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Analysis of the Characteristics of Cellulose Films Generated by Black Organic Tea Kombucha Utilizing Various Carbon Sources

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Abstract

The aim of this study was to evaluate the production of microbial cellulose films (MCFs) in culture media based on Black Organic Tea Kombucha and different carbon sources, using two microbial consortia KN and KD. It highlights the importance of biodegradable materials in light of environmental concerns. The study investigates how microbial strain composition and carbon sources affect MCF properties, focusing on the production efficiency of two microbial consortia using different carbon sources. The objective is to advance sustainable biomaterial development in Vietnam by using kombucha fermentation by-products and encouraging collaboration between consumers and material scientists. The microbial consortia KD and KN produced MCFs with a homogeneous, thick appearance, slight flexibility, and the characteristic brown color of the fermentation medium. The highest yields for KD were 10.2 (g/l) and 0.49 (g/l) (fructose), and were 8.9 (g/l) and 0.32 (g/l) (sucrose) for KN on a wet and dry basis, respectively. This research aims to contribute to waste reduction and the advancement of biomaterials in Vietnam.

Keywords: *Microbial cellulose films (MCFs), SCOBY, Kombucha, Biomaterials, fermentation*

1. Introduction

With the increasing severity of the environmental issue of untreated waste accumulation in recent years, significant focus has been directed into the research and progress of biomaterials [1-2]. Regarding possible alternative materials, cellulose as an abundant and biodegradable source with various other advantageous physical properties is considered highly [3-7]. While cellulose extracted directly from plants is proven to be demanding and inefficient, cellulose obtained from another source, specifically from SCOBY (symbiotic consortium of bacteria and yeasts), has more potential [3-5,7]. Previous studies has shown that microbial cellulose films (MCFs) produced from SCOBY have a higher level of purity, degree of polymerization, crystallinity, mechanical strength, water

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holding capacity, chemical stability, biological adaptability, high productivity, excellent biocompatibility, etc., making them extremely potential [3-5,7]. es is considered highly [3-7]. While cellulose extracted directly from plants is proven to be demanding and inefficient, cellulose obtained from another source, specifically from SCOBY (symbiotic consortium of bacteria and yeasts), has more potential [3-5,7]. Previous studies has shown that microbial cellulose films (MCFs) produced from SCOBY have a higher level of purity, degree of polymerization, crystallinity, mechanical strength, water holding capacity, chemical stability, biological adaptability, high productivity, excellent biocompatibility, etc., making them extremely potential [3-5,7].

Populations of microorganisms that are part of SCOBY normally include different bacteria such as Komagataeibacter xylinus, Acetobacter aceti, Acetobacter pasteurianus, and Gluconobacter oxydans, and yeasts such as Brettanomyces, Zygosaccharomyces, and Saccharomyces [3-5,7,9]. On one hand, Kombucha tea is a slightly alcoholic beverage that was obtained from the fermentation of SCOBY in infused sweetened tea (Camellia sinensis), usually the black tea. To produce final Kombucha, the microorganisms consume the added sugar as the main carbon source. During this process. SCOBY produces a thick cellulosic floating biofilm composed of cellulose, which will eventually thicken, often forming multiple pancake-like layers on the top [8-10]. Eventually, this film is mostly discarded as a by-product. However, it is now under research to be commercially utilized to create a new biomaterial due to its mentioned potential.

The culture medium, kind of sugars, nutrients, pH level, temperature, dissolved oxygen, fermenting duration, and diversity of microbes are some of the elements influencing the SCOBY fermentation process in kombucha [4,5,11]. The lack of productive fermentation systems to boost the yield of MCFs from sugars is one of the primary barriers to the production of large-scale, inexpensive microbial cellulose, causing BC commercialization to remain still in its early stages and is concentrated on high-value niche markets [4,11-12]. Nevertheless, since the focus emphasizes on improving the production yield of MCFs [10,11-16], different carbon sources—including xylose, saccharose, maltose, fructose, cellobiose, mannitol—are taken into account as well as investigated.

