Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(12B): 925-929 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i12.016

Research Article

Changes of anthocyanin, vitamin C content and antioxidant activities of purple cabbage pickle during fermentation process

Weiqin Li, Shaoying Zhang*, Qinya Li
College of Food Science, Shanxi Normal University, Linfen China 041004

***Corresponding author**
Shaoying Zhang
Email: zsynew@163.com

Abstract: Pickle was made using the purple cabbage as raw material to ferment, and the changes of anthocyanin, vitamin C content and antioxidant activities of purple cabbage pickle during fermentation process were investigated. The results showed that with the extension of fermentation time, anthocyanin content decreased, and vitamin C content first increased and then decreased. With regard to antioxidant activity, DPPH radical scavenging ability and reducing power decreased, but Fe^{2+} chelating activity increased with fermentation time extention. From the nutrition perspective, it was suggested that purple cabbage pickle should be eaten as early as possible.
Keywords: purple cabbage; pickle; anthocyanin; vitamin C; antioxidant activity.

INTRODUCTION

Purple cabbage, also known as the purple broccoli, is one of cruciferous plants, grown in many parts of the world with wide distribution. Its leaf epidermal cells are rich in anthocyanins (main ingredients for centaurea cyanus)[1]. Many studies have shown that anthocyanin could resist mutation, prevent cardiovascular disease, protect the liver and inhibit tumor cells and so on[2].

Pickle was prepared using fresh vegetables through fermentation. Beneficial microorganism naturally attached on the surface of the vegetables and produce acid during fermentation. Pickle is a kind of fermentable vegetable by semi-solid fermentation processing by means of lactic acid bacteria. Pickle product is with a long history and unique popularization. It is very crisp, tasty and slightly sour to be good appetizers with high popularity and is rich in vitamins, calcium, phosphorus, iron, amino acids and other nutrients [3]. Lactic acid bacteria fermented pickle can not only enhance appetite but also help digestion, thus having certain health care efficacy. Futhermore, pickle is health food with a variety of adjustment such as antibacterium, antimutations, harening of the arteries resistance, obesity and anti-aging and so on [4].

Purple cabbage is a kind of fiber vegetables with good storage and fresh purple. When made into pickled products, it has unique salty, sweet, sour and hot flavor. Therefore, it is an ideal pickle raw material. This experiment studied the changes of anthocyanin, vitamin C content and antioxidant activities of purple cabbage pickle during fermentation process, which have not been reported so far. So this research might provide references for purple cabbage pickle or other related pickle production.

MATERIALS AND METHODS

Materials and Reagents

Purple cabbage, salt, pepper, Chinese prickly ash and ginger, etc. were purchased from New Milky Way supermarket of Linfen city. Vitamin C, sodium hydroxide, acetic acid, sodium acetate, sodium dihydrogen phosphate, disodium hydrogen phosphate, trichloroacetic acid and ferrous sulfate (analytical grade) were purchased from Kermel Chemical Reagent Co.,Ltd.(Tianjin,China). Hydrochloric acid and methanol were purchased from Chemical Reagent Factory (Luoyang, China). Ferrozine and diphenyl-picryl hydrazide (analytical grade) were purchased from National Pharmaceutical Group Chemical Reagent Co., Ltd.

Instruments

The apparatus used in the experiment were as followed: UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., China; PHS-3C pH meter, Shanghai Scientific Instrument Co., Ltd., China; SHA-C Thermostatic water bath oscillators, Jincheng Guosheng Experiment Instrument Factory, China.

Preparation of Purple Cabbage Pickle

Purple cabbages about 12cm in diameter were stripped away a layer of elder leaves outside and washed with tap water. And they were drained the moisture and longitudinally cut into small pieces from

the root by eighth-excision. 2 kg of purple cabbage was put into each jar. Water was added into the altar until it was 8cm away from top brim of jar. 4% salt and a little of pepper, Chinese prickly ash and ginger were added into the jar. The jar was gently shaken and sealed with water. Purple cabbage was fermented for 7 days at room temperature (25±1)˚C under natural condition [5]. Sample was taken every day during fermentation process, refrigerated storage at -50˚C for analysis.

