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Proximate Composition and Essential Amino Acids Profile of Fermenting Cabbage Leaves to Sauerkraut using Starter Cultures and Spontaneous Fermentation

Tanko, O.O[1], Gberikon, G.M[2], Azua. E.T.[3]

[1]Department of Food Technology, School of Science and Technology Federal Polytechnic Kaura Namoda.Zamfara State

[2]Department of Microbiology, University of Agriculture Makurdi, Benue State

[3]Department of Zoology and Environmental Science, Federal University of Agriculture, Makurdi, Benue State

Abstract: ***Proximate composition and essential amino acids profile of fermenting cabbage to sauerkraut using starter culture and spontaneous fermentation was carried out. Three hundred grams (300g) of cabbage leaves 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. Revalidation of test and standard strains were carried out using standard microbiological and biochemical methods. Cabbage leaves (300g) were prepared for controlled fermentation using starter cultures and spontaneous fermentation. Starter culture (5%) was inoculated into 300g of the cabbage in an earth pot with cover lined with aluminum foil. Fermentation was allowed to progress at room temperature (25±2^{0}C) for 4weeks. Products of fermentation were analyzed for proximate composition and amino acid profiling.Results, showed that at the fourth week of fermentation, fermenting cabbage with standard and test strains had the best values in lysine (2.725g/100g) and (2.685g/100g) respectively. Fiber content of 2.66g/100gand crude protein with a value of 2.1g/100g was highest with standard strain. Moisture content reduced significantly as fermentation progressed to the fourth week. Results of proximate and essential amino acids compositions of sauerkraut (fermented cabbage) was higher with those fermented with starter cultures as opposed to those with spontaneous fermentation and fresh cabbage.Fermentations of vegetables assisted with starter cultures can be encouraged to enhance nutrient compositions and shelf life stability.***

Keywords: Proximate composition, Essential amino acids, Cabbage, Sauerkraut, Fermentation

1. Introduction

In the production of sauerkraut, microbiological information implicated*Lactobacillus* species involvement in fermentation (Dakwa*et al.,* 2005);Dicagno (2013) also reported that fermentation of vegetables can occur spontaneously by the natural lactic acid bacterial surface microflora such as *Lactobacillus*, *LeuconostocPediococcus*, however, the use of starter culture such as *Lactobacillus plantarum, Lactobacillus gasseri* and *Lactobacillusacidophilus* (all probiotic strains) provides consistency and reliability of performance. Dicagno (2013) maintained that, these microflora play a role in the development of flavor, aroma, substrate modification, synthesis of vitamins and other minerals found in sauerkraut. The safety of vegetables fermentation is based on the principle that the food substance overgrown with desirable, edible microorganisms become resistance tofood invasion by spoilage microorganisms causing toxic or poisoning and fermentation involving the production of lactic acid which are generally safe (Anon, 1993). High quality sauerkraut can be produced with a starter culture if equilibrated sodium chloride (NaCl) concentrate is adjusted to 2% and the temperature maintained at 18^{0}C (Pederson and Albury, 2005).

2. 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.

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^{0}C 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 such 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 for Fermentation

Three hundred (300g) of cabbage leaves were cleaned by removing the damaged outer cover, it was washed

thoroughly and shredded. Inoculation of 5% *Lactobacillusacidophilus* as starter culture and introduction of 2% salt (sodium chloride) was carried out.

Preparation of *Lactobacillusacidophilus* 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) (Cockerill, 2012). The inoculum which was 15ml of 24hr old culture formed 5.0% for inoculation.

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

The fermentation process was set up into three sets; set A was 300g of cabbage leaves with 5% standard strain ; set B was 300g of cabbage leaves with 5% test strain; set C was 300g of cabbage leaves allowed to ferment spontaneously. All sets were 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 6weeks in the laboratory at the Department of Microbiology, University of Agriculture, Makurdi.