Moreover, the carbon source used during the fermentation process and the microbial strains that synthesize this polymer determine the properties of MCFs, such as their mechanical properties, water and oxygen vapor transmission rates, as well as their surface area, permeability, level of polymerization, molecular weight, crystallinity index (67%–96%), average crystallite size (5.7–6.4 nm), intrinsic viscosity [11,16]. The primary variations among the examined components are associated with infusions of Kombucha tea [11,13], fermentation periods and circumstances [11,17], and isolated consortia [18].

Ultimately, the microbial consortia and the carbon source utilized to produce this polysaccharide are the primary variables of the MCFs quality. Therefore, the aim of this work was to evaluate the efficiency of two microbial consortia, KN and KD, in producing MCFs in a culture medium using traditional black organic tea and various carbon sources such as glucose, dextrose, fructose, and saccharose. This is a new sector in Vietnam, where the field of biomaterials has a lot of opportunity to grow and kombucha is mostly renowned for its health-related advantages. To enhance our understanding of how Kombucha consumers and material scientists in Vietnam can collaborate to develop a novel waste reduction approach, we aim to utilize the SCOBY, currently available on the Vietnamese open market, and conduct a scientific study.

2. Materials and Methods

2.1. Materials

The black organic tea used as a substrate during the fermentation process was purchased from "FOODPLUS IMPORT AND EXPORT COMPANY LIMITED". On the other hand, sugars such as glucose, dextrose, fructose, and sucrose, were analytical reagents purchased from chemical store in the University of Science and Technology of Hanoi (USTH). The kombucha cultures used as starters for the fermentation process provided by FOODPLUS IMPORT AND EXPORT COMPANY LIMITED with known species of bacteria according to standard regulations of alcoholic beverages in Vietnam. The two microbial consortia are specified in order as KD and KN.

Machinery and equipment:

Refractometer (brix): Brand: Total Meter. Code RHB-92T.

Drying oven in laboratory: Model: 101-2A, Origin: China, Electronic microprocessor controller, Drying temperature: 0 - 250 degrees C, Error: ±1 degrees C, Power source: 220 V - 50Hz.

pH meter: Manufacturer: HORIBA Model: PH1200-S, Origin: Japan.

UV-Vis Spectrophotometer: Model: DR6000, Manufacturer: HACH, Origin: Germany.

2.2. Preparation of Kombucha cultures and Fermentation

This was based on previous studies with some modifications [11,15,18,19]. In this process, four different carbon sources were evaluated: Glucose, dextrose, fructose, and saccharose. The methodology was carried out as described in previous studies but with some modifications related to water temperature, amount of sugar, and fermentation time.

First, the culture necessary for further analysis of the fermentation process was incubated in the first place. Two liters of water were heated until the temperature of the solution reached 85 ◦C. Next, 8g of black organic tea was added to the solution (concentration: 4g tea/liter). The medium (250 mL) was then dispensed into eight glass containers (1L capacity, sterilized by boiling before). Consequently, 12.5 g of sugar was added to each container to obtain two containers for each sugar. The solutions were allowed to settle for 15 min and then moved into a cold bath to reach the room temperature (25±2◦C). Two different kombucha cultures, KD and KN, were used as each SCOBY was cut into four separate smaller cultures. The cultures were placed respectively in the sterilized glass containers with the tea-sugar solution and kept in the covered with gauze for 15 days at room conditions.

After the incubation process, the solution in each container was used to incubate another generation to analyze its fermentation process. 800 mL of water were heated to a temperature of 85 ◦C. Once this temperature was reached, 3.6g of black organic tea was added to the water (4g tea/liter). The medium (100 mL) was then dispensed into glass flasks (250 mL capacity) and inoculated with 10% (V/V) of the KD and KN cultures (10 mL each), obtained during the previous stage. The bottles were covered with gauze and kept in storage at room conditions for 17 days for analysis.

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2.3. Physicochemical Analysis of Fermentations

Determination of pH, Total Acidity, and a Total of Soluble Solids

The pH was determined using a multiparametric pH meter, previously calibrated to a pH of 4.0 and 7.0. Each sample solution was taken out in a tube and allowed to settle for analysis. The result was read among the most stable numbers recorded [11,20].

The content of total soluble solids (TSS) was determined using an analogous hand refractometer and the results were expressed in ◦Brix. A drop of each sample solution was used for analysis [11,20].

