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Research Article

EVALUATION OF MINERALS AND VITAMINS COMPOSITIONS OF FERMENTING CABBAGE LEAVES USING *LACTOBACILLUS* ACIDOPHILUS FOR THE PRODUCTION OF SAUERKRAUT

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A B S T R A C T

Evaluation of minerals and vitamins compositions of fermented cabbage using *Lactobacillus* species for the production of sauerkraut was investigated to ascertain their nutritional levels in the sauerkraut. Cabbage leaves (300g) were purchased from Wurukum Market Makurdi, Benue State, Nigeria. Test strain of *Lactobacillus acidophilus* was obtained from Department of Microbiology, Federal University of Agriculture, Makurdi, while standard strain of *L.acidophilus* was obtained from Veterinary Research Institute Vom. Standard microbiological and biochemical methods were employed to revalidate the strains. Cabbage leaves (300g) were prepared for controlled fermentation using starter cultures and spontaneous fermentation. Starter cultures (5%) test and standard strains were inoculated into 300g of the cabbage. Fermentation was allowed to progress at room temperature (25 ± 2^0C) for 4weeks. Products of fermentation were analyzed for vitamins and mineral compositions using standard methods. Highest values of vitamins E (314.005mg), vitamin K (75.00mg) and C (37.06mg) were recorded with starter assisted fermentations at week four. Calcium recorded highest results for minerals (62.062mg) at the fourth week of fermentation assisted with starter cultures as compared to spontaneous fermentation and fresh cabbage.

INTRODUCTION

In Nigeria, cabbage (*Brassica oleracae*) may account for up to 40% of vegetable requirements which is normally consumed raw or in boiled form but can be fermented to sauerkraut (Philip, 2007). Although the fermentation of cabbage to sauerkraut may be relatively new in Nigeria, it has been a common way of preserving vegetable in the Western World, China and Korea (Aponte *et al*., 2012). Cabbage (*Brassica oleracae*) fermentation by *Lactobacillus* species to sauerkraut improves the nutritive value and increase its shelf-life (Viander *et al.,* 2013). Food and Agricultural Organization (FAO, 2012) described two heterolactic and homolactic species of lactic acid bacteria as *Lactobacillus mesenteriods, Lactobacillus brevis*, and *Pediococcus cerevisiae*, *Lactobacillusplantarum* as primary bacteria present in fermentation. Cabbage (*Brassica oleracae*) fermentation to sauerkraut by lactic acid bacteria is beneficial to human health (Taylor, 2011).

MATERIALS AND METHODS

Sample Collection

Cabbage (*Brassica oleracae*) leaves (300g) were purchased from Wurukum Market Makurdi, Benue State, Nigeria. Samples were packaged in sterile polythene bags and were immediately transported to the Laboratory, Department of Microbiology, Federal University of Agriculture, Makurdi.

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Primary Characterization of Lactobacillus acidophilus isolates

Test strain of *Lactobacillus acidophilus* obtained from the Department of Microbiology, University of Agriculture, Makurdi was revalidated alongside with standard strain obtained from Veterinary Research Institute, Vom by sub-culturing on De Man Rogosa Agar. The strains were incubated under anaerobic conditions at 37^0C for 48hours. Representative colonies which developed on the plates were subjected to initial staining and microscopic examinations. The isolates were subjected to the following biochemical tests suchas catalase test, oxidase test, Indole test, Methyl Red (MR) test, VougesProskauer (VP) test, citrate utilization and carbohydrate fermentation were performed as delineated by Bergey's Manual of Systemic Bacteriology (Hensyl, 1994)

Preparation of Cabbage Leaves for Fermentation

Three hundred grams (300g) of cabbage leaves were cleaned by removing the damaged outer cover, it was washed thoroughly and shredded. Inoculation of 5% *Lactobacillus*

acidophilus as starter culture and introduction of 2.5% salt (sodium chloride) was carried out.

Preparation of Lactobacillus acidophilus Inoculum

The inoculum used for fermentation contained 2.7 x 10^7 cells/ml which was calibrated using McFarland standard (No 7) which was prepared by adding 0.7ml of 1% anhydrous barium chloride ($BaCl_2$) to 9.3ml of 1% sulphuric acid (H_2SO_4) (Cockeril, 2012). The inoculum which was 15ml of 24hr old culture formed 5.0% for fermentation.

