Optimisation of Kombucha fermentation

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DEDICATION

This Thesis is dedicated to my mother, Nanda Navin Gangude, father Navin Sonaji Gangurde, and brother, Akash Navin Gangurde.

Thank you all for your love, support, and guidance!

ABSTRACT

Kombucha is a slightly acidic, self-carbonated and refreshing fermented beverage made from sugared tea by the symbiosis of yeast and acetic acid bacteria (AAB). The symbiotic culture exists in a biofilm commonly called 'symbiotic culture of bacteria and yeast' (SCOBY) and fermented broth. Kombucha is popular in many regions of the world and its production relies on natural fermentation by an undefined complex mixed starter culture. Therefore, the fermentation process results in products of variable quality among the producers and even by the same individual processor. The efficiency of non-alcoholic Kombucha fermentation is determined by the final ethanol content, residual sugar and acidity at the end of fermentation. Kombucha beverages sold in the market are biologically active, therefore the fermentation continues during storage as the yeast and bacteria thrive on the residual sugar present in the final product. During storage, secondary fermentation generates the desired carbonation and also reduces residual sugar in the final products, but the microbial activity may also result in the production of beverages with higher alcohol levels than permissible. In New Zealand, Kombucha is categorised as a non-alcoholic beverage with alcohol of no more than 1.15% ABV or 0.5 % ABV (Australia). Kombucha brewers generally find it difficult to produce consistently high-quality products with low alcohol as stipulated by the Food Standards Australia New Zealand regulations. The present study aimed to optimise the fermentation process using robust experimental plans to produce a stable low alcohol fermented Kombucha with low sugar.

The present research work was conducted in three Phases and the experiments were repeated twice with duplicate analyses. Phase 1 involved selection of the best fermentation conditions to propagate the Kombucha starter culture comprising 'fermented broth and cellulose pellicle (SCOBY)' using fermented broth as the starting material. The factors used in this Phase were sugar (3% and 4.7%, w/v) and tea (0.3% and 0.5%, v/v), *inocula* (12.5% and 20%, v/v), fermentation temperature (22°C and 24°C) and the formulations were fermented for 14 days. To select the best propagation conditions for the growth of starter culture comprising SCOBY and fermented broth, acidity, total soluble solids (TSS), weight of SCOBY and microbial concentrations (yeast, AAB, total counts) were determined during fermentation. Yeast were enumerated using yeast extract glucose chloramphenicol agar (YGC) agar, AAB were enumerated by yeast extract peptone mannitol (YPM), and total counts by plate count agar (PCA). The best fermentation conditions for the growth of the culture were then selected and adopted for use in the subsequent experiments.

Phase 2 investigated the best conditions for the fermentation of Kombucha using a combination of SCOBY and fermented broth as starter culture, in two stages (Stage 1 and Stage 2). Two sugar concentrations (3% and 4.7%, w/v) and two fermentation temperatures (22°C and 24°C) were used in Stage 1 to determine the optimum sugar concentration and fermentation temperature required to ferment Kombucha for 9 days (primary fermentation). Stage 2 investigated the effect of filtration on Kombucha during storage (4°C) for a week, post-primary fermentation (9 days). Physico-chemical (TSS, acidity, colour) and microbiological (yeast, AAB, total counts) characteristics of the beverages were determined in the two stages including the measurement of colour (CM-5 Konica Minolta spectrophotometer Japan). The beverages were also evaluated by 5-7 focus sensory panellists in Stage 1 and consumer sensory panellists (n=35) in Stage 2, using a 9-point hedonic scale.

The storage stability (4 °C) of the selected formulations from Stage 2 (Phase 2) were evaluated in Phase 3 for three weeks (4℃). In addition to the analyses conducted in Phase 2, concentrations of sugars, ethanol organic acids and antioxidants were determined during fermentation and storage. The developed beverages were also evaluated by consumer sensory panellists (n= 108). Results showed that the formulation (Phase 1) that contained 3% sugar, 0.5% tea, 20% fermented broth and fermented at 24℃ produced the best growth of the starter culture.

In Stage 1 (Phase 2), titratable acidity (T.A.) and microbial counts increased ($p<0.05$), while pH, TSS ($p<0.05$) and overall acceptability by a focus sensory panel decreased for samples fermented with low sugar concentration (3%, w/v) at 22℃ and 24℃. In Stage 2, T.A. and microbial counts increased ($p<0.05$), while pH, TSS and consumer sensory scores decreased ($p<0.05$) for the Kombucha samples (filtered and unfiltered) fermented at 24 ℃. Samples (filtered and unfiltered) containing 4.7% sugar and fermented at 22 °C received higher overall consumer sensory scores (7.30±0.32 ;6.98 ±0.19) compared to the sample that contained 4.7% sugar and fermented at 24℃. Therefore, this formulation (4.7% sugar; fermentation temperature 22℃) was selected for further evaluation of the filtered and unfiltered samples in Phase 3.

In Phase 3, the filtered beverage containing 4.7% sugar, 0.5% tea, 20% fermented broth and fermented at 22℃ received the highest sensory scores for overall acceptability (7.26±0.88) after storage for three weeks (4°C). The lower acidity, ethanol, and sugar (sweetness) of filtered samples ($p<0.05$) compared to unfiltered samples may have contributed to the better overall liking by the panellists. The lower levels of organic compounds in the filtered sample may be due to reduced metabolic activities caused by the partial removal of trapped cultures in the matrices of the cellulose during filtration. The colour of the fermented beverages was stable during fermentation and no significant changes were observed during storage for 3 weeks ($p<0.05$). Overall, the filtered sample contained higher concentrations of antioxidants than the unfiltered which may be beneficial to human health.

In conclusion, low alcohol Kombucha was successfully produced after filtration, post-primary fermentation at low temperature. The ethanol content of the filtered beverage was <0.5% ABV, which complied with the Australia NZ Food Standards Authority regulation. Furthermore, the residual sugar in the filtered beverage was markedly lower than reported in previous studies. The optimised beverage was stable during refrigerated storage and was well accepted by sensory panellists. The beverages had a pH<4.6, which is generally considered as safe. Therefore, the optimised Kombucha fermentation process resulting from this study has potential for commercialisation. However, further work is required to integrate the filtration step and scale-up.

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LIST OF ABBREVIATIONS

μg	microgram
a*	redness – greenness
AAB	acetic acid bacteria
ABV	alcohol by volume
ACS	acetyl-COA synthetase
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
AOAC	Association of Official Analytical Chemist
ASH	alcohol dehydrogenase
ATP	Adenosine triphosphate
b*	yellowness – blueness
BC	Bacteria cellulose layer
BT	Black tea
Ca	Calcium
Cfu	Colony forming per Unit
CP	cellulose pellicle
Cu	Copper
DHA	Dihydroxyacetone
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
EMP	Embden- Meyerhof Parnas
FB	Fermented broth
FDA	Food and Drug Administration
Fe	Iron
FKB	Filtered Kombucha Beverage
FSANZ	Food Standards Australia New Zealand
FT	Fermentation temperature
g	gramme
GAE	Gallic acid equivalent
GC	Gas chromatography
HPLC	High performance liquid chromatography
K	Potassium
KHP	Potassium hydrogen phthalate
KTB	Kombucha sugared tea broth
L*	lightness
LAB	lactic acid bacteria
Min	minute
mL	millilitre
mm	millimetre
Mn	manganese
Na	Sodium
NaOH	Sodium hydroxide
NZ	New Zealand

PCA	Plate count agar
PDH	pyruvate dehydrogenase
PSC	pyruvate decarboxylase
RI	refractive index
RO	Reverse Osmosis
ROS	reactive oxygen species
S. cerevisiae	*Saccharomyces cerevisiae*
SCOBY	Symbiotic culture of bacteria and yeast
T.A.	Titratable acidity
TCA	tricarboxylic acid
Temp	Temperature
TFA	trifluoroacetic acid
TSS	Total soluble solids
UKB	Unfiltered Kombucha Beverage
UV	ultra violet
WoB	weight of empty beaker
WS	White sugar
YGC	Yeast extract glucose chloramphenicol
YPM	Yeast peptone mannitol

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Chapter 1. INTRODUCTION

1.1 Background

Demand for fermented functional foods that support health and wellbeing has increased significantly since 1999 (Flachs & Orkin, 2021; Marshall & Mejia, 2011; Vergari et al, 2010). Dairy-based fermented foods date back to 1000 BC and have a long history in the diet of humans as they are presumed to have evolved when pastoral farming commenced. However, dairy based products may not be suitable for lactose-intolerant individuals due to the presence of residual lactose. This has led to rapid development of several plant-based fermented products including the beverage Kombucha which has been made and consumed for over 2000 years (Baschali et al., 2017; Ranadheera et al., 2017; Valero-Cases et al., 2020).

Kombucha is characterised by sourness and fizziness due to the production of organic acids and natural carbonation, respectively, during fermentation (Chakravorty et al., 2019; De Roos & De Vuyst, 2018). The exact origins of Kombucha are not known, but available information traces the beverage to north east China around 220 B.C. (Jayabalan et al., 2014). Kombucha is prepared from tea and sugar (substrates), then fermented by a symbiotic culture of bacteria and yeast. The Kombucha starter culture is commonly called ‘symbiotic culture of bacteria and yeast’ which is abbreviated to SCOBY. The Kombucha starter culture consists of fermented liquid broth and cellulose pellicle layer, which contain the fermenting the microorganisms. The starter culture mainly contains acid-producing acetic acid bacteria (AAB) and osmophilic yeast, with occasional low levels of lactic acid bacteria (LAB) (Villarreal-Soto et al., 2018; Jayabalan et al., 2014; Marsh et al., 2014; Sievers et al., 1995). The substrates which are added during fermentation provide carbon, nitrogen and essential elements required for the growth of fermenting microbes (Jayabalan et al., 2014; Watawana et al., 2017). White (cane) sugar is commonly used in Kombucha fermentation, as it can be easily metabolised by yeast to produce CO_2 and ethanol (Goh et al., 2012). However, other sources of carbon such as honey, jaggery and unrefined sugar have also been used in Kombucha fermentation (Watawana et al., 2017). Black tea used in Kombucha fermentation is a rich source of flavonoids and catechins which may confer health benefits to the consumer (Malbaša et al., 2006; Vinson & Dabbagh, 1998). The symbiotic interaction between the fermenting microbes influences the overall characteristics of Kombucha which include physico-chemical sensory, microbiological properties (May et al., 2019; Sievers et al., 1995; Teoh et al., 2004).

The fermentation of Kombucha is complex, involving a series of biochemical reactions During fermentation, the yeast invertase metabolises the added sucrose into glucose and fructose. Fructose is then metabolised by the yeast to produce ethanol and carbon dioxide via the glycolytic pathway (Villarreal-Soto et al., 2018; Vargas et al.,2021). The ethanol produced and any remaining (residual) fructose are then metabolised by the AAB to form acetic acid (Lin et al.,2013; Villarreal-Soto et al., 2018). Meanwhile, glucose is metabolised by the AAB to produce gluconic acid via the pentose phosphate pathway (Jayabalan et al., 2015). Glucose is also involved in the synthesis of the visible cellulose pellicle layer which is mainly produced by *Acetobacter xylinum* during fermentation (Al-Kalifawi, 2014; Costa et al., 2017). The large variations in the composition of Kombucha starter cultures are attributed to differences in climatic, cultural and geographical conditions (Dufresne & Farnworth, 2000; Toel et al., 2014).

The physio-chemical, microbiological, and sensory properties of Kombucha are generally dependent on several factors such as the composition and concentration of starter cultures, sugar and tea concentrations, fermentation time and temperature (Jayabalan et al., 2014; Jayabalan et al., 2015; Vitas et al., 2018). Thus, optimisation of the fermentation process should be based on a robust empirical study (Villarreal-Soto et al., 2018). A full-bodied Kombucha can be produced within 14 days at fermentation temperatures of 22 to 30°C (Villarreal-Soto et al., 2018; Yavari et al., 2010). The commercial production of Kombucha is currently dominated by small- to medium-scale producers who tend to have better control of the process than household brewers. However, Kombucha brewing is also attracting large commercial producers due to consumer demand for traditional, natural and clean label products. Therefore, Kombucha is viewed as a potential substitute for mainstream artificial, fizzy non-alcoholic drinks which are characterised by E-numbers and high calories.

Despite the popularity of the traditional Kombucha brew, some Kombucha products do not meet the stipulated national regulations (Kim & Adhikari, 2020). This is not unexpected, as fermented Kombucha is a biologically active beverage. While the viable cultures in Kombucha are desirable as some members of the starter cultures have been reported to be probiotics which may confer health benefits to consumers. but they are not expected to increase alcohol during storage. The presence of residual sugar poses challenges as it can lead to increased levels of alcohol due to post-fermentation even at refrigerated temperatures. Therefore, some Kombucha brewers face challenges to produce products that comply with the stipulated regulations. The regulations for the limit of ethanol content in Kombucha varies around the world. In New Zealand, Kombucha is classified as a non-alcoholic beverage with no more than 1.15 % ABV or 0.5 % ABV (Australia) (FSANZ, 2019; Rogan & Healthwatch, 2019; Mutukumira et al. 2020; McIntyre & Sik Jang, 2020). In Canada, Kombucha is also categorised as non-alcoholic product and the ethanol content should not exceed 1.1 % ABV (Jang et al. 2021), whereas in Mexico it is 2.0 % ABV (KBI). In the EU and UK, low alcohol products must be ≤ 1.2% ABV (FSA), whereas the US has stricter regulations (≤ 0.5% ABV) (KBI, FDA).

In an effort to reduce the final ethanol content of Kombucha, some producers may use advanced food processing technologies such as pasteurisation, vacuum distillation of ethanol, and filtration. Application of heat-treatment may result in the loss of viable cultures and hence the perceived health benefits from probiotics (Jayabalan et al., 2008; Kim & Adhikari, 2020). Filtration aids the removal of yeast and bacterial cells, as well as protein and hop substances which can interfere with the clarity and stability of the beverages (Freeman & McKechnie, 2003; Shala et al., 2017; Ubeda & Briones, 1999). While filtration is well-adapted in beer and wine fermentations, its application has been not widely reported for Kombucha production due to the challenges of integrating the technique into the complex traditional fermentation process (Kim & Adhikari, 2020). Currently there is little information on strategies to control ethanol levels in Kombucha during storage, which is compounded by the presence of residual sugar and microbial activity during storage. Thus, the combined effects of live cultures, concentrations of substrates added (sugar and tea) and fermentation conditions need to be optimised to maintain alcohol within regulated levels.

1.2 Aim and objectives

Aim

The main aim of the study was to optimise the production of low alcohol fermented Kombucha.

Objectives

1. To select suitable fermentation conditions to propagate the Kombucha starter culture. The factors (independent variables) comprised; sugar concentration, black tea concentration, fermentation temperature, fermentation time, and concentration of fermented broth. Parameters analysed comprised acidity (pH and T.A.), total soluble solids (TSS), weight of SCOBY, and concentration of yeast, acetic acid bacteria (AAB) and total counts during fermentation for 14 days;

2. To determine the effect of sugar concentration and fermentation temperature on the physico-chemical, microbiological and sensory characteristics of fermented Kombucha;
    a. Parameters analysed were acidity (pH and T.A.), TSS, colour, weight of SCOBY, concentrations of yeast, AAB, and total counts during fermentation for 9 days;
    b. Focus group sensory evaluation of fermented Kombucha was also conducted;

3. To investigate the effect of filtration post-primary fermentation, on microbial counts and the overall characteristics of Kombucha;
    a. Parameters analysed were acidity (pH and T.A.), total soluble solids (TSS), colour, and concentrations of yeast, AAB and total counts during fermentation (9 days) and one week storage (4℃) for filtered and unfiltered samples;
    b. Consumer sensory evaluation for filtered and unfiltered samples;
4. To investigate the storage stability of filtered and unfiltered Kombucha during storage for three weeks at 4℃;
    a. Parameters analysed were acidity (pH and T.A.), total soluble solids (TSS), colour, and concentration of yeast, AAB and total counts during fermentation (9 days) and three-week storage (4℃) for filtered and unfiltered samples;

b. Consumer sensory evaluation for sample (filtered and unfiltered) after Week 1, Week 2 and Week 3 of storage period (4°C); and,

5. To determine concentrations of ethanol, organic acids (acetic acid, gluconic acid and glucuronic acid), sugars (sucrose, fructose, and glucose), and antioxidants (ECG, EGC, EGCG, gallic, theobromine, and caffeine) in Kombucha during fermentation and storage (4°C) using gas chromatography (GC) and high-performance liquid chromatography (HPLC).

Chapter 2. LITERATURE REVIEW

2.1 Background

The origins of fermentation are unknown, but it is believed to have evolved from spontaneous fermentation of foods, with the earliest evidence of alcoholic fermentation of grapes to wine and barley to beer occurring over 5000 years ago (Campbell-Platt, 1994; Prajapati & Nair, 2003). Thus, fermented foods have been part of the human diet for thousands of years (Prajapati & Nair, 2003; Ray & Joshi, 2014). During early times, fermented foods were produced for special occasions such as traditional gatherings, weddings, and funerals. Some products were also produced to improve household food security through preservation for the different seasons, as well as providing variety (Mota de Carvalho et al., 2018). As knowledge about fermentation processes has increased through scientific research, the technology has been transformed into large scale commercial enterprises.

While there have been significant scientific advances in food fermentations in modern times, traditional practices have also developed into commercial enterprises due to the demand for natural functional foods (Corbo et al., 2014; Siro et al., 2008). Traditional or indigenous fermentation mainly involves spontaneous fermentation (wild fermentation) which relies on inherent and environmental microorganisms. Thus, the fermenting microorganisms are unknown and the process is uncontrolled. To improve the overall quality of the products, a previous batch of fermented product is commonly used to initiate fermentation (back-slopping), which is a common technique used to manufacture sourdough bread and fermented drinks (Capozzi et al., 2017; Dimidi et al., 2019; Villarreal-Soto et al., 2018; Voidarou et al., 2021). Back-slopping can also be achieved by using the same fermentation vessels, which aids in establishment of an undefined dominant culture (Dimidi et al., 2019; Villarreal-Soto et al., 2018; Yann & Pauline, 2014). Several products in modern markets have evolved through traditional fermentations and some of them are still being produced using traditional techniques such as vinegar, yogurt, kefir, cheese, sourdough bread, tempeh, kimchi, miso and sauerkraut. The latter products have continued to occupy a niche in the market due to their superior sensory properties (Corbo et al., 2014; Kim & Adhikari, 2020).

The final composition and presence of significant metabolites in fermented foods depends on the complex interactions of the substrate utilized, fermenting microorganisms, fermentation temperature and fermentation time. Generally, food substrates are metabolised by enzymes of the fermenting microorganisms to produce a range of metabolites (ethanol, organic acids, carbon dioxide) and intermediate compounds which are perceived to confer potential health benefits (Junker, 2000; Prajapati & Nair, 2003; Ray & Joshi, 2014; Stanbury, 1988; Tamang et al., 2016).

Fermentation adds value to foods because it contributes to preservation, improves texture, flavour, and aroma, eliminates pathogens and toxic compounds. Fermentation can also increase the nutritional value of the food and create new products (Mota de Carvalho et al., 2018; Voidarou et al., 2021). Based on substrate utilization, fermented foods are classified into several categories including fermented vegetables (kimchi, sauerkraut), fermented soybean and cereals (tempe, miso, doenjang), fermented milk (yogurt, cheese), fermented fish (fish sauce), fermented meat and fermented alcohols (rice wine, beer, vinegar) (Kwon et al., 2014). Regional fermented foods have evolved due to differences in landscapes, weather and cultural practices resulting in traditional products (Table 2.1). The application of fermentation has also grown into pharmaceuticals, biofuels, industrial enzymes, food additives and agricultural industries. For example, penicillin a popular antibiotic, is obtained from *Penicillium chrysogenum* during fermentation (Laich et al., 2002).

Table 2. 1 Some fermented foods from different regions of the world

Region	**Fermented food**
India	Idli, dosas, dhokla
Africa	Injea
America	Buttermilk, sourdough bread
Middle East	Torshi, dugh, kashk, shubatt
Europe	Salami, kvass, skyr
Caucuses	Kefir, yoghurt,
Asia	Kimchi, soy, sauce, miso, Kombucha

(Campbell-Platt, 1994)

Urbanisation and globalisation have created greater awareness about the development and consumption of functional foods to promote better health and wellness (Chakravorty et al., 2019; Corbo et al., 2014). Thus, the consumption of food is not limited to the supply of nutrients and energy to the body; it has expanded to provision of foods with perceived health promoting and medicinal properties (Flachs & Orkin, 2021; Marshall & Mejia, 2011). The term functional foods have been defined in many ways; however, the widely accepted definition is "foods that in addition to nutrients, supply the organism with components that contribute to the prevention of diseases, or to reduce the risk of developing them" (Prado et al., 2008; Siro et al., 2008). Of all functional foods, functional beverages are often favoured due to their convenience and versatile formulations (Figure 2.1) (Carbo et al., 2014; Min et al., 2019; Valero-Cases et al., 2020; Vargas et al ., 2021).

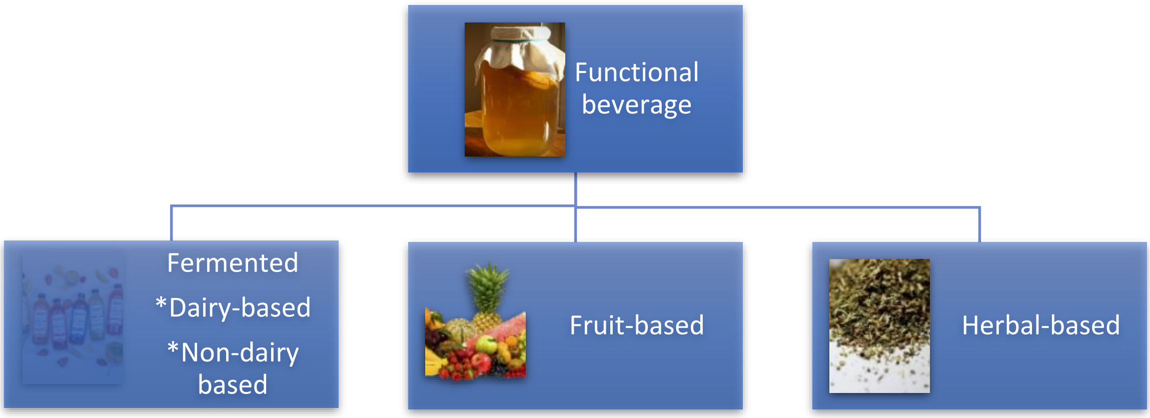


Figure 2. 1 Classification of functional beverages based on their formulations

(Tolun & Altintas, 2019)

Recent advances in the development of low-alcohol and non-alcohol fermented beverages, especially non-dairy based products have gained popularity because of their nutritional and dietary characteristics. Among the range of low-alcoholic fermented beverages, Kombucha is gaining popularity in domestic and international markets because of its low calorie and low alcohol content (Baschali et al., 2017).

2.2 Introduction to Kombucha

Kombucha is a traditional fermented tea beverage which has been consumed for centuries. It is characterised by fizziness, slight sweetness, tartness and it has a unique refreshing flavour. The perceived health promoting attributes of Kombucha have also increased its popularity among consumers (Jayabalan et al., 2014). Therefore, Kombucha brewing has attracted large commercial producers to enter the market, due to consumer demand for functional drinks as well as their desire for natural, clean label products (Chakravorty et al., 2019). Kombucha is therefore viewed as a strong competitor to the mainstream artificial carbonated soft drinks which are characterized by E-numbers and high calories (Watawana, Jayawardena, Gunawardhana, et al., 2015). Kombucha is sold commercially in non-flavoured (original raw flavour) and flavoured-infused forms. Flavoured-infused Kombucha available in the market is made from different substrates such as fruits, herbs, spice, flower, algae, and vegetables (Kim & Adhikari, 2020).

2.2.1 History of kombucha

Kombucha originated in north east China around 220 B.C, and was later brought to Japan by a physician to apparently cure digestive problems of the Emperor Inkyo. The beverage was then introduced to Europe through Russia and Mongolia, and was commonly brewed by small-scale artisans to cure illnesses and as a refreshing drink. As the trade routes extended, Kombucha spread to many parts of the world and has been known by a variety of names such as *Fungus japonicus*, Volga, Cainii Kvass, Tea Kvass, Kombuchaschwamm, and Tschambucco (Laureys et al., 2020; Leal et al., 2018). In the 1960’s and 1970’s Kombucha brewing re-emerged due to its perceived health effects (Dufresne & Farnworth, 2000).

2.2.2 Kombucha preparation

Kombucha is traditionally fermented with brewed tea, sugar and symbiotic cells of yeast and bacteria (SCOBY). Kombucha fermentation has been associated with various names such as “fermented tea”, “pellicle”, “tea mushroom”, “SCOBY”, “biofilm”, “tea fungus” and “tea kvass” (Kim & Adhikari, 2020; Kumar & Joshi, 2016a; May et al., 2019). Although variations exist in the proportions of raw materials used prior to kombucha fermentation, the general fermentation procedure is essentially the same (Figure 2.2). This includes infusion of tea leaves (0.4-0.5%, w/v) in filtered boiled water (80-90℃). Sugar is added (5-15%, w/v) and the tea is allowed to brew for 5-10 minutes. The tea leaves are removed using a tea strainer or coffee filter and the infusion is cooled to 20-22℃ (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). Back-slopping fermentation is initiated by adding previously fermented broth (10-20%, v/v) and SCOBY into the infused sweetened tea. The fermentation vessel is then covered with a clean porous cheese or muslin cloth and the brew is allowed to ferment for approximately two weeks at temperatures ranging from 22 to 28℃ to achieve the desired sensory characteristics (Figure 2.3) (Dutta & Paul, 2019a; Neffe-Skocińska et al., 2017). The fermentation period is variable and is mainly influenced by the activity of the starter culture, type and concentration of substrate utilised, fermentation time and fermentation temperature. The type of fermentation vessel and its dimensions has also been reported to affect the fermentation process (Hassan & AL-Kalifawi, 2014; J. H. Lin et al., 2012).

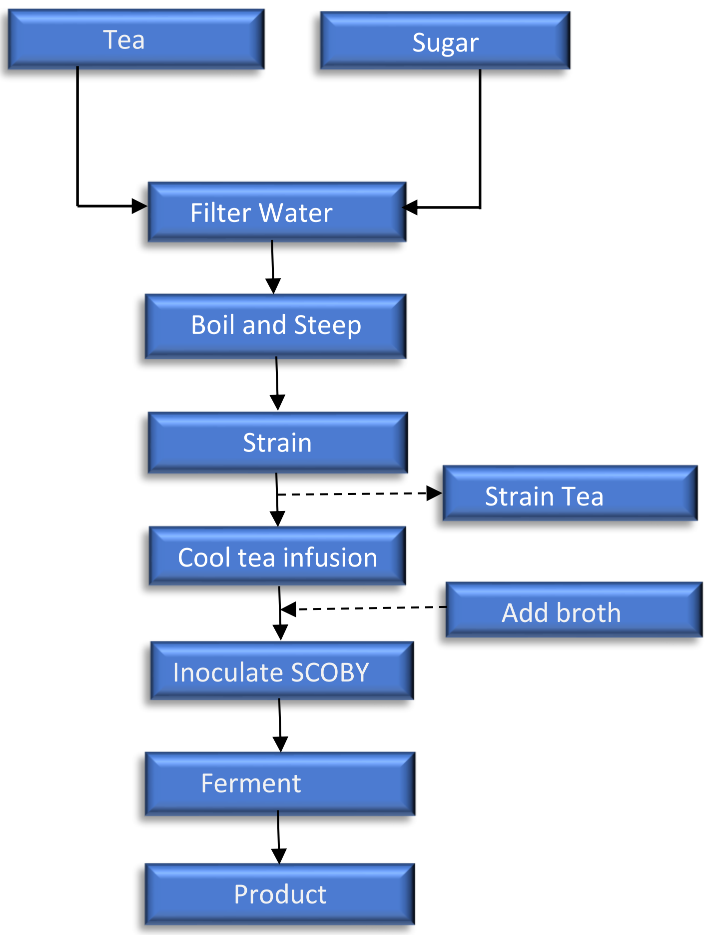


Figure 2. 2 Overview preparation of Kombucha tea

(Kumar & Joshi, 2016b)

During fermentation of Kombucha, it is important to maintain clean working areas and to use sterilised utensils to avoid contamination from unwanted microorganisms (Jayabalan et al., 2014; Laureys et al., 2020; Leal et al., 2018; Nummer, 2013; Villarreal-Soto et al., 2018). The duration of fermentation has a major effect on the colour of the Kombucha, growth of the microbial cellulose, final residual sugar and acidity of the final brew (Chen & Liu, 2000; Iličić et al., 2017). At low temperatures (17 to 20 °C), the growth of yeast dominates over the bacterial growth, therefore the time needed to ferment the Kombucha increases due to a reduction in the fermentation rate. In addition, the cellulose may be not fully developed due to lower microbial activity of AAB during early stages of Kombucha fermentation. In contrast, fermentation at high temperatures (30 to 42 °C) can inhibit the growth of bacteria, and under extreme temperature conditions, the bacteria may die (Neffe-Skocińska et al., 2017). Temperatures in the range of 22-28°C are considered to be optimum for the fermentation of Kombucha.

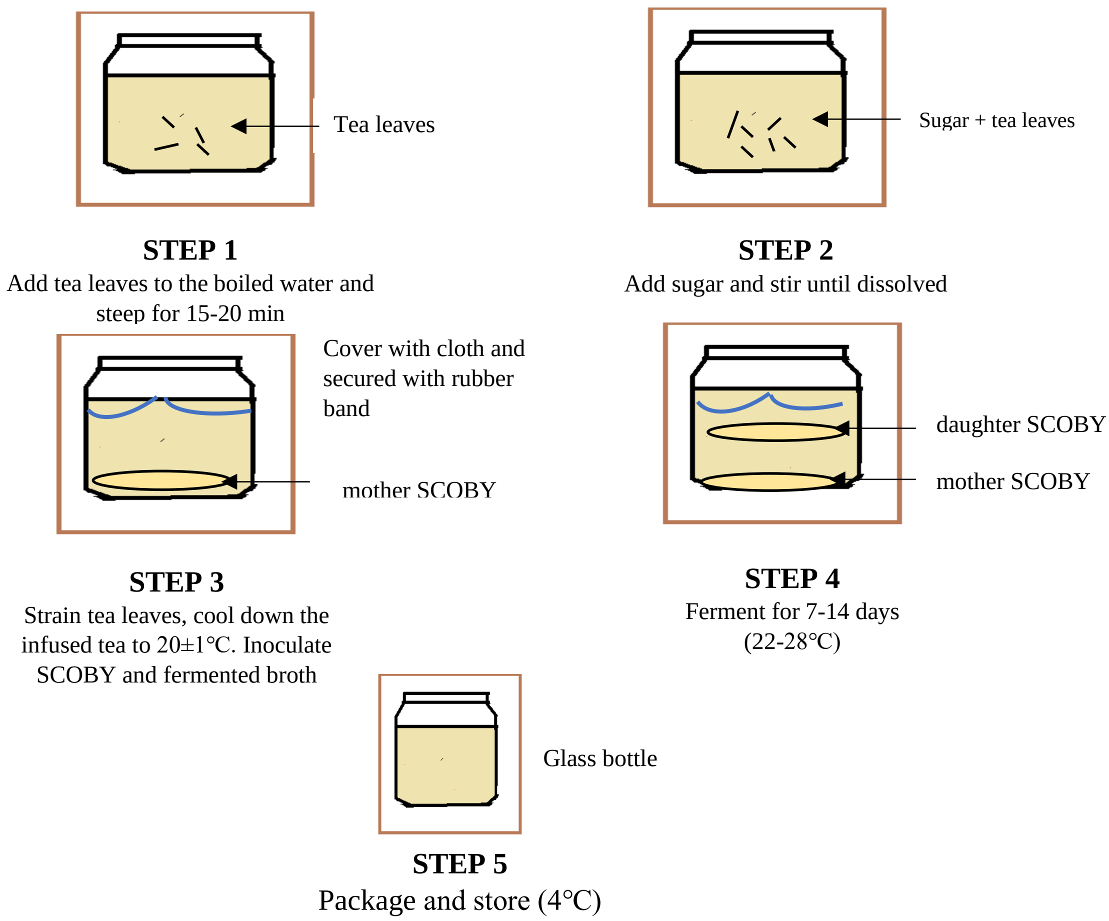


Figure 2. 3 Generalised steps involved in the fermentation of Kombucha

Source: Dutta and Paul (2019a); Jarrell et al. (2000)

Note : SCOBY = symbiotic culture of bacteria and yeast

2.2.3 Metabolites produced during fermentation of Kombucha

Kombucha contains substantial amounts of metabolites (acetic acid, lactic acid, glucuronic acid, gluconic acid, oxalic acid, citric acid, succinic acid and pyruvic acid) which may confer health benefits (Vargas et al., 2021). Other metabolites such as carbon dioxide and ethanol play important roles in the characteristics of the fermented beverage. Carbon dioxide provides the desirable carbonation, while ethanol is oxidised to acetic acid which has major impact on the sensory properties of Kombucha. In addition, the brew contains amino acids (e.g., lysine), minerals (Mn, Ca, Fe, Cu, K, Mn, Zn, Na), vitamins (Vit C, B, B2), fibre, purines, antibiotics and polyphenols (Figure 2.4) (Dutta & Paul, 2019b; Jayabalan et al., 2014; Jayabalan et al., 2017; Leal et al., 2018). Most of the metabolites formed during fermentation of Kombucha originate from the substrate, primarily tea (polyphenols) (Dufresne & Farnworth, 2000; Dutta & Paul, 2019a; Osiripun & Apisittiwong, 2021).

The purported health promoting properties of kombucha are largely based on personal testimonials and observations, with very few scientific studies carried out to demonstrate its prophylactic and therapeutic benefits (Lobo et al., 2017; Xia et al., 2019). The live microbes present in Kombucha are believed to help maintain the balance of vital intestinal microflora in the human body (Watawana, Jayawardena, Gunawardhana, et al., 2015). The antioxidants derived from tea are believed to alleviate allergies, asthma, lung diseases, joint pains, chronic inflammation, auto immune disorders, skin disorders, ageing, dermatitis, and obesity. Hence, the overall chemical composition and live cultures of Kombucha have been reported to contribute to human health (Figure 2.4 and Figure 2.5) (Leal et al., 2018; Lobo et al., 2017). Due to the growing demand and popularity of Kombucha, more focus has been given to *in vitro* and *in vivo* studies investigating the health benefits of consuming Kombucha (Aung & Eun, 2022; Lobo et al., 2017; Marco et al., 2021; Vargas et al., 2021; Vīna et al., 2014).

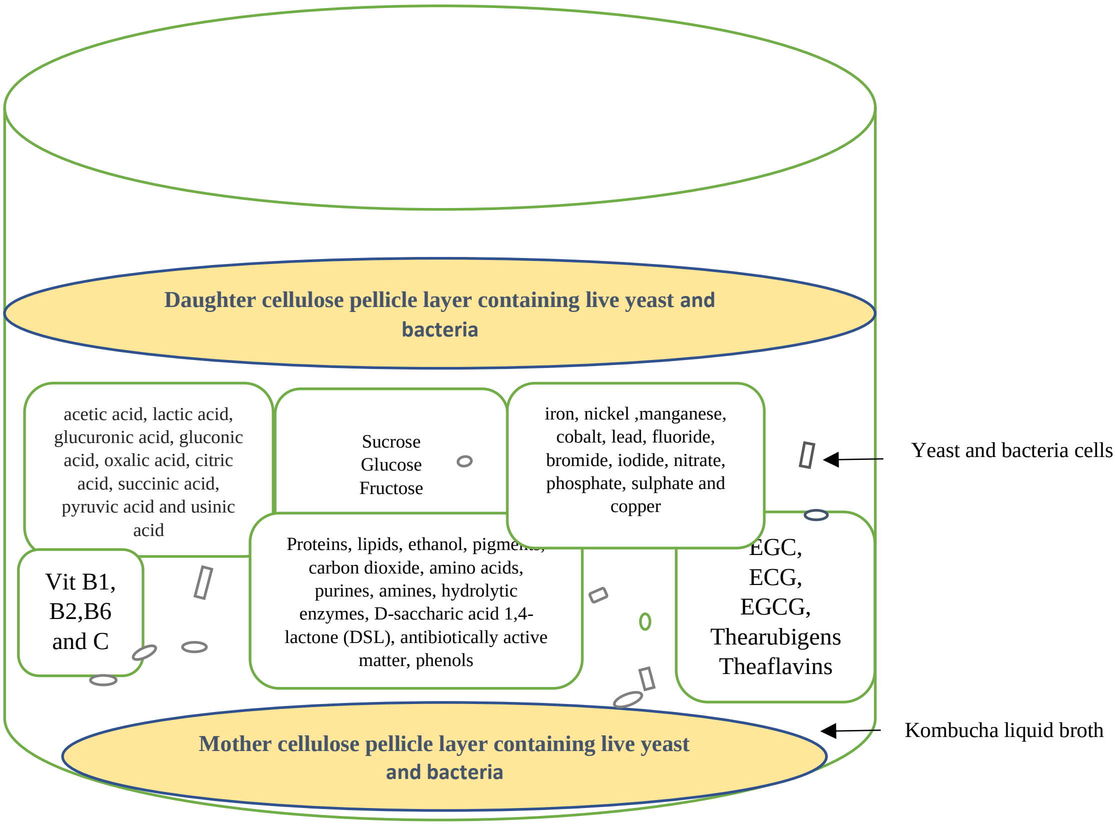


Figure 2. 4 Biological and chemical composition of fermented Kombucha

Note: EGC = epigallocatechin; ECG = epicatechin gallate; EGCG = epigallocatechin gallate

(Jayabalan et al., 2014; Jayabalan et al., 2017; Villarreal-Soto et al., 2018)

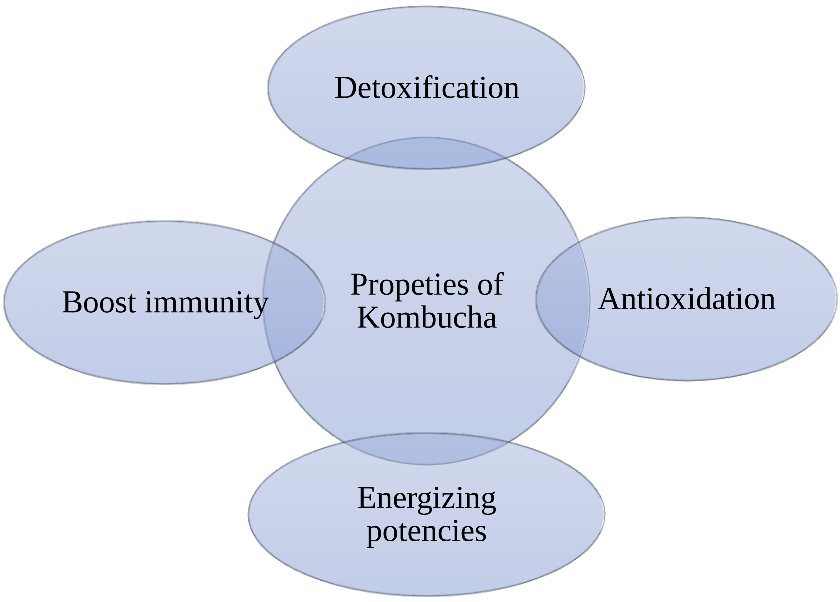


Figure 2. 5 Four essential properties of fermented Kombucha

(Leal et al., 2018)

2.3 Ingredients used in Kombucha brewing

2.3.1 Water

Potable water, well water, spring water and bottled water are commonly used to brew kombucha (Malbaša et al., 2005). Water with dissolved fluoride, chloramine and chlorine contaminants can have negative effects on the growth of the starter cultures during fermentation Well water may be more alkaline, and contain toxins and other pathogens which can also be lethal to Kombucha starter cultures (Shenoy et al., 2019; Toccalino et al., 2012). Therefore, it is important to determine the suitability of the water supply before using it for the production of Kombucha (Jayabalan et al., 2014; Villarreal-Soto et al., 2018).

2.3.2 Tea

Tea; the oldest aromatic beverage consumed in many parts of the world is brewed from the leaves of evergreen shrub, *Camellia sinensis*. Different types of teas are differentiated based on their manufacturing process, with the quality and composition of functional compounds in the teas dependent on factors such as age of the leaves, climate, species and horticultural practices (Gaggia et al., 2019). The most common varieties of tea used to make kombucha include green, black, oolong, white, herbal, pekoe, and red. The teas are differentiated based on their processing steps. Typically, green tea is unfermented, whereas black tea is fermented, oolong tea is semi-fermented (Figure 2.6) (Ren et al., 2020; Watawana et al., 2018). White tea is wilted in natural sunlight and is obtained mainly from leaves and buds of young plants. The process involved in the manufacturing of yellow tea is similar to green tea with the exception of a shorter drying phase in yellow tea (Banerjee,1992).

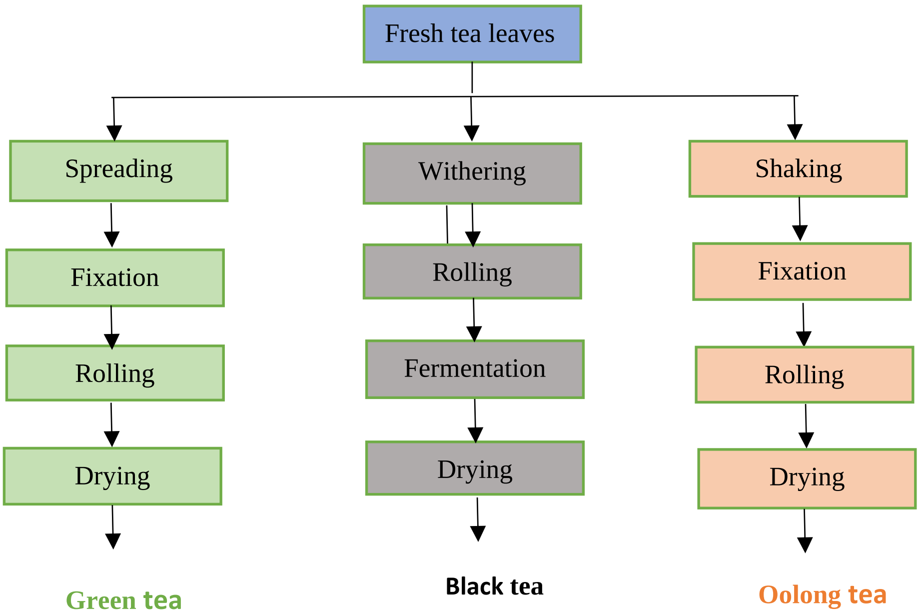


Figure 2. 6 Main processing steps of different types of tea

(Ren et al., 2020)

The growth of the starter culture (SCOBY) in infused sugared tea can be affected by the type and concentration of tea and sugar. The chemical composition of the substrate used influences the final taste and composition of the fermented beverages (Cardoso et al., 2020). Amongst the available varieties of tea, black tea has been widely used to brew Kombucha and is considered as a major source of nitrogen and minerals required for the growth of cultures in Kombucha (Gaggìa et al., 2019; Goh et al., 2012). Black tea contains high polyphenols and catechins (Table 2.2). The polyphenols are beneficial to human health as they can help to remove excess reactive oxygen species (ROS). Whereas, the catechins present in tea are oxidised by enzymes to produce theaflavins and thearubigin pigments which may confer potential health benefits (Banerjee, 1992; Tanaka et al., 2010).

Table 2. 2 Phenolic compounds in black tea

Phenolic compounds	**Concentration (%)**
Flavonoids	70.2
Phenolic acids	18.3
Other polyphenols	8.4
Lignans	2.3
Stilbenes	0.8

(Cardoso et al., 2020)

2.3.3 Sugar

In natural environmental conditions, yeast rely on a broad spectrum of carbon sources including alcohols, polyols, amino acids, and organic acids, but they preferentially metabolise sugars as their main carbon supply (Lončar et al., 2006; Rodrigues et al., 2006). The type and concentration of sugar used affects the growth of the cultures, residual sugar and the final ethanol content during fermentation of Kombucha. Sweeteners such as palm honey, palm sugar, jaggery and aspartame are considered low-cost alternative sweeteners for brewing kombucha. However, the traditional energy source, white sugar (sucrose) which is obtained by bleaching and other processing steps is considered most suitable for Kombucha as it can be easily metabolised by yeast compared to other unrefined sweeteners (Tietze, 1996; Watawana et al., 2017). It is desirable to use the optimum concentration of sugar during Kombucha fermentation to control the production of metabolites (Iličić et al., 2017; Jones & Greenfield, 1982; Muhialdin et al., 2019). During fermentation, sucrose is broken down first by the enzyme invertase into glucose and fructose, and then to ethanol which provides energy to the AAB present in culture. The AAB then oxidise ethanol into acetic acid with the acid produced helping protect the yeast cells from unwanted invaders/pathogens.

2.3.4 Kombucha starter culture

The starter culture used to initiate fermentation of kombucha consists of a cellulose pellicle layer (SCOBY) and fermented broth (Greenwalt et al., 2000; Jarrell et al., 2000). The gelatinous cellulose pellicle layer is an exopolymer made up glucopyranose β 1-4 linkages and has a disc shape or pancake structure. The cellulose is formed at the liquid surface in the fermenting vessel during fermentation (Al-Kalifawi, 2014; Amarasekara et al., 2020; Amarasekara et al., 2020; Shenoy et al., 2019). Previous studies have reported the importance of using fermented broth and the cellulose pellicle layer as a starter culture to initiate Kombucha fermentation (Jarrell et al., 2000; May et al., 2019). A complex interaction takes place between the microbial diversity present in fermented liquid broth and SCOBY which affects the overall sensory profile of Kombucha (Chakravorty et al., 2016; Jayabalan et al., 2014; Lee et al., 2021; Sievers et al., 1995; St-Pierre, 2019; Villarreal-Soto et al., 2018).

The cellulose pellicle produced by bacteria helps to protect the viable cells in the fermented broth from unwanted microorganisms (moulds), pathogens (*Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus)*, and harsh environmental conditions (e.g. ultraviolet radiation, and other environmental challenges; (Villarreal-Soto et al., 2018). SCOBY can also be viewed as a storage reservoir of food resources with potential for greater access to oxygen for the bacteria entrapped in the cellulose pellicle, as AAB are known to be obligate aerobes. The addition of fermented broth (back-slopping) is essential to reduce the acidity of Kombucha (pH<4.6) before fermentation commences (Hassan & Al-Kalifawi, 2014; Jarrell et al., 2016; Jayabalan , 2014). This low acidity is desirable for the growth of yeast and to initiate fermentation of Kombucha. The optimum pH range for growth of yeast is pH 4.0-4.5, and an acidic environment (pH<4.7) also helps to prevent growth of spoilage microorganisms (Laureys et al., 2020; Nummer, 2013). Therefore, fermented broth and SCOBY both play important roles in maintaining the partial aerobic conditions, production of characteristic organic acids and metabolites vital for the production of refreshing Kombucha beverage.

Kombucha fermentation may differ depending on the microbiological composition of SCOBY and the fermented broth (Kumar & Joshi, 2016b; Sievers et al., 1995; Tan et al., 2020). Cellulose yield is affected by fermentation time, size of fermentation vessel (surface area: height ratio), and the initial quantity and composition of the starter culture and, type and concentration of substrate utilized. The production of the cellulose layer increases with increased surface area of the vessel, which results in greater access to oxygen (Gorgieva & Trček, 2019; Hassan & AL-Kalifawi, 2014; J.-H. Lin et al., 2012). Ethanol produced by yeast metabolism of sugar provides energy for the AAB to produce the cellulose biofilm during Kombucha fermentation (May et al., 2019). Two distinct layers of cellulose are formed during fermentation consisting of the top floating layer (‘daughter’ SCOBY), and the bottom layer (‘mother’ SCOBY) (Jarrell et al., 2000). Generally, the mother SCOBY is inoculated at the beginning of fermentation, and the daughter SCOBY develops later as fermentation proceeds. The cellulose first grows outwards to occupy the entire surface area of the fermentation vessel to form a pellicle layer followed by its growth in the downward direction (Figure 2.5). Fermentation time has a significant effect on the thickness of the cellulose layer, which increases as fermentation proceeds. However, the anaerobic environmental conditions and lack of nutrient supply may restrict the growth of the cellulose biofilm (Marsh et al., 2014; Teoh et al., 2004). Variation in the microbiota of the SCOBY have been reported due to differences in climate and geographic conditions (Teoh et al., 2004)**.**

The environment during Kombucha fermentation is favourable for the growth of a variety of different yeast and bacteria species (Table 2.3). Fermentative, acid-tolerant and osmotolerant yeast are the most commonly found yeast species in Kombucha starter cultures, with *Brettanomyces (Dekkera)* an acid-producing species of yeast, *Schizosachharomyces. pombe, Torulaspoa. delbreuckii, and Zygosachharomyces bailii* species with high sugar tolerance level being common yeasts found in kombucha (Marsh et al., 2014; Teoh et al., 2004; Tran et al., 2020a; Villarreal-Soto et al., 2018). Previous studies (Lee et al.,2021, Chakravorty et al., 2016) reported higher bacterial diversity in the fermented liquid broth and cellulose pellicle compared to yeast species. *Acetobacter*, *Gluconacetobacter* and *Lactobacillus* are the three most common genera of bacteria identified in Kombucha (Jarrell et al., 2000; Jayabalan et al., 2014). Of all the AAB, *Gluconacetobacter* is the most common fermentative species (Chakravorty et al., 2016; Teoh et al., 2004; Villarreal-Soto et al., 2018). Synthesis of the cellulose pellicle layer is one of the important roles of bacteria during Kombucha fermentation. Many bacterial species are known to synthesize cellulose pellicles (*Rhizobium*, *Azotobacter*, *Agrobacterium*, *Aerobacter*, *Gluconacetobacter and Salmonella*) but *Acetobacter xylinum* (Gram-negative bacteria, rod shaped) also known as *Gluconacetobacter xylinus* and recently classified as *Komagataeibacter xylinus* is the dominant species involved in the production of the cellulose biofilm in Kombucha (Costa et al., 2017; Treviño-Garza et al., 2020; Zahan et al., 2015).

Table 2. 3 Kombucha Microbiota

Type	Microorganism
Yeast	*Brettanomyces bruxellensis*
	Brettanomyces lambicus
	Brettanomyces intermedius
	Brettanomyces custersii
	Brettanomyces claussenii
	Candida guilliermondii
	Candida stellate
	Kloechkera apiculata
	Pichia membranaefaciens
	Pichia fermentans
	Saccharomyces cerevisiae
	Saccharomyces ludwigii
	Saccharomyces bisporus
	Schizosaccharomyces pombe
	Schizosaccharomyces ludwigii
	Torulaspora delbrueckii
	Torulopsis famata
	Zygosaccharomyces bailii
	Zygosaccharomyces rouxii
	Zygosaccharomyces rouzii
Bacteria	*Acetobacter xylinum*
	Acetobacter xylinoides
	Acetobacter pasteurianus
	Acetobacter ketogenum
	Acetobacter aceti
	Acetobacter nitrogenifigens
	Bacteria xylinum
	Bacteria xylinoides
	Bacteria katogenum
	Bacteria gluconicum
	Gluconacter sp. A4
	Gluconobacter oxydans
	Komagataeibacter

(Mutukumira et al., 2020; Sievers et al., 1995; St-Pierre, 2019; Teoh et al., 2004; Villarreal-Soto et al., 2018)

2.4 Microbial activity of microorganisms during fermentation of Kombucha

2.4.1 Metabolic actvity of yeast

Yeast are non-motile, spherical or oval shaped eukaryotic microorganisms. They are facultative anaerobes with a cell diameter around 8 µm. The growth of yeast is supported by fermentable sugars, minerals, vitamins, amino acids and oxygen, and their nutritional requirements are lower compared to other microbes such as lactic acid bacteria. Yeast metabolises sucrose to produce ethanol and carbon dioxide as shown in Figure 2.7. Based on their metabolic activity, yeast can be classified into three categories: (1) obligate fermentative yeast, e.g. *Rhodotorula glutinis*, (2) obligate respirative yeast involved in respiration, e.g. *Yarrowia lipolyticia*; and (3) facultative fermentative yeast which can perform both fermentation and respiratory activities, e.g. *Dekkera bruxellensis and Saccharomyces cerevisiae* (Kulshrestha et al., 2013).

The metabolism of yeast is affected by two phenomena : the Pasteur effect and the Crabtree effect (De Deken, 1966; Teoh et al., 2004). The inhibition of fermentation in an oxygen-rich medium is referred to as the Pasteur effect, which may be caused by lower efficiency of cells to produce ATP during fermentation compared to respiration. The Pasteur effect in *S. cerevisiae* only takes place when the cells are resting or when the growth rates are lower or in a sugar-limiting continuous culturing condition. Yeast require oxygen to undergo respiratory metabolism, but they do not always perform respiration in the presence of oxygen instead they perform fermentation as long as carbohydrates are available. This phenomenon is referred to as the Crabtree effect (Figure 2.7) (De Deken, 1966; Pfeiffer & Morley, 2014).

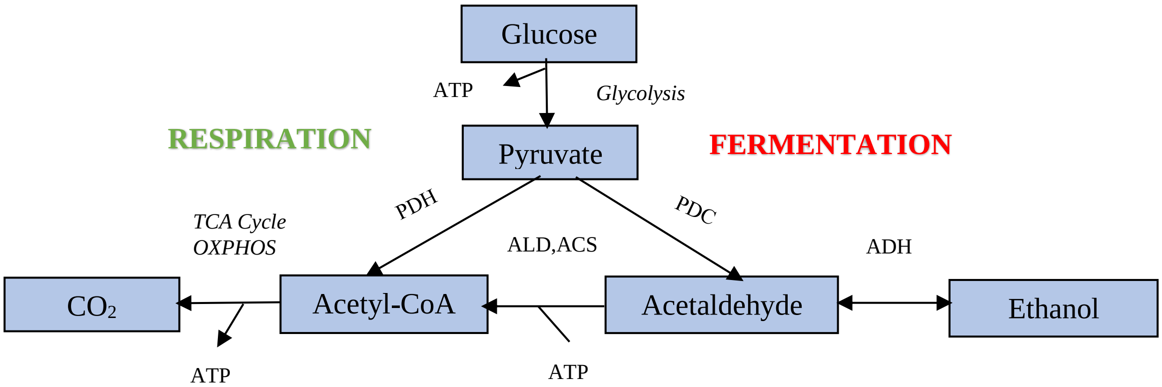


Figure 2. 7 Energy metabolism in yeast

(Pfeiffer & Morley, 2014)

Note: PDH-pyruvate dehydrogenase, PDC-pyruvate decarboxylase, ADH- alcohol dehydrogenase, ALD- acetyl-CoA by aldehyde dehydrogenase, ACS- acetyl- Coa synthetase

Kombucha is made from sugar, and tea, fermented by a symbiotic interaction of yeast and bacteria present in the starter culture. During fermentation, sucrose is hydrolysed to glucose and fructose by the enzyme invertase. Glucose is then transformed to pyruvate via the glycolytic pathway. In the presence of high sugar levels, the cell switches from respiration to the fermentation pathway, in which the pyruvate is converted into acetaldehyde with the help of the enzyme pyruvate decarboxylase (PDC), and then to alcohol (alcohol dehydrogenase, ADH) yielding 2 moles of ATP per molecule. However, in the presence of low sugar levels, the cell switches to the respiration pathway, whereby the pyruvate is converted to acetyl CoA and enters the tricarboxylic acid (TCA) cycle which yields 32 moles of ATP per molecule. In addition, acetyl CoA can also be formed during transformation of acetaldehyde with the aid of acetyl-CoA synthetase (ACS) and acetaldehyde dehydrogenase (ALD) as shown in Figure 2.7. Therefore, the concentration of sugar and oxygen availability strongly influence yeast metabolism during Kombucha fermentation .

During alcoholic fermentation by yeast, by-products such as glycerol, acetic acid, ester compounds along with higher alcohols are produced as a response to high osmotic pressure and to maintain internal redox balance. The environmental and metabolic stresses during alcoholic fermentation can inhibit the growth of yeast cells, thus reducing the final ethanol levels (Figure 2.8) (Bai et al., 2008; Harden, 1923; Moulin et al., 1984). Upon lysis of the yeast cells, intracellular compounds including proteins, lipids, nucleic acids, polysaccharides are broken down by endogenous enzymes, and released into the surrounding medium where they are available to support the growth of AAB, and other bacteria such as LAB which are occasionally present in Kombucha. Therefore, yeast and bacteria mutually benefit each other during Kombucha fermentation.

Figure 2. 8 Environmental and metabolism stresses on the yeast cell

(Bai et al., 2008)

2.4.2 Metabolic activity of Acetic acid bacteria

Acetic acid bacteria are strictly aerobic, gram negative or gram variable microorganisms. They are rod or ellipsoidal shaped cells which appear singly, paired or in clusters, can be motile and are around 1-4 μm long and 0.5μm wide. They are usually referred to as mesophilic microorganisms as their growth is optimal within the temperature range of 25-30 ℃ and pH 5.0-6.5 (Gomes et al., 2018). Under aerobic conditions, AAB oxidise ethanol to acetic acid in two steps. Firstly, alcohol dehydrogenase (ADH) converts ethanol to acetaldehyde, which is then oxidised to acetate by aldehyde dehydrogenase (ALDH). In the bacterial membrane, ADH and ALDH form a multienzyme complex producing acetic acid from ethanol. Meanwhile, glucose is oxidised to gluconic acid and glucuronic acid (Figure 2.9 and Figure 2.10) (De Roos & De Vuyst, 2018; Gomes et al., 2018; Jarrell et al., 2000; Marsh et al., 2014).

Uptake of substrate varies amongst different genera of bacteria. For example, *Glunonobacter* oxidises glucose, sorbitol, gluconic acid and glycerol instead of ethanol. Whereas *Acetobacter* and *Gluconacetobacter* prefer oxidation of ethanol rather than glucose. During fermentation, ethanol and glycerol produced are further metabolized to dihydroxyacetone (DHA), via the Embden-Meyerhof Parnas (EMP) pathway, followed by gluconeogenesis to produce the cellulose pellicle. Acetic acid bacteria lack the enzyme phosphofructokinase; hence glucose is metabolised via the pentose phosphate pathway rather than EMP (Figure 2.9). *Acetobacter*, *Komagataeibacter* and *Gluconacetobacter* utilise the tricarboxylic acid (TCA) cycle to oxidize organic acids (including lactic acid or acetic acid) to carbon dioxide with the help of succinate dehydrogenase and ketoglutarate dehydrogenase. In contrast, *Glunonobacter* lack the enzymes needed to oxidise organic acids to carbon dioxide (May et al., 2019; Pothakos et al., 2016).

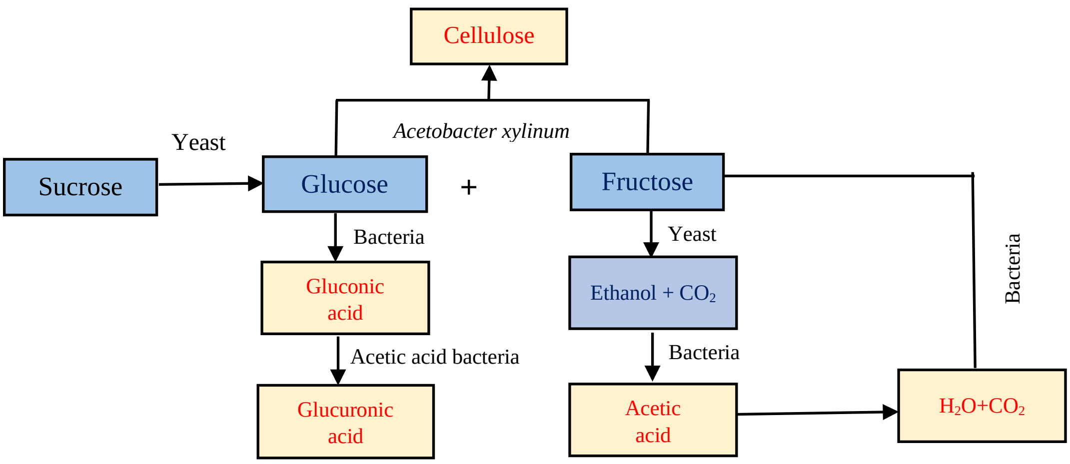


Figure 2. 9 Metabolic activity in Kombucha fermentation (Villarreal-Soto et al., 2018)

Figure 2.10 Biological and chemical pathway involved during fermentation of Kombucha

(Lin et al., 2013)

2.5 Physico-chemical changes during fermentation of Kombucha

2.5.1 Acidity

During fermentation of Kombucha, it is important to monitor acidity as it aids the determination of the endpoint. During fermentation the bacteria convert the alcohol produced by yeast to various organic acids (acetic acid, malic acid, lactic acid, glucuronic acid, gluconic acid and citric acid), which may result in decreased pH and increased titratable acidity (TA) in the Kombucha (Ivanišová et al., 2019; Malbaša et al., 2011; Villarreal-Soto et al., 2018). Previous studies reported that the pH of Kombucha decreased from pH 5 to 2.97, while TA increased by 0.03% to 0.24% during fermentation for 11 to 14 days at 22 - 24°C (Jayabalan et al., 2007; Chen & Liu, 2000). Similar results were reported by Chen & Liu (2000) where TA increased during fermentation for 30 days, and then it decreased when the process was prolonged to 60 days. The changes in acidity (pH and T.A.) may be attributed to production of organic acids during fermentation of

Kombucha. The decrease in rate of production of organic acids in the last 30 days was probably caused by depletion of substrates.

Of the range of organic acids produced during fermentation, acetic acid, gluconic acid and glucuronic acid (Figure 2.11) are important (Jayabalan et al. 2014). Jayabalan et al. (2007) reported increased concentrations of acetic acid (4.69 g/L) and glucuronic acid (1.71 g/L) in Kombucha fermented for 18 days at 24±3℃. A similar result was reported by Chen & Liu (2000) with acetic acid concentrations increasing with fermentation time, and then gradually decreasing (8 g/L) at the end of fermentation (60 days) for Kombucha fermented at 24±3℃. The differences in the results of organic acid content may be attributed to different fermentation times. A previous study reported high gluconic acid concentrations (70.11 g/L), followed by acetic acid (11.15 g/L); with the lowest being glucuronic acid (1.58 g/L) in black tea Kombucha fermented for 15 days at room temperature (Kaewkod et al., 2019). The results of the previous study suggests that the types and concentrations of substrates can affect the levels of organic acids in the final Kombucha product. The differences in the organic acid levels may also be attributed to differences in the fermentation temperatures as higher concentrations of acetic and glucuronic acid concentrations have been reported in samples fermented (10 days) at 25℃ than at 30℃ (Neffe-Skocińska et al., 2017).

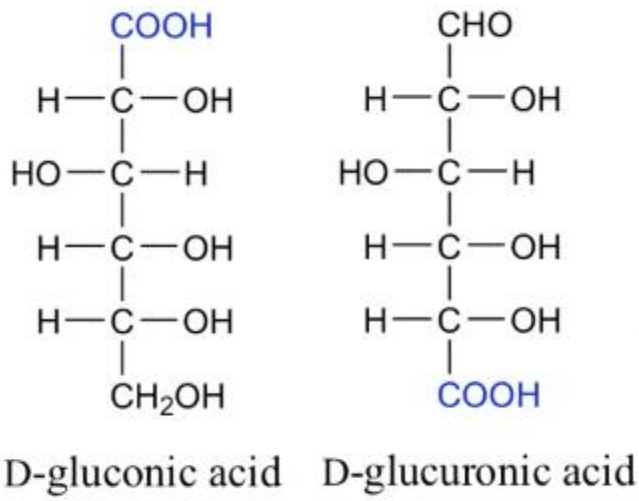


Figure 2.11 Structure of D-gluconic and D-glucuronic acid

(Jayabalan et al. 2014)

2.5.2 Total soluble solids (TSS) and total sugar content

Sucrose is the most vital nutrient in Kombucha fermentation as it provides energy for the growth of the bacteria and yeast in starter cultures. Total soluble solids (TSS), which mainly reflects the sugar content in sample is measured using a refractometer and the results are recorded as °Brix (Gaggia et al., 2019). The final sugar content in Kombucha is dependent on several factors including fermentation time, temperature, initial concentration of sugar and metabolic activity of yeast and bacteria present in the starter cultures. Previous studies have reported decreased sucrose concentrations, and increased glucose and fructose concentrations with fermentation time (Gaggìa et al., 2019a; Jayabalan et al.,2014; May et al., 2019; Neffe-Skocińska et al., 2017; Villarreal-Soto et al., 2018). A study by Neffe-Skocińska et al (2017) reported marked decreases in sucrose (%) (97.6±0.06 - 0.93±0.17) and increases in fructose (1.2±0.01 - 30.09±0.02) and glucose (1.4±0.03 - 37.7±0.04) during fermentation of Kombucha fermentation for 10 days at 25℃. Previous researchers reported higher concentrations of fructose (17.8 -18 g/L) than glucose (9.4 – 10 g/L) in Kombucha fermented for 14 to 15 days at 24±3℃ (Chen & Liu, 2000; Kayisoglu & Soskun., (2020), indicating glucose rather than fructose was the preferred substrate by yeast. A study by Lončar et al. (2006) reported higher reductions of sucrose in samples inoculated with 15% rather than 10% fermented broth, suggesting higher numbers of fermenting microorganisms in the 15% fermented broth.

Previous studies reported decreased sucrose levels concomitant with the decrease in TSS during fermentation of Kombucha (Kayisoglu & Coskun, 2020; Neffe-Skocińska et al., 2017). Others reported that Kombucha fermented with black tea had the highest reduction of TSS (28.3%) compared to Kombucha fermented with other varieties of tea (mint tea, green tea, sage and linden) (Kaewkod et al., 2019). Hence, the type of tea used to make Kombucha can affect the sucrose content of the final product. Kombucha fermented at 25℃ had the highest reduction of sucrose compared to products fermented at 20℃ and 30℃ (Neffe-Skocińska et al., 2017). This may be attributed to higher microbial activity of the fermenting microorganisms in Kombucha fermented at 25℃ than at 20℃ and 30℃.

2.5.3 Colour

Kayisoglu & Coskun (2020) reported differences ($p<0.05$) in colour parameters (L*, a* and b*) of kombucha brewed with different varieties of tea, with significant differences observed at both the beginning and end of fermentation ($p<0.05$). This suggests that the variety of tea and fermentation time can influence the colour of kombucha during fermentation. Kombucha fermented with black tea for 14 days at 24±1°C showed a slight increase in L* (lightness) value from 59.29 to 68.79, with a decrease in a* (redness-greenness) value from 2.22 to 10.05 and b* value (yellowness-blueness) from 68.72 to 59.98. A similar trend was observed by Tarhan (2017) and Harnjez where the L* value increased and a* and b* value decreased with increased fermentation time. The colour changes may be caused by degradation or biotranformation of polyphenols by metabolic action of yeast and bacteria present in Kombucha starter cultures (Betlej et al., 2020; Kayisoglu & Coskun, 2020; Osiripun & Apisittiwong, 2021).

2.5.4 Ethanol content

The sugar is converted to glucose and fructose by yeast, then to ethanol and carbon dioxide during fermentation of Kombucha. (Chen & Liu, 2000; Lai et al., 2019; Sievers et al., 1995; Villarreal-Soto et al., 2018). According to previous studies, ethanol first increases then decreases during fermentation (Chen & Liu.,2020; Tan et al.,2020; Neffe-Skocińska et al.,2017). In a study by Chen & Liu (2000), ethanol increased (5.5 g/L) up to 20 days, and gradually decreased during prolonged fermentation of 60 days at 24±3°C. A similar trend was reported by others (Neffe-Skocińska et al., 2017) where ethanol in the Kombucha initially increased (1.10% ABV), and then decreased during fermentation for 10 days at 25°C. The data presented by Tan al., 2020 also showed reduction in the ethanol during prolonged storage of soursop Kombucha fermented for 21 days at room temperature. The decrease in ethanol concentration may be attributed to the oxidation of ethanol to acetic acid by acetic acid bacteria present in cultures (Chakravorty et al., 2016; Chen & Liu, 2000; Neffe-Skocińska et al., 2017).

Studies by Hillberg (2020) and Guzel-Seydim et al. (2020) showed that ethanol in Kombucha increased (2.8-3.4% v/v) during cold-storage (4°C). Increased ethanol content during storage may be due to post-fermentation of residual sugars by yeast and bacteria present in the beverages (Iličić et al., 2017; Muhialdin et al., 2019).

2.6 Microbial composition of Kombucha during fermentation

Several studies have reported changes in yeast, AAB and total counts during fermentation of Kombucha (Sengun, 2013; Sievers et al., 1995). Chen & Liu (2020) reported marginal increases in yeast and bacterial populations until Day 9 of fermentation, after which the cell counts gradually decreased to the end of fermentation (14 days at 24±3℃). Similar trends have been reported by others (Zhao et al.2018; Aung & Eun, 2022). The gradual decrease in the yeast and total bacteria count at the end of fermentation may be caused by acid shock (low pH), which interferes with the metabolic activity and growth of the microbes (Guan & Liu, 2020; Sousa et al., 2012). Also, insufficient supply of nutrients (starved conditions) and lower availability of dissolved oxygen may also lead to reductions in microbial counts in fermented Kombucha (Chen & Liu ., 2000, May et al.,2019) .

Other studies have reported higher counts of yeast and bacteria at higher fermentation temperature (28℃) than at lower temperature (22℃) during Kombucha fermentation, suggesting that the fermentation temperature has a major effect on the growth of fermenting microorganisms (Aung & Eun., 2022; Neffe-Skocińska et al., 2017; Xia et al., 2019; Zhao et al., 2018). Previous workers reported increased microbial counts up to an optimum sugar level (90-100 g/L), and then decreased with further increases in sugar during fermentation of Kombucha (Goh et al., 2012a.; Hassan & AL-Kalifawi, 2014). Thus, high sugar concentrations can be inhibitory to the metabolic activity of microbes present in cultures, thereby reducing their counts (May et al., 2019; Goh et al., 2012a.; Hassan & AL-Kalifawi, 2014).

The interactions between yeast and bacteria in the fermented broth and cellulose pellicle is responsible for the physio-chemical, microbiological and sensory characteristics of Kombucha. Chen &Liu (2020) reported higher cell counts in the fermented broth than the cellulose pellicle, whereas Reiss (1944) reported higher counts in the pellicle than in the broth. The discrepancy may be due to differences in supply of dissolved oxygen to the cultures embedded in the cellulose matrix. During Kombucha fermentation, alcohol and carbon dioxide are produced by yeast and the carbon dioxide accumulates at the air-liquid interface during prolonged fermentation, thereby creating a gap between the fermented broth and the cellulose pellicle. This may block the transfer of oxygen from the cellulose to the broth and nutrient supply from the broth to the pellicle (Chen & Liu., 2000; Goh et al., 2012a; May et al., 2019; Reiss.,1994). Mostafa et al (2020) reported reductions of cell counts from 6 to 4 log cfu/ml during storage (4°C) for three weeks. The decreases in microbial counts can be caused by insufficient supply of nutrient and partial anaerobic conditions during cold-storage of packaged beverages (Mostafa et al., 2020; May et al.,2019).

2.7 Antioxidant activity

The antioxidant properties of Kombucha originate from the tea used to prepare the sugared tea infusion used for Kombucha fermentation. The main antioxidants comprise of polyphenols especially catechin which belongs to the flavanol group. Antioxidants help in prevention of cancer, obesity, arthritis and boost immunity. In addition to polyphenols, Kombucha contains other nutrients such as vitamin C, B_2, B_6 and catalase which have the ability to trap/scavenge free- radicals (Ahmed et al., 2020; Chakravorty et al., 2016; Jakubczyk et al., 2020; Jayabalan et al., 2008; Watawana, Jayawardena, Ranasinghe, et al., 2015). Chu & Chen (2006) reported DPPH scavenging activities of black tea kombucha increased to about 70% on day 15 of fermentation. Kombucha fermented with black tea had the highest antioxidant activity (89.69%) compared to Kombucha fermented with barley and rice (Ahmed et al., 2020), suggesting that the type of tea along with fermentation time can effect the antioxidant concentration in fermented Kombucha.

Ivanišová et al.(2019) reported higher total polyphenol content of kombucha beverage (412.25 mg GAE/L) than black tea (180.17 mg GAE/L) alone, which may be caused by an increase in the antioxidant properties of Kombucha due to fermentation (Lobo et al., 2017). The metabolic conversion of tea caused by the release of microbial enzyme during fermentation process may contribute towards the increased antioxidant activity in Kombucha (Jakubczyk et al., 2020).

Phenolic compounds are extremely sensitive to light and heat and can be degraded on exposure to harsh environmental conditions (Ahmed et al., 2020; Zafrilla et al., 2003). Heat treatment (100°C and 76°C) has been reported to reduce the total phenolic compounds and free radical scavenging properties of Kombucha by 35.95% and 18.12%, respectively (Ahmed et al.,2020; Jayabalan et al.,2008). However, others (Gramza-

Michałowska et al.,2016), reported no significant changes in the total polyphenol content of black tea Kombucha (5.79 mg GAE/200mL) during 11 days of storage at 28±2°C. The results suggested that storage period had no major effects on the antioxidant concentration of Kombucha.

2.8 Post-fermentation processing

The efficiency of kombucha fermentation is determined by the final acidity, ethanol content and the residual sugar remaining at the end of fermentation. The metabolism of residual sugar is required to generate the desired level of carbonation in the second fermentation and also to reduce the amount of the sugar in the final product (Alderson et al., 2021; Zhang et al., 2021). However, secondary fermentation may result in over production of alcohol to a level higher than permissible in the final product (Talebi et al., 2017; Vohra et al., 2019). Regulations for non-alcoholic beverages vary across the globe. In NZ, Kombucha is categorised as a non-alcoholic beverage with an alcohol content of no more than 1.15 % ABV or 0.5 % ABV (FSANZ, 2019; Mutukumira et al. 2020 ; KBI, 2020; McIntyre & Sik Jang, 2020). To maintain a low alcohol content, some producers use advanced food processing technologies such as high heat treatments, pasteurization, filtration, and the distillation of alcohol (Kim & Adhikari, 2020).

Vacuum distillation technology is used in the wine industry to reduce ethanol in beverages during the fermentation step. Maintaining fluctuating temperatures, high pressure and continuous flow are some of the challenges faced during the vacuum distillation process (Liguori et al., 2018). Heat treatment methods used to reduce ethanol are likely to disrupt the probiotic function of the culture and alter the sensory profile of Kombucha (Kim & Adhikari, 2020). Reduction in antioxidant properties have been reported for Kombucha following preservation by heat treatment (76 and 100°C for 10 min) (Ahmed et al., 2020). A similar study (Jayabalan et al., 2008) also showed reported the negative impact of heat treatment (60, 65 and 68°C for 1 min) on the stability of Kombucha. Filtration is a common technique used to maintain stability, quality and clarity of fermented beverages (beer and wine), with previous studies (Freeman & McKechnie, 2003; Shala et al., 2017; Ubeda & Briones, 1999) reporting the efficiency of filtration by physically removing yeast and bacterial cells, protein, and hop substances that can interfere with the transparency and stability of beverages during storage.

2.9 Sensory evaluation

Kombucha is characterised by a slight sweetness, tartness and effervescence. Sensory attributes of Kombucha are linked to the physical (colour, turbidity), chemical (pH, T.A. and organic acids) and microbiological properties (yeast and bacteria counts) of Kombucha. Occasionally, small amounts of LAB have been reported in Kombucha but their role during fermentation is not clear (Jayabalan et al., 2014; Laureys et al., 2020). The residual sugar present after fermentation is responsible for sweetness; organic acids produced by AAB are responsible for sourness/vinegary taste, and the carbon dioxide generated contributes to the refreshing taste of Kombucha. The acetic acid produced during fermentation is believed to be involved in the aroma profile of Kombucha (Tran et al., 2020b), along with the ester compounds formed by yeasts in the culture (Laureys et al., 2018; Laureys & De Vuyst, 2014; Rodrigues et al., 2006).

Studies by Ayed et al. (2017) and Marsh et al. (2014) have reported that prolonged fermentation times result in a vinegary taste in Kombucha, which were unacceptable to consumers. This is supported by findings that Kombucha fermented at 25℃ for 10 days received higher sensory scores compared to Kombucha fermented at 20℃ and 30℃ for 10 days (Neffe-Skocińska et al., 2017). Thus, the results suggest that duration of fermentation and temperature influence the organoleptic properties of Kombucha.

Zhang et al.(2021), who performed consumer sensory evaluations on tea and herbal-based Kombucha on Day 6, determined the tea-based Kombucha had a lower sensory score than the herbal-based Kombucha, due to the unpleasant acidity produced in former. The possible reasons for undesirable acidity could be excess production of isovaleric acid in tea-based Kombucha (1.2 to 3.9 g/L) compared to herbal-based Kombucha (0.1 to 0.3 g/L) (Zhang et al., 2021), as others have found isovaleric acid (mainly produced by *Brettanomyces*) to be the main contributor of the unpleasant flavour in fermented Kombucha (Cibrario et al., 2019 ; Romano et al., 2009).

2.10 Summary

Kombucha is a traditional beverage prepared using sugar and tea, followed by fermentation by a symbiotic association of yeast and acetic acid bacteria. The popularity of Kombucha has increased due to its natural refreshing properties, appealing sensory properties and its perceived health benefits. Kombucha contains a range of metabolites (organic acids, ethanol, carbon dioxide) and inherent nutrients (polyphenols, amino acids, minerals, vitamins) derived from tea. The final composition of Kombucha beverages may be affected by several factors including type and concentration of substrate (tea and sugar), fermentation time, fermentation temperature and concentration of viable cells in the brewed Kombucha. Typically, the pH of the fermented Kombucha lies below 4.6 which is generally regarded as safe. As a non-alcoholic beverage, Kombucha should meet the regional stipulated regulations for alcohol content. However, most producers find it difficult to meet this requirement due to the complex fermentation of Kombucha using a largely undefined culture. This study attempts to optimise the fermentation of Kombucha to produce a consistent, low alcohol, high-quality beverage.

Chapter 3. MATERIALS AND METHODS

3.1 Description of Experimental Design

This section presents an overview of the experiments used in the present study as shown in Figures 3.1 and 3.2. The study was divided into three integrated phases (Figure 3.1; Figure 3.2). Phase 1 aimed to select the optimum fermentation conditions to propagate the Kombucha starter culture. Eight treatments with four factors (independent variables) comprising initial sugar concentration, initial tea concentration, fermented broth concentration and fermentation temperature were used. Each factor consisted of two levels of each independent variable (sugar; black tea; fermented broth, fermentation temperatures) (Figure 3.2). The responses measured (dependent variables) were acidity (pH and T.A.), total soluble solids, weight of SCOBY and microbiological counts (yeast, acetic acid bacteria and total counts) during fermentation of Kombucha (14 days). The best conditions for growth of the starter culture determined in Phase1 were used to propagate fresh starter culture for the subsequent experiments.

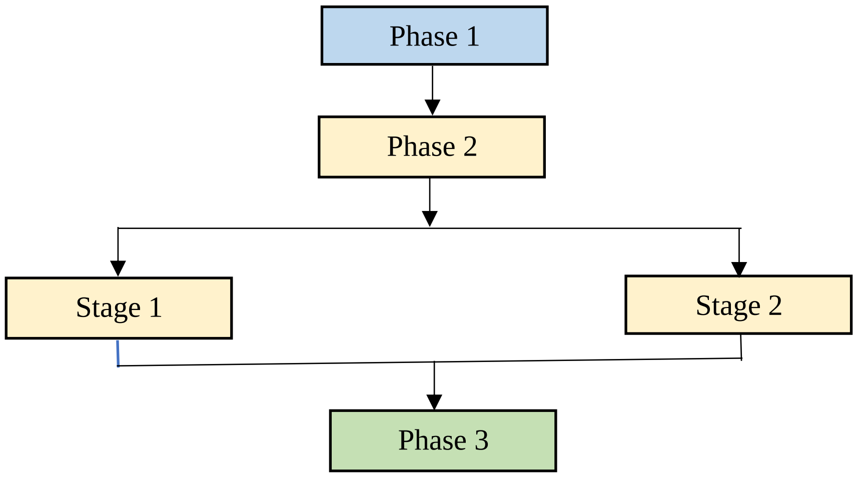


Figure 3. 1 Global overview of the study

Phase 2 aimed to select the most promising formulation for fermentation of Kombucha using two integrated stages. Stage 1 investigated the effect of two sugar concentrations and two fermentation temperatures to select suitable formulation/fermentation conditions for Kombucha production. Stage 2 investigated the effect of filtration post-primary fermentation, on microbial counts and overall impact on the characteristics of fermented Kombucha during storage (4°C) for one week. A focus group of sensory panellists also evaluated the beverages at the end of primary fermentation. The two most promising formulations were selected for further investigation in Phase 3.

Phase 3 investigated the storage stability of the selected formulations (filtered and unfiltered Kombucha) from Phase 2 for three weeks (4°C).

3.2 Description of Materials

The raw materials used to propagate the culture and to ferment the Kombucha beverage were black tea (English breakfast tea, New Zealand), organic white sugar (Natural Sugars Ltd, NZ) and fermented broth (pH 2.86) supplied by Mamas Brew Shop Limited, Auckland, NZ. All chemicals used in this study were of reagent grade or higher. The experiments described in this study were repeated twice and each analysis was conducted twice.

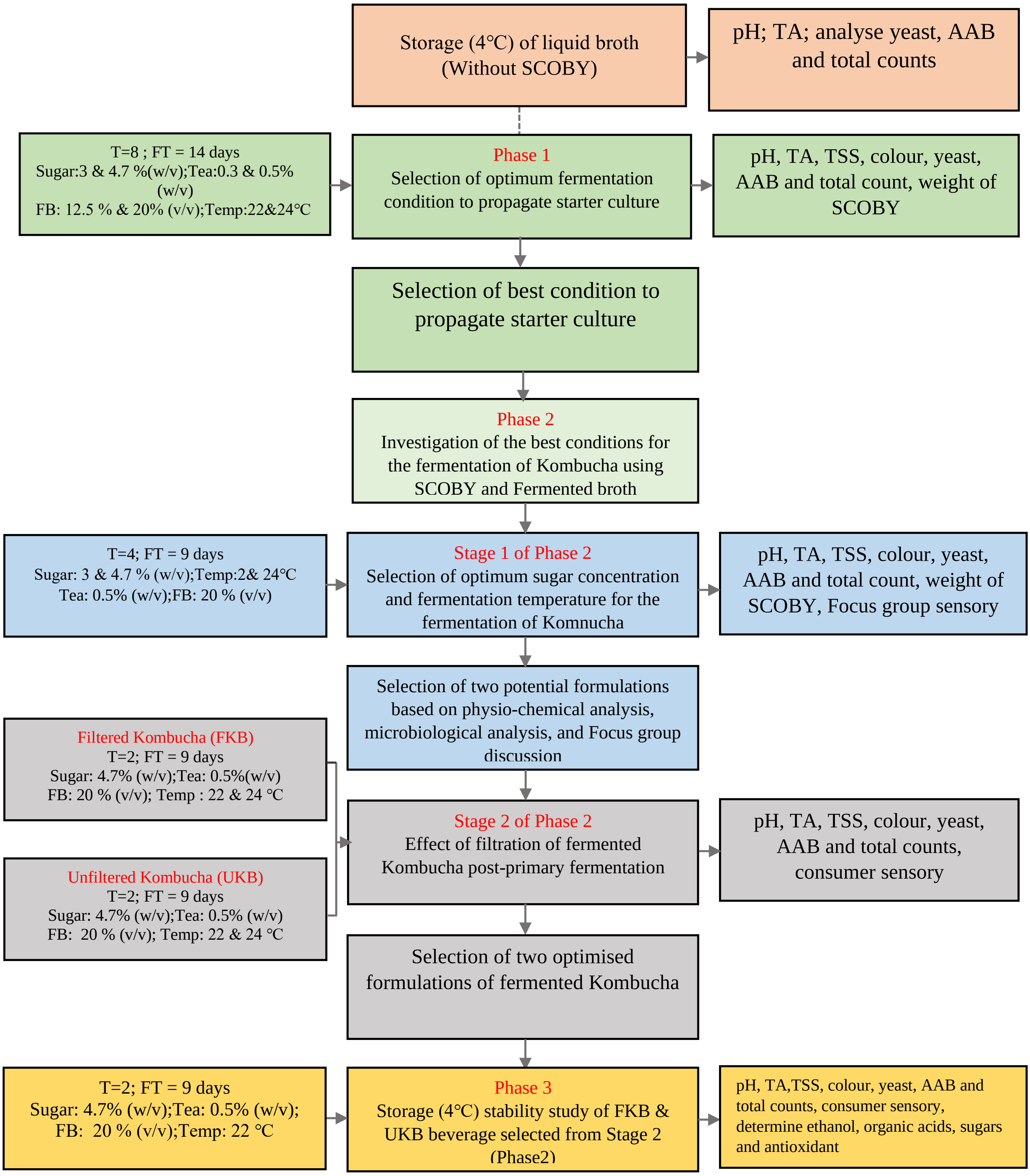


Figure 3. 2 Experimental design to determine conditions for optimum propagation of Kombucha starter culture, fermentation and storage of the fermented beverage

Note: FKB = filtered Kombucha samples; UKB = unfiltered Kombucha samples; FT=fermentation time; FB = fermented broth; TA = titratable acidity; TSS = total soluble solids; Temp = Temperature.

3.3 Experimental design to determine optimum conditions for propagation of the starter culture and fermentation of the Kombucha beverage

3.3.1 Phase 1 Selection of optimum fermentation conditions to propagate starter culture comprising fermented broth with no visible developed SCOBY

The supplied liquid fermented broth (Mamas Brew Shop Limited, Auckland, NZ) containing undeveloped strands of the suspended cellulose pellicle was stored at 4°C until required for propagating starter cultures using the formulations shown in Table 3.1. Propagation of starter cultures was based on the report by Ivanišová et al. (2019) with minor modifications.

Table 3. 1 Experimental design used in Phase 1 to select suitable conditions to propagate Kombucha starter culture

Formulation Group #	Product	RO Water (L)	% BT (w/v)	% WS (w/v)	% FB (v/v)	FT (°C)
I	KB1	1.32	0.3	4.7	12.5	22
	KB2	1.32	0.3	4.7	12.5	24
	KB3	1.32	0.3	3.0	12.5	22
	KB4	1.32	0.3	3.0	12.5	24
II	KB5	1.20	0.5	4.7	20.0	22
	KB6	1.20	0.5	4.7	20.0	24
	KB7	1.20	0.5	3.0	20.0	22
	KB8	1.20	0.5	3.0	20.0	24

Note: RO = reverse osmosis; BT= black tea; WS = organic white sugar ; FB = fermented broth; FT= fermentation temperature; fresh(24 hours) fermented broth was used to inoculate the sugared infusions.

