma to look fresh. They proposed the use of plasma with other non-thermal technologies to achieve greater outcomes. Their results show that they are well compatible with probiotic-bearing products. This research advocates the use of cold plasma in contemporary preservation methods of fruits. It expands the non-thermal applications in indigenous fruits.

5. Donsi and Ferrari (2016):.

Donsi and Ferrari (2016) investigated the ultrasound processing and its impact on fruit-based drinks. They found out that ultrasound facilitates cavitation, which results in reducing microbes and enhancing bioactive extraction. They claimed an improved color stability and retention of nutrients over heat treatments. Ultrasound also raised the level of antioxidant as plant cell walls were broken. The method enhanced uniformity of juices, which is advantageous to the dispersion of probiotics. Nonetheless, too much intensity of ultrasound may ruin texture. The authors suggested parameters that can be controlled in order to process optimally. They propose in their work that indigenous fruits produced with probiotics can be used with ultrasound. This paper encourages the functional drink development with a combination of ultrasound and probiotics.

6. Sharma Devi (2020):

Sharma and Devi (2020) examined the nutritional and therapeutic value of local Indian fruits. They pointed out that fruits such as jamun, bael, amla and wood apple are rich sources of antioxidants and medicinal properties. They used these fruits in their research to explain how the fruits are used in traditional healing. They discovered that indigenous fruits possess high potential of value addition by processing. The authors have pointed out consumer needs of natural and functional products. They proposed the modern preservation technologies to minimize losses after harvesting. Probiotics were also suggested by the study as a supplement to such fruits. Their results justify the exploitation of indigenous fruits in the production of functional products that rely on probiotics.

7. Yadav and Shukla (2021):

Yadav and Shukla (2021) examined the survival of probiotics in fruit beverages that had not undergone thermo-preservation. Their research found that probiotics have better viability when subjected to HPP, PEF and ultrasound. They noted that cell stress was minimized because of absence of high temperatures. The authors also put emphasis on the need to select acid-tolerant strains. They further determined that non-thermal methods avoid destruction of nutrients and this creates an enabling atmosphere to probiotics. The study proved the extension of shelf-life with no loss of quality. Their results reinforce the idea of synergy of probiotics and non-thermal preservation. This confirms the assumption of this research on indigenous fruits.

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8. Zhang et al. (2015):

Zhang et al. (2015) compared the performance of UV-C irradiation on the preservation of fruit juices. They also proved that UV-C efficacy is great (in terms of reduction of surface microorganisms), where sensory quality is not compromised. The long period of treatment, however, may cause a loss of color and vitamin. The authors discovered the best exposure levels that do not degrade phytonutrients. They find UV-C to be an effective preservative technique that is mild. Treatment with UV-C did not affect the viability of probiotics in acidic fruit systems. The paper suggests the implementation of UV-C with other non-thermal technologies. It presents proof of UV-C as an auxiliary device in the indigenous fruit processing.

OBJECTIVES

The main aim of the research is to investigate the viability and stability of the chosen probiotic strains in the case of their inclusion in native fruit matrices. The study is supposed to cognize the effect of non-thermal treatments on the reduction of microbial loads, nutrient preservation, and textual integrity. The other important aim is to determine the sensory acceptability of the probiotic-enriched fruits processed via the various non-thermal methods. The study will also aim to achieve a scientific ground on the development of functional indigenous fruit products by accomplishing these objectives.

One of the secondary goals is to determine the synergistic action of probiotics and non-thermal technologies. This involves the knowledge on the effects of processing conditions on probiotic adherence, fruit biochemical properties and storage behaviour. Another objective of the study is to compare how various technologies perform in the maintenance of nutritional and sensory qualities of fruits. Also in the list of the objectives is the evaluation of shelf-life improvement and suitability to the consumer. Together, these goals assist in constructing a paradigm of functional food production, which is sustainable, minimally processed.