Controlled Fermentation of Cabbage Leaves Using 5% Starter Culture (L.acidophilus)

The fermentation process was set up with the cabbage leaves and the inocula. Starter culture (5%) was inoculated into 300g of the cabbage and was wrapped with sterile aluminum foil and placed in an earthen pot with cover (Gberikon *et al*., 2009). Fermentation was allowed to progress at room temperature (25±2^{0}C) for 4weeks in the laboratory at the Department of Microbiology, University of Agriculture, Makurdi. The treatments were coded T0-T3 as shown below:

T0= FC= Fresh cabbage (300g)
T1=5% Test strain + 300g cabbage leaves
T2= 5% Standard strain + 300g cabbage leaves
T3= Spontaneous fermentation (300g cabbage leaves without inoculum)

Microbiological Monitoring of Fermentation

Microbiological analysis was carried out at interval of 24hrs to monitor the growth of the starter culture from the start to the end of the fermentation process. During the period of fermentation, samples of ten grams each were taken aseptically at intervals of 24hrs and was transferred into 90ml sterile peptone water. The suspension was shaken vigorously to dislodge microorganisms, thus forming the stock concentration. A tenfold serial dilution was carried out. Aliquots of 0.1ml of dilutions 10^{-5} and 10^{-6} were plated in duplicates on DeMan Rogosa medium for isolation and monitoring of starter culture. Nutrient Agar plates (oxiod) and Potato Dextrose Agar were for isolation of contaminants. Plating was done using a hockey glass stick spreader. De Man Rogosa medium was incubated anaerobically for 48 hours at 37^{0}C, while Nutrient Agar plates were incubated at 37^{0}C for 24hours. Potato Dextrose Agar plates were incubated at room temperature (25±2^{0}C) for one week

Analysis of Mineral Contents of Fermented Cabbage (Sauerkraut)

Calcium, iron, magnesium, zinc and iodine were determined using methods of AOAC (2012)

Determination of Vitamins

Vitamins such as vitamins A, C, B_1, B_3, B_6 and B_{12} were determined from methods adopted by AOAC (2012) while Vitamins K and E were determined by methods of AOAC (2012)

Data Analysis

ANOVA post hoc analysis was carried out. Hypotheses were tested using 95% confidence limit (0.05 significance level) at appropriate degree of freedom.

RESULTS AND DISCUSSION

Table 1 Vitamin Profile of Fresh Cabbage and Fermenting Cabbage at Week 1-4

Vitamin mg/100g	FC	TS@2wks	SS@2wks	SP@2wks	SP@4wks	TS@4wks	SS@4wks
A	78.045	78.9	81.385	79.77	78.94	80.42	81.4
C	33.215	33.44	36.495	35.33	34.23	37.78	36.665
B6	0.2	0.235	0.235	0.255	0.215	0.245	0.265
B12	0.03	0.065	0.735	0.715	0.055	0.725	0.765
B1	0.055	0.065	0.06	0.08	0.07	0.065	0.09
E	313.665	313.935	321.935	314.945	316.66	320.19	314.005
K	70.31	72.615	72.34	73.345	72.51	73.045	75.04
B3	0.43	0.533	0.534	0.614	0.454	0.563	0.756

(Vitamin type) = 45764.59, P= 0.000
(Treatment) = 3.13, P= 0.013
Legend:
FC= Fresh cabbage
TS@2wks=Test strain + cabbage at 2 weeks fermentation
SS@2wks= Standard strain + cabbage at 2weeks fermentation
SP@2wks= Spontaneous fermentation at 2weeks
SP@4wks= Spontaneous fermentation at 4 weeks
TS@4wks= Test strain + cabbageat 4weeks fermentation
SS@4wks= Standard strain + cabbageat 4 weeks fermentation