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, ten grams each of the sample was 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. Aliqouts of 0.1ml of dilutions10^{-5} and 10^{-6} was plated in duplicates on DeMan Rogosa medium for isolation and monitoring of starter culture. Nutrient Agar plates (oxiod), Potato Dextrose Agar was used 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.

Proximate Analysis of Sauerkraut

The proximate composition of sauerkraut such as moisture content, ash content, crude protein, crude fats and fiber contents were determined according to the methods of AOAC, (2012).

Determination of Amino Acids Profile

Amino acid composition of sauerkraut samples was determined by using an automated amino acid analyzer (TVA AAAA: 230 dinkjing UAE, 2014), able to determine Sixteen amino acids. Sample (0.5g) soy flour was pasted with 50ml 6N HCl by mortar pestle, filter and filtrate was hydrolyzed for 22-24 hours in a hydrolyzing apparatus. After hydrolyzing HCl was removed from filtrate with distill water for 3-4 times by evaporation in a water bath. After completing the evaporation, the stock solution was prepared and mark up to 25ml in a volumetric flask by using 0.1N HCl. This stock solution was used for the determination of amino acids as outlined by AOAC, (2012).

Data Analysis

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

3. Results and Discussion

Table 1: Essential Amino Acid Profile of Fresh and Fermenting Cabbage at 1-4 Weeks

Amino Acid (g/100g Protein)	FC	TS@2wks	SS@2wks	SP@2wks	SP@4wks	TS@4wks	SS@4wks
Arginine	0.155	0.205	0.215	0.205	0.21	0.22	0.255
Histidine	1.085	1.095	1.09	1.11	1.105	1.12	1.135
Isoleucine	2.165	2.2	2.19	2.19	2.18	2.19	2.21
Leucine	1.28	1.235	1.235	1.32	1.33	1.36	1.367
Lysine	2.335	2.365	2.65	2.66	2.68	2.685	2.725
Methionine	1.325	1.33	1.37	1.385	1.425	1.44	1.456
Phenylalanine	1.1	1.12	1.17	1.2	1.24	1.3	1.34
Threonine	1.09	1.09	1.11	1.13	1.14	1.17	1.175
Valine	1.715	1.825	1.83	1.86	1.86	1.89	1.89
Tryptophan	1.045	1.05	1.05	1.045	1.07	1.08	1.09

(Amino Acid) = 1200.73, P= 0.000
(Treatment) = 9.27, P= 0.000

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

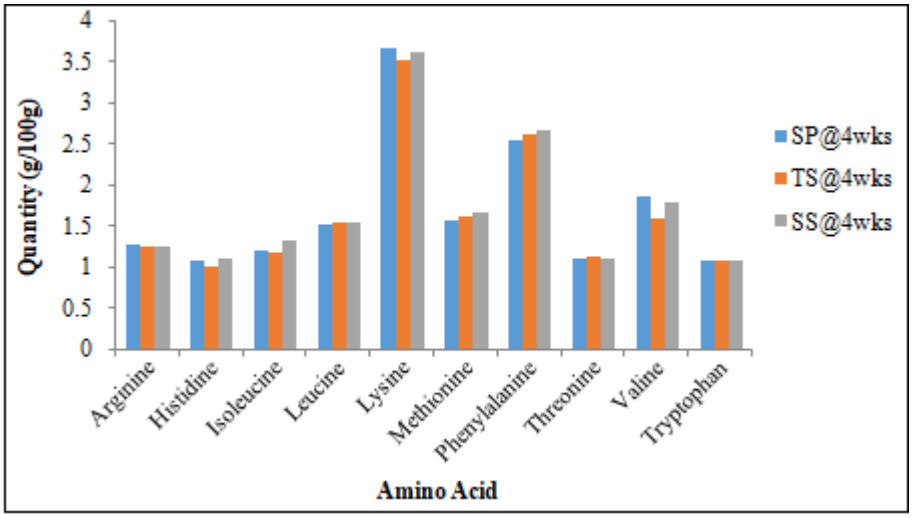

Figure 1: Amino Acids Profile of Four Weeks Fermented Cabbage (Sauerkraut)
F (Fermentation type@4wks) = 0.02 , P= 0.985