Titratable acidity (TA) was determined based on previous studies with some modifications [11,18,19]. Briefly, 5 mL of the fermentation was put in a flask (50mL capacity) and titrated with a standard solution of NaOH (0.1M), with the use of phenolphthalein as an indicator. The results were expressed as a percentage of acetic acid based on the following equation:

acetic acid (%) = (NaOH mL) (M NaOH) (0.06)/(sample mL) × 100

Determination of Total Sugars and Proteins

The determination of total sugar content was carried out using the phenol-sulfuric acid method with some modifications. First, 10 µL of the sample was added to test tubes containing 15 of water. Subsequently, 1 ml of phenol 5% and 5 mL of concentrated sulfuric acid (96%) was added to these mixtures and they were slightly stirred. The solutions were allowed to settle for 10 min at room temperature (25 ± 2 ◦C) and then were vortexed for 30 seconds before being placed in a cold-water bath for 20 min. The samples were analyzed in a spectrophotometer at 472 nm [11,21,22].

The quantification of total proteins was carried out using the Bradford technique. Briefly, Bradford reagent (8 mL, produced according to standard formula) and sample solution (2 mL) were added to the test tubes; the solutions were allowed to settle for 5 min and subsequently, they were measured in a spectrophotometer at 595 nm [11,23-25].

Production of MCFs

The generation of MCFs in the different culture media was monitored [11,25], through observations and changes in the clarity of the culture medium (clear/cloudy) every 3 days, during the 17 days of fermentation.

Determination of Wet Weight, Dry Weight, and Yields of the MCFs

At the end of the fermentation process (day 17), the MCFs produced in the different culture media were carefully removed with the help of a spatula. Moisture excess was removed with paper towels, and the fresh weight was determined on a digital scale. Subsequently, the MCFs were placed in an oven at 45 ± 2 ◦C until a constant dry weight was obtained. Wet weight and dry weight results were expressed in grams [11,13].

3. Results

3.1. pH, TSS and TA

Table 1. Chemical composition of kombucha fermentations with various carbon sources stored at 25 ◦C for 17 days.

pH	Initial (Day 0)		Final (Day 17)	
Carbon Source	pH			
	KĐ	KN	KĐ	KN
Glucose	B3.61 ± 0.01a	A3.25 ± 0.02a	B43.04 ± 0.01b	A2.98 ± 0.07b
Dextrose	AB3.58 ± 0.03a	A3.43 ± 0.01a	A3.07 ± 0.01b	A3.00 ± 0.19b
Fructose	A3.96± 0.00a	B3.79 ± 0.01a	C3.48 ± 0.04b	A3.31 ± 0.26b
Sucrose	AB4.05 ± 0.04a	A3.71 ± 0.01a	D3.32 ± 0.02b	A3.32 ± 0.06a
Carbon Source	TSS (◦Brix)			
	KĐ	KN	KĐ	KN
Glucose	C4.20 ± 0.07a	A4.20 ± 0.35a	A2.90 ± 0.17b	A2.60 ± 0.12b
Dextrose	A4.30 ± 0.03a	A4.00 ± 0.05a	AB2.80 ± 0.59b	B2.90 ± 0.09b
Fructose	A4.80 ± 0.03b	A4.90 ± 0.02a	A3.10 ± 0.11b	AB2.70 ± 0.62b
Sucrose	B4.90 ± 0.01a	A4.90 ± 0.12b	A3.70 ± 0.14a	A2.40 ± 0.12a
Carbon Source	Total Acidity			
	KĐ	KN	KĐ	KN

