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Research Article

Bacteriological and Physicochemical Assessment of Sauerkraut Produced from Cabbage (*Brassica olearaceae* var. capitata) Without Addition of Microorganisms

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ABSTRACT

This study aimed to evaluate the bacteriological profile of sauerkraut produced without addition of microorganisms. Fresh cabbage was purchased from the market and sliced into thin shreds. The shredded cabbage was fermented for 15 days with the addition of sodium chloride 2.25%. During fermentation, samples of brine were collected at different time intervals for biochemical and microbiological analysis. Total sugar in the brine increased slowly to 3.0% on the 8th day and reduced to 1.0% on day 15; reducing sugar also increased slowly to 2.0% on the 8th day and then reduced to 0.2% on the 15th day. Total acidity increased from 0.4% from start of fermentation to 2.3% on the 15th day. The pH of the shredded cabbage at start was 6.8, which decreased to 3.9 on the 15 day. While Vitamin C content varied form 27.2mg/100g from start to 16.8mg/100g. Bacteriological analysis revealed that counts of aerobic mesophilic bacteria and total *Lactobacillus* within limits of established by the legislation. Cultural, morphological and physiological analysis also revealed that *Bacillus, Pseudomonas* and *Micrococcus* were dominant at the initial fermentation, but later, *Leuconostoc* and *Lactobacillus* became dominant. While *Salmonella* and coagulase-positive *Staphylococcus* and coliform bacteria were absent. The results revealed that the production of fermented foods, such as sauerkraut, without addition of microorganisms may provide a product of good microbiological quality if the hygienic-sanitary conditions are properly maintained. This present study revealed that sauerkraut produced without addition of microorganisms is safe and suitable food, and may be a good alternative in gastronomy.

Keywords: Sauerkraut, Cabbage, sodium chloride, *Lactobacillus* sp, fermentation, lactic acid bacteria, vitamin C.

Introduction

Cabbage belongs to the family Brassicaceae (formerly Cruciferae). It is classified as *Brassica oleracea* variety capitata. The wild cabbage is classified as *Brassica oleracea* (Taylor-Davies *et al*., 2008; Rao *et al.*, 2020). Cabbage is the most important commercial vegetables of the Cole crops, which includes cabbage, cauliflower, Brussels sprouts, kale, kohlrabi, collard, broccoli, and many others. It also ranks as one of the most important of all vegetable crops and is universally cultivated as a garden, truck and general farm crop (Shoemaker, 2009). Cabbage is the king of cruciferous vegetables in defending the body against illness. It is as rich in vitamin C as citrus fruits, with all its protective and healing properties (Nixon, 2017).

One of the most important commercial products obtained from brassica vegetables is sauerkraut, which results from the lactic acid fermentation of shredded and salted white cabbage (Alden, 2005).

Sauerkraut is a pickled cabbage salad, a German dish of shredded cabbage fermented in its own juice with salt (Marquis and Robert, 2008). The calorie in sauerkraut is low, vitamin C and other nutrients are preserved and desirable sensory properties are created by a proper fermentation (Enwa, 2014). It is made from cabbage and acidic, which results from natural fermentation by bacteria indigenous to cabbage in the presence of salt. According to Enwa, (2014), cabbage fermentation has been shown to enhance its protective activities.

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The addition of salt is one of the critical point in sauerkraut production due to the type and extent of microbial growth; and the sensory properties of the final product are affected by the amount of salt used (Thakur and Kabir, 2015). The addition of salt favours the growth of lactic acid bacteria and restricts the activities of Gram negative bacteria (Pundir and Jam, 2010). The concentration of Salt had a significant effect on sauerkraut fermentation at early stage. It affects LAB population and metabolic rate which will be reduced and the yield of lactic acid decreased with the increase of salt concentration. According to Xiong *et al.*, (2014) suitable salt concentration can effectively inhibit the proliferation of fungi and *Escherichia coli*. In comparison, high salt concentration delayed the maturation of sauerkraut and inhibited the metabolism of LAB. The development of LAB bacteria with desired properties is of prime importance to the preparation of sauerkraut of consistent quality. The fermentation yield lactic acid as a major product. This lactic acid along with other minor products of fermentation, gives sauerkraut its characteristic flavor and texture. Also, lacto-fermented sauerkraut provides an array of lactobacilli probiotics, vitamin C, dietary folates, manganese and pyridoxine (Enwa, 2014).

Fermentation is one of the oldest processing techniques used to extend the shelf life of perishable food and was particularly important before refrigeration (Swain *et al*., 2014). Fermentation of fruits and vegetables can occur spontaneously by the natural lactic bacterial surface microflora, such as *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. Lactic acid fermentation of vegetables has an industrial importance only for cucumbers, cabbages, and olives. Fermentation play an important role in preservation, production of wholesome nutritious foods in a wide variety of flavors, aromas, and textures which enrich the human diet and remove anti-nutritional factors to make the food safe to eat. The most valuable role of fermentation is that it helps to make the nutrients naturally present in the starting food materials, more palatable and more widely available than would be possible without fermentation (Dimidi *et al.*, 2019).