(a) *Preparation of Kombucha starter using formulations for Group I (Table 3.1)*

For each sample, reverse osmosis (RO) water was boiled, then 1.32 L was transferred into a stainless-steel container and allowed to cool to 80-90 °C at room temperature (22 °C) before 3.6 g black tea leaves (English breakfast tea, New Zealand) was added. The infusion was covered with thin kitchen-type aluminium foil (Briscoes, New Zealand) and allowed to steep for 15 min at room temperature (22°C). The tea leaves were removed by straining using a double mesh ultra-fine 18/10 stainless steel filter (Bialett Industrie, Italy). Organic white sugar (70.5 g) (Natural Sugars Ltd, New Zealand) was added into two infused tea solutions (KB1, KB2) and 45 g of sugar was added to the other two samples (KB3, KB4) (Table 3.1). The infused tea solutions were stirred continuously using a stainless-steel spoon until the sugar had completely dissolved. The sugared solutions were cooled to room temperature (22±1℃) and then transferred to 2-L clip-lidded glass jars (Kmart Ltd, New Zealand). Fermented broth (150 ml) was added to each sugared tea solution to give a final volume of 1.5 L. The glass jars were covered with 30×30 cm perforated multipurpose non-woven cloths (Briscoes Limited, New Zealand) and secured with rubber bands and incubated in water baths at either 22 °C (KB1 and KB3) or 24 °C (KB2 and KB4). The labelled inoculated infused tea mixtures were allowed to ferment aerobically for 14 days (Figure 3.3 and Figure 3.5).

(b) *Preparation of Kombucha starter using formulations in Group II (Table 3.1)*

Kombucha starter culture for the samples in this section were prepared according to the method described in Section 3.1a, except the concentration of black tea leaves and volume of initial fermented broth added to the sweetened tea solutions differed. To propagate the starter culture used for the formulations in Group II (Table 3.1), RO water (1.2 L) was boiled and the following ingredients were sequentially added: black tea leaves (7.5 g), sugar (45 g) for samples KB7 and KB8, while 70.5 g sugar was added to samples KB5 and KB6 (Table 3.1). Fermented broth (300 mL) was added to each infused mixture to make a final volume of 1.5 L. The glass jars containing the inoculated, infused tea solutions were labelled, covered as previously described and allowed to ferment aerobically for 14 days at 22℃ (KB5 and KB7) and 24℃ (KB6 and KB8) (Table 3.1)

During fermentation, samples (20 mL) were withdrawn at days 0, 3, 6, 9, 12 and 14 (Figure 3.1) to determine acidity (pH and titratable acidity) and total soluble solids (TSS). Yeast, AAB and total counts of the fermented broth were determined at Day 0 and Day 14 during fermentation. Microbial counts for the cellulose pellicle were determined at the end of fermentation (Day 14), and the weight of cellulose pellicle was also measured. The optimised formulation for propagating starter culture was used to prepare starter culture for the fermentation of Kombucha beverages in the subsequent experiments.

Figure 3. 3 Kombucha following fermentation in clip-lidded glass jars at 22 and 24°C for 14 days

SCOBY = symbiotic culture of bacteria and yeast

3.3.2 Phase 2 Investigation of the best conditions for the fermentation of Kombucha using a combination of developed SCOBY and fermented broth as the starter culture

Stage 1 Selection of optimum sugar concentration and fermentation temperature for the fermentation of Kombucha

Fresh starter culture for the fermentation of Kombucha was prepared using the optimised conditions determined in Section 3.3.1. In this stage, fermented Kombucha beverages were prepared according to the formulations shown in Table 3.2. The infused sugared samples were inoculated with fermented broth (20%) and SCOBY (2.5%), then fermented for 9 days at 22 and 24 °C (Figure 3.5).

Table 3.2 Experimental design used in stage 1 of Phase 2 to investigate the effect of sugar concentration and fermentation temperature on Kombucha

Product	RO Water (L)	% BT (w/v)	% WS (w/v)	% FB (v/v)	% SCOBY (w/v)	FT (°C)
KB5	1.20	0.5	4.7	20	2.5	22
KB6	1.20	0.5	4.7	20	2.5	24
KB7	1.20	0.5	3	20	2.5	22
KB8	1.20	0.5	3	20	2.5	24

Note: BT= black tea; WS =organic white sugar; FB = fermented broth; FT = fermentation temperature; RO = reverse osmosis.

Samples (20 mL) were withdrawn from the four samples (KB5, KB6, KB7, KB8) (Table 3.2) at 0, 3, 6 and 9 days to determine pH, acidity, specific gravity, colour and microbial counts (AAB, yeast and total counts). The developed SCOBY was weighed at the end of fermentation (Day 9). A focus group of sensory panellists (5-7) who were familiar with fermented Kombucha evaluated the products on Day 9 (Section 3.7). The results obtained in this Stage were used to select two potential formulations which were further investigated in Stage 2 of Phase 2.

Stage 2 Potential of filtration for reducing the microbial counts in Kombucha following fermentation

Stage 2 investigated the potential of using filtration to reduce the fermenting microbial load in the Kombucha samples as well as its impact on the overall characteristics of the products. Based on the preliminary results obtained in the earlier experiments (Stage 1, Phase 2), two promising formulations (KB5, KB6) were selected for further evaluation in this Phase (Stage 2, Phase 2). Sugar concentration (4.7% w/v), tea concentration (0.5% w/v), weight of SCOBY (2.5% w/w) and volume of fermented broth (20%, v/v) were kept constant in this Phase, however two fermentation temperatures (22°C, 24°C) were utilised.

Table 3. 3 Summary of experimental design for Stage 2 of Phase2 to investigate the effect of filtration on Kombucha post-primary fermentation

Product	RO Water (L)	% BT (w/v)	WS (w/v)	FB (v/v)	SCOBY (w/v)	FT (℃)	Treatment
KB5F	1.20	0.5	4.7	20	2.5	22	FKB
KB5U	1.20	0.5	4.7	20	2.5	22	UKB
KB6F	1.20	0.5	4.7	20	2.5	24	FKB
KB6U	1.20	0.5	4.7	20	2.5	24	UKB

Notes: FT= fermentation temperature; FKB = filtered Kombucha beverage; UKB = unfiltered Kombucha beverage.

After fermentation of the Kombucha (1.5L) samples for 9 days at two different temperatures (Table 3.3), 350 mL of each were filtered using a double mesh ultra-fine 18/10 stainless steel filter (10-11 µm, Bialetti Industrie, Italy) and bottled in 500-mL swing capped amber glass bottles (Mighty Ape, New Zealand) (Figure 3.4). The remaining unfiltered Kombucha was transferred into separate labelled swing capped bottles. All bottled samples (filtered and unfiltered) were stored (4 ℃) for one week. The determinations (responses) conducted in Stage2 (Phase 2) were also evaluated in this Phase (Section 3.3.2). In addition, consumer sensory evaluation was conducted after storage (4℃) for one week (Section 3.6), where panellists evaluated the products for appearance, aroma, flavour, sweetness, sourness and overall acceptability.

3.3.3 Phase 3 Stability of filtered and unfiltered Kombucha during prolonged storage (4℃) for 3 weeks

Figure 3. 4 Primary fermentation and storage (swing capped bottles) of fermented Kombucha for three weeks at 4℃

The storage stability of the two optimised formulations from Stage 2 (Phase 2) was studied in Phase 3. The samples were fermented following the method described in Phase 2 (Section 3.3.2), after which the labelled bottles were stored at 4℃ for three weeks. In Phase 3, Kombucha samples were analysed at 0, 3, 6, 9 days (primary fermentation). Thereafter, samples were analysed for concentrations of ethanol, sugar, organic acids, antioxidants, microbiological content and physico-chemical characteristics. Consumer sensory evaluation was performed weekly during storage of the samples.

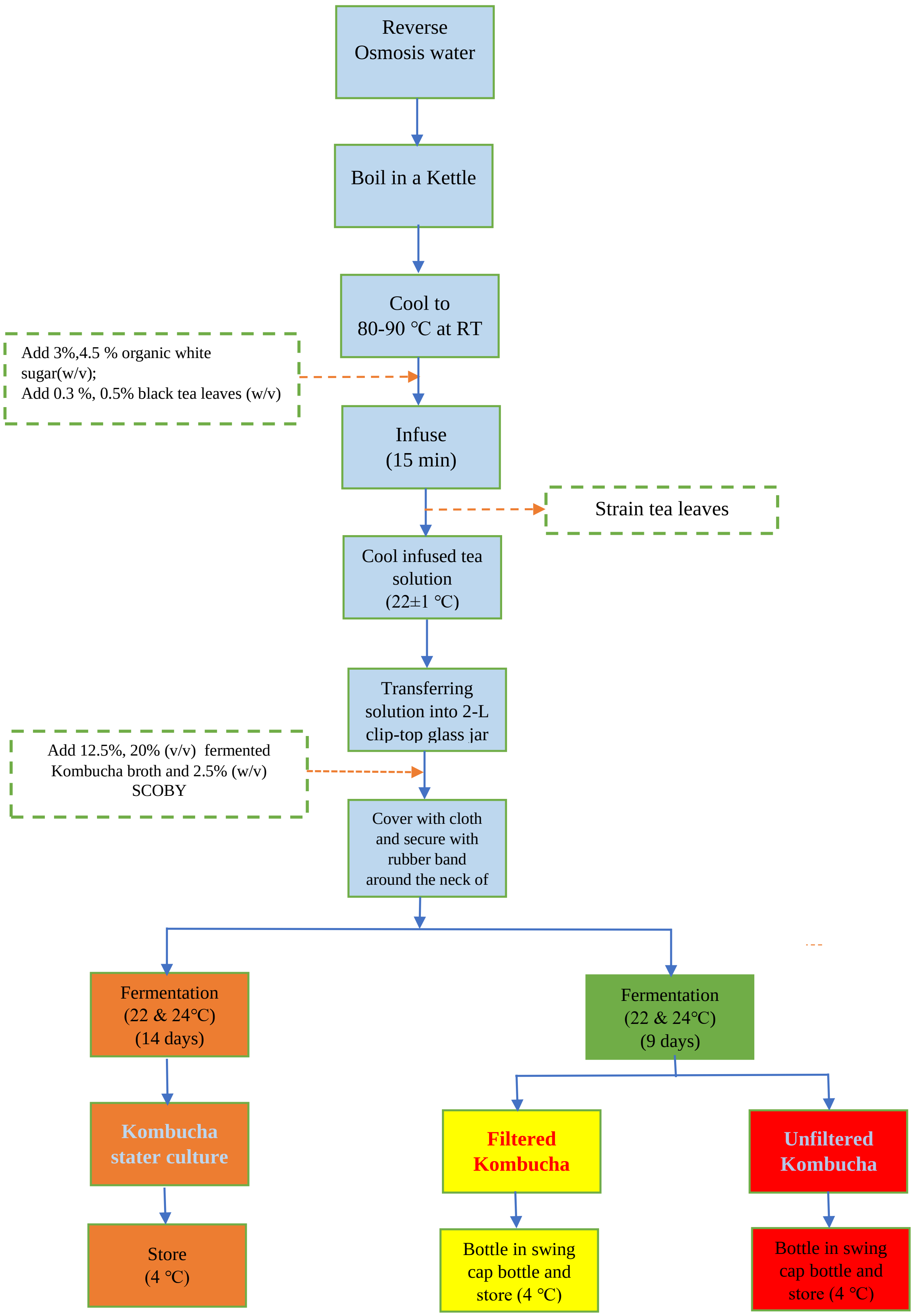


Figure 3. 5 Lab production of Kombucha

Note: RT = room temperature

3.4 Physico-chemical characterisation of fermented Kombucha

3.4.1 Measurement of pH

The pH of Kombucha samples was measured directly using a digital pH meter (Sartorious PB-20, USA) equipped with a glass electrode described by the AOAC method 981.12 (AOAC,2015). Prior to measurement, the pH meter was calibrated using standard buffer solutions at pH 4.0, 7.0 and 10.0 (LabServ, Thermofisher, NZ). The pH glass electrode was rinsed with distilled water between measurements. The pH of the samples was measured in duplicate and the experiments were replicated twice.

3.4.2 Determination of titratable acidity

Standardisation of 0.1 M sodium hydroxide

Standardised sodium hydroxide (0.1M) was prepared according to the AOAC (AOAC 936.16,1990). The following reagents were used to standardise the sodium hydroxide: distilled water, potassium hydrogen phthalate solution (KHP), 1% phenolphthalein solution and 0.1 M sodium hydroxide (NaOH) (Univar, AjaxFinechem Pvt Ltd, NZ). Prior to the standardisation process, 0.15-20 g of dried KHP was weighed using an analytical balance (Sartorius CP 225 D, USA) and dissolved in 50 ml of distilled water in an Erlenmeyer flask. The prepared KHP solution was titrated against 0.1 M NaOH using phenolphthalein indicator until the first persistent pink colour was observed. The amount of NaOH (ml) required was recorded, and the titration was repeated until concordant results were obtained. The concentration of NaOH was calculated using equation 1.

$$M\ NaOH = \frac{m\ KHP}{V\ NaOH \times 0.204229} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [1]$$

Where M NaOH = molarity of NaOH (mol/L); m KHP = mass of KHP (g); V NaOH = volume used in the titration (ml); molecular weight of KHP = 204.23 (g/mol)

Determination of titratable acidity of fermented Kombucha

Acid-base titrations were conducted to determine the titratable acidity of the fermented Kombucha samples using the AOAC method (AOAC 947.05, 2005). The Kombucha samples (10 g) were weighed in 250-mL Erlenmeyer flasks using an analytical balance and mixed thoroughly with 20 ml distilled water. One - three drops of phenolphthalein indicator (1 %) were added to the sample solutions and swirled to mix completely. The diluted samples were titrated against 0.1 M NaOH solution until the first persistent faint pink colour was observed. The volume of NaOH used for the titration was recorded and the concentration of acetic acid was calculated using equation 2.

$$\% \ Acetic\ acid = \frac{volume\ of\ NaOH\ used\ (ml) \times 0.0060}{sample\ weight} \times 100 \qquad [2]$$

1 mL of test sample ≈ 1 g of sample; molecular weight of acetic acid = 60 g/mol; 1 mL 0.1 M NaOH = 0.0060 g acetic acid

3.4.3 Determination of total soluble solids (TSS)

The total soluble solids in the fermented Kombucha samples were measured using a digital refractometer (Atago PR-101, Japan). Prior to measurement, the refractometer was calibrated using distilled water by following the AOAC 932.12 standard method (AOAC, 1990). The results were recorded in °Brix.

3.4.4 Measurement of colour

The colour of the fermented Kombucha samples was measured using a spectrophotometer (CM-5, Konica Minolta, Japan) based on the L*, a*, b* colour system (Jakubczyk et al., 2020; Kayisoglu & Coskun, 2020; Muhialdin et al., 2019; Pathare et al., 2013). Prior to measurement, the spectrophotometer was calibrated following the manufacturer's instructions. For colour measurement of samples, the fermented Kombucha sample (3 mL) was gently pipetted into 4 mL plastic spectrophotometer cuvettes (Sigma Aldrich, NZ) to avoid generation of some bubbles which can interfere with the accuracy of results.

The illuminating D65 artificial daylight at a 10° standard angle was used for the measurement and the responses L*, a*, b* were recorded immediately.

3.4.5. Measurement of wet weight of cellulose pellicle

The wet weight of cellulose pellicle (g/L) during fermentation of black tea Kombucha was measured using previous methods with slight modifications (Goh et al., 2012; Hassan & AL-Kalifawi, 2014). The cellulose pellicle obtained at the end of fermentation in Phase 1 and Phase 2 was calculated using equation 3 and equation 4, respectively.

Phase 1 Kombucha starter culture was propagated with fermented Kombucha broth without visible cellulose pellicle at start of fermentation

$$\text{Weight of wet cellulose pellicle (g/L)} = \text{WoB} + \text{KTB} + \text{CP (end of fermentation)} - \text{WoB} + \text{KTB (start of fermentation)} \ldots\ldots [3]$$

Phase 2 Kombucha beverage was fermented with cellulose pellicle and Kombucha fermented broth at the start of fermentation

$$\text{Weight of wet cellulose pellicle (g/L)} = \text{WoB} + \text{KTB} + \text{CP (end of fermentation)} - \text{WoB} + \text{KTB} + \text{CP (start of fermentation)} \text{------}[4]$$

WoB= weight of empty beaker; CP = cellulose pellicle; KTB = Kombucha sugared tea broth

3.4.6 Analysis of ethanol content by gas chromatography

Analysis of alcohol by volume (% ABV) in the fermented Kombucha was measured using a Shimadzu Gas Chromatograph system model GC-17A (Shimadzu, Japan) according to the method of Liu et al. (2019) with minor modifications. The GC operating conditions are shown in Table 3.4.

Table 3. 4 Gas Chromatography operating conditions for analysis of ethanol

Parameter	Description
Column	DBWAX capillary column (30 m by 0.32 mm) with 0.25-µm stationary phase
Initial GC oven temperature	40 ℃
Split ratio	40:0
Run time	4 min
Injector temperature	150 ℃
Flame ionization detector (FID)	230 ℃
Carrier gas	Nitrogen at 108.1mL/min
Injection volume	1 µL

The column oven temperature programme for the GC system during analysis of ethanol in both the test samples and standards is shown in Figure 3.6. Standard ethanol solutions (0.1%, 0.25% ,0.5% ,0.75%, 1%) were prepared using ethanol (≥98%, Thermo Fisher Scientific, NZ) and freshly distilled water (v/v). The Kombucha samples were filtered through a 0.22 µm syringe filter (Merck, Germany) into 2-mL glass GC vials (Shimadzu, AUS) and kept at -4℃ until required for analysis. The standards were analysed first to generate standard curves followed by test samples. The retention time and peak areas were used for the identification and determination of ABV (%) in the Kombucha samples. Chromatographic data was manually integrated using the Shimadzu GC Solution Software (Shimadzu, Japan) and the standard curve was generated using Microsoft® Excel 2016 (Microsoft Office, CA, USA).

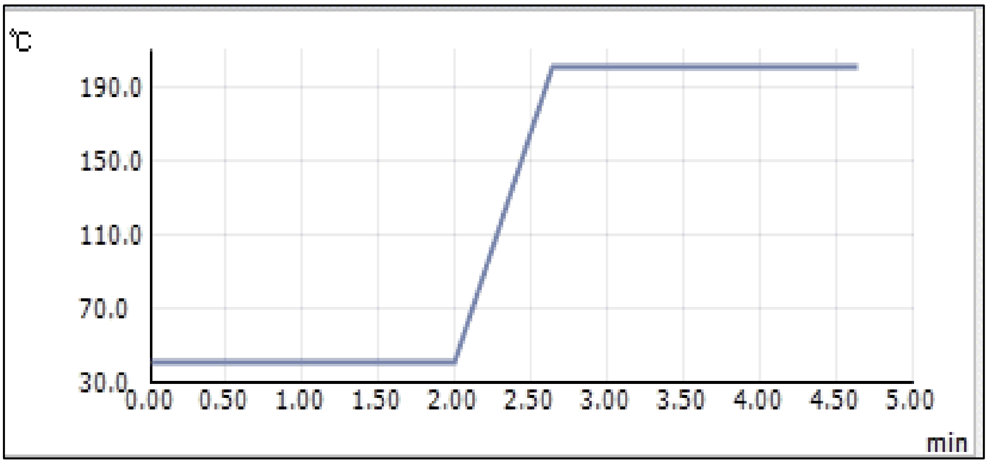


Figure 3. 6 GC programme for column temperature during analysis of ethanol in fermented Kombucha

Injection temperature =150℃; Rate of temperature increase = 250℃/mi

3.4.7 Analysis of organic acids by high performance liquid chromatography

Acetic acid, gluconic acid and glucuronic acid levels in fermented Kombucha samples were analysed using high pressure liquid chromatography (HPLC) following the method of Gaggia et al. (2019) with slight medications. Assays were performed using the Shimadzu HPLC system model LC -10AT which consisted of a column oven (CTO-AS, Shimadzu Corp, Japan), auto-injector (SIL-10A), a system controller (SCL-10A), a dual injection system equipped with an ultra violet (UV) detector (SPD-10A) and a refractive index (RI) detector (RID-10A). The organic acids were separated using a Rezex ROA-organic acid (8% cross-linked resin) column (300 × 7.8 mm) at 25℃. Sulphuric acid (0.05 N) was used as the eluent at a flow rate of 0.4 mL/ min. Prior to analysis, eluent was filtered through a 0.22-µm membrane filter (Merck Millipore, Ltd, Ireland) and degassed using an ultrasonic bath (Bandelin Sonorex Super RK510, Germany) to remove air bubbles**.** A mixed standard solution containing glucuronic acid (98%, Fisher Scientific , UK), gluconic acid (49-53 %, w/v in water, Sigma- Aldrich, USA) and acetic acid (≥ 99.5%, Fisher Scientific , UK) was prepared in distilled water. The concentrations of the three organic acids standards in the mixed solutions were 0.001%, 0.01%, 0.1%, 0.5%, 1%, 2% (w/v). The standards and samples were filtered through a 0.22-µm filter (Merck, Germany) then stored in 2-mL glass vials (Shimadzu Corp, Japan) at -4℃ until required for chromatographic analysis. The sample and standard injection volume was 20 µL, with each injection repeated twice. The organic acids in each sample were identified and quantified based on the retention time and peak area of the standards using the Shimadzu GC Solution Software (Shimadzu, Japan). A standard curve was generated using the Microsoft® Excel 2016 (Microsoft Office, CA, USA).

3.4.8 Analysis of sugars by gas liquid chromatography (GLC)

Derivatised sugars were analysed by an accredited laboratory (AssureQuality; https://www.assurequality.com), using gas chromatography (Medeiros & Simoneit, 2007).

3.4.9 Analysis of antioxidants

The phenolic compounds comprising epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), gallic acid and the alkaloids (theobromine and caffeine) in fermented Kombucha were determined by HPLC according to Malbaša et al. (2014) with minor modifications. Phenolic compounds and alkaloids were separated using a Shimadzu HPLC system (Shimadzu UFLC, Japan), integrated with an auto-sampler (SIL-20ACHT), pump (LC-20 AD), photodiode array detector (SPD-M20A) and 5 µm Grace Smart RP18 column (250 × 4.6 mm) set at 18°C. A discontinuous gradient described by D'Avila (2013) was run from 100% mobile phase A (0.1% TFA + Milli-Q-water) to 100% mobile phase B (0.1% TFA in acetonitrile) at a flow rate of 0.75 mL/min.

Standard stock solutions were prepared as follows: ECG, EGCG, EGC (1 mg/mL) in ethanol (70 %, v/v); Gallic acid (1mg/mL) in Milli -Q water; theobromine (25 µg/mL) and caffeine (126.2 µg/mL) were prepared in methanol. All antioxidant standards, and test samples were filtered (0.20 µm syringe filter; Merck, Germany) and stored in 2-mL glass vials (Shimadzu Corporation, Japan) at -4°C until required for chromatographic determination. The sample and standard injection volume was 20 µL and automatic injections were conducted in duplicate. Identification and analysis of antioxidants in test samples was based on the retention time and peak areas of standards detected at 270 nm. Chromatographic data were manually integrated using Shimadzu Solution Software (Shimadzu, Japan) and the data were used to generate standard curves using Microsoft® Excel 2017 (Microsoft Office, CA, USA).

3.5 Microbiological analysis

3.5.1 Microbial enumeration of Kombucha starter cultures and fermented beverages

The methods of Abel and Andreson (2020) and Tan et al (2020) were used to enumerate yeast, AAB and total counts in the Kombucha starter culture (cellulose pellicle, fermented broth) and Kombucha Beverage with minor modifications. For microbial analysis of the cellulose pellicle (SCOBY), about 10 g of SCOBY (sample) was aseptically weighed into a stomacher bag on an analytical balance (Sartorius CP 225 D, USA). Ninety (90) mL sterile peptone water (0.1%) was weighed into the stomacher bag and the mixture homogenised using a stomacher for 5 minutes and then suitable serial dilutions (10^6) were prepared in peptone water. For the fermented broth and Kombucha beverage sample, 1 mL of liquid sample was transferred into 9 mL peptone water using a sterile micropipette, then suitable serial dilutions of up to 10^6 were prepared and plated on Yeast Extract

Glucone Chloramphenicol (YGC), Yeast Extract Peptone Mannitol (YPM) and Plate Count Agar (PCA) agar by the pour plate method as described in subsequent sections.

Enumeration of yeast

Total viable yeast counts were enumerated on YGC agar (Merck KGaA, Germany). The medium was prepared following the manufacturer's instructions and sterilised in an autoclave (Astell Scientific, UK) at 121℃ for 15 minutes. Suitable dilutions of samples were pour-plated in duplicate and the solidified agar plates were incubated (Clayson IM1000R, Australia) at 25 ℃ for 5 days. After incubation, the grown colonies were counted using a colony counter (Bibby scientific, UK) and data expressed as log cfu/mL.

Enumeration of acetic acid bacteria

The AAB in the Kombucha samples were enumerated on Yeast Peptone Mannitol (YPM) agar containing bacteriological agar (12 g/L) (Thermo Fisher, New Zealand), D-mannitol (25 g/L), peptone (3 g/L) and yeast extract (5 g/L) (Sigma- Aldrich, New Zealand). YPM agar was prepared by mixing all the ingredients and adjusting the final volume to 1 L, and then autoclaving at 121℃ for 15 minutes. Once the medium was cooled (50 ± 2℃), cycloheximide (0.1g/L) was added to inhibit the growth of yeast. The diluted test samples were pour-plated in duplicate and allowed to solidify for 15 minutes, and further incubated (Clayson IM1000R, Australia) for 7 days at 30℃. The enumerated colony forming units were expressed as log CFU/mL.

Enumeration of total counts

Enumeration of total counts was performed on Plate Count Agar (Thermo Fisher, New Zealand). The agar was prepared according to the manufacturer's instructions and sterilised by autoclaving for 15 mins at 121℃. Ten-fold serial dilutions of samples up to 10^6 were prepared. Then 1 mL dilution was pour-plated in duplicate on PCA, allowed to solidify and then incubated at 30 ℃ for 3 days. Grown colonies were counted using a colony counter (Bibby scientific, UK) and expressed as log CFU/mL.

3.5.2. Microbial enumeration of filtered, unfiltered Kombucha and recovered cells during filtration

Following primary fermentation (Section 3.3.2), microbial enumeration of unfiltered and filtered Kombucha beverages was conducted as described in Section 3.6.1. During filtration, some of the yeast and AAB cells embedded in suspended cellulose strands were trapped on the surface of the stainless sieve (Freeman & McKechnie, 2003; Shala et al., 2017). This material was recovered from the surface of the sieve and enumerated by plating on suitable agar.

Microorganisms trapped on the sieve were dislodged by ultrasonication (Joyce et al., 2011; Pitt & Ross, 2003). The stainless-steel filter was inverted in a 2000 mL sterile glass beaker containing 0.1 % peptone water (1 L) and placed in an ultrasonic bath and sonicated (Bandelin Sonorex Super RK510) for 15 minutes at an ultrasonic nominal power of 160 W and peak power of 640W. The peptone water containing suspended cells were serially diluted up to 10^5 and enumeration of yeast, AAB and total counts were conducted according to the procedures described in Section 3.5.1.

3.5.3 Examination of morphology of yeast and AAB cells

Morphological examination of yeast and AAB was conducted for the unfiltered Kombucha samples after primary fermentation at Day 9 (Section 3.3.3). Samples were enumerated for yeast and AAB by the pour-plate method on selective media as described in Section 3.5.1. After incubation, three representative colonies from incubated plates were streaked on YGC and YPM agar for yeast and AAB, respectively. Cell morphology was examined by Gram staining (AAB) and methylene blue (yeast) staining methods.

Examination of Gram stained AAB

After streaking, three representative colonies isolated on YPM agar were Gram-stained by placing one drop of water on a clean glass slide, then transferring a loopful of the bacterial colony onto the slide to prepare a bacterial smear. The smear was air-dried and heat-fixed (3-5 sec). Crystal violet was poured onto the smear and allowed to react for 1 min, then rinsed off with running potable water and excess water was removed using a soft tissue. The smear was flooded with Gram’s iodine (1 min) and washed with water.

Then, 3 to 4 drops of ethanol were added to decolourise the slide, incubated for about 30 seconds and then rinsed with water. Safranin (counterstain) was added to the slide and left for 1 min before washing with water. The slides were air-dried and the Gram-stained isolates were observed under oil immersion (x1000) using the Carl Zeiss Transmission Light Microscope (Model HB0 50/AC, Germany). The shape of the cells was recorded and AxioVision microscope software (Version 4.8.1) was used to measure the size of the AAB cells.

Examination of methylene blue stained yeast

Three representative pure yeast colonies isolated from YGC agar were stained with methylene blue to observe the cell morphology. Prior to staining, the methylene blue stain was prepared by dissolving 0.01 g of methylene blue powder (Sigma Aldrich, New Zealand) and sodium citrate dihydrate (2g) (Sigma Aldrich, New Zealand) in distilled water (10 mL). The solution was mixed thoroughly, and distilled water was added to make up to 100 mL. For staining, one drop of methylene blue was transferred to a clean glass slide and then a young yeast colony (18-24 h) was transferred onto the slide using a sterile loop. A cover slip was gently placed on the glass slide to avoid trapping air bubbles. The shapes and sizes of cells were examined using a Carl Zeiss Transmission Light Microscope under oil immersion (x1000 resolution), AxioVision microscope software (Version 4.8.1).

3.6 Sensory evaluation

Focus groups and consumer sensory evaluation was used to evaluate kombucha during fermentation and storage. Six sensory attributes of fermented Kombucha were assessed: appearance, odour, flavour, sweetness, sourness and overall acceptability (Alderson et al., 2021; Osiripun & Apisittiwong, 2021). Focus group discussions and consumer sensory evaluations were conducted in the Product Development Laboratory (Massey University, Albany campus). Sensory testing conducted in this study was approved by the Massey University Human Ethics Committee (Ethics Approved Number 4000022993). Prior to evaluating the fermented Kombucha, sensory panelists completed a consent form (Appendix B).

Both focus group and consumer sensory panellists were recruited within Massey University, Auckland Campus, and comprised of students, staff and visitors. Each focus group consisted of 6 to 8 multicultural persons who had a food science background, experience in sensory analysis, and were familiar with fermented and non-fermented beverages. Focus group panellists evaluated four fermented Kombucha beverages in Stage 1 of Phase 2. Consumer sensory evaluations were completed by 108 panellists who evaluated the samples using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (Appendix B) (Francisco & Igor, 2021; Osiripun & Apisittiwong, 2021; Ulusoy & Tamer, 2019). For sensory evaluation, the samples were prepared by transferring 15 mL of refrigerated (4°C) fermented Kombucha into 3-digit coded transparent plastic cups (25 mL) (Huhtamaki, NZ). The panelists cleaned their palate with still mineral water before evaluating each sample. Fermented Kombucha samples were evaluated at Week 1 in Stage 2 of Phase 2 (Section 3.3.2). Whereas sensory evaluation of stored samples (4°C) was performed at Week 1, Week 2 and Week 3 for Phase 3 (Section 3.3.3).

3.7 Statistical analysis of data

All experiments described in the present research were repeated twice. For each sample the data for physio-chemical (pH, T.A, TSS, colour, ethanol content, sugar levels, organic acid content and antioxidants), microbiological analysis (mean yeast, AAB and total counts) and sensory analysis was collected in duplicate. Data analyses were performed using Minitab version 18 Statistical Software (Minitab Inc., State College, PA, USA). The data was represented as mean ± SD, and the mean and standard deviations for samples was calculated using Microsoft Excel 2016 (Santa Rose, CA, USA). Graphical representation of data was conducted using Origin 2020 (Origin Lab Corporation, Northampton, MA, USA). General Linear Model (GLM) was to select optimum fermentation condition to propagate the starter culture in Phase 1 based on the mean significant differences ($p<0.05$). GLM was also used in Phase 2 and Phase3 to determine the effect of sugar concentration, fermentation temperature and storage of filtered and unfiltered Kombucha samples on the pH, T.A., colour, microbial counts, ethanol content, sugar levels, organic acid content, antioxidants and consumer sensory analysis. The statistical output is shown in Appendix H.

Chapter 4. RESULTS AND DISCUSSION

4.1 Phase 1 Selection of optimum fermentation conditions to propagate Kombucha starter culture comprising fermented broth with no visible developed SCOBY

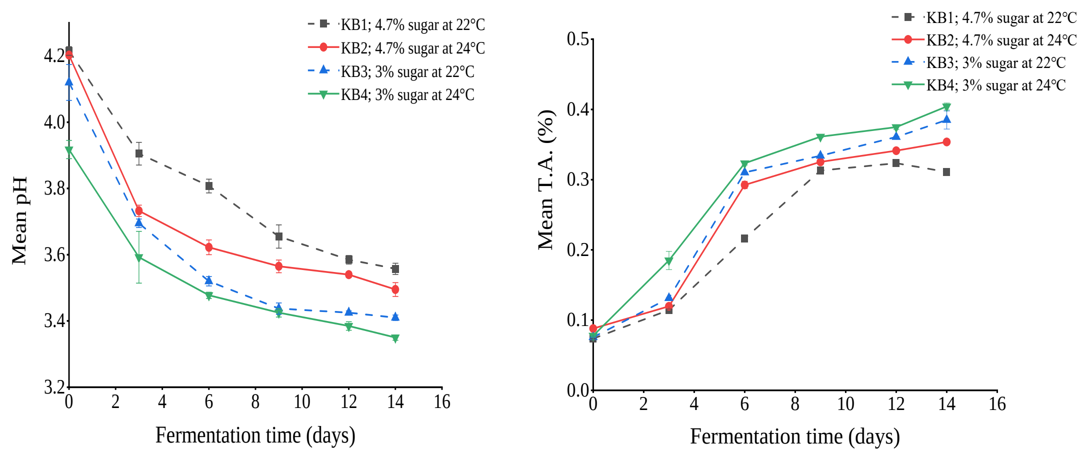
Phase 1 consisted of eight treatments shown in Table 3.1 (Section 3.3.1). Acidity, total soluble solids, weight of SCOBY and total microbial counts were determined to evaluate the performance of the fermentation processes. For this study, the culture used for Kombucha fermentation was propagated from liquid fermented broth which had no visible strands of cellulose pellicle. The liquid fermented broth was propagated to form a culture consisting of the 'broth and developed cellulose pellicle (SCOBY)' which is desirable to optimise the fermentation of Kombucha beverage (Dufresne & Farnworth, 2000; Jayabalan et al., 2014).

4.1.1 Acidity (pH and T.A.) during propagation of the starter culture

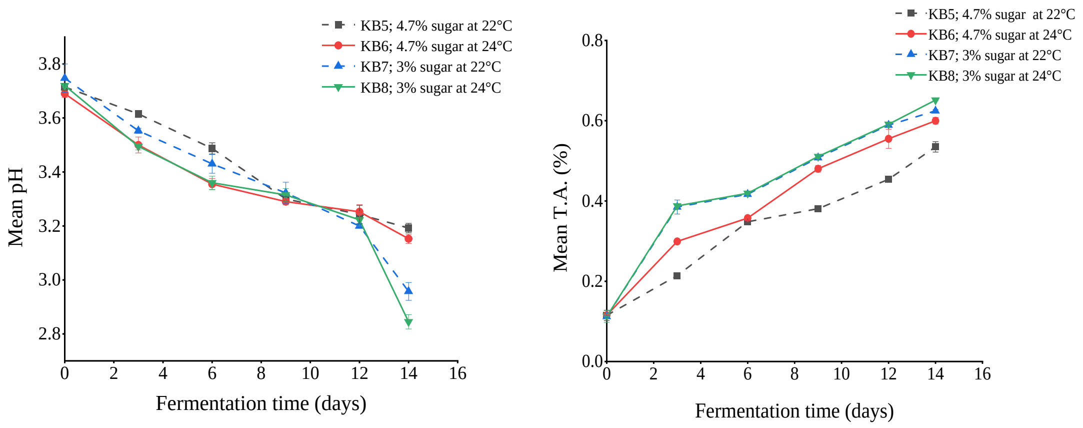
Acidity affects the microbial growth and metabolic activity of the Kombucha starter culture and therefore the synthesis of cellulose (Goh et al., 2012; Hassan & AL-Kalifawi, 2014; Lončar et al., 2006; Malbaša et al., 2008; Reiss, 1994). Consequently, it is important to monitor acidity during Kombucha fermentation. As expected, pH gradually decreased and T.A. steadily increased during fermentation for 14 days at 22℃ and 24℃ ($p<0.05$) (Figures 4.1 a-d). A range of organic acids produced during propagation of the starter culture may be responsible for the increasing acidity in the Kombucha samples (Chen & Liu, 2000; Jayabalan et al., 2007). In the previous studies mentioned here, increased acidity was also reported in Kombucha fermented for 14 and 18 days at 24±3℃.

The initial pH of samples (KB5, KB6, KB7 and KB8) that contained a higher level (20%) of fermented broth (Figure 4.1c) were higher than the pH of samples (KB1, KB2, KB3 and KB4) inoculated with a lower level (12.5%) of the fermented broth (Figure 4.1a). The differences in the initial pH of the samples were attributed to the different quantities of inocula added prior to fermentation (Greenwalt et al., 2000; Loncar et al., 2014). During starter propagation, the pH of all samples decreased until day 9, and then stabilised until the end of fermentation (day 14), except for two products (KB7 and KB8) (Figure 4.1 c). The pH of these two products continued to decrease until the end of fermentation (day

14). The decrease in pH during fermentation reflected the metabolic activity of the fermenting microorganisms present in the starter culture which produce a range of organic acids including acetic acid, gluconic acid, glucuronic acid (Chen & Liu., 2000; Goh et al., 2012; Jayabalan et al., 2007). The pH of the starter culture at the end of fermentation (14 days) ranged from 2.85±0.02 to 3.60±0.01 (Appendix C), which was similar to the study reported by previous researcher (Jakubczyk et al., 2020). This is an ideal pH range as it has been associated with high cell counts of yeast and AAB which are desirable for inoculation into infused tea-sugar solution for Kombucha fermentation.



(a) (b)



(c) (d)

Figure 4. 1 Mean pH and T.A. during propagation of Kombucha starter cultures fermented with 2 levels of sugars, low (a,b) and high (c,d) concentrations of tea and broth at two temperatures for 14 days

KB1 (4.7/22) contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 (4.7/24) contained 4.7% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 (3/22) contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 (3/24) contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 (4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 (4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 (3/22) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 (3/24) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Error bars =±SD; n=8; independent experiments were repeated twice.

The initial T.A. of all samples ranged from 0.07±0.00 to 0.12±0.00, and significantly ($p<0.05$) increased (0.31±0.00 to 0.65±0.01) during fermentation. Changes in T.A. may also be attributed to the conversion of sugar to organic acids by the consortia of yeast and bacterial cells present in the Kombucha starter culture (Malbaša et al., 2008; Woo et al., 2014). Product (KB8) fermented with 3% sugar, 0.5% tea, and 20% fermented broth at 24℃ had the lowest pH (2.84±0.02) and highest T.A. (0.65±0.00) (Figure 4.1c,d). The range of T.A. and pH levels obtained in this study suggested that the performance of the starter cultures used may be comparable to the study by Lončar et al. (2006) who used similar inocula concentrations and fermentation conditions of Kombucha.

4.1.2 Changes in total soluble solids during propagation of the starter culture

The TSS of all samples decreased ($p<0.05$) during the propagation of the Kombucha starter culture from the start (day 0) to end of fermentation (day 14) (Figures 4.2a,b; Appendix H.1). Changes in TSS indicated the presence of microbial activity of starter culture which resulted in increased acidity as reflected by the low pH and high T.A (Section 4.1.1). Reductions in TSS may be attributed to the uptake of nutrients by the micooroganisms present in the fermented broth and cellulose pellicle for their metabolism and growth (Jayabalan et al., 2014; Muhialdin et al., 2019; Villarreal-Soto et al., 2018). Sucrose metabolised into glucose and fructose which are subsequently hydrolysed into range of important metabolic products including organic acids, ethanol, and carbon dioxide (Chen & Liu, 2000; Muhialdin et al., 2019; Rodrigues et al., 2006). The starter cultures also utilise vitamins, proteins and minerals from the tea which also contributes to the reduction in the concentration of TSS (Laureys et al., 2020; Leal et al., 2018).

The initial TSS of samples ranged from 4.15±0.05 to 5.05±0.00 (Figure 4.2a,b), and samples (KB5, KB6, KB7 and KB8) fermented with higher amounts (20%) of fermented broth had lower initial TSS than samples (KB1, KB2, KB3 and KB4) fermented with a lower concentration (12.5%) of the broth. The variations in initial TSS are likely due to different concentrations of substrate and fermented broth used to commence the Kombucha fermentation (Jayabalan et al., 2014; Malbaša et al., 2011). Samples that contained a high concentration of tea (0.5%) (KB5, KB6, KB7 and KB8) and fermented broth (20%) (Figure 4.2b) showed a rapid decrease in TSS than samples with a low concetration of tea (0.3%) (KB1, KB2, KB3 and KB4) and fermented broth (12.5%) (Figure 4.2a). Of all the samples, sample KB8 recorded the highest overall reduction of TSS. The high decrease in TSS of KB8 may be caused by the high metabolic activities in the formulation which contained low sugar (3%) and was fermented at a high temperature. Use of a high concentration of fermented broth (20%) in the inoculum may also have contributed to increased metabolic activities (Jarrell et al., 2000; Lončar et al., 2006; Villarreal-Soto et al., 2018). A high level of inoculum contains a large number of microbial cells which generate rapid metabolism of microorganism at the beginning of fermentation (Lončar et al., 2006).

Overall, the reduction in TSS was higher in samples fermented at the higher temperature (24°C) rather than the lower temperature (22°C) (Figure 4.2 a,b). Luncar (2006) also reported higher decreases in TSS of black tea Kombucha fermented at 30°C than 22 °C for 10 days. The enzyme-catalysed reactions during metabolism of fermenting microorganisms are accelerated at higher fermentation temperatures, which reduce the TSS of samples (Wang et al., 1979; Xia et al., 2019). The optimum temperature for Kombucha fermentation ranges from 22 to 28°C, therefore using temperatures above the optimum range may retard the metabolic activity and fermentation process (Hammel et al., 2016; Jayabalan et al., 2014;Wang et al., 1979). The fermentation temperatures used in this study fall within the 22-28°C range.

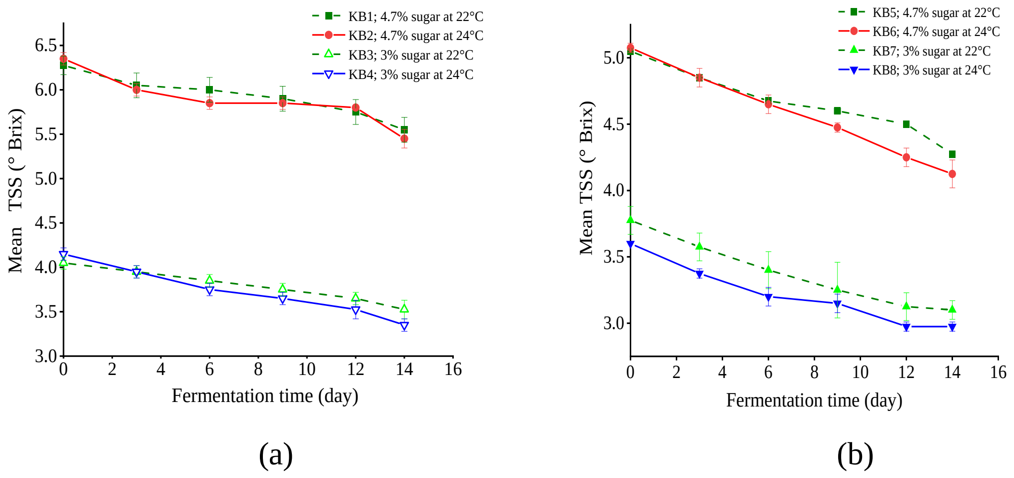


Figure 4. 2 Mean TSS during propagation of Kombucha starter cultures fermented with 2 levels of sugars, low (a) and high (b) concentrations of tea and broth at two temperatures for 14 days

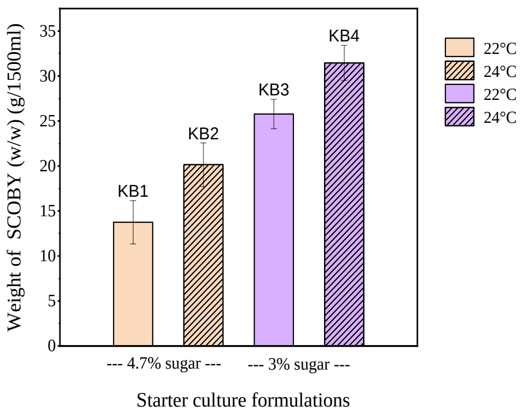
KB1 (4.7/22) contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 (4.7/24) contained 4.7% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 (3/22) contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 (3/24) contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 (4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 (4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 (3/22) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 (3/24) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°CError bars =±SD; n=8; independent experiments were repeated twice.

4.1.3 Weight of the cellulose pellicle layer (SCOBY) during propagation of the starter culture

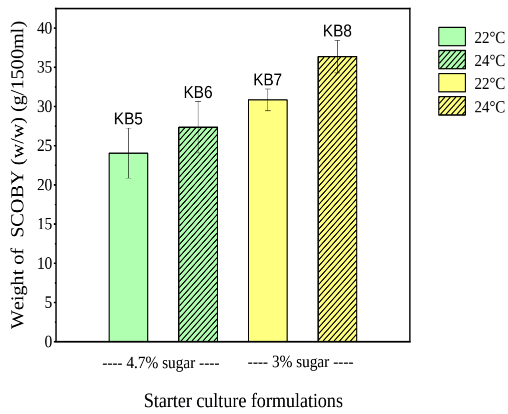
Eight different Kombucha formulations fermented at two temperatures which contained variable levels of sugar, tea, and broth were used to determine the optimum formulation for production of SCOBY. The yield of SCOBY harvested from eight samples (KB1-KB8) during the propagation of the Kombucha starter cultures ranged from 13.75±2.4 g to 36.37±2.07 g (Figure 4.3 a,b). The average yield of SCOBY was higher for samples fermented with high concentrations of tea and broth (Figure 4.3b) than samples fermented with the same amount of sugar but lower concentrations of tea and broth (Figure 4.3a). The results indicate that samples KB5-KB8 (Figure 4.3b) contained more suitable levels of nutrients for the development of cellulose pellicle than samples KB1-KB4 (Figure 4.3a).

The concentration of sugar used in the formulations also impacted on the yield of SCOBY, with samples fermented with the lower sugar level (3%) (KB3, KB4, KB7 and KB8) yielding higher amounts of SCOBY than samples fermented with the higher sugar level (4.7%) (KB1, KB2, KB5 and KB6). The differences in the yield of SCOBY may be attributed to inhibitory compounds and phenolics produced during the fermentation process (Kim et al., 2021). Formation of these compounds can affect the enzymic activities of fermenting bacteria which may hinder cellulose production.

The cell counts in the cellulose were higher for samples fermented at the higher temperature (24℃) than at the lower temperature (22℃) (Figure 4.3), which suggested that the fermentation temperature impacted the yield of cellulose pellicle in Kombucha. Of all the samples, sample KB8 produced the highest yield of cellulose indicating that the fermentation conditions of the formulation were the best for the cultures. KB8 contained lower sugar (3%), higher tea (0.5%) and fermented broth (20%) and was fermented at the higher temperature (24°C).



(a)



(b)

Figure 4.3 Mean weight (w/v) of recovered cellulose pellicle layer (SCOBY) during propagation of Kombucha starter cultures fermented with 2 levels of sugars, low (a) and high (b) concentrations of tea and broth at two temperatures for 14 days

KB1 (4.7/22) contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 (4.7/24) contained 4.7% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 (3/22) contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 (3/24) contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 (4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 (4.7/24) contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 (3/22) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 (3/24) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Error bars =±SD; n=8; independent experiments were repeated twice.

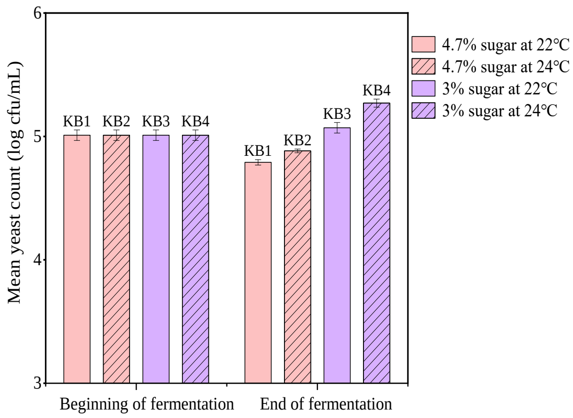
4.1.4 Microbiological composition of fermented broth and harvested cellulose pellicle (SCOBY) during propagation of starter culture

Fermentation is driven by the high microbial activities of the fermenting cultures. In Kombucha fermentation, it is therefore important to determine the viable counts in both the cellulose and the fermented broth (Malbaša et al., 2011; Tran et al., 2020). The complex interaction between the diverse microorganisms present in the fermented broth and cellulose pellicle helps to develop the overall balanced flavour profile of Kombucha (Jayabalan et al., 2014; Sievers et al., 1995; Teoh et al., 2004). Furthermore, cellulose produced by the bacteria protect the viable cells in the fermented broth from harsh environmental conditions such as UV radiation, high hydrostatic pressure and microbial contaminants. The cellulose also serves as a food reservoir and provides access to aeration due to its large surface area. The fermented broth also helps to reduce the initial pH which is desirable for the growth of yeast and helps prevent spoilage by opportunistic microorganisms.

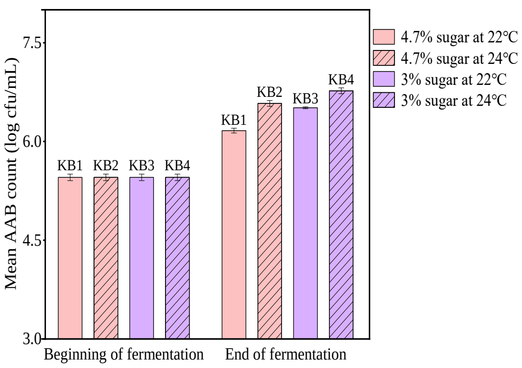
Yeast, AAB and total counts in the fermented broth

Yeast, AAB and total counts increased in the Kombucha broth fermented under variable conditions (tea and sugar concentration, fermented broth and fermentation temperature) at the beginning (day 0) and end of fermentation (day 14) are shown in Figure 4.4 and Figure 4.5 ($p<0.05$). At the end of fermentation, the AAB counts and total counts were higher in the fermented broth than yeast counts. Cell counts of AAB ranged from 6.27±0.05 - 8.45±0.04 log cfu/mL, yeast ranged from 5.07±0.01- 6.43±0.07 and total counts ranged from 6.32±0.06 - 8.51± 0.08 log cfu/mL (Appendix C). The results agreed with Tran et al. (2020) who reported increased AAB population of between 5 log cfu/mL and 7 log cfu/mL for black tea Kombucha fermented for 14 days at 26℃. The results of the present study indicated that the substrate (medium) and fermentation conditions were suitable for the growth of AAB (De Roos & De Vuyst, 2018; Gomes et al., 2018; Tran et al., 2020).Yeast counts increased during fermentation in all samples with the exception of KB5 and KB6, where the counts were slightly lower at the end of fermentation. Similar results have been reported by others (Tran et al.,2020; Chen and Liu 2000) where the yeast counts decreased in black tea Kombucha fermented for 7 and 14 days at 26℃ and 24±3℃, respectively. This decrease in yeast counts during fermentation may be caused by the depletion of nutrients and degeneration of cells (Shenoy et al., 2019; Sievers et al., 1995; Teoh et al., 2004).

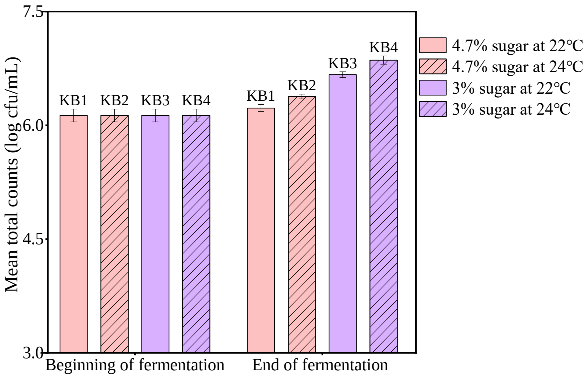
Yeast, AAB and total counts were higher (1-2 log CFU/mL) in samples (KB5, KB5, KB7, KB8) containing higher concentrations of tea (0.5%) and inoculum (fermented broth) (20%) compared to samples (KB1, KB2, KB3, KB4) containing lower concentration of tea (0.3%) and fermented broth (12.5%). The results suggested that differences in microbial counts may be due to different concentrations of substrate and inoculum used at the beginning of fermentation (Figures 4.4 and Figure 4.5). Of the samples, KB8 (3% sugar, 0.5% tea, 20% fermented broth and 24℃) had the highest microbial counts on day 14 (Figure 4.5a,b,c), suggesting that the conditions were more favourable for the growth of yeast and bacterial cells compared to other treatments.



(a) Yeast



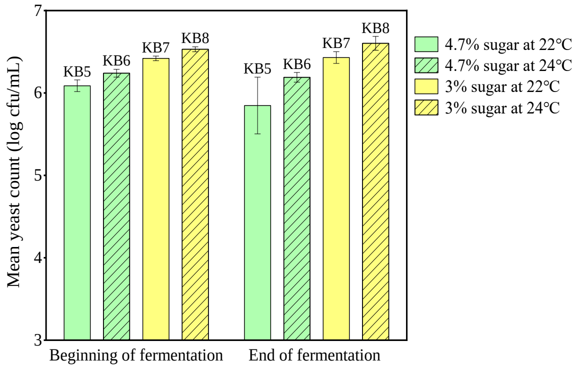
(b) AAB



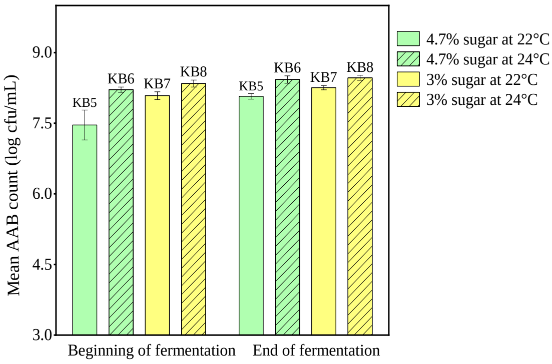
(c) Total counts

Figure 4.4 Mean (log cfu/ml) of yeast (a), AAB (b) and total counts (c) in fermented broth propagated with 2 sugar concentration levels (3%; 4.7%), low concentrations of tea (0.3%) and fermented broth (12.5%) for 14 days at 22 and 24°C

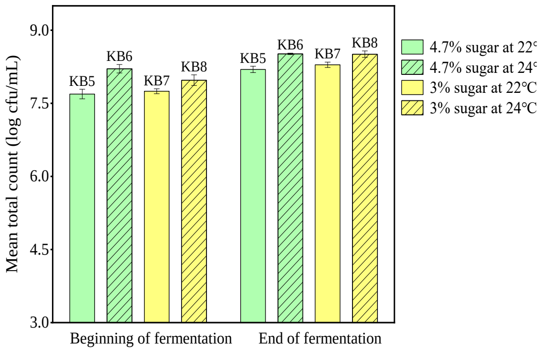
KB1 (4.7/22) contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 (4.7/24) contained 4.7% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 (3/22) contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 (3/24) contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; Error bars =±SD; n=4; independent experiments were repeated twice.



(a) Yeast



(b) AAB



(c) Total counts

Figure 4.5 Mean (log cfu/ml) of yeast (a), AAB (b) and total counts (c) in fermented broth propagated with 2 levels of sugar concentration (3%; 4.7%), high concentrations of tea (0.5%) and fermented broth (20.0 %) for 14 days at 22 and 24°C

KB5 (4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 (4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 (3/22) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 (3/24) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Error bars =±SD; n=4; independent experiments were repeated twice.

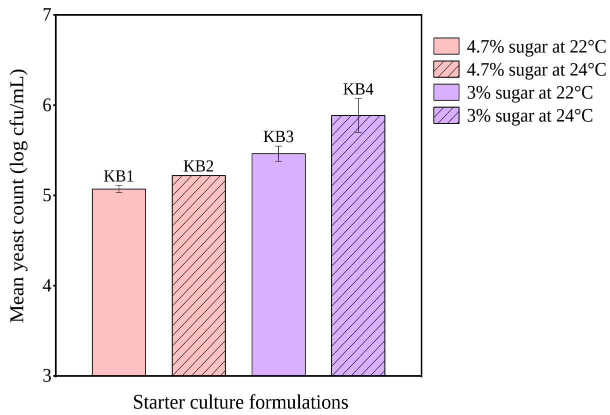
Yeast, AAB and total counts in the cellulose pellicle

In this study the yeast, AAB and total counts of cellulose pellicle were determined only at the end of the fermentation period (day 14) (Figure 4.6 and Figure 4.7). Yeast cell counts (5.07±0.01 - 6.64±0.05) were lower than AAB (6.27±0.04 - 8.45±0.04) and total counts (6.33±0.06 - 8.51±0.08) in both cellulose pellicle and fermented broth ($p<0.05$;

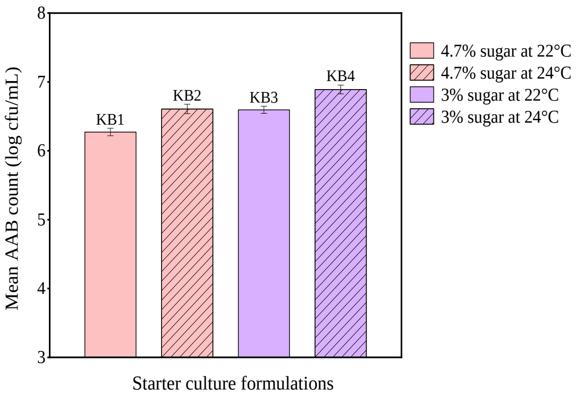
Appendix C). These results are comparable to previous studies (Chakravorty et al., 2016; Tran et al., 2020) which reported lower yeast counts compared to AAB and total counts. Possible explanation for lower yeast count may be due to nutrient competition or acidic stress caused by accumulation of organic acids (Guan & Liu, 2020; Sousa et al., 2012).

Further, the yeast, AAB and total counts were higher (~2 log CFU/ mL) in the cellulose pellicle than in the fermented broth on day 14. The lower yeast, AAB and total counts in fermented broth may be caused by insufficient nutrients and aeration of the medium. As fermentation progresses, carbon dioxide produced by yeast accumulates at the air-liquid interface between the pellicle and broth, which may limit the flow of air with oxygen to the fermenting microbes in the broth, thereby retarding their growth (Chen & Liu, 2000; Jarrell et al., 2000; Jayabalan et al., 2014).

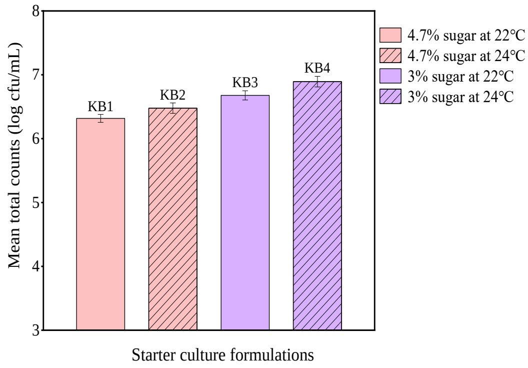
Of the eight samples, KB8 (3% sugar, 0.5% tea, 20% fermented broth and 24℃ temperature) contained the highest AAB counts in the fermented broth and cellulose pellicle. This result was consistent with the weight of SCOBY discussed in Section 4.1.3, where KB8 had the highest SCOBY weight. The results suggested that of the conditions tests, those utilised for fermentation of KB8 were the most favourable for the growth of acetic acid bacteria and development of cellulose pellicle layer.



(a) Yeast



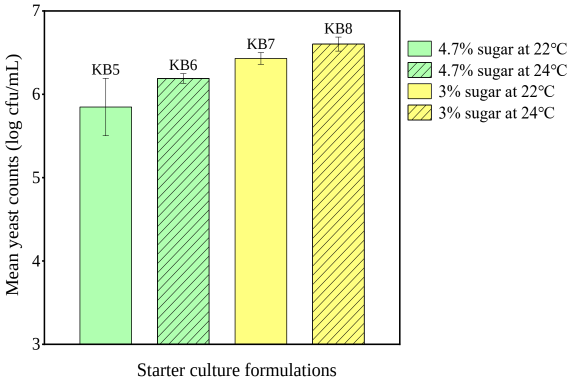
(b) AAB



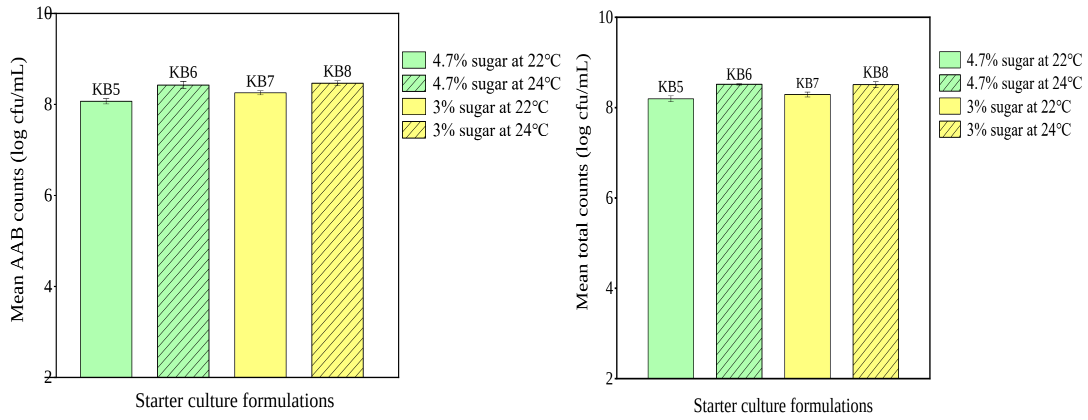
(c) Total counts

Figure 4.6 Mean (log cfu/ml) of yeast (a), AAB (b) and total counts (c) in cellulose pellicle propagated with 2 levels of sugar concentration (3%; 4.7%), low concentrations of tea (0.3%) and fermented broth (12.5%) for 14 days at 22 and 24°C

KB1 (4.7/22) contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 (4.7/24) contained 4.7% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 (3/22) contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 (3/24) contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; Error bars =±SD; n=4; independent experiments were repeated twice.



(a) Yeast



(b) AAB

(c) Total counts

Figure 4.7 Mean (log cfu/ml) of yeast (a), AAB (b) and total counts (c) in cellulose pellicle propagated with 2 levels of sugar concentration (3%; 4.7%), high concentrations of tea (0.5%) and fermented broth (20.0 %) for 14 days at 22 and 24℃

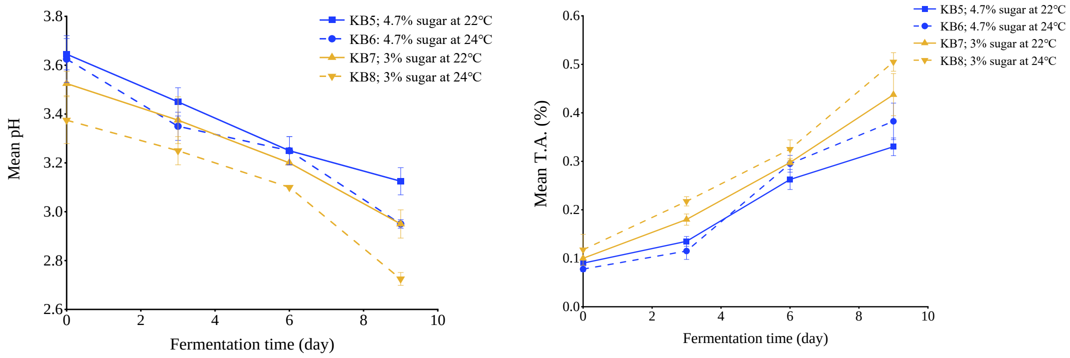
KB5 (4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 (4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 (3/22) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 (3/24) contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Error bars =±SD; n=4; independent experiments were repeated twice.

4.2 Phase 2 Investigation of the best conditions for the fermentation of Kombucha using a combination of developed SCOBY and fermented broth as the starter culture

4.2.1 Stage 1: Selection of optimum sugar concentration and fermentation temperature for the fermentation of Kombucha

4.2.1.1 Acidity (pH and titratable acidity) of Kombucha during fermentation

During fermentation of Kombucha for 9 days at 22℃ and 24℃, the pH decreased gradually while T.A. steadily increased for all samples as shown in Figure 4.8a,b ($p<0.05$). These results were consistent with the decrease in pH and increase in T.A. discussed in the propagation of the starter culture for 14 days in Section 4.1.1 (Phase 1). In the current phase, the final pH of samples ranged from 3.35±0.01 to 3.65±0.06 (Appendix C). The initial and final pH levels of the samples containing the lower concentration of sugar (3%) were lower than the samples containing higher sugar levels (4.7%). The samples fermented at a 24℃ had a higher acidity than those fermented at a 22℃ ($p<0.05$) (Figure 4.8a.b).The results indicated that the acidity of the Kombucha samples were affected by differences in both the levels of sugar used and fermentation temperature (De Filippis et al., 2018; Hammel et al., 2016; Muhialdin et al., 2019). Fermentation of sugar at a higher temperature is generally associated with a faster growth of microorganisms, which increases the acidity of Kombucha as previously discussed in Section 4.1.1 (Aung & Eun.,2021; Lončar et al., 2006; Xia et al 2019).



(a) (b)

Figure 4.8 Mean pH (a) and mean (%) titratable acidity (b) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

4.2.1.2 Total soluble solids of Kombucha during fermentation

TSS in all samples decreased ($p<0.05$) during fermentation of Kombucha for 9 days (Appendix H.2). The decrease in TSS during fermentation (Figure 4.9) was similar to the results of the TSS during propagation of Kombucha starter culture for 14 days at 22°C and 24°C (Section 4.1.2). Reduction of TSS was most likely caused by the metabolism of sugars by yeast and AAB from the starter cultures as reported by others (Chen & Liu, 2000; Muhialdin et al., 2019; Rodrigues et al., 2006). The mean total TSS of samples containing 3% sugar (KB7 and KB8) were lower than those containing higher sugar (4.7%) (KB5 and KB6). Data shown in Figure 4.9 also indicate that the reduction in TSS was higher for samples (KB6 and KB8) fermented at 24°C than samples (KB5 and KB7) fermented at 22°C. These results agreed with previous studies (Aung & Eun, 2021; Luncar, 2006) which reported high reductions of TSS at high fermentation temperatures in fermented black tea Kombucha. Therefore, the higher reduction of TSS in samples

KB6 and KB8 may be attributed to increased fermentation rate during fermentation at elevated temperatures as discussed in Section 4.1.2.

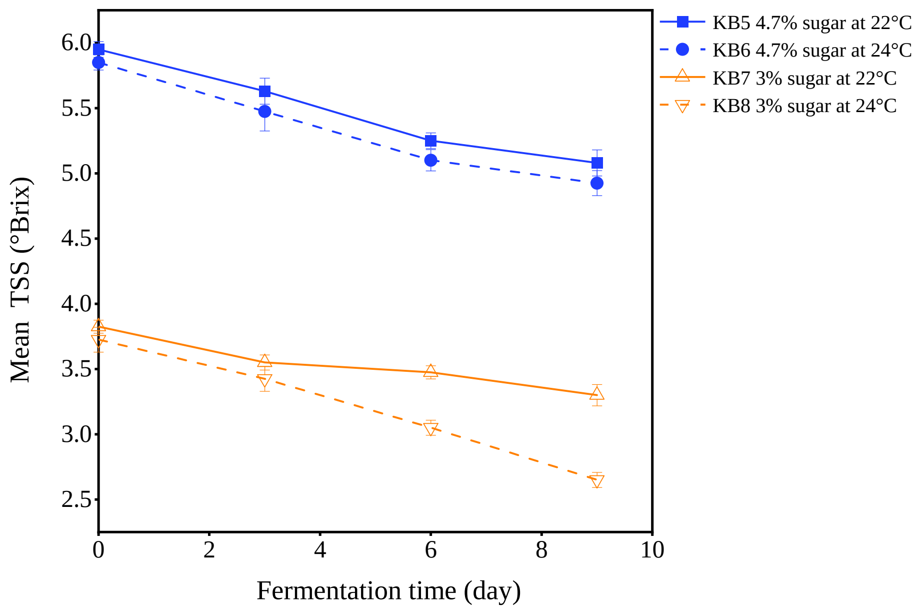


Figure 4.9 Mean TSS (°Brix) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

4.2.1.3 Colour of Kombucha during fermentation

The colour of Kombucha samples during fermentation for 9 days at 22°C and 24°C is shown in Figures 4.10 - 4.12. There were significant ($p>0.05$) colour differences as fermentation progressed (Appendix H.2). The lightness/brightness (L*) of Kombucha samples increased from 78.5±0.5 at day 0 to 84.5±0.5 at the end of fermentation (day 9). Meanwhile, the redness/greenness (a*) and yellowness-blueness (b*) both decreased from the beginning to the end of fermentation. The redness/greenness (a*) decreased from 5.3±0.5 at the beginning to 4.5±0.55 at the end of fermentation; yellowness-blueness (b*) decreased from 51.8±0.96 to 43.0±0.82 from day 0 to day 9, respectively. The differences in the colour indices between products were probably caused by the different formulations, efficiency of equipment used and fermentation temperatures (Pathare et al., 2013; Tamer et al., 2021). Changes in colour indices may be caused by chemical transformation of polyphenols during microbial metabolism and growth (Alderson et al., 2021). Theaburigin, a major component in black tea is partially converted to theaflavin during fermentation, resulting in colour changes of the Kombucha beverages (Alderson et al., 2021; Nurikasari et al., 2017; Yuwanti & Urbahillah, 2020). The results of the present study were similar to a study by Tamer et al. (2021) where the lightness/brightness (L*) increased, while redness-greenness (a*) and yellowness/blueness (b*) decreased for black tea Kombucha fermented at 28±2℃ for 14 days.

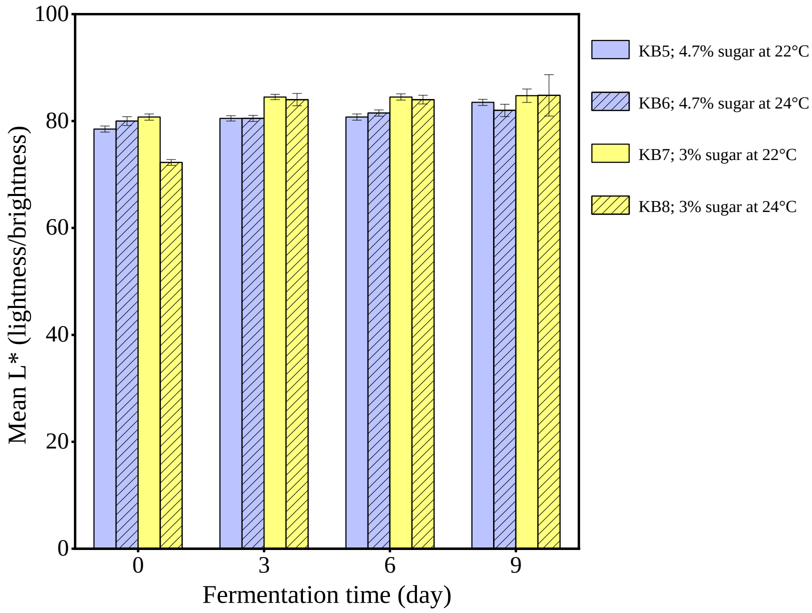


Figure 4.10 Mean L* during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24℃

Error bars =±SD; n=4; independent experiments were repeated twice

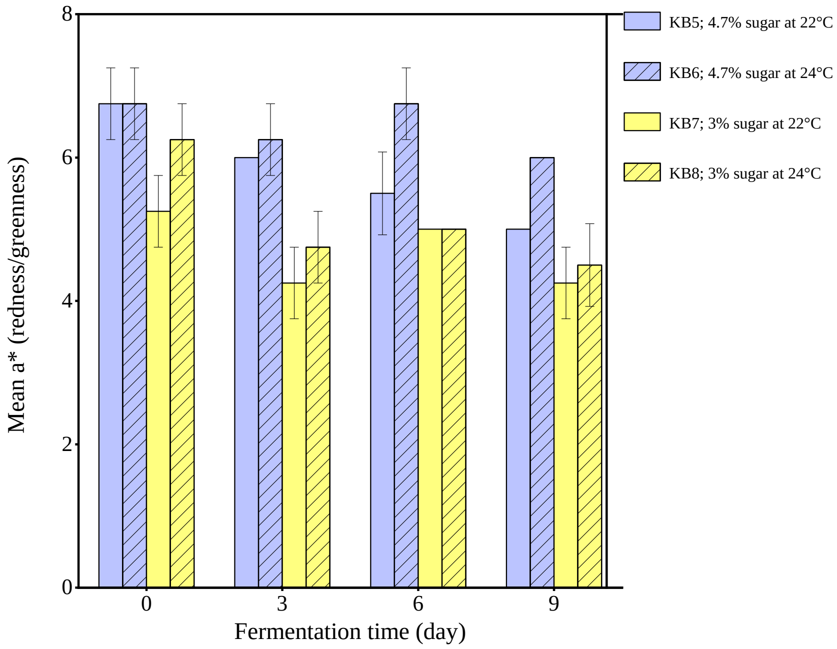


Figure 4.11 Mean a* during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

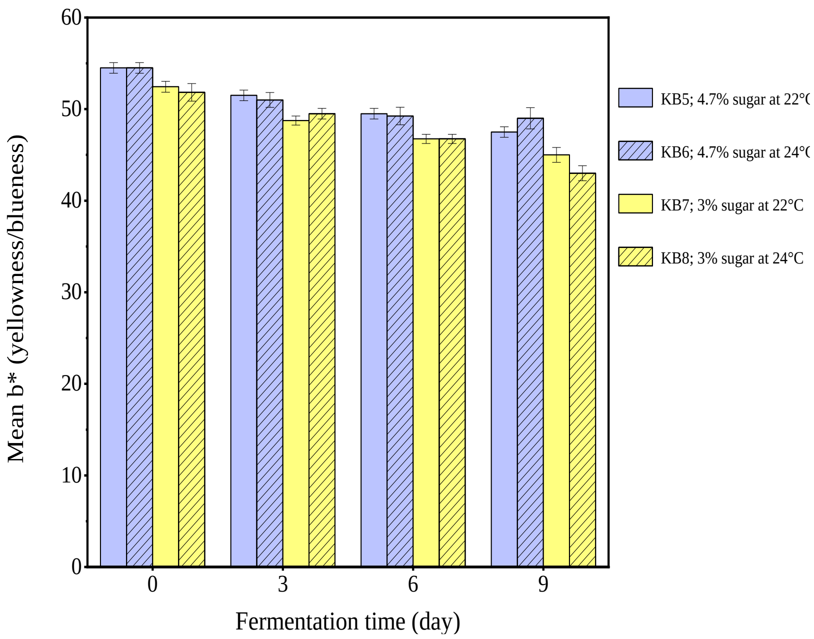


Figure 4.12 Mean b* during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

4.2.1.4 Weight of cellulose pellicle layer (SCOBY) of Kombucha during fermentation

The weight of the cellulose pellicle layer during fermentation of black tea Kombucha for 9 days at 22℃ and 24℃ is shown in Figure 4.13. In this phase, the weight of cellulose (w/v) harvested ranged from 49.15±0.19 to 53.14±0.13 (g/1500 mL) at the end of fermentation (day 9). Variations in the weight of SCOBY for the four samples (KB5 - KB8) may be caused by differences in the formulations and concentration of microorganisms present in the starter culture (Betlej et al., 2020; Hassan & AL-Kalifawi, 2014; Zahan et al., 2015).

The weight of harvested cellulose was higher for samples fermented at 24℃ (KB6 and KB8) than those fermented at the 22℃ (KB5 and KB7) irrespective of differences in the sugar concentrations used (Figure 4.13). Of all the samples, sample KB8 that contained 3% sugar and was fermented at 24℃ recorded the highest weight of cellulose. Fermentation temperatures for Kombucha ranging from 20 to 50℃ have been reported suitable for the synthesis of cellulose (Aung & Eun., 2022; Betlej et al., 2020). However, at temperatures higher than 50℃ the microbial enzymes responsible for cellulose synthesis may be denatured. Hence, fermentation at higher temperatures may not be appropriate for Kombucha fermentation and will be highly dependent on the microbial composition of the stater culture. Further, the synthesis of cellulose by the AAB may also be affected by insufficient supply of nutrients and/or dissolved oxygen which may hinder the fermentation process (Al-Kalifawi, 2014; Laavanya et al., 2021).

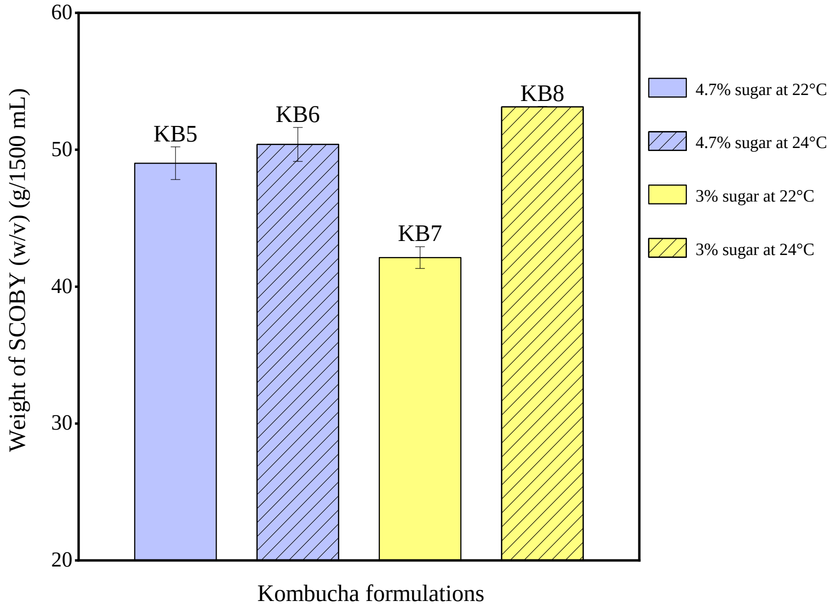


Figure 4.13 Mean weight (w/v) of cellulose pellicle layer (SCOBY) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24℃

Error bars =±SD; n=4; independent experiments were repeated twice.

The weight of harvested cellulose was higher for samples containing 3% rather than 4.7% sugar levels (Figure 4.13), which agreed with other researchers (Al-Kalifawi, 2014; Aung & Eun, 2021). Prevous studies reported decreased synthesis of cellulose with increased concentrations of sugar (5- 19% w/v) which was added during fermentation of Kombucha for 12-20 days at 22℃ to 27℃. Mean weights of cellulose ranging from 13.13 to 61.89 g/1000 mL have been reported in Kombucha fermentations containing 5 to 19 % sugar (Hassan & AL-Kalifawi, 2014; Lin et al., 2012). There was no linkage reported between the yield of cellulose and sensory profiles of the products in the previous studies. However, there is no way of knowing the desired optimum yield that correlates to a successful fermentation producing a highly acceptable beverage. The synthesis of cellulose depends mainly on sufficient supply of carbon (sugar) as fermenting bacteria do not have enough reserves of carbon required for the formation of the pellicle (Aswini et al., 2020; Hassan & AL-Kalifawi, 2014).

4.2.1.5 Microbiological analysis during fermentation of Kombucha

Concentration of yeast, AAB and total counts in fermented liquid samples

The microbial counts (yeast, AAB, total counts) increased in all samples as the fermentation progressed ($p<0.05$) (Figures 4.14 - 4.16;Appendix H.2). Average yeast counts ranged from 6.78±0.01 to 7.21±0.06 log cfu/mL at the end of fermentation (day 9). Compared to the present study, Aung & Eun (2022) reported lower yeast counts (3.00 – 4.60 log cfu/mL) whereas Chen & Liu (2000) reported higher yeast counts (7.50 – 7.90 log cfu/mL) during fermentation of Kombucha for 6-14 days at 24-25°C. In these reports, there is no published data on the relationship between yeast counts and sensory characteristics of the beverages. There is no evidence on the expected number of yeasts counts which can be associated with sensory characteristics of fermented Kombucha. Variations in the yeast counts between the different studies may be attributed to factors such as fermentation conditions, substrate utilised, strains of the microorganisms in the starter as well as concentration of starter culture added.

In the present study, AAB counts ranged from 7.13±0.09 to 7.87±0.02 at the end of fermentation (day 9), while total counts ranged from 7.13±0.05 to 7.90±0.07. These results were slightly higher than another study (Aung & Eun, 2022) which reported AAB counts ranging from 3.0 to 4.0 log cfu/mL at the end of Kombucha fermentation for 8 days at 25°C and 30°C. Similar to the discussion on yeast counts, there seems to be no published studies showing the relationship between optimum AAB counts and organoleptic properties of Kombucha. In Kombucha fermentation, the symbiotic relationship between AAB and yeast is important. AAB are responsible for the development desirable acidity as well as the synthesis of cellulose and the symbiotic growth of yeast (Jarrell et al., 2000; Jayabalan et al., 2014; Sievers et al., 1995; Teoh et al., 2004).

Higher microbial counts were recorded in samples fermented at 24°C (Figure 4.14, Figure 4.15, Figure 4.16) than samples fermented at 22°C. The optimum temperature range for the growth of microbial cells during Kombucha fermentation has recently been reported to be between 22°C and 28°C (Xia et al., 2019, Vargas et al., 2021). In this study, samples fermented at the higher temperature had higher cell counts and acidity indicating higher microbial activity at the elevated temperature. The highest total microbial counts were recorded in the sample that contained high sugar (KB6) (4.7%) and fermented at 24°C, suggesting that the concentration of added sugar also affected the cell counts in Kombucha samples during fermentation (Goh et al., 2012a; Hassan & AL-Kalifawi, 2014).

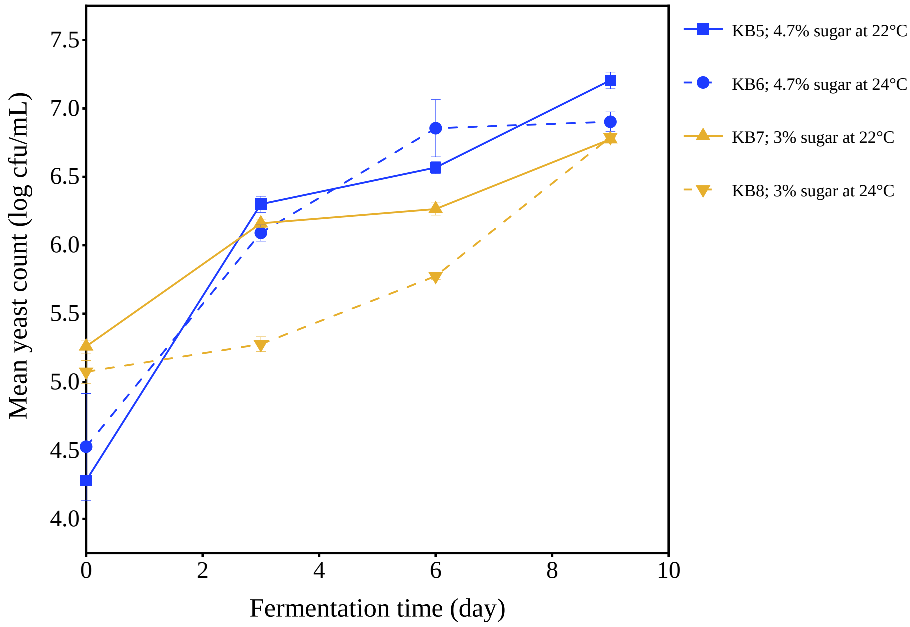


Figure 4.14 Mean yeast counts (log cfu/mL) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

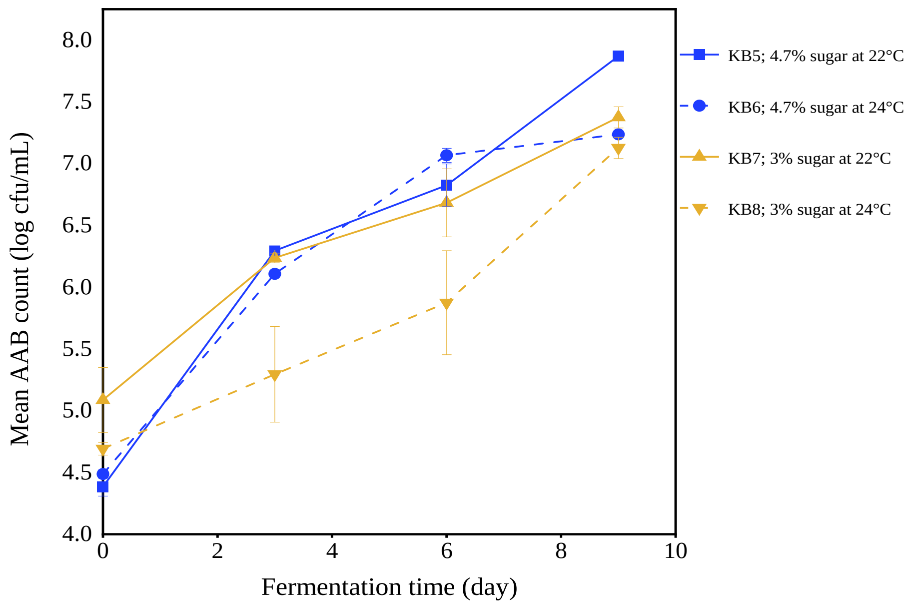


Figure 4.15 Mean AAB counts (log cfu/mL) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

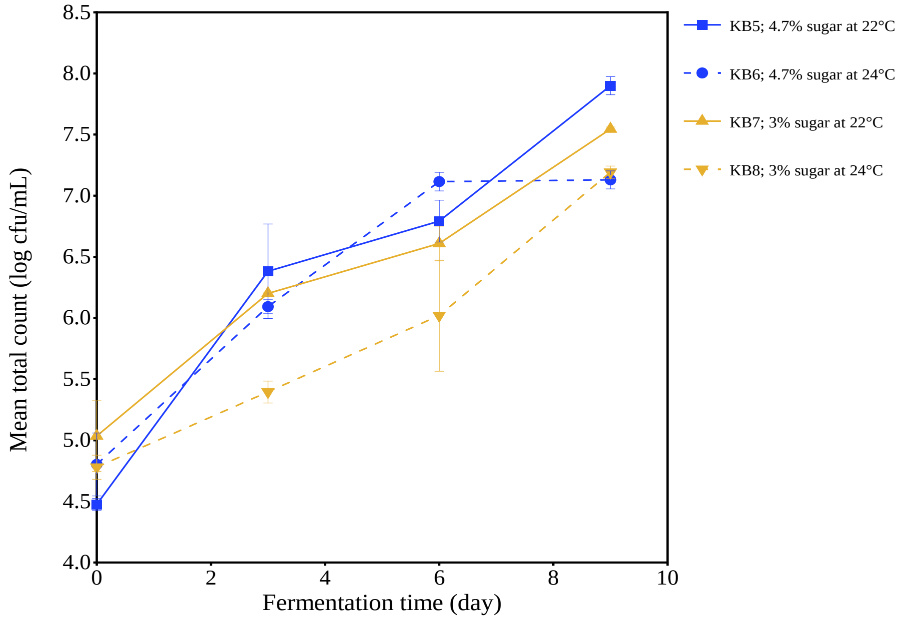


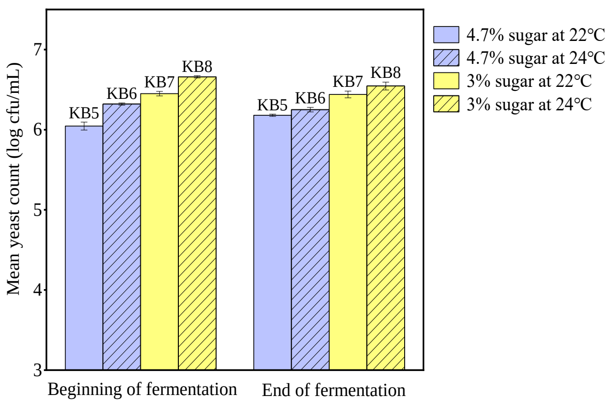
Figure 4.16 Mean total counts (log cfu/mL) during fermentation of Kombucha with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

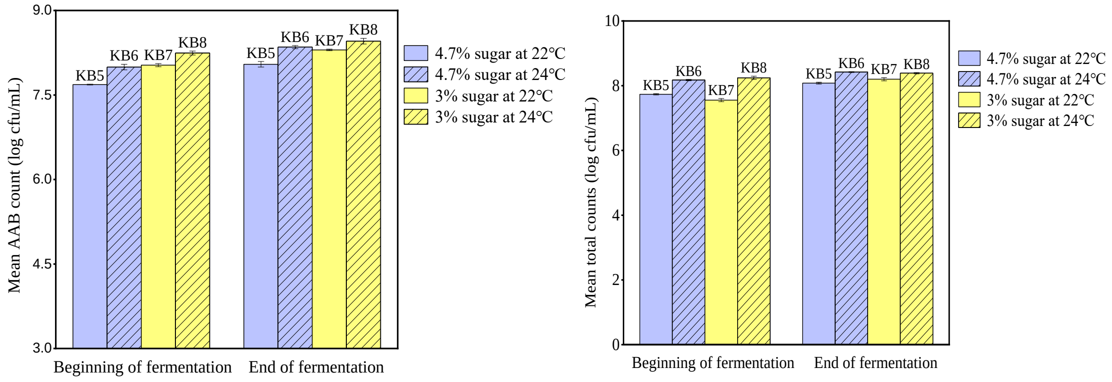
Concentration of yeast, AAB and total counts in the cellulose pellicle

Microbial counts in the cellulose pellicle were higher in samples fermented at 24℃ than at 22℃ regardless of the sugar concentration during fermentation of Kombucha for 9 days ($p<0.05$) (Appendix H.2). In all samples, the concentration of AAB was higher in the cellulose pellicle than in the fermented broth (Figure 4.17). Previous studies reported conflicting results, with some (Chen & Liu ,2000) reporting higher counts in the broth than in the pellicle, and others (Goh et al., 2012; Reiss, 1944) vice versa. The reason why higher microbial counts may be found in the pellicle than in the fermented broth cannot be easily explained. One possible reason may be due to differences in the supply of oxygen for the microbes in the cellulose and broth during fermentation (Chen & Liu, 2000; Goh et al., 2012; Vergas et at., 2021). Once a tight layer of the cellulose pellicle has been formed, oxygen has to diffuse through the pellicle to reach the broth. Therefore the may partially block oxygen flow, thereby starving the microbial cells in the broth (Al-Kalifawi, 2014; Gorgieva & Trček, 2019).