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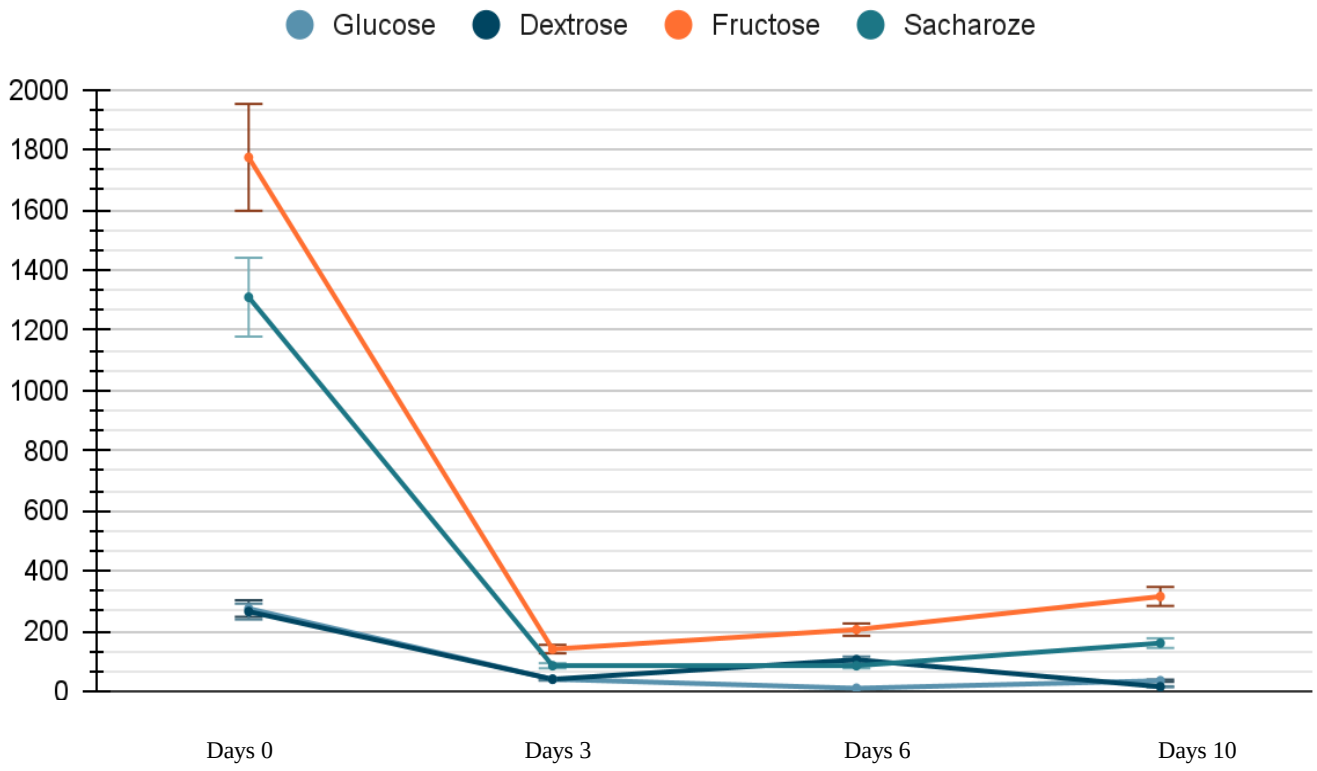
Glucose	A0.25 ± 0.03a	A0.28 ± 0.02a	B2.83 ± 0.08b	AB2.73 ± 0.14b
Dextrose	A0.31 ± 0.03a	A0.30 ± 0.05a	AB2.22 ± 0.59b	B2.24 ± 0.09b
Fructose	A0.18 ± 0.14a	A0.25 ± 0.28a	A1.48 ± 0.06b	A1.79 ± 0.38b
Sucrose	B0.17 ± 0.14a	A0.24 ± 0.57a	A1.40 ± 0.35b	A1.60 ± 0.06a

Note: Mean ± standard deviation. Letters in columns (A, B, C) indicate significant differences between the carbon sources, and letters in rows (a, b) indicate significant differences between the fermentation time (days 0 and 17)