Lactic acid fermentation of cabbage to produce sauerkraut has been universally studied for many years. During fermentation of sauerkraut, lactic acid is produced which acts as a preservative in addition to imparting desired aroma and flavor (Chauhan *et al.*, 2008; An *et al*., 2021). The Production is a means of preserving cabbage in inexpensive, bulk storage. Sauerkraut has many nutritional benefits, it boost immune system and also provides energy (Byers, 2013; Mcallister, 2018). During cooking of cabbage, vitamin C which is an important component of cabbage is destroyed if not processed properly. Howbeit, through fermentation vitamin C and other nutrients can be preserved (Pandey and Garg, 2015).

According to Johanningsmeier *et al.*, (2007), sauerkraut fermentation is consistently initiated by heterofermentative lactic acid bacteria (LAB), primarily *Leuconostoc mesenteroides*. As the pH decreases, *L. mesenteroides* begins to decline in number and the more acid-tolerant homofermentative LAB, predominantly *Lactobacillus plantarum*, increase in cell numbers and completes the fermentation process. The succession of micro-organism produces interesting changes in the sauerkraut during fermentation. Cabbage contains abundant lactic acid bacteria in order to ferment and produce sauerkraut with salt alone. In order to obtain product of the highest quality all those bacteria strains must ferment in a certain sequence. This happens naturally as long as sauerkraut is fermented around 65°F (18°C) (Yang *et al.*, 2020). Due to several health benefits provided, it is significant to use traditional foods manufactured by the natural technology (Marco *et al*., 2017). This present work was designed to produce Sauerkraut by natural fermentation at room temperature over a 15-day fermentation period without the addition of microorganisms.

Materials and Methods

Collection, Shredding and Preparation of Cabbage for Sauerkraut Fermentation

Fresh white cabbage (*Brassica olearaceae* var. capitata) was purchased from the Mile I market in Diobu, Port-Harcourt, Nigeria and washed thoroughly before further processing. All the equipment, working surfaces were washed with clean water and sanitized.

Cabbage was washed with water and damaged outer green leaves were removed. The cabbage heads were trimmed and cores of cabbage were removed.

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Adopting the method of Penas *et al*., (2017), the cabbage was cut into thin shreds of about 3-4 mm thickness, using a sharp knife. The shredded cabbage were mixed with 2.25% (w/w) food grade salt (NaCl) and kept in four different 1L sterilized glass bottles in triplicate. After some minutes, the cabbage started releasing water that was enough to cover the cabbage entirely. Afterward, the water released was poured into a jar and then covered with a lid. Fermentation was conducted at approximately 21°C. Fermentation was done in room temperature by varying brine concentration that lasted for 15 days. Successful fermentation was determined by a final pH below 3.6. Fermentation broth samples were immediately collected in triplicate using Pasteur pipettes from the fermenting sauerkraut (0 hr) and at after Days 2, 4, 6, 8, 10, 14 and 15.

Microbiological Analysis

Enumeration of Microorganisms

Samples of fermenting sauerkraut brine were withdrawn in triplicate from each bottle using 1 ml sterile glass pipettes. Samples were diluted by serial dilution technique and 0.1 ml aliquot of appropriate dilution was spread on two types of media in triplicate for the determination of naturally occurring bacteria and aerobic bacteria. The plates containing Rogosa SL agar medium for naturally occurring bacteria (lactic acid bacteria) were incubated at 37ºC while the plates containing plate count agar (PCA) medium for aerobic bacteria were incubated at 37ºC in an incubator for 24 hr or till the appearance of visible colonies. The number of colonies that appeared on two types of media after 24 hours of incubation was counted from plates containing 100-200 colonies and recorded as Colony forming units per ml of fermentation broth or per gram of Sauerkraut.

Biochemical Tests

Bacterial isolates were characterized by methods described in Manual of Microbiological, Microbiological Laboratory Manual and Microbiological methods. The biochemical tests used were Indole production, Methyl red reaction, Voges-proskauer test, Catalase test, Gelatin liquefaction, Nitrate reduction, Citrate utilization, Sugar fermentation (acid and gas production), oxidase test, Esculin hydrolysis, Growth on nutrient agar with 7.5% NaCl.

Determination of coliforms

To ensure the safety of the final product coliform bacteria were determined by MPN. The selective media used was MacConkey medium that contain bile salt inhibitory for the growth of non-intestinal lactose fermenting bacteria. Statistical method was employed to estimate most probable number of coliforms.