The counts in the cellulose pellicle were higher in samples fermented at the higher temperature (24℃) and low sugar concentration (3%) rather than the lower temperature (22℃) and high sugar concentration (4.7%) (Figure 4.17 a, b).



Yeast



AAB Total counts

Figure 4.17 Mean yeast, AAB and total counts (log cfu/mL) in the cellulose pellicle at the beginning and end of fermentation with 2 levels of sugar, 0.5% tea and 20% fermented broth for 9 days at 22 and 24°C

Error bars =±SD; n=4; independent experiments were repeated twice.

4.2.1.6 Focus group sensory evaluation of fermented Kombucha

In stage 1 (Phase 2), four formulations (KB5, KB6, KB7 and KB8) of Kombucha fermented for 9 days at 22°C or 24°C were evaluated for appearance, aroma, flavour, sweetness, sourness and overall acceptability (Appendix C). There were apparent differences in sensory characteristics between the fermented Kombucha containing 3% sugar and 4.7% sugar, with results indicating that fermentation temperatures and sugar concentrations impacted on the sensory profiles. This may be attributable to differences in their levels of cell counts, organic acids, ethanol and carbon dioxide produced during fermentation (Ahmed et al., 2020; Aung & Eun, 2021).

Overall, there were no marked differences in the appearance of any of the four fermented Kombucha samples (KB5, KB6, KB7 and KB8). The samples were described as being a transparent ‘light-yellow’ with a refreshing vinegary aroma. Release of gas bubbles was observed in all samples, especially those fermented at 24°C (KB6 and KB8), indicating active fermentation in these two samples. Consumers liked the fizziness of the Kombucha beverage which is likely caused by the evolution of carbon dioxide bubbles during the metabolism of sugar by yeast (Ahmed et al., 2020; Alderson et al., 2021). In the present study, yeast counts were higher for samples fermented at 24°C (Section 4.2.1.5), and the fizziness of these beverages was more pronounced, possibly due to higher microbial activity (Al-Kalifawi, 2014; Aung & Eun, 2021; Woo et al., 2014).

The four fermented samples were described as 'refreshing'. The sample fermented with 3% added sugar was described as having lower sweetness and high sourness compared to the formulation that contained 4.7% added sugar. The sweetness and sourness of the samples is probably due to the acidity (pH and T.A.), TSS and residual sugar at the end of fermentation (Section 4.2.1.1; Section 4.2.1.2). The two samples (KB7 and KB8) containing low sugar (3%) received lower acceptability scores and were described as having a vinegary flavour and overpowering aroma with a pungent and astringent taste. The vinegary flavour may be due to the accumulation of different organic acids produced during fermentation. The organic acids produced tend to mask the sweetness of the beverages while increasing their sourness and vinegary taste (Ahmed et al., 2020; Chakravorty et al., 2016; Jayabalan et al., 2015; Teoh et al., 2004; Tran et al., 2020). There were no marked differences in the sensory attributes evaluated by a focus group for samples KB5 and KB6 which contained 4.7% sugar and were fermented at 22°C and 24°C.

Based on their acceptable sensory attributes, physico-chemical and microbiological properties Kombucha samples KB5 and KB6 were selected (Section 4.2.1.1 to Section 4.2.1.6), for further investigation. The two selected samples had characteristics typical of Kombucha that are desirable to consumers including the acidity (pH = 2.80 - 3.00; T.A. = 0.32 - 0.51) and colour. The selected fermented samples contained high viable microbial cell counts ($\geq 10^6$ cfu/mL) whcih are desired by consumers (Kim & Adhikari, 2020; Marco et al., 2021; Vargas et al., 2021).

4.2.2 Stage 2 Potential of filtration for reducing the microbial counts in Kombucha following fermentation

Filtration, which removes viable fermenting microbes has been used during the preparation of fermented beverages to control alcohol levels, extend shelf life and improve organoleptic characteristics (Freeman & McKechnie, 2003; Shala et al., 2017a). In this study, filtration was used at the end of fermentation (day 9) to partially remove the fermenting yeast and bacteria before bottling to control levels of alcohol and stability of fermented samples during storage. Stage 2 (Phase 2) consisted of four treatments with response factors of acidity, totals soluble solids, colour, consumer sensory and microbial counts which were determined for the unfiltered (control) and filtered samples stored for one week (4°C).

4.2.2.1 Acidity (pH and T.A.) of Kombucha during fermentation and storage (4°C) for one week

The pH of the four samples (filtered and unfiltered) decreased during fermentation for 9 days (3.60±0.01 - 3.64±0.03), with further decreases (2.95±0.02 - 3.17±0.01) during week one of storage (4°C) ($p<0.05$). Meanwhile, T.A. increased during fermentation for 9 days (0.07±0.00 to 0.08±0.01 %), and further increased to 0.35±0.00 – 0.48±0.01 % during storage for one week (Figure 4.18) ($p<0.05$).

The acidity (pH and T.A.) of the unfiltered samples were higher than the filtered samples after one week storage (4°C) (Figure 4.18) ($p<0.05$). The minimum pH (2.95±0.02) and the highest T.A. (0.48±0.01) were recorded for the unfiltered sample fermented at 24°C (KB6U). The continued reduction of pH with corresponding increases in T.A. during cold-storage (4°C) can be explained by post-fermentation of residual sugar into organic acids and other organic compounds. At the end of storage for one week, the acidity of the unfiltered samples (KB5U; KB6U) was higher than the filtered samples (KB5F; KB6F) ($p<0.05$) (Figure 4.18; Appendix H.3). The presence of higher concentrations of fermenting microorganisms in the unfiltered samples may be responsible for the higher acidity of the unfiltered sample (Ahmed et al., 2020; Chen & Liu, 2000). The results of this study agreed with previous researchers, who reported increased acidity of unfiltered samples (control) during cold-storage (Abbo et al., 2006; Fadaei et al., 2022a). The results of the current study suggested that the high microbial counts and their increased post-fermentation activity might have resulted in increased acidity of unfiltered Kombucha samples during storage (4°C).

While there are no published studies that have reported the use of filtration to reduce fermenting microbial cells in Kombucha and its effect on acidity, the technique has been applied in beer and wine production (Joshi et al., 2017; Shala et al., 2017a; Ubeda & Briones, 1999). Thus, in the absence of product specific information, the effect of filtration on the acidity of Kombucha obtained in this study was compared to the acidity of beers and wines post-filtration. The microbial counts in filtered samples were lower after filtration, which may have resulted in reduced post-metabolic activity of fermenting microbes leading to lower concentrations of organic acids produced during the one-week storage (4°C) . In this study, the lower acidity of filtered rather than unfiltered samples may be attributed to differences in the microbial counts of the samples.

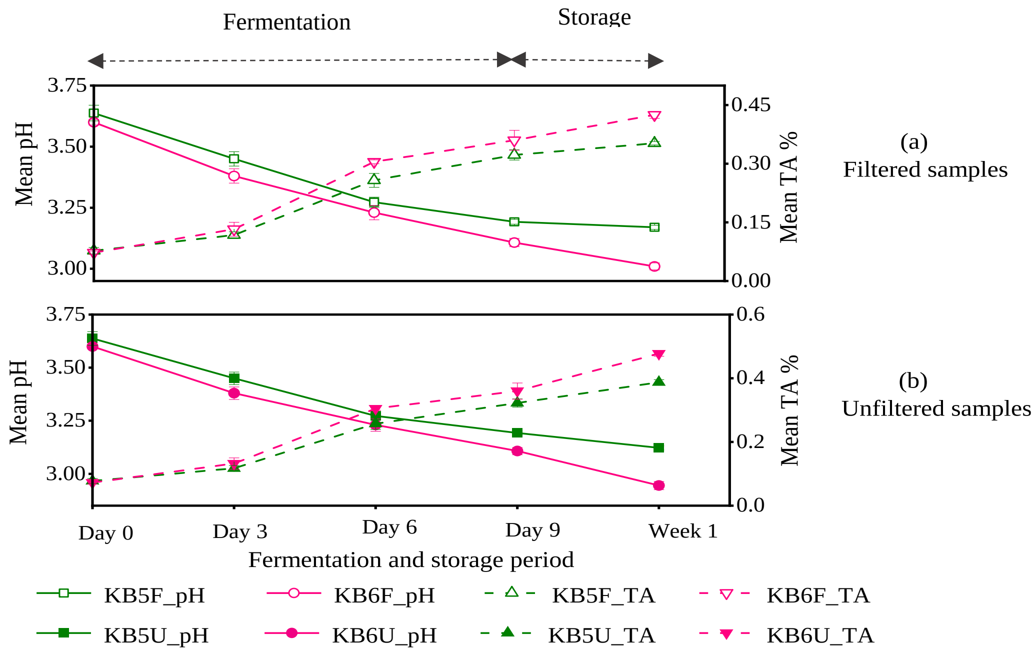


Figure 4.18 Mean pH and mean titratable acidity (%) during fermentation of Kombucha with 4.7% sugar at two temperatures (22°C; 24°C) for 9 days and storage (filtered and unfiltered samples) for one week (4°C)

(a) Samples were filtered after fermentation (KB5F; KB6F); (b) Unfiltered samples (KB5U; KB6U)

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample) ; KB6F(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (filtered sample); KB6U(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (unfiltered sample); Error bars =±SD; n=4; independent experiments were repeated twice.

Samples fermented at 24°C (KB6F and KB6U) had higher T.A. and lower pH values than samples fermented at 22°C (KB5F and KB5U) (Figure 4.18). This result is consistent with earlier discussions (Section 4.2.1), where samples fermented at a higher temperature had higher acidity.

4.2.2.2 Total soluble solids of Kombucha during fermentation and storage (4°C) for one week

TSS of all samples decreased during fermentation (9 days) and storage for a week ($p<0.05$) (Figure 4.19; Appendix H.3). Unfiltered samples (KB5U and KB6U) had lower TSS than filtered samples (KB5F and KB6F) at the end of the storage period (4°C). The lower TSS in the unfiltered samples at the end of storage was reflected by the higher acidity and microbial counts in the respective samples (Sections 4.2.2.1 and 4.2.2.4). The high microbial counts (yeast, AAB and total counts) in the unfiltered samples (KB5U; KB6U) probably resulted in increased post-fermentation microbial activities, leading to reduced TSS and increased acidity compared to the filtered samples (Chen & Liu,2000; Muhialdin et al.,2019; Rodrigues et al., 2006).

Kombucha samples fermented at 24°C recorded higher reductions in TSS compared to the samples fermented at 22°C during fermentation and storage as shown in Figure 4.19.

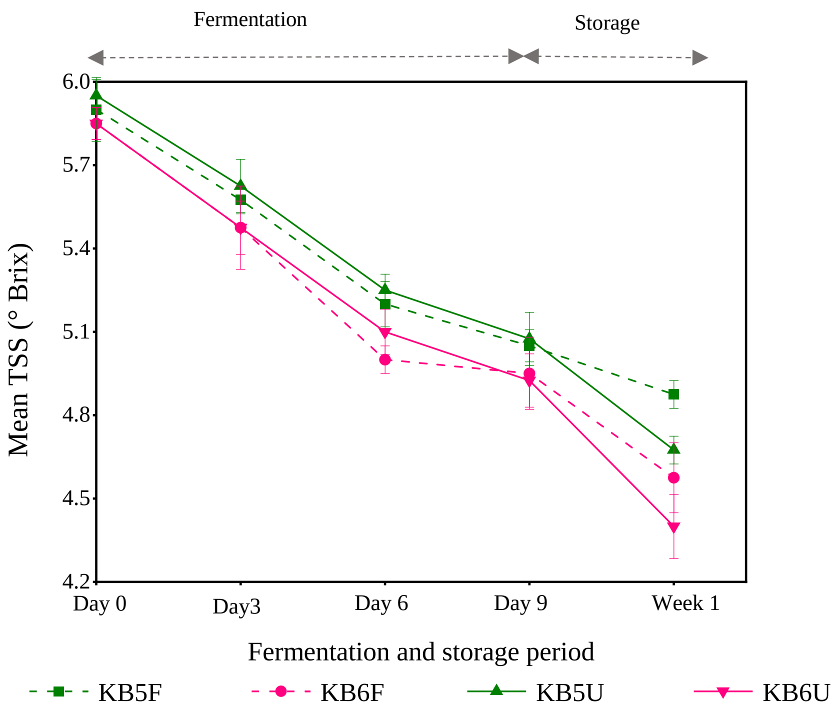


Figure 4.19 Mean TSS (°Brix) during fermentation of Kombucha with 4.7% sugar at two temperatures (22°C; 24°C) for 9 days and storage (filtered and unfiltered samples) for one week (4°C)

(a) Samples were filtered after fermentation (KB5F; KB6F); (b) Unfiltered samples (KB5U; KB6U)

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample) ; KB6F(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (filtered sample); KB6U(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (unfiltered sample); Error bars =±SD; n=4; independent experiments were repeated twice.

4.2.2.3 Colour of Kombucha samples during fermentation and storage (4℃) for one week

There were significant differences ($p<0.05$) in the colour (L*, a*, b*) of the filtered (KB5F; KB6F) and unfiltered samples (KB5U; KB6U) during storage (4°C) for one week (Figure 4.20; Appendix H.3). The results indicated that the storage time had significant ($p<0.05$) impact on the colour profile of both filtered and unfiltered Kombucha samples during storage (post-fermentation). The lightness/brightness (L*) of the samples increased from 78.5±0.58 at the start of fermentation (day 0) to 84.3±0.50 at the end of fermentation (day 9), and then decreased to 83.5±0.58 at the end of storage (week 1) ($p<0.05$). The redness/ greenness (a*) decreased from 5.95±0.06 at the start (day 0) to 5.50±0.06 at day 9, and then reduced to 5.13±0.2 at end of the 1-week storage period. Meanwhile, the yellowness/blueness (b*) of the samples decreased from 53.50±0.57 to 47.50±0.57 at the beginning of fermentation (day 0) to the end (day 9), and then remained stable during the storage period for both samples (filtered and unfiltered) ($p<0.05$).

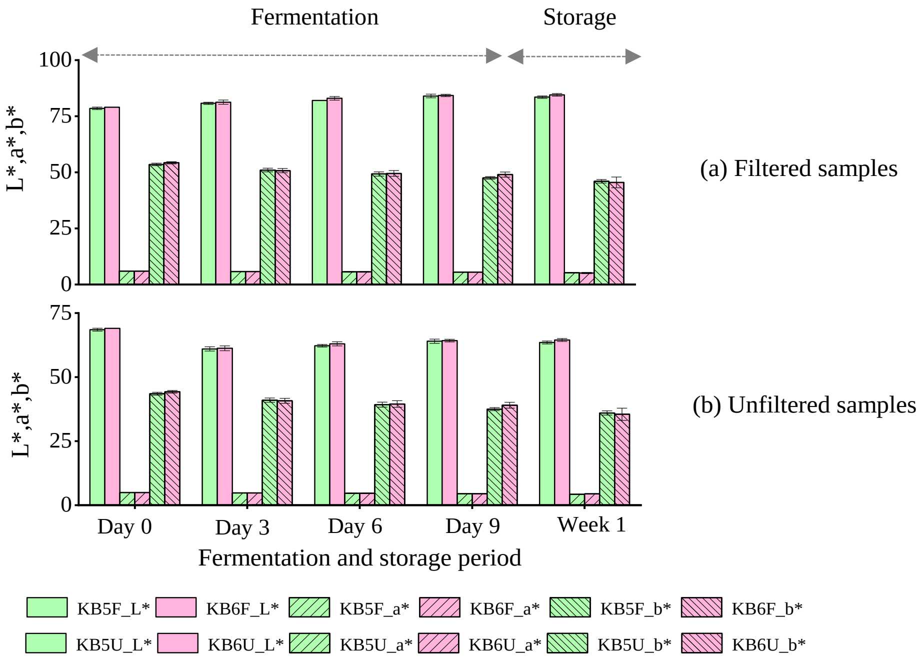


Figure 4.20 Mean colour coordinates (L*, a*, b*) during fermentation of Kombucha with 4.7% sugar at two temperatures (22°C; 24°C) for 9 days and storage (filtered and unfiltered samples) for one week (4°C)

(a) Samples were filtered after fermentation (KB5F; KB6F); (b) Unfiltered samples (KB5U; KB6U)

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample) ; KB6F(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (filtered sample); KB6U(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (unfiltered sample); Error bars =±SD; n=4; independent experiments were repeated twice.

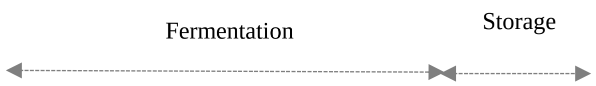
Previous studies did not report any changes in the hue (L*, a*, b*) of the Kombucha samples during storage (Watawana et al., 2016; Tamer et al., 2021). However, the observed minor changes in the colour of the Kombucha samples obtained in this study during fermentation may have been the consequence of chemical modifications or transformation of polyphenols during post-fermentation activity. As previously mentioned, theaburigin present in the samples can be partially transformed into theaflavin resulting in colour changes (Alderson et al., 2021; Nurikasari et al., 2017; Yuwanti & Urbahillah, 2020).

4.2.2.4 Microbiological analysis of Kombucha samples during fermentation and storage (4℃) for one week

Concentration of yeast, AAB and total counts in filtered and unfiltered samples

Yeast, AAB and total counts of samples increased from day 0 to day 9 during fermentation (22℃ and 24℃) as previously observed in Section 4.2.1.5. As expected, the microbial counts (yeast, AAB and total counts) in the filtered samples (KB5F; KB6F) at day 9 (end of fermentation) were lower than those of the unfiltered samples (KB5U; KB6U). This is due to the partial removal of yeast and bacterial cells by the filtration process which was carried out immediately before bottling/ storage of Kombucha samples (Flores-García et al., 2019; Freeman & McKechnie, 2003; May et al., 2019; Shala et al., 2017a; Tran et al., 2020). During filtration of the Kombucha samples, the liquid fermented broth passed through the filter and the resultant retentate (residue) was probably a suspension of cellulose pellicle with microbial cells trapped on the filter. The cells partially removed by filtration were likely to have come from the suspended solid cellulose pellicles trapped in the filters rather than the broth, since the pore size of the double-sided filter (10 to 11 µm) used was much larger than the diameter of the yeast and AAB cells (1.35 – 4.26 µm) as discussed in the subsequent section 4.3.4b (Phase 3).

Compared to the filtered samples, the yeast counts in unfiltered samples increased during storage (4℃) for one week ($p<0.05$) (Figure 4.21). The high yeast counts in unfiltered samples were related to the lower TSS of the sample, indicating that the yeast continued to metabolise sugar during cold-storage (Abbo et al., 2006; Fadaei et al., 2022b; La Torre et al., 2021). The AAB and total counts in the unfiltered samples were also higher than in the filtered samples following storage for one week. Higher AAB and total counts suggested increased post-fermentation activity in the unfiltered samples. Whereas, lower AAB and total counts in filtered samples are likely the consequence of the removal of fermenting microbes by the filtration carried out following fermentation.



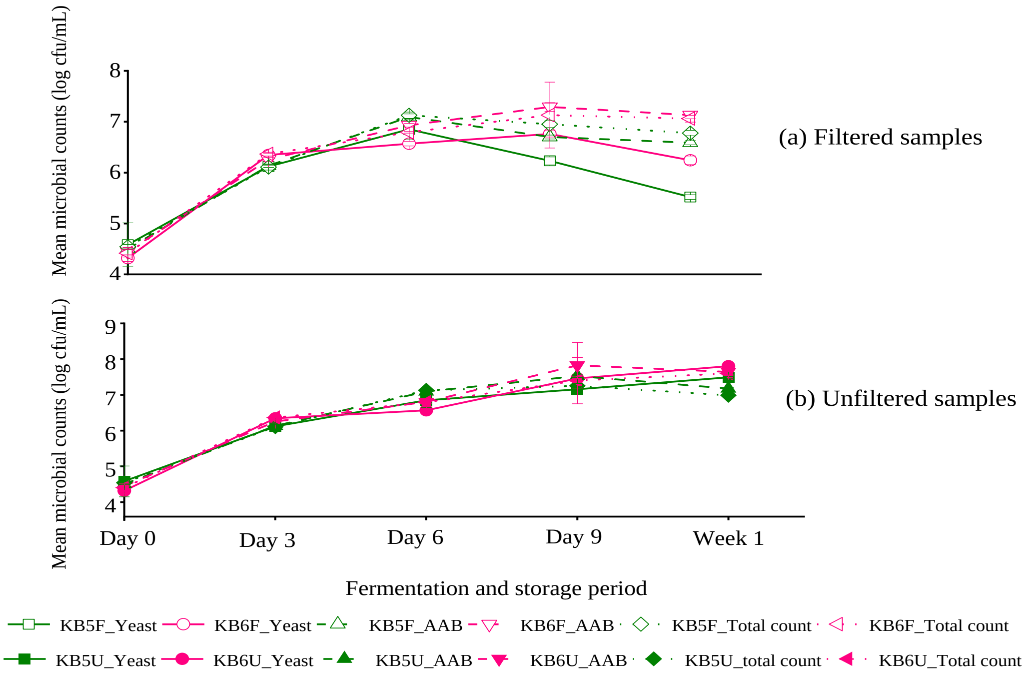


Figure 4.21 Mean microbial counts (log cfu/mL) during fermentation of Kombucha with 4.7% sugar at two temperatures (22°C; 24°C) for 9 days and storage (filtered and unfiltered samples) for one week (4°C)

(a) Samples were filtered after fermentation (KB5F; KB6F); (b) Unfiltered samples (KB5U; KB6U)

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample) ; KB6F(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (filtered sample); KB6U(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C (unfiltered sample); Error bars =±SD; n=4; independent experiments were repeated twice.

Trisnatia et al. (2018) reported no significant effect of cold-storage (4°C) on viable cell counts in unfiltered Kombucha samples after 28 days, which is in contrast to our findings. However, the results of the present study agreed with Sheehan et al. (2007) who reported decreased viable cells in unfiltered fermented Kombucha at pH 2.5 and 3.7 during cold-storage ($p<0.05$). The reduction of microbial cells in unfiltered samples cannot be explained easily as many factors are responsible such as depletion of nutrients due to competition, insufficient dissolved oxygen, low pH and ethanol stress affected (Fadaei et al., 2022b; Freeman & McKechnie, 2003; Shala et al., 2017b).

4.2.2.5 Consumer sensory evaluation of Kombucha during fermentation and storage for a week (4℃)

Consumer sensory panellists (n=35) used a 9-point scale to evaluate the filtered (KB5F; KB6F) and unfiltered (KB5U; KB6U) samples in Stage 2 (Phase 2) for appearance, aroma, flavour, sweetness, sourness and overall acceptability to select the most promising formulations for stability testing in Phase 3. Overall, acceptability sensory scores of the filtered samples were higher compared to the unfiltered samples following storage (4°C) for one week (Figure 4.22; Appendix C). The higher scores may be linked to the high acidity, low TSS, and high cell counts in the unfiltered samples compared to the filtered samples post-fermentation.

The unfiltered sample (KB6U), fermented at 24℃ received the lowest sensory scores for appearance (5.8±0.10) and aroma (5.6±0.08), which may be attributed to their distinct 'vinegary-like' aroma and 'yeasty odour' which were disliked by the panellists. The yeasty odour was possibly caused by the formation of esters (ethyl decanoate, ethyl hexaoate, isomyl acetate and ethyl octanoate) due to the high yeast activity (Section 4.2.2.4) in the unfiltered sample (Laureys et al., 2018, Laureys & De Vuyst., 2014; Rodrigues et al., 2016). The lower sensory scores for turbidity of the unfiltered sample (KB6U) was probably caused by residual suspended cellulose, which was not liked by the consumers. In contrast to the unfiltered samples, the appearance scores of the filtered samples were higher, which was apparent as suspended cellulose strands were removed by the filtration process pre-bottling, thereby making the beverage less turbid.

The filtered sample (KB5F, pH 3.17, TSS 4.9 °Brix) containing 4.7% sugar and femented at 22℃ received the highest overall acceptability scores of all samples tested. Whereas, the unfiltered samples (KB6U, pH 2.95, TSS 4.4° Brix) containing the same initial amount of sugar (4.7%) but fermented at 24℃ received the lowest overall acceptability scores. In addition, the unfiltered sample KB6U also received lower sensory scores for flavour, sweetness and sourness compared to the other samples (Figure 4.22). Therefore, the results suggested that fermentation temperature(22℃) and filtration may improve the overall acceptability of black tea fermented Kombucha. The sourness of the unfiltered product (KB6U) increased and sweetness reduced on fementation at 24℃, which may be due to the higher production of organic acids by yeast and bacteria consortia present in the culture (Ahmed et al., 2020; Neffe-Skocińska et al., 2017; Tamer et al., 2021). To support this, in the present study unfiltered samples contained higher concentrations of yeast and bacteria cells, which metabolise sugar into organic acids, thereby increasing the overall acidity of the unfiltered samples compared to filtered samples (Ahmed et al., 2020; Neffe-Skocińska et al., 2017; Tamer et al., 2021; Trisnawita et al., 2018; Ubeda & Briones, 1999).

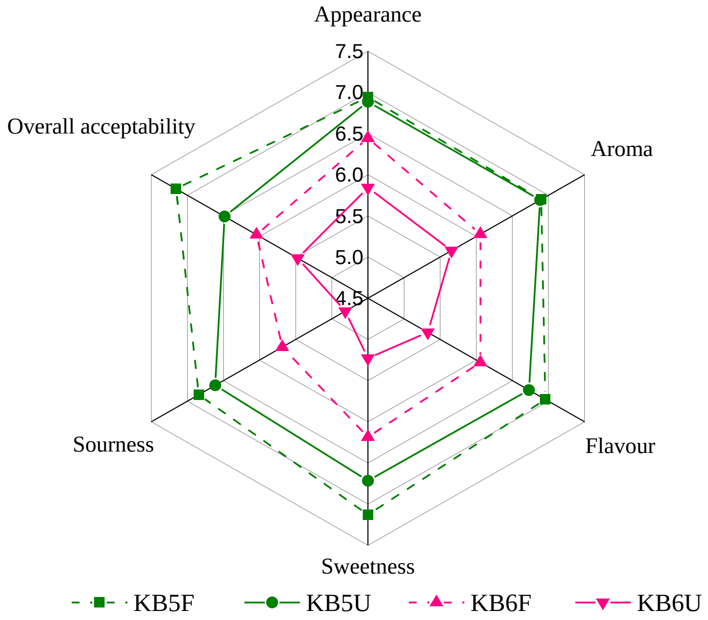


Figure 4.22 Mean consumer sensory evaluation of filtered (KB5F; KB6F) and unfiltered (KB5U; KB6U) fermented Kombucha after one week storage at 4℃

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Unfiltered sample) ; KB6F(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (Filtered sample); KB6U(4.7/24) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (Unfiltered sample); n=4; independent experiments were repeated twice.

Of the four samples investigated in Stage 2, two (KB5F and KB5U) were selected for stability testing during prolonged storage for 3 weeks (Phase 3) at 4 °C. These two samples were selected on the basis of their overall higher mean sensory scores as determined by 35 panellists.

4.3 Phase 3 Stability of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

Phase 3 investigated the physico-chemical, microbiological and sensory characteristics of filtered and unfiltered fermented Kombucha beverages (KB5F and KB5U) during prolonged storage for three weeks at 4°C.

4.3.1 Acidity (pH and T.A.) of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

The acidity of fermented Kombucha increased ($p<0.05$) during prolonged storage (4°C) for three weeks (Figure 4.23; Appendix H.4). The increased acidity indicated the post-fermentation of residual sugar into organic acids during storage of samples (4°C) (Abbo et al., 2006; Gaggia et al., 2019). Others have also reported (Neffe-Skocińska et al., 2017; Tamer et al.,2021; Watawana et al., 2015) post-fermentation during extended storage (4°C) resulting in increased acidity in Kombucha samples.

At the end of the three-week storage periods, the acidity of the unfiltered sample (KB5U) was higher than the filtered sample (KB5F) ($p<0.05$; Figure 4.23). The differences in the acidity of filtered and unfiltered samples were probably due to the reduction of microbial activity due to the lower number of cells in the filtered sample (KB5F) post-fermentation. The results discussed in the subsequent sections (4.3.4 and 4.3.7) showed that there was partial removal of the fermenting microorganisms in the filtered sample, as shown by the lower cell counts in the filtered sample (KB5F) than the unfiltered sample post-fermentation and during storage. Therefore, the activity of higher cell counts may be the cause of higher acidity (low pH; high T.A.) in the unfiltered sample as shown in Figure 4.23.

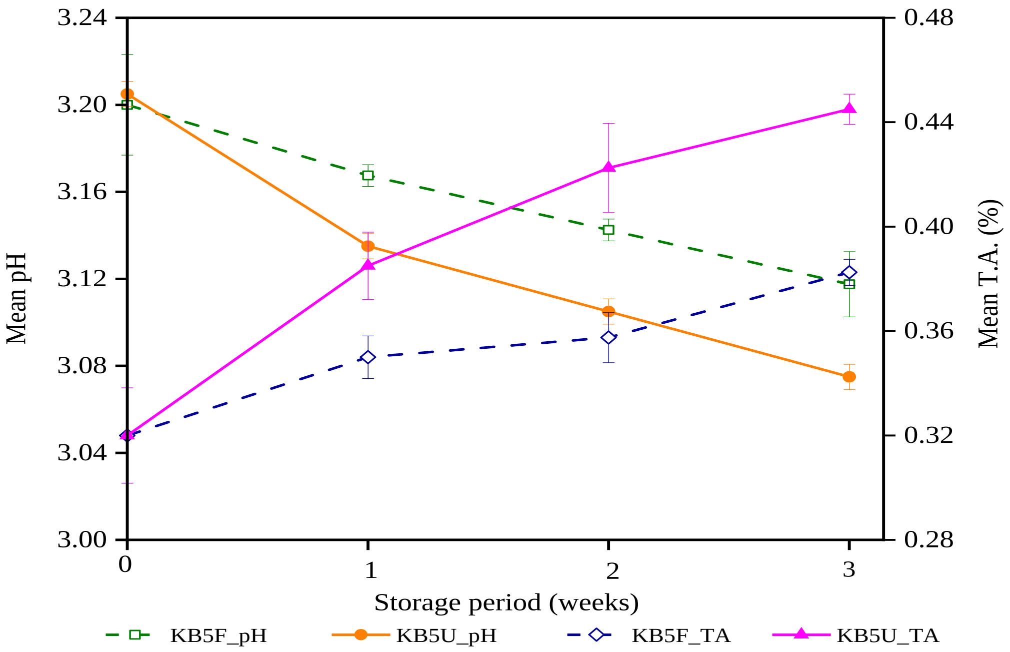


Figure 4.23 Mean pH and mean titratable acidity (%) of filtered (KB5F) and unfiltered (KB5U) Kombucha samples during prolonged storage (4℃) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); Error bars =±SD; n=2; independent experiments were repeated twice.

In the present study, the pH of Kombucha samples (filtered and unfiltered) at the end of the 3-week storage (4℃) ranged from 3.08±0.00 - 3.12±0.01, while T.A. ranged from 0.38±0.00 % to 0.45±0.00 %. The pH of the unfiltered sample was different to previous studies which reported lower pH levels ranging from 2.77 to 2.95 during cold storage for three to eight weeks (4 °C) (Aung & Eun, 2021; La Torre et al., 2021; Neffe-Skocińska et al., 2017). With respect to the acidity of the filtered sample, there is no conclusive published data on the effect of filtration on Kombucha post-fermentation. Therefore, the differences in acidity (pH; T.A.) of the present study compared to previous reports may be due to several factors including the filtration process, different fermentation conditions, fermentation substrates and storage temperature used (Chen & Liu, 2000; Jayabalan et al., 2014; May et al., 2019).

4.3.2 Total soluble solids of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

Total soluble solids of Kombucha decreased ($p<0.05$) in all samples during prolonged storage (4℃) as shown in Figure 4.24. The decreases in TSS suggested that yeast and acetic acid bacteria present in the beverages continued to utilise residual sugars for their growth during cold storage (Laureys et al., 2020; May et al., 2019; Teoh et al., 2004).

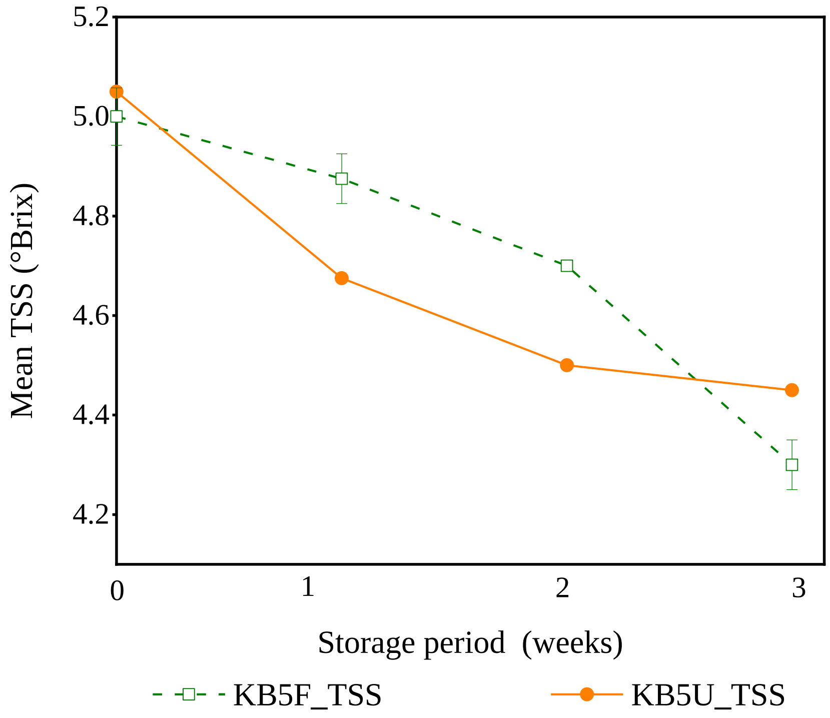


Figure 4.24 Mean TSS (°Brix) of filtered (KB5F) and unfiltered (KB5U) Kombucha samples during prolonged storage (4℃) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); Error bars =±SD; n=2; independent experiments were repeated twice.

During the first 2 weeks of prolonged storage (4℃), the TSS of the unfiltered samples were significantly ($p<0.05$) lower than the filtered sample (Figure 4.24). Thereafter, a rapid decrease in TSS of the filtered sample was observed in week 3 of storage compared to the unfiltered sample ($p<0.05$). The lower TSS in the filtered sample (KB5F) than the unfiltered sample (KB5U) was consistent with the lower sucrose levels of the filtered sample observed in week 3 of storage (Section 4.3.6). Figure 4.24 also showed that in week 3 the reduction of TSS of the unfiltered sample (KB5U) was lower than in weeks 1 and 2 of the same sample. This observation may be due to several reasons. Firstly, there was probably high AAB metabolic activity in week 3 resulting in the production of high acid in the unfiltered sample. It may be postulated that the acid levels (T.A = 0.45±0.00 %) inactivated the microbial cells especially those of the yeast in the unfiltered sample. Additionally, high concentrations of organic acids are likely to induce stress on yeast cells and/or can be lethal (Guan & Liu, 2020; Sousa et al., 2012). Stressed yeast cells can lose their ability to metabolise sugar which may partially explain the presence of higher TSS in the unfiltered sample compared to the filtered sample in week 3 of prolonged storage (4°C).

4.3.3 Colour of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

There were no significant changes ($p<0.05$) in the measured colour attributes of filtered (KB5F) and unfiltered (KB5U) Kombucha samples during prolonged storage (4°C) (Figure 4.25). Thus, prolonged storage of filtered and unfiltered Kombucha had no major effect on the colour of the samples. It is also interesting to note that the colour attributes of both samples were stable during storage despite post-fermentation by the fermenting microorganisms ($p<0.05$) indicating the stability of the colour components at low pH.

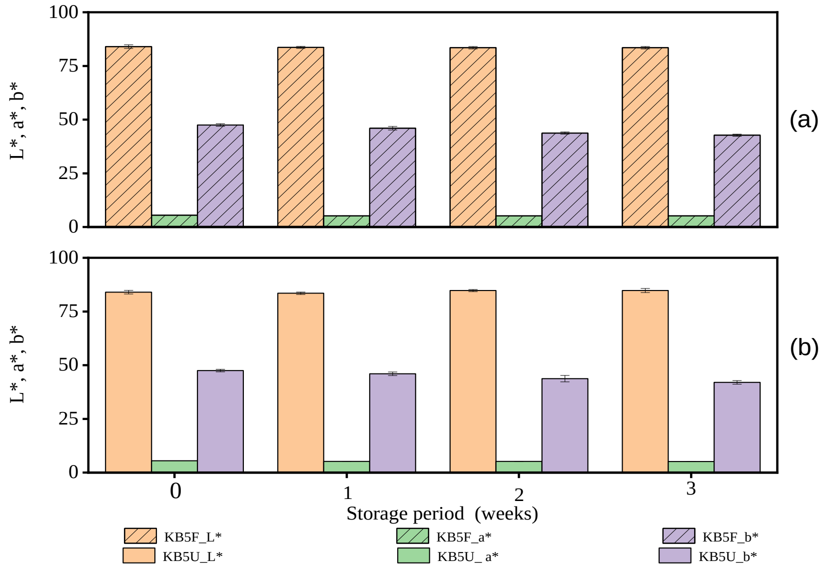


Figure 4.25 Mean colour coordinates (L*, a*, b*) of filtered (KB5F, a) and unfiltered (KB5U, b) Kombucha samples during prolonged storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); Error bars = ±SD; n=2; independent experiments were repeated twice.

4.3.4 Microbiological analysis of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

a) Concentration of yeast, AAB and total counts in Kombucha beverage during prolonged storage

The microbial counts of filtered (KB5F) and unfiltered (KB5U) fermented Kombucha decreased during prolonged storage ($p<0.05$) (Figure 4.26: Appendix H.4). At the beginning of storage (week 0), yeast counts were about one log lower (6.89±0.07 log cfu/ml) than the AAB and total counts (7.23±0.03 log cfu/ml and 7.15±0.06 log cfu/mL, respectively) for all samples. The filtered sample (KB5F) had lower yeast counts at the beginning of the storage period than the unfiltered sample (KB5U), due to the partial removal of microbial cells by the filtration process which was carried out following fermentation (Section 4.2.2.4).

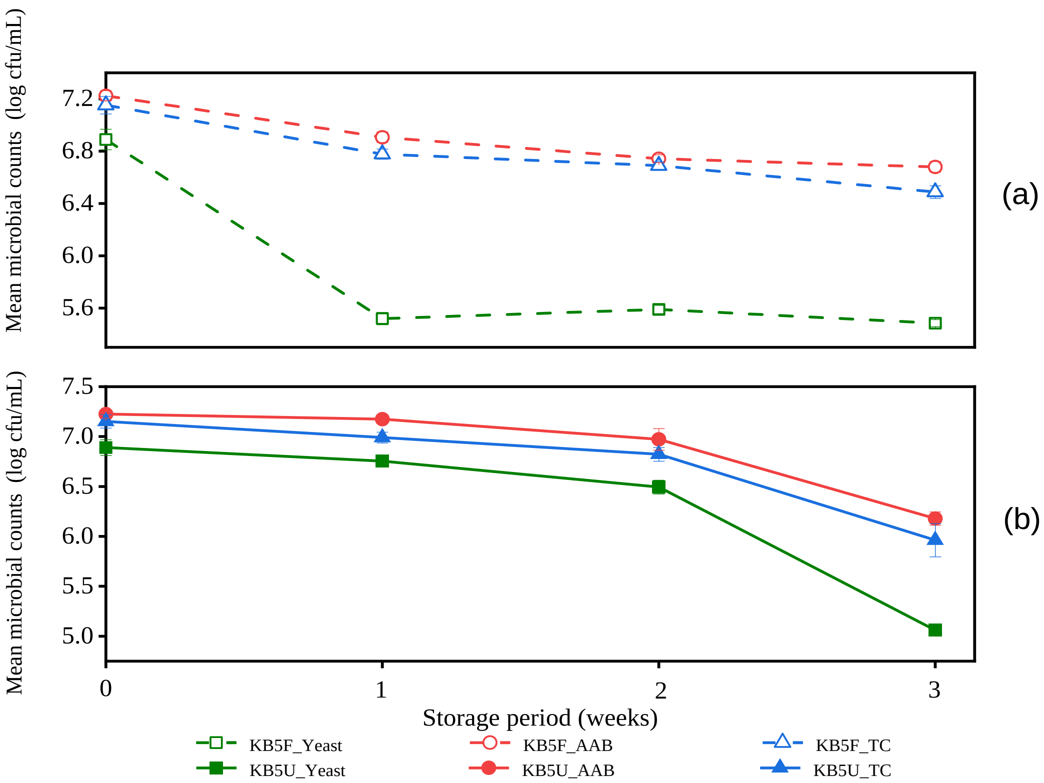


Figure 4.26 Mean microbial counts (log cfu/mL) in filtered (KB5F, a) and unfiltered (KB5U, b) Kombucha samples during prolonged storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

The yeast counts for the filtered sample decreased after week 1 and remained stable for the last two weeks of storage. For the unfiltered sample, yeast counts steadily decreased in the first two weeks, followed by steep reduction in week 3 of storage (4℃) (Figure 4.26). Both the AAB and total counts in the filtered sample (KB5F) decreased steadily during storage. For the unfiltered sample (KB5U), the AAB and total counts decreased during the first two weeks of storage (4℃), then decreased rapidly in the last week ($p<0.05$). The reductions in microbial counts after week 2 in the unfiltered sample might have been caused by acid shock (low pH) as discussed in the subsequent Section 4.3.7. Another possible reason for the reduction of microbial counts may be due to the depletion of nutrients and/or insufficient aeration. Carbon dioxide released during fermentation can be a major inhibitor for the growth of yeast and bacterial cells, especially AAB which are aerobic in nature (Jayabalan et al., 2014; Jones & Greenfield, 1982; Kallel et al., 2012).

b) Concentration of yeast, AAB and total counts in the recovered residue during filtration of Kombucha

After fermentation, samples were filtered using a stainless-steel filter (10 to 11 µm) before packaging and storage in swing-cap tight bottles. Mean cell counts of yeast, AAB and total counts for the cells recovered from the filter were 5.13 log cfu/mL, 5.58 log cfu/mL and 5.06 log cfu/mL, respectively (Appendix C). The average size of the yeast cells (1.35 to 7.05 µm) and AAB (2.4 to 4.26 µm) in the Kombucha samples were smaller than the size of the filter used in the study as shown in Figure 4.27 and Figure 4.28. This indicates that any individual free cells of the microorganisms would not be removed by the filter.

As previously discussed (Section 4.3.4a), microbial counts of the filtered (KB5F) sample were lower than the unfiltered (KB5U) sample at the beginning of storage, indicating that some yeast and bacteria cells were removed during filtration. Microbial cells embedded in the suspended strands of cellulose pellicle were most likely removed during filtration (May et al., 2019; St-Pierre, 2019). As fermentation progresses, young cellulose strands can remain suspended in the broth which may impact on the appearance and the overall sensory profile of fermented Kombucha (Costa et al., 2017; Lin et al., 2013; May et al., 2019). Therefore, filtration after fermentation may aid in improving the appearance of fermented Kombucha, however, there are no comparative published data on the application of filtration in Kombucha production. Filtration has been used to remove yeast and bacteria during the fermentation of wine and beer to maintain the alcohol limit, extend shelf life and improve sensory characteristics (Freeman & McKechnie.,2003; Shala et al.,2017; Ubeda & Briones.,1999). The present study agrees with previous reports on wine and beer manufacturing, where the reduction of microbial counts in filtered Kombucha samples post-fermentation may have contributed to the reduction of ethanol during prolonged storage (4°C) (Section 4.3.5).

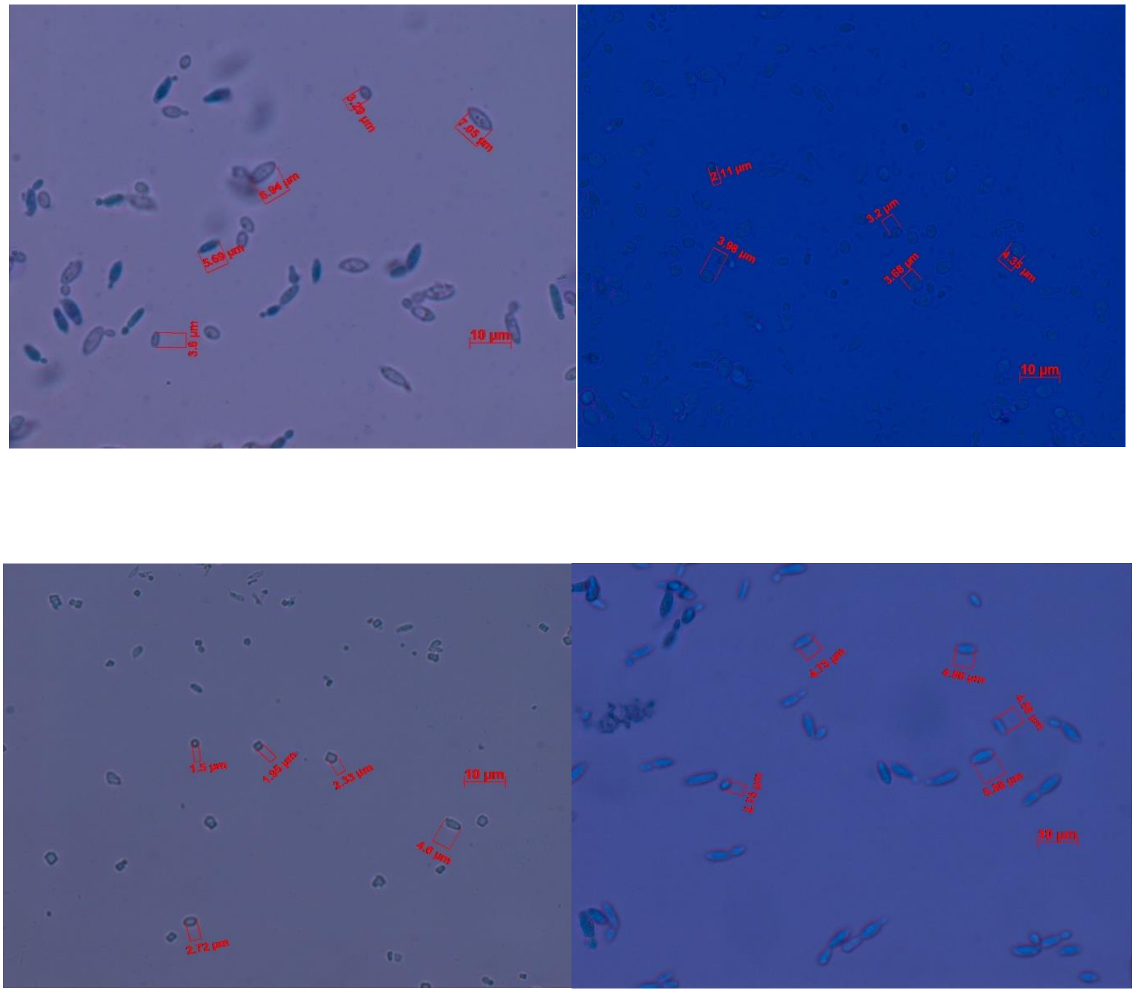


Figure 4.27 Morphology of methylene blue stained yeast cells grown on Yeast Extract Glucose Chloramphenicol (YGC) agar

The morphology of yeast cells was observed using methylene blue staining method under oil immersion at x1000 magnification.

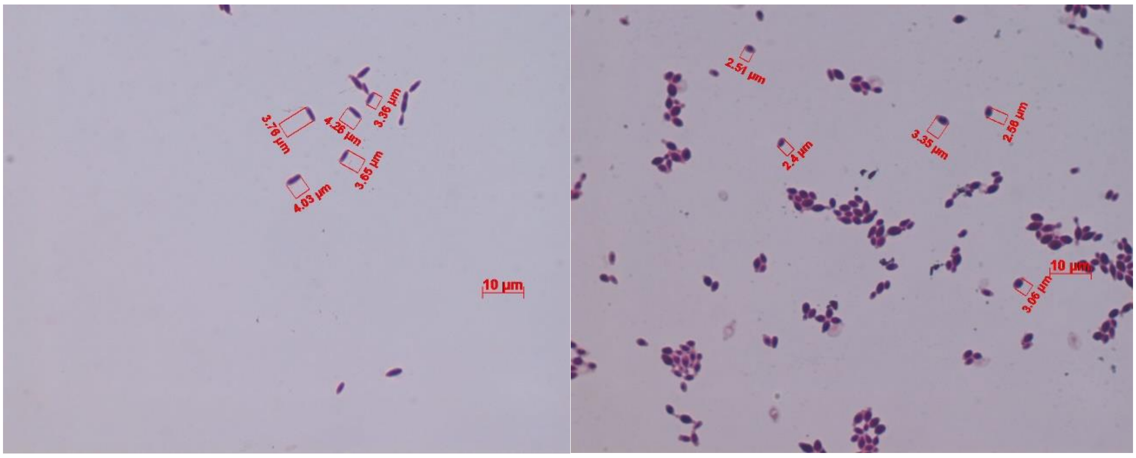


Figure 4.28 Morphology of Gram stained AAB cells grown on Yeast Extract Peptone Mannitol (YPM) agar

The morphology of AAB cells were observed using Gram-staining method under oil immersion at x1000 magnification.

4.3.5 Ethanol of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

Ethanol concentrations of the two samples (KB5F; KB5U) were similar during primary fermentation for 9 days at 22°C (Appendix D). Thereafter, ethanol in the unfiltered sample rapidly increased during prolonged storage (4°C) for 3 weeks ($p<0.05$). In contrast, ethanol in the filtered sample remained stable during week 1, then gradually increased in week 2, and finally decreased in week 3 of storage. At the end of the prolonged storage, ethanol levels in the filtered sample were lower (0.29±0.07%) than the unfiltered sample (0.53±0.10 %) ($p<0.05$) as shown in Figure 4.29. The higher ethanol content in the unfiltered sample during prolonged storage was probably due to increased post-fermentation activity of yeast. The results of the present work agree with previous reports (Talebi et al.,2017; Hillberg,2020) which reported increased ethanol levels due to post-fermentation in cold-stored Kombucha samples for 4 weeks.

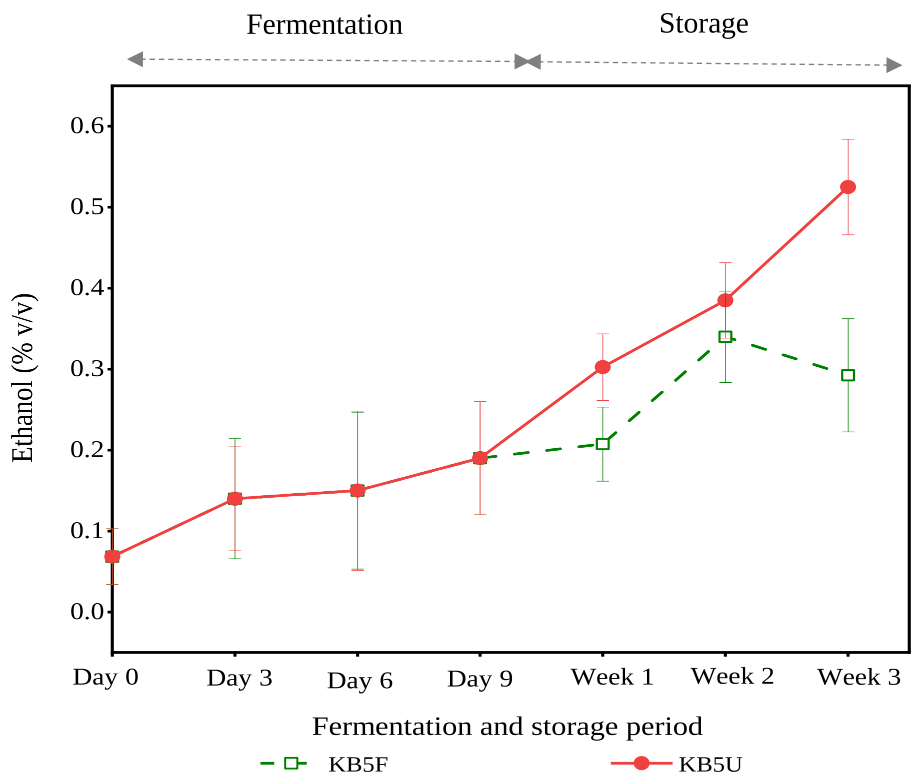


Figure 4.29 Mean concentration (%,v/v) of ethanol in filtered (KB5F) and unfiltered (KB5U) Kombucha samples during fermentation and storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

4.3.6 Sugars in filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

The sucrose concentration in Kombucha samples (KB5F, KB5U) decreased, while fructose and glucose concentration increased during fermentation (9 days) and prolonged storage (4°C) ($p<0.05$; Appendix F). The reduction of sucrose during fermentation and storage is likely associated with the hydrolysis of the sugar into glucose and fructose (Iličić et al., 2017; Muhialdin et al., 2019; Neffe-Skocińska et al., 2017; Rodrigues et al., 2006). The pattern of sucrose reduction in the two samples (KB5F; KB5U) during prolonged storage was consistent with their respective TSS (Section 4.3.2), where the reduction of sucrose for the unfiltered sample was higher than the filtered sample until week 2 of storage (Figure 4.30). Thereafter, in week 3, a rapid reduction in sucrose was observed in the filtered sample compared to the unfiltered sample ($p<0.05$). Possible explanations for the lower reduction in sucrose in the unfiltered sample in week 3 of prolonged storage were explained in Section 4.3.2. The sensitivity of the yeast strains to the levels of acid and ethanol in the samples may have contributed to the lower hydrolysis of sucrose into glucose and fructose thereby affecting the rate of fermentation (Berthels et al., 2004).

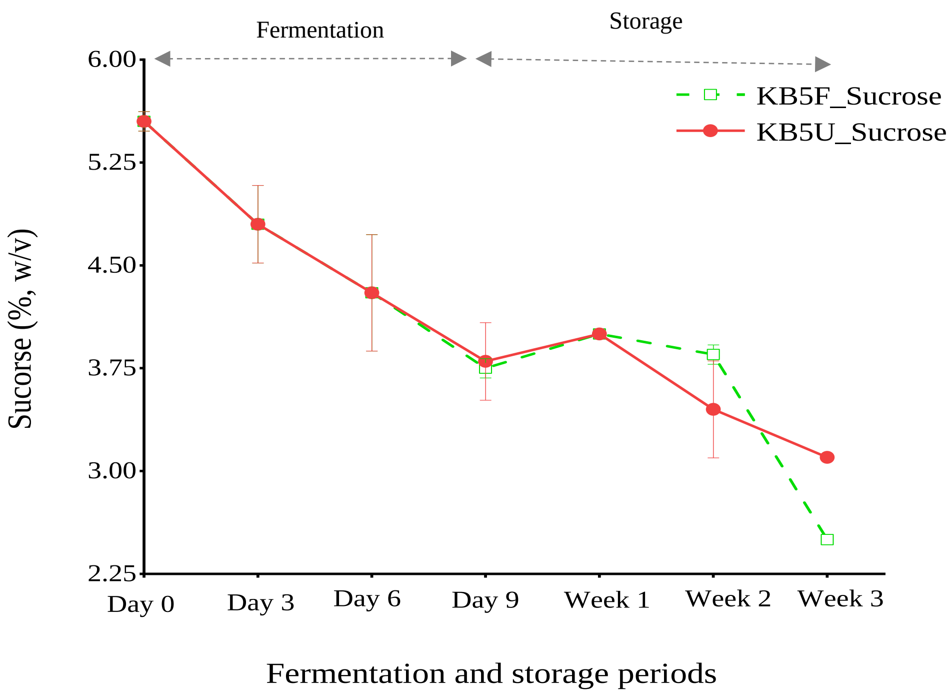


Figure 4.30 Mean concentration (%,v/v) of sucrose in filtered (KB5F) and unfiltered (KB5U) Kombucha samples during fermentation and storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

The higher concentration of glucose than fructose at the end of week 3 storage (4°C) suggested that fructose rather than glucose was the preferred carbon source of yeast cells ($p<0.05$). This result agreed with previous studies, where a higher content of glucose than fructose was reported during cold-storage of Kombucha (Iličić et al., 2017; Muhialdin et al., 2019; Neffe-Skocińska et al., 2017; Rodrigues et al., 2006). With respect to the filtered (KB5F) and unfiltered samples (KB5U), the unfiltered sample had lower concentrations of glucose and fructose than the filtered samples at the end of the week 3 storage period (Figure 4.31). The differences in the glucose and fructose levels may be attributed to varying populations and composition of the yeast-bacteria consortium present during fermentation and cold-storage of the Kombucha samples (Rodrigues et al., 2006; Iličić et al., 2017).

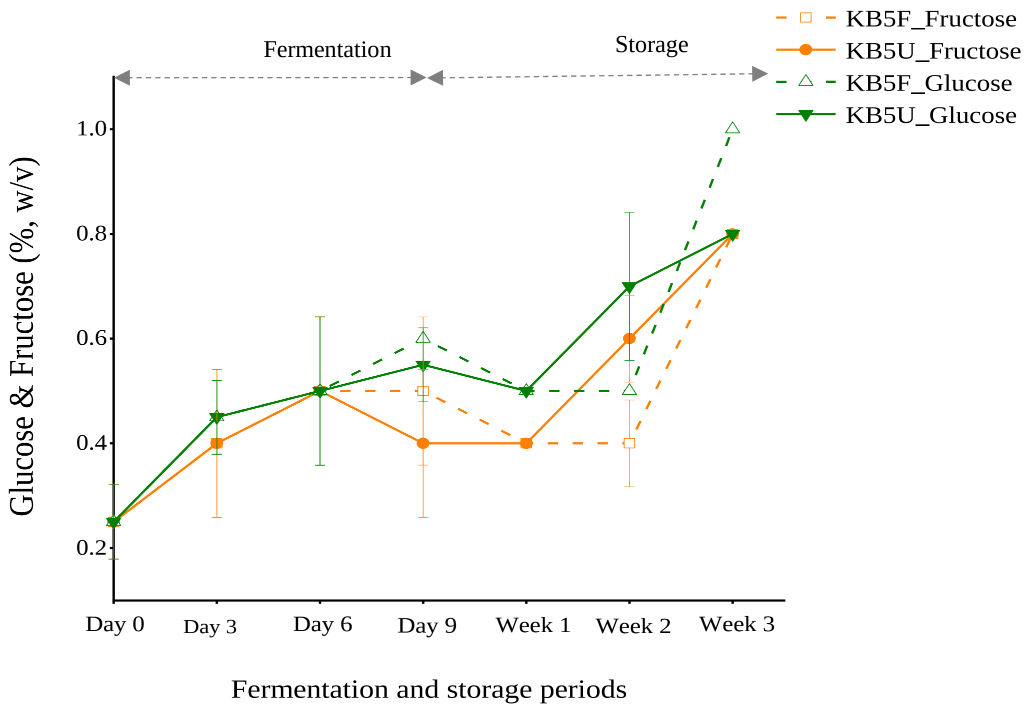


Figure 4.31 Mean concentration (%,v/v) of glucose and fructose in filtered (KB5F) and unfiltered (KB5U) Kombucha samples during fermentation and storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

4.3.7 Organic acids of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

The changes in the levels of major organic acids (acetic acid, glucuronic and gluconic) during fermentation for 9 days and prolonged storage (4°C) for 3 weeks are shown in Figure 4.32 and Figure 4.33. The concentrations of organic acids increased during fermentation and prolonged storage in all samples ($p<0.05$; Appendix E). The dominant organic acid found in the two samples (KB5F; KB5U) at the end of week 3 was acetic acid, followed by glucuronic acid and gluconic acid. The concentration of acetic acid (0.13±0.05 to 0.24±0.05 %w/v) obtained in this study was similar to that reported by Neffe-Skocińska et al. (2017), but lower than the range (0.54 to 0.8 %, w/v) reported by

Kallel et al. (2012). The differences in the levels of acetic acids may be due to the filtration step used in this study along with varying fermentation conditions and microbial cultures. Further, in present study the unfiltered sample had higher acetic acid content than the filtered sample at the end of prolonged storage ($p<0.05$; Figure 4.32). The presence of high acetic acid levels may have had a negative impact on the sensory attributes of Kombucha due its contribution to the vinegary taste (Zou, 2021).

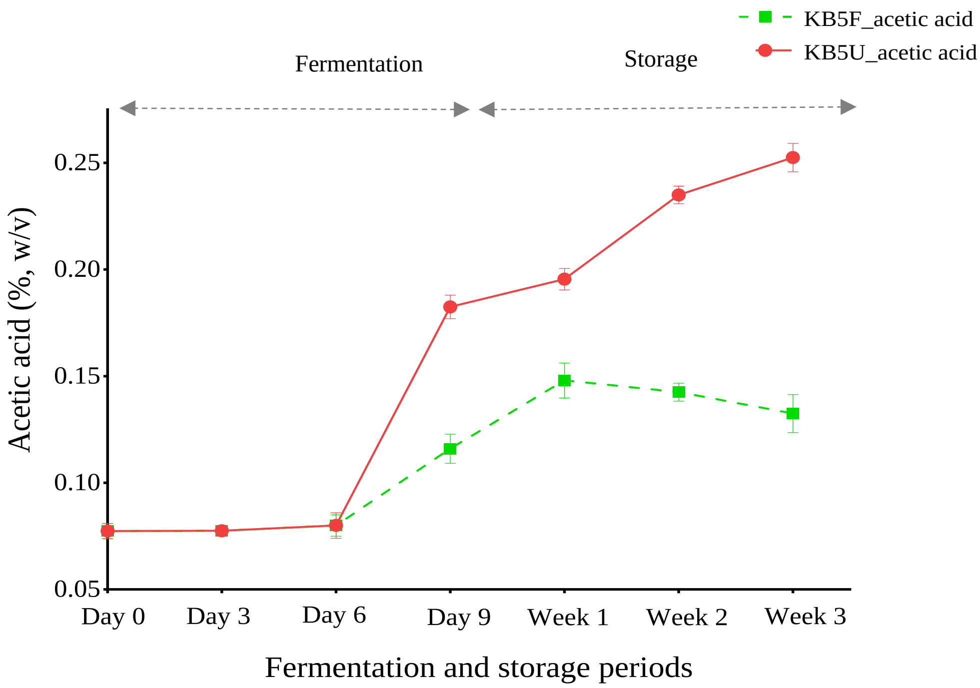


Figure 4.32 Mean concentration (%,v/v) of acetic acid in filtered (KB5F) and unfiltered (KB5U) Kombucha samples during fermentation and storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

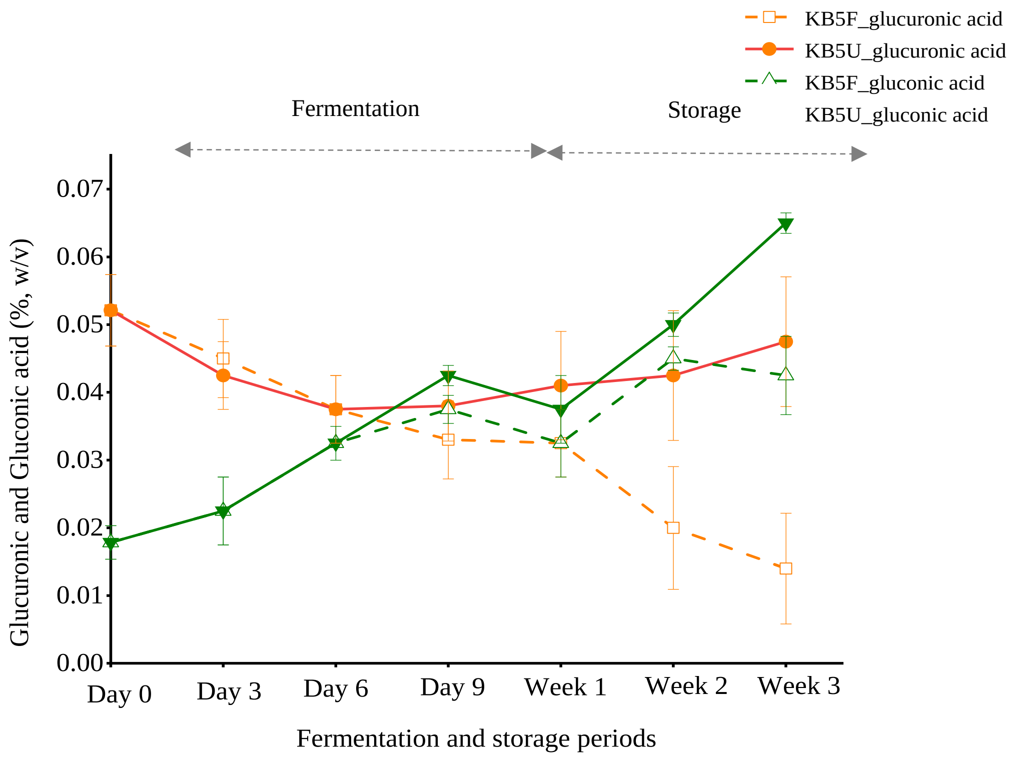


Figure 4.33 Mean concentration (%,v/v) of gluconic and glucuronic acid in filtered (KB5F) and unfiltered (KB5U) Kombucha samples during fermentation and storage (4℃) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); Error bars =± SD; n=2; independent experiments were repeated twice.

The glucuronic acid content in the unfiltered sample (0.04±0.01 %, w/v) was higher than that of the filtered sample (0.02±0.00 %, w/v) at the end of week 3 ($p<0.05$; Figure 4.33). Similarly, the gluconic acid content of the unfiltered sample (0.04±0.01 %, w/v) was also higher than the unfiltered sample (0.07±0.02 %, w/v) in week 3 ($p<0.05$; Figure 4.33). The differences in the gluconic and glucuronic acid levels of the filtered and unfiltered samples may be associated with the filtration step used in the former (sample), which removed some fermenting microbes before bottling/ post-fermentation (Laureys et al., 2020; Chen & Liu., 2000). The presence of lower cell counts in the filtered sample compared to the unfiltered sample may produce lower levels of organic acids as previously discussed in Section 4.3.1

4.3.8 Antioxidant levels in filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

Concentrations of phenolic (gallic acid, EGCG, ECG, EGC) and methylxanthine compounds (caffeine and theobromine) during fermentation and prolonged cold-storage (4°C) are shown in Figure 4.34. In the two samples (KB5F; KB5U), the concentration of gallic acid was the highest, followed by caffeine, with theobromine being the lowest ($p<0.05$; Figure 4.34).

Concentrations of phenolic and methylxanthine compounds decreased from day 0 to day 9 of fermentation in both filtered and unfiltered samples. This result is in contrast to previous researchers who reported increased concentration of antioxidants at the end of Kombucha fermentation (Osiripun & Apisittiwong, 2021; Zafrilla et al., 2003). One possible reason for the decrease in the concentrations of antioxidants in the unfiltered sample (KB5U) on day 9 was probably due to the high acidity of the unfiltered Kombucha. The mean pH of the unfiltered sample (3.08±0.00) in the present study was much lower than the mean pH (4.6) required for the stability of catechins (EGC, ECG and EGCG) (Tue et al., 2005; Li et al., 2012). Thus, high acidity may be the primary reason for the breakdown of antioxidants in the unfiltered sample. Further, antioxidant compounds are reported to be sensitive to heat and light which could also result in the degradation of antioxidants by oxidation and hydrolysis (Ahmed et al., 2020; Jakubczyk et al., 2020; Zafrilla et al., 2003).

For the filtered sample (KB5F), the reduction in phenolic and methylxanthine compounds on day 9 may be attributed primarily to the filtration step used prior to bottling, which may have potentially filtered out some antioxidant compounds. Acidity, light, and heat can also affect the stability of oxidants (Ahmed et al., 2020; Jakubczyk et al., 2020; Zafrilla et al., 2003). However, there are no published data on the effect of filtration on concentrations of antioxidants in Kombucha.

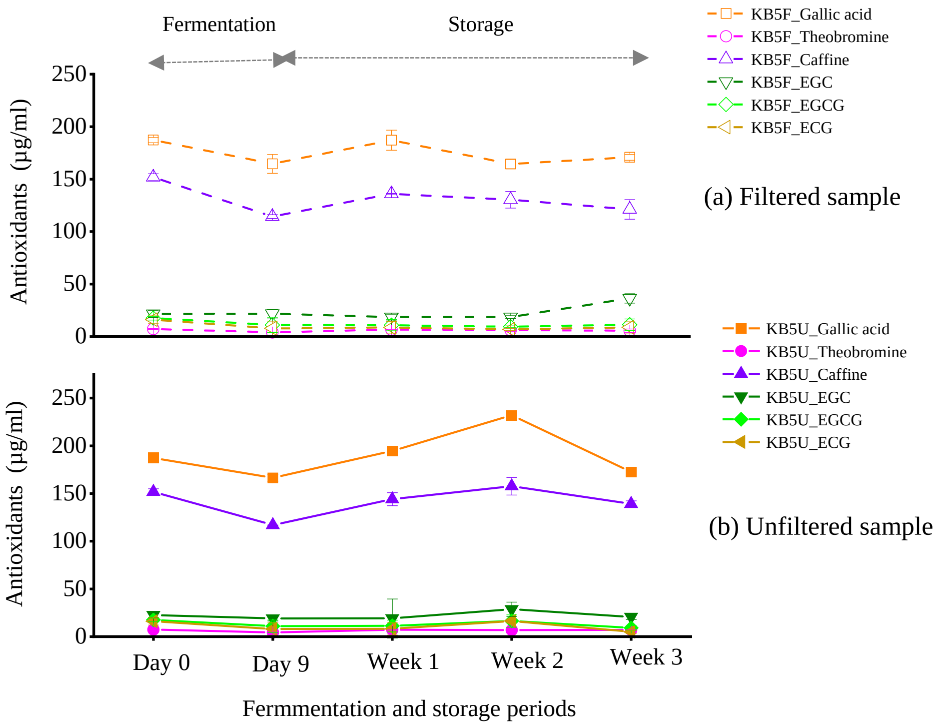


Figure 4.34 Mean concentration (µg/mL) of antioxidants in filtered (KB5F, a) Kombucha and unfiltered (KB5U, b) samples during fermentation and prolonged storage (4℃) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); Error bars =± SD; n=2; experiments were repeated twice.

The mean concentrations of phenolic and methylxanthine compounds in both samples (KB5F; KB5U) were variable during first the two weeks, with no significant ($p<0.05$) decreases in week 3 of storage (4℃) (Appendix G). Moreover, the increased concentration of gallic acid and caffeine in week 1 of storage in both samples may be linked to post-fermentation activities of Kombucha cultures as they possess the enzyme (phytase) required to breakdown the cellulosic chain of *Camellia sinensis* and generate phenolic compounds (Watawana et al., 2015). The increase in the concentrations of antioxidant compounds during storage may also be caused by the release of catechins by acid-sensitive microbial cells under acidic conditions (Ivanišová et al., 2019; Osiripun & Apisittiwong, 2021; Watawana et al., 2015).

4.3.9 Consumer sensory evaluation of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

Consumer sensory panellists (n=108) evaluated filtered and unfiltered Kombucha samples using the 9-point hedonic scale during storage (4°C) for three weeks (Figure 4.35 and Figure 4.36). The sensory panellists evaluated the samples for appearance, aroma, flavour, sweetness, sourness and overall acceptability. Overall, the sensory scores for the filtered sample (KB5F) were higher than the unfiltered sample (KB5U) ($p<0.05$; Appendix H.4).

For both filtered and unfiltered Kombucha samples (KB5F and KB5U), the sensory scores for appearance decreased during cold-storage (4°C), while the scores for the other sensory attributes steadily increased. The lower appearance scores of these two samples (KB5F; KB5U) compared to other attributes may be associated with the cloudiness or lack of clarity, which can be caused by the production of the cellulosic structure of *Acetobacter xylinum* during post-fermentation at 4°C (Al-Kalifawi, 2014; Gramza-Michałowska et al., 2016; Treviño-Garza et al., 2020). Although the cloudy appearance of Kombucha may not appeal to some consumers, it is desired by others as it serves to confirm the presence of viable cultures perceived to have health benefits (Flores-García et al., 2019; Marco et al., 2021; Valero-Cases et al., 2020).

The lowest mean score for aroma (5.63±0.90) was received by the unfiltered sample probably due to its ‘vinegary’ and ‘yeasty odour’ ($p<0.05$). Higher yeast counts in the unfiltered sample probably resulted in higher production of ester compounds which are characterised by the yeasty odour (Laureys et al., 2018; Laureys & De Vuyst, 2014; Rodrigues et al., 2016). Furthermore, high yeast counts may also lead to higher accumulation of organic acids which could be responsible for the vinegary odour of unfiltered sample during prolonged storage (4°C) (Aung, 2022; Ubeda, 1999; Zou, 2021).

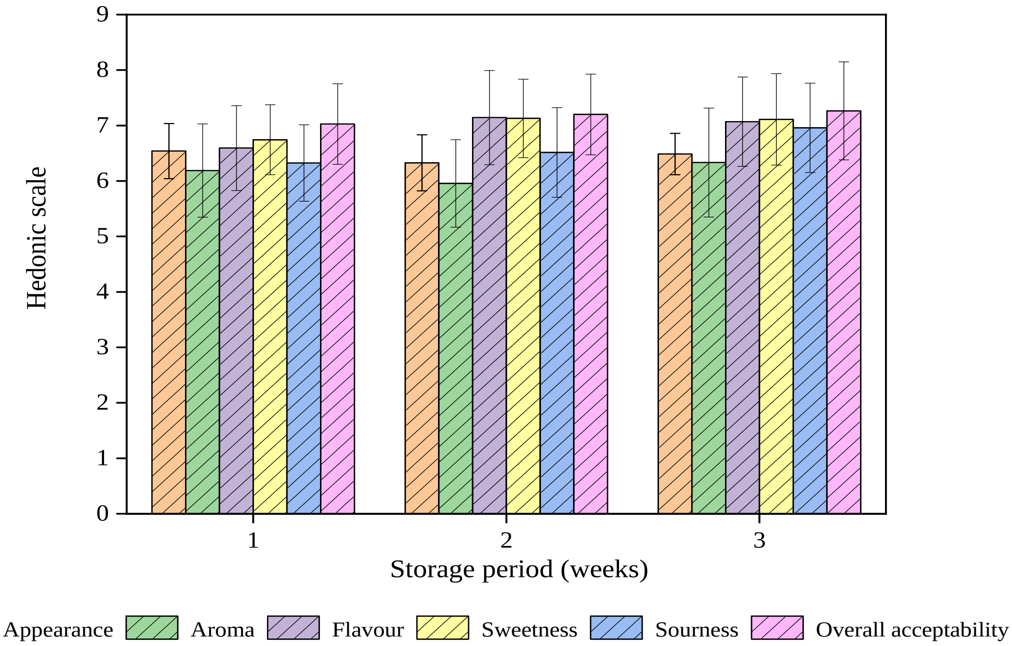


Figure 4.35 Mean consumer sensory evaluation scores of filtered Kombucha samples (KB5F) during prolonged storage (4°C) for three weeks

KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); Error bars = ± SD; n=2; independent experiments were repeated twice.

At the end of week 3 of prolonged storage, sensory scores for sourness and sweetness of the filtered sample were higher than for the unfiltered sample, which may have been affected by the presence of high concentrations of organic acids ($p<0.05$). As discussed earlier in Section 4.3.7, the level of acetic acid was higher in the unfiltered sample which could have contributed to the overpowering vinegary taste, due to increased acidity and reduced sweetness of the sample. In addition to this, the slightly bitter flavour of the unfiltered sample was possibly caused by tea alkaloids (Gramza-Michałowska et al., 2016). No bitter after-taste was reported for the filtered samples, which may be due to the production of amino acids during fermentation which possibly masked the bitterness of tea alkaloids (Gramza-Michałowska et al., 2016). Overall, based on the consumer sensory results of samples following storage for three weeks at 4°C, the slightly sour, fruitier taste and natural sparkling of the filtered sample (KB5F) was highly acceptable (7.26±0.88) consumer scores.

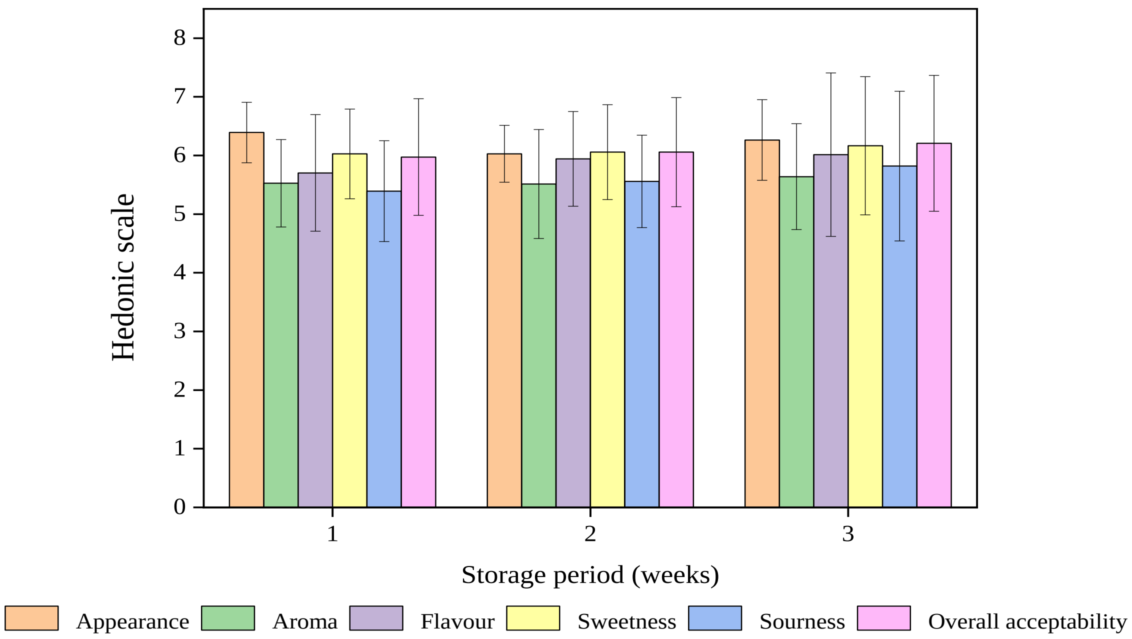


Figure 4.36 Mean consumer sensory evaluation scores of unfiltered Kombucha samples (KB5U) during prolonged storage (4℃) for three weeks

KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); Error bars = ± SD; n=2; independent experiments were repeated twice.

Chapter 5. OVERALL CONCLUSIONS

A suitable sugar-tea formulation and optimum fermentation conditions were identified for the propagation of the Kombucha starter culture. In this study, sugar concentration and temperature had significant effects ($p<0.05$) on fermentation of Kombucha. Filtered Kombucha with low alcohol (<0.5%) was stable during storage and the beverage received the highest overall acceptability consumer sensory scores of those tested. During storage for three weeks (4°C), the acidity, microbial counts and sugars decreased, while the organic acids analysed increased. The fermented brew contained appreciable quantities of antioxidants and the colour was stable during fermentation and storage (4°C). The residual sugar present in the filtered beverages was markedly lower than results reported in previous studies. The results of the optimised production of Kombucha showed good potential for scale-up and commercialisation.

Chapter 6. RECOMMENDATIONS

In the present study, the optimised production of low alcohol Kombucha beverage was developed by filtration using a domestic double-sieve stainless coffee filter. There is need to further test the performance of the optimised production process using other sieves. Also, work needs to be conducted to determine the integration of the sieving processing in a commercial plant.

The microbial community of the Kombucha starter culture used in the study is unknown and complex. It would be desirable to identify the symbiotic culture of yeast and acetic acid bacteria in the fermented broth and cellulose pellicle to better understand the fermentation kinetics of the microorganisms. Although LAB have rarely been reported in Kombucha, it would be interesting to confirm whether any lactic acid bacteria are present in the culture used.

Due to its inherent acidity, Kombucha (pH = ≤ 4.6) falls under the shelf-stable category of foods. However, the antimicrobial capability of the fermented beverage could be challenged against a range of potential pathogens.

Kombucha is perceived to confer beneficial effects on human health mainly based on personal testimonials (Vargas et al., 2021). It is important to conduct both *in vivo* and in vitro studies to substantiate these perceptions.

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APPENDIX

A. Composition of agar media for microbiological analysis

Table A.1 List of ingredients of agar media

Product	**Ingredients**	**Composition (g/L)**
YGC agar (1.16000.0500), Merck K GAA	Yeast extract D (+) glucose Chloramphenicol Agar	5.0 20.0 0.1 14.9
YPM agar (Thermo Fisher, New Zealand)	Yeast extract Peptone Mannitol Agar	5.0 3.0 25.0 12.0
PCA agar (Thermo Fisher, New Zealand)	Enzymatic Digest of Casein/tryptone Yeast extract Glucose Agar	5.0 2.5 1.0 15.0

B. Sensory evaluation questionnaire

INFORMATION SHEET

I am Ashwini Navin Gangurde, a Master of Food Technology student at the School of Food and Advanced Technology (SFAT), Albany, Massey University. My research investigates the optimisation of Kombucha fermentation to produce consistent high-quality products which meet the stipulated regulations.

You are being invited to evaluate the sensory characteristics of freshly fermented Kombucha. The objective of this part of the study is to evaluate the level of acceptance of the prepared fermented Kombucha by consumers.

Participant involvement

Your participation involves evaluating the Kombucha samples which may take 5-7 minutes. The fermented Kombucha contains the following ingredients : organic white organic sugar, organic black tea, natural Kombucha starter culture, boiled and cooled water.

You should not participate, if you are allergic or may be affected by the consumptions of any of the listed ingredients. In the unlikely event of any adverse reaction, medical assistance will be provided. You may advise one of the researchers of any potentially relevant cultural, religious or ethical benefits which may prevent you from consuming the food under consideration.

The information collected in this study will not be linked to any individual's identity and will be used to complete the postgraduate degree research project. In case you wish to receive a summary of the findings once data analysis has been completed, please provide your email address.

You have no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular questions;
- Withdraw from the study (at any time);
- Ask any questions about the study at any time during your participation;

Ethics Approved number 4000022993

- Provide information on the understanding that your name will not be used unless you give permission to the researchers.

Project Contacts

- Ashwini Gangurde (Master of Food Technology student): ag1714@gmail.com
- Dr. Tony Mutukumira (supervisor): a.n.mutukumira@massey.ac.nz

This project has been evaluated by peer review and judged to be low risk (Massey University Ethics 4000022993). Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) name above are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you wish to raise with someone other than researcher(s), please contact Professor John O'Neill, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz.