MATERIALS AND METHODS

Native fruits like jamun, bael, Indian gooseberry and wood apple were chosen because of their rich antioxidant and nutrient values. Fruits were cleansed, disinfected and treated to get pulp in hygienic laboratory conditions. Strains of probiotics such as L. plantarum, L. rhamnosus and B. bifidum were grown in MRS broth and put to the fruit samples in 108-109 CFU/mL. Some of the non-thermal treatments were HPP at 400-600 MPa, PEF at 30-40 kV/cm, ultrasound at 20-25 kHz, UV-C light at 254 nm as well as cold plasma via a dielectric barrier discharge system.

Physicochemical AOAC methods were applied to the samples to run physicochemical analyses, including pH, titratable acidity, TSS, ascorbic acid, color (L, a, b), and viscosity. Microbial tests included total plate count and probiotic viability tests at a storage period of 28 days under refrigerated conditions. The sensory assessment was conducted on a semi-trained panel on a 9 point hedonic scale. The determination of statistical significance was performed using ANOVA with the p less than 0.05. The consistency of the methodology was achieved because the experiments were repeated three times and the standardized protoc was used.

RESULTS

Findings indicated that non-thermal treatments maintained the physicochemical integrity of native fruits in a much better way compared to thermal controls. The retention of ascorbic acid, phenols, and anthocyanins in HPP-treated samples was the greatest. PEF and ultrasound reduced enzyme browning and preserved natural fruity aroma. The UV-C and cold plasma were effective in reducing the amount of microbes but exhibited minor surface discoloration. In general, non-thermal methods enhanced texture,

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color, and nutrients.

The highest survivability of the probiotics was observed in HPP-treated samples throughout the storage time of 28 days. L. plantarum was more tolerant to acidic environments, as it is naturally resistant to acidic environments. The level of microbial spoilage in all the treated samples was greatly reduced, extending the shelf-life up to 28 days. High acceptability of samples that had HPP, PEF and ultrasound treatment was evident by sensory evaluation. Fruits enriched with probiotics retained the desirable aroma, flavor, and appearance during the storage.

DISCUSSION

The findings confirm other studies that show that non-thermal technologies are more effective in preserving nutritional and sensory properties compared to thermal. The native fruits offered a favorable environment to probiotics, which increased the functional value. HPP became the best technology since it preserves vitamins, pigments and probiotic viability at the same time. PEF and ultrasound showed potential results by preserving structural properties and enhancement of homogenization.

The synergy that is being noted between probiotics and non-thermal technologies indicates that there is high potential of coming up with the next generation functional foods. Probiotic strains were more successful as a result of the work of non-thermal techniques that ensured protection against the effect of heat. The long shelf-life, greater safety and better nutritional composition indicate the possibility of commercial viability. The results justify the use of non-thermal technologies in food processing in order to be sustainable.

CONCLUSION

It is concluded that probiotics plus non-thermal preservation methods are very effective in improving the quality, safety, and functionality of native fruits. Non-thermal methods had a positive impact on preserving nutrients and reducing spoilage microorganisms and sustaining probiotic activity. This synergy fosters the production of minimally processed products that are health promoting fruit products.

These results indicate that there exists an effective scientific groundwork on functional indigenous fruit-based foods having longer shelf-life. The study adds value to new processing techniques, which may be applied in the commercial and industrial contexts. In general, the research provides a solid background to be used in advancing the field of functional food.

FUTURE SCOPE AND RECOMMENDATIONS.

The future studies should be aimed at the scaling of non-thermal technologies into the industrial use and assessment of cost-effectiveness. Other indigenous fruits and encapsulated probiotics may also be investigated and, therefore, make the product more differentiated. Moreover, stability studies should be conducted in the long term to determine nutrient storage within long storage periods.

Regulatory assessments (FSSAI, CODEX), packaging innovations and consumer acceptance investigations should be sought as well. The functionality could be enhanced by investigating hybrid preservation technologies, which comprise two or more of the non-thermal methods. These suggestions form the points of expanding this study to commercial markets.

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