LIQUID GOLD IN A BOTTLE

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Abstract

Kombucha has transitioned from an ancient type of fermented tea to an emerging category of functional beverages being consumed on a large scale. This reflects the increasing popularity of healthy probiotic-like beverages. Berries contribute a variety of phytochemicals including anthocyanins, flavanols, and phenolic acids. The inclusion of these phytochemicals in kombucha formulations leads to products with enhanced antioxidant potential as well as additional health benefits. Berrybased kombuchas exhibit greater antioxidant activity compared to other kombucha products and also exhibit a phenolic profile unique to this beverage. Some of these differences may be attributed to the multiple biotransformation processes that occur during fermentation, as well as potential interactions between the berryderived components and microbial metabolites formed during the fermentation of kombucha. The current body of knowledge is compiled in this review and it provides insight into how to achieve universal health-promoting products through selecting appropriate substrates; understanding fermentation dynamics; identifying phenolic transformations; and developing beneficial functional antioxidant properties, while addressing important legal and regulatory issues. The inclusion of various berries to Kombucha yields Important antioxidant activity versus traditional products. Kombucha undergoes qualitative changes due to microbial biotransformation including Hydrolysis, glycosylation , and esterification of berry polyphenols. Berry compounds interact with Acetic Acid , gluconic acid , & glucuronic acids of Kombucha leading to efficient synergism which results in stability, increased bio-accessibility, and improved flavour characteristics (Tartness & aroma). In addition to changes in composition, berry-kombucha contains significantly higher amounts of total phenolics, greater DPPH, ABTS and FRAP scavenging activities, and evidence of antibacterial activity against foodborne pathogens, indicating that this product may be considered as a "next generation" functional beverage.

Keywords: Berry-kombucha, antioxidant biotransformation, SCOBY fermentation, polyphenol glycosylation, functional beverage regulation.