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction.

I understand that I have the right to withdraw from the study at any time and decline my answers.

I agree to voluntarily participate in this study under the condition set out in the Information Sheet.

Signature : ______________________________

Date : ______________________________

Full Name (Printed): ______________________________

Ethics Approved Number 4000022993

SENSORY ACCEPTANCE TEST

You will be given 4 coded samples of freshly fermented Kombucha for sensory evaluation. Please taste the samples one at a time and indicate how much you like/ dislike each product by ticking ☑ in the appropriate box. Please **rinse your mouth thoroughly** between each sample.

You may taste the sample more than once.

PROJECT : Optimisation of Kombucha fermentation **SAMPLE CODE :**

Note: Each sample must be evaluated on different form supplied.

Attribute	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like Very much	Like extremely
Appearance/ Colour									
Aroma									
Flavour									
Sweetness									
Sourness									
Overall acceptability									

Comment:

C. Data Analysis (Raw data)

Table C.1 Characteristic of Kombucha during propagation of starter culture in Phase 1

Product	Replication	Duplicate	Fermentation time (days)	pH	TA%	TSS °Brix
KB1	1	1	1	4.22	0.07	6.40
KB1	1	2	1	4.21	0.08	6.30
KB1	1	1	2	3.90	0.11	6.20
KB1	1	2	2	3.86	0.12	6.10
KB1	1	1	3	3.83	0.21	6.10
KB1	1	2	3	3.82	0.25	6.10
KB1	1	1	4	3.69	0.31	6.00
KB1	1	2	4	3.68	0.33	6.00
KB1	1	1	5	3.58	0.35	5.90
KB1	1	2	5	3.57	0.35	5.80
KB1	1	1	6	3.55	0.39	5.60
KB1	1	2	6	3.54	0.38	5.60
KB1	2	1	1	4.23	0.08	6.20
KB1	2	2	1	4.22	0.07	6.20
KB1	2	1	2	3.94	0.12	5.90
KB1	2	2	2	3.92	0.12	6.00
KB1	2	1	3	3.79	0.22	5.90
KB1	2	2	3	3.79	0.26	5.90
KB1	2	1	4	3.62	0.32	5.80
KB1	2	2	4	3.63	0.36	5.80
KB1	2	1	5	3.59	0.37	5.60
KB1	2	2	5	3.60	0.37	5.70
KB1	2	1	6	3.56	0.39	5.50
KB1	2	2	6	3.58	0.38	5.50
KB2	1	1	1	4.20	0.09	6.40
KB2	1	2	1	4.21	0.09	6.40
KB2	1	1	2	3.75	0.13	6.10
KB2	1	2	2	3.74	0.13	6.10
KB2	1	1	3	3.60	0.30	5.90
KB2	1	2	3	3.61	0.29	5.90
KB2	1	1	4	3.59	0.35	5.90
KB2	1	2	4	3.57	0.36	5.90
KB2	1	1	5	3.55	0.39	5.80
KB2	1	2	5	3.54	0.39	5.80
KB2	1	1	6	3.52	0.44	5.50
KB2	1	2	6	3.50	0.44	5.60
KB2	2	1	1	4.20	0.09	6.30
KB2	2	2	1	4.20	0.09	6.30
KB2	2	1	2	3.73	0.13	6.00
KB2	2	2	2	3.71	0.14	6.00
KB2	2	1	3	3.65	0.30	5.80
KB2	2	2	3	3.63	0.29	5.80
KB2	2	1	4	3.55	0.35	5.80
KB2	2	2	4	3.55	0.36	5.80
KB2	2	1	5	3.53	0.39	5.80
KB2	2	2	5	3.54	0.41	5.80
KB2	2	1	6	3.49	0.43	5.40
KB2	2	2	6	3.47	0.43	5.40

Product	Replication	Duplicate	Fermentation time (days)	pH	TA%	TSS °Brix
KB3	1	1	1	4.08	0.75	4.10
KB3	1	2	1	4.10	0.79	4.10
KB3	1	1	2	3.71	0.13	4.00
KB3	1	2	2	3.70	0.15	4.00
KB3	1	1	3	3.52	0.28	3.90
KB3	1	2	3	3.50	0.28	3.90
KB3	1	1	4	3.46	0.35	3.80
KB3	1	2	4	3.44	0.34	3.80
KB3	1	1	5	3.43	0.35	3.70
KB3	1	2	5	3.43	0.34	3.70
KB3	1	1	6	3.41	0.46	3.60
KB3	1	2	6	3.40	0.47	3.60
KB3	2	1	1	4.10	0.10	4.00
KB3	2	2	1	4.20	0.13	4.00
KB3	2	1	2	3.69	0.19	3.90
KB3	2	2	2	3.68	0.20	3.90
KB3	2	1	3	3.53	0.21	3.80
KB3	2	2	3	3.53	0.30	3.80
KB3	2	1	4	3.43	0.37	3.70
KB3	2	2	4	3.42	0.37	3.70
KB3	2	1	5	3.42	0.40	3.60
KB3	2	2	5	3.42	0.41	3.60
KB3	2	1	6	3.42	0.49	3.50
KB3	2	2	6	3.41	0.48	3.40
KB4	1	1	1	3.95	0.10	4.10
KB4	1	2	1	3.93	0.13	4.10
KB4	1	1	2	3.53	0.17	3.90
KB4	1	2	2	3.54	0.20	3.90
KB4	1	1	3	3.49	0.29	3.80
KB4	1	2	3	3.48	0.30	3.80
KB4	1	1	4	3.44	0.38	3.70
KB4	1	2	4	3.43	0.36	3.70
KB4	1	1	5	3.38	0.40	3.60
KB4	1	2	5	3.40	0.41	3.60
KB4	1	1	6	3.35	0.49	3.40
KB4	1	2	6	3.36	0.46	3.40
KB4	2	1	1	3.93	0.10	4.20
KB4	2	2	1	3.90	0.14	4.20
KB4	2	1	2	3.70	0.19	4.00
KB4	2	2	2	3.60	0.22	4.00
KB4	2	1	3	3.47	0.30	3.70
KB4	2	2	3	3.47	0.30	3.70
KB4	2	1	4	3.42	0.39	3.60
KB4	2	2	4	3.41	0.37	3.60
KB4	2	1	5	3.39	0.42	3.50
KB4	2	2	5	3.37	0.43	3.40
KB4	2	1	6	3.34	0.51	3.30
KB4	2	2	6	3.35	0.51	3.30

Product	Replication	Duplicate	Fermentation time (days)	pH	TA%	TSS °Brix
KB5	1	1	1	3.73	0.10	5.10
KB5	1	2	1	3.70	0.13	5.10
KB5	1	1	2	3.62	0.17	4.90
KB5	1	2	2	3.61	0.20	4.90
KB5	1	1	3	3.47	0.29	4.70
KB5	1	2	3	3.50	0.30	4.80
KB5	1	1	4	3.29	0.38	4.70
KB5	1	2	4	3.30	0.36	4.70
KB5	1	1	5	3.21	0.40	4.60
KB5	1	2	5	3.24	0.41	4.50
KB5	1	1	6	3.20	0.54	4.40
KB5	1	2	6	3.17	0.54	4.30
KB5	2	1	1	3.72	0.10	5.00
KB5	2	2	1	3.71	0.14	5.00
KB5	2	1	2	3.63	0.19	4.80
KB5	2	2	2	3.60	0.22	4.80
KB5	2	1	3	3.47	0.30	4.60
KB5	2	2	3	3.51	0.30	4.60
KB5	2	1	4	3.30	0.39	4.50
KB5	2	2	4	3.31	0.37	4.50
KB5	2	1	5	3.23	0.42	4.50
KB5	2	2	5	3.29	0.43	4.40
KB5	2	1	6	3.21	0.54	4.20
KB5	2	2	6	3.19	0.53	4.20
KB6	1	1	1	3.69	0.10	5.10
KB6	1	2	1	3.67	0.09	5.00
KB6	1	1	2	3.48	0.16	4.90
KB6	1	2	2	3.47	0.14	4.90
KB6	1	1	3	3.33	0.27	4.70
KB6	1	2	3	3.35	0.28	4.70
KB6	1	1	4	3.28	0.35	4.50
KB6	1	2	4	3.28	0.34	4.50
KB6	1	1	5	3.28	0.49	4.30
KB6	1	2	5	3.27	0.51	4.30
KB6	1	1	6	3.17	0.57	4.20
KB6	1	2	6	3.13	0.56	4.20
KB6	2	1	1	3.70	0.10	5.10
KB6	2	2	1	3.70	0.09	5.10
KB6	2	1	2	3.53	0.17	4.80
KB6	2	2	2	3.52	0.15	4.80
KB6	2	1	3	3.38	0.28	4.60
KB6	2	2	3	3.36	0.29	4.60
KB6	2	1	4	3.30	0.36	4.50
KB6	2	2	4	3.30	0.33	4.40
KB6	2	1	5	3.23	0.41	4.20
KB6	2	2	5	3.23	0.44	4.20
KB6	2	1	6	3.16	0.57	4.10
KB6	2	2	6	3.15	0.57	4.00

Product	Replication	Duplicate	Fermentation time (days)	pH	TA%	TSS °Brix
KB7	1	1	1	3.67	0.10	3.70
KB7	1	2	1	3.78	0.12	3.70
KB7	1	1	2	3.55	0.19	3.50
KB7	1	2	2	3.56	0.18	3.50
KB7	1	1	3	3.48	0.26	3.30
KB7	1	2	3	3.42	0.30	3.30
KB7	1	1	4	3.38	0.43	3.10
KB7	1	2	4	3.31	0.48	3.10
KB7	1	1	5	3.21	0.52	3.10
KB7	1	2	5	3.20	0.51	3.00
KB7	1	1	6	2.98	0.59	3.00
KB7	1	2	6	2.96	0.60	3.10
KB7	2	1	1	3.78	0.10	3.90
KB7	2	2	1	3.76	0.13	3.80
KB7	2	1	2	3.56	0.18	3.70
KB7	2	2	2	3.54	0.18	3.60
KB7	2	1	3	3.42	0.28	3.50
KB7	2	2	3	3.40	0.32	3.50
KB7	2	1	4	3.31	0.47	3.40
KB7	2	2	4	3.29	0.49	3.40
KB7	2	1	5	3.20	0.53	3.20
KB7	2	2	5	3.19	0.52	3.20
KB7	2	1	6	2.98	0.60	3.20
KB7	2	2	6	2.91	0.61	3.10
KB8	1	1	1	3.71	0.09	3.60
KB8	1	2	1	3.72	0.09	3.60
KB8	1	1	2	3.49	0.21	3.40
KB8	1	2	2	3.48	0.21	3.30
KB8	1	1	3	3.34	0.31	3.30
KB8	1	2	3	3.34	0.31	3.20
KB8	1	1	4	3.29	0.49	3.20
KB8	1	2	4	3.30	0.47	3.20
KB8	1	1	5	3.21	0.53	3.00
KB8	1	2	5	3.20	0.57	3.00
KB8	1	1	6	2.88	0.64	3.00
KB8	1	2	6	2.82	0.64	3.00
KB8	2	1	1	3.71	0.15	3.60
KB8	2	2	1	3.73	0.14	3.60
KB8	2	1	2	3.51	0.22	3.40
KB8	2	2	2	3.50	0.23	3.40
KB8	2	1	3	3.39	0.33	3.20
KB8	2	2	3	3.37	0.35	3.10
KB8	2	1	4	3.33	0.43	3.10
KB8	2	2	4	3.34	0.48	3.10
KB8	2	1	5	3.25	0.54	2.90
KB8	2	2	5	3.23	0.58	3.00
KB8	2	1	6	2.83	0.63	2.90
KB8	2	2	6	2.85	0.63	3.00

Note: KB1 contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 contained 4.7% sugar 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Fermentation time: 1= Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9, 5= Day 12, 6 = Day 14.

Table C.2 Microbial counts of fermented broth during propagation of Kombucha starter culture in Phase 1

Product	Replication	Duplicate	Fermentation time (days)	Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB1	1	1	1	4.81	6.16	6.23
KB1	1	2	1	4.80	6.21	6.29
KB1	1	1	2	5.01	6.18	6.31
KB1	1	2	2	5.12	6.3	6.22
KB1	2	1	1	4.76	6.11	6.21
KB1	2	2	1	4.79	6.18	6.18
KB1	2	1	2	5.08	6.32	6.38
KB1	2	2	2	5.07	6.29	6.36
KB2	1	1	1	4.87	6.59	6.35
KB2	1	2	1	4.90	6.63	6.42
KB2	1	1	2	5.18	6.51	6.41
KB2	1	2	2	5.23	6.58	6.52
KB2	2	1	1	4.89	6.51	6.39
KB2	2	2	1	4.87	6.58	6.36
KB2	2	1	2	5.21	6.65	6.59
KB2	2	2	2	5.26	6.69	6.39
KB3	1	1	1	5.08	6.53	6.63
KB3	1	2	1	5.11	6.52	6.67
KB3	1	1	2	5.32	6.62	6.71
KB3	1	2	2	5.49	6.52	6.78
KB3	2	1	1	5.01	6.49	6.72
KB3	2	2	1	5.08	6.51	6.65
KB3	2	1	2	5.51	6.58	6.60
KB3	2	2	2	5.53	6.66	6.62
KB4	1	1	1	5.27	6.71	6.87
KB4	1	2	1	5.23	6.83	6.8
KB4	1	1	2	5.67	6.83	6.83
KB4	1	2	2	5.73	6.84	6.93
KB4	2	1	1	5.31	6.75	6.93
KB4	2	2	1	5.27	6.77	6.84
KB4	2	1	2	6.03	6.90	6.80
KB4	2	2	2	6.11	6.99	7.01

Product	Replication	Duplicate	Fermentation time (days)	Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB5	1	1	1	6.06	7.26	7.62
KB5	1	2	1	6.19	7.33	7.81
KB5	1	1	2	6.16	8.11	8.11
KB5	1	2	2	6.13	8.01	8.21
KB5	2	1	1	6.03	8.01	7.73
KB5	2	2	1	6.07	7.25	7.60
KB5	2	1	2	5.58	8.13	8.29
KB5	2	2	2	5.52	8.03	8.17
KB6	1	1	1	6.27	8.17	8.22
KB6	1	2	1	6.29	8.19	8.32
KB6	1	1	2	6.25	8.50	8.52
KB6	1	2	2	6.21	8.32	8.49
KB6	2	1	1	6.19	8.31	8.11
KB6	2	2	1	6.21	8.18	8.19
KB6	2	1	2	6.11	8.42	8.52
KB6	2	2	2	6.19	8.47	8.53
KB7	1	1	1	6.40	8.01	7.71
KB7	1	2	1	6.42	8.11	7.78
KB7	1	1	2	6.37	8.23	8.23
KB7	1	2	2	6.39	8.25	8.25
KB7	2	1	1	6.46	8.21	7.80
KB7	2	2	1	6.40	8.01	7.70
KB7	2	1	2	6.43	8.22	8.31
KB7	2	2	2	6.53	8.32	8.37
KB8	1	1	1	6.51	8.22	7.97
KB8	1	2	1	6.55	8.39	7.83
KB8	1	1	2	6.54	8.51	8.61
KB8	1	2	2	6.52	8.44	8.52
KB8	2	1	1	6.50	8.41	8.09
KB8	2	2	1	6.56	8.35	8.01
KB8	2	1	2	6.67	8.44	8.47
KB8	2	2	2	6.68	8.40	8.43

Note: KB1 contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 contained 4.7% sugar 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Fermentation time: 1= Day 0, 2 = Day 14.

Table C.3 Microbial counts of cellulose pellicle during propagation of Kombucha starter culture (Day 14) in Phase 1

		Yeast count (log cfu/ml)		AAB count (log cfu/ml)		Total count (log cfu/ml)	
Product	Duplicate	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
KB1	1	4.78	4.84	6.11	6.21	6.18	6.28
KB1	2	4.72	4.86	6.17	6.19	6.15	6.2
KB2	1	4.83	4.91	6.66	6.52	6.32	6.37
KB2	2	4.85	4.88	6.68	6.48	6.3	6.41
KB3	1	5.04	5.11	6.38	6.68	6.74	6.51
KB3	2	5.07	5.09	6.4	6.62	6.72	6.58
KB4	1	5.21	5.33	6.76	6.81	6.89	6.84
KB4	2	5.23	5.31	6.79	6.71	6.86	6.81
KB5	1	5.93	6.19	8.21	8.29	8.24	8.25
KB5	2	6.01	6.17	8.19	8.27	8.31	8.33
KB6	1	6.26	6.27	8.57	8.51	8.47	8.49
KB6	2	6.19	6.23	8.51	8.49	8.40	8.44
KB7	1	6.39	6.41	8.44	8.41	8.30	8.32
KB7	2	6.36	6.44	8.49	3.43	8.35	8.38
KB8	1	6.68	6.51	8.56	8.68	8.63	8.51
KB8	2	6.67	6.53	8.72	8.62	8.69	8.55

Note: KB1 contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 contained 4.7% sugar 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C.

Table C.4 Weight of cellulose pellicle during propagation of Kombucha starter culture in Phase 1

Product	**Replication**	**Fermentation time (days)**	**Weight of cellulose pellicle (g/1500mL)**
KB1	1	14	12.05
KB1	2	14	15.45
KB2	1	14	18.43
KB2	2	14	21.86
KB3	1	14	24.62
KB3	2	14	26.93
KB4	1	14	30.08
KB4	2	14	32.84
KB5	1	14	26.31
KB5	2	14	21.8
KB6	1	14	25.04
KB6	2	14	29.68
KB7	1	14	31.83
KB7	2	14	29.86
KB8	1	14	31.83
KB8	2	14	37.83

Note: KB1 contained 4.7% sugar, 0.3% tea, and 12.5% fermented broth and was fermented at 22°C; KB2 contained 4.7% sugar 0.3% tea, 12.5% fermented broth and was fermented at 24°C; KB3 contained 3% sugar, 0.3% tea, 12.5% fermented broth and was fermented at 22°C; KB4 contained 3% sugar 0.3% tea, 12.5 % fermented broth and was fermented at 24°C; KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C.

Table C.5 Characteristics of Kombucha during fermentation for 9 days in Stage 1 of Phase 2

										Fermented broth		
Product	Replication	Duplicate	Fermentation time (days)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB5	1	1	1	3.6	0.09	5.9	83.0	7.00	55.00	4.18	4.45	4.56
KB5	1	2	1	3.6	0.09	6.0	83.0	7.00	55.00	4.20	4.47	4.60
KB5	1	1	2	3.4	0.13	5.6	81.0	6.00	52.00	6.09	6.08	6.06
KB5	1	2	2	3.4	0.15	5.5	81.0	6.00	52.00	6.01	6.10	6.04
KB5	1	1	3	3.2	0.28	5.3	81.0	6.00	50.00	6.69	7.03	7.04
KB5	1	2	3	3.2	0.28	5.2	81.0	6.00	50.00	6.66	7.01	7.06
KB5	1	1	4	3.1	0.35	5.2	79.0	6.00	48.00	6.93	7.24	7.08
KB5	1	2	4	3.0	0.34	5.0	79.0	6.00	48.00	6.89	7.23	7.10
KB5	2	1	1	3.7	0.09	6.0	84.0	6.00	54.00	4.84	4.52	5.02
KB5	2	2	1	3.7	0.09	5.9	84.0	7.00	54.00	4.89	4.51	5.03
KB5	2	1	2	3.5	0.13	5.7	81.0	6.00	51.00	6.11	6.13	6.10
KB5	2	2	2	3.5	0.13	5.7	80.0	6.00	51.00	6.15	6.12	6.17
KB5	2	1	3	3.3	0.25	5.2	80.0	5.00	49.00	7.01	7.10	7.17
KB5	2	2	3	3.3	0.24	5.3	80.0	5.00	49.00	7.06	7.13	7.19
KB5	2	1	4	3.2	0.32	5.1	78.0	5.00	47.00	6.98	7.27	7.10
KB5	2	2	4	3.2	0.31	5.0	78.0	5.00	47.00	6.81	7.21	7.24
KB6	1	1	1	3.6	0.08	5.8	82.0	7.00	55.00	4.28	4.32	4.42
KB6	1	2	1	3.5	0.08	5.9	81.0	6.00	55.00	4.24	4.45	4.50
KB6	1	1	2	3.3	0.13	5.3	82.0	6.00	52.00	6.24	6.30	6.34
KB6	1	2	2	3.3	0.13	5.4	82.0	7.00	50.00	6.26	6.29	6.36
KB6	1	1	3	3.2	0.31	5.1	81.0	7.00	50.00	6.56	6.84	6.79
KB6	1	2	3	3.2	0.31	5.0	81.0	7.00	50.00	6.51	6.81	6.77
KB6	1	1	4	3.1	0.35	4.8	81.0	6.00	50.00	7.16	7.89	7.85
KB6	1	2	4	2.7	0.35	4.9	81.0	6.00	50.00	7.15	7.85	7.83
KB6	2	1	1	3.7	0.08	5.9	83.0	7.00	54.00	4.31	4.51	4.45
KB6	2	2	1	3.7	0.07	5.8	82.0	7.00	54.00	4.29	4.49	4.53
KB6	2	1	2	3.4	0.10	5.6	81.0	6.00	51.00	6.36	6.29	6.41
KB6	2	2	2	3.4	0.10	5.6	81.0	6.00	51.00	6.34	6.27	6.42
KB6	2	1	3	3.3	0.28	5.2	80.0	6.00	49.00	6.59	6.82	6.81
KB6	2	2	3	3.3	0.28	5.1	80.0	7.00	48.00	6.61	6.80	6.80
KB6	2	1	4	3.0	0.42	5.0	79.0	6.00	48.00	7.28	7.91	7.93
KB6	2	2	4	3.0	0.41	5.0	79.0	6.00	48.00	7.23	8.05	7.99

Product	Replication	Duplicate	Fermentation time (days)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth: Yeast count log cfu/ml	Fermented broth: AAB count log cfu/ml	Fermented broth: Total count log cfu/ml
KB7	1	1	1	3.6	0.10	3.8	85.0	5.00	52.00	5.05	4.64	4.71
KB7	1	2	1	3.5	0.10	3.8	84.0	6.00	52.00	4.98	4.66	4.69
KB7	1	1	2	3.5	0.17	3.6	85.0	4.00	49.00	5.28	5.24	5.34
KB7	1	2	2	3.4	0.17	3.6	85.0	4.00	49.00	5.20	5.30	5.31
KB7	1	1	3	3.2	0.29	3.5	85.0	5.00	47.00	5.75	5.83	5.99
KB7	1	2	3	3.2	0.30	3.5	85.0	5.00	47.00	5.76	5.85	5.98
KB7	1	1	4	3.0	0.40	3.4	82.0	4.00	46.00	6.77	7.09	7.18
KB7	1	2	4	3.0	0.40	3.3	79.0	5.00	45.00	6.76	7.12	7.21
KB7	2	1	1	3.5	0.10	3.9	85.0	5.00	53.36	5.18	4.75	4.82
KB7	2	2	1	3.5	0.10	3.8	84.0	5.00	52.51	5.09	4.72	4.90
KB7	2	1	2	3.3	0.19	3.5	85.0	5.00	48.00	5.31	5.33	5.42
KB7	2	2	2	3.3	0.19	3.5	84.0	4.00	49.00	5.32	5.31	5.51
KB7	2	1	3	3.2	0.30	3.4	84.0	5.00	47.00	5.79	5.90	6.03
KB7	2	2	3	3.2	0.30	3.5	84.0	5.00	46.00	5.80	5.92	6.08
KB7	2	1	4	2.9	0.47	3.3	81.0	4.00	45.00	6.81	7.05	7.13
KB7	2	2	4	2.8	0.48	3.2	81.0	4.00	44.00	6.82	7.25	7.25
KB8	1	1	1	3.5	0.09	3.7	84.0	6.00	51.00	5.21	5.05	5.01
KB8	1	2	1	3.4	0.09	3.8	83.0	6.00	51.00	5.23	5.09	5.06
KB8	1	1	2	3.3	0.21	3.5	85.0	4.00	49.00	6.12	6.22	6.21
KB8	1	2	2	3.3	0.21	3.4	85.0	5.00	49.00	6.18	6.20	6.19
KB8	1	1	3	3.1	0.31	3.0	85.0	5.00	47.00	6.25	6.67	6.60
KB8	1	2	3	3.1	0.31	3.0	84.0	5.00	47.00	6.21	6.65	6.60
KB8	1	1	4	3.1	0.49	2.7	75.0	5.00	44.00	6.79	7.30	7.56
KB8	1	2	4	2.7	0.49	2.6	68.0	5.00	42.00	6.78	7.31	7.53
KB8	2	1	1	3.3	0.15	3.6	84.1	6.00	52.67	5.29	5.11	5.01
KB8	2	2	1	3.3	0.14	3.8	84.1	7.00	52.64	5.31	5.10	5.06
KB8	2	1	2	3.2	0.22	3.5	83.0	5.00	50.00	6.19	6.29	6.21
KB8	2	2	2	3.2	0.23	3.3	83.0	5.00	50.00	6.15	6.24	6.19
KB8	2	1	3	3.1	0.33	3.1	84.0	4.00	46.00	6.31	6.71	6.63
KB8	2	2	3	3.1	0.35	3.1	83.0	4.00	47.00	6.29	6.70	6.61
KB8	2	1	4	2.5	0.51	2.7	76.0	5.00	43.00	6.77	7.48	7.56
KB8	2	2	4	2.6	0.53	2.6	70.0	5.00	43.00	6.76	7.41	7.53

Note: KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C; Fermentation time: 1 = Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9.

Table C.6 Microbial counts of cellulose pellicle during fermentation of Kombucha for 9 days in Stage 1 of Phase 2

	Yeast count log cfu/mL				AAB count log cfu/mL				Total count log cfu/mL			
Product	Day 0		Day 14		Day 0		Day 14		Day 0		Day 14	
KB5	6.01	6.08	6.19	6.17	7.68	7.69	8.01	8.08	7.72	7.75	8.06	8.1
KB6	6.31	6.33	6.27	6.23	7.96	8.03	8.33	8.37	8.16	8.19	8.44	8.46
KB7	6.43	6.47	6.41	6.47	8.01	8.05	8.29	8.31	7.59	7.52	8.11	8.18
KB8	6.65	6.67	6.51	6.58	8.22	8.27	8.42	8.49	8.28	8.21	8.32	8.35

Note: KB5 contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB6 contained 4.7% sugar,0.5% tea, 20% fermented broth and was fermented at 24°C; KB7 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C; KB8 contained 3% sugar, 0.5% tea, 20% fermented broth and was fermented at 24°C.

Table C.7 Characteristics of filtered and unfiltered Kombucha post-primary fermentation in Stage 2 of Phase 2

Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth Yeast count log cfu/ml	Fermented broth AAB count log cfu/ml	Fermented broth Total count log cfu/ml
KB5F	1	1	1	3.61	0.07	6.0	79.0	6.00	54.00	4.19	4.48	4.52
KB5F	1	2	1	3.61	0.07	6.0	78.0	5.90	53.00	4.23	4.49	4.61
KB5F	1	1	2	3.47	0.12	5.5	81.0	5.80	52.00	6.11	6.18	6.13
KB5F	1	2	2	3.48	0.11	5.6	80.0	5.70	51.00	6.09	6.10	6.04
KB5F	1	1	3	3.29	0.27	5.2	82.0	5.78	50.00	6.71	7.14	7.09
KB5F	1	2	3	3.29	0.28	5.3	82.0	5.50	50.00	6.64	7.01	7.06
KB5F	1	1	4	3.20	0.33	5.1	84.0	5.50	48.00	6.93	7.24	7.17
KB5F	1	2	4	3.20	0.34	5.0	84.0	5.50	48.00	6.84	7.18	7.10
KB5F	1	1	5	3.18	0.36	4.9	84.0	5.20	47.00	5.50	6.86	6.83
KB5F	1	2	5	3.17	0.35	4.9	83.0	5.20	46.00	5.59	6.89	6.78
KB5F	2	1	1	3.67	0.08	5.8	79.0	6.00	53.00	4.93	4.52	4.51
KB5F	2	2	1	3.66	0.09	5.8	78.0	5.90	54.00	4.98	4.51	4.53
KB5F	2	1	2	3.42	0.11	5.6	81.0	5.80	51.00	6.15	6.14	6.09
KB5F	2	2	2	3.43	0.13	5.6	82.0	5.70	5.00	6.13	6.12	6.17
KB5F	2	1	3	3.25	0.24	5.2	82.0	5.78	49.00	7.01	7.10	7.17
KB5F	2	2	3	3.26	0.24	5.1	83.0	5.50	48.00	7.04	7.13	7.19
KB5F	2	1	4	3.18	0.31	5.0	83.0	5.50	47.00	6.98	7.27	7.10
KB5F	2	2	4	3.19	0.31	5.0	85.0	5.50	47.00	6.81	7.21	7.24
KB5F	2	1	5	3.16	0.35	4.8	84.0	5.30	46.00	5.50	6.93	6.76
KB5F	2	2	5	3.17	0.35	4.9	83.0	5.20	45.00	5.49	6.95	6.74

Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB6F	1	1	1	3.59	0.07	5.9	79.0	6.00	54.00	4.32	4.33	4.42
KB6F	1	2	1	3.59	0.07	5.9	79.0	5.90	55.00	4.24	4.45	4.50
KB6F	1	1	2	3.36	0.11	5.4	80.0	5.80	52.00	6.31	6.30	6.32
KB6F	1	2	2	3.35	0.12	5.4	82.0	5.70	50.00	6.32	6.21	6.36
KB6F	1	1	3	3.21	0.30	5.1	83.0	5.78	51.00	6.56	6.84	6.79
KB6F	1	2	3	3.20	0.30	5.1	84.0	5.50	50.00	6.51	6.81	6.77
KB6F	1	1	4	3.10	0.34	4.8	85.0	5.50	50.00	7.16	7.89	7.85
KB6F	1	2	4	3.09	0.33	4.9	84.0	5.50	50.00	7.15	7.85	7.83
KB6F	1	1	5	3.00	0.41	4.7	84.0	5.10	49.00	6.13	7.21	6.87
KB6F	1	2	5	3.00	0.43	4.6	85.0	5.00	44.00	6.26	7.05	6.83
KB6F	2	1	1	3.61	0.08	5.8	79.0	6.00	54.00	4.40	4.52	4.33
KB6F	2	2	1	3.61	0.07	5.8	79.0	5.90	54.00	4.32	4.49	4.36
KB6F	2	1	2	3.40	0.15	5.5	82.0	5.80	5.00	6.36	6.24	6.41
KB6F	2	2	2	3.41	0.15	5.6	81.0	5.70	51.00	6.39	6.27	6.37
KB6F	2	1	3	3.26	0.31	5.1	83.0	5.78	49.00	6.59	6.75	6.81
KB6F	2	2	3	3.25	0.31	5.1	82.0	5.50	48.00	6.61	6.80	6.80
KB6F	2	1	4	3.11	0.39	5.0	84.0	5.50	48.00	7.28	7.91	7.93
KB6F	2	2	4	3.13	0.38	5.1	84.0	5.50	48.00	7.23	8.05	7.99
KB6F	2	1	5	3.01	0.43	4.6	84.0	5.00	45.00	6.29	7.09	6.85
KB6F	2	2	5	3.03	0.43	4.4	85.0	4.90	44.00	6.32	7.17	6.89

Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB5U	1	1	1	3.61	0.07	5.9	79.0	6.00	54.00	4.19	4.48	4.52
KB5U	1	2	1	3.61	0.07	6.0	78.0	5.90	53.00	4.23	4.49	4.61
KB5U	1	1	2	3.47	0.12	5.6	81.0	5.80	52.00	6.11	6.18	6.13
KB5U	1	2	2	3.48	0.11	5.5	80.0	5.70	51.00	6.09	6.10	6.04
KB5U	1	1	3	3.29	0.27	5.3	82.0	5.78	50.00	6.71	7.14	7.09
KB5U	1	2	3	3.29	0.28	5.2	82.0	5.50	50.00	6.64	7.01	7.06
KB5U	1	1	4	3.20	0.33	5.2	84.0	5.50	48.00	6.93	7.24	7.17
KB5U	1	2	4	3.20	0.34	5.0	84.0	5.50	48.00	6.84	7.18	7.10
KB5U	1	1	5	3.13	0.39	4.7	84.0	5.20	47.00	6.73	7.19	7.01
KB5U	1	2	5	3.13	0.40	4.7	83.0	5.20	46.00	6.79	7.21	6.91
KB5U	2	1	1	3.67	0.08	6.0	79.0	6.00	53.00	4.93	4.52	4.51
KB5U	2	2	1	3.66	0.09	5.9	78.0	5.90	54.00	4.98	4.51	4.53
KB5U	2	1	2	3.42	0.11	5.7	81.0	5.80	51.00	6.15	6.14	6.09
KB5U	2	2	2	3.43	0.13	5.7	82.0	5.70	5.00	6.13	6.12	6.17
KB5U	2	1	3	3.25	0.24	5.2	82.0	5.78	49.00	7.01	7.10	7.17
KB5U	2	2	3	3.26	0.24	5.3	83.0	5.50	48.00	7.04	7.13	7.19
KB5U	2	1	4	3.18	0.31	5.1	83.0	5.50	47.00	6.98	7.27	7.10
KB5U	2	2	4	3.19	0.31	5.0	85.0	5.50	47.00	6.81	7.21	7.24
KB5U	2	1	5	3.12	0.38	4.6	84.0	5.30	46.00	6.74	7.17	7.03
KB5U	2	2	5	3.11	0.38	4.7	83.0	5.20	45.00	6.76	7.13	7.01

										Fermented broth		
Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB6U	1	1	1	3.59	0.07	5.8	79.0	6.00	54.00	4.32	4.33	4.42
KB6U	1	2	1	3.59	0.07	5.9	79.0	5.90	55.00	4.24	4.45	4.50
KB6U	1	1	2	3.36	0.11	5.3	80.0	5.80	52.00	6.31	6.30	6.32
KB6U	1	2	2	3.35	0.12	5.4	82.0	5.70	50.00	6.32	6.21	6.36
KB6U	1	1	3	3.21	0.30	5.1	83.0	5.78	51.00	6.56	6.84	6.79
KB6U	1	2	3	3.20	0.30	5.0	84.0	5.50	50.00	6.51	6.81	6.77
KB6U	1	1	4	3.10	0.34	4.8	85.0	5.50	50.00	7.16	7.89	7.85
KB6U	1	2	4	3.09	0.33	4.9	84.0	5.50	50.00	7.15	7.85	7.83
KB6U	1	1	5	2.97	0.47	4.5	84.0	5.10	49.00	7.01	7.69	7.55
KB6U	1	2	5	2.95	0.47	4.3	85.0	5.00	44.00	7.05	7.67	7.54
KB6U	2	1	1	3.61	0.08	5.9	79.0	6.00	54.00	4.40	4.52	4.33
KB6U	2	2	1	3.61	0.07	5.8	79.0	5.90	54.00	4.32	4.49	4.36
KB6U	2	1	2	3.40	0.15	5.6	82.0	5.80	5.00	6.36	6.24	6.41
KB6U	2	2	2	3.41	0.15	5.6	81.0	5.70	51.00	6.39	6.27	6.37
KB6U	2	1	3	3.26	0.31	5.2	83.0	5.78	49.00	6.59	6.75	6.81
KB6U	2	2	3	3.25	0.31	5.1	82.0	5.50	48.00	6.61	6.80	6.80
KB6U	2	1	4	3.11	0.39	5.0	84.0	5.50	48.00	7.28	7.91	7.93
KB6U	2	2	4	3.13	0.38	5.0	84.0	5.50	48.00	7.23	8.05	7.99
KB6U	2	1	5	2.93	0.49	4.3	84.0	5.00	45.00	7.21	7.85	7.68
KB6U	2	2	5	2.93	0.48	4.5	85.0	4.90	44.00	7.19	7.77	7.63

Note: KB5F contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample) ; KB6F contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (filtered sample); KB6U contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 24℃ (unfiltered sample); F = filtered sample; U = unfiltered sample; Fermentation time : 1 = Day 0, 2 = Day 3, 3 = Day 6, 4 = Day, 5 = Week 1.

Table C.8 Consumer sensory analysis of filtered and unfiltered Kombucha post-primary fermentation in Stage 2 of Phase 2

Panellist	Product	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Product	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	5	7	7	6	8	8	KB6F	5	4	7	7	8	7
2	KB5F	5	5	7	7	7	7	KB6F	6	3	7	7	7	7
3	KB5F	8	7	7	7	6	6	KB6F	8	7	7	8	7	8
4	KB5F	8	8	9	7	7	9	KB6F	8	7	7	7	8	8
5	KB5F	7	5	6	6	5	6	KB6F	6	5	6	7	4	6
6	KB5F	6	7	6	5	7	7	KB6F	7	6	6	5	6	6
7	KB5F	8	8	8	7	8	9	KB6F	8	3	4	5	6	4
8	KB5F	8	8	8	9	8	9	KB6F	8	7	8	8	8	9
9	KB5F	6	5	5	6	5	6	KB6F	6	5	5	6	7	6
10	KB5F	5	4	5	5	5	5	KB6F	4	3	3	3	3	4
11	KB5F	7	6	5	5	6	6	KB6F	7	7	*	6	6	6
12	KB5F	7	5	7	6	6	7	KB6F	6	6	7	6	5	7
13	KB5F	7	7	7	7	7	7	KB6F	6	5	7	8	8	7
14	KB5F	8	7	8	8	8	8	KB6F	7	8	8	8	8	7
15	KB5F	6	7	7	7	7	8	KB6F	7	7	7	7	8	7
16	KB5F	5	4	6	6	4	6	KB6F	7	7	6	5	5	5
17	KB5F	5	5	5	5	5	6	KB6F	5	5	5	5	7	5
18	KB5F	6	7	7	7	8	7	KB6F	6	8	8	8	7	8
19	KB5F	9	5	9	8	7	8	KB6F	7	6	6	7	5	7
20	KB5F	7	7	7	6	7	7	KB6F	6	6	4	4	4	4
21	KB5F	5	4	4	6	7	6	KB6F	7	8	7	8	2	8
22	KB5F	8	5	8	8	5	8	KB6F	8	4	4	4	4	7
23	KB5F	4	6	7	8	5	8	KB6F	4	3	5	5	5	6
24	KB5F	5	6	6	7	5	6	KB6F	5	5	7	7	5	7
25	KB5F	5	8	8	8	8	8	KB6F	5	5	6	6	6	6
26	KB5F	7	5	7	6	6	7	KB6F	7	5	7	6	5	7
27	KB5F	8	5	7	8	8	8	KB6F	8	4	4	6	5	6
28	KB5F	6	6	5	7	6	7	KB6F	7	7	7	7	6	7
29	KB5F	8	8	7	7	9	7	KB6F	8	7	7	6	6	7
30	KB5F	6	8	8	8	8	8	KB6F	3	4	4	4	4	4
31	KB5F	7	5	5	5	6	6	KB6F	7	4	3	3	3	4
32	KB5F	6	4	4	5	5	5	KB6F	5	7	6	7	3	7
33	KB5F	7	4	4	5	5	5	KB6F	5	6	6	6	6	7
34	KB5F	7	8	7	4	4	6	KB6F	7	8	7	6	6	7
35	KB5F	6	5	5	5	5	6	KB6F	5	5	7	7	7	7

Panellist	Product	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Product	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	5	6	4	4	4	5	KB6U	5	5	5	5	5	5
2	KB5U	7	4	4	4	5	5	KB6U	5	5	5	5	5	4
3	KB5U	6	5	4	6	4	6	KB6U	6	6	3	4	3	4
4	KB5U	8	8	6	7	7	8	KB6U	9	8	9	7	8	9
5	KB5U	5	5	4	6	6	5	KB6U	5	4	6	6	5	5
6	KB5U	7	6	5	5	6	5	KB6U	5	3	4	5	4	4
7	KB5U	8	5	7	7	8	7	KB6U	6	4	2	5	5	4
8	KB5U	5	6	4	4	4	5	KB6U	4	8	6	4	2	5
9	KB5U	4	3	3	6	4	5	KB6U	6	5	6	6	6	6
10	KB5U	4	2	5	5	5	5	KB6U	5	3	3	4	4	4
11	KB5U	7	7	6	6	7	6	KB6U	7	6	5	5	5	5
12	KB5U	6	6	7	8	8	8	KB6U	6	6	4	4	3	4
13	KB5U	7	7	7	7	8	7	KB6U	8	6	8	8	8	8
14	KB5U	8	8	8	8	8	8	KB6U	6	7	8	8	8	7
15	KB5U	7	7	7	7	7	8	KB6U	7	7	7	7	7	7
16	KB5U	7	7	6	6	6	7	KB6U	7	7	6	5	5	6
17	KB5U	5	6	5	4	5	5	KB6U	5	5	5	3	2	4
18	KB5U	6	7	8	8	7	7	KB6U	7	8	7	7	8	7
19	KB5U	7	7	6	7	4	6	KB6U	7	8	8	7	7	8
20	KB5U	7	7	7	6	6	6	KB6U	7	7	8	8	8	8
21	KB5U	5	5	7	8	5	7	KB6U	5	2	2	2	8	3
22	KB5U	8	8	8	8	5	8	KB6U	8	4	4	5	4	7
23	KB5U	4	8	7	5	8	7	KB6U	4	2	3	5	4	4
24	KB5U	5	4	6	6	7	6	KB6U	6	6	4	4	5	4
25	KB5U	5	7	7	7	7	7	KB6U	5	5	8	8	8	8
26	KB5U	8	9	8	9	5	8	KB6U	7	7	7	6	6	7
27	KB5U	9	7	6	5	5	7	KB6U	8	8	4	5	6	6
28	KB5U	7	6	7	8	7	7	KB6U	7	8	7	7	6	6
29	KB5U	8	6	3	5	2	4	KB6U	8	6	4	5	3	5
30	KB5U	6	6	5	6	4	6	KB6U	6	5	6	5	6	6
31	KB5U	7	4	7	4	6	7	KB6U	6	5	6	6	6	6
32	KB5U	4	6	7	4	2	5	KB6U	7	6	7	7	5	7
33	KB5U	5	4	5	4	6	4	KB6U	5	4	7	7	7	7
34	KB5U	7	8	7	7	7	7	KB6U	7	8	7	8	8	7
35	KB5U	5	6	5	6	5	5	KB6U	5	5	4	5	4	4

Table C.9 Characteristics of filtered and unfiltered Kombucha during storage (4°C) for three weeks in Phase 3

Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB5F	1	1	1	3.63	0.09	6.1	78.0	6.10	55.00	4.19	4.48	4.52
KB5F	1	2	1	3.62	0.08	6.0	78.0	5.90	54.00	4.23	4.49	4.61
KB5F	1	1	2	3.49	0.10	5.5	81.0	5.80	53.00	6.11	6.18	6.13
KB5F	1	2	2	3.48	0.11	5.6	80.0	5.70	52.00	6.09	6.10	6.04
KB5F	1	1	3	3.30	0.27	5.2	82.0	5.78	50.00	6.71	7.14	7.09
KB5F	1	2	3	3.28	0.29	5.3	82.0	5.50	51.00	6.64	7.01	7.06
KB5F	1	1	4	3.22	0.33	5.1	84.0	5.50	48.00	6.93	7.24	7.17
KB5F	1	2	4	3.22	0.34	5.0	84.0	5.50	48.00	6.84	7.18	7.10
KB5F	1	1	5	3.17	0.36	4.7	84.0	5.20	47.00	5.50	6.86	6.83
KB5F	1	2	5	3.17	0.35	4.7	83.0	5.20	46.00	5.59	6.89	6.78
KB5F	1	1	6	3.14	0.36	4.5	83.0	5.19	44.00	5.57	6.73	6.67
KB5F	1	2	6	3.15	0.37	4.5	84.0	5.18	43.00	5.56	6.71	6.73
KB5F	1	1	7	3.13	0.38	4.3	84.0	5.17	43.00	5.50	6.69	6.51
KB5F	1	2	7	3.11	0.38	4.4	84.0	5.18	43.00	5.51	6.62	6.54
KB5F	2	1	1	3.67	0.07	5.9	79.0	6.00	55.00	4.93	4.52	4.51
KB5F	2	2	1	3.68	0.08	6.0	78.0	5.90	54.00	4.98	4.51	4.53
KB5F	2	1	2	3.41	0.11	5.6	81.0	5.80	53.00	6.15	6.14	6.09
KB5F	2	2	2	3.43	0.12	5.7	82.0	5.70	52.00	6.13	6.12	6.17
KB5F	2	1	3	3.25	0.22	5.2	82.0	5.78	49.00	7.01	7.10	7.17
KB5F	2	2	3	3.26	0.23	5.1	83.0	5.50	48.00	7.04	7.13	7.19
KB5F	2	1	4	3.18	0.31	5.1	83.0	5.50	47.00	6.98	7.27	7.10
KB5F	2	2	4	3.18	0.30	5.0	85.0	5.50	47.00	6.81	7.21	7.24
KB5F	2	1	5	3.16	0.35	4.6	84.0	5.30	46.00	5.50	6.93	6.76
KB5F	2	2	5	3.17	0.34	4.7	83.0	5.20	45.00	5.49	6.95	6.74
KB5F	2	1	6	3.14	0.35	4.5	83.5	5.18	44.00	5.63	6.75	6.69
KB5F	2	2	6	3.14	0.35	4.5	83.0	5.19	44.00	5.60	6.78	6.67
KB5F	2	1	7	3.13	0.38	4.3	83.0	5.18	42.00	5.45	6.71	6.47
KB5F	2	2	7	3.10	0.39	4.3	83.0	5.18	43.00	5.48	6.70	6.43

Product	Replication	Duplicate	Fermentation time (day)	pH	TA %	TSS °Brix	Colour(L)	Colour(a)	Colour(b)	Fermented broth Yeast count log cfu/ml	AAB count log cfu/ml	Total count log cfu/ml
KB5U	1	1	1	3.60	0.07	5.8	79.0	6.00	56.00	4.19	4.48	4.52
KB5U	1	2	1	3.61	0.09	6.0	78.0	5.90	55.00	4.23	4.49	4.61
KB5U	1	1	2	3.49	0.13	5.5	81.0	5.80	52.00	6.11	6.18	6.13
KB5U	1	2	2	3.48	0.12	5.5	80.0	5.70	51.00	6.09	6.10	6.04
KB5U	1	1	3	3.27	0.29	5.3	82.0	5.78	50.00	6.71	7.14	7.09
KB5U	1	2	3	3.28	0.28	5.2	82.0	5.50	50.00	6.64	7.01	7.06
KB5U	1	1	4	3.20	0.33	5.0	84.0	5.50	48.00	6.93	7.24	7.17
KB5U	1	2	4	3.21	0.34	5.0	84.0	5.50	48.00	6.84	7.18	7.10
KB5U	1	1	5	3.13	0.39	4.9	84.0	5.20	47.00	6.73	7.19	7.01
KB5U	1	2	5	3.14	0.40	4.9	83.0	5.20	46.00	6.79	7.21	6.91
KB5U	1	1	6	3.11	0.43	4.8	84.0	5.19	45.00	6.45	7.01	6.73
KB5U	1	2	6	3.10	0.44	4.8	85.0	5.18	45.00	6.43	7.09	6.82
KB5U	1	1	7	3.08	0.44	4.8	85.0	5.16	43.00	5.01	6.12	6.08
KB5U	1	2	7	3.07	0.45	4.7	84.0	5.17	42.00	5.09	6.13	6.13
KB5U	2	1	1	3.65	0.09	5.8	79.0	6.00	57.00	4.93	4.52	4.51
KB5U	2	2	1	3.66	0.09	5.9	78.0	5.90	56.00	4.98	4.51	4.53
KB5U	2	1	2	3.41	0.10	5.8	81.0	5.80	53.00	6.15	6.14	6.09
KB5U	2	2	2	3.43	0.12	5.7	82.0	5.70	52.00	6.13	6.12	6.17
KB5U	2	1	3	3.25	0.24	5.2	82.0	5.78	49.00	7.01	6.05	5.94
KB5U	2	2	3	3.26	0.23	5.3	83.0	5.50	48.00	7.04	6.03	5.98
KB5U	2	1	4	3.20	0.31	5.0	83.0	5.50	47.00	6.98	7.27	7.10
KB5U	2	2	4	3.21	0.30	5.0	85.0	5.50	47.00	6.81	7.21	7.24
KB5U	2	1	5	3.13	0.38	4.8	84.0	5.30	46.00	6.74	7.17	7.03
KB5U	2	2	5	3.14	0.37	4.9	83.0	5.20	45.00	6.76	7.13	7.01
KB5U	2	1	6	3.11	0.40	4.7	85.0	5.19	43.00	6.52	6.96	6.85
KB5U	2	2	6	3.10	0.42	4.9	85.0	5.18	42.00	6.58	6.83	6.89
KB5U	2	1	7	3.08	0.45	4.7	86.0	5.17	41.00	5.06	6.21	5.80
KB5U	2	2	7	3.07	0.44	4.6	84.0	5.16	42.00	5.08	6.26	5.84

Note: KB5F contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (filtered sample); KB5U contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (unfiltered sample); F = filtered sample; U = unfiltered sample; Fermentation time : 1 = Day 0, 2 = Day 3, 3 = Day 6, 4 = Day, 5 = Week 1, 6 = Week 2, 7= Week 3.

Table C.10 Consumer sensory analysis of filtered and unfiltered Kombucha during storage (4°C) for three weeks in Phase 3

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	1	1	7	6	7	8	8	8
2	KB5F	1	1	7	7	7	7	7	7
3	KB5F	1	1	5	4	7	6	*	9
4	KB5F	1	1	5	4	6	6	6	6
5	KB5F	1	1	8	8	8	7	7	8
6	KB5F	1	1	5	7	7	8	7	7
7	KB5F	1	1	5	4	6	6	4	6
8	KB5F	1	1	8	8	8	9	8	9
9	KB5F	1	1	7	5	6	6	5	7
10	KB5F	1	1	7	6	7	7	5	7
11	KB5F	1	1	5	6	7	8	8	8
12	KB5F	1	1	9	3	6	6	6	5
13	KB5F	1	1	7	5	8	8	5	8
14	KB5F	1	1	7	7	8	8	8	8
15	KB5F	1	1	5	3	6	6	4	7
16	KB5F	1	1	5	6	7	8	7	7
17	KB5F	1	1	8	7	8	8	8	8
18	KB5F	1	1	4	6	4	4	6	6
19	KB5F	1	1	9	9	9	9	9	9
20	KB5F	1	1	7	5	6	6	3	6
21	KB5F	1	1	8	9	8	8	6	8
22	KB5F	1	1	5	6	3	3	5	5
23	KB5F	1	1	5	5	7	7	6	8
24	KB5F	1	1	6	6	6	6	6	6
25	KB5F	1	1	7	6	6	6	6	6
26	KB5F	1	1	5	6	6	5	6	6
27	KB5F	1	1	8	7	6	8	8	7
28	KB5F	1	1	6	4	8	8	8	8
29	KB5F	1	1	8	7	6	7	7	7
30	KB5F	1	1	7	5	6	5	5	7
31	KB5F	1	1	9	8	9	9	9	9
32	KB5F	1	1	7	7	8	8	7	9
33	KB5F	1	1	7	6	7	7	7	7
34	KB5F	1	1	9	9	7	7	7	8
35	KB5F	1	1	9	7	9	9	9	9
36	KB5F	1	1	6	6	6	6	6	6
37	KB5F	1	1	6	5	6	6	7	7

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	1	2	5	6	7	8	7	7
2	KB5F	1	2	7	7	7	7	7	8
3	KB5F	1	2	5	4	6	6	6	6
4	KB5F	1	2	6	3	5	6	6	6
5	KB5F	1	2	8	5	6	6	6	7
6	KB5F	1	2	5	8	7	7	7	7
7	KB5F	1	2	5	5	4	5	4	4
8	KB5F	1	2	8	8	8	9	8	9
9	KB5F	1	2	5	5	6	6	4	7
10	KB5F	1	2	7	6	6	7	5	7
11	KB5F	1	2	5	3	4	6	6	5
12	KB5F	1	2	9	6	6	6	6	6
13	KB5F	1	2	7	8	7	7	5	7
14	KB5F	1	2	7	8	8	8	7	8
15	KB5F	1	2	5	6	6	6	5	7
16	KB5F	1	2	6	6	8	8	8	8
17	KB5F	1	2	8	9	6	6	5	7
18	KB5F	1	2	6	7	5	7	7	7
19	KB5F	1	2	9	8	7	7	7	8
20	KB5F	1	2	7	5	5	6	3	5
21	KB5F	1	2	7	5	7	7	7	8
22	KB5F	1	2	6	5	7	5	7	7
23	KB5F	1	2	5	7	6	5	4	5
24	KB5F	1	2	6	6	6	6	6	6
25	KB5F	1	2	5	6	5	5	4	5
26	KB5F	1	2	6	6	7	5	6	7
27	KB5F	1	2	5	5	5	5	5	5
28	KB5F	1	2	5	7	5	7	7	7
29	KB5F	1	2	7	8	8	8	8	8
30	KB5F	1	2	5	5	6	5	4	5
31	KB5F	1	2	7	7	7	7	7	7
32	KB5F	1	2	7	6	8	8	8	8
33	KB5F	1	2	6	7	7	7	7	7
34	KB5F	1	2	9	9	9	9	9	9
35	KB5F	1	2	8	9	6	6	5	7
36	KB5F	1	2	5	5	7	7	6	7
37	KB5F	1	2	7	7	6	7	7	7

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	2	1	8	3	8	8	7	7
2	KB5F	2	1	5	5	7	8	5	7
3	KB5F	2	1	5	6	7	7	6	7
4	KB5F	2	1	7	6	7	8	5	7
5	KB5F	2	1	6	5	8	7	6	8
6	KB5F	2	1	5	2	7	8	5	7
7	KB5F	2	1	7	7	8	8	8	8
8	KB5F	2	1	5	6	6	7	5	6
9	KB5F	2	1	7	5	8	8	5	8
10	KB5F	2	1	5	4	6	6	6	6
11	KB5F	2	1	7	8	9	9	8	9
12	KB5F	2	1	8	6	*	5	5	5
13	KB5F	2	1	9	7	9	9	9	9
14	KB5F	2	1	5	5	7	7	7	7
15	KB5F	2	1	8	8	8	7	8	9
16	KB5F	2	1	6	6	6	6	6	6
17	KB5F	2	1	8	7	9	9	9	9
18	KB5F	2	1	5	6	6	6	6	6
19	KB5F	2	1	7	8	9	8	9	9
20	KB5F	2	1	5	4	8	7	8	8
21	KB5F	2	1	5	6	8	6	6	8
22	KB5F	2	1	5	3	8	6	7	8
23	KB5F	2	1	8	5	8	8	8	8
24	KB5F	2	1	8	6	8	8	6	8
25	KB5F	2	1	5	4	4	7	4	5
26	KB5F	2	1	5	5	6	8	8	7
27	KB5F	2	1	7	5	4	6	6	6
28	KB5F	2	1	7	6	8	8	7	8
29	KB5F	2	1	6	7	8	7	7	7
30	KB5F	2	1	7	7	8	8	8	8
31	KB5F	2	1	5	7	6	6	6	6
32	KB5F	2	1	8	9	5	6	5	6
33	KB5F	2	1	7	4	6	6	5	5
34	KB5F	2	1	8	8	8	7	8	9
35	KB5F	2	1	5	5	7	7	5	6

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	2	2	7	5	7	6	6	7
2	KB5F	2	2	4	5	4	7	5	6
3	KB5F	2	2	5	7	8	8	7	8
4	KB5F	2	2	7	5	5	5	5	7
5	KB5F	2	2	8	5	7	7	8	8
6	KB5F	2	2	5	5	6	6	6	6
7	KB5F	2	2	5	4	6	7	4	5
8	KB5F	2	2	6	6	7	7	5	6
9	KB5F	2	2	8	5	8	8	5	9
10	KB5F	2	2	5	6	7	7	7	7
11	KB5F	2	2	8	8	9	9	8	9
12	KB5F	2	2	6	4	8	8	8	8
13	KB5F	2	2	8	5	8	8	8	9
14	KB5F	2	2	5	6	7	6	6	6
15	KB5F	2	2	9	9	9	9	9	9
16	KB5F	2	2	7	7	7	7	7	7
17	KB5F	2	2	8	9	6	8	5	7
18	KB5F	2	2	5	7	7	7	7	7
19	KB5F	2	2	8	8	7	7	7	7
20	KB5F	2	2	5	8	8	7	8	8
21	KB5F	2	2	5	6	7	6	5	7
22	KB5F	2	2	5	5	7	5	5	5
23	KB5F	2	2	7	6	7	6	6	6
24	KB5F	2	2	5	8	7	8	6	7
25	KB5F	2	2	5	5	7	7	5	6
26	KB5F	2	2	4	4	8	8	8	7
27	KB5F	2	2	6	5	4	3	6	6
28	KB5F	2	2	7	7	8	8	5	7
29	KB5F	2	2	6	5	5	6	7	6
30	KB5F	2	2	8	8	8	8	7	8
31	KB5F	2	2	6	7	7	7	7	7
32	KB5F	2	2	7	8	8	8	8	8
33	KB5F	2	2	6	4	8	8	8	8
34	KB5F	2	2	8	8	7	8	8	9
35	KB5F	2	2	5	6	8	7	5	8

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	3	1	8	5	5	5	6	9
2	KB5F	3	1	9	9	9	9	9	9
3	KB5F	3	1	7	7	7	7	7	7
4	KB5F	3	1	8	8	8	7	8	8
5	KB5F	3	1	5	5	6	7	7	7
6	KB5F	3	1	5	4	7	9	9	7
7	KB5F	3	1	7	8	8	8	7	8
8	KB5F	3	1	7	6	8	8	8	8
9	KB5F	3	1	5	7	6	9	7	8
10	KB5F	3	1	9	7	6	6	6	8
11	KB5F	3	1	8	5	8	8	5	8
12	KB5F	3	1	7	6	7	7	8	9
13	KB5F	3	1	5	5	6	7	7	6
14	KB5F	3	1	5	7	7	8	5	7
15	KB5F	3	1	5	5	7	5	5	7
16	KB5F	3	1	3	6	7	6	8	7
17	KB5F	3	1	6	6	7	7	6	7
18	KB5F	3	1	7	5	8	9	6	7
19	KB5F	3	1	5	6	7	6	7	7
20	KB5F	3	1	8	8	6	8	7	8
21	KB5F	3	1	8	8	8	9	9	9
22	KB5F	3	1	8	5	8	8	8	8
23	KB5F	3	1	7	4	6	6	6	7
24	KB5F	3	1	9	5	7	5	7	8
25	KB5F	3	1	5	6	7	7	7	7
26	KB5F	3	1	7	6	7	7	8	8
27	KB5F	3	1	5	7	6	8	8	7
28	KB5F	3	1	7	8	6	6	7	7
29	KB5F	3	1	6	7	8	6	6	6
30	KB5F	3	1	7	4	7	6	5	6
31	KB5F	3	1	6	6	7	7	5	6
32	KB5F	3	1	7	7	6	5	8	6
33	KB5F	3	1	6	7	7	6	6	7
34	KB5F	3	1	7	6	6	*	*	5
35	KB5F	3	1	5	5	6	6	5	6
36	KB5F	3	1	7	7	7	7	7	7

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5F	3	2	3	5	6	5	5	5
2	KB5F	3	2	8	7	9	9	9	9
3	KB5F	3	2	6	7	6	6	6	6
4	KB5F	3	2	8	8	8	8	8	8
5	KB5F	3	2	5	9	9	9	9	9
6	KB5F	3	2	5	*	9	9	9	9
7	KB5F	3	2	6	5	5	6	7	5
8	KB5F	3	2	7	6	6	6	6	6
9	KB5F	3	2	5	7	9	9	9	9
10	KB5F	3	2	8	5	6	6	6	7
11	KB5F	3	2	7	8	8	8	9	8
12	KB5F	3	2	8	7	6	6	6	5
13	KB5F	3	2	6	6	7	6	6	7
14	KB5F	3	2	5	8	9	9	8	9
15	KB5F	3	2	5	6	7	5	6	6
16	KB5F	3	2	6	3	7	7	8	7
17	KB5F	3	2	6	6	5	7	6	6
18	KB5F	3	2	8	5	6	5	5	7
19	KB5F	3	2	5	7	8	7	8	8
20	KB5F	3	2	8	7	6	7	6	7
21	KB5F	3	2	8	8	8	8	8	8
22	KB5F	3	2	8	8	8	8	8	8
23	KB5F	3	2	7	6	8	7	8	8
24	KB5F	3	2	9	9	9	9	9	9
25	KB5F	3	2	5	5	7	6	6	6
26	KB5F	3	2	7	9	9	9	9	9
27	KB5F	3	2	5	5	5	5	6	6
28	KB5F	3	2	7	6	9	9	9	9
29	KB5F	3	2	6	5	7	7	7	6
30	KB5F	3	2	7	8	7	8	7	8
31	KB5F	3	2	*	5	7	7	5	7
32	KB5F	3	2	8	7	8	8	5	7
33	KB5F	3	2	5	6	6	7	5	6
34	KB5F	3	2	7	8	8	8	8	8
35	KB5F	3	2	5	5	6	7	5	7
36	KB5F	3	2	6	7	6	6	6	6

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	1	1	5	5	7	8	8	8
2	KB5U	1	1	6	6	6	6	6	6
3	KB5U	1	1	5	5	6	6	6	6
4	KB5U	1	1	6	5	6	6	7	6
5	KB5U	1	1	8	5	7	7	6	8
6	KB5U	1	1	5	8	7	7	6	7
7	KB5U	1	1	5	5	4	5	3	3
8	KB5U	1	1	7	8	7	8	7	8
9	KB5U	1	1	7	5	7	7	6	6
10	KB5U	1	1	7	6	6	7	5	7
11	KB5U	1	1	5	5	4	4	4	4
12	KB5U	1	1	9	3	6	6	6	7
13	KB5U	1	1	7	6	8	7	5	8
14	KB5U	1	1	8	7	8	8	7	8
15	KB5U	1	1	5	3	7	7	5	7
16	KB5U	1	1	4	4	8	7	7	7
17	KB5U	1	1	8	6	7	8	7	7
18	KB5U	1	1	4	7	4	7	7	6
19	KB5U	1	1	9	8	7	7	7	8
20	KB5U	1	1	7	5	7	7	6	7
21	KB5U	1	1	7	5	8	7	5	7
22	KB5U	1	1	7	5	3	3	3	4
23	KB5U	1	1	5	5	4	4	2	5
24	KB5U	1	1	7	7	7	7	7	7
25	KB5U	1	1	7	4	4	6	4	4
26	KB5U	1	1	6	6	6	6	5	6
27	KB5U	1	1	8	4	2	4	2	4
28	KB5U	1	1	5	5	7	7	7	7
29	KB5U	1	1	8	8	8	8	8	8
30	KB5U	1	1	7	5	5	6	5	5
31	KB5U	1	1	7	8	7	8	8	8
32	KB5U	1	1	7	8	7	6	7	7
33	KB5U	1	1	6	6	7	7	7	7
34	KB5U	1	1	7	7	7	7	7	7
35	KB5U	1	1	8	6	7	8	7	7
36	KB5U	1	1	5	5	3	5	3	3
37	KB5U	1	1	5	3	5	4	4	3

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	1	2	5	4	7	8	8	8
2	KB5U	1	2	6	6	6	6	6	6
3	KB5U	1	2	5	5	6	6	6	7
4	KB5U	1	2	4	3	4	5	4	4
5	KB5U	1	2	8	7	4	5	5	5
6	KB5U	1	2	5	4	4	4	4	4
7	KB5U	1	2	5	5	2	5	2	2
8	KB5U	1	2	8	8	7	8	6	7
9	KB5U	1	2	7	5	7	7	5	8
10	KB5U	1	2	7	6	7	7	5	7
11	KB5U	1	2	5	5	3	4	4	4
12	KB5U	1	2	3	3	3	3	3	2
13	KB5U	1	2	7	5	4	4	4	4
14	KB5U	1	2	8	8	8	8	8	8
15	KB5U	1	2	5	4	6	6	4	6
16	KB5U	1	2	6	3	4	4	3	3
17	KB5U	1	2	9	7	6	7	7	8
18	KB5U	1	2	4	7	7	7	7	7
19	KB5U	1	2	8	4	4	4	4	6
20	KB5U	1	2	6	5	6	6	5	6
21	KB5U	1	2	8	8	8	6	6	7
22	KB5U	1	2	7	5	6	5	7	7
23	KB5U	1	2	5	5	4	5	3	5
24	KB5U	1	2	5	5	5	5	5	5
25	KB5U	1	2	5	4	4	6	4	4
26	KB5U	1	2	6	6	6	5	5	7
27	KB5U	1	2	7	6	4	4	3	4
28	KB5U	1	2	5	3	3	3	3	3
29	KB5U	1	2	7	6	6	5	5	5
30	KB5U	1	2	7	4	4	6	5	6
31	KB5U	1	2	8	8	8	7	7	8
32	KB5U	1	2	8	4	5	6	6	6
33	KB5U	1	2	6	6	7	7	7	7
34	KB5U	1	2	9	8	6	6	6	6
35	KB5U	1	2	9	7	6	7	7	8
36	KB5U	1	2	5	5	3	5	2	3
37	KB5U	1	2	6	6	6	6	6	6

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	2	1	8	8	6	6	3	6
2	KB5U	2	1	5	4	6	7	5	7
3	KB5U	2	1	5	4	3	3	3	3
4	KB5U	2	1	5	4	4	5	3	4
5	KB5U	2	1	2	2	6	6	6	6
6	KB5U	2	1	5	6	6	6	6	5
7	KB5U	2	1	5	6	5	4	4	4
8	KB5U	2	1	6	6	6	6	5	6
9	KB5U	2	1	7	2	6	6	5	7
10	KB5U	2	1	5	6	7	7	7	7
11	KB5U	2	1	7	8	8	7	8	8
12	KB5U	2	1	5	4	3	2	3	3
13	KB5U	2	1	9	5	6	6	7	8
14	KB5U	2	1	5	6	6	6	6	6
15	KB5U	2	1	8	8	7	9	8	9
16	KB5U	2	1	8	8	8	8	8	8
17	KB5U	2	1	8	7	7	8	7	7
18	KB5U	2	1	5	3	3	6	5	3
19	KB5U	2	1	8	8	8	8	7	7
20	KB5U	2	1	5	6	8	7	8	8
21	KB5U	2	1	7	4	5	4	5	6
22	KB5U	2	1	5	4	7	6	5	7
23	KB5U	2	1	7	6	8	7	6	6
24	KB5U	2	1	5	6	3	4	5	5
25	KB5U	2	1	5	5	5	7	3	5
26	KB5U	2	1	8	3	4	4	3	3
27	KB5U	2	1	3	4	5	5	4	5
28	KB5U	2	1	7	8	7	8	7	8
29	KB5U	2	1	6	7	7	7	6	6
30	KB5U	2	1	8	8	7	7	8	8
31	KB5U	2	1	6	7	5	5	6	5
32	KB5U	2	1	7	8	7	7	7	7
33	KB5U	2	1	6	6	7	7	6	7
34	KB5U	2	1	6	6	6	6	6	6
35	KB5U	2	1	5	3	5	5	5	5

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	2	2	7	3	4	5	3	4
2	KB5U	2	2	6	6	8	8	7	8
3	KB5U	2	2	5	4	4	4	3	4
4	KB5U	2	2	7	5	7	7	5	7
5	KB5U	2	2	8	7	8	9	8	9
6	KB5U	2	2	5	4	6	6	6	6
7	KB5U	2	2	5	3	3	3	3	4
8	KB5U	2	2	6	5	6	6	6	6
9	KB5U	2	2	7	3	7	7	4	7
10	KB5U	2	2	5	5	7	7	7	7
11	KB5U	2	2	7	7	7	7	6	7
12	KB5U	2	2	5	5	4	3	3	4
13	KB5U	2	2	8	6	6	7	6	8
14	KB5U	2	2	5	5	6	6	6	6
15	KB5U	2	2	8	8	7	8	8	8
16	KB5U	2	2	7	7	7	7	7	7
17	KB5U	2	2	8	7	8	8	8	8
18	KB5U	2	2	7	6	7	8	8	7
19	KB5U	2	2	8	8	9	9	9	9
20	KB5U	2	2	5	7	7	6	7	7
21	KB5U	2	2	5	4	6	6	4	4
22	KB5U	2	2	5	5	5	5	5	5
23	KB5U	2	2	4	6	6	4	7	8
24	KB5U	2	2	5	6	3	3	3	3
25	KB5U	2	2	5	5	5	7	2	5
26	KB5U	2	2	6	4	6	7	4	5
27	KB5U	2	2	3	4	4	4	4	4
28	KB5U	2	2	7	8	7	5	6	7
29	KB5U	2	2	6	5	7	6	8	7
30	KB5U	2	2	7	7	7	7	7	7
31	KB5U	2	2	6	5	7	7	7	7
32	KB5U	2	2	6	4	4	5	3	5
33	KB5U	2	2	6	4	4	5	3	4
34	KB5U	2	2	5	6	6	6	6	6
35	KB5U	2	2	5	6	4	4	4	3

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	3	1	8	6	5	5	3	4
2	KB5U	3	1	7	4	5	6	3	5
3	KB5U	3	1	6	7	7	7	8	7
4	KB5U	3	1	8	8	5	5	5	6
5	KB5U	3	1	5	4	3	3	3	6
6	KB5U	3	1	5	3	8	9	9	7
7	KB5U	3	1	6	5	5	4	3	5
8	KB5U	3	1	7	4	4	4	4	4
9	KB5U	3	1	5	7	7	8	9	8
10	KB5U	3	1	8	4	6	6	4	6
11	KB5U	3	1	8	5	8	8	8	8
12	KB5U	3	1	8	7	6	4	8	6
13	KB5U	3	1	5	2	3	5	6	4
14	KB5U	3	1	5	7	8	6	8	8
15	KB5U	3	1	6	5	6	6	6	6
16	KB5U	3	1	5	3	6	6	5	5
17	KB5U	3	1	6	6	5	6	6	6
18	KB5U	3	1	4	7	8	8	5	7
19	KB5U	3	1	4	6	7	6	8	6
20	KB5U	3	1	8	7	8	8	8	8
21	KB5U	3	1	7	7	4	6	6	6
22	KB5U	3	1	8	7	7	8	8	7
23	KB5U	3	1	7	5	6	8	7	7
24	KB5U	3	1	9	7	3	5	5	5
25	KB5U	3	1	4	5	4	4	5	6
26	KB5U	3	1	8	7	4	4	3	4
27	KB5U	3	1	5	7	8	7	7	7
28	KB5U	3	1	7	6	7	7	7	8
29	KB5U	3	1	7	5	8	6	5	6
30	KB5U	3	1	7	6	8	7	7	8
31	KB5U	3	1	6	4	4	6	4	4
32	KB5U	3	1	6	7	8	8	7	8
33	KB5U	3	1	7	8	7	6	8	8
34	KB5U	3	1	5	5	5	5	5	5
35	KB5U	3	1	5	3	2	7	3	4
36	KB5U	3	1	6	6	5	5	5	6

Panellist	Product	Week	Replication	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	KB5U	3	2	9	5	7	7	7	7
2	KB5U	3	2	4	5	4	4	3	3
3	KB5U	3	2	7	8	7	7	7	7
4	KB5U	3	2	8	8	8	8	7	8
5	KB5U	3	2	5	5	7	6	*	7
6	KB5U	3	2	5	2	6	4	4	4
7	KB5U	3	2	7	7	7	7	7	7
8	KB5U	3	2	3	6	6	7	7	6
9	KB5U	3	2	5	8	7	9	8	8
10	KB5U	3	2	8	4	6	6	6	8
11	KB5U	3	2	5	5	4	6	4	6
12	KB5U	3	2	8	7	3	3	4	3
13	KB5U	3	2	5	4	7	7	7	7
14	KB5U	3	2	8	9	9	8	8	9
15	KB5U	3	2	5	7	7	7	6	7
16	KB5U	3	2	5	3	6	7	6	5
17	KB5U	3	2	5	6	7	7	7	7
18	KB5U	3	2	7	4	5	4	4	5
19	KB5U	3	2	5	5	6	7	6	7
20	KB5U	3	2	8	8	9	8	9	8
21	KB5U	3	2	9	8	7	8	8	7
22	KB5U	3	2	8	8	7	8	4	6
23	KB5U	3	2	8	8	9	8	8	9
24	KB5U	3	2	8	5	9	9	9	9
25	KB5U	3	2	4	3	2	5	5	5
26	KB5U	3	2	7	4	7	7	7	7
27	KB5U	3	2	4	3	4	5	3	3
28	KB5U	3	2	8	5	7	7	6	7
29	KB5U	3	2	7	7	7	6	4	6
30	KB5U	3	2	6	6	5	5	5	6
31	KB5U	3	2	6	4	5	6	5	5
32	KB5U	3	2	6	8	5	3	7	6
33	KB5U	3	2	4	5	6	4	5	5
34	KB5U	3	2	4	4	4	4	4	4
35	KB5U	3	2	6	4	6	6	3	6
36	KB5U	3	2	6	6	5	5	5	6

Note: KB5F contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (filtered sample); KB5U contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (unfiltered sample); F = filtered sample; U = unfiltered sample.

D. Ethanol (GC)

D.1 Ethanol standard curve (GC)

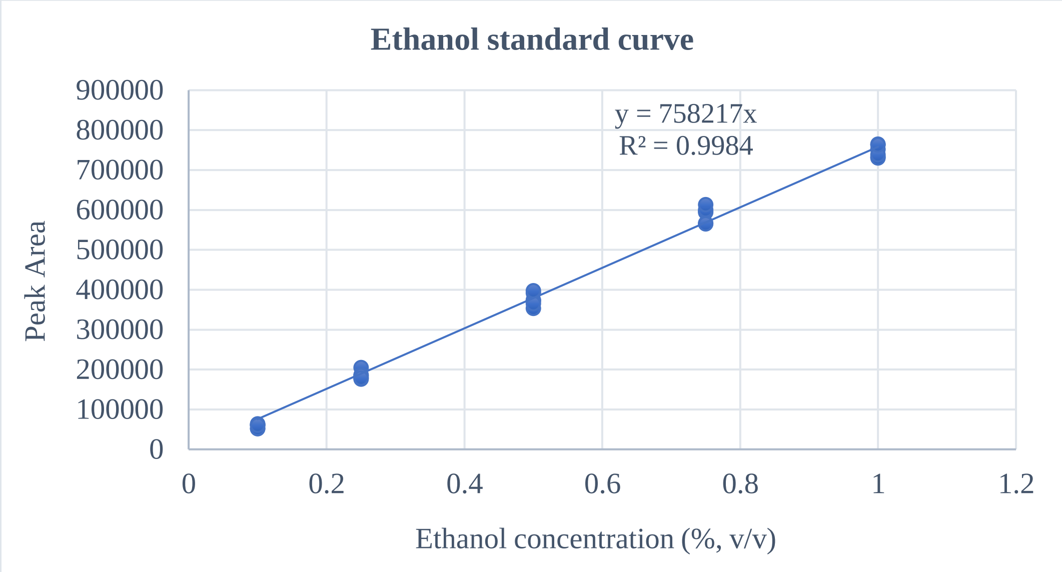


Table D.2 Ethanol standard peak area and retention time (GC)

Standard : Ethanol Mean retention time : 4 min

Concentration (%)	Peak area	Concentration (%)	Peak area
0.1	62264	0.5	392118
0.1	64231	0.5	365278
0.1	59721	0.5	397791
0.1	51629	0.75	594087
0.1	52818	0.75	596289
0.1	60428	0.75	600574
0.25	205027	0.75	613024
0.25	180983	0.75	566725
0.25	176531	0.75	564802
0.25	186629	1	752358
0.25	185297	1	733177
0.25	178570	1	764939
0.5	372621	1	730242
0.5	353043	1	738708
0.5	374588	1	763292

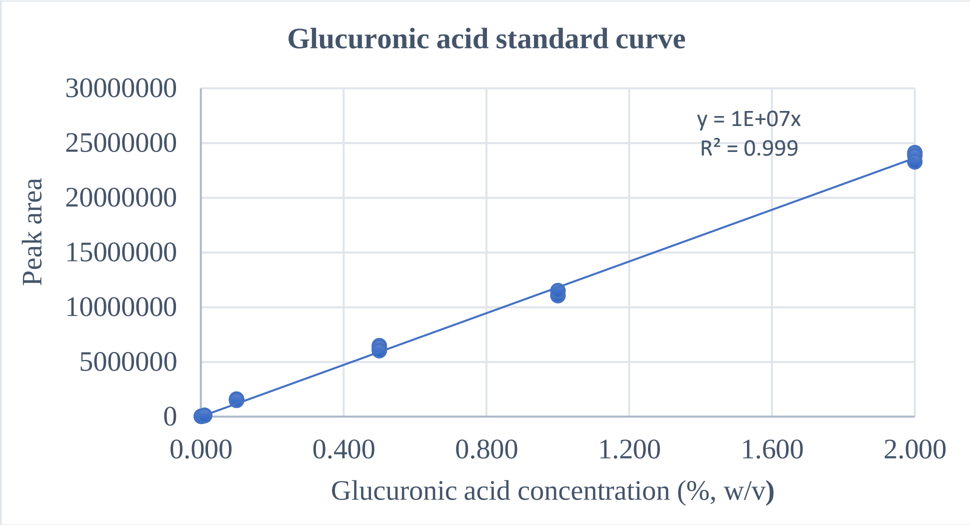
Table D.3 Ethanol concentration of filtered and unfiltered Kombucha during storage (4℃) for three weeks in Phase 3

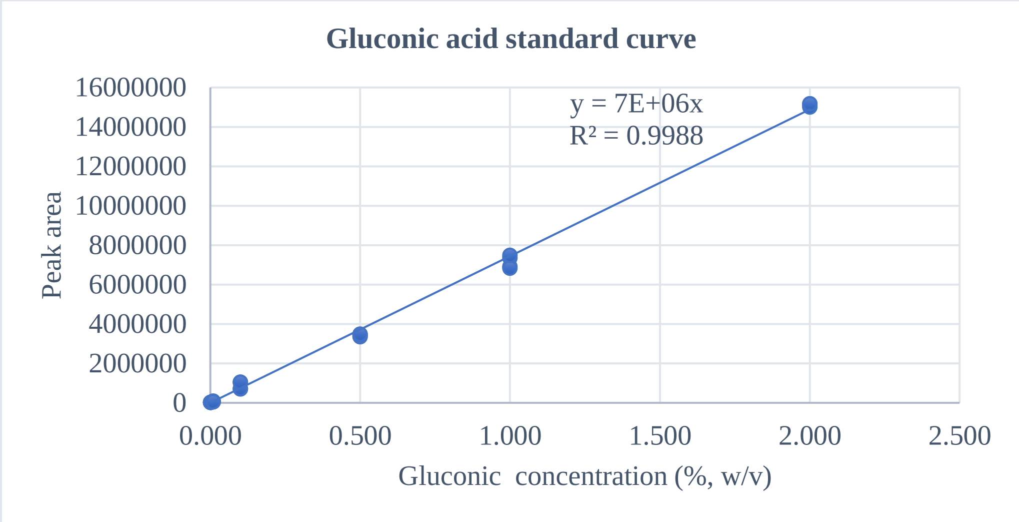
Product	Replication	Duplicate	Fermentation time	Peak area	Ethanol concentration (%v/v)
KB5F	1	1	1	38266	0.05
KB5F	1	2	1	38412	0.05
KB5F	1	1	2	51077	0.07
KB5F	1	2	2	37655	0.05
KB5F	1	1	3	48021	0.06
KB5F	1	2	3	66459	0.09
KB5F	1	1	4	135540	0.18
KB5F	1	2	4	216913	0.29
KB5F	1	1	5	119005	0.16
KB5F	1	2	5	138405	0.18
KB5F	1	1	6	365213	0.48
KB5F	1	2	6	353361	0.47
KB5F	1	1	7	251286	0.33
KB5F	1	2	7	210604	0.28
KB5F	2	1	1	38266	0.05
KB5F	2	2	1	38412	0.05
KB5F	2	1	2	51077	0.07
KB5F	2	2	2	37655	0.05
KB5F	2	1	3	48021	0.06
KB5F	2	2	3	66459	0.09
KB5F	2	1	4	135540	0.18
KB5F	2	2	4	216913	0.29
KB5F	2	1	5	175992	0.23
KB5F	2	2	5	197415	0.26
KB5F	2	1	6	147445	0.19
KB5F	2	2	6	163806	0.22
KB5F	2	1	7	155059	0.20
KB5F	2	2	7	275415	0.36
KB5U	1	1	1	38266	0.05
KB5U	1	2	1	38412	0.05
KB5U	1	1	2	51077	0.07
KB5U	1	2	2	37655	0.05
KB5U	1	1	3	48021	0.06
KB5U	1	2	3	66459	0.09
KB5U	1	1	4	135540	0.18
KB5U	1	2	4	216913	0.29
KB5U	1	1	5	190345	0.25
KB5U	1	2	5	232386	0.31
KB5U	1	1	6	329655	0.43
KB5U	1	2	6	173851	0.40
KB5U	1	1	7	314005	0.41
KB5U	1	2	7	433160	0.57
KB5U	2	1	1	38266	0.05
KB5U	2	2	1	38412	0.05
KB5U	2	1	2	51077	0.07
KB5U	2	2	2	37655	0.05
KB5U	2	1	3	48021	0.06
KB5U	2	2	3	66459	0.09
KB5U	2	1	4	135540	0.18
KB5U	2	2	4	216913	0.29
KB5U	2	1	5	264327	0.35
KB5U	2	2	5	230627	0.30
KB5U	2	1	6	142072	0.39
KB5U	2	2	6	164748	0.32
KB5U	2	1	7	484791	0.64
KB5U	2	2	7	365989	0.48

Note: KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Unfiltered sample); Fermentation time: 1= Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9, 5 = Week 1, 6 = Week 2, 7 = Week 3.

E. Organic acids (HPLC)

E.1 Organic acids standard curve (HPLC)





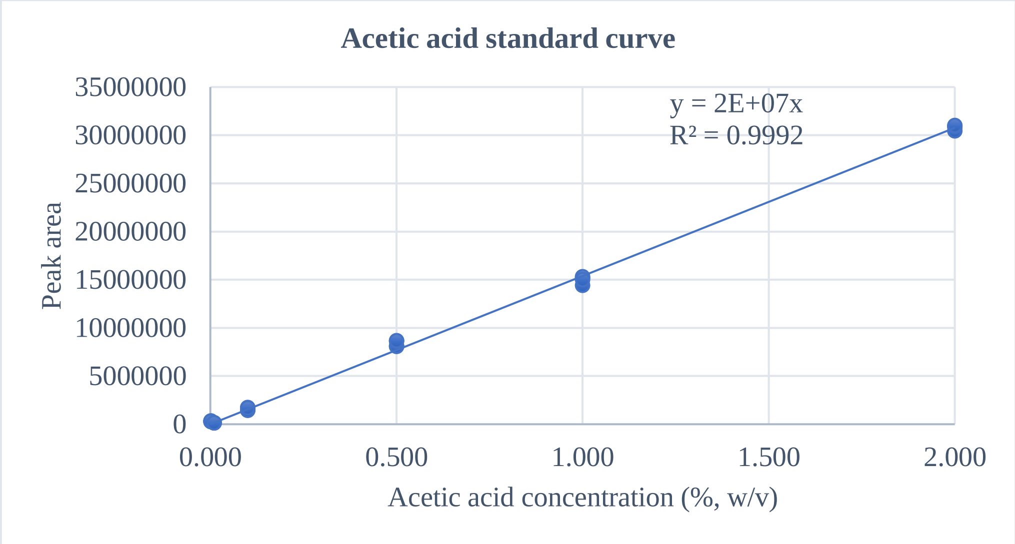


Table E.2 Organic acids standard peak area and retention time (HPLC)

Mean retention time (min)		
Glucuronic acid	Gluconic acid	Acetic acid
14.34	16.14	25.55

Glucuronic acid standard		Gluconic acid standard		Acetic acid standard	
Concentration (%, w/v)	Peak Area	Concentration (%, w/v)	Peak Area	Concentration (%,w/v)	Peak Area
0.001	7969	0.001	9706	0.001	326681
0.001	4955	0.001	9430	0.001	265026
0.001	8626	0.001	8650	0.001	259097
0.001	5232	0.001	10723	0.001	286751
0.010	107413	0.010	74410	0.010	140404
0.010	108105	0.010	48833	0.010	134256
0.010	92174	0.010	53812	0.010	149992
0.010	82996	0.010	53421	0.010	140022
0.1	1492090	0.1	701827	0.1	1454193
0.1	1595718	0.1	707583	0.1	1439421
0.1	1578271	0.1	1036291	0.1	1732471
0.1	1471583	0.1	1035809	0.1	1697844
0.5	6473347	0.5	3341693	0.5	8106468
0.5	6137408	0.5	3384799	0.5	8053121
0.5	6369240	0.5	3468605	0.5	8631713
0.5	6006337	0.5	3482482	0.5	8662290
1	11041804	1	6913086	1	14406498
1	11077997	1	7349238	1	15211270
1	11438547	1	6829157	1	15319272
1	11522705	1	7476564	1	15006704
2	23990602	2	15075383	2	30851502
2	24108474	2	15161087	2	30425321
2	23807800	2	15006220	2	30927567
2	23275250	2	15173467	2	30998849

Table E.3 Organic acid concentration of filtered and unfiltered Kombucha during storage during storage (4℃) for three weeks in Phase 3

Product	Replication	Duplicate	Fermentation time	Glucuronic acid		Gluconic acid		Acetic acid	
				Peak area	Conc (%, w/v)	Peak area	Conc (%,w/v)	Peak area	Conc (%, w/v)
KB5F	1	1	1	660736	0.049	40652	0.016	2148089	0.130
KB5F	1	2	1	658271	0.049	41208	0.016	1164174	0.065
KB5F	1	1	2	515233	0.037	55438	0.018	1920005	0.115
KB5F	1	2	2	546622	0.039	63647	0.019	1359103	0.078
KB5F	1	1	3	533702	0.038	98626	0.023	1442479	0.083
KB5F	1	2	3	537899	0.039	92802	0.023	1344786	0.080
KB5F	1	1	4	497851	0.035	167105	0.033	2323941	0.141
KB5F	1	2	4	449340	0.031	169770	0.033	2375222	0.144
KB5F	1	1	5	406582	0.027	207749	0.038	2373749	0.144
KB5F	1	2	5	449858	0.031	194157	0.036	2505275	0.153
KB5F	1	1	6	425155	0.029	189344	0.036	2702928	0.166
KB5F	1	2	6	389107	0.026	188529	0.035	2688741	0.165
KB5F	1	1	7	2955283	0.244	419235	0.066	1468530	0.085
KB5F	1	2	7	3035975	0.251	419174	0.066	1477959	0.086
KB5F	2	1	1	663191	0.049	40652	0.016	1086654	0.060
KB5F	2	2	1	732907	0.055	41208	0.016	925303	0.049
KB5F	2	1	2	640993	0.047	120711	0.026	1090208	0.060
KB5F	2	2	2	611854	0.045	106446	0.024	1058671	0.058
KB5F	2	1	3	459679	0.032	80918	0.021	1387962	0.080
KB5F	2	2	3	522337	0.037	87493	0.022	1334237	0.076
KB5F	2	1	4	452186	0.031	405484	0.064	2600037	0.159
KB5F	2	2	4	472599	0.033	375364	0.060	2514930	0.154
KB5F	2	1	5	452186	0.031	163264	0.032	2524550	0.154
KB5F	2	2	5	472599	0.033	187614	0.035	2649880	0.162
KB5F	2	1	6	2662326	0.219	443863	0.069	2662326	0.163
KB5F	2	2	6	1452074	0.116	173722	0.033	1452074	0.084
KB5F	2	1	7	2855283	0.236	408128	0.065	2855283	0.176
KB5F	2	2	7	2835975	0.234	407452	0.065	2835975	0.175
KB5U	1	1	1	660736	0.049	40652	0.016	2148089	0.130
KB5U	1	2	1	658271	0.049	41208	0.016	1164174	0.065
KB5U	1	1	2	515233	0.037	55438	0.018	1920005	0.115
KB5U	1	2	2	546622	0.039	63647	0.019	1359103	0.078
KB5U	1	1	3	533702	0.038	98626	0.023	1442479	0.083
KB5U	1	2	3	537899	0.039	92802	0.023	3244786	0.201
KB5U	1	1	4	467441	0.033	416575	0.066	3709926	0.232
KB5U	1	2	4	424212	0.029	390922	0.062	3645889	0.228
KB5U	1	1	5	467594	0.033	162441	0.032	2418860	0.147
KB5U	1	2	5	494802	0.035	188321	0.035	2278391	0.138

KB5U	1	1	6	371580	0.025	383678	0.061	3636494	0.227
KB5U	1	2	6	345259	0.022	378484	0.061	3616705	0.226
KB5U	1	1	7	317524	0.020	334526	0.055	2996770	0.185
KB5U	1	2	7	318625	0.020	338721	0.055	4791790	0.303
KB5U	2	1	1	663191	0.049	40652	0.016	1086654	0.060
KB5U	2	2	1	732907	0.055	41208	0.016	925303	0.049
KB5U	2	1	2	640993	0.047	120711	0.026	1090208	0.060
KB5U	2	2	2	611854	0.045	106446	0.024	1058671	0.058
KB5U	2	1	3	459679	0.032	80918	0.021	1387962	0.080
KB5U	2	2	3	522337	0.037	87493	0.022	1334237	0.076
KB5U	2	1	4	571402	0.042	165378	0.032	2296579	0.139
KB5U	2	2	4	621199	0.046	126877	0.027	2194633	0.133
KB5U	2	1	5	469389	0.033	150094	0.030	2281862	0.138
KB5U	2	2	5	482493	0.034	150685	0.030	2317277	0.141
KB5U	2	1	6	443318	0.031	167536	0.033	4645807	0.293
KB5U	2	2	6	515681	0.037	172889	0.033	3048607	0.189
KB5U	2	1	7	483896	0.034	157998	0.031	2548533	0.156
KB5U	2	2	7	528245	0.038	147120	0.030	2551350	0.156

Note: KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22℃ (Unfiltered sample); Fermentation time: 1= Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9, 5 = Week 1, 6 = Week 2, 7 = Week 3.