Enumeration of coliform Bacteria

Samples of brine were withdrawn in triplicate from each bottle using 1 ml sterile glass pipettes. Coliforms were determined by the most probable number (MPN) method after inoculating the MacConkey's broth tube (using 10.1 and 0.1 ml of brine as inoculum in triplicate). These samples were further examined to confirm the test by serial dilution technique and 0.1 ml aliquot of appropriate dilution was spread on EMB media in triplicates. The plates were incubated at 37ºC in a desiccator for 24 hr.

Chemical Analysis of Sauerkraut Brine

Five gram of Cabbage sample was taken and crushed in a mortar using pestle; 10 ml of water was added to it. The crushed product was filtered through Whatman filter no. 20 and the filtrate was analyzed for total sugar, reducing sugar, total acids, volatile acids, pH and vitamin C content.

Determination of Total/Reducing Sugar

Total sugar and reducing sugar were determined by using Lane and Eynon method. 10 g of ground sample was poured in 100 ml volumetric flask and half of volume was made up with distilled water. Then the solution was neutralized by NaOH and carrez I and carrez II was added respectively at the interval of 1 min and the volume was made up to 100 ml using distilled water. The prepared sample was filtered and 10 ml of aliquot was taken for titration with Fehling's solution as described by Chaves-López, *et al*., (2014).

Determination of Total Acids

The total acidity expressed as (%) lactic acid was determined by titrating 10 ml of undiluted sample with a 0.1 N NaOH solution at each sampling time. The titration continues until a light pink colour persisted. Total acid as percent of lactic acid were calculated using the formula:
1 ml of 0.1 N NaOH = 9 mg of Lactic acid.

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Determination of Ascorbic Acid (Vitamin C)

One g of sample was macerated in pestle mortar with 5 ml of 3 percent metaphosphoric acid. It was filtered through Whatman filter paper No. 20 and volume was made to 10 ml with 3 percent metaphosphoric acid. Five ml of aliquot was titrated against 2,6-dichlorophenol-indophenol dye till light pink colour appeared.

Determination of pH

The pH of the brine from the sauerkraut fermentation at different stages was determined by using digital pH meter. The pH meter was calibrated at pH 7 and 4 using citrate- phosphate and phthalate buffer respectively before measuring pH.

Results

The set up of the Sauerkraut Fermentation for this present study is shown in Plate 1. In general, the cabbage was observed to be fresh without any change in natural colour.

The result of the number of bacteria on nutrient agar media at the start of fermentation to the end was presented in Table 1. While the viable counts of bacteria in sauerkraut observed by serial dilution agar plate technique were presented in Table 2.

Plate 1: Sauerkraut Fermentation

The morphological, cultural and biochemical characteristics of the six major types of colonies observed during different stage of fermentation were recorded are described in Table 3. From this analysis it was observed that different bacterial groups identified were Bacillus sp, *Staphylococcus aureus, Leuconostoc mesenteriodes, Lactobacillus brevis, L. fermentum,* and *L. plantarum*.

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Table 1: Values of pH, % lactic acid and microbial load (CFU/ml) analysis of sauerkraut during fermentation

Fermentation Time (Days)	pH	% Lactic acid	Lactic acid bacteria (CFU/ml)	Aerobic bacteria (CFU/ml)	Microorganism
0	6.8	0.05	1.30×10^3	1.60×10^3	*Lactobacillus brevis*
2	6.0	0.15	7.60×10^5	1.70×10^3	*Lactobacillus plantarum*
4	5.5	0.20	1.00×10^6	2.80×10^3	*Lactobacillus fermentum*
6	5.3	0.55	2.80×10^7	1.60×10^3	*Leuconostoc mesenteroides*
8	5.0	1.60	1.80×10^7	1.40×10^3	*Bacillus* sp.
10	4.8	1.65	1.60×10^6	1.00×10^2	*Staphylococcus aureus*
12	4.5	1.68	1.00×10^6	1.70×10^1	
14	4.0	1.75	1.00×10^5	1.50×10^1	
15	3.9	1.82	1.00×10^5	1.40×10^1	

Table 2: Types of Bacteria and viable mean count in Sauerkraut observed by agar plate technique

Lactic acid bacteria (LAB)	LAB Count (CFU/ml)	Aerobic bacteria	Aerobic bacteria Count (CFU/ml)
Leuconostoc mesenteroides	7.60×10^5	*Bacillus* sp.	1.60×10^3
Lactobacillus brevis	2.80×10^5	*Staphylococcus aureus*	1.45×10^3
Lactobacillus plantarum	1.45×10^5		
Lactobacillus fermentum	1.45×10^5		

Table 3: Morphological, Cultural and Physiological Characteristics of different Bacteria isolated from Sauerkraut during Fermentation