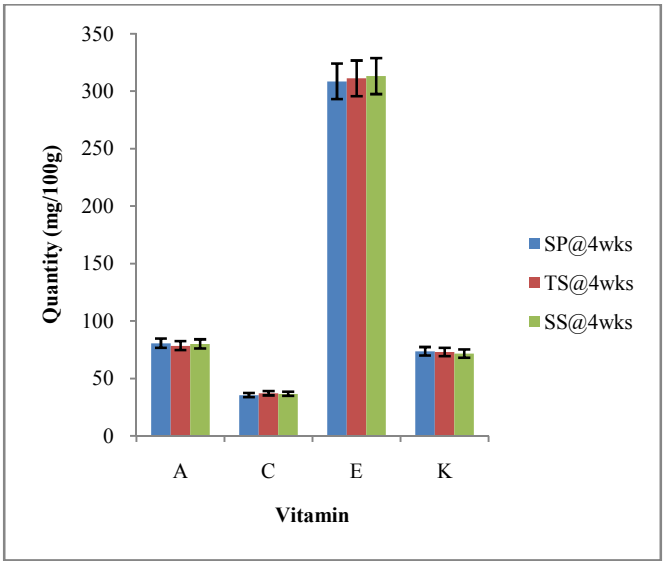

Figure 1 Selected Vitamins in Four Weeks Fermenting Cabbage to Sauerkraut

F (Fermentation type) =0.00, P= 1.000
Legend:
SP@4wks= Spontaneous fermentation at 4 weeks
TS@4wks= Test strain at 4weeks fermentation
SS@4wks= Standard strain at 4 weeks fermentation

Table 2 Selected Minerals in Fresh and fermenting Cabbage from Week 1-4

Mineral (mg/100g)	FC	TS@2wks	SS@2wks	SP@2wks	SP@4wks	TS@4wks	SS@4wks
Iron	0.63	0.685	1.665	2.135	0.73	1.695	2.145
Iodine	2.345	2.4	2.69	2.39	2.415	2.46	2.96
Calcium	53.88	5 2.69	53.715	61.325	53.925	53.98	62.065
Magnesium	0.2	0.185	0.215	0.365	0.225	0.34	0.375
Zinc	0.135	0.235	0.28	0.365	0.225	0.24	0.356

(Mineral type) = 37.79, P= 0.000
(Treatment) = 1.07, P= 0.410

Legend:
FC= Fresh cabbage
TS@2wks=Test strain + cabbage at 2 weeks fermentation
SS@2wks= Standard strain + cabbage at 2weeks fermentation
SP@2wks= Spontaneous fermentation at 2weeks
SP@4wks= Spontaneous fermentation at 4 weeks
TS@4wks= Test strain + cabbage at 4weeks fermentation
SS@4wks= Standard strain + cabbage at 4 weeks fermentation

DISCUSSION

Nutritional qualities and shelf life stability of sauerkraut obtained during fermentation of cabbage using 5% *Lactobacillus acidophilus* as starter cultures under different test conditions increased tremendously (Jagannath *et al*., 2011; Gberikon and Agbulu, 2015). It also aligns with the view of Achi (2005) that traditional fermented foods can be enhanced through microbial fermentation. Hence, microorganisms are indispensable as they have huge roles to play in food production and preservation (Dakwa, 2005). Fresh cabbage analyzed was rich in vitamins and minerals. Highest values of vitamins were recorded in vitaminsE, A, K and C at week four. This is in agreement with the findings of Gberikon and Agbulu (2015) that starter cultures when used in fermentation enhances overall nutritive values. In all the vitamins B complex recorded lowest values as compared to other vitamins analyzed. Starter culture induced sauerkraut yielded higherquantities of vitamins than fresh cabbage and spontaneous fermentation. Values of minerals were higher in fermentation assisted with starter cultures than in fresh cabbage and spontaneous fermentation. The level of calcium increased as fermentation progresses, at the fourth week, very high levels of calcium was recorded followed by iodine. It has been confirmed that the use of starter culture such as *L.acidophilus* provides consistency and reliability of performance as it plays a role in substrate modification and synthesis of vitamins and other minerals found in sauerkraut (Dicagno, 2013).

CONCLUSION

It was concluded from results of this findings that fermentation of cabbage for production of sauerkraut at week four with standard and test strains yielded more nutritional values than spontaneous fermentation. It has been established in this work that starter cultures in food fermentation industry can help address food insecurity and malnutrition in many parts of Nigeria.

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