3.2. Total of Sugars and Protein Content

Total of Sugars (ug/ml) with Type KN



Total of Sugars (ug/ml) with Type KD

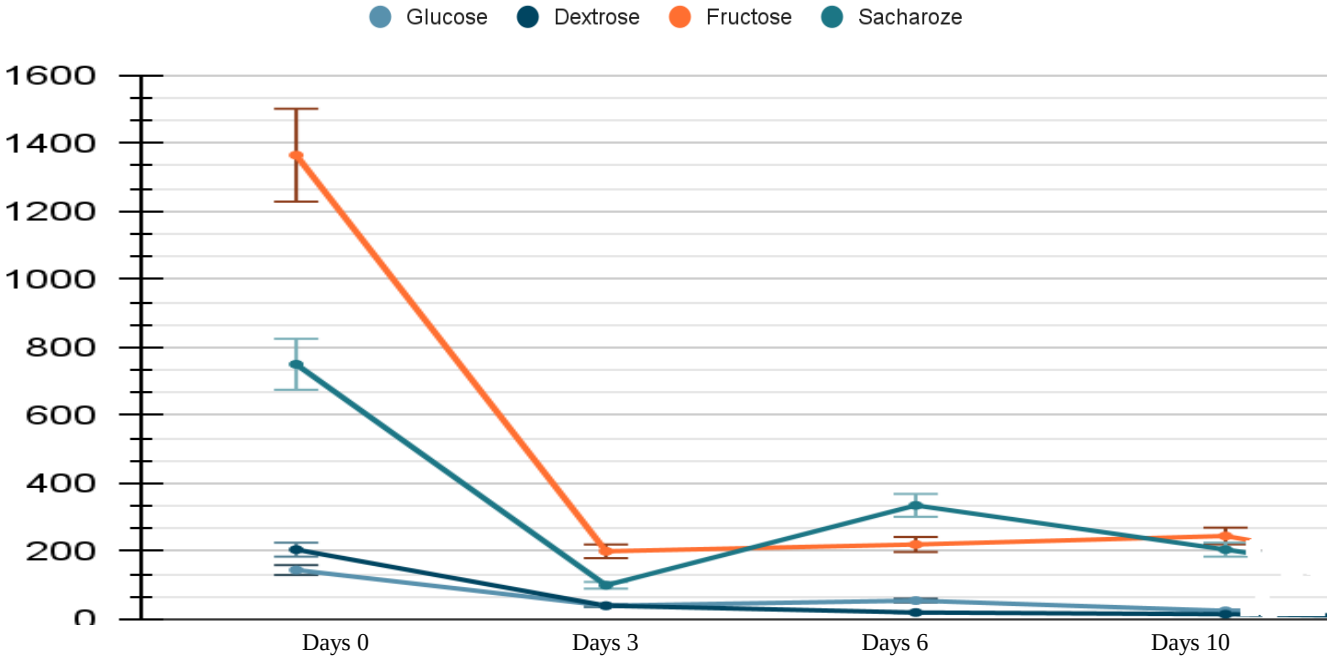


Fig.01

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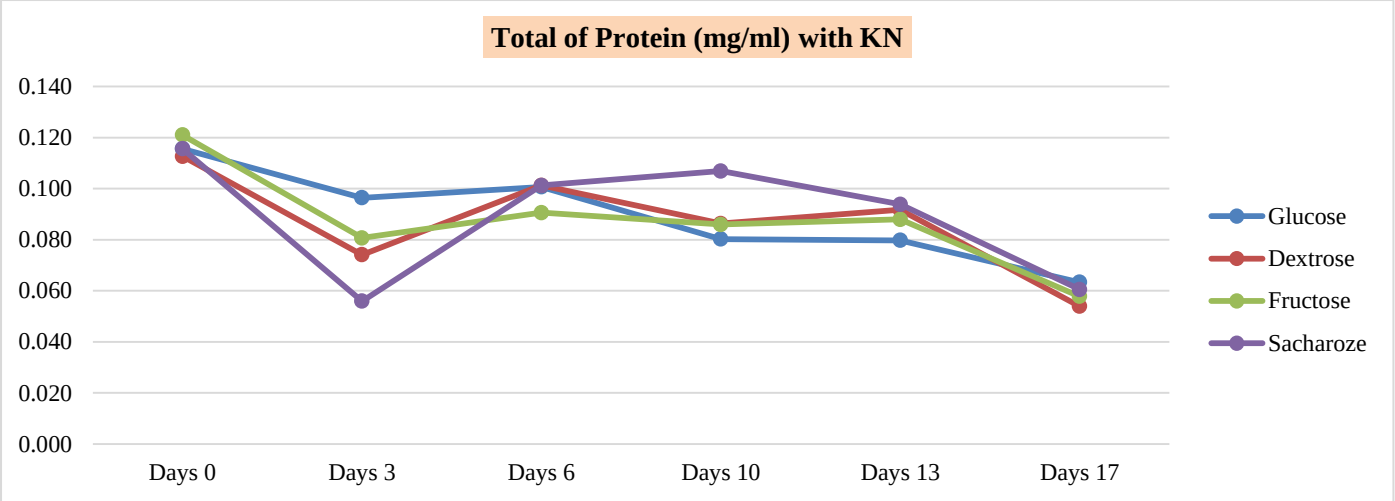