Colonial Characteristics	Circular raised, white and large	Wrinkled, white and large	Circular, white and large	Reddish, circular and small	Circular, raised, white and Small
Cell morphology	large rods	small rods	cocci	cocco bacilli	large rods
Gram reaction	+	-	+	+	+
Spore formation	+	-	-	-	-
Growth in 2.5% salt concentration	-	-	-	+	-
Sugar fermentation					
Glucose	+	-	+	+	+
Fructose	+	-	-	+	+
Sucrose	-	-	+	+	+
Maltose	+	-	+	+	+
Lactose	-	-	-	+	+
Catalase Test	+	+	-	-	-
Oxidase Test	+	-	-	-	-
Indole Production	-	-	-	-	-
Methyl Red	+	-	-	+	+
Voges Proskauer reaction	+	-	-	-	-
gelatine hydrolysis	+	+	-	-	-
esculin hydrolysis	+	+	-	+	+
citrate agar growth	+	+	-	+	+
nitrate reduction	+	+	-	-	+
Suspected organism	***Bacillus* sp.**	***Pseudomonas* sp.**	***Micrococcus* sp.**	***Leuconostoc* sp.**	***Lactobacillus* sp.**

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Results for total sugar, reducing sugar, total acidity, pH and ascorbic acid (Vitamin C) analyzed the brine released during the fermentation and storage, as shown in Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5 respectively.

The soluble sugar was released slowly into the brine solution and increased from 1.5% to 3.0% on the 8th day of fermentation. After which the amount of total sugar started to reduce to 1.0% on the 15 day of fermentation with the addition Sodium chloride.