F. Sugars (GC)

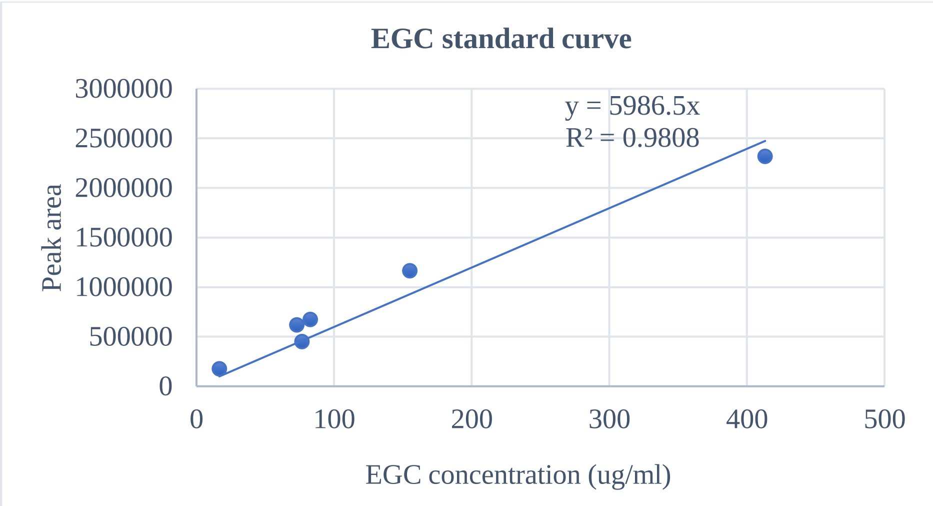
Table F.1 Sugar concentration of filtered and unfiltered Kombucha during storage (4°C) for three weeks in Phase 3

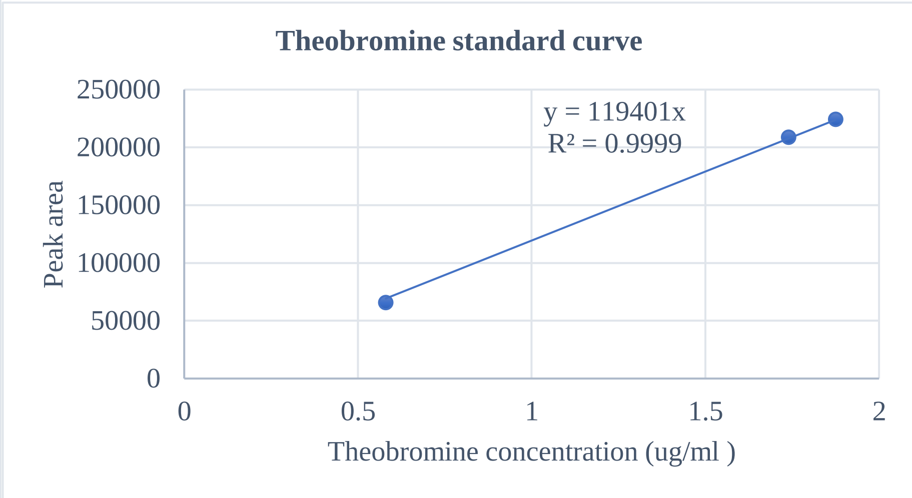
Product code	Replication	Fermentation time	Fructose (%.w/v)	Glucose (%.w/v)	Sucrose (%.w/v)
KB5F	1	1	0.30	0.30	5.50
KB5F	1	2	0.30	0.40	5.00
KB5F	1	3	0.40	0.40	4.60
KB5F	1	4	0.50	0.60	3.80
KB5F	1	5	0.40	0.50	4.00
KB5F	1	6	0.40	0.50	3.80
KB5F	1	7	0.80	1.00	2.50
KB5F	2	1	0.20	0.20	5.60
KB5F	2	2	0.50	0.50	4.60
KB5F	2	3	0.60	0.60	4.00
KB5F	2	4	0.50	0.60	3.70
KB5F	2	5	0.40	0.50	4.00
KB5F	2	6	0.40	0.50	3.90
KB5F	2	7	0.80	1.00	2.50
KB5U	1	1	0.30	0.30	5.50
KB5U	1	2	0.30	0.40	5.00
KB5U	1	3	0.40	0.40	4.60
KB5U	1	4	0.50	0.60	3.60
KB5U	1	5	0.40	0.50	4.00
KB5U	1	6	0.80	0.80	3.20
KB5U	1	7	0.80	0.80	3.10
KB5U	2	1	0.20	0.20	5.60
KB5U	2	2	0.50	0.50	4.60
KB5U	2	3	0.60	0.60	4.00
KB5U	2	4	0.30	0.50	4.00
KB5U	2	5	0.40	0.50	4.00
KB5U	2	6	0.40	0.60	3.70
KB5U	2	7	0.80	0.80	3.10

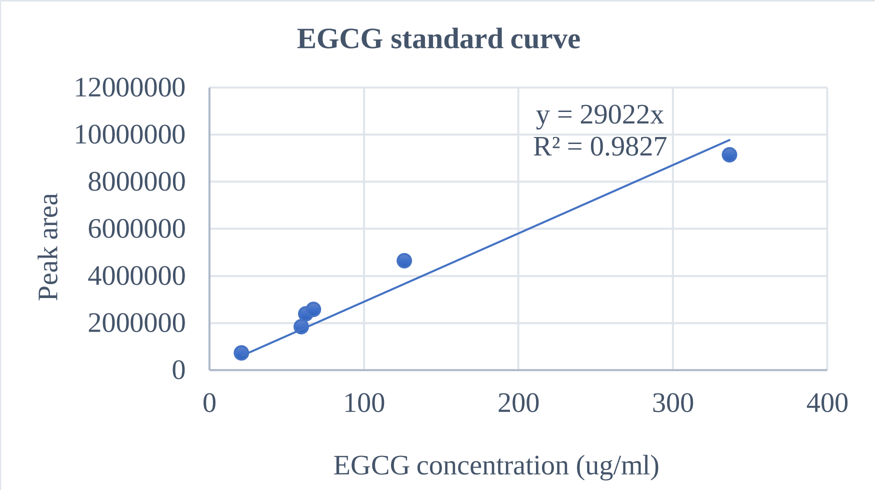
Note: KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (Unfiltered sample); Fermentation time: 1= Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9, 5 = Week 1, 6 = Week 2, 7 = Week 3.

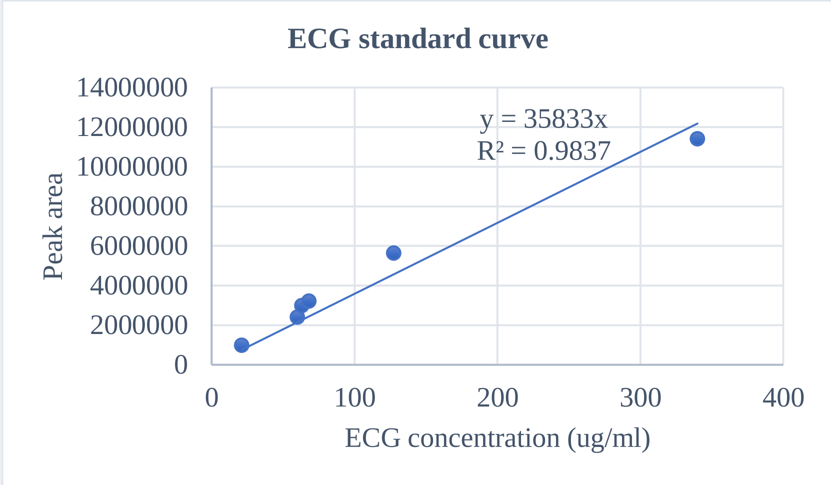
G Antioxidants (HPLC)

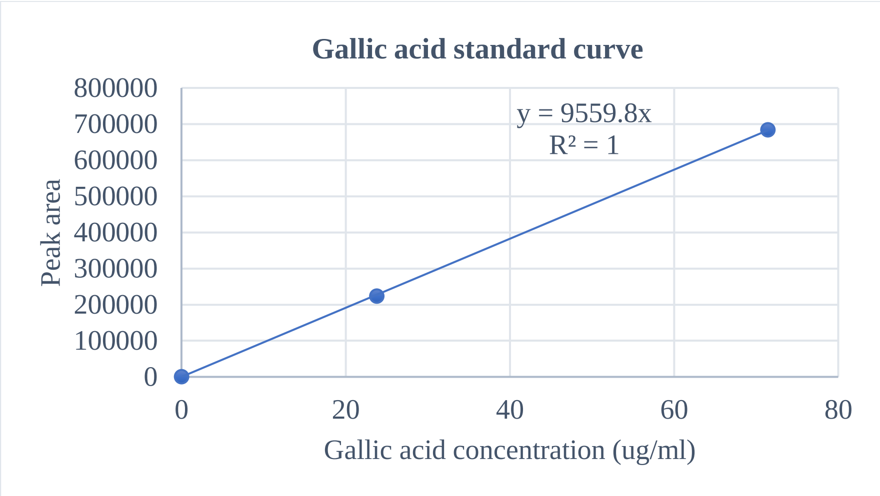
G.1 Antioxidants standard curve (HPLC)






EGCG standard curve
y = 29022x
R² = 0.9827
Peak area
12000000
10000000
8000000
6000000
4000000
2000000
0
0
100
200
300
400
EGCG concentration (ug/ml)


ECG standard curve
y = 35833x
R² = 0.9837
Peak area
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
100
200
300
400
ECG concentration (ug/ml)


Gallic acid standard curve
y = 9559.8x
R² = 1
Peak area
800000
700000
600000
500000
400000
300000
200000
100000
0
0
20
40
60
80
Gallic acid concentration (ug/ml)

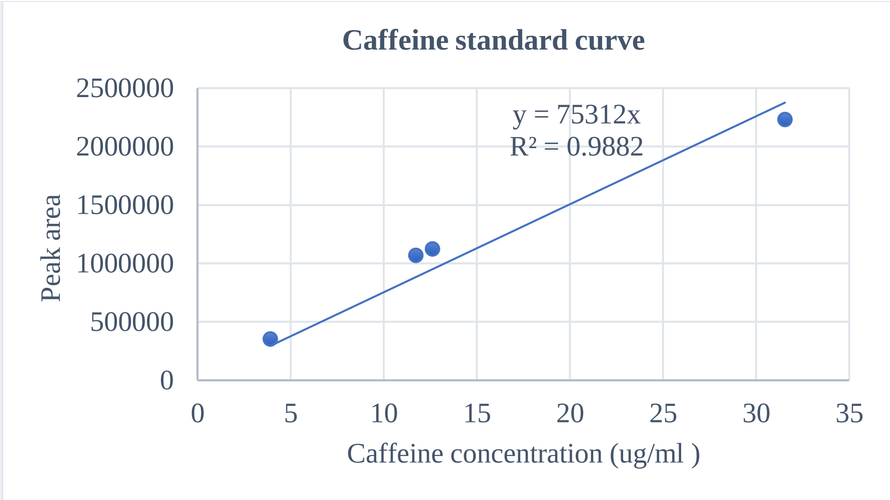

Caffeine standard curve
y = 75312x
R² = 0.9882
Peak area
2500000
2000000
1500000
1000000
500000
0
0
5
10
15
20
25
30
35
Caffeine concentration (ug/ml)

Table G.2 Antioxidant concentration of filtered and unfiltered Kombucha during storage (4°C) for three weeks in Phase 3

Product code	Replication	Duplicate	Fermentation time	Gallic acid		Theobromine		Caffeine	
				Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)
KB5F	1	1	1.0	1682161	175.96	817945	6.85	10580259	140.49
KB5F	1	2	1.0	1691810	176.97	856388	7.17	10563100	140.26
KB5F	1	1	2.0	1729100	180.87	525100	4.40	8751979	116.21
KB5F	1	2	2.0	1728558	180.82	475528	3.98	8711274	115.67
KB5F	1	1	3.0	1856313	194.18	800172	6.70	10230804	135.85
KB5F	1	2	3.0	1877955	196.44	755144	6.32	10286578	136.59
KB5F	1	1	4.0	1620869	169.55	803857	6.73	10345552	137.37
KB5F	1	2	4.0	1592360	166.57	804063	6.73	10333150	137.20
KB5F	1	1	5.0	1521800	159.19	776730	6.51	10095347	134.05
KB5F	1	2	5.0	1395782	146.01	480866	4.03	8402781	111.57
KB5F	2	1	1.0	1893009	198.02	883468	7.40	12253538	162.70
KB5F	2	2	1.0	1893434	198.06	947556	7.94	12351823	164.01
KB5F	2	1	2.0	1395536	145.98	464683	3.89	8492080	112.76
KB5F	2	2	2.0	1439776	150.61	462178	3.87	8507253	112.96
KB5F	2	1	3.0	1722453	180.18	96525	7.00	10247463	136.07
KB5F	2	2	3.0	1699748	177.80	97131	6.89	10229198	135.82
KB5F	2	1	4.0	1536146	160.69	539794	7.37	9300773	123.50
KB5F	2	2	4.0	1536692	160.75	118708	4.54	9310426	123.62
KB5F	2	1	5.0	1798575	188.14	143717	4.43	8983325	119.28
KB5F	2	2	5.0	1816388	190.00	526314	7.66	9031540	119.92
KB5U	1	1	1.0	817945	198.02	817945	7.40	10580259	162.70
KB5U	1	2	1.0	856388	198.06	856388	7.94	10563100	164.01
KB5U	1	1	2.0	1703589	178.20	485333	4.06	8833701	117.29
KB5U	1	2	2.0	1704882	178.34	602608	5.05	8799638	116.84
KB5U	1	1	3.0	1892762	178.20	836517	4.06	10418536	117.29

Product code	Replication	Duplicate	Fermentation time	Gallic acid		Theobromine		Caffeine	
				Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)
KB5U	1	2	3.0	1897452	178.34	777062	5.05	10404369	116.84
KB5U	1	1	4.0	2337489	244.51	993317	8.32	12472615	165.61
KB5U	1	2	4.0	2339042	244.67	925916	7.75	12479179	165.70
KB5U	1	1	5.0	2029048	212.25	851475	7.13	12614180	167.49
KB5U	1	2	5.0	1781265	186.33	865650	7.25	12559120	166.76
KB5U	2	1	1.0	817945	198.02	817945	7.40	12614180	162.70
KB5U	2	2	1.0	856388	198.06	856388	7.94	12559120	164.01
KB5U	2	1	2.0	1479785	154.79	491446	4.12	8805883	116.93
KB5U	2	2	2.0	1477310	154.53	552079	4.62	8782334	116.61
KB5U	2	1	3.0	1828324	191.25	904750	7.58	11275265	149.71
KB5U	2	2	3.0	1822518	190.64	913108	7.65	11325142	150.38
KB5U	2	1	4.0	2095210	219.17	674189	5.65	11267051	149.60
KB5U	2	2	4.0	2100528	219.73	664551	5.57	11293605	149.96
KB5U	2	1	5.0	1395782	146.01	819665	6.86	8402781	111.57
KB5U	2	2	5.0	1397101	146.14	826911	6.93	8364886	111.07

Product	Replication	Duplicate	Fermentation time	EGC		EGCG		ECG	
				Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)
KB5F	1	1	1.0	101878	17.02	477961	16.47	484691	13.53
KB5F	1	2	1.0	128126	21.40	454621	15.66	483384	13.49
KB5F	1	1	2.0	150964	25.22	491727	16.94	459385	12.82
KB5F	1	2	2.0	151437	25.30	505431	17.42	528750	14.76
KB5F	1	1	3.0	126282	21.09	445526	15.35	482114	13.45
KB5F	1	2	3.0	125578	20.98	454768	15.67	502911	14.03
KB5F	1	1	4.0	105654	17.65	291985	10.06	267815	7.47
KB5F	1	2	4.0	104415	17.44	301653	10.39	270079	7.54
KB5F	1	1	5.0	89813	15.00	186697	6.43	180228	5.03
KB5F	1	2	5.0	112787	18.84	197844	6.82	130665	3.65
KB5F	2	1	1.0	151010	25.23	547468	18.86	575329	16.06
KB5F	2	2	1.0	136122	22.74	559065	19.26	767755	21.43
KB5F	2	1	2.0	108850	18.18	141537	4.88	75419	2.10
KB5F	2	2	2.0	110439	18.45	145801	5.02	77683	2.17
KB5F	2	1	3.0	96525	16.12	185910	6.41	124295	3.47
KB5F	2	2	3.0	97131	16.23	187388	6.46	126244	3.52
KB5F	2	1	4.0	539794	90.17	251360	8.66	263586	7.36
KB5F	2	2	4.0	118708	19.83	254679	8.78	191330	5.34
KB5F	2	1	5.0	143717	24.01	492130	16.96	433161	12.09
KB5F	2	2	5.0	526314	87.92	439959	15.16	526893	14.70
KB5U	1	1	1.0	101878	25.23	477961	18.86	484691	16.06
KB5U	1	2	1.0	128126	22.74	454621	19.26	483384	21.43
KB5U	1	1	2.0	154318	25.78	470620	16.22	575329	11.64
KB5U	1	2	2.0	156104	26.08	495030	17.06	767755	15.55
KB5U	1	1	3.0	130498	25.78	482967	16.22	85240	11.64
KB5U	1	2	3.0	128484	26.08	481872	17.06	88304	15.55
KB5U	1	1	4.0	157123	26.25	599853	20.67	96559	18.28
KB5U	1	2	4.0	157514	26.31	588121	20.26	96320	18.24
KB5U	1	1	5.0	149236	24.93	326571	11.25	518593	6.79
KB5U	1	2	5.0	148240	24.76	328744	11.33	527677	6.57
KB5U	2	1	1.0	151010	25.23	547468	18.86	484691	16.06
KB5U	2	2	1.0	136122	22.74	559065	19.26	483384	21.43
KB5U	2	1	2.0	115205	19.24	153797	5.30	85240	2.38
KB5U	2	2	2.0	114273	19.09	152569	5.26	88304	2.46
KB5U	2	1	3.0	115007	19.21	171862	5.92	96559	2.69
KB5U	2	2	3.0	114646	19.15	170053	5.86	96320	2.69
KB5U	2	1	4.0	172778	28.86	533671	18.39	518593	14.47
KB5U	2	2	4.0	172413	28.80	527109	18.16	527677	14.73
KB5U	2	1	5.0	480866	80.33	197844	6.82	130665	3.65
KB5U	2	2	5.0	471635	78.78	215760	7.43	129713	3.62

Note: KB5F(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (Filtered sample); KB5U(4.7/22) contained 4.7% sugar, 0.5% tea, 20% fermented broth and was fermented at 22°C (Unfiltered sample); Fermentation time: 1= Day 0, 2 = Day 3, 3 = Day 6, 4 = Day 9, 5 = Week 1, 6 = Week 2, 7 = Week 3

H Statistical Output

H.1 Phase 1: Selection of optimum fermentation condition to propagate starter culture comprising fermented broth with no visible developed SCOBY

General Linear Model: pH versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	8	KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	6	0, 3, 6, 9, 12, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	2.39452	0.342074	660.75	0.000
Fermentation time (day)	5	4.55898	0.911797	1761.22	0.000
Product*Fermentation time (day)	35	0.41584	0.011881	22.95	0.000
Error	48	0.02485	0.000518		
Total	95	7.39419			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0227532	99.66%	99.33%	98.66%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.51531	0.00232	1513.76	0.000	
Product					
KB1	0.27635	0.00614	44.98	0.000	1.75
KB2	0.17969	0.00614	29.25	0.000	1.75
KB3	0.08802	0.00614	14.33	0.000	1.75
KB4	0.01385	0.00614	2.25	0.029	1.75
KB5	-0.08615	0.00614	-14.02	0.000	1.75
KB6	-0.14031	0.00614	-22.84	0.000	1.75
KB7	-0.14448	0.00614	-23.52	0.000	1.75
Fermentation time (day)					
0	0.40469	0.00519	77.93	0.000	1.67
3	0.12406	0.00519	23.89	0.000	1.67
6	-0.00656	0.00519	-1.26	0.212	1.67
9	-0.09844	0.00519	-18.96	0.000	1.67
12	-0.15594	0.00519	-30.03	0.000	1.67
Product*Fermentation time (day)					
KB1 0	0.0286	0.0137	2.09	0.042	2.92
KB1 3	-0.0107	0.0137	-0.78	0.439	2.92
KB1 6	0.0249	0.0137	1.81	0.076	2.92
KB1 9	-0.0332	0.0137	-2.42	0.019	2.92
KB1 12	-0.0457	0.0137	-3.33	0.002	2.92
KB2 0	0.1053	0.0137	7.67	0.000	2.92
KB2 3	-0.0841	0.0137	-6.12	0.000	2.92
KB2 6	-0.0634	0.0137	-4.62	0.000	2.92
KB2 9	-0.0316	0.0137	-2.30	0.026	2.92
KB2 12	0.0059	0.0137	0.43	0.668	2.92
KB3 0	0.1120	0.0137	8.15	0.000	2.92

KB3 3	-0.0274	0.0137	-1.99	0.052	2.92
KB3 6	-0.0768	0.0137	-5.59	0.000	2.92
KB3 9	-0.0649	0.0137	-4.72	0.000	2.92
KB3 12	-0.0224	0.0137	-1.63	0.110	2.92
KB4 0	-0.0039	0.0137	-0.28	0.780	2.92
KB4 3	-0.0582	0.0137	-4.24	0.000	2.92
KB4 6	-0.0426	0.0137	-3.10	0.003	2.92
KB4 9	-0.0007	0.0137	-0.05	0.958	2.92
KB4 12	0.0118	0.0137	0.86	0.396	2.92
KB5 0	-0.1139	0.0137	-8.29	0.000	2.92
KB5 3	0.0668	0.0137	4.86	0.000	2.92
KB5 6	0.0674	0.0137	4.91	0.000	2.92
KB5 9	-0.0257	0.0137	-1.87	0.067	2.92
KB5 12	-0.0282	0.0137	-2.05	0.045	2.92
KB6 0	-0.0897	0.0137	-6.53	0.000	2.92
KB6 3	0.0059	0.0137	0.43	0.668	2.92
KB6 6	-0.0134	0.0137	-0.98	0.333	2.92
KB6 9	0.0134	0.0137	0.98	0.333	2.92
KB6 12	0.0359	0.0137	2.62	0.012	2.92
KB7 0	-0.0255	0.0137	-1.86	0.069	2.92
KB7 3	0.0601	0.0137	4.37	0.000	2.92
KB7 6	0.0657	0.0137	4.78	0.000	2.92
KB7 9	0.0526	0.0137	3.83	0.000	2.92
KB7 12	-0.0099	0.0137	-0.72	0.475	2.92

Regression Equation

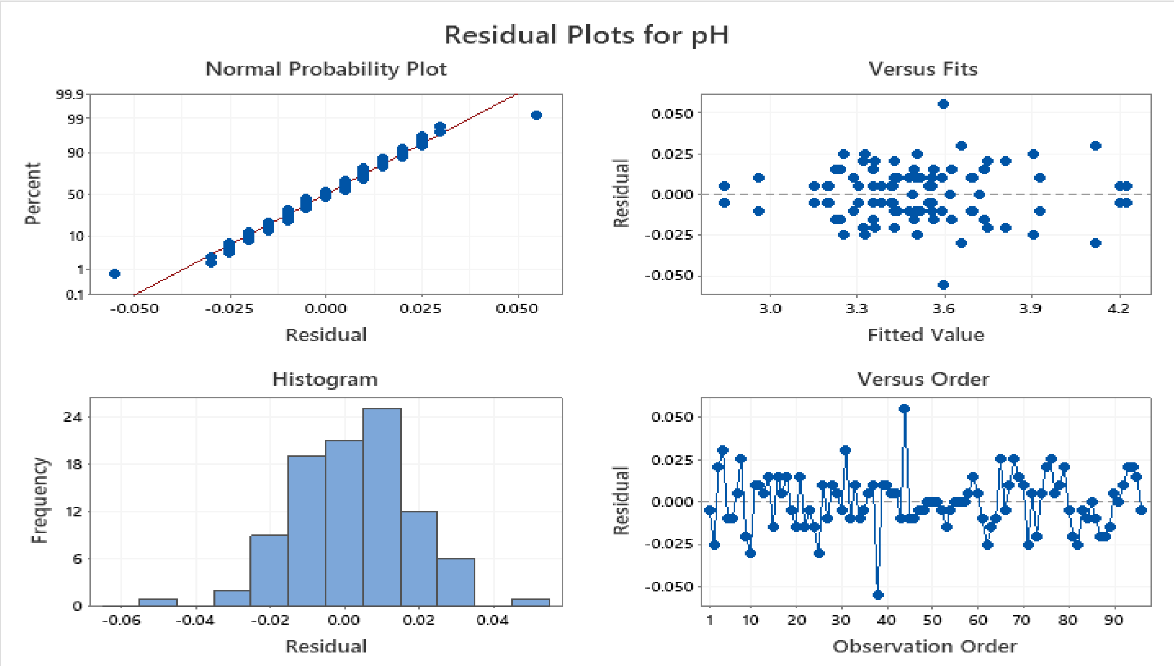
pH = 3.51531 + 0.27635 Product_KB1 + 0.17969 Product_KB2 + 0.08802 Product_KB3
+ 0.01385 Product_KB4 - 0.08615 Product_KB5 - 0.14031 Product_KB6
- 0.14448 Product_KB7
- 0.18698 Product_KB8 + 0.40469 Fermentation time (day)_0
+ 0.12406 Fermentation time (day)_3 - 0.00656 Fermentation time (day)_6
- 0.09844 Fermentation time (day)_9 - 0.15594 Fermentation time (day)_12
- 0.26781 Fermentation time (day)_14
+ 0.0286 Product*Fermentation time (day)_KB1 0
- 0.0107 Product*Fermentation time (day)_KB1 3
+ 0.0249 Product*Fermentation time (day)_KB1 6
- 0.0332 Product*Fermentation time (day)_KB1 9
- 0.0457 Product*Fermentation time (day)_KB1 12
+ 0.0361 Product*Fermentation time (day)_KB1 14
+ 0.1053 Product*Fermentation time (day)_KB2 0
- 0.0841 Product*Fermentation time (day)_KB2 3
- 0.0634 Product*Fermentation time (day)_KB2 6
- 0.0316 Product*Fermentation time (day)_KB2 9
+ 0.0059 Product*Fermentation time (day)_KB2 12
+ 0.0678 Product*Fermentation time (day)_KB2 14
+ 0.1120 Product*Fermentation time (day)_KB3 0
- 0.0274 Product*Fermentation time (day)_KB3 3
- 0.0768 Product*Fermentation time (day)_KB3 6
- 0.0649 Product*Fermentation time (day)_KB3 9
- 0.0224 Product*Fermentation time (day)_KB3 12
+ 0.0795 Product*Fermentation time (day)_KB3 14
- 0.0039 Product*Fermentation time (day)_KB4 0
- 0.0582 Product*Fermentation time (day)_KB4 3
- 0.0426 Product*Fermentation time (day)_KB4 6
- 0.0007 Product*Fermentation time (day)_KB4 9
+ 0.0118 Product*Fermentation time (day)_KB4 12
+ 0.0936 Product*Fermentation time (day)_KB4 14
- 0.1139 Product*Fermentation time (day)_KB5 0
+ 0.0668 Product*Fermentation time (day)_KB5 3
+ 0.0674 Product*Fermentation time (day)_KB5 6
- 0.0257 Product*Fermentation time (day)_KB5 9
- 0.0282 Product*Fermentation time (day)_KB5 12
+ 0.0336 Product*Fermentation time (day)_KB5 14
- 0.0897 Product*Fermentation time (day)_KB6 0
+ 0.0059 Product*Fermentation time (day)_KB6 3
- 0.0134 Product*Fermentation time (day)_KB6 6
+ 0.0134 Product*Fermentation time (day)_KB6 9
+ 0.0359 Product*Fermentation time (day)_KB6 12
+ 0.0478 Product*Fermentation time (day)_KB6 14
- 0.0255 Product*Fermentation time (day)_KB7 0

+ 0.0601 Product*Fermentation time (day)_KB7 3
+ 0.0657 Product*Fermentation time (day)_KB7 6
+ 0.0526 Product*Fermentation time (day)_KB7 9
- 0.0099 Product*Fermentation time (day)_KB7 12
- 0.1430 Product*Fermentation time (day)_KB7 14
- 0.0130 Product*Fermentation time (day)_KB8 0
+ 0.0476 Product*Fermentation time (day)_KB8 3
+ 0.0382 Product*Fermentation time (day)_KB8 6
+ 0.0901 Product*Fermentation time (day)_KB8 9
+ 0.0526 Product*Fermentation time (day)_KB8 12
- 0.2155 Product*Fermentation time (day)_KB8 14

Fits and Diagnostics for Unusual Observations

Obs	pH	Fit	Resid	Std Resid
38	3.5400	3.5950	-0.0550	-3.42 R
44	3.6500	3.5950	0.0550	3.42 R

R Large residual



General Linear Model: Titratable acidity% versus Product and Fermentation time (day)

Method

Factor coding	(-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	8	KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	6	0, 3, 6, 9, 12, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	0.15775	0.022536	81.95	0.000
Fermentation time (day)	5	2.05126	0.410252	1491.83	0.000
Product*Fermentation time (day)	35	0.08179	0.002337	8.50	0.000
Error	48	0.01320	0.000275		
Total	95	2.30400			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0165831	99.43%	98.87%	97.71%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.31750	0.00169	187.59	0.000	
Product					
KB1	-0.06500	0.00448	-14.52	0.000	1.75
KB2	-0.03500	0.00448	-7.82	0.000	1.75
KB3	-0.02500	0.00448	-5.58	0.000	1.75
KB4	0.00083	0.00448	0.19	0.853	1.75
KB5	0.00833	0.00448	1.86	0.069	1.75
KB6	-0.00000	0.00448	-0.00	1.000	1.75
KB7	0.04583	0.00448	10.24	0.000	1.75
Fermentation time (day)					
0	-0.21438	0.00378	-56.64	0.000	1.67
3	-0.14562	0.00378	-38.48	0.000	1.67
6	-0.03125	0.00378	-8.26	0.000	1.67
9	0.06750	0.00378	17.84	0.000	1.67
12	0.12250	0.00378	32.37	0.000	1.67
Product*Fermentation time (day)					
KB1 0	0.0419	0.0100	4.18	0.000	2.92
KB1 3	0.0131	0.0100	1.31	0.196	2.92
KB1 6	0.0137	0.0100	1.37	0.176	2.92
KB1 9	0.0100	0.0100	1.00	0.323	2.92
KB1 12	-0.0150	0.0100	-1.50	0.141	2.92
KB2 0	0.0219	0.0100	2.18	0.034	2.92
KB2 3	-0.0069	0.0100	-0.69	0.496	2.92
KB2 6	0.0387	0.0100	3.87	0.000	2.92
KB2 9	0.0050	0.0100	0.50	0.620	2.92
KB2 12	-0.0100	0.0100	-1.00	0.323	2.92
KB3 0	0.0169	0.0100	1.69	0.098	2.92
KB3 3	0.0231	0.0100	2.31	0.025	2.92
KB3 6	0.0088	0.0100	0.87	0.387	2.92
KB3 9	0.0000	0.0100	0.00	1.000	2.92
KB3 12	-0.0350	0.0100	-3.50	0.001	2.92
KB4 0	0.0160	0.0100	1.60	0.116	2.92
KB4 3	0.0273	0.0100	2.73	0.009	2.92
KB4 6	0.0129	0.0100	1.29	0.203	2.92
KB4 9	-0.0108	0.0100	-1.08	0.285	2.92
KB4 12	-0.0208	0.0100	-2.08	0.043	2.92
KB5 0	0.0085	0.0100	0.85	0.398	2.92
KB5 3	0.0198	0.0100	1.98	0.054	2.92
KB5 6	0.0054	0.0100	0.54	0.591	2.92
KB5 9	-0.0183	0.0100	-1.83	0.073	2.92
KB5 12	-0.0283	0.0100	-2.83	0.007	2.92
KB6 0	-0.0131	0.0100	-1.31	0.196	2.92

KB6 3	-0.0169	0.0100	-1.69	0.098	2.92
KB6 6	-0.0062	0.0100	-0.62	0.535	2.92
KB6 9	-0.0400	0.0100	-3.99	0.000	2.92
KB6 12	0.0250	0.0100	2.50	0.016	2.92
KB7 0	-0.0390	0.0100	-3.89	0.000	2.92
KB7 3	-0.0377	0.0100	-3.77	0.000	2.92
KB7 6	-0.0421	0.0100	-4.20	0.000	2.92
KB7 9	0.0392	0.0100	3.91	0.000	2.92
KB7 12	0.0392	0.0100	3.91	0.000	2.92

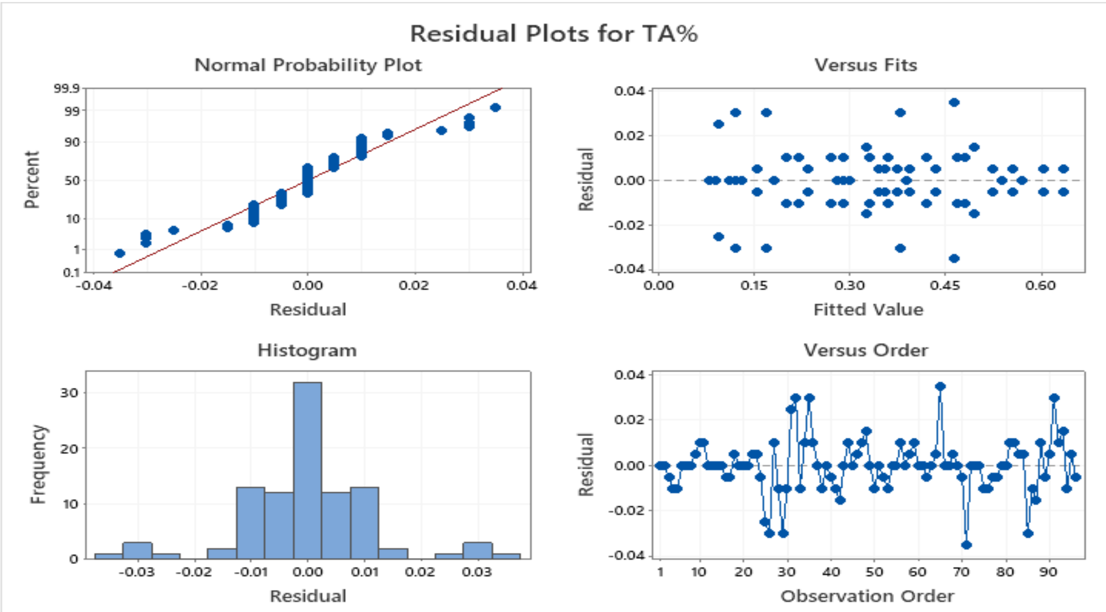
Regression Equation

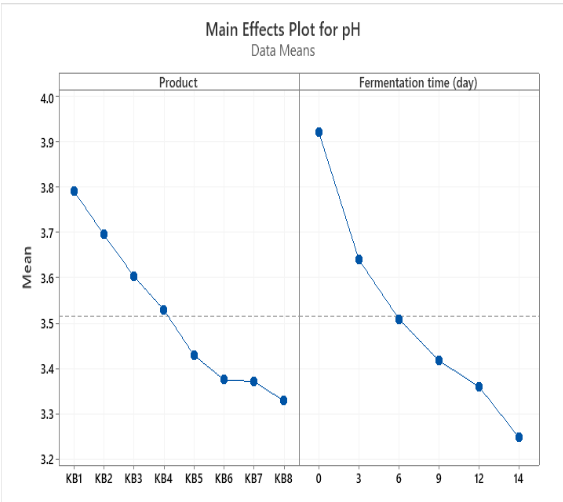
TA% = 0.31750 - 0.06500 Product_KB1 - 0.03500 Product_KB2 - 0.02500 Product_KB3
+ 0.00083 Product_KB4 + 0.00833 Product_KB5 - 0.00000 Product_KB6
+ 0.04583 Product_KB7
+ 0.07000 Product_KB8 - 0.21438 Fermentation time (day)_0
- 0.14562 Fermentation time (day)_3 - 0.03125 Fermentation time (day)_6
+ 0.06750 Fermentation time (day)_9 + 0.12250 Fermentation time (day)_12
+ 0.20125 Fermentation time (day)_14
+ 0.0419 Product*Fermentation time (day)_KB1 0
+ 0.0131 Product*Fermentation time (day)_KB1 3
+ 0.0137 Product*Fermentation time (day)_KB1 6
+ 0.0100 Product*Fermentation time (day)_KB1 9
- 0.0150 Product*Fermentation time (day)_KB1 12
- 0.0638 Product*Fermentation time (day)_KB1 14
+ 0.0219 Product*Fermentation time (day)_KB2 0
- 0.0069 Product*Fermentation time (day)_KB2 3
+ 0.0387 Product*Fermentation time (day)_KB2 6
+ 0.0050 Product*Fermentation time (day)_KB2 9
- 0.0100 Product*Fermentation time (day)_KB2 12
- 0.0488 Product*Fermentation time (day)_KB2 14
+ 0.0169 Product*Fermentation time (day)_KB3 0
+ 0.0231 Product*Fermentation time (day)_KB3 3
+ 0.0088 Product*Fermentation time (day)_KB3 6
+ 0.0000 Product*Fermentation time (day)_KB3 9
- 0.0350 Product*Fermentation time (day)_KB3 12
- 0.0138 Product*Fermentation time (day)_KB3 14
+ 0.0160 Product*Fermentation time (day)_KB4 0
+ 0.0273 Product*Fermentation time (day)_KB4 3
+ 0.0129 Product*Fermentation time (day)_KB4 6
- 0.0108 Product*Fermentation time (day)_KB4 9
- 0.0208 Product*Fermentation time (day)_KB4 12
- 0.0246 Product*Fermentation time (day)_KB4 14
+ 0.0085 Product*Fermentation time (day)_KB5 0
+ 0.0198 Product*Fermentation time (day)_KB5 3
+ 0.0054 Product*Fermentation time (day)_KB5 6
- 0.0183 Product*Fermentation time (day)_KB5 9
- 0.0283 Product*Fermentation time (day)_KB5 12
+ 0.0129 Product*Fermentation time (day)_KB5 14
- 0.0131 Product*Fermentation time (day)_KB6 0
- 0.0169 Product*Fermentation time (day)_KB6 3
- 0.0062 Product*Fermentation time (day)_KB6 6
- 0.0400 Product*Fermentation time (day)_KB6 9
+ 0.0250 Product*Fermentation time (day)_KB6 12
+ 0.0513 Product*Fermentation time (day)_KB6 14
- 0.0390 Product*Fermentation time (day)_KB7 0
- 0.0377 Product*Fermentation time (day)_KB7 3
- 0.0421 Product*Fermentation time (day)_KB7 6
+ 0.0392 Product*Fermentation time (day)_KB7 9
+ 0.0392 Product*Fermentation time (day)_KB7 12
+ 0.0404 Product*Fermentation time (day)_KB7 14
- 0.0531 Product*Fermentation time (day)_KB8 0
- 0.0219 Product*Fermentation time (day)_KB8 3
- 0.0313 Product*Fermentation time (day)_KB8 6
+ 0.0150 Product*Fermentation time (day)_KB8 9
+ 0.0450 Product*Fermentation time (day)_KB8 12
+ 0.0463 Product*Fermentation time (day)_KB8 14

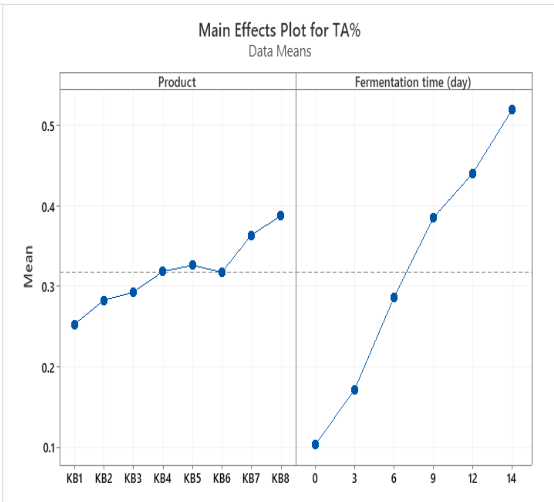
Fits and Diagnostics for Unusual Observations

Obs	TA%	Fit	Resid	Std Resid
25	0.0700	0.0950	-0.0250	-2.13 R
26	0.1400	0.1700	-0.0300	-2.56 R
29	0.3500	0.3800	-0.0300	-2.56 R
31	0.1200	0.0950	0.0250	2.13 R
32	0.2000	0.1700	0.0300	2.56 R
35	0.4100	0.3800	0.0300	2.56 R
65	0.5000	0.4650	0.0350	2.98 R
71	0.4300	0.4650	-0.0350	-2.98 R
85	0.0900	0.1200	-0.0300	-2.56 R
91	0.1500	0.1200	0.0300	2.56 R

R Large residual







General Linear Model: TSS °Brix versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	8	KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	6	0, 3, 6, 9, 12, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	95.166	13.5951	1616.26	0.000
Fermentation time (day)	5	5.710	1.1419	135.76	0.000
Product*Fermentation time (day)	35	0.300	0.0086	1.02	0.468
Error	48	0.404	0.0084		
Total	95	101.579			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0917140	99.60%	99.21%	98.41%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.39427	0.00936	469.45	0.000	
Product					
KB1	1.5266	0.0248	61.64	0.000	1.75
KB2	1.5016	0.0248	60.63	0.000	1.75
KB3	-0.5984	0.0248	-24.16	0.000	1.75
KB4	-0.6651	0.0248	-26.86	0.000	1.75
KB5	0.2641	0.0248	10.66	0.000	1.75
KB6	0.1766	0.0248	7.13	0.000	1.75
KB7	-1.0234	0.0248	-41.32	0.000	1.75
Fermentation time (day)					
0	0.3964	0.0209	18.94	0.000	1.67
3	0.1870	0.0209	8.93	0.000	1.67
6	0.0276	0.0209	1.32	0.193	1.67
9	-0.0661	0.0209	-3.16	0.003	1.67
12	-0.1974	0.0209	-9.43	0.000	1.67
Product*Fermentation time (day)					
KB1 0	-0.0422	0.0554	-0.76	0.450	2.92
KB1 3	-0.0578	0.0554	-1.04	0.302	2.92
KB1 6	0.0516	0.0554	0.93	0.356	2.92
KB1 9	0.0453	0.0554	0.82	0.417	2.92
KB1 12	0.0266	0.0554	0.48	0.634	2.92
KB2 0	0.0578	0.0554	1.04	0.302	2.92
KB2 3	-0.0328	0.0554	-0.59	0.556	2.92
KB2 6	-0.0734	0.0554	-1.33	0.191	2.92
KB2 9	0.0203	0.0554	0.37	0.715	2.92
KB2 12	0.1016	0.0554	1.83	0.073	2.92
KB3 0	-0.1422	0.0554	-2.57	0.013	2.92
KB3 3	-0.0328	0.0554	-0.59	0.556	2.92
KB3 6	0.0266	0.0554	0.48	0.634	2.92
KB3 9	0.0203	0.0554	0.37	0.715	2.92
KB3 12	0.0516	0.0554	0.93	0.356	2.92
KB4 0	0.0245	0.0554	0.44	0.660	2.92
KB4 3	0.0339	0.0554	0.61	0.544	2.92
KB4 6	-0.0068	0.0554	-0.12	0.903	2.92
KB4 9	-0.0130	0.0554	-0.24	0.815	2.92
KB4 12	-0.0068	0.0554	-0.12	0.903	2.92
KB5 0	-0.0047	0.0554	-0.08	0.933	2.92
KB5 3	0.0047	0.0554	0.08	0.933	2.92

KB5 6	-0.0109	0.0554	-0.20	0.844	2.92
KB5 9	0.0078	0.0554	0.14	0.888	2.92
KB5 12	0.0391	0.0554	0.71	0.484	2.92
KB6 0	0.1078	0.0554	1.95	0.057	2.92
KB6 3	0.0922	0.0554	1.66	0.102	2.92
KB6 6	0.0516	0.0554	0.93	0.356	2.92
KB6 9	-0.0297	0.0554	-0.54	0.594	2.92
KB6 12	-0.1234	0.0554	-2.23	0.031	2.92
KB7 0	0.0078	0.0554	0.14	0.888	2.92
KB7 3	0.0172	0.0554	0.31	0.758	2.92
KB7 6	0.0016	0.0554	0.03	0.978	2.92
KB7 9	-0.0547	0.0554	-0.99	0.328	2.92
KB7 12	-0.0484	0.0554	-0.87	0.386	2.92

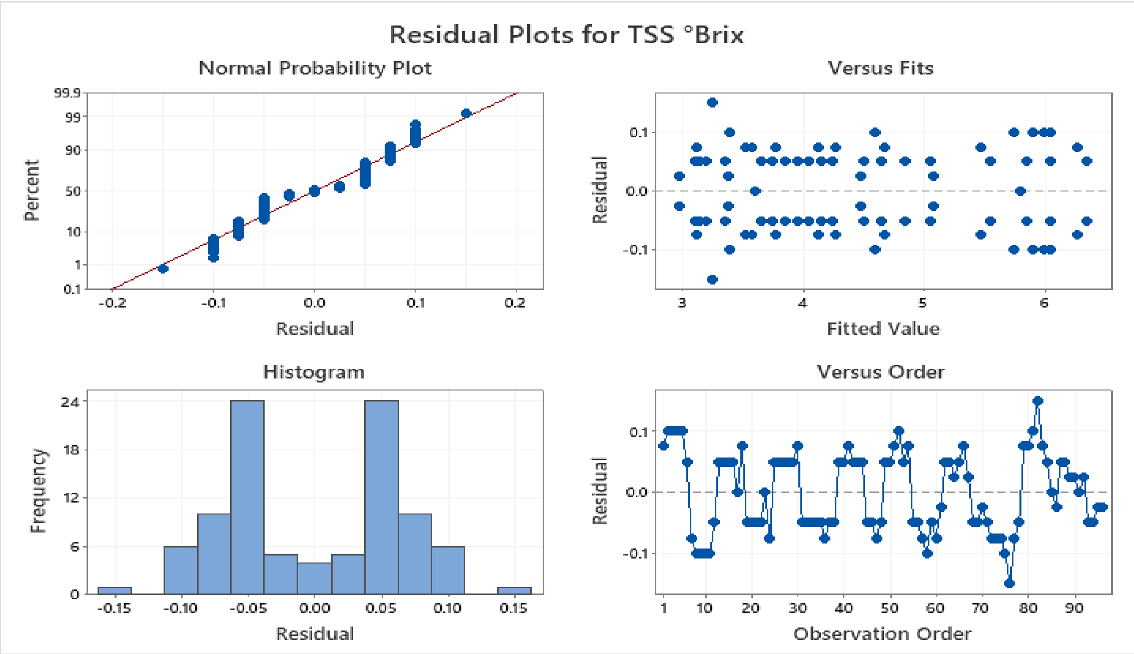
Regression Equation

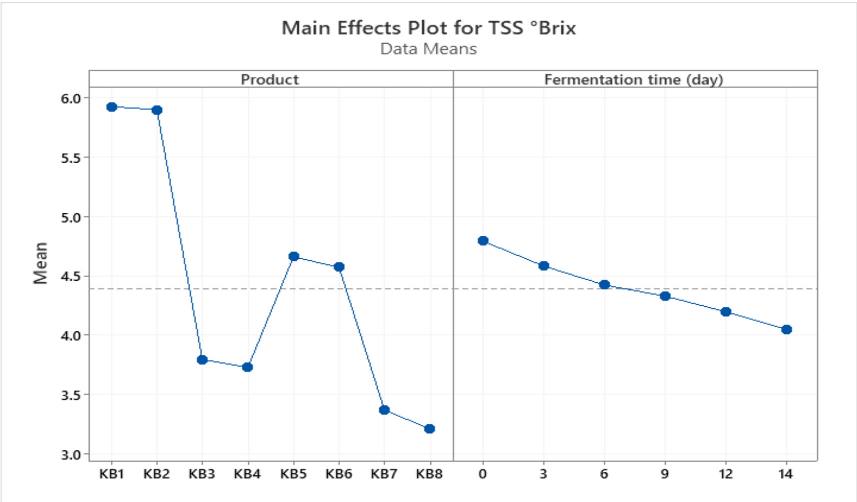
TSS °Brix = 4.39427 + 1.5266 Product_KB1 + 1.5016 Product_KB2
- 0.5984 Product_KB3
- 0.6651 Product_KB4 + 0.2641 Product_KB5 + 0.1766 Product_KB6
- 1.0234 Product_KB7 - 1.1818 Product_KB8
+ 0.3964 Fermentation time (day)_0
+ 0.1870 Fermentation time (day)_3 + 0.0276 Fermentation time (day)_6
- 0.0661 Fermentation time (day)_9 - 0.1974 Fermentation time (day)_12
- 0.3474 Fermentation time (day)_14
- 0.0422 Product*Fermentation time (day)_KB1
0 - 0.0578 Product*Fermentation time (day)_KB1 3
+ 0.0516 Product*Fermentation time (day)_KB1 6
+ 0.0453 Product*Fermentation time (day)_KB1 9
+ 0.0266 Product*Fermentation time (day)_KB1 12
- 0.0234 Product*Fermentation time (day)_KB1 14
+ 0.0578 Product*Fermentation time (day)_KB2 0
- 0.0328 Product*Fermentation time (day)_KB2 3
- 0.0734 Product*Fermentation time (day)_KB2 6
+ 0.0203 Product*Fermentation time (day)_KB2 9
+ 0.1016 Product*Fermentation time (day)_KB2 12
- 0.0734 Product*Fermentation time (day)_KB2 14
- 0.1422 Product*Fermentation time (day)_KB3 0
- 0.0328 Product*Fermentation time (day)_KB3 3
+ 0.0266 Product*Fermentation time (day)_KB3 6
+ 0.0203 Product*Fermentation time (day)_KB3 9
+ 0.0516 Product*Fermentation time (day)_KB3 12
+ 0.0766 Product*Fermentation time (day)_KB3 14
+ 0.0245 Product*Fermentation time (day)_KB4 0
+ 0.0339 Product*Fermentation time (day)_KB4 3
- 0.0068 Product*Fermentation time (day)_KB4 6
- 0.0130 Product*Fermentation time (day)_KB4 9
- 0.0068 Product*Fermentation time (day)_KB4 12
- 0.0318 Product*Fermentation time (day)_KB4 14
- 0.0047 Product*Fermentation time (day)_KB5 0
+ 0.0047 Product*Fermentation time (day)_KB5 3
- 0.0109 Product*Fermentation time (day)_KB5 6
+ 0.0078 Product*Fermentation time (day)_KB5 9
+ 0.0391 Product*Fermentation time (day)_KB5 12
- 0.0359 Product*Fermentation time (day)_KB5 14
+ 0.1078 Product*Fermentation time (day)_KB6 0
+ 0.0922 Product*Fermentation time (day)_KB6 3
+ 0.0516 Product*Fermentation time (day)_KB6 6
- 0.0297 Product*Fermentation time (day)_KB6 9
- 0.1234 Product*Fermentation time (day)_KB6 12
- 0.0984 Product*Fermentation time (day)_KB6 14
+ 0.0078 Product*Fermentation time (day)_KB7 0
+ 0.0172 Product*Fermentation time (day)_KB7 3
+ 0.0016 Product*Fermentation time (day)_KB7 6
- 0.0547 Product*Fermentation time (day)_KB7 9
- 0.0484 Product*Fermentation time (day)_KB7 12
+ 0.0766 Product*Fermentation time (day)_KB7 14
- 0.0089 Product*Fermentation time (day)_KB8 0
- 0.0245 Product*Fermentation time (day)_KB8 3
- 0.0401 Product*Fermentation time (day)_KB8 6
+ 0.0036 Product*Fermentation time (day)_KB8 9
- 0.0401 Product*Fermentation time (day)_KB8 12
+ 0.1099 Product*Fermentation time (day)_KB8 14

Fits and Diagnostics for Unusual Observations

Obs	TSS °Brix	Fit	Resid	Std Resid
76	3.1000	3.2500	-0.1500	-2.31 R
82	3.4000	3.2500	0.1500	2.31 R

R Large residual





General Linear Model: Yeast (Fermented broth) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	8	KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	2	0, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	11.2394	1.60562	91.75	0.000
Fermentation time (day)	1	0.2512	0.25116	14.35	0.002
Product*Fermentation time (day)	7	0.5389	0.07698	4.40	0.007
Error	16	0.2800	0.01750		
Total	31	12.3094			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.132291	97.73%	95.59%	90.90%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.7498	0.0234	245.87	0.000	
Product					
KB1	-0.8198	0.0619	-13.25	0.000	1.75
KB2	-0.6986	0.0619	-11.29	0.000	1.75
KB3	-0.4836	0.0619	-7.82	0.000	1.75
KB4	-0.1723	0.0619	-2.79	0.013	1.75
KB5	0.2177	0.0619	3.52	0.003	1.75
KB6	0.4652	0.0619	7.52	0.000	1.75
KB7	0.6752	0.0619	10.91	0.000	1.75
Fermentation time (day)					
0	-0.0886	0.0234	-3.79	0.002	1.00
Product*Fermentation time (day)					
KB1 0	-0.0514	0.0619	-0.83	0.418	1.75
KB2 0	-0.0802	0.0619	-1.30	0.214	1.75
KB3 0	-0.1077	0.0619	-1.74	0.101	1.75
KB4 0	-0.2189	0.0619	-3.54	0.003	1.75
KB5 0	0.2086	0.0619	3.37	0.004	1.75
KB6 0	0.1136	0.0619	1.84	0.085	1.75
KB7 0	0.0836	0.0619	1.35	0.195	1.75

Regression Equation

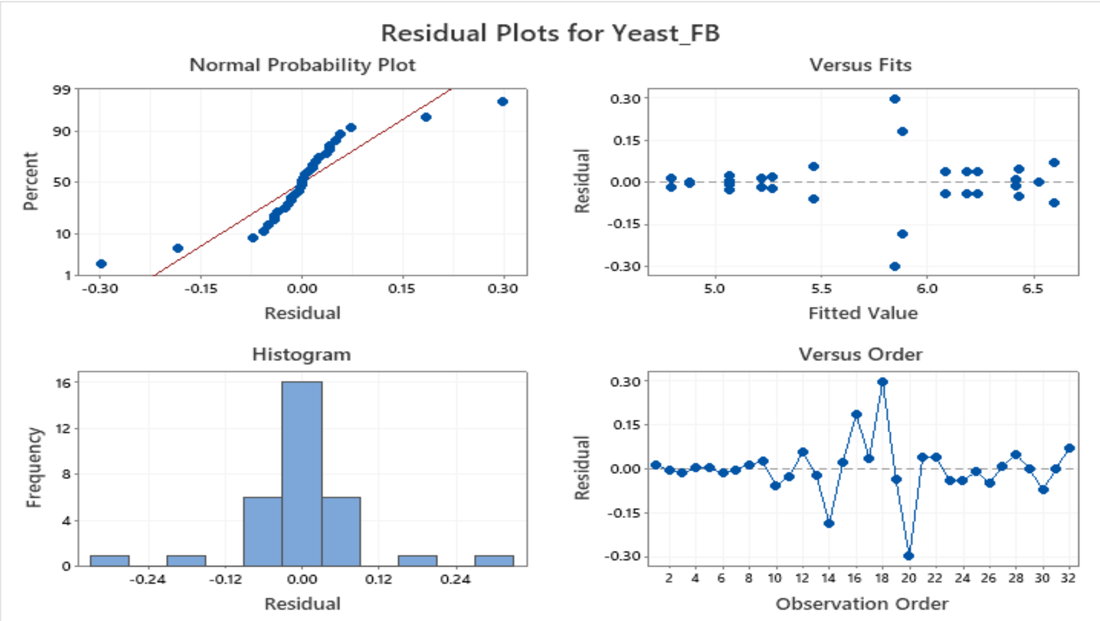
Yeast_FB = 5.7498 - 0.8198 Product_KB1 - 0.6986 Product_KB2
- 0.4836 Product_KB3
- 0.1723 Product_KB4 + 0.2177 Product_KB5 + 0.4652 Product_KB6
+ 0.6752 Product_KB7 + 0.8164 Product_KB8
- 0.0886 Fermentation time (day)_0
+ 0.0886 Fermentation time (day)_14
- 0.0514 Product*Fermentation time (day)_KB1 0
+ 0.0514 Product*Fermentation time (day)_KB1 14
- 0.0802 Product*Fermentation time (day)_KB2 0
+ 0.0802 Product*Fermentation time (day)_KB2 14
- 0.1077 Product*Fermentation time (day)_KB3 0

+ 0.1077 Product*Fermentation time (day)_KB3 14
- 0.2189 Product*Fermentation time (day)_KB4 0
+ 0.2189 Product*Fermentation time (day)_KB4 14
+ 0.2086 Product*Fermentation time (day)_KB5 0
- 0.2086 Product*Fermentation time (day)_KB5 14
+ 0.1136 Product*Fermentation time (day)_KB6 0
- 0.1136 Product*Fermentation time (day)_KB6 14
+ 0.0836 Product*Fermentation time (day)_KB7 0
- 0.0836 Product*Fermentation time (day)_KB7 14
+ 0.0523 Product*Fermentation time (day)_KB8 0
- 0.0523 Product*Fermentation time (day)_KB8 14

Fits and Diagnostics for Unusual Observations

Obs	Yeast_FB	Fit	Resid	Std Resid
18	6.1450	5.8475	0.2975	3.18 R
20	5.5500	5.8475	-0.2975	-3.18 R

R Large residual



General Linear Model: AAB (Fermented broth) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	8	KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	2	0, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	22.5556	3.22223	602.55	0.000
Fermentation time (day)	1	0.2601	0.26010	48.64	0.000
Product*Fermentation time (day)	7	0.2300	0.03286	6.14	0.001
Error	16	0.0856	0.00535		

Total	31	23.1313

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0731277	99.63%	99.28%	98.52%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.3555	0.0129	568.99	0.000	
Product					
KB1	-1.1367	0.0342	-33.24	0.000	1.75
KB2	-0.7630	0.0342	-22.31	0.000	1.75
KB3	-0.8017	0.0342	-23.44	0.000	1.75
KB4	-0.5280	0.0342	-15.44	0.000	1.75
KB5	0.4108	0.0342	12.01	0.000	1.75
KB6	0.9645	0.0342	28.20	0.000	1.75
KB7	0.8145	0.0342	23.82	0.000	1.75
Fermentation time (day)					
0	-0.0902	0.0129	-6.97	0.000	1.00
Product*Fermentation time (day)					
KB1 0	0.0364	0.0342	1.06	0.303	1.75
KB2 0	0.0752	0.0342	2.20	0.043	1.75
KB3 0	0.0489	0.0342	1.43	0.172	1.75
KB4 0	0.0277	0.0342	0.81	0.431	1.75
KB5 0	-0.2136	0.0342	-6.25	0.000	1.75
KB6 0	-0.0173	0.0342	-0.51	0.619	1.75
KB7 0	0.0052	0.0342	0.15	0.882	1.75

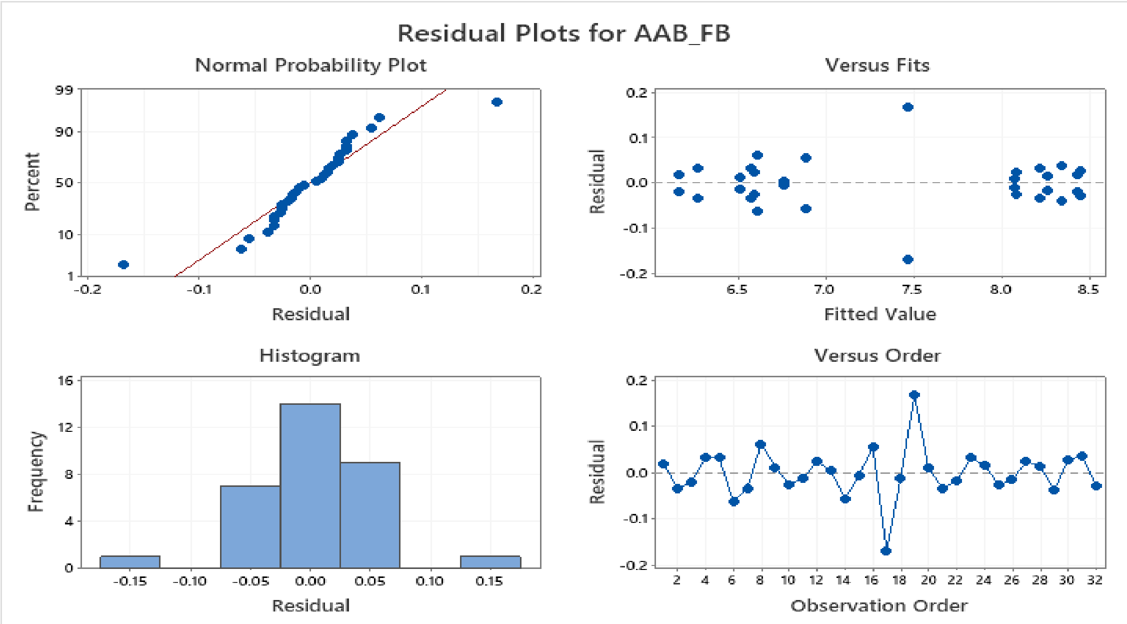
Regression Equation

AAB_FB = 7.3555 - 1.1367 Product_KB1 - 0.7630 Product_KB2 - 0.8017 Product_KB3
- 0.5280 Product_KB4 + 0.4108 Product_KB5 + 0.9645 Product_KB6
+ 0.8145 Product_KB7
+ 1.0395 Product_KB8 - 0.0902 Fermentation time (day)_0
+ 0.0902 Fermentation time (day)_14
+ 0.0364 Product*Fermentation time (day)_KB1 0
- 0.0364 Product*Fermentation time (day)_KB1 14
+ 0.0752 Product*Fermentation time (day)_KB2 0
- 0.0752 Product*Fermentation time (day)_KB2 14
+ 0.0489 Product*Fermentation time (day)_KB3 0
- 0.0489 Product*Fermentation time (day)_KB3 14
+ 0.0277 Product*Fermentation time (day)_KB4 0
- 0.0277 Product*Fermentation time (day)_KB4 14
- 0.2136 Product*Fermentation time (day)_KB5 0
+ 0.2136 Product*Fermentation time (day)_KB5 14
- 0.0173 Product*Fermentation time (day)_KB6 0
+ 0.0173 Product*Fermentation time (day)_KB6 14
+ 0.0052 Product*Fermentation time (day)_KB7 0
- 0.0052 Product*Fermentation time (day)_KB7 14
+ 0.0377 Product*Fermentation time (day)_KB8 0
- 0.0377 Product*Fermentation time (day)_KB8 14

Fits and Diagnostics for Unusual Observations

Obs	AAB_FB	Fit	Resid	Std Resid	
17	7.2950	7.4625	-0.1675	-3.24	R
19	7.6300	7.4625	0.1675	3.24	R

R Large residual



General Linear Model: Total count (Fermented broth)versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	8 KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	2 0, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	7	21.2436	3.03479	911.86	0.000
Fermentation time (day)	1	0.5592	0.55915	168.01	0.000
Product*Fermentation time (day)	7	0.3855	0.05507	16.55	0.000
Error	16	0.0533	0.00333		
Total	31	22.2415			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0576899	99.76%	99.54%	99.04%

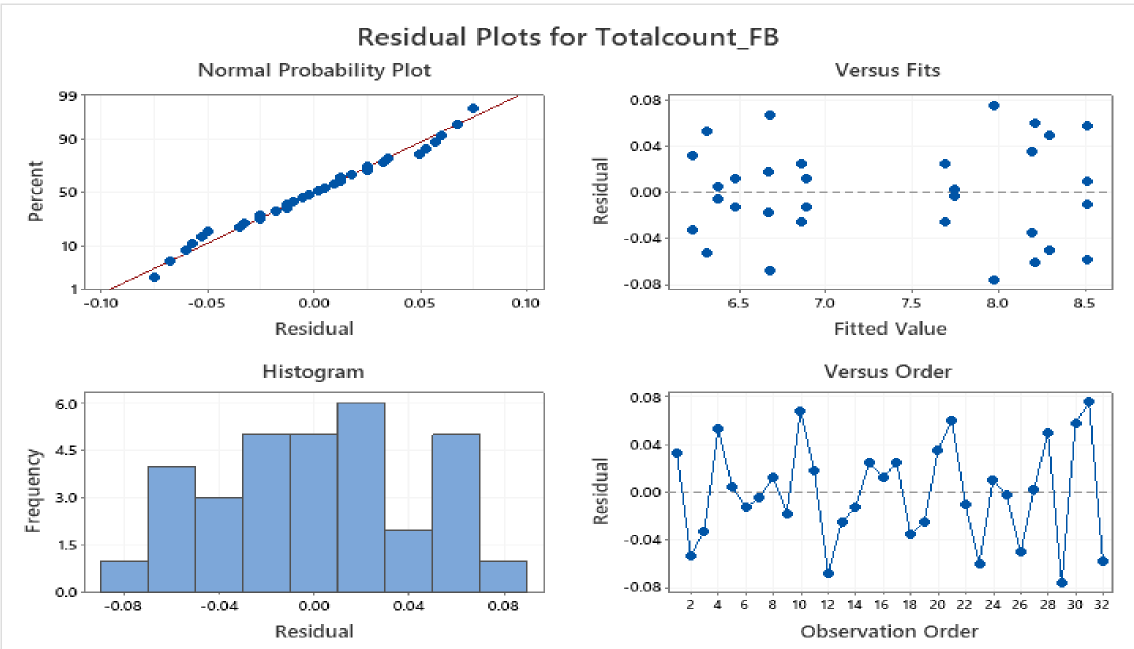
Coefficients

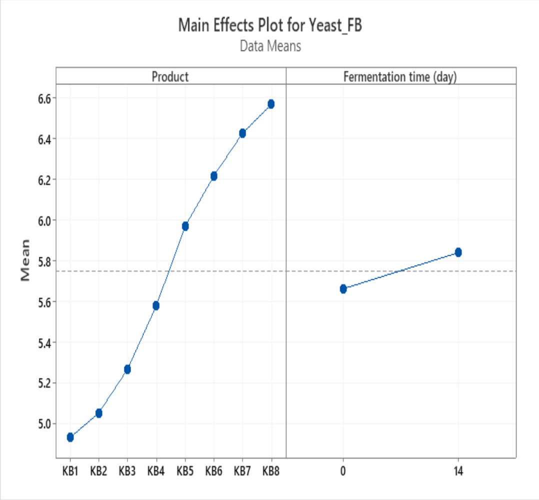
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.3519	0.0102	720.90	0.000	
Product					
KB1	-1.0794	0.0270	-40.00	0.000	1.75
KB2	-0.9231	0.0270	-34.21	0.000	1.75
KB3	-0.6794	0.0270	-25.18	0.000	1.75
KB4	-0.4756	0.0270	-17.63	0.000	1.75
KB5	0.5906	0.0270	21.89	0.000	1.75

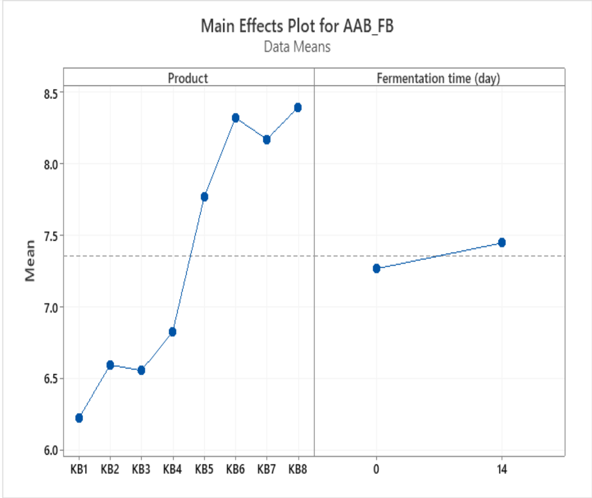
KB6	1.0106	0.0270	37.46	0.000	1.75
KB7	0.6669	0.0270	24.72	0.000	1.75
Fermentation time (day)					
0	-0.1322	0.0102	-12.96	0.000	1.00
Product*Fermentation time (day)					
KB1 0	0.0872	0.0270	3.23	0.005	1.75
KB2 0	0.0834	0.0270	3.09	0.007	1.75
KB3 0	0.1272	0.0270	4.71	0.000	1.75
KB4 0	0.1159	0.0270	4.30	0.001	1.75
KB5 0	-0.1203	0.0270	-4.46	0.000	1.75
KB6 0	-0.0203	0.0270	-0.75	0.462	1.75
KB7 0	-0.1391	0.0270	-5.15	0.000	1.75

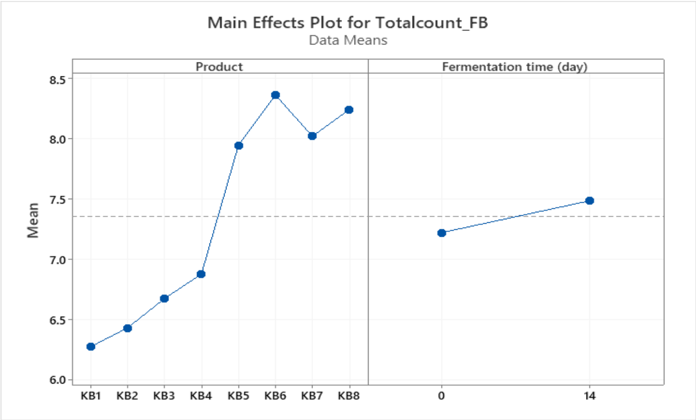
Regression Equation

Totalcount_FB = 7.3519 - 1.0794 Product_KB1 - 0.9231 Product_KB2
- 0.6794 Product_KB3
- 0.4756 Product_KB4 + 0.5906 Product_KB5 + 1.0106 Product_KB6
+ 0.6669 Product_KB7 + 0.8894 Product_KB8
- 0.1322 Fermentation time (day)_0
+ 0.1322 Fermentation time (day)_14
+ 0.0872 Product*Fermentation time (day)_KB1 0
- 0.0872 Product*Fermentation time (day)_KB1 14
+ 0.0834 Product*Fermentation time (day)_KB2 0
- 0.0834 Product*Fermentation time (day)_KB2 14
+ 0.1272 Product*Fermentation time (day)_KB3 0
- 0.1272 Product*Fermentation time (day)_KB3 14
+ 0.1159 Product*Fermentation time (day)_KB4 0
- 0.1159 Product*Fermentation time (day)_KB4 14
- 0.1203 Product*Fermentation time (day)_KB5 0
+ 0.1203 Product*Fermentation time (day)_KB5 14
- 0.0203 Product*Fermentation time (day)_KB6 0
+ 0.0203 Product*Fermentation time (day)_KB6 14
- 0.1391 Product*Fermentation time (day)_KB7 0
+ 0.1391 Product*Fermentation time (day)_KB7 14
- 0.1341 Product*Fermentation time (day)_KB8 0
+ 0.1341 Product*Fermentation time (day)_KB8 14









Phase 2: Investigation of the best conditions for the fermentation of Kombucha using a combination of developed SCOBY and fermented broth as the starter culture

H.2. Stage 1 (Phase 2): Selection of optimum sugar concentration and fermentation temperature for the fermentation of Kombucha

General Linear Model: pH versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.22840	0.076132	9.61	0.001
Fermentation time (day)	3	1.65185	0.550617	69.50	0.000
Product*Fermentation time (day)	9	0.05278	0.005864	0.74	0.669
Error	16	0.12676	0.007923		
Total	31	2.05979			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0890093	93.85%	88.08%	75.38%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.2561	0.0157	206.94	0.000	
Product					
KB5	0.1008	0.0273	3.70	0.002	1.50
KB6	0.0364	0.0273	1.34	0.200	1.50
KB7	-0.0067	0.0273	-0.25	0.808	1.50
Fermentation time (day)					
0.0	0.2814	0.0273	10.33	0.000	1.50
3.0	0.1127	0.0273	4.13	0.001	1.50
6.0	-0.0611	0.0273	-2.24	0.040	1.50
Product*Fermentation time (day)					
KB5 0.0	-0.0183	0.0472	-0.39	0.704	2.25
KB5 3.0	-0.0295	0.0472	-0.63	0.540	2.25
KB5 6.0	-0.0483	0.0472	-1.02	0.322	2.25
KB6 0.0	0.0436	0.0472	0.92	0.369	2.25
KB6 3.0	-0.0277	0.0472	-0.59	0.566	2.25
KB6 6.0	-0.0039	0.0472	-0.08	0.935	2.25
KB7 0.0	-0.0158	0.0472	-0.33	0.742	2.25
KB7 3.0	0.0280	0.0472	0.59	0.562	2.25
KB7 6.0	-0.0033	0.0472	-0.07	0.945	2.25

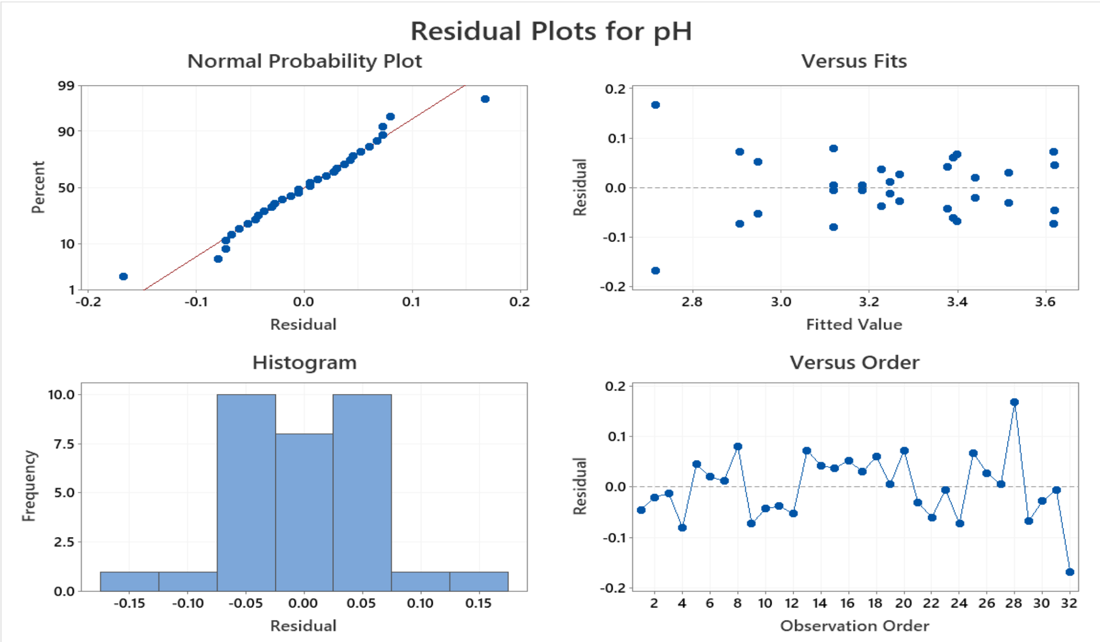
Regression Equation

pH = 3.2561 + 0.1008 Product code_KB5 + 0.0364 Product code_KB6
- 0.0067 Product code_KB7
- 0.1305 Product code_KB8 + 0.2814 Fermentation time (day)_0.0
+ 0.1127 Fermentation time (day)_3.0 - 0.0611 Fermentation time (day)_6.0
- 0.3330 Fermentation time (day)_9.0
- 0.0183 Product code*Fermentation time (day)_KB5
0.0 - 0.0295 Product code*Fermentation time (day)_KB5 3.0
- 0.0483 Product code*Fermentation time (day)_KB5 6.0
+ 0.0961 Product code*Fermentation time (day)_KB5 9.0
+ 0.0436 Product code*Fermentation time (day)_KB6 0.0
- 0.0277 Product code*Fermentation time (day)_KB6 3.0
- 0.0039 Product code*Fermentation time (day)_KB6 6.0
- 0.0120 Product code*Fermentation time (day)_KB6 9.0
- 0.0158 Product code*Fermentation time (day)_KB7 0.0
+ 0.0280 Product code*Fermentation time (day)_KB7 3.0
- 0.0033 Product code*Fermentation time (day)_KB7 6.0
- 0.0089 Product code*Fermentation time (day)_KB7 9.0
- 0.0095 Product code*Fermentation time (day)_KB8 0.0
+ 0.0292 Product code*Fermentation time (day)_KB8 3.0
+ 0.0555 Product code*Fermentation time (day)_KB8 6.0
- 0.0752 Product code*Fermentation time (day)_KB8 9.0

Fits and Diagnostics for Unusual Observations

Obs	pH	Fit	Resid	Std Resid
28	2.8850	2.7175	0.1675	2.66 R
32	2.5500	2.7175	-0.1675	-2.66 R

R Large residual



General Linear Model: Titratable acidity % versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.036634	0.012211	20.17	0.000
Fermentation time (day)	3	0.479759	0.159920	264.13	0.000
Product *Fermentation time (day)	9	0.015451	0.001717	2.84	0.033
Error	16	0.009687	0.000605		
Total	31	0.541530			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0246063	98.21%	96.53%	92.84%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.24172	0.00435	55.57	0.000	
Product					
KB5	-0.03734	0.00753	-4.96	0.000	1.50
KB6	-0.02422	0.00753	-3.21	0.005	1.50
KB7	0.01203	0.00753	1.60	0.130	1.50
Fermentation time (day)					
0.0	-0.14547	0.00753	-19.31	0.000	1.50
3.0	-0.07984	0.00753	-10.60	0.000	1.50
6.0	0.05328	0.00753	7.07	0.000	1.50
Product *Fermentation time (day)					
KB5 0.0	0.0311	0.0130	2.38	0.030	2.25
KB5 3.0	0.0105	0.0130	0.80	0.434	2.25
KB5 6.0	0.0048	0.0130	0.37	0.715	2.25
KB6 0.0	0.0055	0.0130	0.42	0.681	2.25
KB6 3.0	-0.0227	0.0130	-1.74	0.102	2.25
KB6 6.0	0.0242	0.0130	1.86	0.082	2.25
KB7 0.0	-0.0083	0.0130	-0.63	0.535	2.25
KB7 3.0	0.0061	0.0130	0.47	0.647	2.25
KB7 6.0	-0.0095	0.0130	-0.73	0.476	2.25

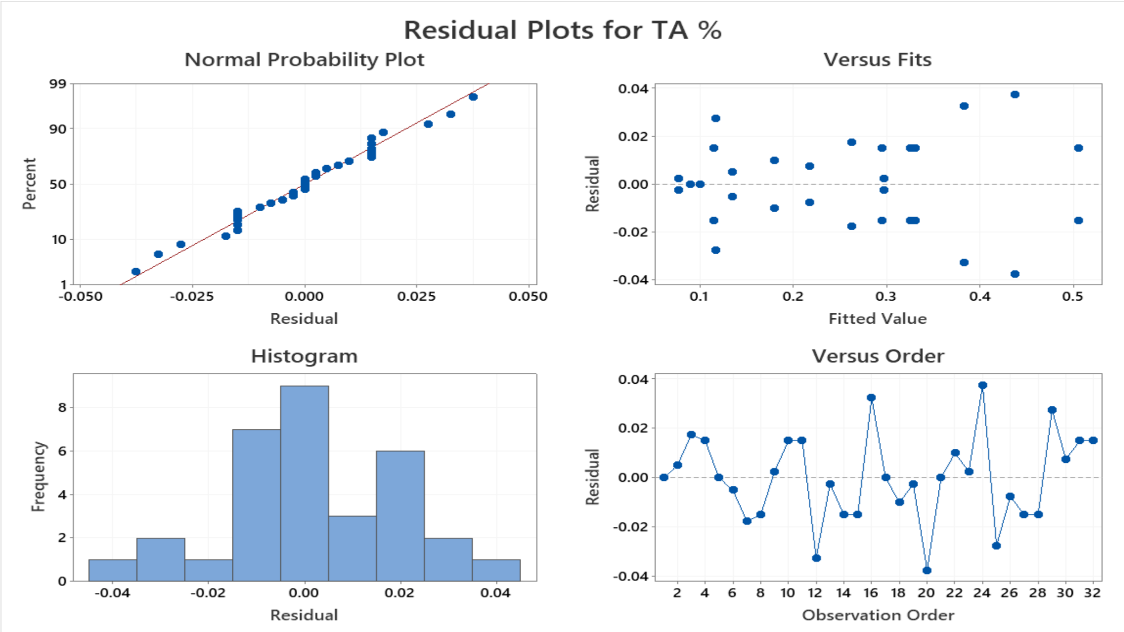
Regression Equation

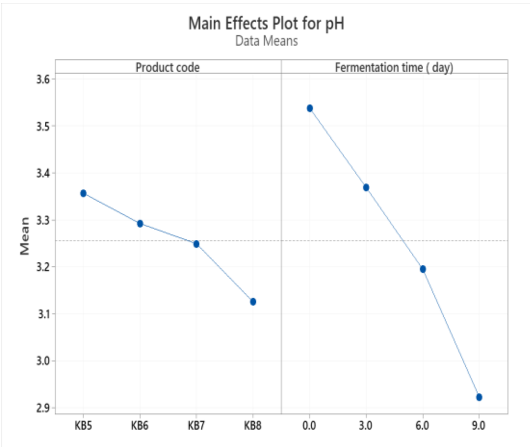
TA % = 0.24172 - 0.03734 Product code_KB5 - 0.02422 Product code_KB6
+ 0.01203 Product code_KB7 + 0.04953 Product code_KB8
- 0.14547 Fermentation time (day)_0.0
- 0.07984 Fermentation time (day)_3.0
+ 0.05328 Fermentation time (day)_6.0
+ 0.17203 Fermentation time (day)_9.0
+ 0.0311 Product code*Fermentation time (day)_KB5 0.0
+ 0.0105 Product code*Fermentation time (day)_KB5 3.0
+ 0.0048 Product code*Fermentation time (day)_KB5 6.0
- 0.0464 Product code*Fermentation time (day)_KB5 9.0
+ 0.0055 Product code*Fermentation time (day)_KB6 0.0
- 0.0227 Product code*Fermentation time (day)_KB6 3.0
+ 0.0242 Product code*Fermentation time (day)_KB6 6.0
- 0.0070 Product code*Fermentation time (day)_KB6 9.0
- 0.0083 Product code*Fermentation time (day)_KB7 0.0
+ 0.0061 Product code*Fermentation time (day)_KB7 3.0
- 0.0095 Product code*Fermentation time (day)_KB7 6.0
+ 0.0117 Product code*Fermentation time (day)_KB7 9.0
- 0.0283 Product code*Fermentation time (day)_KB8 0.0
+ 0.0061 Product code*Fermentation time (day)_KB8 3.0
- 0.0195 Product code*Fermentation time (day)_KB8 6.0
+ 0.0417 Product code*Fermentation time (day)_KB8 9.0

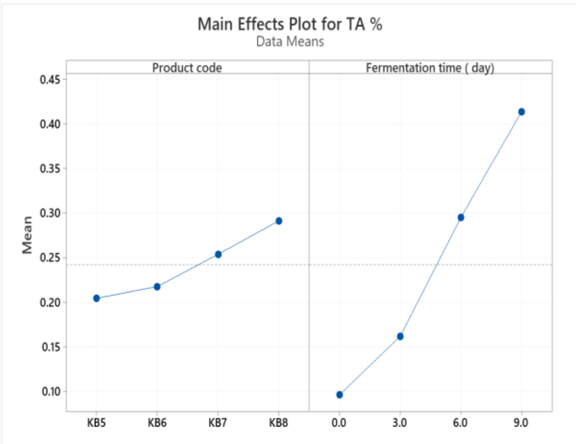
Fits and Diagnostics for Unusual Observations