Graphical Representation

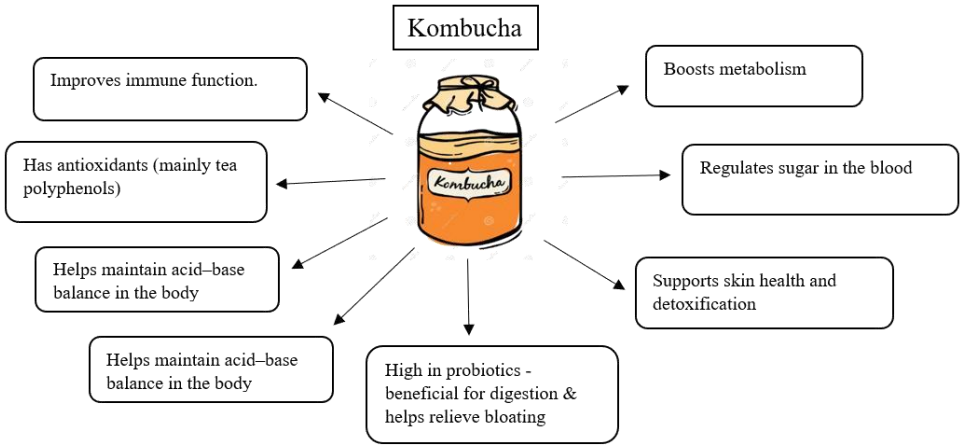

Fig. 1 Kombucha health benefits

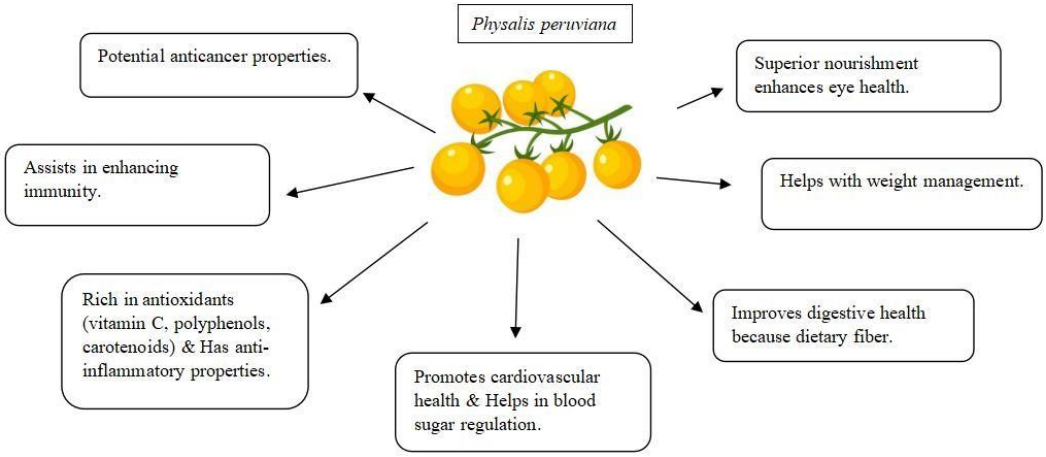

Fig. 2 *Physalis peruviana* health benefits

Introduction

Functional beverages, previously predicted to reach an estimated $158 billion USD valuation in 2023, and projected to increase up to an estimated value of $234 billion USD by 2032, are currently experiencing rapid growth within the global beverage industry[1]. Many of these products have experienced a significant boom in popularity among consumers due to an increase in the demand for more healthoriented products as viable substitutes for sugary carbonated soft drinks. The current demand for healthier drink options has arisen primarily from consumers who care about their health and well-being, such as millennials and Gen Z[2]. These segments of the population are increasingly choosing to replace traditional sodas with functional beverages designed to target specific areas of health or fitness, while still providing an option that has fewer calories than a conventional carbonated soft drink. Examples of the targeted areas include enhanced immunity, improved digestive health, and antioxidant benefits[3].

Kombucha's growth across the globe has been exponential (CAGR) with increases ranging from 16-25%, and the fastest development has occurred within the Asia Pacific region. The unique salience of kombucha as a beverage exists because it draws upon traditional physiological benefits associated with fermentation and has revolutionized how consumers view kombucha as

an avenue to detoxify themselves and achieve better overall health[2]. Kombucha gained popularity due to the success of GT Dave's manufacturing efforts in the 1990's in response to increased consumer interest in immune modulating therapies during the HIV/AID's pandemic. The commercial viability of kombucha can be demonstrated by many recent acquisitions by large corporations: Coca-Cola Co. purchased Health-Ade for over $500 million in late 2020; PepsiCo acquired KeVita for $250 million in 2016 and Keurig Dr. Pepper made strategic investments totaling $300 million into health and wellness products[3].

The sensory properties of kombucha (e.g., its mild effervescence, low alcohol content [<0.5% ABV] and many different flavour categories) allow for its easy distribution through retail supermarkets (92% penetration), specialty coffee shops, and online[4]. However, because kombucha is cold-brewed from black and green tea, there are some drawbacks associated with using these types of tea substrates. The limitations of using black and green tea for kombucha production include limited flavour variation, leading to consumer fatigue; the moderate amount of polyphenols (50-150 mg of gallic acid equivalents per litre); and the vulnerability of the tea supply chain to disruptions caused by climate change on tea growing regions. Examples of non-tea alternatives developed to solve some of these issues include fruit juices, botanical extracts, and cold-brewed extracts. Among these, berries are exceptional. Berries consist of great amounts of oxygen radical absorbance capacity (ORAC), naturally acid, and highly pigmented, which boosts their nutritional and organoleptic worth[5]. Kombucha has been around for thousands of years, with a history dating back to the Qin Dynasty in northeast China (220 BCE), where it was known as Ling Chi, or "Tea of Immortality." While the veracity of this account is debatable, it is said that the legendary first emperor of China, Qin Shi Huangdi, consumed this drink in the hope of achieving immortal life through alchemical means. We do know that by 414 CE, this fermented tea beverage had made its way to Japan and Korea as Chong Jun Cha via the Silk Road[6]. According to Japanese tradition, the name "kombu-cha" (kombu meaning "doctor" and cha meaning "tea") refers to a doctor named Kombu who allegedly cured Emperor Ingyo. Russian versions known as "Tea Kvass" became wellknown by the 19th century due to its advantages for metabolism and digestion, especially in Eastern European cultures[7].