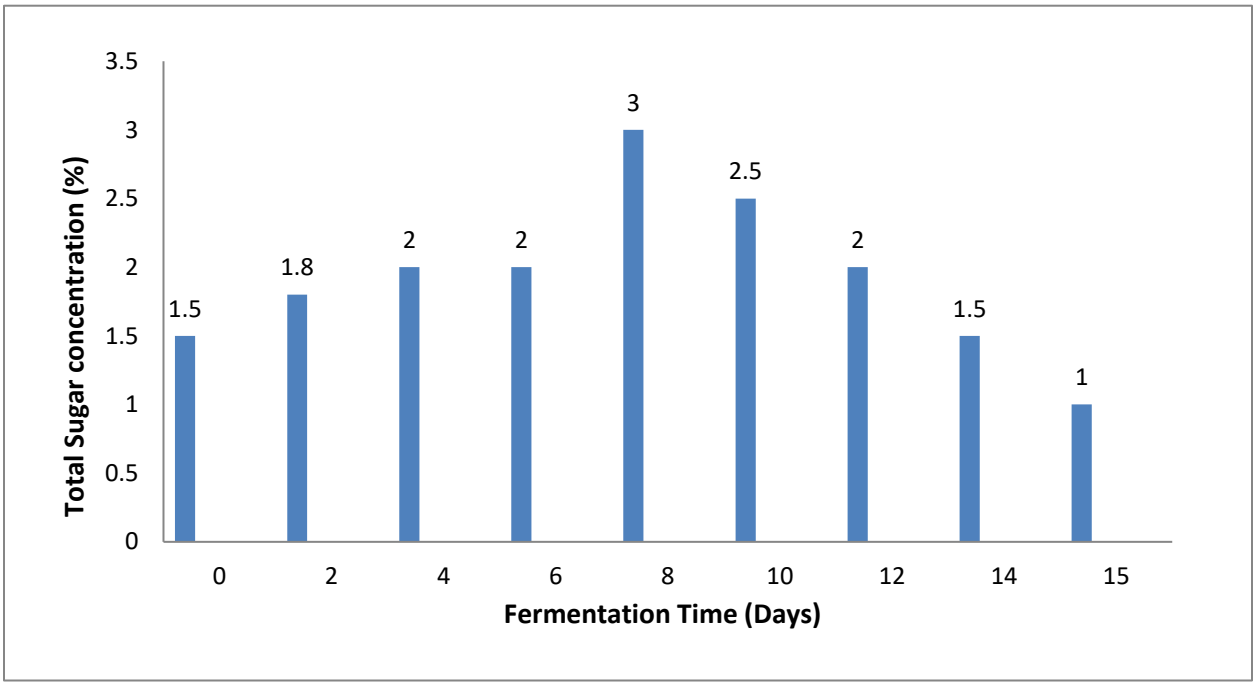

Fig. 1: Changes in Total Sugar concentration during Sauerkraut fermentation

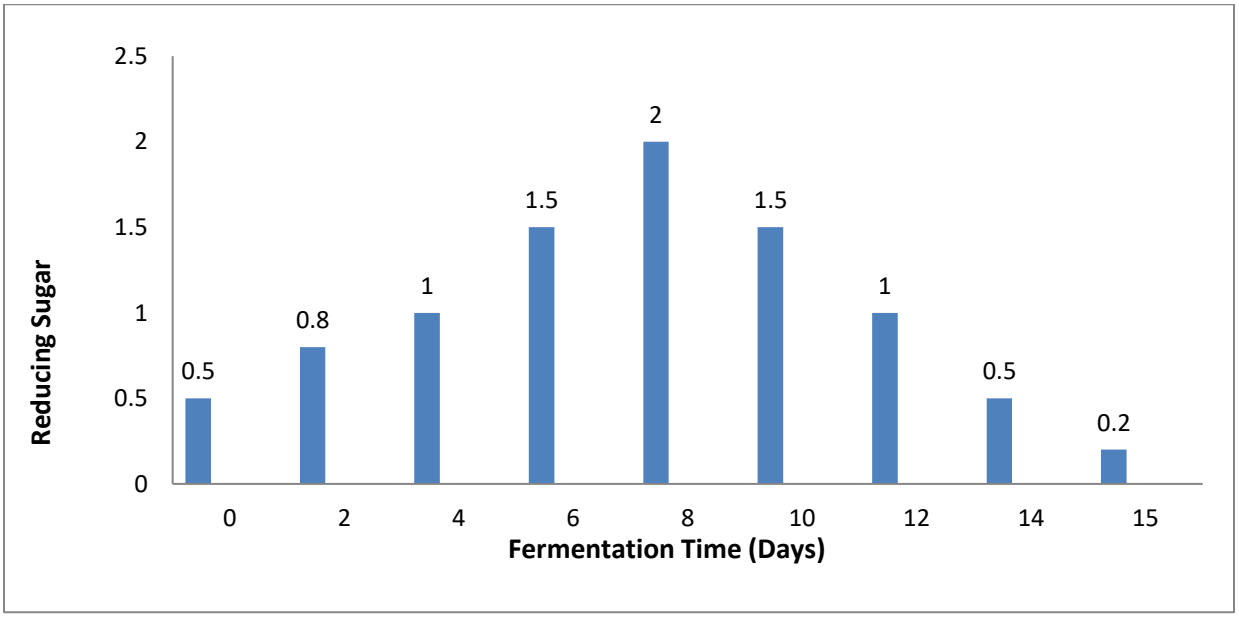