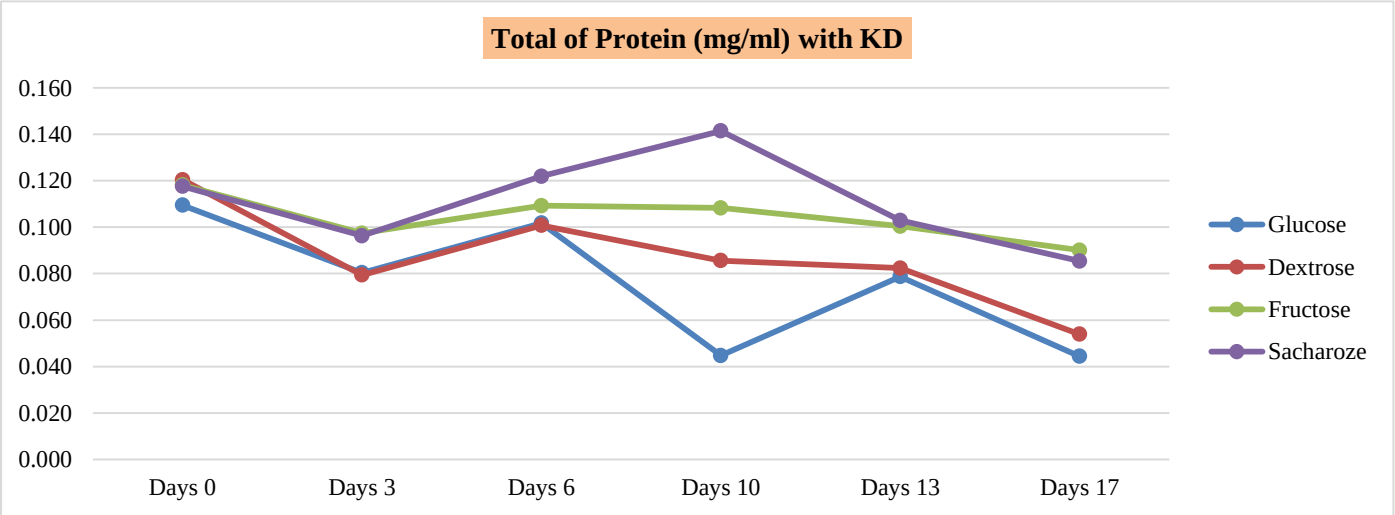

Fig.02

3.3. Formation of MCFs

Table 2. Changes in the clarity level of kombucha fermentations with various carbon sources stored at 25 ◦C for 17 days.

	KD			
Storage (Days)	Glucose	Dextrose	Fructose	Sucrose
0	Very Clear	Very Clear	Very Clear	Very Clear
3	Turbid	Clear	Turbid	Clear/Turbid
6	Turbid	Turbid	Turbid	Turbid
10	Turbid	Very Turbid	Turbid	Very Turbid
13	Very Turbid	Very Turbid	Very Turbid	Very Turbid
17	Very Turbid	Very Turbid	Very Turbid	Very Turbid
	KN			
Storage (Days)	Glucose	Dextrose	Fructose	Sucrose
0	Very Clear	Very Clear	Very Clear	Very Clear
3	Clear/Turbid	Turbid	Clear	Clear/Turbid
6	Turbid	Clear/Turbid	Clear/Turbid	Turbid
10	Very Turbid	Turbid	Turbid	Very Turbid
13	Very Turbid	Very Turbid	Very Turbid	Very Turbid
17	Very Turbid	Very Turbid	Very Turbid	Very Turbid

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3.4. MCFs Yields

Yields of the microbial cellulose films obtained from KD and KN consortia after 17 days of fermentation at 25◦C.