The 1950s saw a renewed interest for kombucha in post World War II Europe with German physician Rudolf Sklenar suggesting that kombucha can help fight against cancer along with many other ailments. While many people believe this, there has been insufficient reliable clinical data to support these statements. In the late 1990's due to the rise of interest in natural healing methods and immune support during the HIV/AIDs epidemic GT Dave founded a company called GT's Living Foods. GT's Living Foods is credited with being one of the first companies to successfully create a modern kombucha beverage in the United States enhancing kombucha's commercial growth. As a result, kombucha became a commercial product growing from a little known ancient remedy to a multi-billion dollar industry forecasted to reach approximately $3.6 billion by 2024[8]. The resurgence of kombucha parallels the industrial revitalization in Southeast Asia. Through its evolution from traditional medicinal uses to becoming one of the fastest-growing 'health drinks' kombucha has maintained a balance between ancient brewing methods and contemporary health research conducted in South Korea, Japan, and India[9]. This indicates that kombucha has been around for a long time and has adapted to a variety of countries, cultures,

and different age groups, as well as to changes in health care practices from being considered a mystical elixir to a basic product in GNC or Whole Foods.

Physalis peruviana is an excellent fruit for kombucha production and is particularly well suited to the tropical climate of places like Haryana, India. The benefits of using this fruit in the form of kombucha, rather than the traditional method of using tea leaves, comes from the fact that it contains a unique blend of beneficial components. These are flavonoids (30 to 50 mg/100 g), ellagic acid, anthocyanins (including delphinidin and cyanidin derivatives), flavanols (including kaempferol and quercetin), and vitamin C (20 to 40 mg /100 g). The unique combination of these nutrients gives perennial fruit based kombucha up to 3 to 5 times higher ORAC Values than regular green and black tea[10]. This unique combination of nutrients combines with the limitations associated with tea-based kombucha, where the levels of polyphenols are typically low (50 to 150 mg GAE/L) and there is limited variety in flavours. *Physalis* has a natural Brix of 12 to 16 degrees and a pH level of 3.2 to 3.8, making it ideal for using with SCOBYs. Additionally, it has a lot of sugars that allow for the F1 fermentation on the SCOBY while offsetting the acidity. The sweet-sour flavour of the juice will reduce the amount of artificial flavouring that is typically added during the F2 phase of production. Initial research on fruit based kombucha indicates numerous benefits. Using the juice of fruit allows the SCOBY to produce changes that increase the growth of the bacteria Komagataeibacter, resulting in a more efficient formation of cellulose pellicles. Furthermore, using the juice from *Physalis* to produce kombucha will increase the antioxidant levels by 20 to 60%, through the development of glycosylated flavonoids, as well as create new compounds of ellagic acid-glucuronide that are more bioavailable[11].

The fermentation process through which Kombucha is produced produces a wide variety of metabolites (100+) which can be classified as follows: organic acids such as acetic acid, gluconic acid, and glucuronic acid (which are responsible for their antimicrobial properties, as well as stabilizing the pH), polyphenols produced through the fermentation of tea catechins (where many antioxidants are produced), B-vitamins (which are produced by yeast), hydrolytic enzymes, and trace minerals such as zinc, iron, manganese[12]. The benefits documented in vitro include a high level of antioxidant activity (measured by the DPPH and ORAC assays), antihyperglycemic properties, and hepatoprotective properties due to glucuronic acid's (detoxification) and synergistic effects with polyphenols on gut microbiota. While some studies have shown a low level of clinical evidence to date[13], limited human studies have shown a small but significant reduction in LDL cholesterol levels and better blood glucose control after eating; however, larger clinical trials will be necessary to confirm this. In addition, kombucha's safety profile is generally regarded as good, provided fermented products maintain an acid pH (<4.2) to prevent pathogenic growth, and that monitoring occurs for the accumulation of ethanol (>0.5% for alcohol) or contamination (e.g., Aspergillus Molds)[14].

Methodology

This review is based on an analysis of previously existing literature on the preparation and fermentation process of kombucha in a traditional way. The sources evaluated include peer-reviewed articles and authoritative publications on kombucha and its methods of fermentation.