Obs	TA %	Fit	Resid	Std Resid
20	0.4000	0.4375	-0.0375	-2.16 R
24	0.4750	0.4375	0.0375	2.16 R

R Large residual







General Linear Model: TSS °Brix versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	33.5059	11.1686	2233.73	0.000
Fermentation time (day)	3	3.2653	1.0884	217.69	0.000
Product *Fermentation time (day)	9	0.2559	0.0284	5.69	0.001
Error	16	0.0800	0.0050		
Total	31	37.1072			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0707107	99.78%	99.58%	99.14%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.3906	0.0125	351.25	0.000	
Product					
KB5	1.0844	0.0217	50.09	0.000	1.50
KB6	0.9469	0.0217	43.73	0.000	1.50
KB7	-0.8531	0.0217	-39.40	0.000	1.50
Fermentation time (day)					
0.0	0.4469	0.0217	20.64	0.000	1.50
3.0	0.1281	0.0217	5.92	0.000	1.50
6.0	-0.1719	0.0217	-7.94	0.000	1.50
Product *Fermentation time (day)					
KB5 0.0	0.0281	0.0375	0.75	0.464	2.25
KB5 3.0	0.0219	0.0375	0.58	0.568	2.25
KB5 6.0	-0.0531	0.0375	-1.42	0.176	2.25
KB6 0.0	0.0656	0.0375	1.75	0.099	2.25
KB6 3.0	0.0094	0.0375	0.25	0.806	2.25
KB6 6.0	-0.0656	0.0375	-1.75	0.099	2.25
KB7 0.0	-0.1594	0.0375	-4.25	0.001	2.25
KB7 3.0	-0.1156	0.0375	-3.08	0.007	2.25
KB7 6.0	0.1094	0.0375	2.92	0.010	2.25

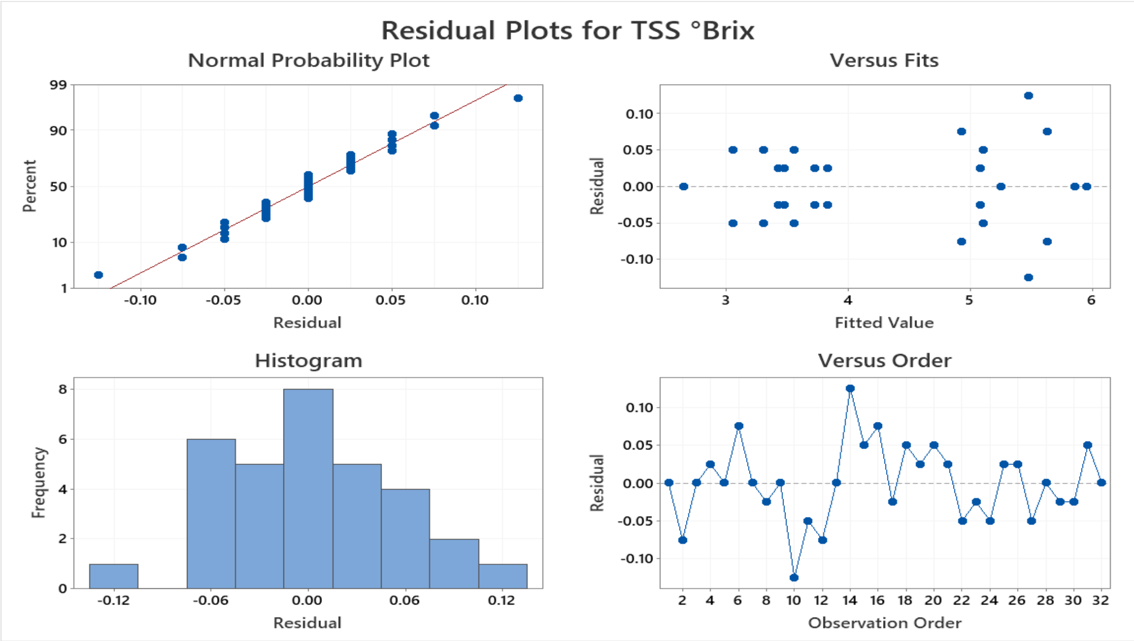
Regression Equation

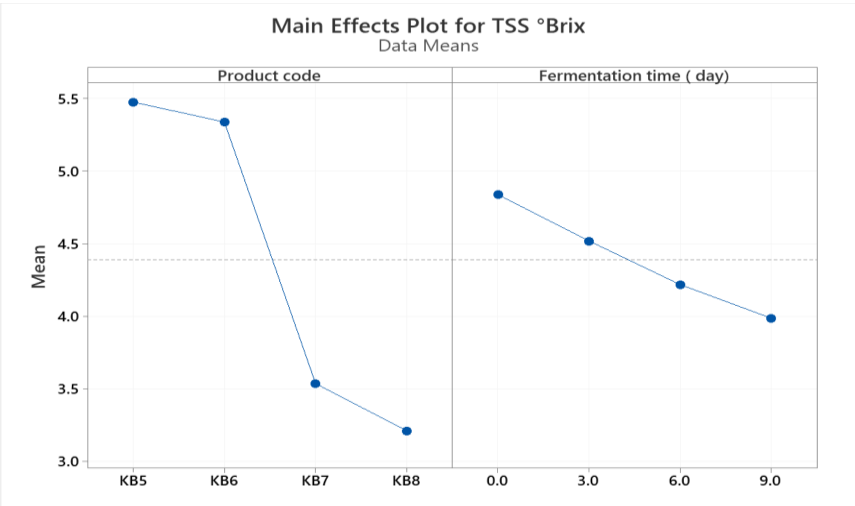
TSS °Brix = 4.3906 + 1.0844 Product_KB5 + 0.9469 Product _KB6
- 0.8531 Product code_KB7 - 1.1781 Product code_KB8
+ 0.4469 Fermentation time (day)_0.0
+ 0.1281 Fermentation time (day)_3.0
- 0.1719 Fermentation time (day)_6.0
- 0.4031 Fermentation time (day)_9.0
+ 0.0281 Product code*Fermentation time (day)_KB5 0.0
+ 0.0219 Product code*Fermentation time (day)_KB5 3.0
- 0.0531 Product code*Fermentation time (day)_KB5 6.0
+ 0.0031 Product code*Fermentation time (day)_KB5 9.0
+ 0.0656 Product code*Fermentation time (day)_KB6 0.0
+ 0.0094 Product code*Fermentation time (day)_KB6 3.0
- 0.0656 Product code*Fermentation time (day)_KB6 6.0
- 0.0094 Product code*Fermentation time (day)_KB6 9.0
- 0.1594 Product code*Fermentation time (day)_KB7 0.0
- 0.1156 Product code*Fermentation time (day)_KB7 3.0
+ 0.1094 Product code*Fermentation time (day)_KB7 6.0
+ 0.1656 Product code*Fermentation time (day)_KB7 9.0
+ 0.0656 Product code*Fermentation time (day)_KB8 0.0
+ 0.0844 Product code*Fermentation time (day)_KB8 3.0
+ 0.0094 Product code*Fermentation time (day)_KB8 6.0
- 0.1594 Product code*Fermentation time (day)_KB8 9.0

Fits and Diagnostics for Unusual Observations

Obs	TSS °Brix	Fit	Resid	Std Resid
10	5.3500	5.4750	-0.1250	-2.50 R
14	5.6000	5.4750	0.1250	2.50 R

R Large residual





General Linear Model: Colour(L) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	43.421	14.4735	23.95	0.000
Fermentation time (day)	3	153.710	51.2366	84.77	0.000
Product *Fermentation time (day)	9	103.479	11.4977	19.02	0.000
Error	16	9.671	0.6044		

Total	31	310.281

Model Summary

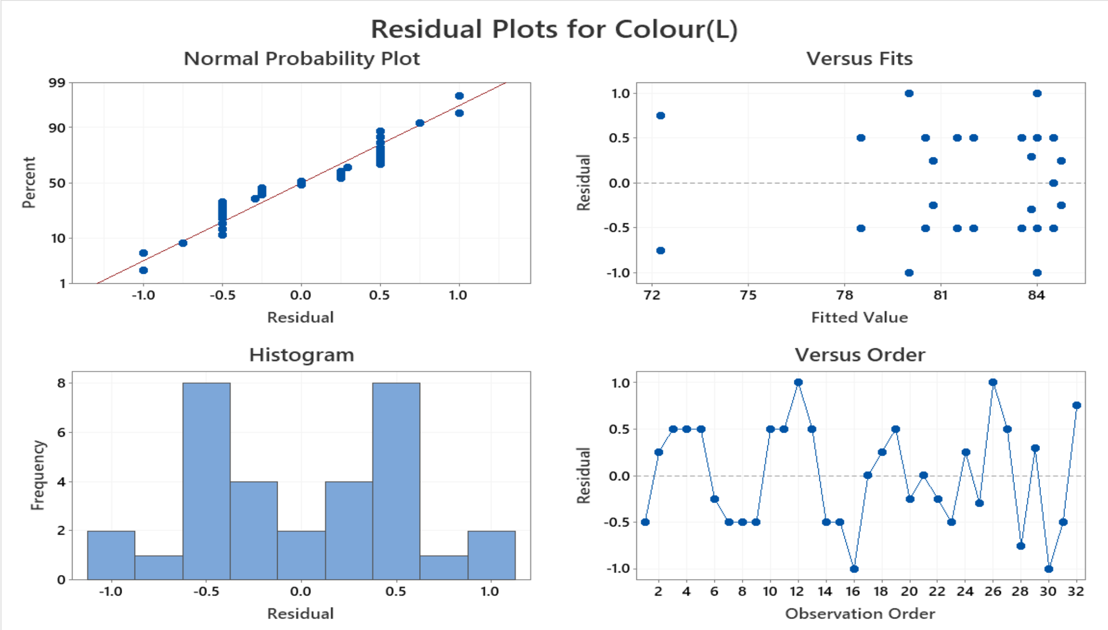
S	R-sq	R-sq(adj)	R-sq(pred)
0.777460	96.88%	93.96%	87.53%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	81.612	0.137	593.81	0.000	
Product					
KB5	-0.800	0.238	-3.36	0.004	1.50
KB6	-0.612	0.238	-2.57	0.021	1.50
KB7	2.013	0.238	8.46	0.000	1.50
Fermentation time (day)					
0.0	1.836	0.238	7.71	0.000	1.50
3.0	1.138	0.238	4.78	0.000	1.50
6.0	0.763	0.238	3.21	0.006	1.50
Product *Fermentation time (day)					
KB5 0.0	0.851	0.412	2.06	0.056	2.25
KB5 3.0	-1.200	0.412	-2.91	0.010	2.25
KB5 6.0	-1.075	0.412	-2.61	0.019	2.25
KB6 0.0	-0.836	0.412	-2.03	0.060	2.25
KB6 3.0	-0.638	0.412	-1.55	0.141	2.25
KB6 6.0	-1.263	0.412	-3.06	0.007	2.25
KB7 0.0	-0.961	0.412	-2.33	0.033	2.25
KB7 3.0	-0.013	0.412	-0.03	0.975	2.25
KB7 6.0	0.112	0.412	0.27	0.789	2.25

Regression Equation

Colour(L) = 81.612 - 0.800 Product code_KB5 - 0.612 Product code_KB6
+ 2.013 Product code_KB7
- 0.601 Product code_KB8 + 1.836 Fermentation time (day)_0.0
+ 1.138 Fermentation time (day)_3.0 + 0.763 Fermentation time (day)_6.0
- 3.737 Fermentation time (day)_9.0
+ 0.851 Product code*Fermentation time (day)_KB5 0.0
- 1.200 Product code*Fermentation time (day)_KB5 3.0
- 1.075 Product code*Fermentation time (day)_KB5 6.0
+ 1.425 Product code*Fermentation time (day)_KB5 9.0
- 0.836 Product code*Fermentation time (day)_KB6 0.0
- 0.638 Product code*Fermentation time (day)_KB6 3.0
- 1.263 Product code*Fermentation time (day)_KB6 6.0
+ 2.737 Product code*Fermentation time (day)_KB6 9.0
- 0.961 Product code*Fermentation time (day)_KB7 0.0
- 0.013 Product code*Fermentation time (day)_KB7 3.0
+ 0.112 Product code*Fermentation time (day)_KB7 6.0
+ 0.862 Product code*Fermentation time (day)_KB7 9.0
+ 0.946 Product code*Fermentation time (day)_KB8 0.0
+ 1.851 Product code*Fermentation time (day)_KB8 3.0
+ 2.226 Product code*Fermentation time (day)_KB8 6.0
- 5.024 Product code*Fermentation time (day)_KB8 9.0



General Linear Model: Colour(a) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product code	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	14.898	4.9661	30.27	0.000
Fermentation time (day)	3	5.523	1.8411	11.22	0.000
Product *Fermentation time (day)	9	2.633	0.2925	1.78	0.150
Error	16	2.625	0.1641		
Total	31	25.680			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.405046	89.78%	80.19%	59.11%

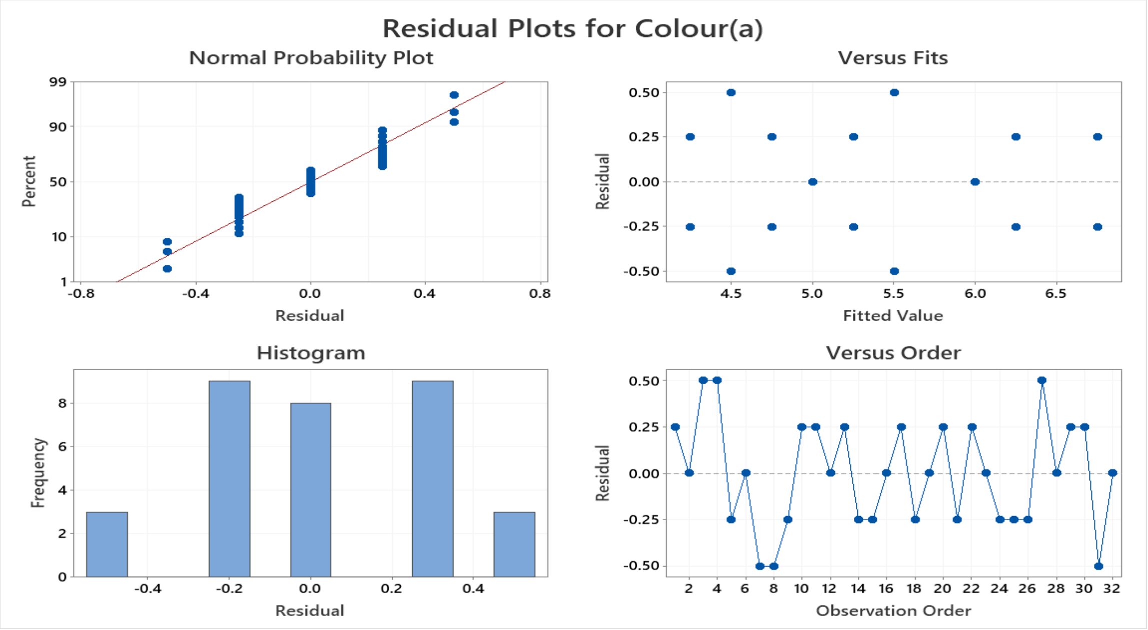
Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.5469	0.0716	77.47	0.000	
Product					
KB5	0.391	0.124	3.15	0.006	1.50
KB6	0.891	0.124	7.18	0.000	1.50
KB7	-0.859	0.124	-6.93	0.000	1.50
Fermentation time (day)					
0.0	0.703	0.124	5.67	0.000	1.50
3.0	-0.234	0.124	-1.89	0.077	1.50
6.0	-0.109	0.124	-0.88	0.391	1.50
Product *Fermentation time (day)					
KB5 0.0	0.109	0.215	0.51	0.618	2.25

KB5 3.0	0.297	0.215	1.38	0.186	2.25
KB5 6.0	-0.328	0.215	-1.53	0.146	2.25
KB6 0.0	-0.391	0.215	-1.82	0.088	2.25
KB6 3.0	0.047	0.215	0.22	0.830	2.25
KB6 6.0	0.422	0.215	1.96	0.067	2.25
KB7 0.0	-0.141	0.215	-0.65	0.522	2.25
KB7 3.0	-0.203	0.215	-0.95	0.358	2.25
KB7 6.0	0.422	0.215	1.96	0.067	2.25

Regression Equation

Colour(a) = 5.5469 + 0.391 Product code_KB5 + 0.891 Product code_KB6
- 0.859 Product code_KB7
- 0.422 Product code_KB8 + 0.703 Fermentation time (day)_0.0
- 0.234 Fermentation time (day)_3.0 - 0.109 Fermentation time (day)_6.0
- 0.359 Fermentation time (day)_9.0
+ 0.109 Product code*Fermentation time (day)_KB5 0.0
+ 0.297 Product code*Fermentation time (day)_KB5 3.0
- 0.328 Product code*Fermentation time (day)_KB5 6.0
- 0.078 Product code*Fermentation time (day)_KB5 9.0
- 0.391 Product code*Fermentation time (day)_KB6 0.0
+ 0.047 Product code*Fermentation time (day)_KB6 3.0
+ 0.422 Product code*Fermentation time (day)_KB6 6.0
- 0.078 Product code*Fermentation time (day)_KB6 9.0
- 0.141 Product code*Fermentation time (day)_KB7 0.0
- 0.203 Product code*Fermentation time (day)_KB7 3.0
+ 0.422 Product code*Fermentation time (day)_KB7 6.0
- 0.078 Product code*Fermentation time (day)_KB7 9.0
+ 0.422 Product code*Fermentation time (day)_KB8 0.0
- 0.141 Product code*Fermentation time (day)_KB8 3.0
- 0.516 Product code*Fermentation time (day)_KB8 6.0
+ 0.234 Product code*Fermentation time (day)_KB8 9.0



General Linear Model: Colour(b) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product code	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	65.473	21.8244	39.65	0.000
Fermentation time (day)	3	228.225	76.0748	138.21	0.000
Product *Fermentation time (day)	9	12.067	1.3408	2.44	0.058
Error	16	8.807	0.5504		
Total	31	314.572			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.741899	97.20%	94.58%	88.80%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	49.425	0.131	376.85	0.000	
Product code					
KB5	1.325	0.227	5.83	0.000	1.50
KB6	1.513	0.227	6.66	0.000	1.50
KB7	-1.183	0.227	-5.21	0.000	1.50
Fermentation time (day)					
0.0	3.899	0.227	17.16	0.000	1.50
3.0	0.763	0.227	3.36	0.004	1.50
6.0	-1.362	0.227	-6.00	0.000	1.50
Product code*Fermentation time (day)					
KB5 0.0	-0.149	0.393	-0.38	0.710	2.25
KB5 3.0	-0.013	0.393	-0.03	0.974	2.25
KB5 6.0	0.112	0.393	0.29	0.779	2.25
KB6 0.0	-0.337	0.393	-0.86	0.405	2.25
KB6 3.0	-0.700	0.393	-1.78	0.094	2.25
KB6 6.0	-0.325	0.393	-0.83	0.421	2.25
KB7 0.0	0.327	0.393	0.83	0.419	2.25
KB7 3.0	-0.255	0.393	-0.65	0.527	2.25
KB7 6.0	-0.130	0.393	-0.33	0.746	2.25

Regression Equation

Colour(b) = 49.425 + 1.325 Product code_KB5 + 1.513 Product code_KB6
- 1.183 Product code_KB7
- 1.655 Product code_KB8 + 3.899 Fermentation time (day)_0.0
+ 0.763 Fermentation time (day)_3.0 - 1.362 Fermentation time (day)_6.0
- 3.300 Fermentation time (day)_9.0
- 0.149 Product code*Fermentation time (day)_KB5 0.0
- 0.013 Product code*Fermentation time (day)_KB5 3.0
+ 0.112 Product code*Fermentation time (day)_KB5 6.0
+ 0.050 Product code*Fermentation time (day)_KB5 9.0
- 0.337 Product code*Fermentation time (day)_KB6 0.0
- 0.700 Product code*Fermentation time (day)_KB6 3.0
- 0.325 Product code*Fermentation time (day)_KB6 6.0
+ 1.362 Product code*Fermentation time (day)_KB6 9.0
+ 0.327 Product code*Fermentation time (day)_KB7 0.0
- 0.255 Product code*Fermentation time (day)_KB7 3.0
- 0.130 Product code*Fermentation time (day)_KB7 6.0
+ 0.058 Product code*Fermentation time (day)_KB7 9.0
+ 0.159 Product code*Fermentation time (day)_KB8 0.0
+ 0.968 Product code*Fermentation time (day)_KB8 3.0
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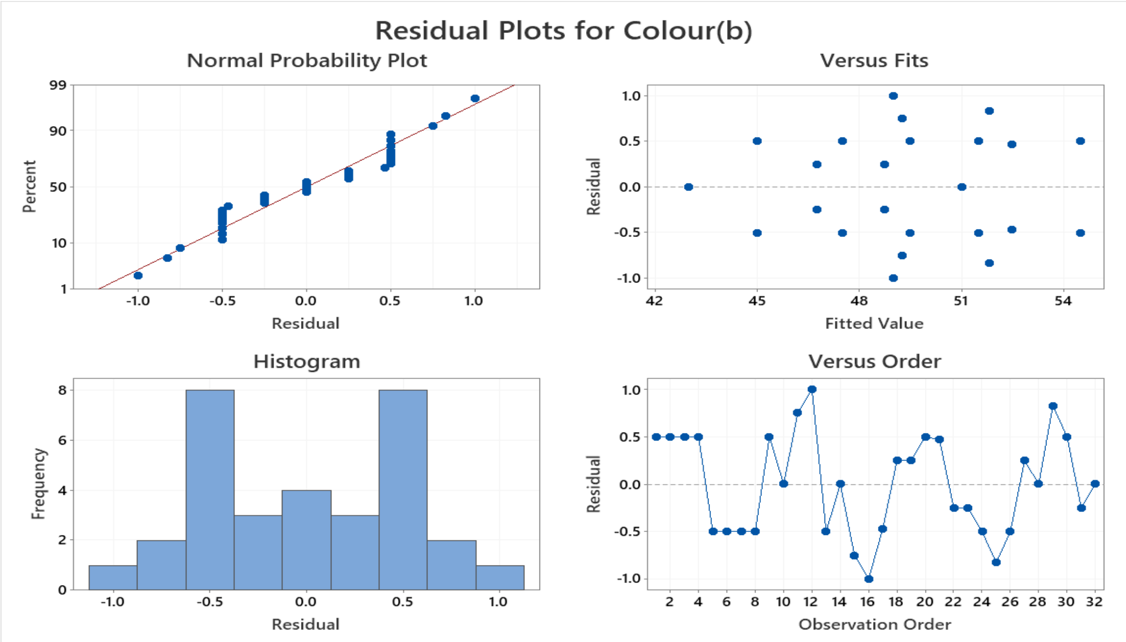
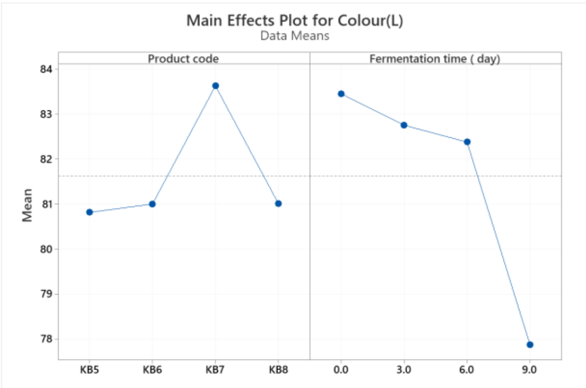
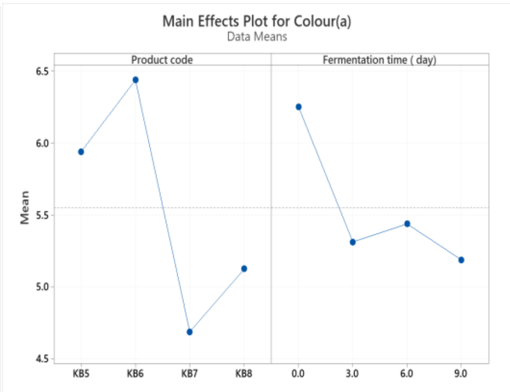
- 1.470 Product code*Fermentation time (day)_KB8 9.0


Residual Plots for Colour(b)
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Main Effects Plot for Colour(L)
Data Means
Product code
Fermentation time (day)
Mean
KB5
KB6
KB7
KB8
0.0
3.0
6.0
9.0


Main Effects Plot for Colour(a)
Data Means
Product code
Fermentation time (day)
Mean
KB5
KB6
KB7
KB8
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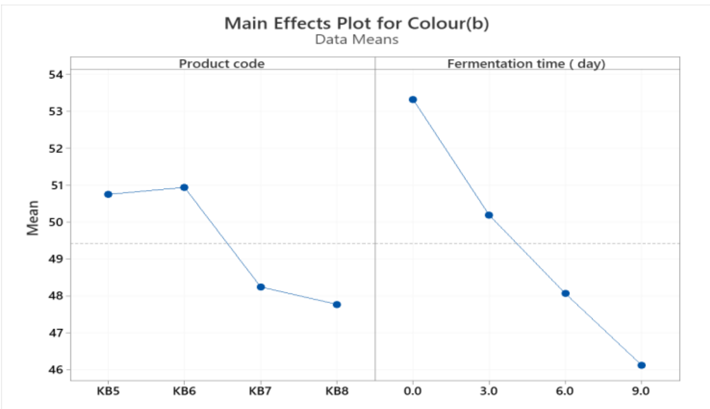
3.0
6.0
9.0


Main Effects Plot for Colour(b)
Data Means
Product code
Fermentation time (day)
Mean
KB5
KB6
KB7
KB8
0.0
3.0
6.0
9.0

General Linear Model: Yeast count (Fermented broth) log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.8228	0.27426	13.42	0.000
Fermentation time (day)	3	19.6242	6.54141	320.12	0.000
Product*Fermentation time (day)	9	3.2332	0.35924	17.58	0.000
Error	16	0.3270	0.02043		
Total	31	24.0071			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.142949	98.64%	97.36%	94.55%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.0066	0.0253	237.70	0.000	
Product					
KB5	0.0816	0.0438	1.86	0.081	1.50
KB6	0.0872	0.0438	1.99	0.064	1.50
KB7	0.1084	0.0438	2.48	0.025	1.50
Fermentation time (day)					
0.0	-1.2209	0.0438	-27.90	0.000	1.50
3.0	-0.0497	0.0438	-1.14	0.273	1.50
6.0	0.3591	0.0438	8.20	0.000	1.50
Product*Fermentation time (day)					
KB5 0.0	-0.5872	0.0758	-7.75	0.000	2.25
KB5 3.0	0.2616	0.0758	3.45	0.003	2.25
KB5 6.0	0.1203	0.0758	1.59	0.132	2.25
KB6 0.0	-0.3453	0.0758	-4.55	0.000	2.25
KB6 3.0	0.0459	0.0758	0.61	0.553	2.25
KB6 6.0	0.4022	0.0758	5.31	0.000	2.25
KB7 0.0	0.3659	0.0758	4.83	0.000	2.25
KB7 3.0	0.0947	0.0758	1.25	0.230	2.25
KB7 6.0	-0.2091	0.0758	-2.76	0.014	2.25

Regression Equation

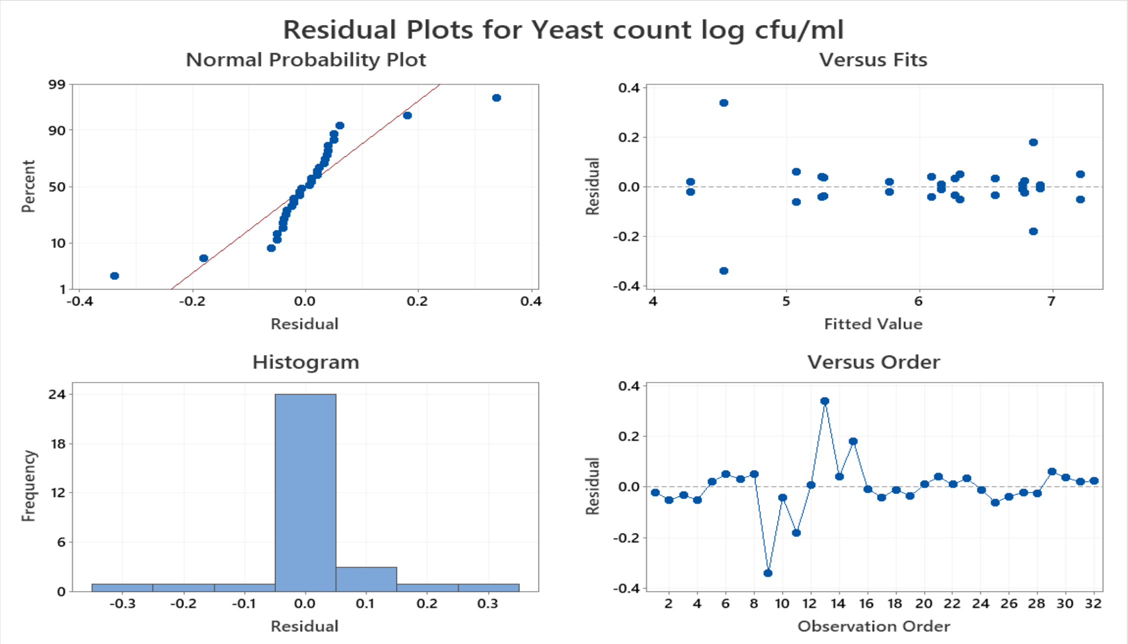
Yeast count log cfu/ml = 6.0066 + 0.0816 Product code_KB5
+ 0.0872 Product code_KB6
+ 0.1084 Product code_KB7 - 0.2772 Product code_KB8
- 1.2209 Fermentation time (day)_0.0
- 0.0497 Fermentation time (day)_3.0
+ 0.3591 Fermentation time (day)_6.0
+ 0.9116 Fermentation time (day)_9.0
- 0.5872 Product code*Fermentation time (day)_KB5 0.0
+ 0.2616 Product code*Fermentation time (day)_KB5 3.0
+ 0.1203 Product code*Fermentation time (day)_KB5 6.0
+ 0.2053 Product code*Fermentation time (day)_KB5 9.0
- 0.3453 Product code*Fermentation time (day)_KB6 0.0
+ 0.0459 Product code*Fermentation time (day)_KB6 3.0
+ 0.4022 Product code*Fermentation time (day)_KB6 6.0

- 0.1028 Product code*Fermentation time (day)_KB6 9.0
+ 0.3659 Product code*Fermentation time (day)_KB7 0.0
+ 0.0947 Product code*Fermentation time (day)_KB7 3.0
- 0.2091 Product code*Fermentation time (day)_KB7 6.0
- 0.2516 Product code*Fermentation time (day)_KB7 9.0
+ 0.5666 Product code*Fermentation time (day)_KB8 0.0
- 0.4022 Product code*Fermentation time (day)_KB8 3.0
- 0.3134 Product code*Fermentation time (day)_KB8 6.0
+ 0.1491 Product code*Fermentation time (day)_KB8 9.0

Fits and Diagnostics for Unusual Observations

Obs	Yeast count log cfu/ml	Fit	Resid	Std Resid
9	4.190	4.527	-0.337	-3.34 R
13	4.865	4.527	0.338	3.34 R

R Large residual



General Linear Model: AAB count (Fermented broth) log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding	(-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4	0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	2.0130	0.6710	263.13	0.000
Fermentation time (day)	3	32.0818	10.6939	4193.70	0.000

Product *Fermentation time (day)	9	2.1464	0.2385	93.53	0.000
Error	16	0.0408	0.0026		
Total	31	36.2820			

Model Summary

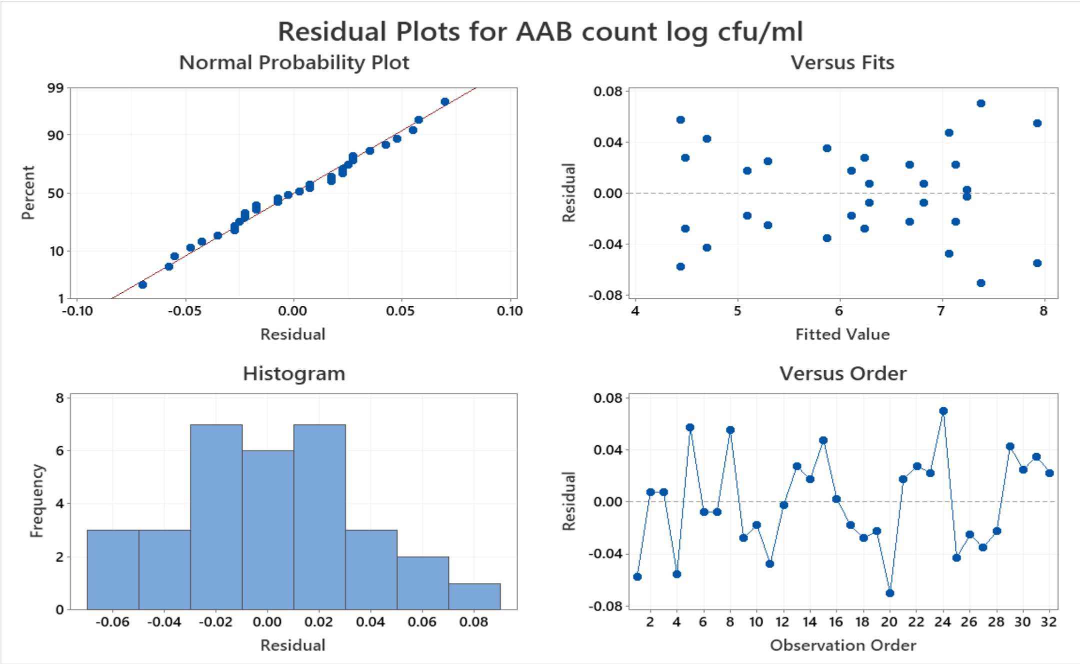
S	R-sq	R-sq(adj)	R-sq(pred)
0.0504975	99.89%	99.78%	99.55%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.17156	0.00893	691.35	0.000	
Product					
KB5	0.1966	0.0155	12.71	0.000	1.50
KB6	0.0534	0.0155	3.46	0.003	1.50
KB7	0.1741	0.0155	11.26	0.000	1.50
Fermentation time (day)					
0.0	-1.4941	0.0155	-96.63	0.000	1.50
3.0	-0.1897	0.0155	-12.27	0.000	1.50
6.0	0.4391	0.0155	28.40	0.000	1.50
Product *Fermentation time (day)					
KB5 0.0	-0.4316	0.0268	-16.11	0.000	2.25
KB5 3.0	0.1091	0.0268	4.07	0.001	2.25
KB5 6.0	0.0103	0.0268	0.39	0.705	2.25
KB6 0.0	-0.2434	0.0268	-9.09	0.000	2.25
KB6 3.0	0.0722	0.0268	2.70	0.016	2.25
KB6 6.0	0.4034	0.0268	15.06	0.000	2.25
KB7 0.0	0.2359	0.0268	8.81	0.000	2.25
KB7 3.0	0.0816	0.0268	3.05	0.008	2.25
KB7 6.0	-0.1022	0.0268	-3.82	0.002	2.25

Regression Equation

AAB count log cfu/ml = 6.17156 + 0.1966 Product code_KB5
+ 0.0534 Product code_KB6
+ 0.1741 Product code_KB7 - 0.4241 Product code_KB8
- 1.4941 Fermentation time (day)_0.0
- 0.1897 Fermentation time (day)_3.0
+ 0.4391 Fermentation time (day)_6.0
+ 1.2447 Fermentation time (day)_9.0
- 0.4316 Product code*Fermentation time (day)_KB5 0.0
+ 0.1091 Product code*Fermentation time (day)_KB5 3.0
+ 0.0103 Product code*Fermentation time (day)_KB5 6.0
+ 0.3122 Product code*Fermentation time (day)_KB5 9.0
- 0.2434 Product code*Fermentation time (day)_KB6 0.0
+ 0.0722 Product code*Fermentation time (day)_KB6 3.0
+ 0.4034 Product code*Fermentation time (day)_KB6 6.0
- 0.2322 Product code*Fermentation time (day)_KB6 9.0
+ 0.2359 Product code*Fermentation time (day)_KB7 0.0
+ 0.0816 Product code*Fermentation time (day)_KB7 3.0
- 0.1022 Product code*Fermentation time (day)_KB7 6.0
- 0.2153 Product code*Fermentation time (day)_KB7 9.0
+ 0.4391 Product code*Fermentation time (day)_KB8 0.0
- 0.2628 Product code*Fermentation time (day)_KB8 3.0
- 0.3116 Product code*Fermentation time (day)_KB8 6.0
+ 0.1353 Product code*Fermentation time (day)_KB8 9.0



General Linear Model: Total count (Fermented broth) log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	4 KB5, KB6, KB7, KB8
Fermentation time (day)	Fixed	4 0.0, 3.0, 6.0, 9.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	1.4965	0.4988	53.42	0.000
Fermentation time (day)	3	30.3761	10.1254	1084.29	0.000
Product *Fermentation time (day)	9	1.9687	0.2187	23.42	0.000
Error	16	0.1494	0.0093		
Total	31	33.9907			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0966348	99.56%	99.15%	98.24%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.2161	0.0171	363.88	0.000	
Product code					
KB5	0.1714	0.0296	5.79	0.000	1.50
KB6	0.0689	0.0296	2.33	0.033	1.50

KB7	0.1289	0.0296	4.36	0.000	1.50
Fermentation time (day)					
0.0	-1.4430	0.0296	-48.77	0.000	1.50
3.0	-0.1986	0.0296	-6.71	0.000	1.50
6.0	0.4158	0.0296	14.05	0.000	1.50
Product code*Fermentation time (day)					
KB5 0.0	-0.4695	0.0512	-9.16	0.000	2.25
KB5 3.0	0.1936	0.0512	3.78	0.002	2.25
KB5 6.0	-0.0108	0.0512	-0.21	0.836	2.25
KB6 0.0	-0.0395	0.0512	-0.77	0.452	2.25
KB6 3.0	0.0061	0.0512	0.12	0.907	2.25
KB6 6.0	0.4142	0.0512	8.08	0.000	2.25
KB7 0.0	0.1330	0.0512	2.59	0.020	2.25
KB7 3.0	0.0536	0.0512	1.05	0.311	2.25
KB7 6.0	-0.1608	0.0512	-3.14	0.006	2.25

Regression Equation

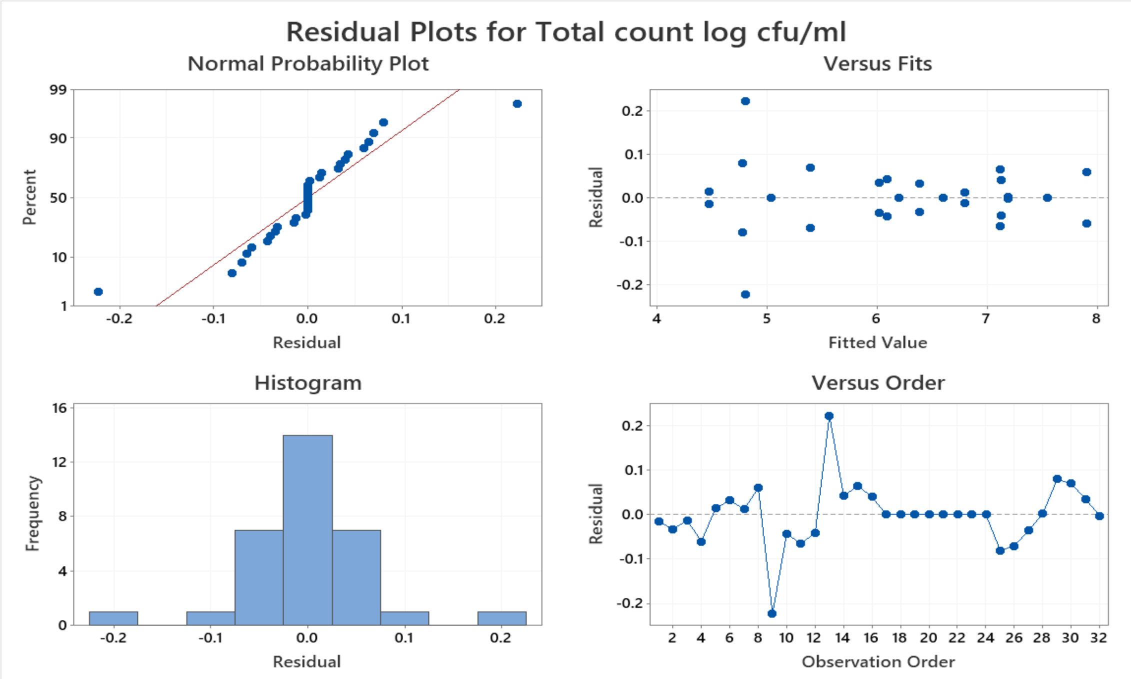
Total count log cfu/ml = 6.2161 + 0.1714 Product code_KB5
+ 0.0689 Product code_KB6
+ 0.1289 Product code_KB7
- 0.3692 Product code_KB8
- 1.4430 Fermentation time (day)_0.0
- 0.1986 Fermentation time (day)_3.0
+ 0.4158 Fermentation time (day)_6.0
+ 1.2258 Fermentation time (day)_9.0
- 0.4695 Product code*Fermentation time (day)_KB5 0.0
+ 0.1936 Product code*Fermentation time (day)_KB5 3.0
- 0.0108 Product code*Fermentation time (day)_KB5 6.0
+ 0.2867 Product code*Fermentation time (day)_KB5 9.0
- 0.0395 Product code*Fermentation time (day)_KB6 0.0
+ 0.0061 Product code*Fermentation time (day)_KB6 3.0
+ 0.4142 Product code*Fermentation time (day)_KB6 6.0
- 0.3808 Product code*Fermentation time (day)_KB6 9.0
+ 0.1330 Product code*Fermentation time (day)_KB7 0.0
+ 0.0536 Product code*Fermentation time (day)_KB7 3.0
- 0.1608 Product code*Fermentation time (day)_KB7 6.0
- 0.0258 Product code*Fermentation time (day)_KB7 9.0
+ 0.3761 Product code*Fermentation time (day)_KB8 0.0
- 0.2533 Product code*Fermentation time (day)_KB8 3.0
- 0.2427 Product code*Fermentation time (day)_KB8 6.0
+ 0.1198 Product code*Fermentation time (day)_KB8 9.0

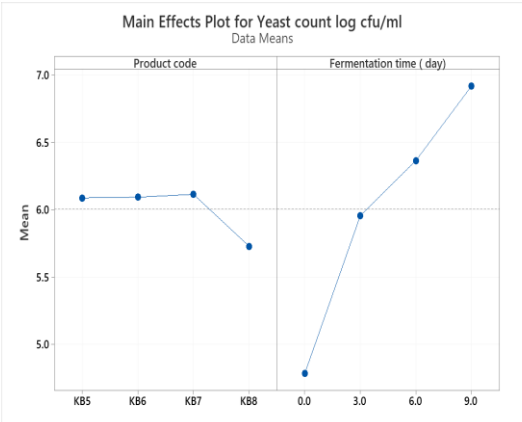
Fits and Diagnostics for Unusual Observations

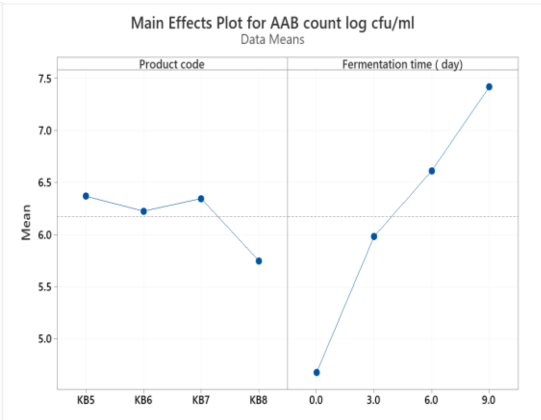
Obs	Total count log cfu/ml	Fit	Resid	Std Resid	
9	4.5800	4.8025	-0.2225	-3.26	R

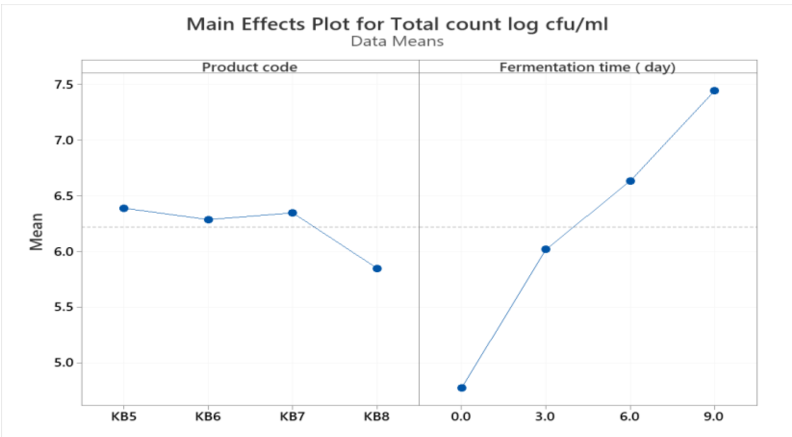
13	5.0250	4.8025	0.2225	3.26 R

R Large residual









H.3 Stage 2 (Phase 2): Potential of filtration for reducing the microbial counts in Kombucha following fermentation

General Linear Model: pH versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.06650	0.022167	32.10	0.000
Fermentation time (day)	4	1.57124	0.392809	568.77	0.000
Product *Fermentation time (day)	12	0.02761	0.002301	3.33	0.009
Error	20	0.01381	0.000691		
Total	39	1.67916			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0262797	99.18%	98.40%	96.71%

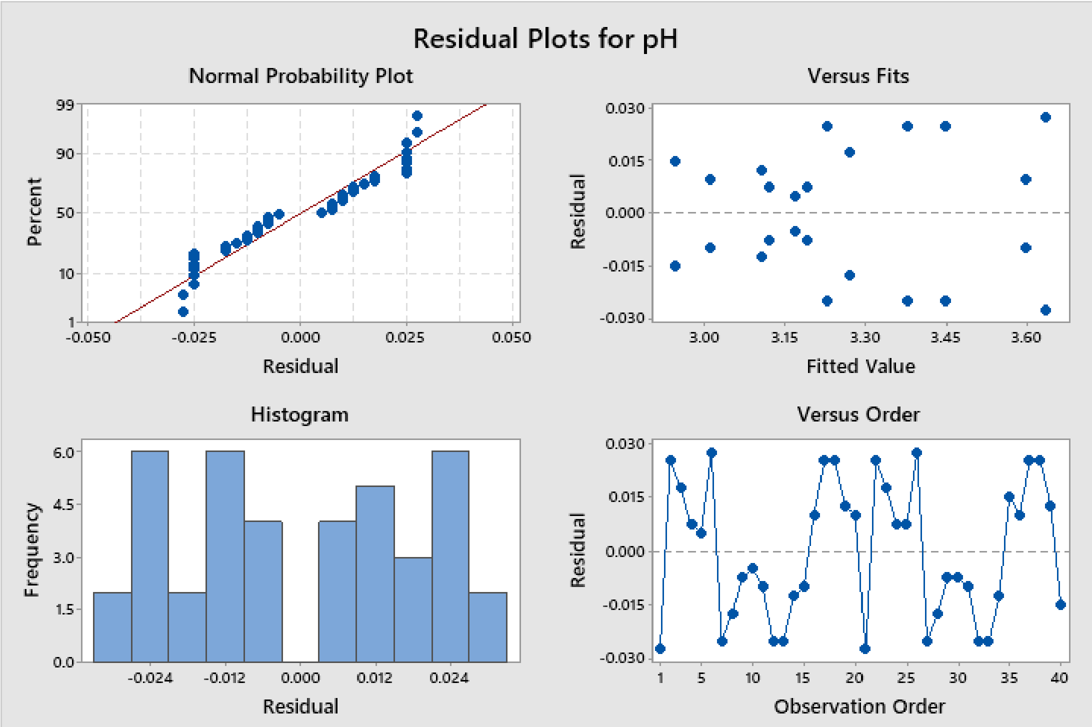
Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.29937	0.00416	794.04	0.000	
Product code					
KB5F	0.04513	0.00720	6.27	0.000	1.50
KB5U	0.03563	0.00720	4.95	0.000	1.50
KB6F	-0.03388	0.00720	-4.71	0.000	1.50
Fermentationt time (day)					
0.0	0.31938	0.00831	38.43	0.000	1.60
3.0	0.11563	0.00831	13.91	0.000	1.60
6.0	-0.04812	0.00831	-5.79	0.000	1.60
9.0	-0.14938	0.00831	-17.97	0.000	1.60
Product code*Fermentationt time (day)					
KB5F 0.0	-0.0264	0.0144	-1.83	0.082	2.40
KB5F 3.0	-0.0101	0.0144	-0.70	0.490	2.40
KB5F 6.0	-0.0239	0.0144	-1.66	0.113	2.40
KB5F 9.0	-0.0026	0.0144	-0.18	0.857	2.40
KB5U 0.0	-0.0169	0.0144	-1.17	0.255	2.40
KB5U 3.0	-0.0006	0.0144	-0.04	0.966	2.40
KB5U 6.0	-0.0144	0.0144	-1.00	0.330	2.40
KB5U 9.0	0.0069	0.0144	0.48	0.638	2.40
KB6F 0.0	0.0151	0.0144	1.05	0.306	2.40
KB6F 3.0	-0.0011	0.0144	-0.08	0.938	2.40
KB6F 6.0	0.0126	0.0144	0.88	0.391	2.40
KB6F 9.0	-0.0086	0.0144	-0.60	0.556	2.40

Regression Equation

pH = 3.29937 + 0.04513 Product code_KB5F + 0.03563 Product code_KB5U
- 0.03388 Product code_KB6F - 0.04687 Product code_KB6U
+ 0.31938 Fermentationt time (day)_0.0

+ 0.11563 Fermentation time (day)_3.0
- 0.04812 Fermentation time (day)_6.0
- 0.14938 Fermentation time (day)_9.0
- 0.23750 Fermentation time (day)_16.0
- 0.0264 Product code*Fermentation time (day)_KB5F 0.0
- 0.0101 Product code*Fermentation time (day)_KB5F 3.0
- 0.0239 Product code*Fermentation time (day)_KB5F 6.0
- 0.0026 Product code*Fermentation time (day)_KB5F 9.0
+ 0.0630 Product code*Fermentation time (day)_KB5F 16.0
- 0.0169 Product code*Fermentation time (day)_KB5U 0.0
- 0.0006 Product code*Fermentation time (day)_KB5U 3.0
- 0.0144 Product code*Fermentation time (day)_KB5U 6.0
+ 0.0069 Product code*Fermentation time (day)_KB5U 9.0
+ 0.0250 Product code*Fermentation time (day)_KB5U 16.0
+ 0.0151 Product code*Fermentation time (day)_KB6F 0.0
- 0.0011 Product code*Fermentation time (day)_KB6F 3.0
+ 0.0126 Product code*Fermentation time (day)_KB6F 6.0
- 0.0086 Product code*Fermentation time (day)_KB6F 9.0
- 0.0180 Product code*Fermentation time (day)_KB6F 16.0
+ 0.0281 Product code*Fermentation time (day)_KB6U 0.0
+ 0.0119 Product code*Fermentation time (day)_KB6U 3.0
+ 0.0256 Product code*Fermentation time (day)_KB6U 6.0
+ 0.0044 Product code*Fermentation time (day)_KB6U 9.0
- 0.0700 Product code*Fermentation time (day)_KB6U 16.0



General Linear Model: Titratable acidity % versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product code	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.013222	0.004407	14.13	0.000
Fermentation time (day)	4	0.650373	0.162593	521.34	0.000
Product *Fermentation time (day)	12	0.011787	0.000982	3.15	0.011
Error	20	0.006238	0.000312		
Total	39	0.681619			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0176600	99.08%	98.22%	96.34%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.24663	0.00279	88.32	0.000	
Product					
KB5F	- 0.02113	0.00484	-4.37	0.000	1.50
KB5U	- 0.01412	0.00484	-2.92	0.008	1.50
KB6F	0.01238	0.00484	2.56	0.019	1.50
Fermentation time (day)					
0.0	- 0.17162	0.00558	-30.73	0.000	1.60
3.0	- 0.12163	0.00558	-21.78	0.000	1.60
6.0	0.03462	0.00558	6.20	0.000	1.60
9.0	0.09463	0.00558	16.94	0.000	1.60
Product *Fermentation time (day)					
KB5F 0.0	0.02362	0.00967	2.44	0.024	2.40
KB5F 3.0	0.01363	0.00967	1.41	0.174	2.40
KB5F 6.0	- 0.00262	0.00967	-0.27	0.789	2.40
KB5F 9.0	0.00238	0.00967	0.25	0.809	2.40
KB5U 0.0	0.01663	0.00967	1.72	0.101	2.40
KB5U 3.0	0.00662	0.00967	0.68	0.501	2.40
KB5U 6.0	- 0.00963	0.00967	-1.00	0.332	2.40
KB5U 9.0	- 0.00463	0.00967	-0.48	0.638	2.40
KB6F 0.0	- 0.01488	0.00967	-1.54	0.140	2.40
KB6F 3.0	- 0.00488	0.00967	-0.50	0.620	2.40
KB6F 6.0	0.01137	0.00967	1.18	0.253	2.40
KB6F 9.0	0.00638	0.00967	0.66	0.517	2.40

Regression Equation

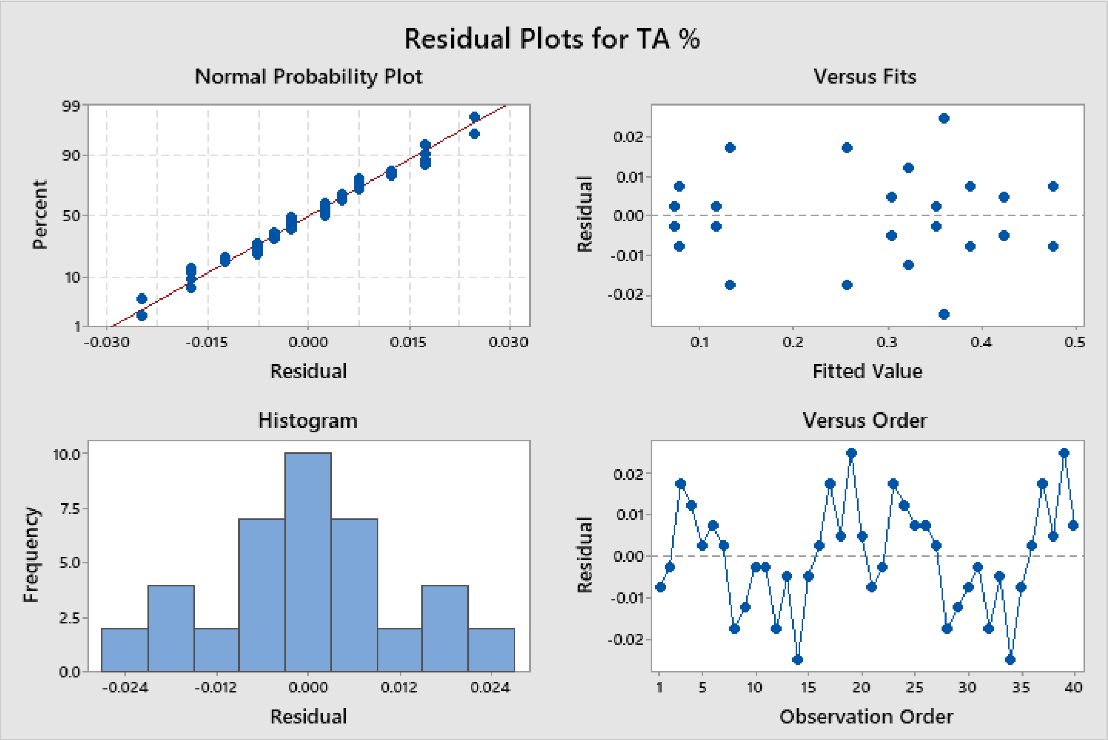
TA % = 0.24663 - 0.02113 Product code_KB5F - 0.01412 Product code_KB5U
+ 0.01238 Product code_KB6F + 0.02288 Product code_KB6U
- 0.17162 Fermentationt time (day)_0.0
- 0.12163 Fermentation time (day)_3.0
+ 0.03462 Fermentation time (day)_6.0
+ 0.09463 Fermentation time (day)_9.0
+ 0.16400 Fermentation time (day)_16.0
+ 0.02362 Product code*Fermentation time (day)_KB5F 0.0
+ 0.01363 Product code*Fermentation time (day)_KB5F 3.0
- 0.00262 Product code*Fermentation time (day)_KB5F 6.0
+ 0.00238 Product code*Fermentation time (day)_KB5F 9.0
- 0.03700 Product code*Fermentation time (day)_KB5F 16.0
+ 0.01663 Product code*Fermentation time (day)_KB5U 0.0
+ 0.00662 Product code*Fermentation time (day)_KB5U 3.0
- 0.00963 Product code*Fermentation time (day)_KB5U 6.0
- 0.00463 Product code*Fermentation time (day)_KB5U 9.0
- 0.00900 Product code*Fermentation time (day)_KB5U 16.0
- 0.01488 Product code*Fermentation time (day)_KB6F 0.0

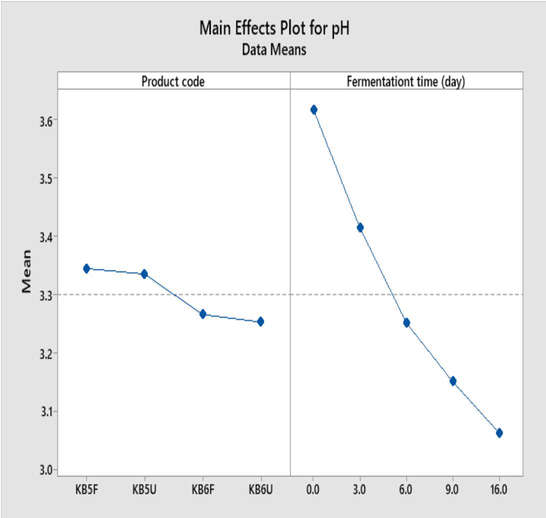
- 0.00488 Product code*Fermentation time (day)_KB6F 3.0
+ 0.01137 Product code*Fermentation time (day)_KB6F 6.0
+ 0.00638 Product code*Fermentation time (day)_KB6F 9.0
+ 0.00200 Product code*Fermentation time (day)_KB6F W1
- 0.02537 Product code*Fermentation time (day)_KB6U 0.0
- 0.01537 Product code*Fermentation time (day)_KB6U 3.0
+ 0.00088 Product code*Fermentation time (day)_KB6U 6.0
- 0.00413 Product code*Fermentation time (day)_KB6U 9.0
+ 0.04400 Product code*Fermentation time (day)_KB6U W1

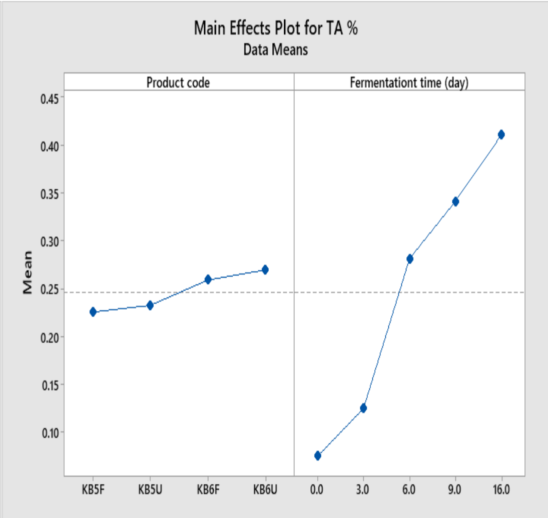
Fits and Diagnostics for Unusual Observations

Orbs	TA %	Fit	Reside	Std Reside
14	0.3350	0.3600	-0.0250	-2.00 R
19	0.3850	0.3600	0.0250	2.00 R
34	0.3350	0.3600	-0.0250	-2.00 R
39	0.3850	0.3600	0.0250	2.00 R

R Large residual







General Linear Model: TSS °Brix versus Product code and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product code	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Ad SS	Adj MS	F-Value	P-Value
Product	3	0.2183	0.07275	10.58	0.000
Fermentation time (day)	4	7.5596	1.88991	274.90	0.000
Product *Fermentation time (day)	12	0.1274	0.01061	1.54	0.189
Error	20	0.1375	0.00688		
Total	39	8.0427			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0829156	98.29%	96.67%	93.16%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.2425	0.0131	399.88	0.000	
Product					
KB5F	0.0725	0.0227	3.19	0.005	1.50
KB5U	0.0725	0.0227	3.19	0.005	1.50
KB6F	-0.0525	0.0227	-2.31	0.032	1.50
Fermentation time (day)					
0.0	0.6450	0.0262	24.60	0.000	1.60
3.0	0.2950	0.0262	11.25	0.000	1.60
6.0	-0.0800	0.0262	-3.05	0.006	1.60
9.0	-0.2488	0.0262	-9.49	0.000	1.60
Product *Fermentation time (day)					
KB5F 0.0	-0.0600	0.0454	-1.32	0.201	2.40
KB5F 3.0	-0.0350	0.0454	-0.77	0.450	2.40
KB5F 6.0	-0.0350	0.0454	-0.77	0.450	2.40

KB5F 9.0	-0.0412	0.0454	-0.91	0.375	2.40
KB5U 0.0	-0.0100	0.0454	-0.22	0.828	2.40
KB5U 3.0	0.0150	0.0454	0.33	0.745	2.40
KB5U 6.0	0.0150	0.0454	0.33	0.745	2.40
KB5U 9.0	0.0087	0.0454	0.19	0.849	2.40
KB6F 0.0	0.0150	0.0454	0.33	0.745	2.40
KB6F 3.0	-0.0100	0.0454	-0.22	0.828	2.40
KB6F 6.0	-0.0100	0.0454	-0.22	0.828	2.40
KB6F 9.0	0.0087	0.0454	0.19	0.849	2.40

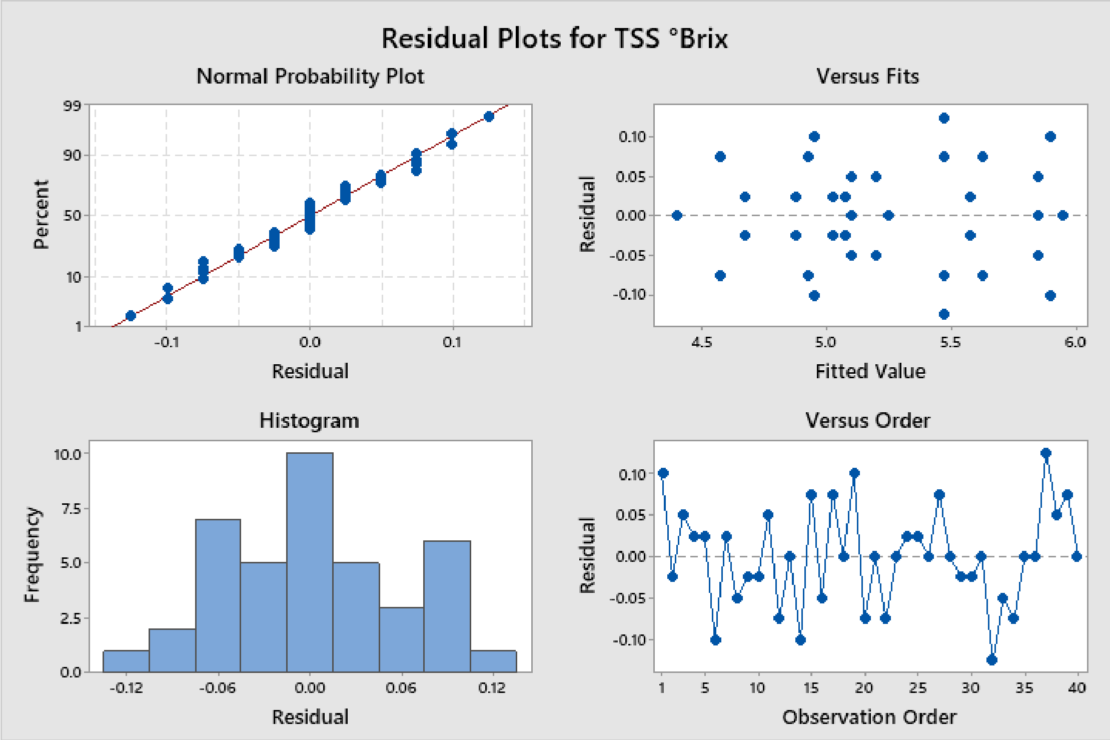
Regression Equation

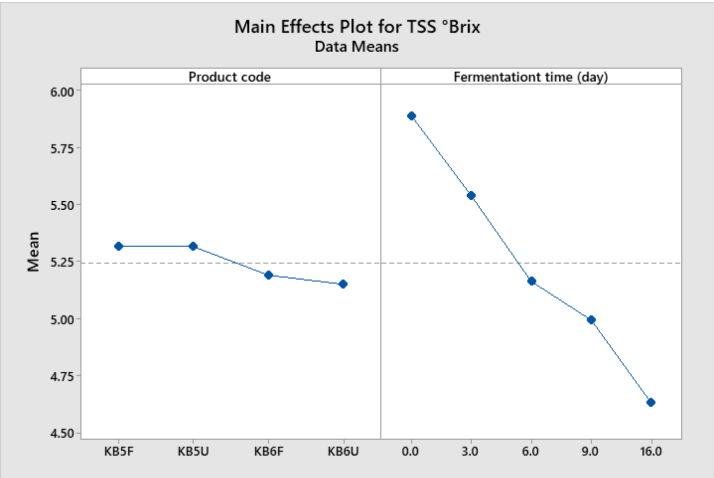
TSS °Brix = 5.2425 + 0.0725 Product code_KB5F + 0.0725 Product code_KB5U
- 0.0525 Product code_KB6F - 0.0925 Product code_KB6U
+ 0.6450 Fermentationt time (day)_0.0
+ 0.2950 Fermentationt time (day)_3.0
- 0.0800 Fermentation time (day)_6.0
- 0.2488 Fermentation time (day)_9.0
- 0.6112 Fermentation time (day)_16.0
- 0.0600 Product code*Fermentation time (day)_KB5F 0.0
- 0.0350 Product code*Fermentation time (day)_KB5F 3.0
- 0.0350 Product code*Fermentation time (day)_KB5F 6.0
- 0.0412 Product code*Fermentation time (day)_KB5F 9.0
+ 0.1712 Product code*Fermentation time (day)_KB5F 16.0
- 0.0100 Product code*Fermentation time (day)_KB5U 0.0
+ 0.0150 Product code*Fermentation time (day)_KB5U 3.0
+ 0.0150 Product code*Fermentation time (day)_KB5U 6.0
+ 0.0087 Product code*Fermentation time (day)_KB5U 9.0
- 0.0287 Product code*Fermentation time (day)_KB5U 16.0
+ 0.0150 Product code*Fermentation time (day)_KB6F 0.0
- 0.0100 Product code*Fermentation time (day)_KB6F 3.0
- 0.0100 Product code*Fermentation time (day)_KB6F 6.0
+ 0.0087 Product code*Fermentation time (day)_KB6F 9.0
- 0.0038 Product code*Fermentation time (day)_KB6F 16.0
+ 0.0550 Product code*Fermentation time (day)_KB6U 0.0
+ 0.0300 Product code*Fermentation time (day)_KB6U 3.0
+ 0.0300 Product code*Fermentation time (day)_KB6U 6.0
+ 0.0238 Product code*Fermentation time (day)_KB6U 9.0
- 0.1387 Product code*Fermentation time (day)_KB6U 16.0

Fits and Diagnostics for Unusual Observations

Orbs	TSS °Brix	Fit	Reside	Std Reside
32	5.3500	5.4750	-0.1250	-2.13 R
37	5.6000	5.4750	0.1250	2.13 R

R Large residual





General Linear Model: Colour(L) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	4 KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5 0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Ad SS	Adj MS	F-Value	P-Value
Product	3	3.025	1.0083	7.33	0.002
Fermentation time (day)	4	161.250	40.3125	293.18	0.000
Product *Fermentation time (day)	12	0.850	0.0708	0.52	0.880
Error	20	2.750	0.1375		
Total	39	167.875			

Model Summary

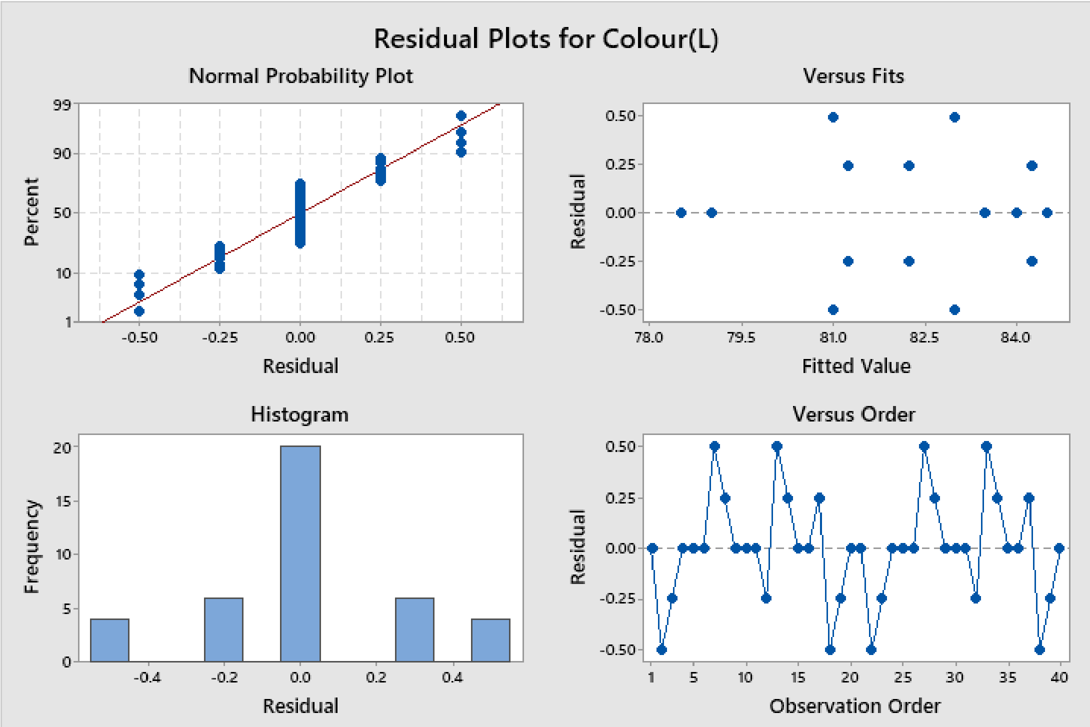
S	R-sq	R-sq(adj)	R-sq(pred)
0.370810	98.36%	96.81%	93.45%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	82.1250	0.0586	1400.73	0.000	
Product					
KB5F	-0.275	0.102	-2.71	0.014	1.50
KB5U	-0.275	0.102	-2.71	0.014	1.50
KB6F	0.275	0.102	2.71	0.014	1.50
Fermentation time (day)					
0.0	-3.375	0.117	-28.78	0.000	1.60
3.0	-1.000	0.117	-8.53	0.000	1.60
6.0	0.500	0.117	4.26	0.000	1.60
9.0	2.000	0.117	17.06	0.000	1.60
Product*Fermentation time (day)					
KB5F 0.0	0.025	0.203	0.12	0.903	2.40
KB5F 3.0	0.150	0.203	0.74	0.469	2.40
KB5F 6.0	-0.100	0.203	-0.49	0.628	2.40
KB5F 9.0	0.150	0.203	0.74	0.469	2.40
KB5U 0.0	0.025	0.203	0.12	0.903	2.40
KB5U 3.0	0.150	0.203	0.74	0.469	2.40
KB5U 6.0	-0.100	0.203	-0.49	0.628	2.40
KB5U 9.0	0.150	0.203	0.74	0.469	2.40
KB6F 0.0	-0.025	0.203	-0.12	0.903	2.40
KB6F 3.0	-0.150	0.203	-0.74	0.469	2.40
KB6F 6.0	0.100	0.203	0.49	0.628	2.40
KB6F 9.0	-0.150	0.203	-0.74	0.469	2.40

Regression Equation

Colour(L) = 82.1250 - 0.275 Product code_KB5F - 0.275 Product code_KB5U
+ 0.275 Product code_KB6F + 0.275 Product code_KB6U
- 3.375 Fermentation time (day)_0.0
- 1.000 Fermentation time (day)_3.0
+ 0.500 Fermentationt time (day)_6.0
+ 2.000 Fermentationt time (day)_9.0
+ 1.875 Fermentationt time (day)_16.0
+ 0.025 Product code*Fermentation time (day)_KB5F 0.0
+ 0.150 Product code*Fermentation time (day)_KB5F 3.0
- 0.100 Product code*Fermentation time (day)_KB5F 6.0
+ 0.150 Product code*Fermentation time (day)_KB5F 9.0
- 0.225 Product code*Fermentation time (day)_KB5F 16.0
+ 0.025 Product code*Fermentation time (day)_KB5U 0.0
+ 0.150 Product code*Fermentation time (day)_KB5U 3.0
- 0.100 Product code*Fermentation time (day)_KB5U 6.0
+ 0.150 Product code*Fermentation time (day)_KB5U 9.0
- 0.225 Product code*Fermentation time (day)_KB5U 16.0
- 0.025 Product code*Fermentation time (day)_KB6F 0.0
- 0.150 Product code*Fermentation time (day)_KB6F 3.0
+ 0.100 Product code*Fermentation time (day)_KB6F 6.0
- 0.150 Product code*Fermentation time (day)_KB6F 9.0
+ 0.225 Product code*Fermentation time (day)_KB6F 16.0
- 0.025 Product code*Fermentation time (day)_KB6U 0.0
- 0.150 Product code*Fermentation time (day)_KB6U 3.0
+ 0.100 Product code*Fermentation time (day)_KB6U 6.0
- 0.150 Product code*Fermentation time (day)_KB6U 9.0
+ 0.225 Product code*Fermentation time (day)_KB6U 16.0



General Linear Model: Colour(a) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product code	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Ad SS	Adj MS	F-Value	P-Value
Product	3	0.02025	0.006750	10.80	0.000
Fermentation time (day)	4	3.15044	0.787610	1260.18	0.000
Product*Fermentation time (day)	12	0.08100	0.006750	10.80	0.000
Error	20	0.01250	0.000625		
Total	39	3.26419			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.025	99.62%	99.25%	98.47%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.59050	0.00395	1414.30	0.000	
Product					
KB5F	0.02250	0.00685	3.29	0.004	1.50
KB5U	0.02250	0.00685	3.29	0.004	1.50

KB6F	-0.02250	0.00685	-3.29	0.004	1.50
Fermentationt time (day)					
0.0	0.35950	0.00791	45.47	0.000	1.60
3.0	0.15950	0.00791	20.18	0.000	1.60
6.0	0.04950	0.00791	6.26	0.000	1.60
9.0	-0.09050	0.00791	-11.45	0.000	1.60
Product code*Fermentation time (day)					
KB5F 0.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5F 3.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5F 6.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5F 9.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5U 0.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5U 3.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5U 6.0	-0.0225	0.0137	-1.64	0.116	2.40
KB5U 9.0	-0.0225	0.0137	-1.64	0.116	2.40
KB6F 0.0	0.0225	0.0137	1.64	0.116	2.40
KB6F 3.0	0.0225	0.0137	1.64	0.116	2.40
KB6F 6.0	0.0225	0.0137	1.64	0.116	2.40
KB6F 9.0	0.0225	0.0137	1.64	0.116	2.40

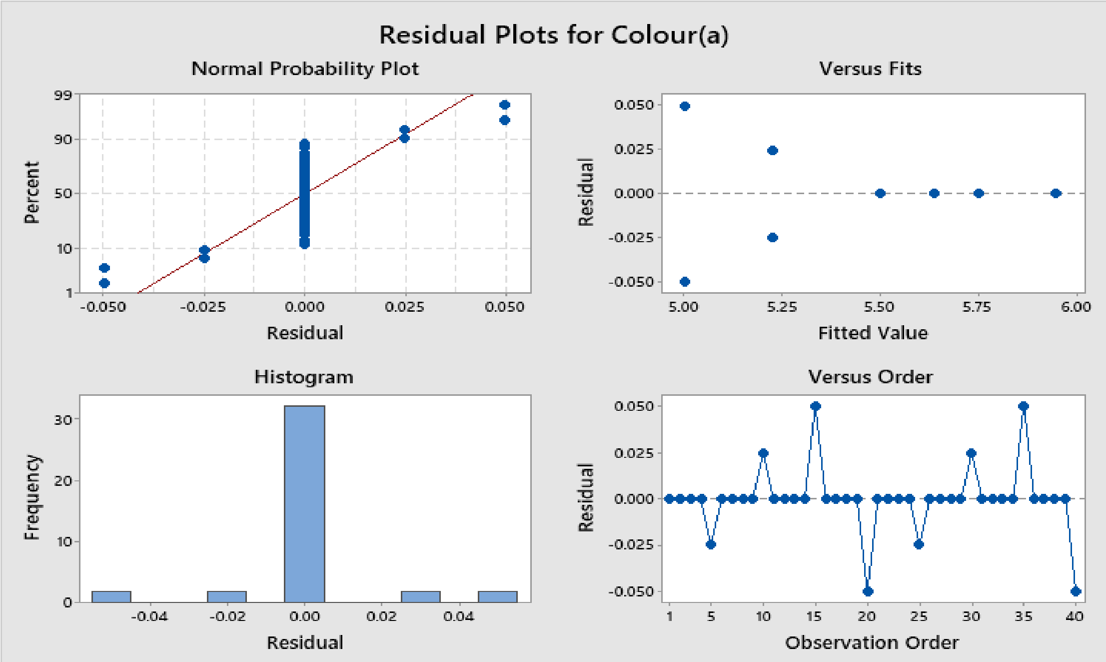
Regression Equation

Colour(a) = 5.59050 + 0.02250 Product code_KB5F
+ 0.02250 Product code_KB5U
- 0.02250 Product code_KB6F - 0.02250 Product code_KB6U
+ 0.35950 Fermentation time (day)_0.0
+ 0.15950 Fermentation time (day)_3.0
+ 0.04950 Fermentation time (day)_6.0
- 0.09050 Fermentation time (day)_9.0
- 0.47800 Fermentation time (day)_16.0
- 0.0225 Product code*Fermentation time (day)_KB5F 0.0
- 0.0225 Product code*Fermentation time (day)_KB5F 3.0
- 0.0225 Product code*Fermentation time (day)_KB5F 6.0
- 0.0225 Product code*Fermentation time (day)_KB5F 9.0
+ 0.0900 Product code*Fermentation time (day)_KB5F 16.0
- 0.0225 Product code*Fermentation time (day)_KB5U 0.0
- 0.0225 Product code*Fermentation time (day)_KB5U 3.0
- 0.0225 Product code*Fermentation time (day)_KB5U 6.0
- 0.0225 Product code*Fermentation time (day)_KB5U 9.0
+ 0.0900 Product code*Fermentation time (day)_KB5U 16.0
+ 0.0225 Product code*Fermentation time (day)_KB6F 0.0
+ 0.0225 Product code*Fermentation time (day)_KB6F 3.0
+ 0.0225 Product code*Fermentation time (day)_KB6F 6.0
+ 0.0225 Product code*Fermentation time (day)_KB6F 9.0
- 0.0900 Product code*Fermentation time (day)_KB6F 16.0
+ 0.0225 Product code*Fermentation time (day)_KB6U 0.0
+ 0.0225 Product code*Fermentation time (day)_KB6U 3.0
+ 0.0225 Product code*Fermentation time (day)_KB6U 6.0
+ 0.0225 Product code*Fermentation time (day)_KB6U 9.0
- 0.0900 Product code*Fermentation time (day)_KB6U 16.0

Fits and Diagnostics for Unusual Observations

Obs	Colour(a)	Fit	Resid	Std Resid
15	5.0500	5.0000	0.0500	2.83 R
20	4.9500	5.0000	-0.0500	-2.83 R
35	5.0500	5.0000	0.0500	2.83 R
40	4.9500	5.0000	-0.0500	-2.83 R

R Large residual



General Linear Model: Colour(b) versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	4 KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5 0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	1.22	0.408	0.01	0.999
Fermentation time (day)	4	877.75	219.437	4.00	0.015
Product *Fermentation time (day)	12	5.15	0.429	0.01	1.000
Error	20	1097.75	54.887		
Total	39	1981.87			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.40861	44.61%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	47.38	1.17	40.44	0.000	
Product code					
KB5F	-0.17	2.03	-0.09	0.932	1.50
KB5U	-0.18	2.03	-0.09	0.932	1.50

KB6F	0.17	2.03	0.09	0.932	1.50
Fermentation time (day)					
0.0	6.50	2.34	2.77	0.012	1.60
3.0	-7.75	2.34	-3.31	0.004	1.60
6.0	2.00	2.34	0.85	0.403	1.60
9.0	0.88	2.34	0.37	0.713	1.60
Product code*Fermentation time (day)					
KB5F 0.0	-0.20	4.06	-0.05	0.961	2.40
KB5F 3.0	0.30	4.06	0.07	0.942	2.40
KB5F 6.0	0.05	4.06	0.01	0.990	2.40
KB5F 9.0	-0.57	4.06	-0.14	0.889	2.40
KB5U 0.0	-0.20	4.06	-0.05	0.961	2.40
KB5U 3.0	0.30	4.06	0.07	0.942	2.40
KB5U 6.0	0.05	4.06	0.01	0.990	2.40
KB5U 9.0	-0.58	4.06	-0.14	0.889	2.40
KB6F 0.0	0.20	4.06	0.05	0.961	2.40
KB6F 3.0	-0.30	4.06	-0.07	0.942	2.40
KB6F 6.0	-0.05	4.06	-0.01	0.990	2.40
KB6F 9.0	0.58	4.06	0.14	0.889	2.40

Regression Equation

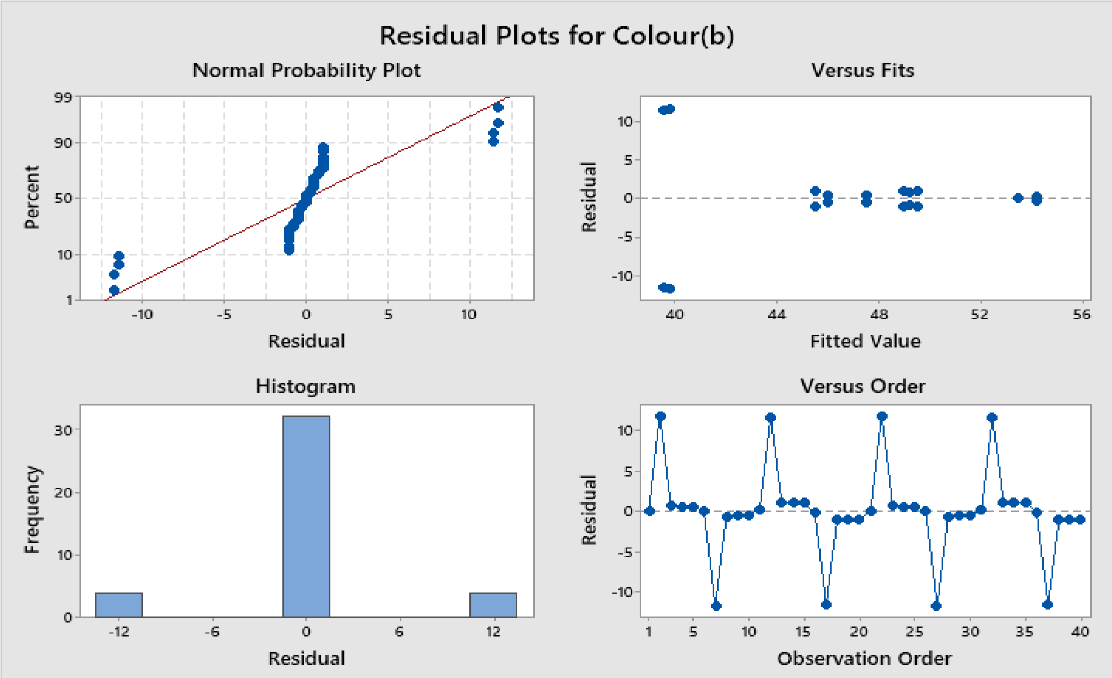
Colour(b) = 47.38 - 0.17 Product code_KB5F - 0.18 Product code_KB5U
+ 0.17 Product code_KB6F
+ 0.17 Product code_KB6U + 6.50 Fermentation time (day)_0.0
- 7.75 Fermentation time (day)_3.0
+ 2.00 Fermentation time (day)_6.0
+ 0.88 Fermentation time (day)_9.0
- 1.63 Fermentation time (day)_16.0
- 0.20 Product code*Fermentation time (day)_KB5F 0.0
+ 0.30 Product code*Fermentation time (day)_KB5F 3.0
+ 0.05 Product code*Fermentation time (day)_KB5F 6.0
- 0.57 Product code*Fermentation time (day)_KB5F 9.0
+ 0.42 Product code*Fermentationt time (day)_KB5F 16.0
- 0.20 Product code*Fermentation time (day)_KB5U 0.0
+ 0.30 Product code*Fermentation time (day)_KB5U 3.0
+ 0.05 Product code*Fermentation time (day)_KB5U 6.0
- 0.58 Product code*Fermentation time (day)_KB5U 9.0
+ 0.43 Product code*Fermentation time (day)_KB5U 16.0
+ 0.20 Product code*Fermentation time (day)_KB6F 0.0
- 0.30 Product code*Fermentation time (day)_KB6F 3.0
- 0.05 Product code*Fermentation time (day)_KB6F 6.0
+ 0.58 Product code*Fermentation time (day)_KB6F 9.0
- 0.43 Product code*Fermentation time (day)_KB6F 16.0
+ 0.20 Product code*Fermentation time (day)_KB6U 0.0
- 0.30 Product code*Fermentation time (day)_KB6U 3.0
- 0.05 Product code*Fermentation time (day)_KB6U 6.0
+ 0.57 Product code*Fermentation time (day)_KB6U 9.0
- 0.42 Product code*Fermentation time (day)_KB6U 16.0

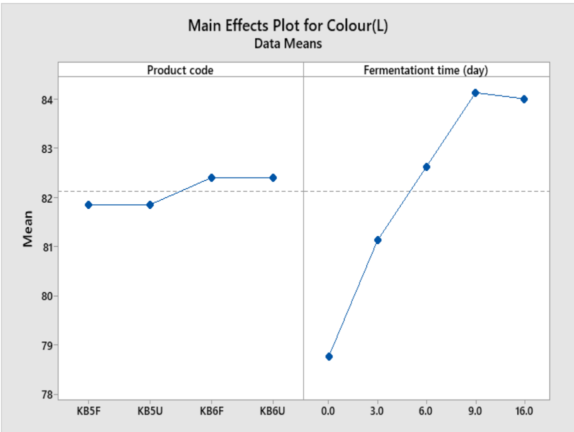
Fits and Diagnostics for Unusual Observations