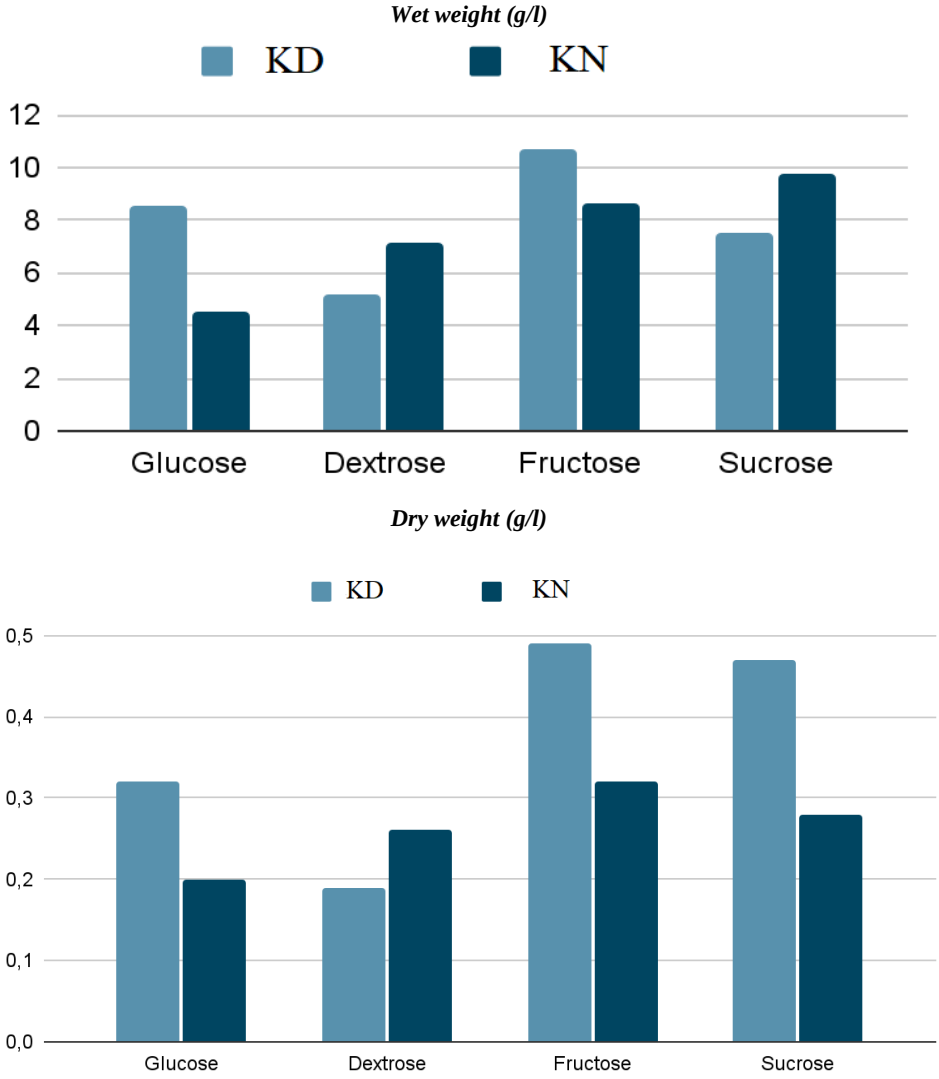

Fig.3

4. Conclusion

In general, during the fermentation process of the KD and KN consortia in the black organic tea with glucose, dextrose, fructose, and saccharose, a reduction in the content of TSS and pH was observed, as well as an increase in the acidity content, indicating the consumption of the carbon source and the development of acetic acid-producing bacteria. Furthermore, alterations in the overall sugar concentration throughout fermentation signified the use of various carbon sources and the synthesis of microbial cellulose in the culture media. Similarly, the alterations observed in protein composition indicated the utilization of the nitrogen source and the microbial proliferation during the fermentation process. During days 10 and 13, turbidity and cellulose production increased in the distinct culture media; the preferred substrate for KD was fructose, while for KN was sucrose, followed by dextrose and glucose in both consortia.

Additionally, KD and KN produced MCFs with a homogeneous, thick appearance, slight flexibility, and the characteristic brown color of the fermentation medium. The highest yields for KD were 10.2 (g/l) and 0.49 (g/l) (fructose), and were 8.9 (g/l) and 0.32 (g/l) (sucrose) for KN on a wet and dry basis, respectively. Finally, further investigation into the MCFs made by the KD and KN consortia is required to learn about their mechanical, chemical, and physical characteristics so that we can assess their potential uses and find ways to enhance them.

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