Kombucha is prepared by the process of fermenting a sweetened substrate using a SYMBIOTIC CULTURE OF BACTERIA AND YEAST, commonly referred as SCOBY [15]. First, the fermentation medium is prepared and cooled to room temperature. This medium is then inoculated with SCOBY. To initiate the fermentation process, a portion of previously fermented medium is added.

Primarily, the fermentation process takes place in a steady and aerobic environment for a defined period, during which the microbial blend metabolizes sugars in the medium and produces organic acids, carbon dioxide, ethanol, and other bioactive compounds. These factors are responsible for the reduction of pH, with the development of desired sensory and functional properties of kombucha. In some traditional brewing techniques, there may be a secondary fermentation process after the primary fermentation. In this process, fruits/berries can be added to the resulting liquid in order to further develop its flavor, aroma, and bioactive properties [16]. This process is normally shorter than the primary fermentation process.

The methodological framework presented in the current review represents traditional preparation methods of kombucha as reported in the scientific literature, forming the basis for the fermentation process and functional modifications.

Evaluation of physiochemical properties

The pH of Kombucha samples are routinely tested during fermentation. This routine testing helps us to track how acidic the kombucha is as it ferments. The pH level indicates how many organic acids are produced by the microorganism, which affects both the flavor and the safety of the finished product [17].

Total soluble solids (TSS) measures the amount of sugar that has been used up by the yeast during fermentation. TSS is commonly noted on the scale of °Brix, and is routinely measured using refractometry to follow fermentation dynamics [17].

Total phenolic and total flavonoid contents

The total phenolic content and total flavonoid content are measured using a colorimetric method such as the Folin-Ciocalteu method (phenolic) and the aluminum chloride method (flavonoid), respectively. The phenolic and flavonoid compounds found in kombucha throughout the fermentation process yield numerous health-related antioxidant properties [17].

In Vitro Antioxidant Activity

Antioxidant capacity is evaluated through free radical scavenging assays, such as DPPH, ABTS, and FRAP to determine the potential for kombucha extracts to neutralize free radicals. These methods collectively offer valuable information on the in vitro antioxidant strength of bioactive compounds formed during the process of kombucha production [18].

High-Performance Liquid Chromatography Analysis

High-performance liquid chromatography (HPLC) is to determine the quantity of organic acids and other by-products of fermentation present in kombucha. HPLC will help identify the chemical composition of kombucha and how it relates to its functional properties [18].

Antimicrobial Activity

Antimicrobial activity assays are done by measuring the presence of a dilution broth where the Kombucha sample directly inhibits the growth of microbial pathogens through a quantitative dilution method. Both measurements reflect the functional benefit of the beverage against microbial pathogens [18][19].

Anti-Inflammatory Activity

The MTT cell viability assay measures the ability of a kombucha sample to suppress inflammation through measuring the expression of inflammatory mediators such as Nitric Oxide. This reflects the immunomodulatory effects of Kombucha Metabolites [18].

Research Gap

Physalis Peruviana has potential in areas of food, medicine, and other uses; however there has not been much clinical research completed on it's bioactive compounds (i.e. withanolids, polyphenols) for standardization, validation and large-scale use. Use of this fruit has received some interest from developers of functional foods; however there remain significant opportunities for use of its byproducts (i.e. calyx pectin) as both food preservative and potential nutraceuticals.It is used for developing pharmaceuticals has not resulted in isolating important pharmacologically active compounds with rigorous testing, and as such there has been limited research into using the fruit as functional foods. There is an important need to study its by-products (i.e. seeds and pomace) and their properties in comparison to the fruit in order to further understand their functional food application potential. There is also a high level of challenge created by the perishable nature, after it has been harvested, along with the lack of opportunity for consumers to consume approximately 50% of the non-usable fruit and what remains after processing into dehydrate or pectin-based products. For the formulation of food products (nectars with the highest concentration of antioxidant activity) surface methodology will assist in determining the most effective processing techniques [23]. Traditional medicinal use has typically been for treatment of gastrointestinal, dermatological or metabolic disorders; therefore, it is crucial to isolate and evaluate pharmacological activity of the phytochemicals prior to conducting clinical trials. There remain insufficient studies investigating the interaction of combinations of plants and their effects on drug pharmacology, along with limited data for dosing, posology and new delivery methods beyond oral decoction (witch's brew). The development of new medications for cancer, diabetes and cardiovascular disease is a current priority with little evidence of efficiently and effectively extracting this fruit [24]. This fruit grows best in sandy loam soils with good drainage and a pH range of 5.5 to 6.8, and requires between 1,000 and 2,000 millimeters of rainfall. There is currently no research about how to provide irrigated or optimized