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: Proximate Analysis of Fresh and Fermenting Cabbage at week 1-4

Proximate composition (g/100g)	FC	TS@2wks	SS@2wks	SP@2wks	SP@4wks	TS@4wks	SS@4wks
Moisture	69.365	58.41	68.21	61.61	43.675	46.535	45.93
Protein	1.1	1.2	1.175	1.3	1.83	2.1	2.035
Fat	0.225	0.235	0.23	0.23	0.325	0.3	0.31
Fiber	2.63	2.61	2.61	2.585	2.575	2.61	2.66
Ash	1.39	1.01	1.005	1.035	1.04	1.065	1.055

(Proximate type) = 175.52, P=0.000
(Treatment) = 0.91, P= 0.503
Legend:
FC= Fresh cabbage
TS@2wks=Test strain at 2 weeks fermentation
SS@2wks= Standard strain at 2weeks fermentation
SP@2wks= Spontaneous fermentation at 2weeks
SP@4wks= Spontaneous fermentation at 4 weeks
TS@4wks= Test strain at 4weeks fermentation
SS@4wks= Standard strain at 4 weeks fermentation

4. Discussion

The use of starter cultures in the fermentation of vegetables in this study has enhanced the nutritional qualities and shelf life stability of cabbage tremendously. As fermentation progressed to the fourth week, values of essential amino acids in fermenting cabbage increased to a significant level with test and standard strains as compared to spontaneous fermentation. The increase in amino acid is as a result of fermentation activities by fermenting organisms. This is in agreement with the work of Gberikon*et al*., (2009) who also recorded increased values of essential amino acids in the fermentation of legume seeds.It was shown that lysine, isoleucine and valine had high values of amino acid in the fermenting cabbage. The values are higher than reports obtained in the fermentation of other vegetables and pulses (Aponte *et al*., 2012).

Report on proximate analysis of cabbage leaveshas confirmed previous works reporting the enrichment when fermented (Jagannath *et al*., 2011; Gberikon and Agbulu, 2015). Fresh cabbage containing 69.4% moisture and reduced significantly to 45.9. Increased values of crude protein and dietary fiber was recorded.

Test strain of*L.acidophilus* yielded the maximum protein content at the fourth week offermentation. Fat was low in fresh cabbage but well enhanced as fermentation progresses. This also agrees with other reports that fermentation of vegetables provides value addition in nutritional contents (Jagannath*et al*.,2011). The highest quantity of dietary fiber was found within four weeks of sauerkraut produced by standard strainof*L. acidophilus*.Protein (2.1g/100g) was high as fermentation progressed to the fourth week and fat (0.31g/100g) recorded lowest values at week four. The new products formed after fermentation of cabbage are considered safe for consumption. According to Anon (1996), the safety of the vegetable fermentation is based on the principle that the food substance with desirable microorganisms becomes resistance to food invasion by

spoilage microorganisms causing toxic or poisoning. Again, fermentation involves the production of lactic acid which are generally safe. Gberikon and Agbulu (2015) discovered faster fermentation of *Glycine max* with higher nutritional values using starter culture of *Bacillus* than spontaneous fermentation. According to the authors, the benefits of utilizing starter cultures in food industry are enormous as they hasten fermentation processes and ensure quality product by nutrient enhancement.

5. Conclusion

Based on the findings of this study, it was concluded that fermentation of cabbage to sauerkraut with starter cultures of *L.acidophilus* had the highest values of amino acids and proximate composition as compared to cabbage allowed to ferment spontaneously. The use of starter culture in fermentation of vegetables to enhance nutritional compositions is a welcome development in the food industry.

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