Fig. 2: Changes in Reducing Sugar concentration during Sauerkraut fermentation

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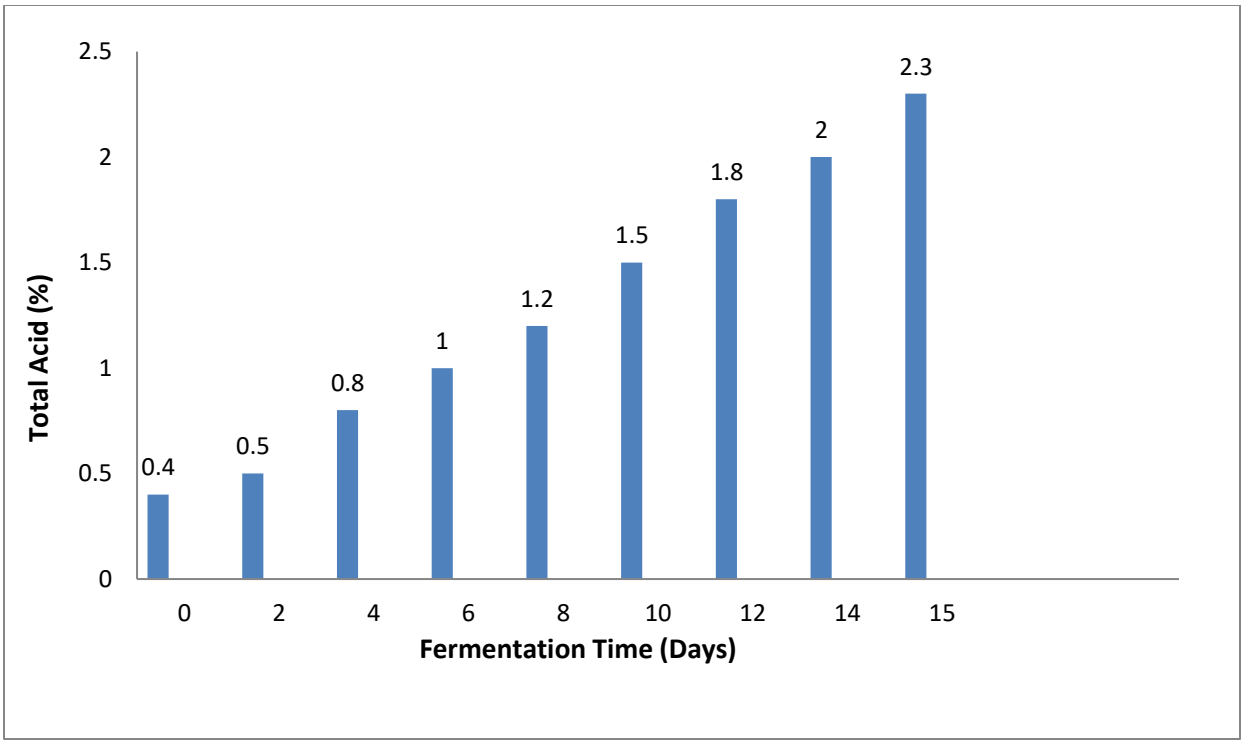