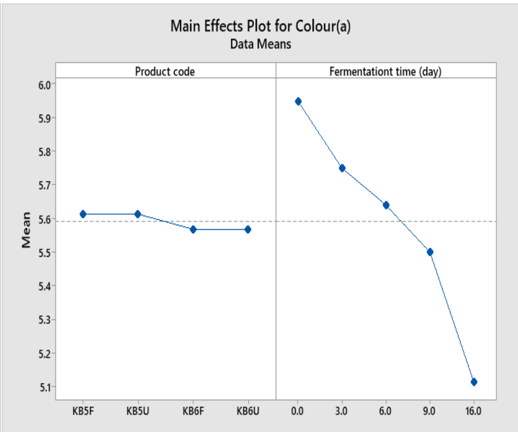
Obs	Colour(b)	Fit	Resid	Std Resid	
2	51.50	39.75	11.75	2.24	R
7	28.00	39.75	-11.75	-2.24	R
12	51.00	39.50	11.50	2.20	R
17	28.00	39.50	-11.50	-2.20	R
22	51.50	39.75	11.75	2.24	R
27	28.00	39.75	-11.75	-2.24	R

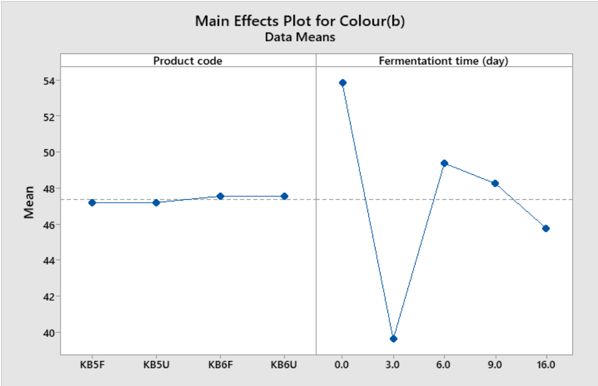
32	51.00	39.50	11.50	2.20 R
37	28.00	39.50	-11.50	-2.20 R

R Large residual









General Linear Model: Yeast count log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.5713	0.19044	5.25	0.008
Fermentatation time (day)	4	32.6069	8.15173	224.80	0.000
Product *Fermentation time (day)	12	2.8933	0.24111	6.65	0.000
Error	20	0.7252	0.03626		
Total	39	36.7968			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.190427	98.03%	96.16%	92.12%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.1700	0.0301	204.92	0.000	
Product					
KB5F	-0.1775	0.0522	-3.40	0.003	1.50
KB5U	0.0695	0.0522	1.33	0.198	1.50
KB6F	-0.0325	0.0522	-0.62	0.540	1.50
Fermentation time (day)					
0.0	-1.7188	0.0602	-28.54	0.000	1.60
3.0	0.0625	0.0602	1.04	0.312	1.60
6.0	0.5388	0.0602	8.95	0.000	1.60
9.0	0.8775	0.0602	14.57	0.000	1.60
Product *Fermentation time (day)					
KB5F 0.0	0.309	0.104	2.96	0.008	2.40
KB5F 3.0	0.065	0.104	0.62	0.540	2.40
KB5F 6.0	0.319	0.104	3.06	0.006	2.40
KB5F 9.0	0.020	0.104	0.19	0.850	2.40
KB5U 0.0	0.062	0.104	0.59	0.560	2.40
KB5U 3.0	-0.182	0.104	-1.74	0.096	2.40
KB5U 6.0	0.072	0.104	0.69	0.499	2.40
KB5U 9.0	-0.227	0.104	-2.18	0.042	2.40
KB6F 0.0	-0.099	0.104	-0.95	0.355	2.40
KB6F 3.0	0.145	0.104	1.39	0.180	2.40
KB6F 6.0	-0.109	0.104	-1.04	0.310	2.40
KB6F 9.0	0.190	0.104	1.82	0.084	2.40

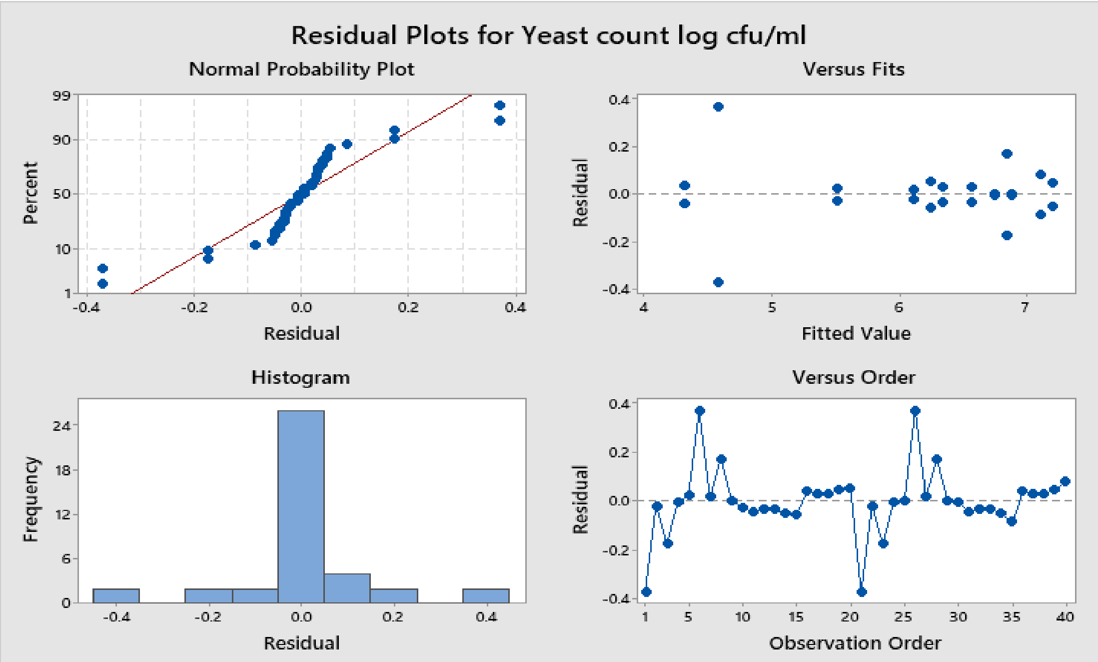
Regression Equation

Yeast count log cfu/ml = 6.1700 - 0.1775 Product code_KB5F
+ 0.0695 Product code_KB5U
- 0.0325 Product code_KB6F + 0.1405 Product code_KB6U
- 1.7188 Fermentation time (day)_0.0
+ 0.0625 Fermentation time (day)_3.0
+ 0.5388 Fermentation time (day)_6.0
+ 0.8775 Fermentation time (day)_9.0
+ 0.2400 Fermentation time (day)_16.0
+ 0.309 Product code*Fermentation time (day)_KB5F 0.0
+ 0.065 Product code*Fermentation time (day)_KB5F 3.0
+ 0.319 Product code*Fermentation time (day)_KB5F 6.0
+ 0.020 Product code*Fermentation time (day)_KB5F 9.0
- 0.713 Product code*Fermentation time (day)_KB5F 16.0
+ 0.062 Product code*Fermentation time (day)_KB5U 0.0
- 0.182 Product code*Fermentation time (day)_KB5U 3.0
+ 0.072 Product code*Fermentation time (day)_KB5U 6.0
- 0.227 Product code*Fermentation time (day)_KB5U 9.0
+ 0.275 Product code*Fermentation time (day)_KB5U 16.0
- 0.099 Product code*Fermentation time (day)_KB6F 0.0
+ 0.145 Product code*Fermentation time (day)_KB6F 3.0
- 0.109 Product code*Fermentation time (day)_KB6F 6.0
+ 0.190 Product code*Fermentation time (day)_KB6F 9.0
- 0.127 Product code*Fermentations time (day)_KB6F 16.0
- 0.272 Product code*Fermentation time (day)_KB6U 0.0
- 0.028 Product code*Fermentation time (day)_KB6U 3.0
- 0.282 Product code*Fermentation time (day)_KB6U 6.0
+ 0.017 Product code*Fermentation time (day)_KB6U 9.0
+ 0.565 Product code*Fermentation time (day)_KB6U 16.0

Fits and Diagnostics for Unusual Observations

Obs	Yeast count log cfu/ml	Fit	Resid	Std Resid
1	4.210	4.582	-0.372	-2.77 R
6	4.955	4.582	0.373	2.77 R
21	4.210	4.582	-0.372	-2.77 R
26	4.955	4.582	0.373	2.77 R

R Large residual



General Linear Model: AAB count log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, W1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.3918	0.1306	60.56	0.000
Fermentation time (day)	4	48.8025	12.2006	5656.62	0.000
Product *Fermentation time (day)	12	1.5603	0.1300	60.28	0.000
Error	20	0.0431	0.0022		
Total	39	50.7978			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0464422	99.92%	99.83%	99.66%

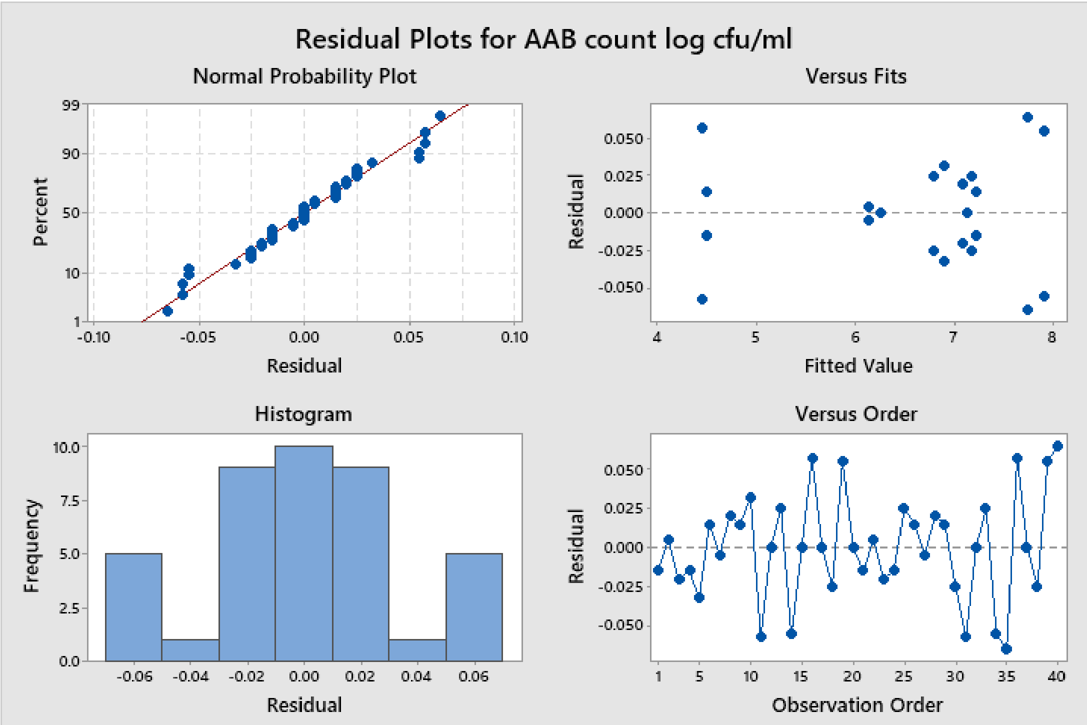
Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.48612	0.00734	883.29	0.000	
Product code					
KB5F	-0.1136	0.0127	-8.93	0.000	1.50
KB5U	-0.0601	0.0127	-4.73	0.000	1.50
KB6F	0.0254	0.0127	2.00	0.060	1.50
Fermentation time (day)					
0.0	-2.0124	0.0147	-137.02	0.000	1.60
3.0	-0.2911	0.0147	-19.82	0.000	1.60
6.0	0.4614	0.0147	31.42	0.000	1.60
9.0	1.0889	0.0147	74.14	0.000	1.60
Product code*Fermentation time (day)					
KB5F 0.0	0.1399	0.0254	5.50	0.000	2.40
KB5F 3.0	0.0536	0.0254	2.11	0.048	2.40
KB5F 6.0	0.2611	0.0254	10.27	0.000	2.40
KB5F 9.0	-0.2364	0.0254	-9.29	0.000	2.40
KB5U 0.0	0.0864	0.0254	3.40	0.003	2.40
KB5U 3.0	0.0001	0.0254	0.00	0.996	2.40
KB5U 6.0	0.2076	0.0254	8.16	0.000	2.40
KB5U 9.0	-0.2899	0.0254	-11.40	0.000	2.40
KB6F 0.0	-0.0516	0.0254	-2.03	0.056	2.40
KB6F 3.0	0.0346	0.0254	1.36	0.189	2.40
KB6F 6.0	-0.1729	0.0254	-6.80	0.000	2.40
KB6F 9.0	0.3246	0.0254	12.76	0.000	2.40

Regression Equation

AAB count log cfu/ml = 6.48612 - 0.1136 Product code_KB5F
- 0.0601 Product code_KB5U
+ 0.0254 Product code_KB6F + 0.1484 Product code_KB6U
- 2.0124 Fermentation time (day)_0.0
- 0.2911 Fermentation time (day)_3.0
+ 0.4614 Fermentation time (day)_6.0
+ 1.0889 Fermentation time (day)_9.0
+ 0.7533 Fermentation time (day)_16.0
+ 0.1399 Product code*Fermentation time (day)_KB5F 0.0
+ 0.0536 Product code*Fermentation time (day)_KB5F 3.0
+ 0.2611 Product code*Fermentation time (day)_KB5F 6.0
- 0.2364 Product code*Fermentation time (day)_KB5F 9.0
- 0.2183 Product code*Fermentation time (day)_KB5F 16.0
+ 0.0864 Product code*Fermentation time (day)_KB5U 0.0
+ 0.0001 Product code*Fermentation time (day)_KB5U 3.0
+ 0.2076 Product code*Fermentation time (day)_KB5U 6.0
- 0.2899 Product code*Fermentation time (day)_KB5U 9.0

- 0.0042 Product code*Fermentation time (day)_KB5U 16.0
- 0.0516 Product code*Fermentation time (day)_KB6F 0.0
+ 0.0346 Product code*Fermentation time (day)_KB6F 3.0
- 0.1729 Product code*Fermentation time (day)_KB6F 6.0
+ 0.3246 Product code*Fermentation time (day)_KB6F 9.0
- 0.1347 Product code*Fermentation time (day)_KB6F 16.0
- 0.1746 Product code*Fermentation time (day)_KB6U 0.0
- 0.0884 Product code*Fermentation time (day)_KB6U 3.0
- 0.2959 Product code*Fermentation time (day)_KB6U 6.0
+ 0.2016 Product code*Fermentation time (day)_KB6U 9.0
+ 0.3572 Product code*Fermentation time (day)_KB6U 16.0



General Linear Model: Total count log cfu/ml versus Product and Fermentation time (day)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U
Fermentation time (day)	Fixed	5	0.0, 3.0, 6.0, 9.0, 16.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	0.4257	0.1419	50.13	0.000
Fermentation time (day)	4	45.9432	11.4858	4057.69	0.000
Product *Fermentation time (day)	12	1.9206	0.1601	56.54	0.000
Error	20	0.0566	0.0028		
Total	39	48.3462			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0532036	99.88%	99.77%	99.53%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.45037	0.00841	766.79	0.000	
Product					
KB5F	-0.1089	0.0146	-7.47	0.000	1.50
KB5U	-0.0664	0.0146	-4.56	0.000	1.50
KB6F	0.0136	0.0146	0.94	0.361	1.50
Fermentation time (day)					
0.0	-1.9779	0.0168	-117.56	0.000	1.60
3.0	-0.2141	0.0168	-12.73	0.000	1.60
6.0	0.5096	0.0168	30.29	0.000	1.60
9.0	1.0759	0.0168	63.95	0.000	1.60
Product *Fermentation time (day)					
KB5F 0.0	0.1789	0.0291	6.14	0.000	2.40
KB5F 3.0	-0.0199	0.0291	-0.68	0.503	2.40
KB5F 6.0	0.2764	0.0291	9.48	0.000	2.40
KB5F 9.0	-0.2649	0.0291	-9.09	0.000	2.40
KB5U 0.0	0.1364	0.0291	4.68	0.000	2.40
KB5U 3.0	-0.0624	0.0291	-2.14	0.045	2.40
KB5U 6.0	0.2339	0.0291	8.03	0.000	2.40
KB5U 9.0	-0.3074	0.0291	-10.55	0.000	2.40
KB6F 0.0	-0.0836	0.0291	-2.87	0.009	2.40
KB6F 3.0	0.1151	0.0291	3.95	0.001	2.40
KB6F 6.0	-0.1811	0.0291	-6.22	0.000	2.40
KB6F 9.0	0.3601	0.0291	12.36	0.000	2.40

Regression Equation

Total count log cfu/ml = 6.45037 - 0.1089 Product code_KB5F
- 0.0664 Product code_KB5U
+ 0.0136 Product code_KB6F + 0.1616 Product code_KB6U
- 1.9779 Fermentation time (day)_0.0
- 0.2141 Fermentation time (day)_3.0
+ 0.5096 Fermentation time (day)_6.0
+ 1.0759 Fermentation time (day)_9.0
+ 0.6065 Fermentation time (day)_16.0
+ 0.1789 Product code*Fermentation time (day)_KB5F 0.0
- 0.0199 Product code*Fermentation time (day)_KB5F 3.0
+ 0.2764 Product code*Fermentation time (day)_KB5F 6.0
- 0.2649 Product code*Fermentation time (day)_KB5F 9.0
- 0.1705 Product code*Fermentation time (day)_KB5F 16.0
+ 0.1364 Product code*Fermentation time (day)_KB5U 0.0
- 0.0624 Product code*Fermentation time (day)_KB5U 3.0
+ 0.2339 Product code*Fermentation time (day)_KB5U 6.0
- 0.3074 Product code*Fermentation time (day)_KB5U 9.0
- 0.0005 Product code*Fermentation time (day)_KB5U 16.0
- 0.0836 Product code*Fermentation time (day)_KB6F 0.0
+ 0.1151 Product code*Fermentation time (day)_KB6F 3.0
- 0.1811 Product code*Fermentation time (day)_KB6F 6.0
+ 0.3601 Product code*Fermentation time (day)_KB6F 9.0
- 0.2105 Product code*Fermentation time (day)_KB6F 16.0
- 0.2316 Product code*Fermentation time (day)_KB6U 0.0
- 0.0329 Product code*Fermentation time (day)_KB6U 3.0
- 0.3291 Product code*Fermentation time (day)_KB6U 6.0
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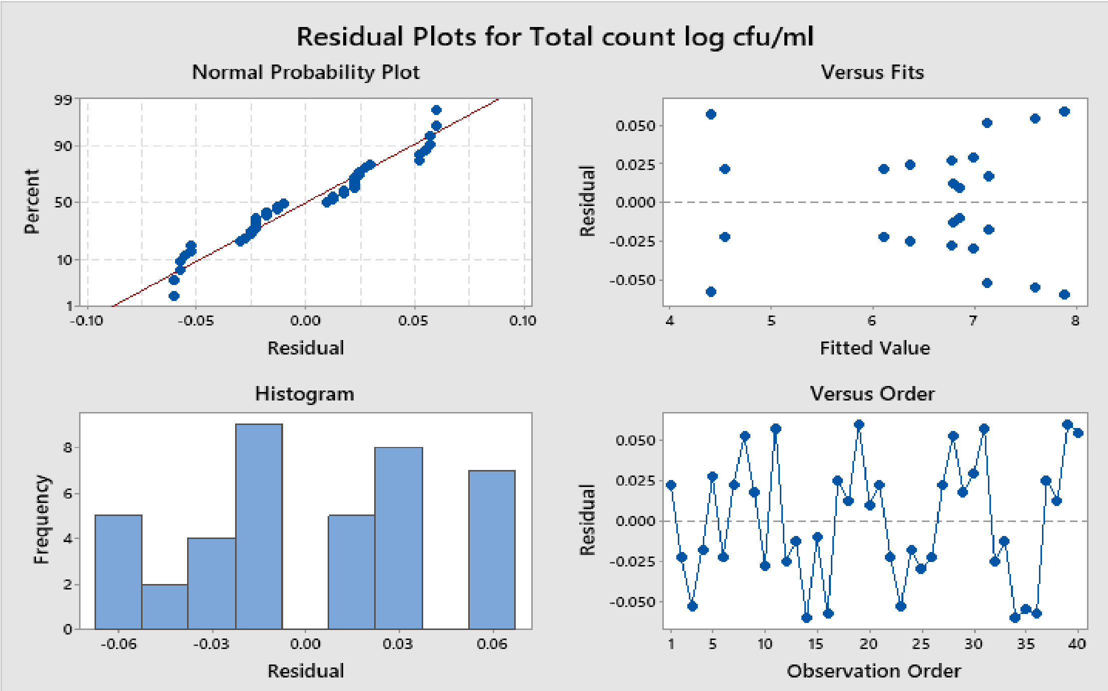
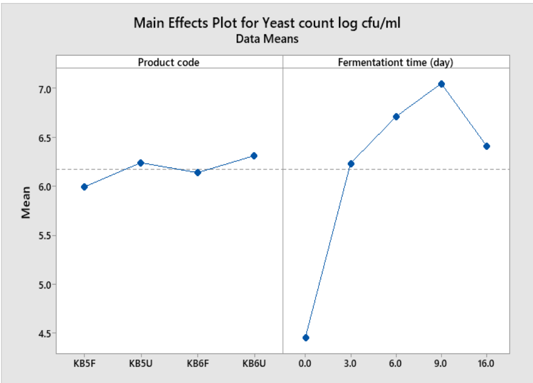
+ 0.3815 Product code*Fermentation time (day)_KB6U 16.0


Residual Plots for Total count log cfu/ml
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Main Effects Plot for Yeast count log cfu/ml
Data Means
Product code
Fermentationt time (day)
Mean
KB5F
KB5U
KB6F
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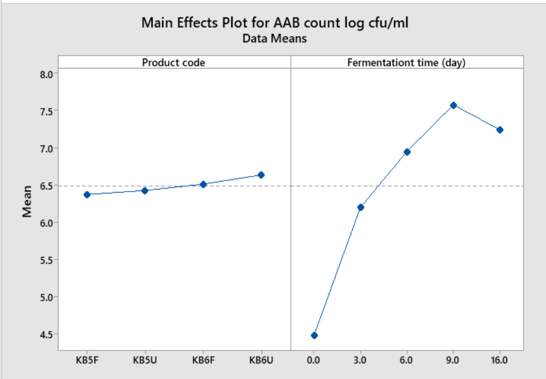
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Data Means
Product code
Fermentationt time (day)
Mean
KB5F
KB5U
KB6F
KB6U
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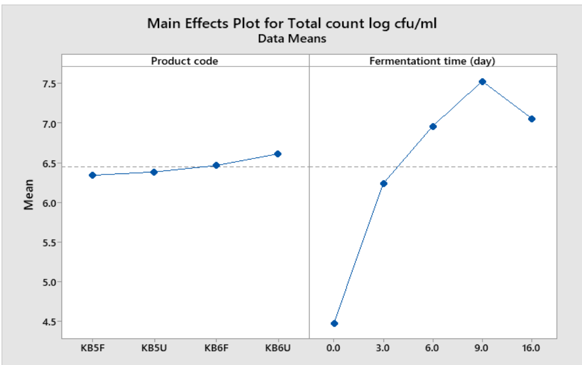
Main Effects Plot for Total count log cfu/ml
Data Means
Product code
Fermentationt time (day)
Mean
KB5F
KB5U
KB6F
KB6U
0.0
3.0
6.0
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16.0

General Linear Model: Appearance versus Product

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	1.964	0.6548	0.39	0.763
Error	136	230.571	1.6954		
Total	139	232.536			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.30207	0.84%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.321	0.110	57.44	0.000	
Product					
KB5F	0.193	0.191	1.01	0.313	1.50
KB5U	-0.064	0.191	-0.34	0.736	1.50
KB6F	-0.007	0.191	-0.04	0.970	1.50

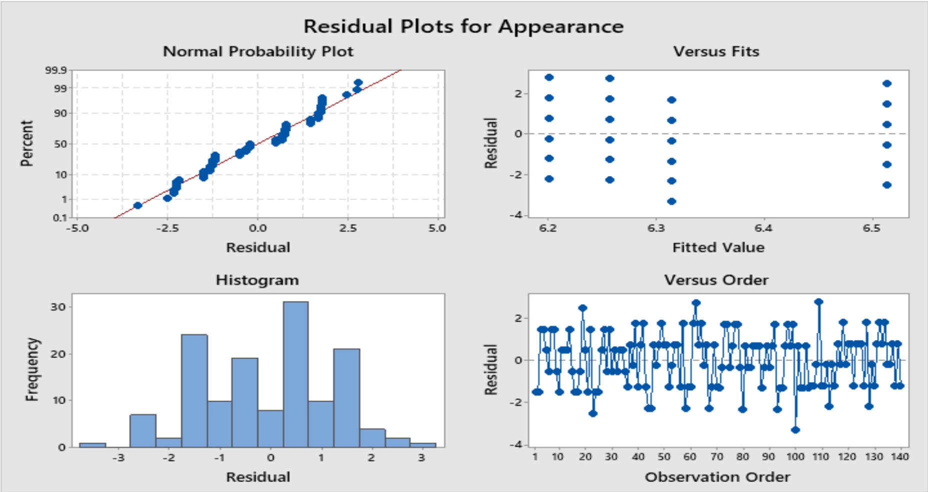
Regression Equation

Appearance = 6.321 + 0.193 Product_KB5F - 0.064 Product_KB5U
- 0.007 Product_KB6F
- 0.121 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Appearance	Fit	Resid	Std Resid
62	9.000	6.257	2.743	2.14 R
100	3.000	6.314	-3.314	-2.58 R
109	9.000	6.200	2.800	2.18 R

R Large residual



General Linear Model: Aroma versus Product

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	5.714	1.905	0.77	0.514
Error	136	337.429	2.481		
Total	139	343.143			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.57515	1.67%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.857	0.133	44.00	0.000	
Product					
KB5F	0.171	0.231	0.74	0.458	1.50
KB5U	0.229	0.231	0.99	0.323	1.50
KB6F	-0.229	0.231	-0.99	0.323	1.50

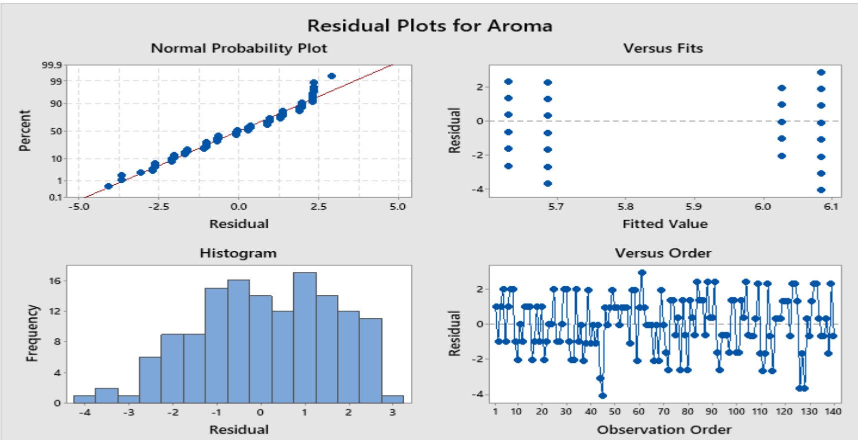
Regression Equation

Aroma = 5.857 + 0.171 Product_KB5F + 0.229 Product_KB5U
- 0.229 Product_KB6F
- 0.171 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Aroma	Fit	Resid	Std Resid	
45	2.000	6.086	-4.086	-2.63	R
126	2.000	5.686	-3.686	-2.37	R
128	2.000	5.686	-3.686	-2.37	R

R Large residual



General Linear Model: Flavour versus Product

Method

Factor coding	(-1, 0, +1)
Rows unused	1

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	15.80	5.267	2.19	0.092
Error	135	324.17	2.401		
Total	138	339.97			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.54960	4.65%	2.53%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.014	0.131	45.76	0.000	
Product					
KB5F	0.500	0.227	2.20	0.029	1.50
KB5U	-0.072	0.227	-0.32	0.753	1.50
KB6F	0.015	0.229	0.07	0.948	1.51

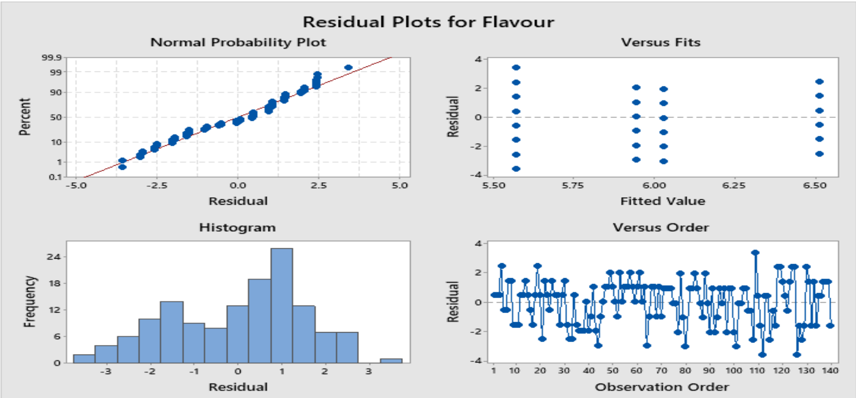
Regression Equation

Flavour = 6.014 + 0.500 Product_KB5F - 0.072 Product_KB5U
+ 0.015 Product_KB6F
- 0.443 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Flavour	Fit	Resid	Std Resid	
109	9.000	5.571	3.429	2.24	R
112	2.000	5.571	-3.571	-2.34	R
126	2.000	5.571	-3.571	-2.34	R

R Large residual



General Linear Model: Sweetness versus Product

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	12.14	4.045	2.03	0.113
Error	136	271.66	1.997		
Total	139	283.79			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.41332	4.28%	2.16%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.093	0.119	51.01	0.000	
Product					
KB5F	0.393	0.207	1.90	0.060	1.50
KB5U	-0.007	0.207	-0.03	0.973	1.50
KB6F	0.050	0.207	0.24	0.809	1.50

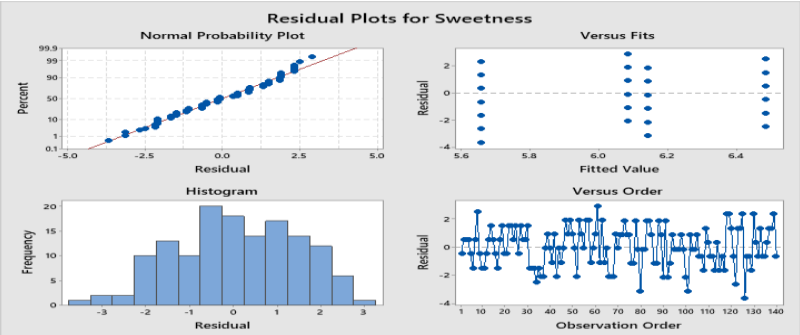
Regression Equation

Sweetness = 6.093 + 0.393 Product_KB5F - 0.007 Product_KB5U
+ 0.050 Product_KB6F
- 0.436 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Sweetness	Fit	Resid	Std Resid
61	9.000	6.086	2.914	2.09 R
80	3.000	6.143	-3.143	-2.26 R
101	3.000	6.143	-3.143	-2.26 R
126	2.000	5.657	-3.657	-2.63 R

R Large residual



General Linear Model: Sourness versus Product

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	14.08	4.693	1.78	0.154
Error	136	359.14	2.641		
Total	139	373.22			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.62504	3.77%	1.65%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.836	0.137	42.49	0.000	
Product					
KB5F	0.536	0.238	2.25	0.026	1.50
KB5U	-0.121	0.238	-0.51	0.611	1.50
KB6F	-0.121	0.238	-0.51	0.611	1.50

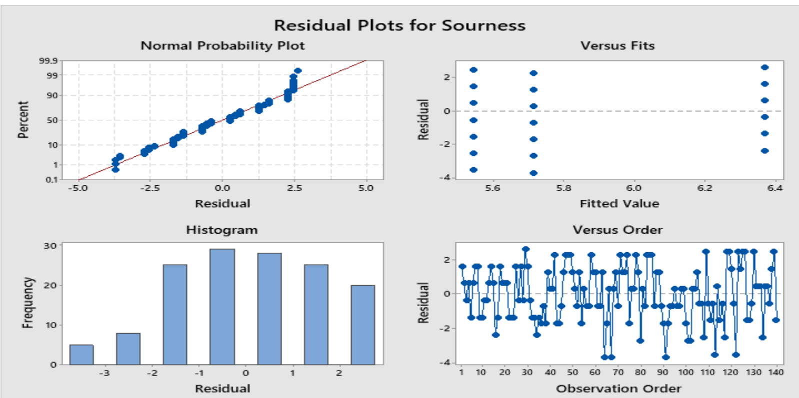
Regression Equation

Sourness = 5.836 + 0.536 Product_KB5F - 0.121 Product_KB5U
- 0.121 Product_KB6F
- 0.293 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Sourness	Fit	Resid	Std Resid
64	2.000	5.714	-3.714	-2.32 R
67	2.000	5.714	-3.714	-2.32 R
91	2.000	5.714	-3.714	-2.32 R
113	2.000	5.543	-3.543	-2.21 R
122	2.000	5.543	-3.543	-2.21 R

R Large residual



General Linear Model: Overall acceptability versus Product

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	4	KB5F, KB5U, KB6F, KB6U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	3	25.71	8.571	4.94	0.003
Error	136	235.83	1.734		
Total	139	261.54			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.31683	9.83%	7.84%	4.45%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.343	0.111	56.99	0.000	
Product					
KB5F	0.600	0.193	3.11	0.002	1.50
KB5U	-0.086	0.193	-0.44	0.657	1.50
KB6F	0.086	0.193	0.44	0.657	1.50

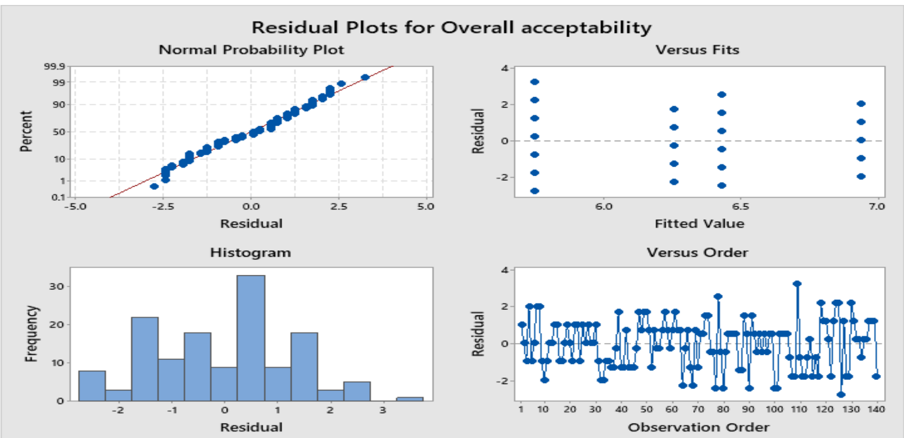
Regression Equation

Overall acceptability = 6.343 + 0.600 Product_KB5F - 0.086 Product_KB5U
+ 0.086 Product_KB6F
- 0.600 Product_KB6U

Fits and Diagnostics for Unusual Observations

Obs	Overall acceptability	Fit	Resid	Std Resid
109	9.000	5.743	3.257	2.51 R
126	3.000	5.743	-2.743	-2.11 R

R Large residual



H.4 Phase 3: Stability of filtered and unfiltered Kombucha during prolonged storage (4°C) for 3 weeks

General Linear Model: pH versus Product and Storage period (weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.002500	0.002500	22.22	0.002
Storage period(weeks)	3	0.029950	0.009983	88.74	0.000
Product*Storage period(weeks)	3	0.001750	0.000583	5.19	0.028
Error	8	0.000900	0.000112		
Total	15	0.035100			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0106066	97.44%	95.19%	89.74%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.14250	0.00265	1185.11	0.000	
Product					
KB5F	0.01250	0.00265	4.71	0.002	1.00
Storage period(weeks)					
0.0	0.06250	0.00459	13.61	0.000	1.50
1.0	0.01250	0.00459	2.72	0.026	1.50
2.0	-0.02000	0.00459	-4.35	0.002	1.50
Product*Storage period(weeks)					
KB5F 0.0	-0.01750	0.00459	-3.81	0.005	1.50
KB5F 1.0	0.00250	0.00459	0.54	0.601	1.50
KB5F 2.0	0.01000	0.00459	2.18	0.061	1.50

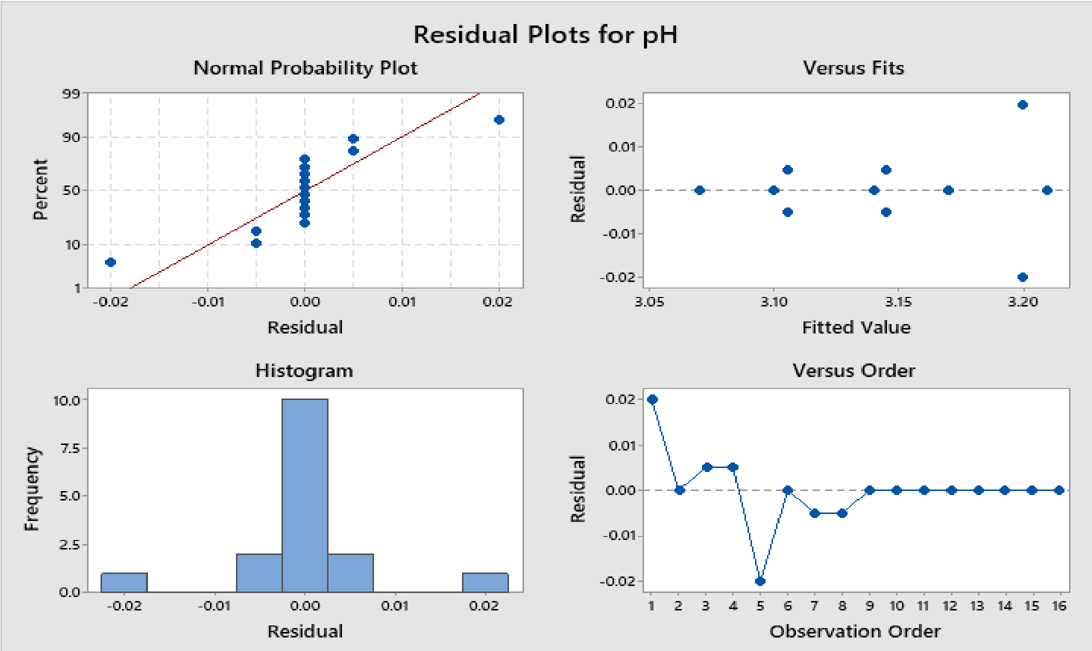
Regression Equation

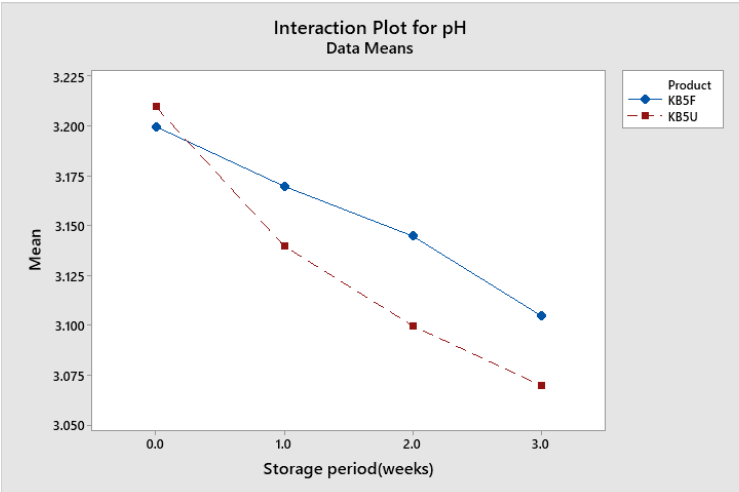
pH = 3.14250 + 0.01250 Product_KB5F - 0.01250 Product_KB5U
+ 0.06250 Storage period(weeks)_0.0
+ 0.01250 Storage period(weeks)_1.0
- 0.02000 Storage period(weeks)_2.0
- 0.05500 Storage period(weeks)_3.0
- 0.01750 Product*Storage period(weeks)_KB5F 0.0
+ 0.00250 Product*Storage period(weeks)_KB5F 1.0
+ 0.01000 Product*Storage period(weeks)_KB5F 2.0
+ 0.00500 Product*Storage period(weeks)_KB5F 3.0
+ 0.01750 Product*Storage period(weeks)_KB5U 0.0
- 0.00250 Product*Storage period(weeks)_KB5U 1.0
- 0.01000 Product*Storage period(weeks)_KB5U 2.0
- 0.00500 Product*Storage period(weeks)_KB5U 3.0

Fits and Diagnostics for Unusual Observations

Obs	pH	Fit	Resid	Std Resid
1	3.22000	3.20000	0.02000	2.67 R
5	3.18000	3.20000	-0.02000	-2.67 R

R Large residual





General Linear Model: Titratable acidity (%) versus Product, Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.007225	0.007225	22.23	0.002
Storage period(weeks)	3	0.020475	0.006825	21.00	0.000
Product*Storage period(weeks)	3	0.002875	0.000958	2.95	0.098
Error	8	0.002600	0.000325		
Total	15	0.033175			

Model Summary

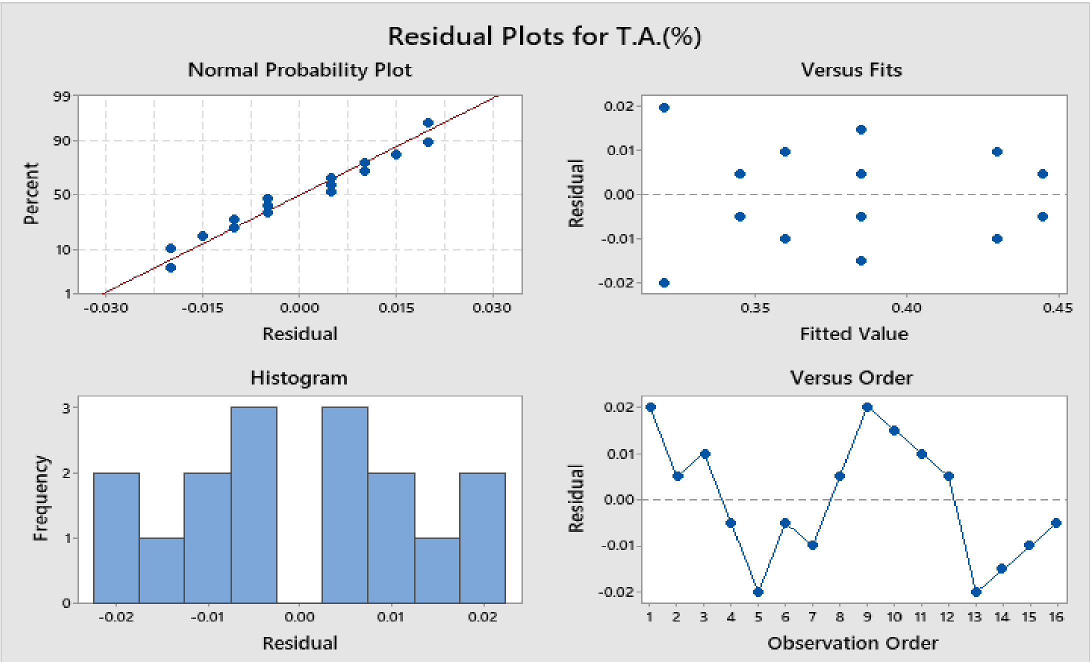
S	R-sq	R-sq(adj)	R-sq(pred)
0.0180278	92.16%	85.31%	68.65%

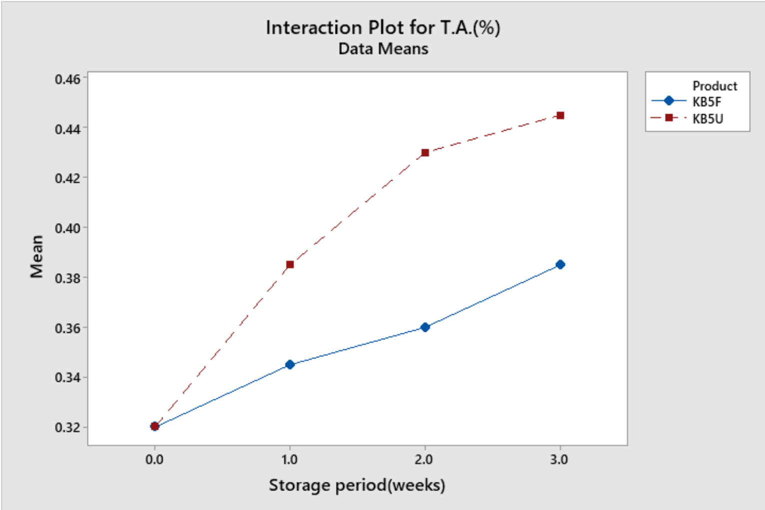
Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.37375	0.00451	82.93	0.000	
Product					
KB5F	-0.02125	0.00451	-4.71	0.002	1.00
Storage period(weeks)					
0.0	-0.05375	0.00781	-6.89	0.000	1.50
1.0	-0.00875	0.00781	-1.12	0.295	1.50
2.0	0.02125	0.00781	2.72	0.026	1.50
Product*Storage period(weeks)					
KB5F 0.0	0.02125	0.00781	2.72	0.026	1.50
KB5F 1.0	0.00125	0.00781	0.16	0.877	1.50
KB5F 2.0	-0.01375	0.00781	-1.76	0.116	1.50

Regression Equation

T.A.(%) = 0.37375 - 0.02125 Product_KB5F + 0.02125 Product_KB5U
- 0.05375 Storage period(weeks)_0.0
- 0.00875 Storage period(weeks)_1.0
+ 0.02125 Storage period(weeks)_2.0
+ 0.04125 Storage period(weeks)_3.0
+ 0.02125 Product*Storage period(weeks)_KB5F 0.0
+ 0.00125 Product*Storage period(weeks)_KB5F 1.0
- 0.01375 Product*Storage period(weeks)_KB5F 2.0
- 0.00875 Product*Storage period(weeks)_KB5F 3.0
- 0.02125 Product*Storage period(weeks)_KB5U 0.0
- 0.00125 Product*Storage period(weeks)_KB5U 1.0
+ 0.01375 Product*Storage period(weeks)_KB5U 2.0
+ 0.00875 Product*Storage period(weeks)_KB5U 3.0





General Linear Model: TSS (° Brix) versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.007225	0.007225	22.23	0.002
Storage period(weeks)	3	0.020475	0.006825	21.00	0.000
Product*Storage period(weeks)	3	0.002875	0.000958	2.95	0.098
Error	8	0.002600	0.000325		
Total	15	0.033175			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0180278	92.16%	85.31%	68.65%

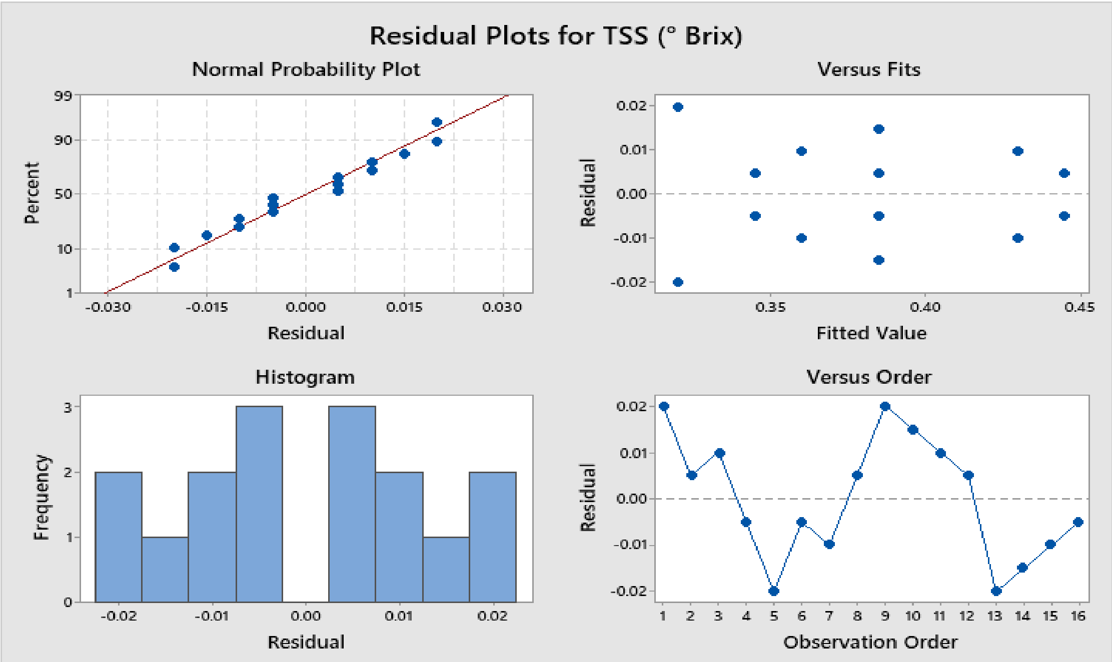
Coefficients

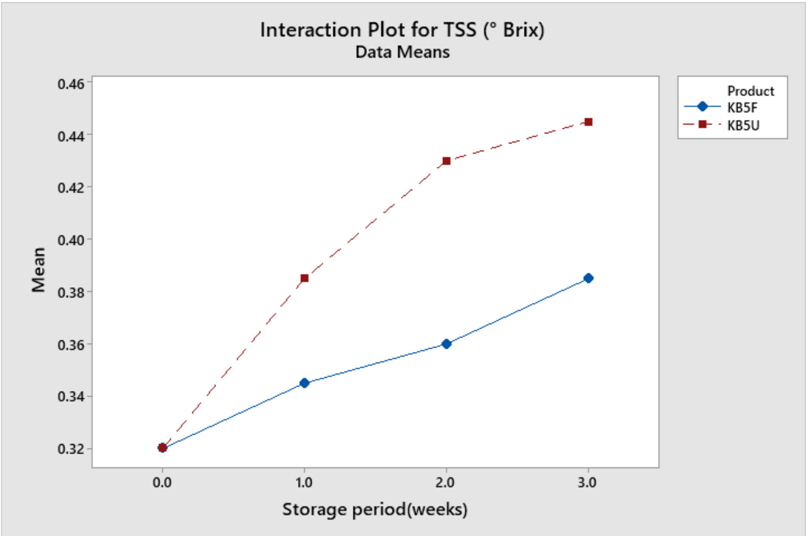
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.37375	0.00451	82.93	0.000	
Product					
KB5F	-0.02125	0.00451	-4.71	0.002	1.00
Storage period(weeks)					
0.0	-0.05375	0.00781	-6.89	0.000	1.50
1.0	-0.00875	0.00781	-1.12	0.295	1.50
2.0	0.02125	0.00781	2.72	0.026	1.50
Product*Storage period(weeks)					
KB5F 0.0	0.02125	0.00781	2.72	0.026	1.50
KB5F 1.0	0.00125	0.00781	0.16	0.877	1.50
KB5F 2.0	-0.01375	0.00781	-1.76	0.116	1.50

Regression Equation

TSS (° Brix) = 0.37375 - 0.02125 Product_KB5F + 0.02125 Product_KB5U - 0.05375 Storage period(weeks)_0.0

- 0.00875 Storage period(weeks)_1.0
+ 0.02125 Storage period(weeks)_2.0
+ 0.04125 Storage period(weeks)_3.0
+ 0.02125 Product*Storage period(weeks)_KB5F 0.0
+ 0.00125 Product*Storage period(weeks)_KB5F 1.0
- 0.01375 Product*Storage period(weeks)_KB5F 2.0
- 0.00875 Product*Storage period(weeks)_KB5F 3.0
- 0.02125 Product*Storage period(weeks)_KB5U 0.0
- 0.00125 Product*Storage period(weeks)_KB5U 1.0
+ 0.01375 Product*Storage period(weeks)_KB5U 2.0
+ 0.00875 Product*Storage period(weeks)_KB5U 3.0





General Linear Model: L* versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	1.000	1.0000	4.00	0.081
Storage period(weeks)	3	5.250	1.7500	7.00	0.013
Product*Storage period(weeks)	3	1.500	0.5000	2.00	0.193
Error	8	2.000	0.2500		
Total	15	9.750			

Model Summary

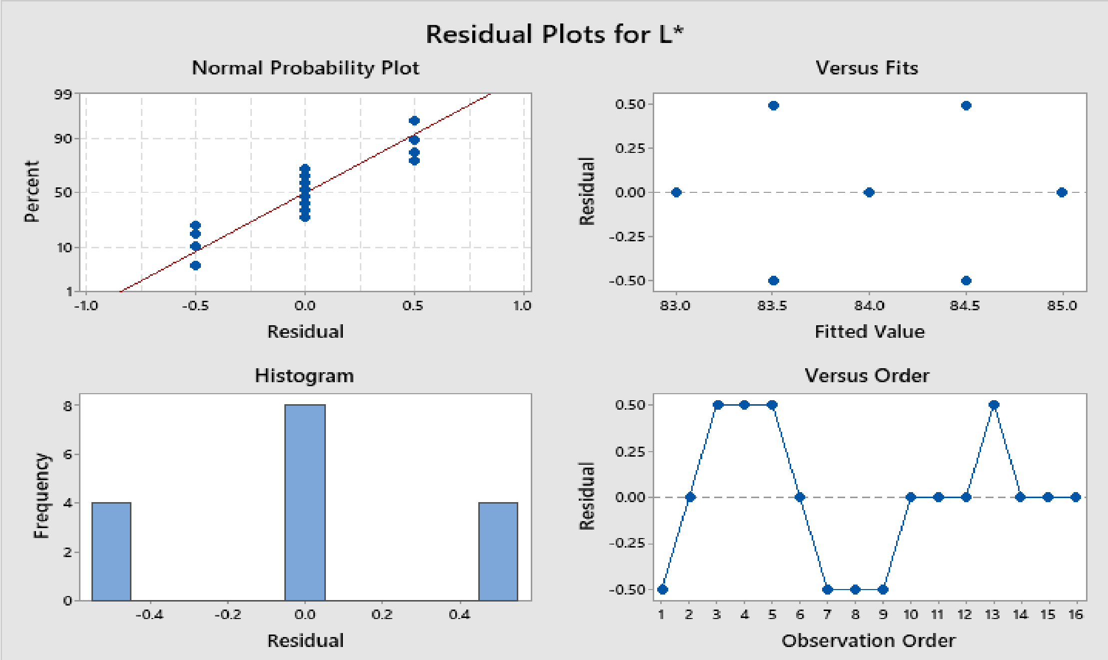
S	R-sq	R-sq(adj)	R-sq(pred)
0.5	79.49%	61.54%	17.95%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	83.875	0.125	671.00	0.000	
Product					
KB5F	-0.250	0.125	-2.00	0.081	1.00
Storage period(weeks)					
0.0	0.625	0.217	2.89	0.020	1.50
1.0	-0.875	0.217	-4.04	0.004	1.50
2.0	0.375	0.217	1.73	0.122	1.50
Product*Storage period(weeks)					
KB5F 0.0	0.250	0.217	1.15	0.282	1.50
KB5F 1.0	0.250	0.217	1.15	0.282	1.50
KB5F 2.0	-0.500	0.217	-2.31	0.050	1.50

Regression Equation

L* = 83.875 - 0.250 Product_KB5F + 0.250 Product_KB5U
+ 0.625 Storage period(weeks)_0.0
- 0.875 Storage period(weeks)_1.0 + 0.375 Storage period(weeks)_2.0
- 0.125 Storage period(weeks)_3.0 + 0.250 Product*Storage period(weeks)_KB5F 0.0
+ 0.250 Product*Storage period(weeks)_KB5F 1.0
- 0.500 Product*Storage period(weeks)_KB5F 2.0
- 0.000 Product*Storage period(weeks)_KB5F 3.0
- 0.250 Product*Storage period(weeks)_KB5U 0.0
- 0.250 Product*Storage period(weeks)_KB5U 1.0
+ 0.500 Product*Storage period(weeks)_KB5U 2.0
+ 0.000 Product*Storage period(weeks)_KB5U 3.0



General Linear Model: a* versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	2 KB5F, KB5U
Storage period(weeks)	Fixed	4 0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.000100	0.000100	8.00	0.022
Storage period(weeks)	3	0.299225	0.099742	7979.33	0.000
Product*Storage period(weeks)	3	0.000150	0.000050	4.00	0.052
Error	8	0.000100	0.000013		
Total	15	0.299575			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0035355	99.97%	99.94%	99.87%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.26375	0.00088	5955.25	0.000	
Product					
KB5F	0.002500	0.000884	2.83	0.022	1.00
Storage period(weeks)					
0.0	0.23625	0.00153	154.32	0.000	1.50
1.0	-0.06375	0.00153	-41.64	0.000	1.50
2.0	-0.08125	0.00153	-53.07	0.000	1.50
Product*Storage period(weeks)					
KB5F 0.0	-0.00250	0.00153	-1.63	0.141	1.50
KB5F 1.0	-0.00250	0.00153	-1.63	0.141	1.50
KB5F 2.0	0.00000	0.00153	0.00	1.000	1.50

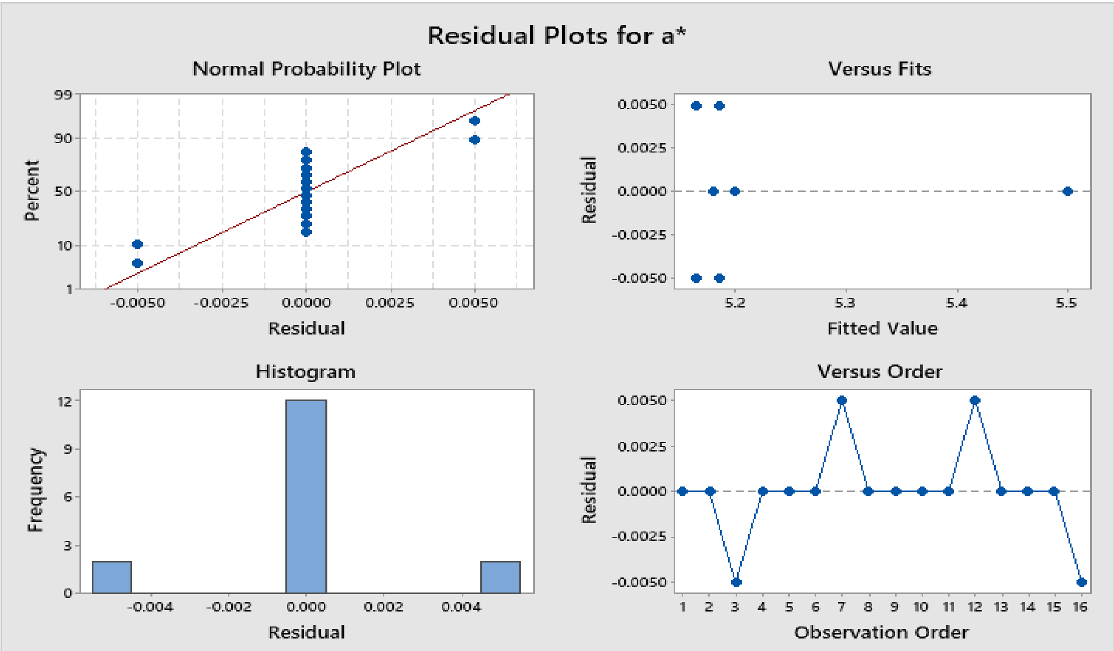
Regression Equation

a* = 5.26375 + 0.002500 Product_KB5F - 0.002500 Product_KB5U
+ 0.23625 Storage period(weeks)_0.0
- 0.06375 Storage period(weeks)_1.0
- 0.08125 Storage period(weeks)_2.0
- 0.09125 Storage period(weeks)_3.0
- 0.00250 Product*Storage period(weeks)_KB5F 0.0
- 0.00250 Product*Storage period(weeks)_KB5F 1.0
+ 0.00000 Product*Storage period(weeks)_KB5F 2.0
+ 0.00500 Product*Storage period(weeks)_KB5F 3.0
+ 0.00250 Product*Storage period(weeks)_KB5U 0.0
+ 0.00250 Product*Storage period(weeks)_KB5U 1.0
- 0.00000 Product*Storage period(weeks)_KB5U 2.0
- 0.00500 Product*Storage period(weeks)_KB5U 3.0

Fits and Diagnostics for Unusual Observations

Obs	a*	Fit	Resid	Std Resid
3	5.18000	5.18500	-0.00500	-2.00 R
12	5.17000	5.16500	0.00500	2.00 R

R Large residual



General Linear Model: b* versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.2500	0.2500	0.29	0.608
Storage period(weeks)	3	59.0000	19.6667	22.48	0.000
Product*Storage period(weeks)	3	0.7500	0.2500	0.29	0.835
Error	8	7.0000	0.8750		
Total	15	67.0000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.935414	89.55%	80.41%	58.21%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	44.750	0.234	191.36	0.000	
Product					
KB5F	0.125	0.234	0.53	0.608	1.00
Storage period(weeks)					
0.0	2.750	0.405	6.79	0.000	1.50
1.0	0.750	0.405	1.85	0.101	1.50
2.0	-1.250	0.405	-3.09	0.015	1.50
Product*Storage period(weeks)					
KB5F 0.0	-0.125	0.405	-0.31	0.766	1.50
KB5F 1.0	-0.125	0.405	-0.31	0.766	1.50
KB5F 2.0	-0.125	0.405	-0.31	0.766	1.50

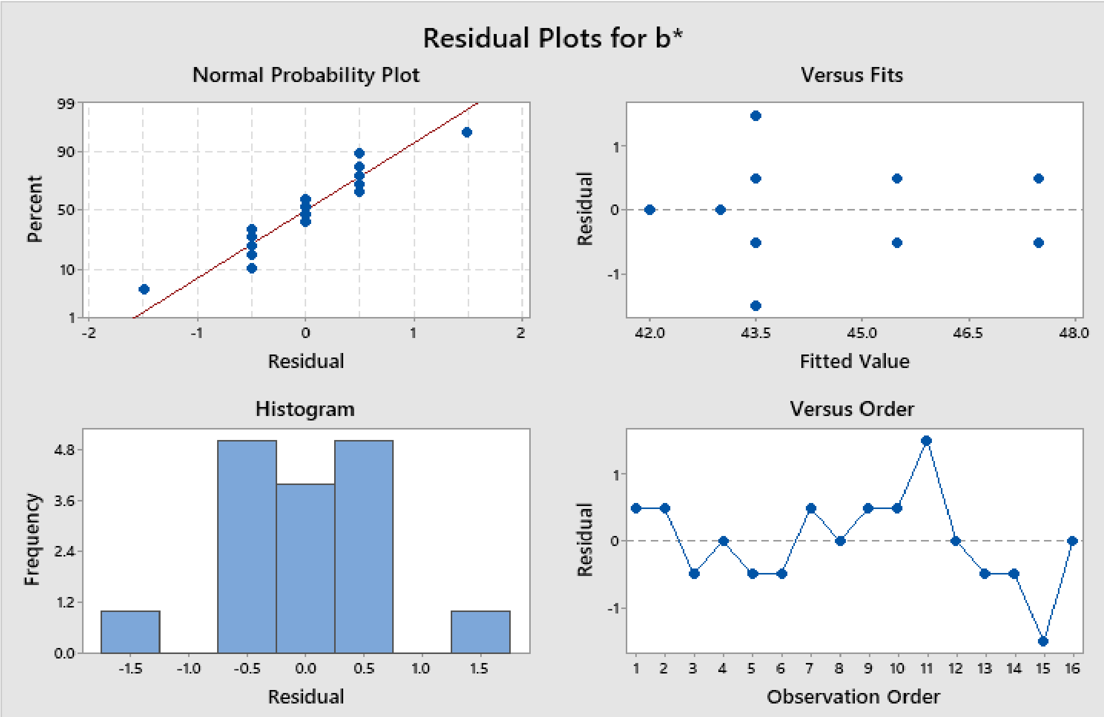
Regression Equation

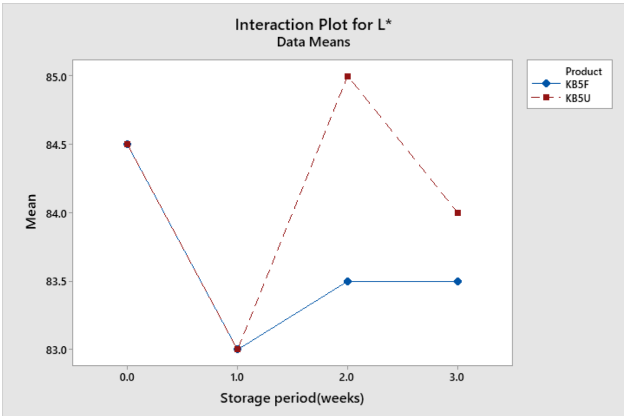
b* = 44.750 + 0.125 Product_KB5F - 0.125 Product_KB5U
+ 2.750 Storage period(weeks)_0.0
+ 0.750 Storage period(weeks)_1.0 - 1.250 Storage period(weeks)_2.0
- 2.250 Storage period(weeks)_3.0 - 0.125 Product*Storage period(weeks)_KB5F 0.0
- 0.125 Product*Storage period(weeks)_KB5F 1.0
- 0.125 Product*Storage period(weeks)_KB5F 2.0
+ 0.375 Product*Storage period(weeks)_KB5F 3.0
+ 0.125 Product*Storage period(weeks)_KB5U 0.0
+ 0.125 Product*Storage period(weeks)_KB5U 1.0
+ 0.125 Product*Storage period(weeks)_KB5U 2.0
- 0.375 Product*Storage period(weeks)_KB5U 3.0

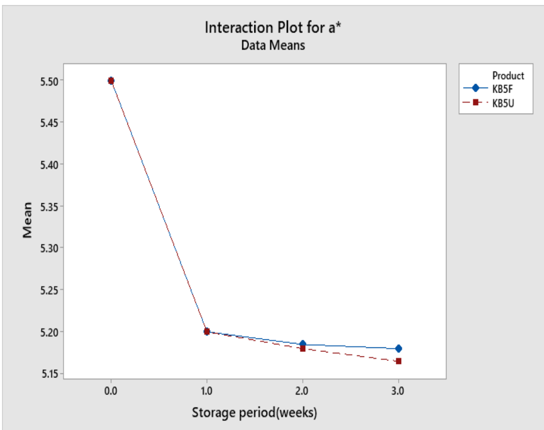
Fits and Diagnostics for Unusual Observations

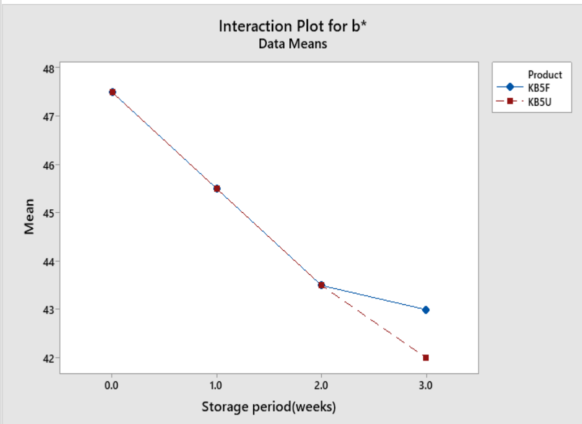
Obs	b*	Fit	Resid	Std Resid
11	45.000	43.500	1.500	2.27 R
15	42.000	43.500	-1.500	-2.27 R

R Large residual


Residual Plots for b*
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Interaction Plot for L*
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U


Interaction Plot for a*
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U


Interaction Plot for b*
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U

General Linear Model: Yeast versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Storage period(weeks)	Fixed	4	0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.76562	0.76562	324.07	0.000
Storage period(weeks)	3	4.74613	1.58204	669.65	0.000
Product*Storage period(weeks)	3	1.78333	0.59444	251.62	0.000
Error	8	0.01890	0.00236		
Total	15	7.31398			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0486056	99.74%	99.52%	98.97%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.0788	0.0122	500.25	0.000	
Product					
KB5F	-0.2187	0.0122	-18.00	0.000	1.00
Storage period(weeks)					
0.0	0.7462	0.0210	35.46	0.000	1.50
1.0	0.0787	0.0210	3.74	0.006	1.50
2.0	-0.0363	0.0210	-1.72	0.123	1.50
Product*Storage period(weeks)					
KB5F 0.0	0.2187	0.0210	10.39	0.000	1.50
KB5F 1.0	-0.3988	0.0210	-18.95	0.000	1.50
KB5F 2.0	-0.2437	0.0210	-11.58	0.000	1.50

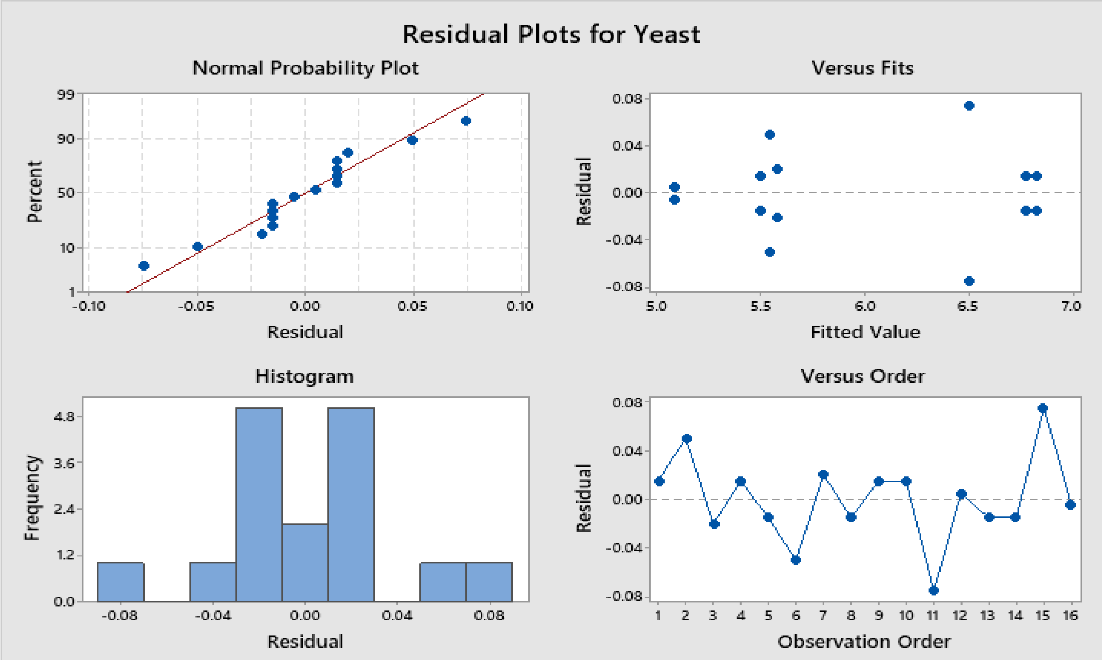
Regression Equation

Yeast = 6.0788 - 0.2187 Product_KB5F + 0.2187 Product_KB5U
+ 0.7462 Storage period(weeks)_0.0
+ 0.0787 Storage period(weeks)_1.0 - 0.0363 Storage period(weeks)_2.0
- 0.7887 Storage period(weeks)_3.0 + 0.2187 Product*Storage period(weeks)_KB5F 0.0
- 0.3988 Product*Storage period(weeks)_KB5F 1.0
- 0.2437 Product*Storage period(weeks)_KB5F 2.0
+ 0.4238 Product*Storage period(weeks)_KB5F 3.0
- 0.2187 Product*Storage period(weeks)_KB5U 0.0
+ 0.3988 Product*Storage period(weeks)_KB5U 1.0
+ 0.2437 Product*Storage period(weeks)_KB5U 2.0
- 0.4238 Product*Storage period(weeks)_KB5U 3.0

Fits and Diagnostics for Unusual Observations

Obs	Yeast	Fit	Resid	Std Resid	
11	6.4300	6.5050	-0.0750	-2.18	R
15	6.5800	6.5050	0.0750	2.18	R

R Large residual



General Linear Model: AAB versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	2 KB5F, KB5U
Storage period(weeks)	Fixed	4 0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.00000	0.000000	0.00	1.000
Storage period(weeks)	3	1.32785	0.442617	65.82	0.000
Product*Storage period(weeks)	3	0.32495	0.108317	16.11	0.001
Error	8	0.05380	0.006725		
Total	15	1.70660			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0820061	96.85%	94.09%	87.39%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.8800	0.0205	335.58	0.000	
Product					
KB5F	0.0000	0.0205	0.00	1.000	1.00
Storage period(weeks)					
0.0	0.3150	0.0355	8.87	0.000	1.50
1.0	0.1650	0.0355	4.65	0.002	1.50
2.0	-0.0275	0.0355	-0.77	0.461	1.50
Product*Storage period(weeks)					
KB5F 0.0	0.0000	0.0355	0.00	1.000	1.50
KB5F 1.0	-0.1250	0.0355	-3.52	0.008	1.50
KB5F 2.0	-0.1075	0.0355	-3.03	0.016	1.50

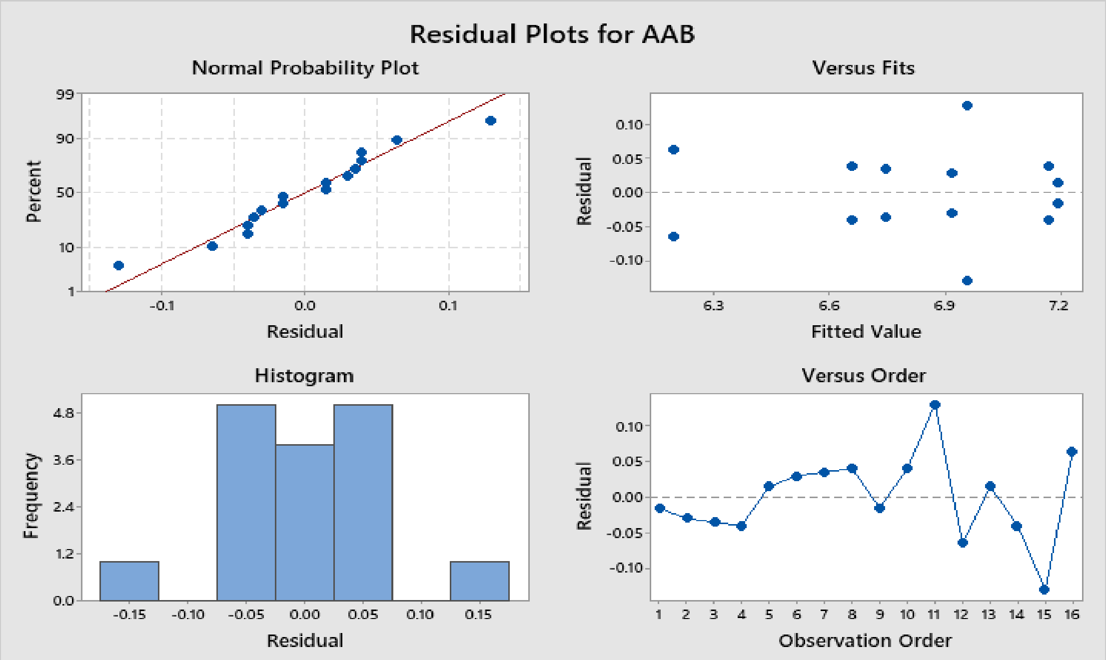
Regression Equation

AAB = 6.8800 + 0.0000 Product_KB5F - 0.0000 Product_KB5U
+ 0.3150 Storage period(weeks)_0.0
+ 0.1650 Storage period(weeks)_1.0 - 0.0275 Storage period(weeks)_2.0
- 0.4525 Storage period(weeks)_3.0 + 0.0 Product*Storage period(weeks)_KB5F 0.0
- 0.1250 Product*Storage period(weeks)_KB5F 1.0
- 0.1075 Product*Storage period(weeks)_KB5F 2.0
+ 0.2325 Product*Storage period(weeks)_KB5F 3.0
+ 0.0 Product*Storage period(weeks)_KB5U 0.0
+ 0.1250 Product*Storage period(weeks)_KB5U 1.0
+ 0.1075 Product*Storage period(weeks)_KB5U 2.0
- 0.2325 Product*Storage period(weeks)_KB5U 3.0

Fits and Diagnostics for Unusual Observations

Obs	AAB	Fit	Resid	Std Resid
11	7.0900	6.9600	0.1300	2.24 R
15	6.8300	6.9600	-0.1300	-2.24 R

R Large residual



General Linear Model: Total counts versus Product and Storage period(weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	2 KB5F, KB5U
Storage period(weeks)	Fixed	4 0.0, 1.0, 2.0, 3.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.00526	0.005256	0.54	0.483
Storage period(weeks)	3	1.81612	0.605373	62.29	0.000

Product*Storage period(weeks)	3	0.30877	0.102923	10.59	0.004
Error	8	0.07775	0.009719		
Total	15	2.20789			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0985837	96.48%	93.40%	85.91%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.7606	0.0246	274.31	0.000	
Product					
KB5F	0.0181	0.0246	0.74	0.483	1.00
Storage period(weeks)					
0.0	0.4094	0.0427	9.59	0.000	1.50
1.0	0.0994	0.0427	2.33	0.048	1.50
2.0	0.0169	0.0427	0.40	0.703	1.50
Product*Storage period(weeks)					
KB5F 0.0	-0.0181	0.0427	-0.42	0.682	1.50
KB5F 1.0	-0.1181	0.0427	-2.77	0.024	1.50
KB5F 2.0	-0.0956	0.0427	-2.24	0.055	1.50

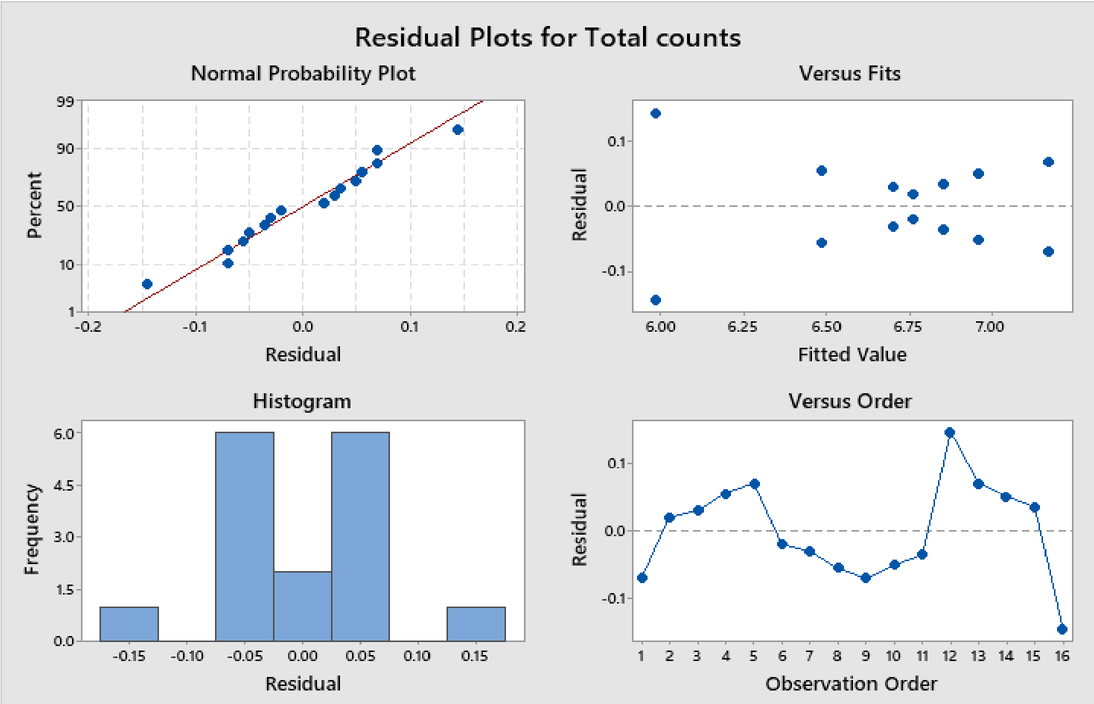
Regression Equation

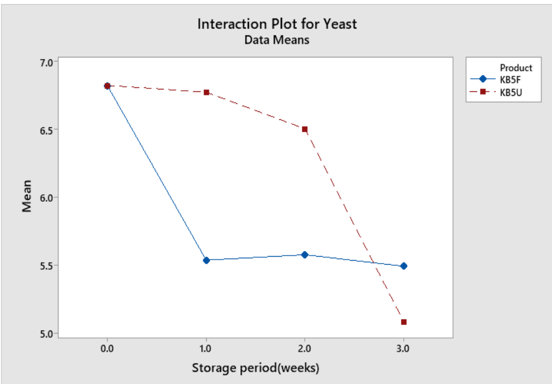
Total counts = 6.7606 + 0.0181 Product_KB5F - 0.0181 Product_KB5U
+ 0.4094 Storage period(weeks)_0.0
+ 0.0994 Storage period(weeks)_1.0
+ 0.0169 Storage period(weeks)_2.0
- 0.5256 Storage period(weeks)_3.0
- 0.0181 Product*Storage period(weeks)_KB5F 0.0
- 0.1181 Product*Storage period(weeks)_KB5F 1.0
- 0.0956 Product*Storage period(weeks)_KB5F 2.0
+ 0.2319 Product*Storage period(weeks)_KB5F 3.0
+ 0.0181 Product*Storage period(weeks)_KB5U 0.0
+ 0.1181 Product*Storage period(weeks)_KB5U 1.0
+ 0.0956 Product*Storage period(weeks)_KB5U 2.0
- 0.2319 Product*Storage period(weeks)_KB5U 3.0

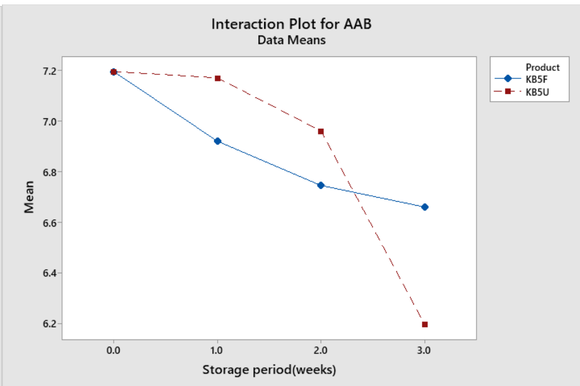
Fits and Diagnostics for Unusual Observations

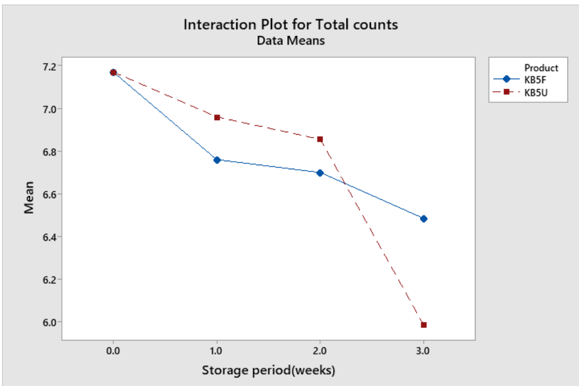
Obs	Total counts	Fit	Resid	Std Resid
12	6.1300	5.9850	0.1450	2.08 R
16	5.8400	5.9850	-0.1450	-2.08 R

R Large residual


Residual Plots for Total counts
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Interaction Plot for Yeast
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U


Interaction Plot for AAB
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U


Interaction Plot for Total counts
Data Means
Mean
Storage period(weeks)
Product
KB5F
KB5U

General Linear Model: Ethanol concentration (%v/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.03964	0.039645	10.20	0.003
Fermentation time	6	1.05322	0.175536	45.17	0.000
Product*Fermentation time	6	0.09057	0.015095	3.88	0.004
Error	42	0.16322	0.003886		
Total	55	1.34666			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0623403	87.88%	84.13%	78.45%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.20661	0.00833	24.80	0.000	
Product					
KB5F	-0.02661	0.00833	-3.19	0.003	1.00
Fermentation time					
1	-0.1566	0.0204	-7.67	0.000	1.71
2	-0.1466	0.0204	-7.18	0.000	1.71
3	-0.1316	0.0204	-6.45	0.000	1.71
4	0.0284	0.0204	1.39	0.171	1.71
5	0.0484	0.0204	2.37	0.022	1.71
6	0.1559	0.0204	7.64	0.000	1.71
Product*Fermentation time					
KB5F 1	0.0266	0.0204	1.30	0.199	1.71
KB5F 2	0.0266	0.0204	1.30	0.199	1.71
KB5F 3	0.0266	0.0204	1.30	0.199	1.71
KB5F 4	0.0266	0.0204	1.30	0.199	1.71
KB5F 5	-0.0209	0.0204	-1.02	0.312	1.71
KB5F 6	0.0041	0.0204	0.20	0.841	1.71

Regression Equation

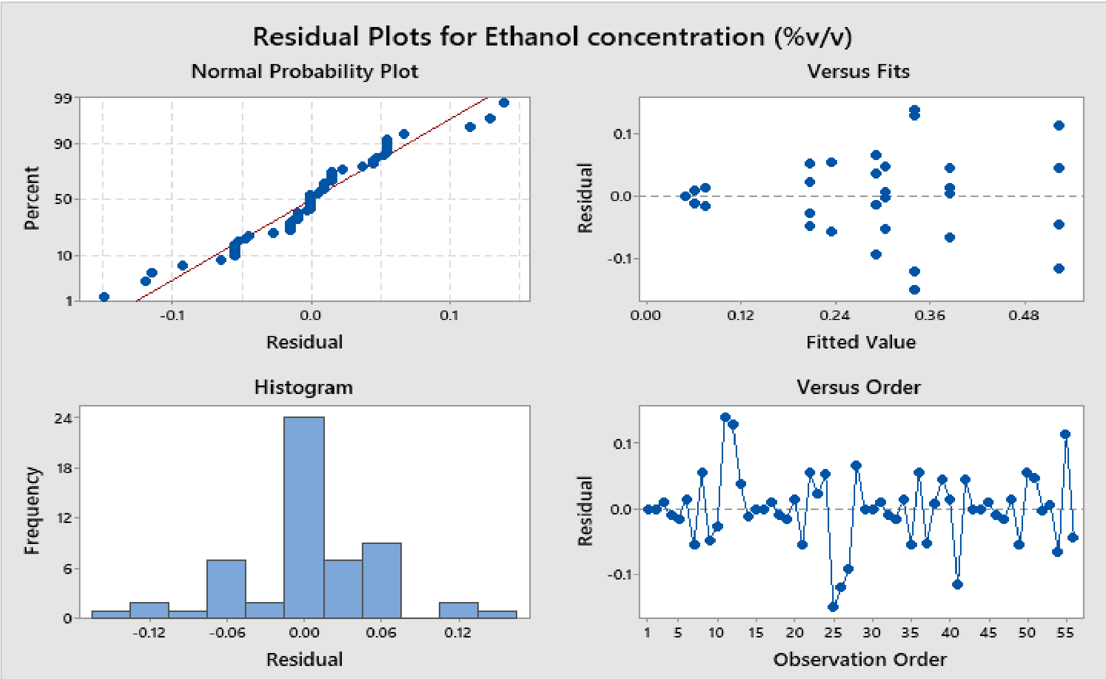
Ethanol concentration (%v/v) = 0.20661 - 0.02661 Product_KB5F
+ 0.02661 Product_KB5U
- 0.1566 Fermentation time_1
- 0.1466 Fermentation time_2
- 0.1316 Fermentation time_3
+ 0.0284 Fermentation time_4
+ 0.0484 Fermentation time_5
+ 0.1559 Fermentation time_6
+ 0.2021 Fermentation time_7
+ 0.0266 Product*Fermentation time_KB5F 1
+ 0.0266 Product*Fermentation time_KB5F 2
+ 0.0266 Product*Fermentation time_KB5F 3
+ 0.0266 Product*Fermentation time_KB5F 4
- 0.0209 Product*Fermentation time_KB5F 5
+ 0.0041 Product*Fermentation time_KB5F 6
- 0.0896 Product*Fermentation time_KB5F 7
- 0.0266 Product*Fermentation time_KB5U 1
- 0.0266 Product*Fermentation time_KB5U 2
- 0.0266 Product*Fermentation time_KB5U 3