fertilizers in areas outside the Andes. Research regarding post-harvest losses is very limited, and it is estimated that 50% of the Colombian yield is not suitable for export due to the variability in size of the fruit. There needs to be more field trials for the cultivation, trellising and adaptation of micro-climate for global expansion [25]. Bioactive compounds and chilling tolerance remain under-emphasized through CRISPR technology and other methods using genetic transformation on available genomic resources and transcriptomic resources for biodiversity. Cold-stressed fruit has improved quality with the application of foliar amino acids; however, breeding for the robustness of this in zone 8 continues to be unintegrated into breeding programmes. There is a need for additional multi-omic research to enhance the phytomedicinal properties of plants using model systems [26].

There has been a lack of research on certain areas of Kombucha, for instance scientific study in food processing and pharmaceuticals, and many of the related disciplines, such as Biomaterials and Sustainable Technology due to issues with standardisation and therefore little follow-up research, have resulted in Kombucha's potential remaining largely unexplored. Recent bibliometric approaches have highlighted an increasing breadth of research regarding the exploitation of SCOBY, and its isolation, through pharmacologic validation of its uses. Issues remain Open concerning standardisation in fermentation; these include questions regarding the degree of uniformity of microbial flora diversity, as well as the role of the substrate in defining important characteristics of the product. The life-cycle analysis of the Kombucha fermentation process is still in its infancy. For example, while there is growing interest in the use of bacterial cellulose from Kombucha as an alternative to plastics for packaging, the scope of sustainable production continues to be far from a reality. There is a growing interest in developing alternative initial feedstocks for the Kombucha fermentation process (i.e. utilisation of alternative substrates such as fruits and milk) but there is currently little information available about their metabolite pathways of activity. While there is already interest in exploring the pharmaceutical properties of Kombucha bioactive components (such as antioxidant activity), confirmation of the pharmacologic action of purified bioactive components (e.g. probiotics) in humans remains to be demonstrated. The fermentation process and the medicinal use of fermentation to change and regulate metabolic activity have not been extensively researched. The existing evidence from using such medicinal practices only exists in the early stages of research into the types of medicinal products containing non-viable living microorganisms. The microbial classification scheme, as well as the SCOBY, is lagging behind in phenotyping. The application of nanocellulose produced from SCOBY is being utilized for biomaterials; however, very little research has been conducted into its potential to be produced on a large scale together with other biomaterials. The patenting process shows that there are innovative ways to manufacture these materials using fermentation; however, there currently does not exist sufficient global collaboration or an evaluation of the potential environmental impacts created during the production process [20][21][22].

Conclusion

It is an alternative fermentation substrate for kombucha due to its higher ORAC values (3-5 times greater), natural acidic composition (pH 3.2–3.8), and abundance of bioactive compounds (flavonoids, ellagic acid, and Vitamin C), in addition to allowing for the incorporation of

glycosylated metabolites produced by the SCOBY and the improved pellicle production by Komagataeibacter [21][27].

Although there is a strong alignment between the fruit and kombucha (i.e., both have the potential to result in a 20-60% increase in antioxidant capacity), there have been no published studies investigating the integration of these two products. This lack of study creates a critical research opportunity for future studies to assess the F1/F2 fermentation responses under PRISMA guidelines at a temperature of 25 °C for 7 to 14 days at a Brix of 12 to 16, and to perform HPLC bioaccessibility testing and RCTs to support claims of glycemic/hapatoprotective activity of kombucha produced in tropic agricultural settings such as Haryana. By working together (i.e., combining Andean Agronomy, fermentation omics/fermentation biosafety, and BET biosafety research/implementation) to develop scalable functional beverages, the two products can be marketed to meet growing demands for both the kombucha market (currently worth $3.6 billion) and the untapped goldenberry market (currently, it is estimated that only 50% of post-harvest yield is being utilized) [28].

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