Fig. 3: Changes in Total Acidity in Sauerkraut Fermentation

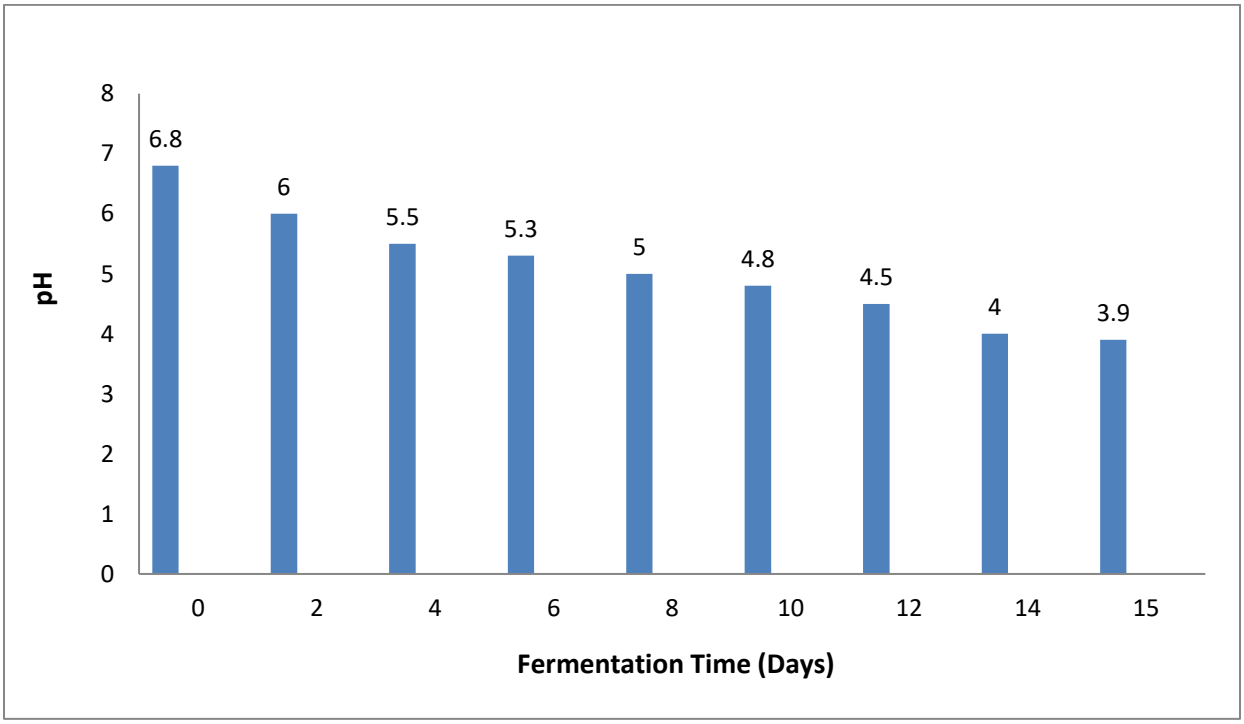

Fig. 4: Changes in pH in Sauerkraut Fermentation

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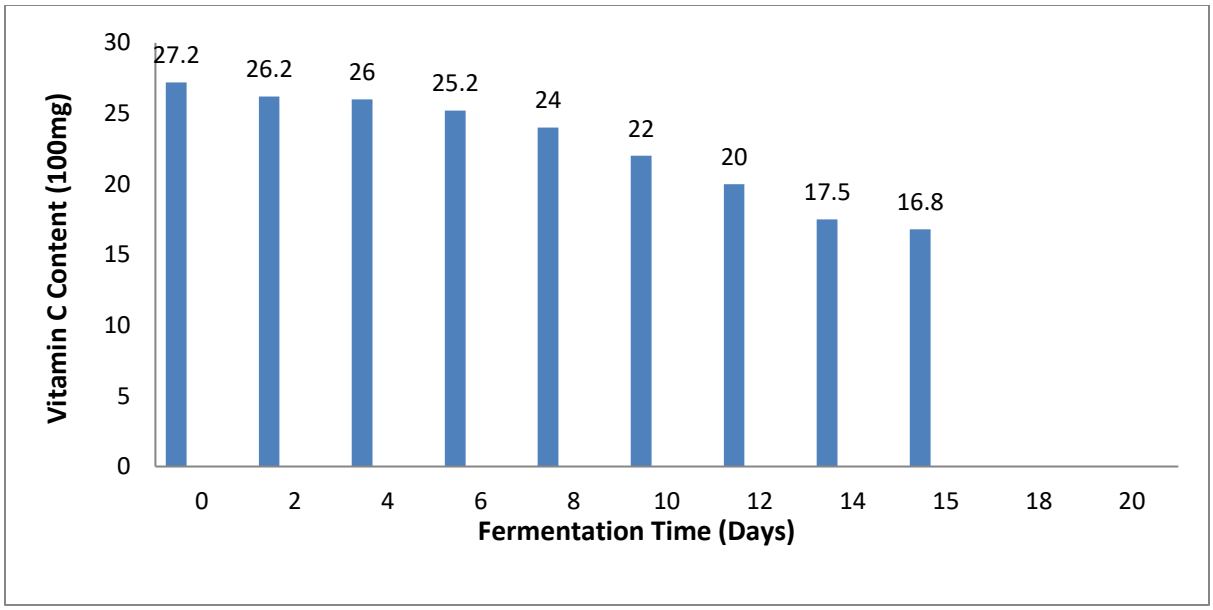

Fig. 5: Effect of Fermentation on Ascorbic Acid (Vitamin C) content in Sauerkraut

Discussion

The fermentation of vegetable is one of the important method of food preservation and retention of nutritional qualities of vegetables during summer time. This present study has revealed some microorganisms and physicochemical constituents associated with the fermentation of Cabbage (*Brassica olearaceae* var. capitata) without addition of microorganisms to produce Sauerkraut. The fermentation was carried out for 15 days till the maximum acidity was achieved. During the course of fermentation it was found that brine was released from the shredded cabbage after addition of Sodium chloride. In general, the cabbage was observed to be fresh without any change in natural colour (Plate 1). According to Yang *et al*., (2020), addition of salt is a key parameter in sauerkraut fermentation. Sufficient quantity of salt inhibit the growth of spoilage bacteria, and in the early stages of fermentation, salt tends to draws nutrients from raw cabbage and provides a suitable substrate for microbial growth (Tereshonok et al., 2019). The addition of salt into cabbage will combine with the acids produced by fermentation to inhibit growth of microorganisms that are not important in sauerkraut fermentation and then delay the enzymatic softening of sauerkraut (Pere-Diaz *et al*., 2020).

The different bacterial groups isolated from the fermented Cabbage Sauerkraut were and identified as Bacillus sp, *Staphylococcus aureus, Leuconostoc mesenteriodes, Lactobacillus brevis, L. fermentum,* and *L. plantarum*. The microflora of natural surface of cabbage is observed to be dominated by bacteria such as *Bacillus, Pseudomonas* and *Micrococcus* according to the findings of Chin *et al*., (2006). It has also been reported that prior to fermentation, fresh fruits and vegetables harbor a variety of microorganisms, including aerobic microorganisms responsible for spoilage like *Pseudomonas, Micrococcus, Bacillus, Erwinia* and *Enterobacter* as well as yeasts and molds (Chin *et al*., 2006). As the fermentation continued the LAB became dominant in natural fermentation of sauerkraut.

The brining of vegetables for fermentation results in the production of organic acids by lactic acid bacteria (LAB) and variety of microbial compounds (Chaves-Milet, *et al*., 2014). Because LAB are more resistant to acid than the spoilage microbiota, they dominate the brined vegetable fermentation. *Leuconostoc* sp, are important in the initiation of the fermentation of vegetables, later other LAB will start to dominant. These microbial sequences are well known and form the basis of fermentation and preservation of vegetables.

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Swain *et al.*, (2014) have reported that Sauerkraut fermentation is initiated by *Leuconostoc mesentroides* followed by *Lactobacillus brevis, Pediococcus cerevisae* and *L. plantarum.*

Similarly, Karovicova and Kohajdova (2003) reported that *Leuconostoc mesentroides* depicted a shorter lag period and fast generation time when grown in cabbage juice as compared to any other microorganisms. During fermentation, greater numbers of exogenous microorganisms that cannot tolerate a highly acidic environment are temporarily inhibited due to the accumulation of acid, and as the microorganisms adapt to the acidic and saline environment, the microbial diversity will rise subsequently (Zhu *et al*., 2018).