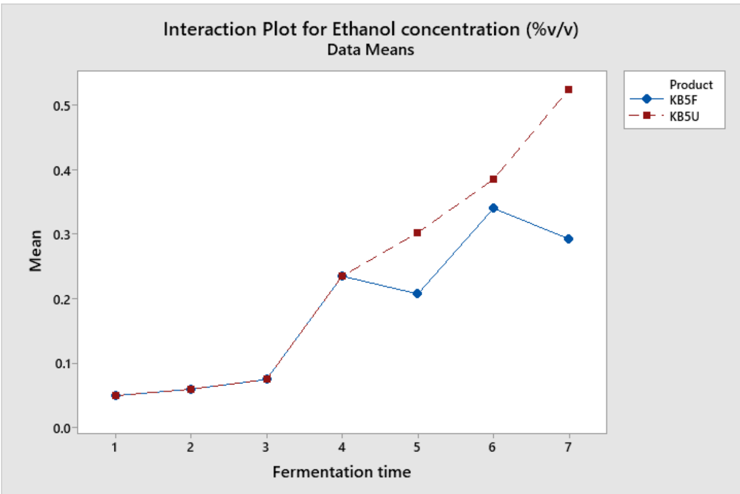
- 0.0266 Product*Fermentation time_KB5U 4
+ 0.0209 Product*Fermentation time_KB5U 5
- 0.0041 Product*Fermentation time_KB5U 6
+ 0.0896 Product*Fermentation time_KB5U 7

Fits and Diagnostics for Unusual Observations

Obs	Ethanol concentration (%v/v)	Fit	Resid	Std Resid
11	0.4800	0.3400	0.1400	2.59 R
12	0.4700	0.3400	0.1300	2.41 R
25	0.1900	0.3400	-0.1500	-2.78 R
26	0.2200	0.3400	-0.1200	-2.22 R
41	0.4100	0.5250	-0.1150	-2.13 R
55	0.6400	0.5250	0.1150	2.13 R

R Large residual





General Linear Model: Sucrose (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.0089	0.00893	0.17	0.688
Fermentation time	6	18.5450	3.09083	58.08	0.000
Product*Fermentation time	6	0.5136	0.08560	1.61	0.217
Error	14	0.7450	0.05321		
Total	27	19.8125			

Model Summary

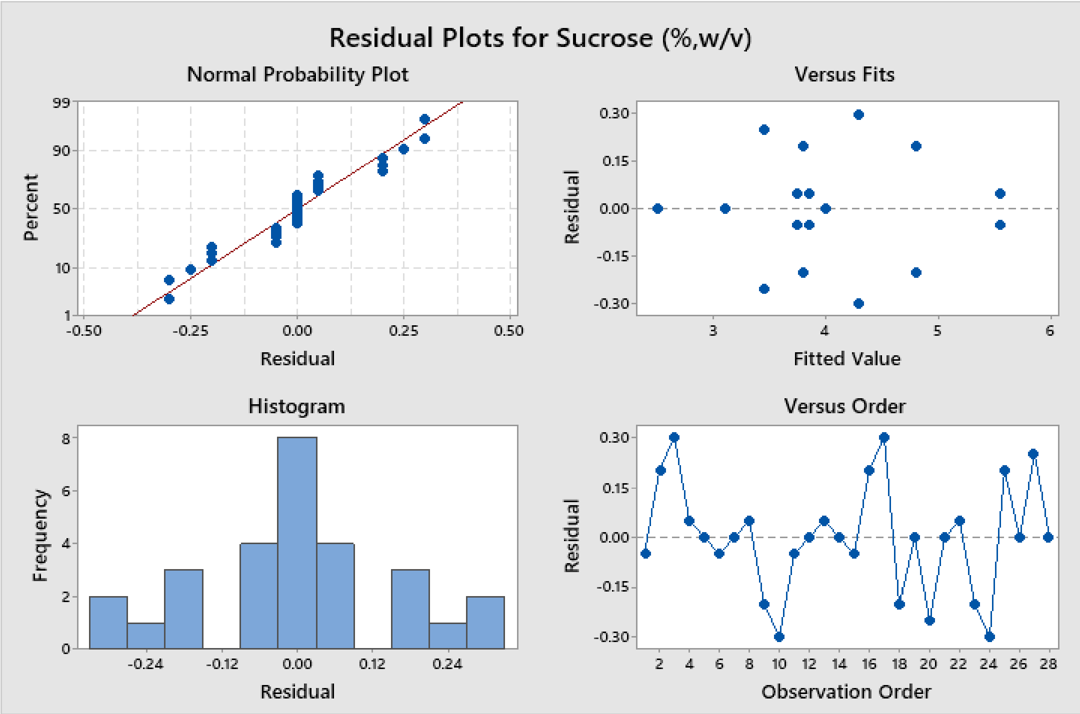
S	R-sq	R-sq(adj)	R-sq(pred)
0.230682	96.24%	92.75%	84.96%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.1250	0.0436	94.62	0.000	
Product					
KB5F	-0.0179	0.0436	-0.41	0.688	1.00
Fermentation time					
1	1.425	0.107	13.34	0.000	1.71
2	0.675	0.107	6.32	0.000	1.71
3	0.175	0.107	1.64	0.124	1.71
4	-0.350	0.107	-3.28	0.006	1.71
5	-0.125	0.107	-1.17	0.261	1.71
6	-0.475	0.107	-4.45	0.001	1.71
Product*Fermentation time					
KB5F 1	0.018	0.107	0.17	0.870	1.71
KB5F 2	0.018	0.107	0.17	0.870	1.71
KB5F 3	0.018	0.107	0.17	0.870	1.71
KB5F 4	-0.007	0.107	-0.07	0.948	1.71
KB5F 5	0.018	0.107	0.17	0.870	1.71
KB5F 6	0.218	0.107	2.04	0.061	1.71

Regression Equation

Sucrose (%,w/v) = 4.1250 - 0.0179 Product_KB5F + 0.0179 Product_KB5U
+ 1.425 Fermentation time_1 + 0.675 Fermentation time_2
+ 0.175 Fermentation time_3 - 0.350 Fermentation time_4
- 0.125 Fermentation time_5 - 0.475 Fermentation time_6
- 1.325 Fermentation time_7
+ 0.018 Product*Fermentation time_KB5F 1
+ 0.018 Product*Fermentation time_KB5F 2
+ 0.018 Product*Fermentation time_KB5F 3
- 0.007 Product*Fermentation time_KB5F 4
+ 0.018 Product*Fermentation time_KB5F 5
+ 0.218 Product*Fermentation time_KB5F 6
- 0.282 Product*Fermentation time_KB5F 7
- 0.018 Product*Fermentation time_KB5U 1
- 0.018 Product*Fermentation time_KB5U 2
- 0.018 Product*Fermentation time_KB5U 3
+ 0.007 Product*Fermentation time_KB5U 4
- 0.018 Product*Fermentation time_KB5U 5
- 0.218 Product*Fermentation time_KB5U 6
+ 0.282 Product*Fermentation time_KB5U 7



General Linear Model: Glucose (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Product	Fixed	2 KB5F, KB5U
Fermentation time	Fixed	7 1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.00036	0.000357	0.06	0.812
Fermentation time	6	0.91929	0.153214	25.24	0.000
Product*Fermentation time	6	0.08214	0.013690	2.25	0.099
Error	14	0.08500	0.006071		
Total	27	1.08679			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0779194	92.18%	84.92%	68.72%

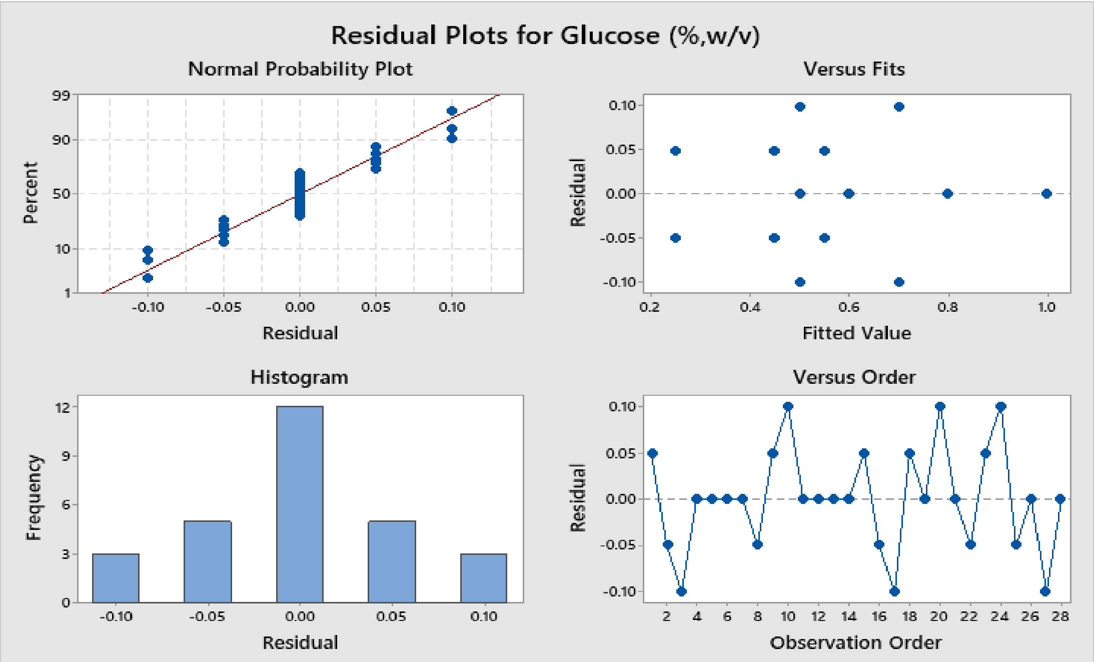
Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.5393	0.0147	36.62	0.000	
Product					
KB5F	0.0036	0.0147	0.24	0.812	1.00
Fermentation time					
1	-0.2893	0.0361	-8.02	0.000	1.71
2	-0.0893	0.0361	-2.48	0.027	1.71
3	-0.0393	0.0361	-1.09	0.294	1.71

4	0.0357	0.0361	0.99	0.339	1.71
5	-0.0393	0.0361	-1.09	0.294	1.71
6	0.0607	0.0361	1.68	0.114	1.71
Product*Fermentation time					
KB5F 1	-0.0036	0.0361	-0.10	0.923	1.71
KB5F 2	-0.0036	0.0361	-0.10	0.923	1.71
KB5F 3	-0.0036	0.0361	-0.10	0.923	1.71
KB5F 4	0.0214	0.0361	0.59	0.562	1.71
KB5F 5	-0.0036	0.0361	-0.10	0.923	1.71
KB5F 6	-0.1036	0.0361	-2.87	0.012	1.71

Regression Equation

Glucose (%,w/v) = 0.5393 + 0.0036 Product_KB5F - 0.0036 Product_KB5U
- 0.2893 Fermentation time_1 - 0.0893 Fermentation time_2
- 0.0393 Fermentation time_3 + 0.0357 Fermentation time_4
- 0.0393 Fermentation time_5 + 0.0607 Fermentation time_6
+ 0.3607 Fermentation time_7
- 0.0036 Product*Fermentation time_KB5F 1
- 0.0036 Product*Fermentation time_KB5F 2
- 0.0036 Product*Fermentation time_KB5F 3
+ 0.0214 Product*Fermentation time_KB5F 4
- 0.0036 Product*Fermentation time_KB5F 5
- 0.1036 Product*Fermentation time_KB5F 6
+ 0.0964 Product*Fermentation time_KB5F 7
+ 0.0036 Product*Fermentation time_KB5U 1
+ 0.0036 Product*Fermentation time_KB5U 2
+ 0.0036 Product*Fermentation time_KB5U 3
- 0.0214 Product*Fermentation time_KB5U 4
+ 0.0036 Product*Fermentation time_KB5U 5
+ 0.1036 Product*Fermentation time_KB5U 6
- 0.0964 Product*Fermentation time_KB5U 7



General Linear Model: Fructose (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U

Fermentation time	Fixed	7 1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.001429	0.001429	0.11	0.750
Fermentation time	6	0.677143	0.112857	8.32	0.001
Product*Fermentation time	6	0.048571	0.008095	0.60	0.729
Error	14	0.190000	0.013571		
Total	27	0.917143			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.116496	79.28%	60.05%	17.13%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.4714	0.0220	21.41	0.000	
Product					
KB5F	-0.0071	0.0220	-0.32	0.750	1.00
Fermentation time					
1	-0.2214	0.0539	-4.11	0.001	1.71
2	-0.0714	0.0539	-1.32	0.207	1.71
3	0.0286	0.0539	0.53	0.605	1.71
4	-0.0214	0.0539	-0.40	0.697	1.71
5	-0.0714	0.0539	-1.32	0.207	1.71
6	0.0286	0.0539	0.53	0.605	1.71
Product*Fermentation time					
KB5F 1	0.0071	0.0539	0.13	0.897	1.71
KB5F 2	0.0071	0.0539	0.13	0.897	1.71
KB5F 3	0.0071	0.0539	0.13	0.897	1.71
KB5F 4	0.0571	0.0539	1.06	0.307	1.71
KB5F 5	0.0071	0.0539	0.13	0.897	1.71
KB5F 6	-0.0929	0.0539	-1.72	0.107	1.71

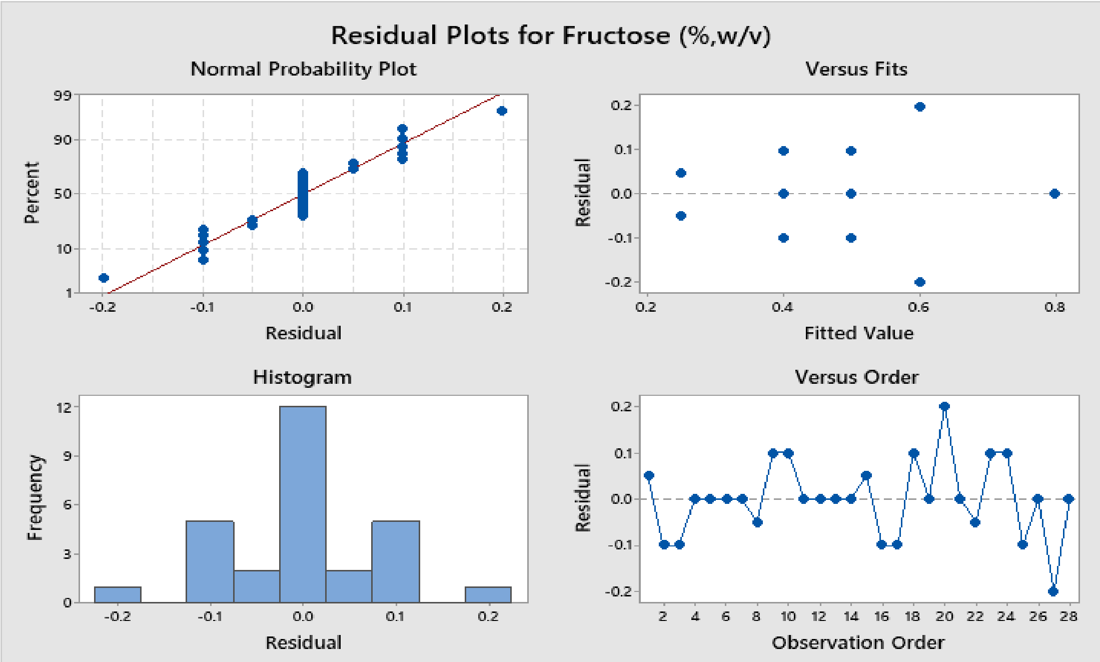
Regression Equation

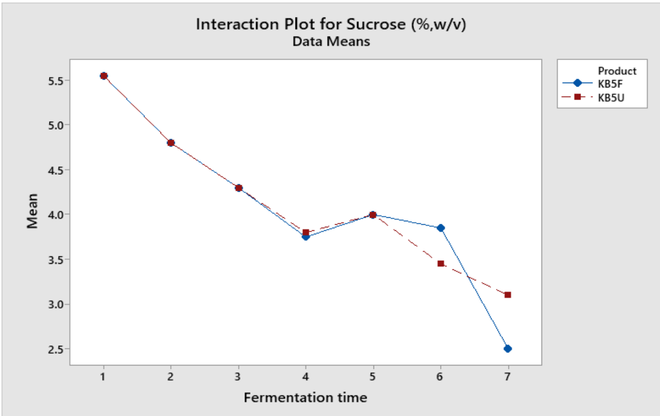
Fructose (%,w/v) = 0.4714 - 0.0071 Product_KB5F + 0.0071 Product_KB5U
- 0.2214 Fermentation time_1 - 0.0714 Fermentation time_2
+ 0.0286 Fermentation time_3 - 0.0214 Fermentation time_4
- 0.0714 Fermentation time_5 + 0.0286 Fermentation time_6
+ 0.3286 Fermentation time_7
+ 0.0071 Product*Fermentation time_KB5F 1
+ 0.0071 Product*Fermentation time_KB5F 2
+ 0.0071 Product*Fermentation time_KB5F 3
+ 0.0571 Product*Fermentation time_KB5F 4
+ 0.0071 Product*Fermentation time_KB5F 5
- 0.0929 Product*Fermentation time_KB5F 6
+ 0.0071 Product*Fermentation time_KB5F 7
- 0.0071 Product*Fermentation time_KB5U 1
- 0.0071 Product*Fermentation time_KB5U 2
- 0.0071 Product*Fermentation time_KB5U 3
- 0.0571 Product*Fermentation time_KB5U 4
- 0.0071 Product*Fermentation time_KB5U 5
+ 0.0929 Product*Fermentation time_KB5U 6
- 0.0071 Product*Fermentation time_KB5U 7

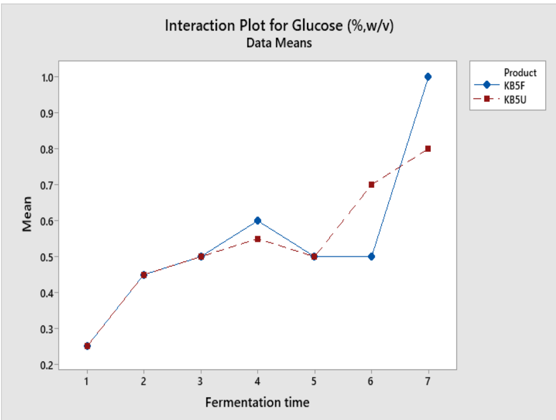
Fits and Diagnostics for Unusual Observations

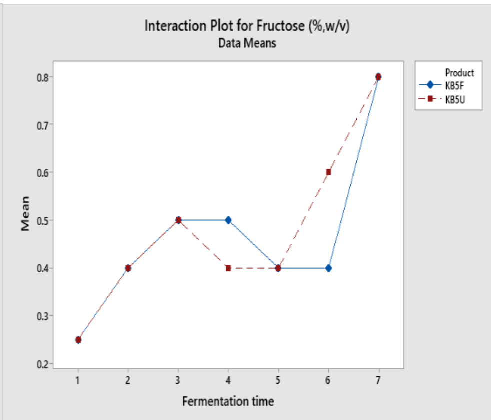
Obs	Fructose (%,w/v)	Fit	Resid	Std Resid
20	0.8000	0.6000	0.2000	2.43 R
27	0.4000	0.6000	-0.2000	-2.43 R

R Large residual


Residual Plots for Fructose (%,w/v)
Normal Probability Plot
Versus Fits
Percent
Residual
Residual
Fitted Value
Histogram
Versus Order
Frequency
Residual
Residual
Observation Order


Interaction Plot for Sucrose (%,w/v)
Data Means
Product
KB5F
KB5U
Mean
Fermentation time


Interaction Plot for Glucose (%,w/v)
Data Means
Product
KB5F
KB5U
Mean
Fermentation time


Interaction Plot for Fructose (%,w/v)
Data Means
Product
KB5F
KB5U
Mean
Fermentation time

General Linear Model: Glucuronic acid (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.008774	0.008774	26.06	0.000
Fermentation time	6	0.031562	0.005260	15.62	0.000
Product*Fermentation time	6	0.038674	0.006446	19.14	0.000
Error	14	0.004714	0.000337		
Total	27	0.083725			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0183500	94.37%	89.14%	77.48%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.05521	0.00347	15.92	0.000	
Product					
KB5F	0.01770	0.00347	5.10	0.000	1.00
Fermentation time					
1	-0.00313	0.00849	-0.37	0.718	1.71
2	-0.01303	0.00849	-1.53	0.147	1.71
3	-0.01721	0.00849	-2.03	0.062	1.71
4	-0.02046	0.00849	-2.41	0.030	1.71
5	-0.02190	0.00849	-2.58	0.022	1.71
6	-0.00484	0.00849	-0.57	0.578	1.71
Product*Fermentation time					
KB5F 1	-0.01770	0.00849	-2.08	0.056	1.71
KB5F 2	-0.01770	0.00849	-2.08	0.056	1.71
KB5F 3	-0.01770	0.00849	-2.08	0.056	1.71
KB5F 4	-0.02033	0.00849	-2.39	0.031	1.71
KB5F 5	-0.01887	0.00849	-2.22	0.043	1.71
KB5F 6	0.00314	0.00849	0.37	0.717	1.71

Regression Equation

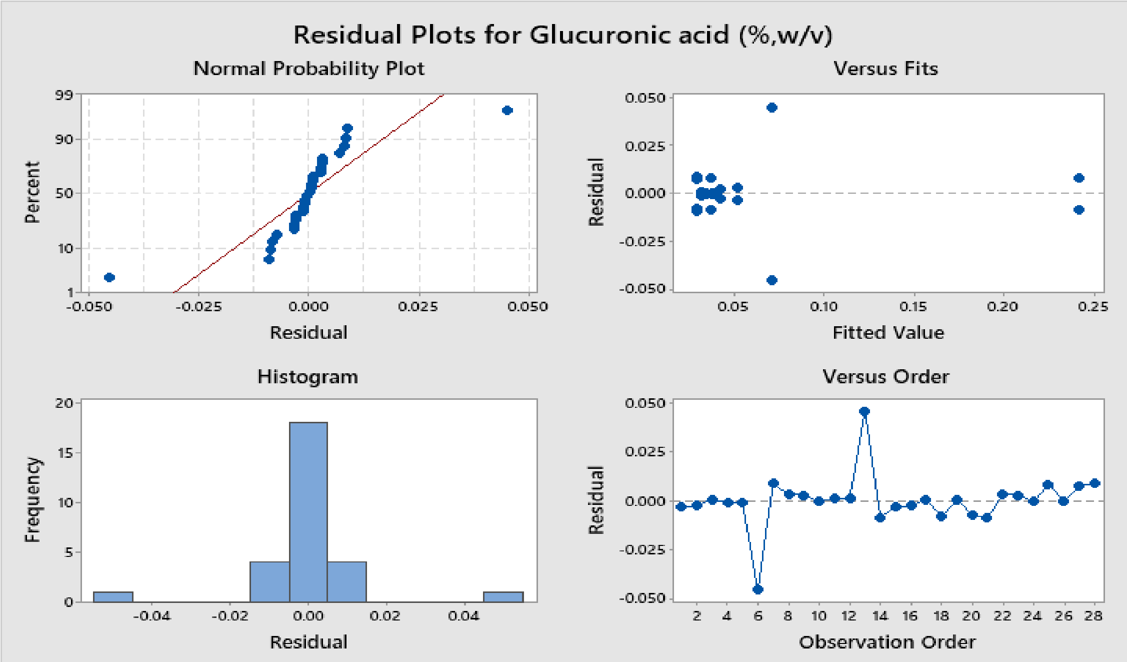
Glucuronic acid (%,w/v) = 0.05521 + 0.01770 Product_KB5F - 0.01770 Product_KB5U
- 0.00313 Fermentation time_1
- 0.01303 Fermentation time_2
- 0.01721 Fermentation time_3
- 0.02046 Fermentation time_4
- 0.02190 Fermentation time_5
- 0.00484 Fermentation time_6
+ 0.08058 Fermentation time_7
- 0.01770 Product*Fermentation time_KB5F 1
- 0.01770 Product*Fermentation time_KB5F 2
- 0.01770 Product*Fermentation time_KB5F 3
- 0.02033 Product*Fermentation time_KB5F 4
- 0.01887 Product*Fermentation time_KB5F 5
+ 0.00314 Product*Fermentation time_KB5F 6
+ 0.08916 Product*Fermentation time_KB5F 7
+ 0.01770 Product*Fermentation time_KB5U 1
+ 0.01770 Product*Fermentation time_KB5U 2
+ 0.01770 Product*Fermentation time_KB5U 3
+ 0.02033 Product*Fermentation time_KB5U 4
+ 0.01887 Product*Fermentation time_KB5U 5

- 0.00314 Product*Fermentation time_KB5U 6
- 0.08916 Product*Fermentation time_KB5U 7

Fits and Diagnostics for Unusual Observations

Obs	Glucuronic acid (%,w/v)	Fit	Resid	Std Resid
6	0.0260	0.0712	-0.0452	-3.48 R
13	0.1164	0.0712	0.0452	3.48 R

R Large residual



General Linear Model: Gluconic acid (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.000031	0.000031	0.25	0.625
Fermentation time	6	0.004823	0.000804	6.44	0.002
Product*Fermentation time	6	0.000656	0.000109	0.88	0.537
Error	14	0.001749	0.000125		
Total	27	0.007260			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0111770	75.91%	53.54%	3.64%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.03351	0.00211	15.86	0.000	
Product					
KB5F	0.00106	0.00211	0.50	0.625	1.00
Fermentation time					
1	-0.01773	0.00517	-3.43	0.004	1.71
2	-0.01188	0.00517	-2.30	0.038	1.71
3	-0.01120	0.00517	-2.17	0.048	1.71
4	0.01222	0.00517	2.36	0.033	1.71
5	0.00081	0.00517	0.16	0.878	1.71
6	0.00724	0.00517	1.40	0.183	1.71
Product*Fermentation time					
KB5F 1	-0.00106	0.00517	-0.20	0.841	1.71
KB5F 2	-0.00106	0.00517	-0.20	0.841	1.71
KB5F 3	-0.00106	0.00517	-0.20	0.841	1.71
KB5F 4	-0.00014	0.00517	-0.03	0.978	1.71
KB5F 5	0.00037	0.00517	0.07	0.944	1.71
KB5F 6	-0.00736	0.00517	-1.42	0.177	1.71

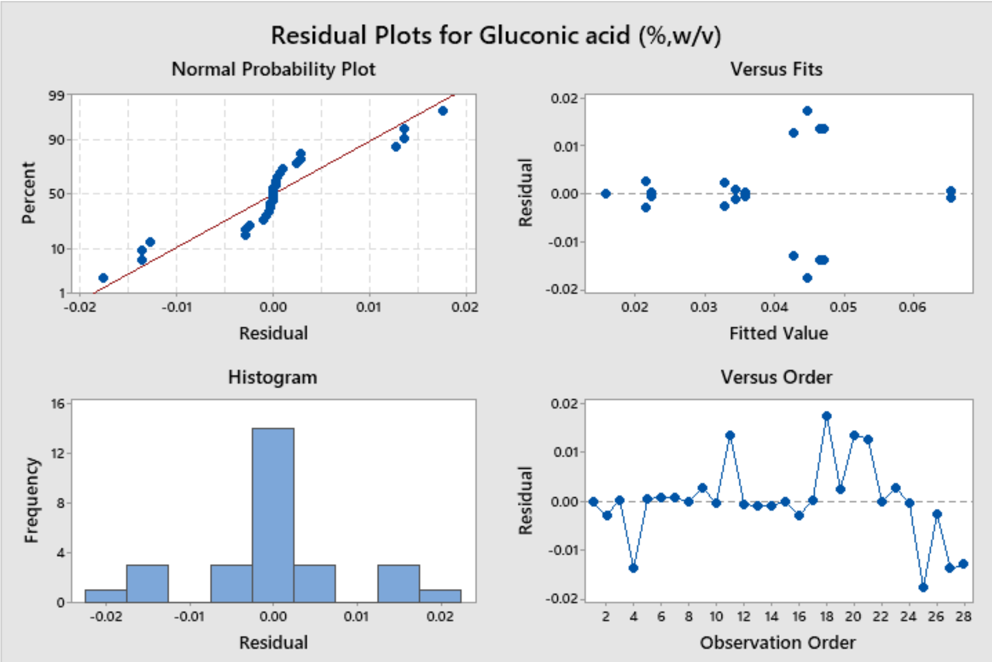
Regression Equation

Gluconic acid (%,w/v) = 0.03351 + 0.00106 Product_KB5F - 0.00106 Product_KB5U
- 0.01773 Fermentation time_1
- 0.01188 Fermentation time_2
- 0.01120 Fermentation time_3
+ 0.01222 Fermentation time_4
+ 0.00081 Fermentation time_5
+ 0.00724 Fermentation time_6
+ 0.02055 Fermentation time_7
- 0.00106 Product*Fermentation time_KB5F 1
- 0.00106 Product*Fermentation time_KB5F 2
- 0.00106 Product*Fermentation time_KB5F 3
- 0.00014 Product*Fermentation time_KB5F 4
+ 0.00037 Product*Fermentation time_KB5F 5
- 0.00736 Product*Fermentation time_KB5F 6
+ 0.01031 Product*Fermentation time_KB5F 7
+ 0.00106 Product*Fermentation time_KB5U 1
+ 0.00106 Product*Fermentation time_KB5U 2
+ 0.00106 Product*Fermentation time_KB5U 3
+ 0.00014 Product*Fermentation time_KB5U 4
- 0.00037 Product*Fermentation time_KB5U 5
+ 0.00736 Product*Fermentation time_KB5U 6
- 0.01031 Product*Fermentation time_KB5U 7

Fits and Diagnostics for Unusual Observations

Obs	Gluconic acid (%,w/v)	Fit	Resid	Std Resid
18	0.06243	0.04482	0.01761	2.23 R
25	0.02720	0.04482	-0.01761	-2.23 R

R Large residual



General Linear Model: Acetic acid (%,w/v) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	0.009315	0.009315	4.10	0.062
Fermentation time	6	0.059351	0.009892	4.36	0.011
Product*Fermentation time	6	0.012346	0.002058	0.91	0.517
Error	14	0.031768	0.002269		
Total	27	0.112780			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0476357	71.83%	45.68%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.12747	0.00900	14.16	0.000	
Product					
KB5F	- 0.01824	0.00900	-2.03	0.062	1.00
Fermentation time					
1	-0.0702	0.0221	-3.18	0.007	1.71

2	-0.0594	0.0221	-2.70	0.017	1.71
3	-0.0190	0.0221	-0.86	0.403	1.71
4	0.0371	0.0221	1.68	0.115	1.71
5	0.0210	0.0221	0.95	0.356	1.71
6	0.0383	0.0221	1.74	0.104	1.71
Product*Fermentation time					
KB5F 1	0.0182	0.0221	0.83	0.422	1.71
KB5F 2	0.0182	0.0221	0.83	0.422	1.71
KB5F 3	-0.0121	0.0221	-0.55	0.592	1.71
KB5F 4	0.0027	0.0221	0.12	0.905	1.71
KB5F 5	0.0274	0.0221	1.24	0.234	1.71
KB5F 6	-0.0231	0.0221	-1.05	0.313	1.71

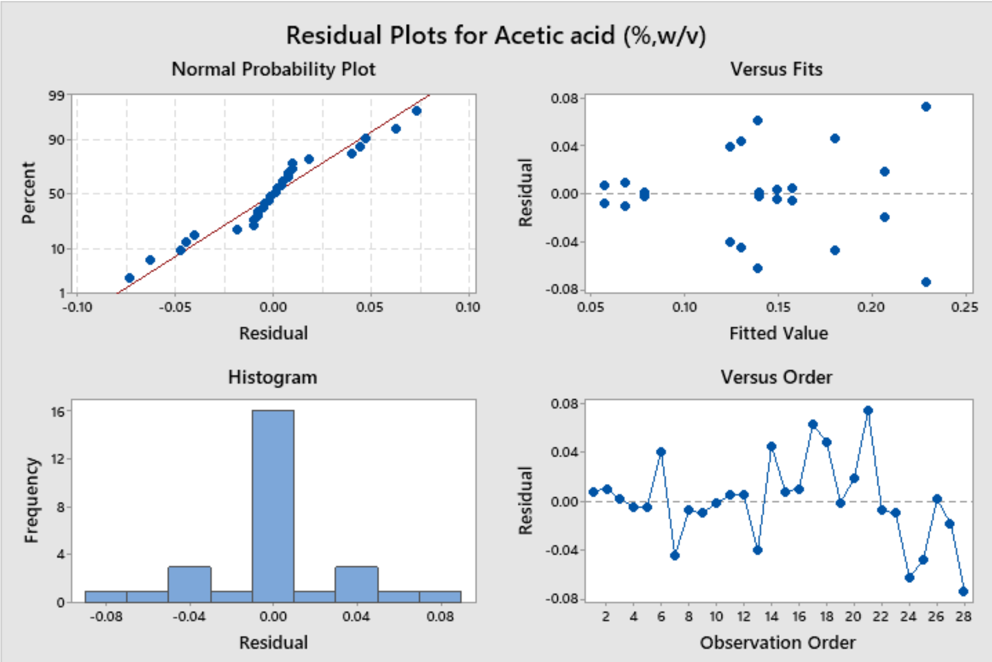
Regression Equation

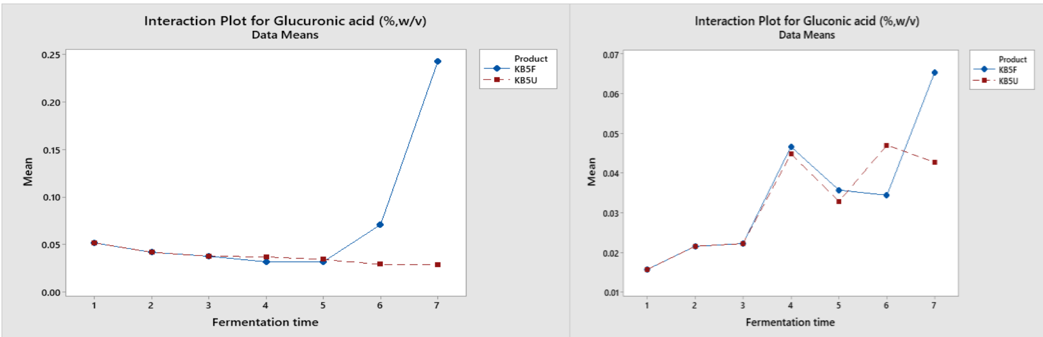
Acetic acid (%,w/v) = 0.12747 - 0.01824 Product_KB5F + 0.01824 Product_KB5U
- 0.0702 Fermentation time_1 - 0.0594 Fermentation time_2
- 0.0190 Fermentation time_3 + 0.0371 Fermentation time_4
+ 0.0210 Fermentation time_5 + 0.0383 Fermentation time_6
+ 0.0522 Fermentation time_7
+ 0.0182 Product*Fermentation time_KB5F 1
+ 0.0182 Product*Fermentation time_KB5F 2
- 0.0121 Product*Fermentation time_KB5F 3
+ 0.0027 Product*Fermentation time_KB5F 4
+ 0.0274 Product*Fermentation time_KB5F 5
- 0.0231 Product*Fermentation time_KB5F 6
- 0.0314 Product*Fermentation time_KB5F 7
- 0.0182 Product*Fermentation time_KB5U 1
- 0.0182 Product*Fermentation time_KB5U 2
+ 0.0121 Product*Fermentation time_KB5U 3
- 0.0027 Product*Fermentation time_KB5U 4
- 0.0274 Product*Fermentation time_KB5U 5
+ 0.0231 Product*Fermentation time_KB5U 6
+ 0.0314 Product*Fermentation time_KB5U 7

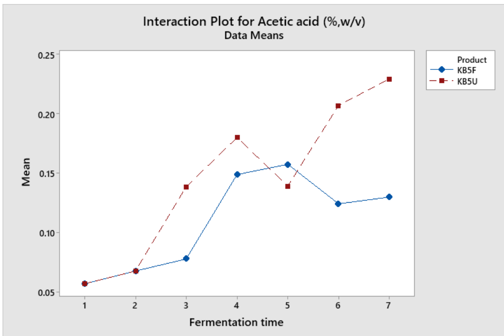
Fits and Diagnostics for Unusual Observations

Obs	Acetic acid (%,w/v)	Fit	Resid	Std Resid
21	0.3027	0.2293	0.0734	2.18 R
28	0.1559	0.2293	-0.0734	-2.18 R

R Large residual







General Linear Model: Gallic acid(µg/ml) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding	(-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	1137	1137.5	3.43	0.094
Fermentation time	4	3439	859.7	2.59	0.101
Product*Fermentation time	4	3682	920.6	2.78	0.087
Error	10	3315	331.5		
Total	19	11574			

Model Summary

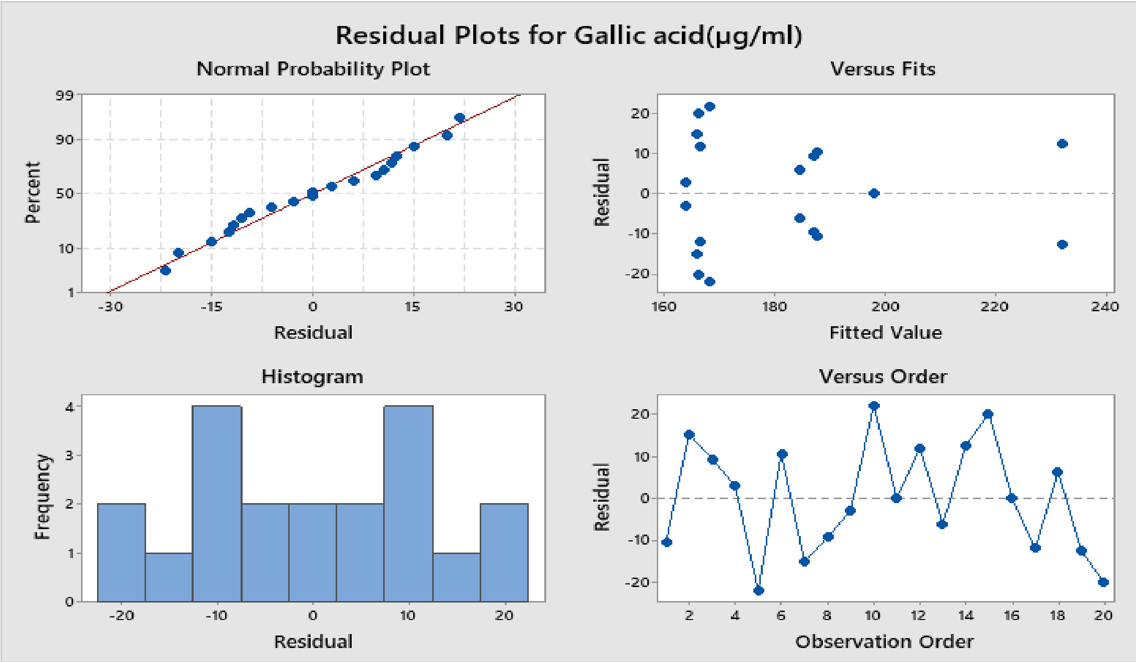
S	R-sq	R-sq(adj)	R-sq(pred)
18.2071	71.36%	45.58%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	181.94	4.07	44.69	0.000	
Product					
KB5F	-7.54	4.07	-1.85	0.094	1.00
Fermentation time					
1.0	10.85	8.14	1.33	0.212	1.60
2.0	-15.87	8.14	-1.95	0.080	1.60
3.0	3.86	8.14	0.47	0.645	1.60
4.0	15.98	8.14	1.96	0.078	1.60
Product*Fermentation time					
KB5F 1.0	2.27	8.14	0.28	0.786	1.60
KB5F 2.0	7.18	8.14	0.88	0.399	1.60
KB5F 3.0	8.86	8.14	1.09	0.302	1.60
KB5F 4.0	-26.73	8.14	-3.28	0.008	1.60

Regression Equation

Gallic acid(µg/ml) = 181.94 - 7.54 Product_KB5F + 7.54 Product_KB5U
+ 10.85 Fermentation time_1.0 - 15.87 Fermentation time_2.0
+ 3.86 Fermentation time_3.0 + 15.98 Fermentation time_4.0
- 14.82 Fermentation time_5.0 + 2.27 Product*Fermentation time_KB5F 1.0
+ 7.18 Product*Fermentation time_KB5F 2.0
+ 8.86 Product*Fermentation time_KB5F 3.0
- 26.73 Product*Fermentation time_KB5F 4.0
+ 8.43 Product*Fermentation time_KB5F 5.0
- 2.27 Product*Fermentation time_KB5U 1.0
- 7.18 Product*Fermentation time_KB5U 2.0
- 8.86 Product*Fermentation time_KB5U 3.0
+ 26.73 Product*Fermentation time_KB5U 4.0
- 8.43 Product*Fermentation time_KB5U 5.0



General Linear Model: Theobromine(μg/ml) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding	(-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	2.170	2.1699	1.41	0.262
Fermentation time	4	23.346	5.8365	3.80	0.040
Product*Fermentation time	4	1.458	0.3646	0.24	0.911
Error	10	15.375	1.5375		
Total	19	42.349			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.23994	63.70%	31.02%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.244	0.277	22.52	0.000	
Product					
KB5F	-0.329	0.277	-1.19	0.262	1.00
Fermentation time					
1.0	1.501	0.555	2.71	0.022	1.60

2.0	-1.863	0.555	-3.36	0.007	1.60
3.0	0.234	0.555	0.42	0.682	1.60
4.0	-0.094	0.555	-0.17	0.869	1.60
Product*Fermentation time					
KB5F 1.0	0.139	0.555	0.25	0.808	1.60
KB5F 2.0	-0.125	0.555	-0.23	0.826	1.60
KB5F 3.0	0.460	0.555	0.83	0.426	1.60
KB5F 4.0	-0.181	0.555	-0.33	0.751	1.60

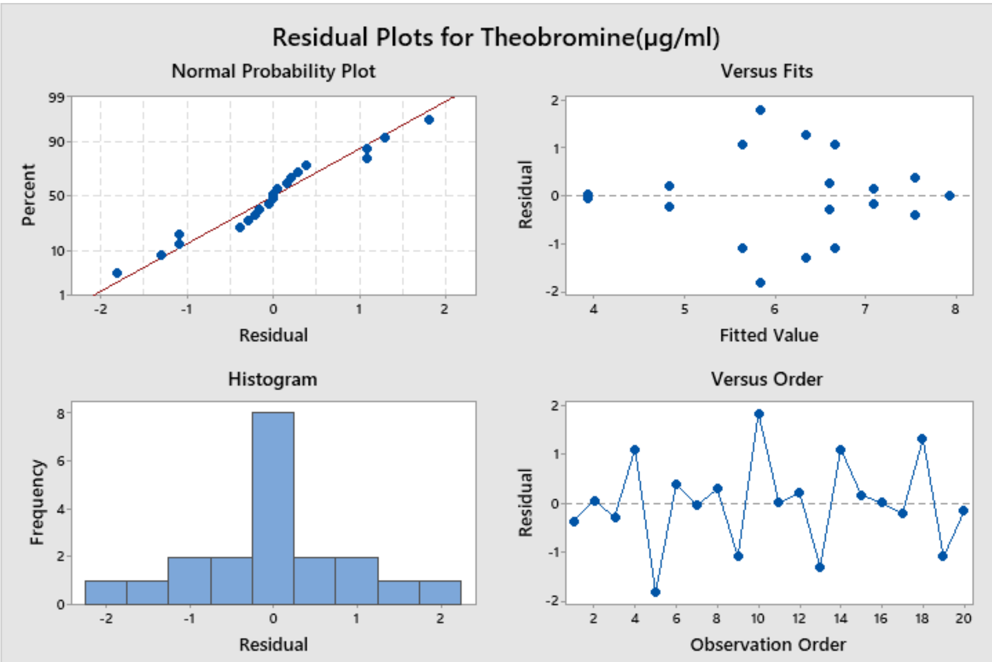
Regression Equation

Theobromine(µg/ml) = 6.244 - 0.329 Product_KB5F + 0.329 Product_KB5U
+ 1.501 Fermentation time_1.0 - 1.863 Fermentation time_2.0
+ 0.234 Fermentation time_3.0 - 0.094 Fermentation time_4.0
+ 0.222 Fermentation time_5.0 + 0.139 Product*Fermentation time_KB5F 1.0
- 0.125 Product*Fermentation time_KB5F 2.0
+ 0.460 Product*Fermentation time_KB5F 3.0
- 0.181 Product*Fermentation time_KB5F 4.0
- 0.293 Product*Fermentation time_KB5F 5.0
- 0.139 Product*Fermentation time_KB5U 1.0
+ 0.125 Product*Fermentation time_KB5U 2.0
- 0.460 Product*Fermentation time_KB5U 3.0
+ 0.181 Product*Fermentation time_KB5U 4.0
+ 0.293 Product*Fermentation time_KB5U 5.0

Fits and Diagnostics for Unusual Observations

Obs	Theobromine(µg/ml)	Fit	Resid	Std Resid
5	4.027	5.844	-1.817	-2.07 R
10	7.661	5.844	1.817	2.07 R

R Large residual



General Linear Model: Caffeine(µg/ml) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	775.6	775.6	2.93	0.118
Fermentation time	4	4195.3	1048.8	3.96	0.035
Product*Fermentation time	4	666.3	166.6	0.63	0.653
Error	10	2650.0	265.0		
Total	19	8287.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
16.2789	68.02%	39.24%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	135.99	3.64	37.36	0.000	
Product					
KB5F	-6.23	3.64	-1.71	0.118	1.00
Fermentation time					
1.0	22.08	7.28	3.03	0.013	1.60
2.0	-20.47	7.28	-2.81	0.018	1.60
3.0	-1.08	7.28	-0.15	0.885	1.60
4.0	8.13	7.28	1.12	0.290	1.60
Product*Fermentation time					
KB5F 1.0	0.29	7.28	0.04	0.969	1.60
KB5F 2.0	5.02	7.28	0.69	0.506	1.60
KB5F 3.0	7.53	7.28	1.03	0.326	1.60
KB5F 4.0	-7.48	7.28	-1.03	0.328	1.60

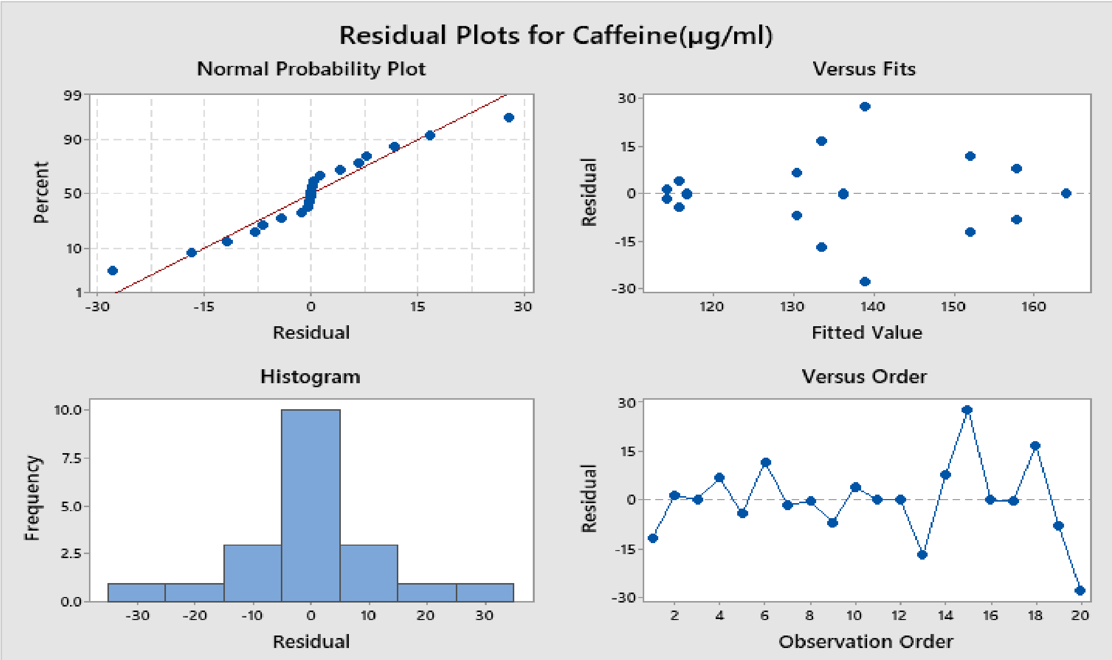
Regression Equation

Caffeine(µg/ml) = 135.99 - 6.23 Product_KB5F + 6.23 Product_KB5U
+ 22.08 Fermentation time_1.0 - 20.47 Fermentation time_2.0
- 1.08 Fermentation time_3.0 + 8.13 Fermentation time_4.0
- 8.66 Fermentation time_5.0 + 0.29 Product*Fermentation time_KB5F 1.0
+ 5.02 Product*Fermentation time_KB5F 2.0
+ 7.53 Product*Fermentation time_KB5F 3.0
- 7.48 Product*Fermentation time_KB5F 4.0
- 5.36 Product*Fermentation time_KB5F 5.0
- 0.29 Product*Fermentation time_KB5U 1.0
- 5.02 Product*Fermentation time_KB5U 2.0
- 7.53 Product*Fermentation time_KB5U 3.0
+ 7.48 Product*Fermentation time_KB5U 4.0
+ 5.36 Product*Fermentation time_KB5U 5.0

Fits and Diagnostics for Unusual Observations

Obs	Caffeine(µg/ml)	Fit	Resid	Std Resid
15	166.8	138.9	27.8	2.42 R
20	111.1	138.9	-27.8	-2.42 R

R Large residual



General Linear Model: EGC(µg/ml) versus Product, Fermentation time and storage period (Weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	32.28	32.28	0.08	0.780
Fermentation time	4	2988.56	747.14	1.90	0.187
Product*Fermentation time	4	66.92	16.73	0.04	0.996
Error	10	3934.88	393.49		
Total	19	7022.64			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
19.8365	43.97%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF

Constant	28.18	4.44	6.35	0.000	
Product					
KB5F	-1.27	4.44	-0.29	0.780	1.00
Fermentation time					
1.0	-5.78	8.87	-0.65	0.530	1.60
2.0	-5.95	8.87	-0.67	0.517	1.60
3.0	-7.57	8.87	-0.85	0.413	1.60
4.0	-5.09	8.87	-0.57	0.579	1.60
Product*Fermentation time					
KB5F 1.0	0.94	8.87	0.11	0.918	1.60
KB5F 2.0	0.92	8.87	0.10	0.920	1.60
KB5F 3.0	-0.74	8.87	-0.08	0.936	1.60
KB5F 4.0	-3.19	8.87	-0.36	0.727	1.60

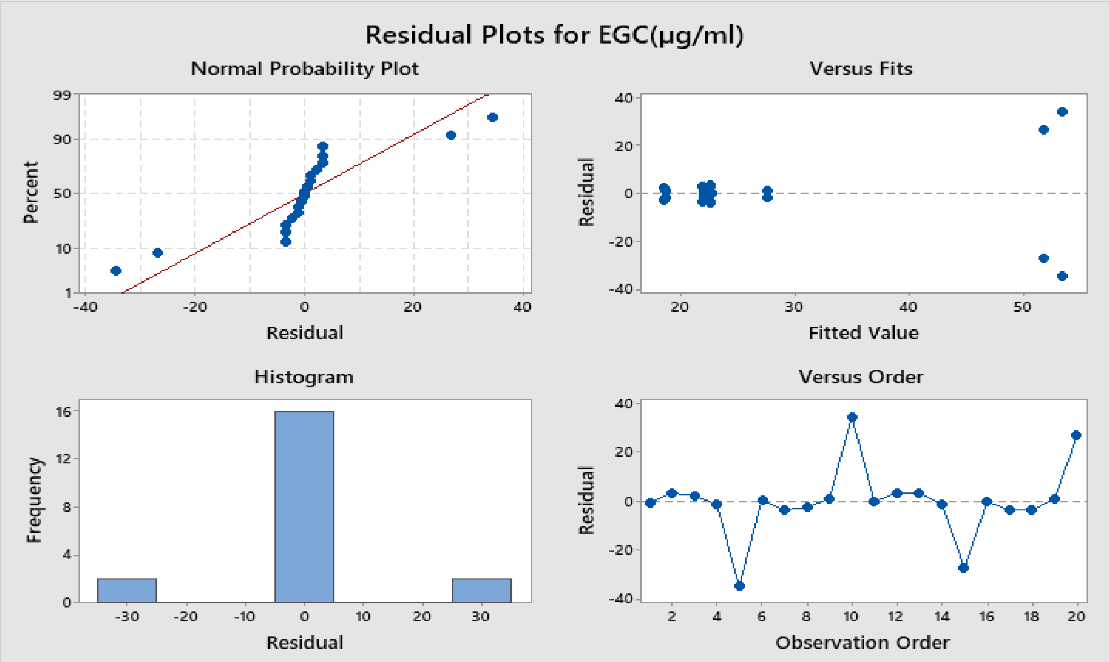
Regression Equation

EGC(µg/ml) = 28.18 - 1.27 Product_KB5F + 1.27 Product_KB5U - 5.78 Fermentation time_1.0
- 5.95 Fermentation time_2.0 - 7.57 Fermentation time_3.0
- 5.09 Fermentation time_4.0 + 24.39 Fermentation time_5.0
+ 0.94 Product*Fermentation time_KB5F 1.0
+ 0.92 Product*Fermentation time_KB5F
2.0 - 0.74 Product*Fermentation time_KB5F 3.0
- 3.19 Product*Fermentation time_KB5F 4.0
+ 2.07 Product*Fermentation time_KB5F
5.0 - 0.94 Product*Fermentation time_KB5U 1.0
- 0.92 Product*Fermentation time_KB5U 2.0
+ 0.74 Product*Fermentation time_KB5U
3.0 + 3.19 Product*Fermentation time_KB5U 4.0
- 2.07 Product*Fermentation time_KB5U 5.0

Fits and Diagnostics for Unusual Observations

Obs	EGC(µg/ml)	Fit	Resid	Std Resid
5	18.8	53.4	-34.5	-2.46 R
10	87.9	53.4	34.5	2.46 R

R Large residual



General Linear Model: EGCG(µg/ml) versus Product, Fermentation time and storage period (weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	20.62	20.62	0.68	0.429
Fermentation time	4	179.72	44.93	1.48	0.280
Product*Fermentation time	4	78.08	19.52	0.64	0.645
Error	10	303.90	30.39		
Total	19	582.32			

Model Summary

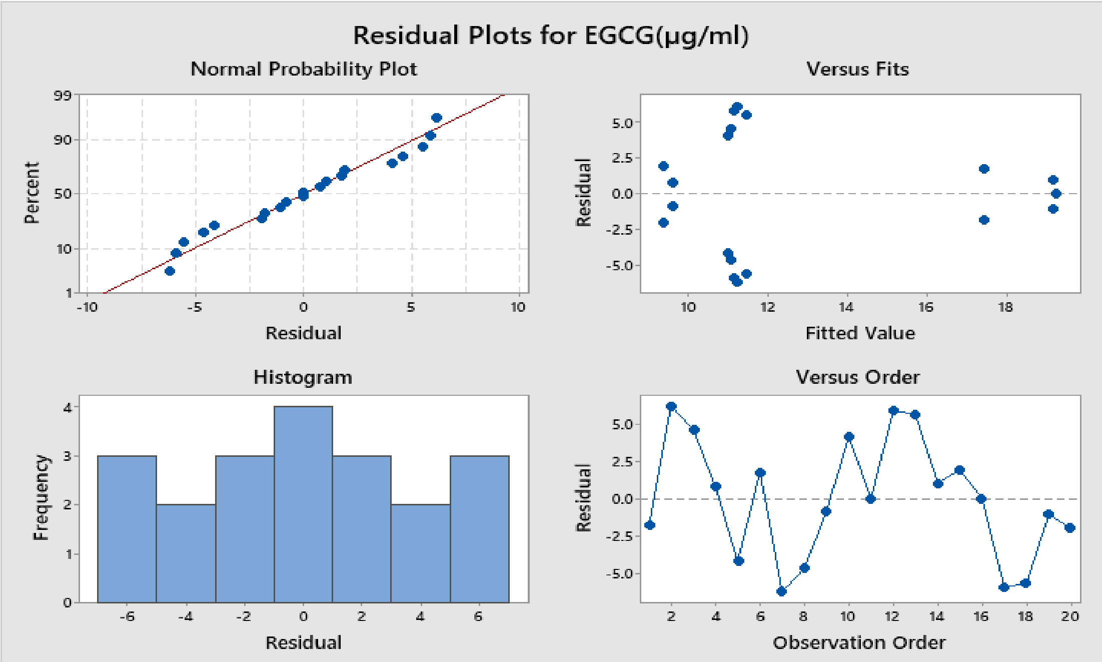
S	R-sq	R-sq(adj)	R-sq(pred)
5.51273	47.81%	0.84%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13.08	1.23	10.61	0.000	
Product					
KB5F	-1.02	1.23	-0.82	0.429	1.00
Fermentation time					
1.0	5.28	2.47	2.14	0.058	1.60
2.0	-1.89	2.47	-0.77	0.461	1.60
3.0	-1.82	2.47	-0.74	0.478	1.60
4.0	1.32	2.47	0.54	0.604	1.60
Product*Fermentation time					
KB5F 1.0	0.12	2.47	0.05	0.964	1.60
KB5F 2.0	1.05	2.47	0.42	0.680	1.60
KB5F 3.0	0.82	2.47	0.33	0.747	1.60
KB5F 4.0	-3.80	2.47	-1.54	0.154	1.60

Regression Equation

EGCG(µg/ml) = 13.08 - 1.02 Product_KB5F + 1.02 Product_KB5U + 5.28 Fermentation time_1.0
- 1.89 Fermentation time_2.0 - 1.82 Fermentation time_3.0
+ 1.32 Fermentation time_4.0 - 2.89 Fermentation time_5.0
+ 0.12 Product*Fermentation time_KB5F 1.0
+ 1.05 Product*Fermentation time_KB5F
2.0 + 0.82 Product*Fermentation time_KB5F 3.0
- 3.80 Product*Fermentation time_KB5F 4.0
+ 1.82 Product*Fermentation time_KB5F
5.0 - 0.12 Product*Fermentation time_KB5U 1.0
- 1.05 Product*Fermentation time_KB5U 2.0
- 0.82 Product*Fermentation time_KB5U
3.0 + 3.80 Product*Fermentation time_KB5U 4.0
- 1.82 Product*Fermentation time_KB5U 5.0



General Linear Model: ECG(µg/ml) versus Product, Fermentation time and storage period (weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Fermentation time	Fixed	5	1.0, 2.0, 3.0, 4.0, 5.0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	23.41	23.41	0.57	0.466
Fermentation time	4	382.56	95.64	2.34	0.125
Product*Fermentation time	4	110.24	27.56	0.67	0.625
Error	10	408.46	40.85		
Total	19	924.67			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6.39106	55.83%	16.07%	0.00%

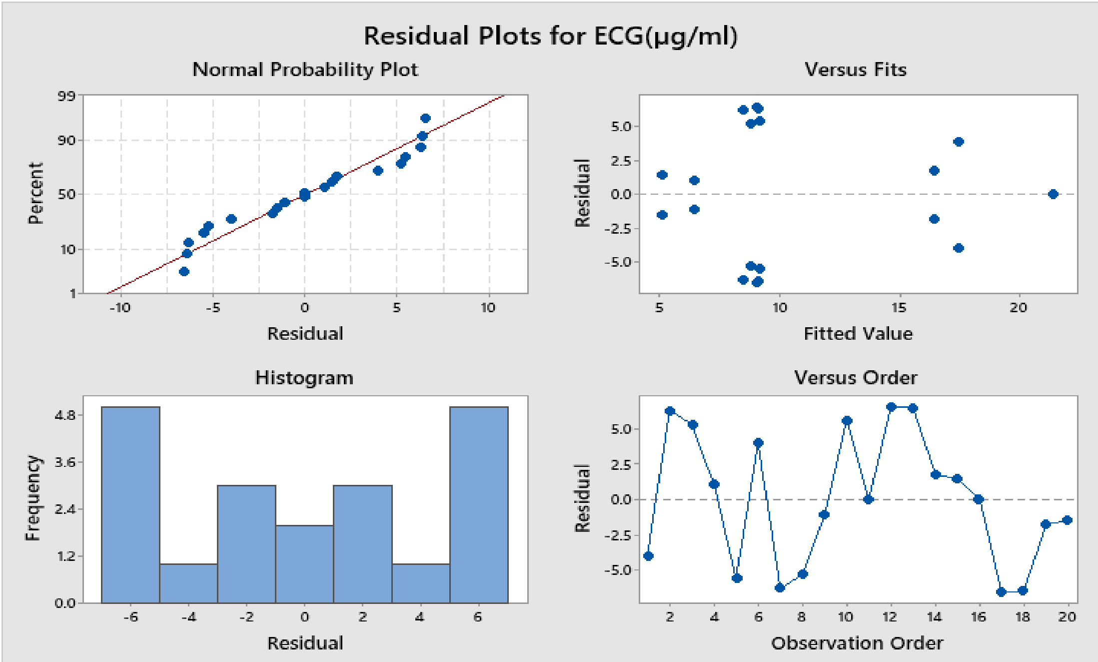
Coefficients

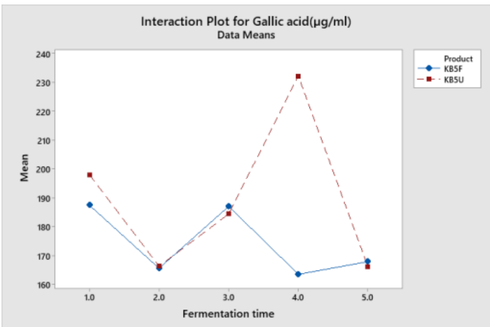
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11.14	1.43	7.80	0.000	
Product					
KB5F	-1.08	1.43	-0.76	0.466	1.00
Fermentation time					
1.0	8.30	2.86	2.90	0.016	1.60
2.0	-2.41	2.86	-0.84	0.419	1.60
3.0	-2.19	2.86	-0.77	0.460	1.60
4.0	0.32	2.86	0.11	0.914	1.60
Product*Fermentation time					
KB5F 1.0	-0.90	2.86	-0.32	0.759	1.60

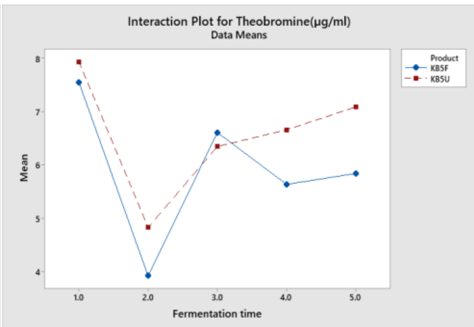
KB5F 2.0	0.81	2.86	0.28	0.783	1.60
KB5F 3.0	0.91	2.86	0.32	0.756	1.60
KB5F 4.0	-3.94	2.86	-1.38	0.198	1.60

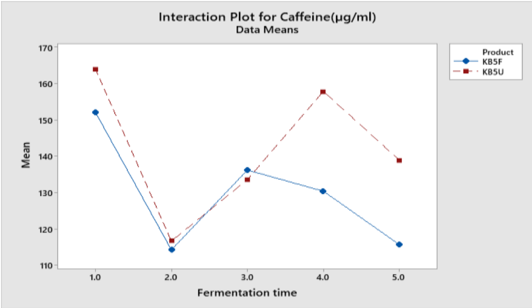
Regression Equation

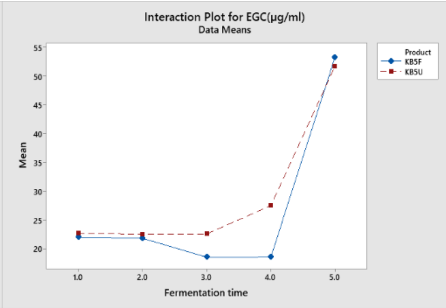
ECG(µg/ml) = 11.14 - 1.08 Product_KB5F + 1.08 Product_KB5U + 8.30 Fermentation time_1.0
- 2.41 Fermentation time_2.0 - 2.19 Fermentation time_3.0
+ 0.32 Fermentation time_4.0 - 4.01 Fermentation time_5.0
- 0.90 Product*Fermentation time_KB5F 1.0
+ 0.81 Product*Fermentation time_KB5F
2.0 + 0.91 Product*Fermentation time_KB5F 3.0
- 3.94 Product*Fermentation time_KB5F 4.0
+ 3.12 Product*Fermentation time_KB5F
5.0 + 0.90 Product*Fermentation time_KB5U 1.0
- 0.81 Product*Fermentation time_KB5U 2.0
- 0.91 Product*Fermentation time_KB5U
3.0 + 3.94 Product*Fermentation time_KB5U 4.0
- 3.12 Product*Fermentation time_KB5U 5.0

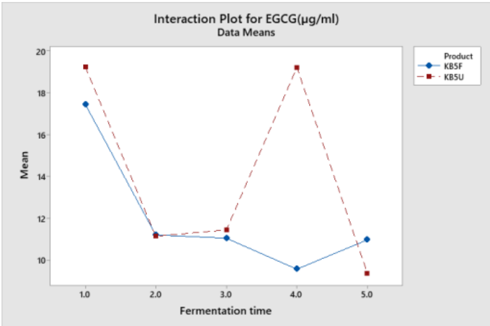


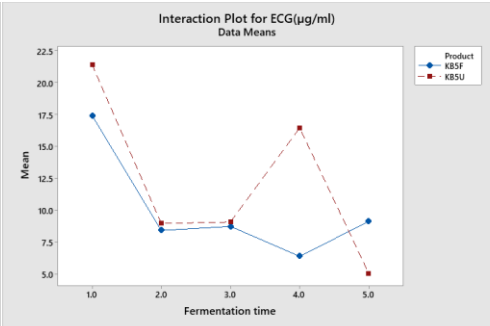












General Linear Model: Appearance versus Product and Storage period (weeks)

Method

Factor coding	(-1, 0, +1)
Rows unused	1

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	5.496	5.4959	2.76	0.008
Week	2	6.211	3.1057	1.56	0.212
Product*Week	2	0.413	0.2066	0.10	0.902
Error	425	847.132	1.9933		
Total	430	859.179			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.41183	1.40%	0.24%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.3411	0.0680	93.22	0.000	
Product					
KB5F	0.1130	0.0680	1.66	0.098	1.00
Week					
1	0.1251	0.0955	1.31	0.191	1.33
2	-0.1625	0.0968	-1.68	0.094	1.33
Product*Week					

KB5F 1	-0.0386	0.0955	-0.40	0.686	1.33
KB5F 2	0.0370	0.0968	0.38	0.702	1.33

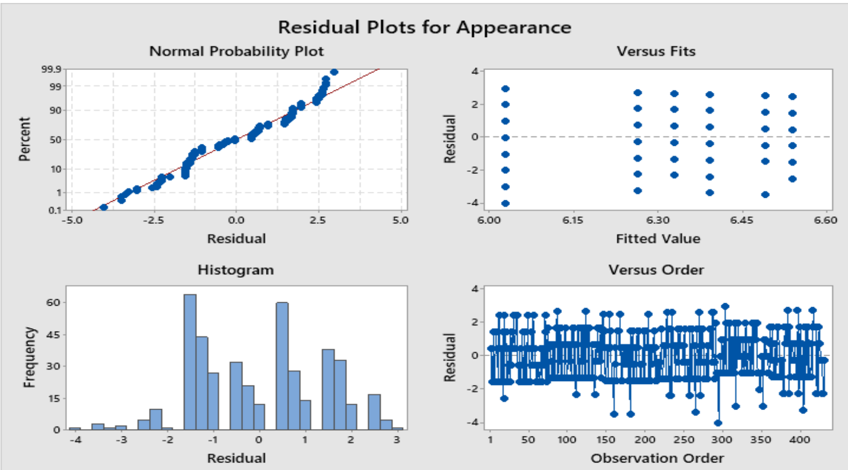
Regression Equation

Appearance = 6.3411 + 0.1130 Product_KB5F - 0.1130 Product_KB5U
+ 0.1251 Week_1
- 0.1625 Week_2 + 0.0374 Week_3
- 0.0386 Product*Week_KB5F 1
+ 0.0370 Product*Week_KB5F 2
+ 0.0016 Product*Week_KB5F 3
+ 0.0386 Product*Week_KB5U 1
- 0.0370 Product*Week_KB5U 2
- 0.0016 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Appearance	Fit	Resid	Std Resid	
160	3.000	6.493	-3.493	-2.49	R
181	3.000	6.493	-3.493	-2.49	R
265	3.000	6.392	-3.392	-2.42	R
295	2.000	6.029	-4.029	-2.87	R
303	9.000	6.029	2.971	2.12	R
317	3.000	6.029	-3.029	-2.16	R
352	3.000	6.029	-3.029	-2.16	R
404	3.000	6.264	-3.264	-2.33	R

R Large residual



General Linear Model: Aroma versus Product and Storage period (weeks)

Method

Factor coding	(-1, 0, +1)
Rows unused	1

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	40.17	40.1741	16.62	0.000
Week	2	5.05	2.5258	1.04	0.353
Product*Week	2	1.57	0.7849	0.32	0.723

Error	425	1027.24	2.4170
Total	430	1074.19	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.55469	4.37%	3.25%	1.65%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.8655	0.0749	78.30	0.000	
Product					
KB5F	0.3054	0.0749	4.08	0.000	1.00
Week					
1	-0.007	0.105	-0.07	0.944	1.33
2	-0.130	0.107	-1.22	0.224	1.33
Product*Week					
KB5F 1	0.026	0.105	0.24	0.807	1.33
KB5F 2	-0.084	0.107	-0.79	0.431	1.33

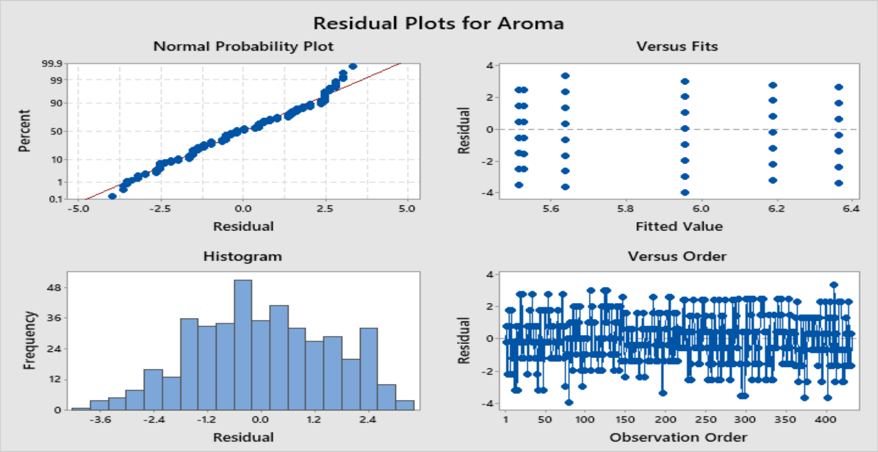
Regression Equation

Aroma = 5.8655 + 0.3054 Product_KB5F - 0.3054 Product_KB5U - 0.007 Week_1
- 0.130 Week_2
+ 0.137 Week_3 + 0.026 Product*Week_KB5F 1 - 0.084 Product*Week_KB5F 2
+ 0.058 Product*Week_KB5F 3 - 0.026 Product*Week_KB5U 1
+ 0.084 Product*Week_KB5U 2
- 0.058 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Aroma	Fit	Resid	Std Resid	
12	3.000	6.189	-3.189	-2.07	R
15	3.000	6.189	-3.189	-2.07	R
41	3.000	6.189	-3.189	-2.07	R
48	3.000	6.189	-3.189	-2.07	R
80	2.000	5.957	-3.957	-2.56	R
196	3.000	6.366	-3.366	-2.18	R
295	2.000	5.514	-3.514	-2.28	R
299	2.000	5.514	-3.514	-2.28	R
373	2.000	5.639	-3.639	-2.36	R
402	2.000	5.639	-3.639	-2.36	R
410	9.000	5.639	3.361	2.18	R

R Large residual



General Linear Model: Flavour versus Product and Storage period (weeks)

Method

Factor coding	(-1, 0, +1)
Rows unused	1

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	117.59	117.588	57.28	0.000
Week	2	14.82	7.412	3.61	0.028
Product*Week	2	1.58	0.788	0.38	0.681
Error	425	872.53	2.053		
Total	430	1005.75			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.43284	13.25%	12.23%	10.78%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.4090	0.0690	92.83	0.000	
Product					
KB5F	0.5225	0.0690	7.57	0.000	1.00
Week					
1	-0.2603	0.0969	-2.69	0.008	1.34
2	0.1277	0.0984	1.30	0.195	1.34
Product*Week					
KB5F 1	-0.0766	0.0969	-0.79	0.430	1.34
KB5F 2	0.0713	0.0984	0.72	0.469	1.34

Regression Equation

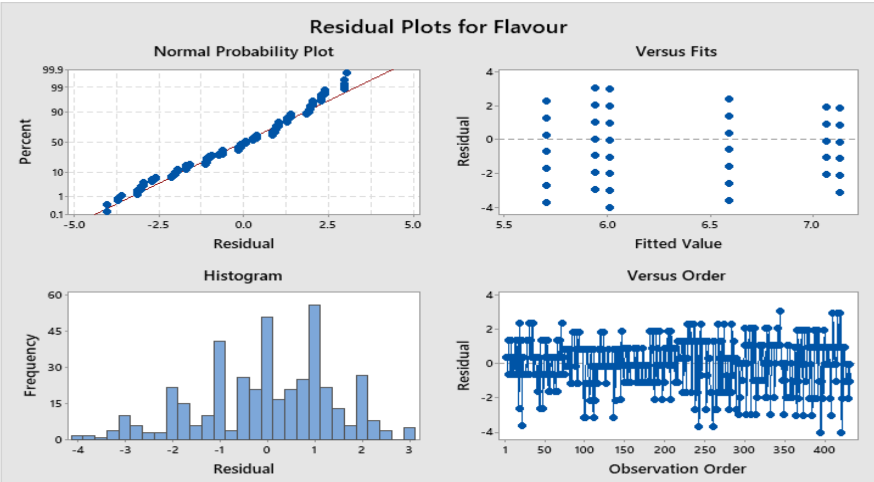
Flavour = 6.4090 + 0.5225 Product_KB5F - 0.5225 Product_KB5U - 0.2603 Week_1
+ 0.1277 Week_2
+ 0.1327 Week_3 - 0.0766 Product*Week_KB5F 1
+ 0.0713 Product*Week_KB5F 2
+ 0.0053 Product*Week_KB5F 3 + 0.0766 Product*Week_KB5U 1
- 0.0713 Product*Week_KB5U 2 - 0.0053 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Flavour	Fit	Resid	Std Resid	
22	3.000	6.595	-3.595	-2.53	R
99	4.000	7.130	-3.130	-2.20	R
101	4.000	7.130	-3.130	-2.20	R
111	4.000	7.130	-3.130	-2.20	R
136	4.000	7.130	-3.130	-2.20	R
243	2.000	5.703	-3.703	-2.60	R
260	2.000	5.703	-3.703	-2.60	R
293	3.000	5.943	-2.943	-2.07	R
302	3.000	5.943	-2.943	-2.07	R
308	3.000	5.943	-2.943	-2.07	R
314	3.000	5.943	-2.943	-2.07	R
332	3.000	5.943	-2.943	-2.07	R
344	9.000	5.943	3.057	2.15	R
349	3.000	5.943	-2.943	-2.07	R
365	3.000	6.014	-3.014	-2.12	R

373	3.000	6.014	-3.014	-2.12 R
384	3.000	6.014	-3.014	-2.12 R
395	2.000	6.014	-4.014	-2.82 R
408	3.000	6.014	-3.014	-2.12 R
410	9.000	6.014	2.986	2.10 R
416	9.000	6.014	2.986	2.10 R
419	9.000	6.014	2.986	2.10 R
420	9.000	6.014	2.986	2.10 R
421	2.000	6.014	-4.014	-2.82 R

R Large residual



General Linear Model: Sweetness versus Product and Storage period (weeks)

Method

Factor coding	(-1, 0, +1)
Rows unused	1

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	88.499	88.499	47.02	0.000
Week	2	5.156	2.578	1.37	0.255
Product*Week	2	2.314	1.157	0.61	0.541
Error	425	799.992	1.882		
Total	430	895.262			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.37198	10.64%	9.59%	8.10%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.5369	0.0661	98.89	0.000	
Product					
KB5F	0.4533	0.0661	6.86	0.000	1.00

Week					
1	-0.1517	0.0928	-1.64	0.103	1.33
2	0.0560	0.0941	0.60	0.552	1.33
Product*Week					
KB5F 1	-0.0952	0.0928	-1.03	0.306	1.33
KB5F 2	0.0825	0.0941	0.88	0.381	1.33

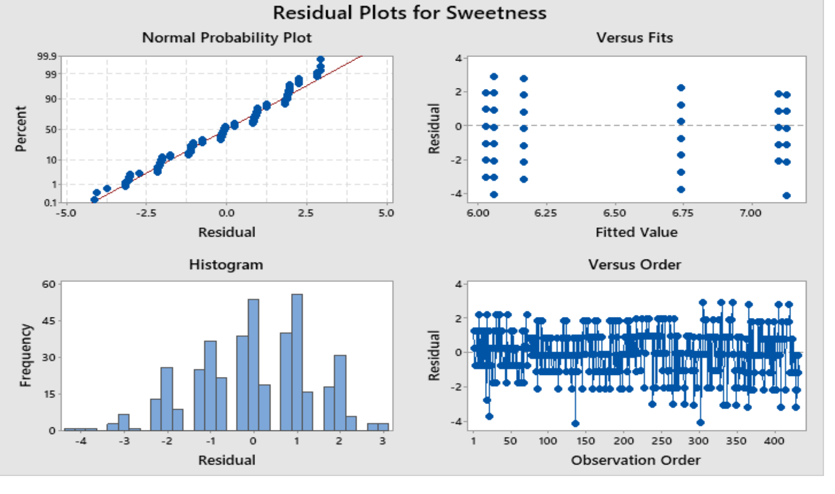
Regression Equation

Sweetness = 6.5369 + 0.4533 Product_KB5F - 0.4533 Product_KB5U
- 0.1517 Week_1
+ 0.0560 Week_2 + 0.0958 Week_3
- 0.0952 Product*Week_KB5F 1
+ 0.0825 Product*Week_KB5F 2
+ 0.0127 Product*Week_KB5F 3
+ 0.0952 Product*Week_KB5U 1
- 0.0825 Product*Week_KB5U 2
- 0.0127 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Sweetness	Fit	Resid	Std Resid	
18	4.000	6.743	-2.743	-2.01	R
22	3.000	6.743	-3.743	-2.75	R
136	3.000	7.129	-4.129	-3.03	R
238	3.000	6.027	-3.027	-2.22	R
265	3.000	6.027	-3.027	-2.22	R
281	3.000	6.027	-3.027	-2.22	R
293	3.000	6.057	-3.057	-2.24	R
302	2.000	6.057	-4.057	-2.98	R
305	9.000	6.057	2.943	2.16	R
330	9.000	6.057	2.943	2.16	R
332	3.000	6.057	-3.057	-2.24	R
337	3.000	6.057	-3.057	-2.24	R
344	9.000	6.057	2.943	2.16	R
349	3.000	6.057	-3.057	-2.24	R
365	3.000	6.167	-3.167	-2.32	R
366	9.000	6.167	2.833	2.08	R
405	9.000	6.167	2.833	2.08	R
408	3.000	6.167	-3.167	-2.32	R
420	9.000	6.167	2.833	2.08	R
428	3.000	6.167	-3.167	-2.32	R

R Large residual



General Linear Model: Sourness versus Product and Storage period (weeks)

Method

Factor coding	(-1, 0, +1)
Rows unused	3

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	105.67	105.674	42.46	0.000
Week	2	21.92	10.959	4.40	0.013
Product*Week	2	0.46	0.229	0.09	0.912
Error	423	1052.87	2.489		
Total	428	1181.08			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.57767	10.86%	9.80%	8.31%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.0992	0.0762	80.05	0.000	
Product					
KB5F	0.4964	0.0762	6.52	0.000	1.00
Week					
1	-0.239	0.107	-2.23	0.026	1.33
2	-0.063	0.108	-0.59	0.558	1.33
Product*Week					
KB5F 1	-0.028	0.107	-0.26	0.794	1.33
KB5F 2	-0.018	0.108	-0.16	0.869	1.33

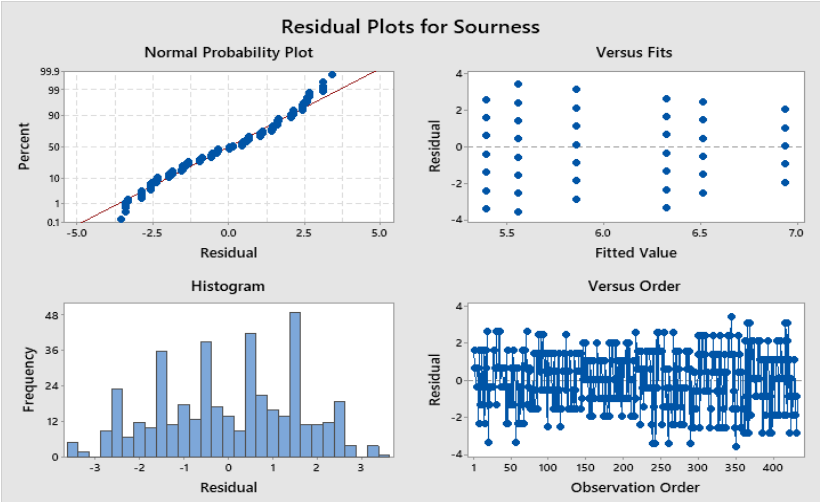
Regression Equation

Sourness = 6.0992 + 0.4964 Product_KB5F - 0.4964 Product_KB5U - 0.239 Week_1
- 0.063 Week_2
+ 0.302 Week_3 - 0.028 Product*Week_KB5F 1 - 0.018 Product*Week_KB5F
2
+ 0.046 Product*Week_KB5F 3 + 0.028 Product*Week_KB5U 1
+ 0.018 Product*Week_KB5U
2 - 0.046 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Sourness	Fit	Resid	Std Resid	
20	3.000	6.329	-3.329	-2.12	R
57	3.000	6.329	-3.329	-2.12	R
239	2.000	5.392	-3.392	-2.16	R
243	2.000	5.392	-3.392	-2.16	R
260	2.000	5.392	-3.392	-2.16	R
289	2.000	5.392	-3.392	-2.16	R
344	9.000	5.557	3.443	2.20	R
350	2.000	5.557	-3.557	-2.27	R
366	9.000	5.859	3.141	2.00	R
369	9.000	5.859	3.141	2.00	R
416	9.000	5.859	3.141	2.00	R
420	9.000	5.859	3.141	2.00	R

R Large residual



General Linear Model: Overall acceptability versus Product and Storage period (weeks)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Product	Fixed	2	KB5F, KB5U
Week	Fixed	3	1, 2, 3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Product	1	126.877	126.877	63.83	0.000
Week	2	4.083	2.041	1.03	0.359
Product*Week	2	0.184	0.092	0.05	0.955
Error	426	846.724	1.988		
Total	431	977.741			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.40983	13.40%	12.38%	10.94%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.6216	0.0678	97.59	0.000	
Product					
KB5F	0.5421	0.0678	7.99	0.000	1.00
Week					
1	-0.1216	0.0953	-1.28	0.203	1.33
2	0.0070	0.0966	0.07	0.942	1.33
Product*Week					
KB5F 1	-0.0151	0.0953	-0.16	0.875	1.33
KB5F 2	0.0294	0.0966	0.30	0.761	1.33

Regression Equation

Overall acceptability	= 6.6216 + 0.5421 Product_KB5F - 0.5421 Product_KB5U - 0.1216 Week_1 + 0.0070 Week_2 + 0.1146 Week_3 - 0.0151 Product*Week_KB5F 1 + 0.0294 Product*Week_KB5F 2 - 0.0143 Product*Week_KB5F 3 + 0.0151 Product*Week_KB5U 1 - 0.0294 Product*Week_KB5U 2 + 0.0143 Product*Week_KB5U 3

Fits and Diagnostics for Unusual Observations

Obs	Overall acceptability	Fit	Resid	Std Resid
44	4.000	7.027	-3.027	-2.16 R
223	3.000	5.973	-2.973	-2.12 R
252	3.000	5.973	-2.973	-2.12 R
253	3.000	5.973	-2.973	-2.12 R
260	2.000	5.973	-3.973	-2.84 R
265	2.000	5.973	-3.973	-2.84 R
269	3.000	5.973	-2.973	-2.12 R
281	3.000	5.973	-2.973	-2.12 R
289	3.000	5.973	-2.973	-2.12 R
293	3.000	6.057	-3.057	-2.18 R
302	3.000	6.057	-3.057	-2.18 R
305	9.000	6.057	2.943	2.10 R
308	3.000	6.057	-3.057	-2.18 R
316	3.000	6.057	-3.057	-2.18 R
330	9.000	6.057	2.943	2.10 R
344	9.000	6.057	2.943	2.10 R
349	3.000	6.057	-3.057	-2.18 R
360	3.000	6.057	-3.057	-2.18 R
398	3.000	6.208	-3.208	-2.29 R
408	3.000	6.208	-3.208	-2.29 R
423	3.000	6.208	-3.208	-2.29 R

R Large residual