Lactobacillus spp. observed in this work can produce various antimicrobial substances, including lactic acid and bacteriocins, which inhibit pathogen growth and spoilage microorganisms and therefore reduce the proportions of other genera, as also noted in the findings of Yang et al., (2020), which is also consistent with the pH and total values measured in this study. Yang *et al*., (2020), showed that natural fermentation leads to sharp reductions in Proteobacteria. Also the findings of Zhou *et al.*, (2018), showed that Lactobacillus dominated in the later stages of fermentation, which is capable of producing carbohydrate-active enzymes that will promote carbohydrate hydrolysis. The same work of Zhu *et al*., (2018) showed that the microbial diversity of sauerkraut depends not only on the fermentation process but also on the fermenting agent and fermentation environment.

No acid and gas formation was observed in the MacConkey's broth tubes (using 10, 1 and 0.1 ml of brine as inoculum in triplicate), indicating the absence of coliforms. Similarly, the confirmatory test on EMB agar plate gave negative results. No colony having Green metallic sheen was observed on the EMB agar plates. Coliforms such as *Escherichia coli* which form the natural microflora of irrigation water are generally present on the surface of the most of the vegetables. Since coliforms was absent after the fermentation time of 15 days of sauerkraut, it is safe for human consumption.

Elevated total acid contents greatly accelerated the initiation of fermentation; this is in agreement with the work of Martorana *et al.,* (2017).

A shorter process of acidification leads to faster conversion of sauerkraut, fewer commercial losses and lower production costs (Martorana *et al*., 2017). Reducing sugars are the main carbohydrates converted into lactic acid and sauerkraut flavor and aroma (Kohajdova *et al.,* 2004).

This present study also reported the trend of biochemical changes that occur during the fermentation of Cabbage into Sauerkraut in terms of total sugar, reducing sugar, total acids, volatile acids, pH and vitamin C contents were almost in agreement with several published literature such as Rhee *et al.*, (2011); Xiong *et al*., (2004), Chin *et al*., (2006) and Breidt *et al*., (2007).

The soluble sugar was released slowly into the brine solution and increased from 1.5% to 3.0% on the 8th day of fermentation. After which the amount of total sugar started to reduce to 1.0% on the 15 day of fermentation with the addition Sodium chloride (Fig. 1). According to Xiong *et al.,* (2014), cabbage typically contains 4 to 5% sugar, consisting of about 2.5% glucose and 2% fructose. During fermentation sugar present in cabbage diffuses in brine and concentration of sugar increases in the brine. The same drift of sugar change was observed in this present investigation. The initial total sugar was found to be low, but as the fermentation proceeded the soluble sugars of cabbage released in the brine and total sugar increased from 1.5% to 3.0%.

Reducing sugar of Sauerkraut brine showed that, after the start of fermentation reducing sugar increase from 0.8% to a range of 1.5-2.0% on the 6thand 8th day of start of fermentation. This afterward, decreased to the range of 0.5% to 0.2% on the 14th and 15th day of fermentation (Fig. 2).

The amount of total acids present in brine collected during Sauerkraut fermentation was analyzed. The initial total acid was found to be 0.4%, increased to 1.2% on day 8; and 23% on the 15th day (Fig. 3).

The pH of the Sauerkraut which was almost neutral (6.8) at the start of fermentation decreased slowly and steadily to 3.9 on the 15 day of fermentation (Fig. 4). The pattern of changes observed in terms of pH is typically similar in most of the vegetable fermentation done by Breidt *et al*., (2007).

Ascorbic acid (Vitamin C) is one of the most essential nutrients of cabbage that needs to be preserved.

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The initial vitamin C content of shredded cabbage was 27.2 mg/100mg of cabbage. This afterward, decreased from 27.2mg/100g to 16.8mg/100g at the end of sauerkraut fermentation for 15 days with the addition of Sodium chloride (Fig. 5). According to Chin *et al*., (2006) vitamin C of vegetables can be preserved by microbiological fermentation. In the present investigation, decrease in vitamin C content was obvious but still the vitamin C was preserved during fermentation because the decrease was not much.

In conclusion, findings on the Cabbage produced in this study showed that, Total acidity increases with fermentation time interval, pH of sauerkraut decreases with fermentation time interval. Vitamin C degraded as the fermentation time increased while reducing sugar increases with fermentation time interval and later reduced at longer time interval. Total sugar of sauerkraut fermentation increases with fermentation time interval and also decreases with longer time interval. Coagulase-positive *Staphylococcus*, *Salmonella*, and coliform bacteria were absent. Counts of aerobic mesophilic bacteria and total lactobacillus are within microbiological limits. Production of fermented foods, such as sauerkraut, without addition of microorganisms may provide a product of good microbiological quality if the hygienic-sanitary conditions are properly maintained. Sauerkraut prepared by fermentation was safe for human consumption. Sauerkraut produced without addition of microorganisms is a safe and suitable food, and may be a good alternative to gastronomy.

It is recommended that Sauerkraut can be fermented in 2.25% brine concentration for 15 days to give an acceptable quality without addition of microorganisms. The temperature of fermentation may be controlled during natural sauerkraut fermentation.

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