Determination of Nutrients

Determination of anthocyanin

Anthocyanin content was evaluated by pH differential method [6]. 1ml of appropriately diluted purple cabbage juice was respectively added into 10 ml of 0.25 M potassium chloride solution (pH 1.0) or 0.4 M sodium acetate buffer solution (pH 4.5), and their absorbance values were measured at 510 and 700nm after 15 min of incubation at 23˚C . 1% HCl-methanol solution served as the blank control, four values were A510 (pH1.0), A700 (pH1.0), A510 (pH4.5), A700 (pH4.5) respectively. The content of total anthocyanin was expressed as follows: Anthocyanin content(mg/L) = $[(\Delta A \times Mw)/(\varepsilon \times 1)] \times D_f \times 1000$, where$\Delta A$= (A510-A700) pH1.0 − (A510 -A700) pH 4.5, Mw is relative molecular mass of cornflower glucoside (484.82g/mol),ε is the molar extinction coefficient of cornflower glucoside (24825 mol^{-1}) and D_f is dilution factor (the total dilution multiple of sample).

Determination of ascorbic acid content

100 μg/ml ascorbic acid sample was prepared by mixing 10 mg of ascorbic acid, 2 ml of 10% hydrochloric acid and distilled water up to 100 ml, and the same method was used to prepare 2μg/ml, 4μg/ml, 6μg/ml, 8μg/ml, 10μg/ml ascorbic acid samples. The absorbance of ascorbic acid standard series solutions was determined at 243 nm with distilled water as blank sample. Standard curve was illustrated according to ascorbic acid concentration as the abscissa and corresponding absorbance as the ordinate [7]. By linear regression analysis, the regression equation of ascorbic acid concentration (x) and its absorbance value at 243 nm (y) is y = 0.0773 x - 0.0015, the correlation coefficient R^2 = 0.9999.

10 g of purple cabbage pickle was chopped and put into the mortar in ice bath, 5 ml of 1% hydrochloric acid was added to grind homogenate, transfer the slurry to 50 ml of volumetric flask and dilute to the scale, then centrifuge for 10 minutes at 3000 r/min in the centrifugal tube. In the first group, 0.5 ml of the supernatant from different concentrations of ascorbic acid samples were extracted in turn, 1.5 ml of 10% hydrochloric acid was added each, then dilute with distilled water to 50 ml volumetric flask. For the second group, the differences from the former were that 10 ml of distilled water and 3.5ml of 1mol/L NaOH were added to the each supernatant to react for 15 min, continue to add 3.5ml of 10% hydrochloric acid and blend. Distilled water was used as the blank control. The absorbance was measured at 243nm. The content of ascorbic acid in the samples was calculated from the absorbance difference between the test sample and that with alkali treatment. The ascorbic acid content = $M \times 100 \times 50/W_{total}$, where M is the content of ascorbic acid from the standard curve (μg) , W_{total} is the sample weight (g).

Determination of antioxidant activities

Determination of DPPH free radical scavenging activity

1.0mL of appropriately diluted pickle juice was added to 4.0 mL of DPPH ($120\mu mol \cdot L^{-1}$) in methanol. Shake well and place for 75 min. Its absorbance value A_1 at 517 nm was determined. In addition, the absorbance A_2 of the pickle juice without DPPH and the value A_0 of the mixture of 4.0 mL of DPPH in methanol with 1.0ml of distilled water at 517 nm were also measured [8]. The scavenging rate of DPPH radicals was calculated as scavenging rate (%) = $[1-(A1- A_2)/ A_0] \times 100\%$.

Determination of reducing power

A 0.5 mL aliquot of appropriately diluted pickle juice was mixed with 2.5 mL of phosphate bufer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide in 20 mL test tubes. The mixtures were incubated for 20 min in water bath of 50 ˚C . After cooling, 1 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation. The upper layer (2.5 mL) was mixed with 2.5 m L of distilled water and 1 mL of 0.1% ferricchloride. The reaction lasted for 10 min. Afterward, the absorbance was measured at 700nm [9].

2.5.3. Determination of Fe^{2+} chelating activity

1.0 mL of pickle juice was mixed with 2.0 mL of 0.2% $FeSO_4$. After 30 min of incubation at 37˚C , 0.5 ml of 0.3% ferrozine was added and reacted for 10 min at 37˚C . The absorbance of the Fe^{2+}-ferrozine complex was measured at 510 nm. The chelating activity of the pickle juice for Fe^{2+} was calculated as chelating rate (%) = $[1-(A_1- A_2)/ A_0] \times 100\%$, where A_0 was the absorbance of the control (blank, without pickle juice), A_1 was the absorbance of pickle juice in the presence of ferrozine and A_2 was the absorbance of pickle juice without ferrozine [10].

Statistical Analysis

The data were processed by analysis of variance using DPS7.05 statistical software (Refine Information Tech. Co., Ltd., Hangzhou, China).

RESULTS AND ANALYSIS

Changes of vitamin C and anthocyanin content

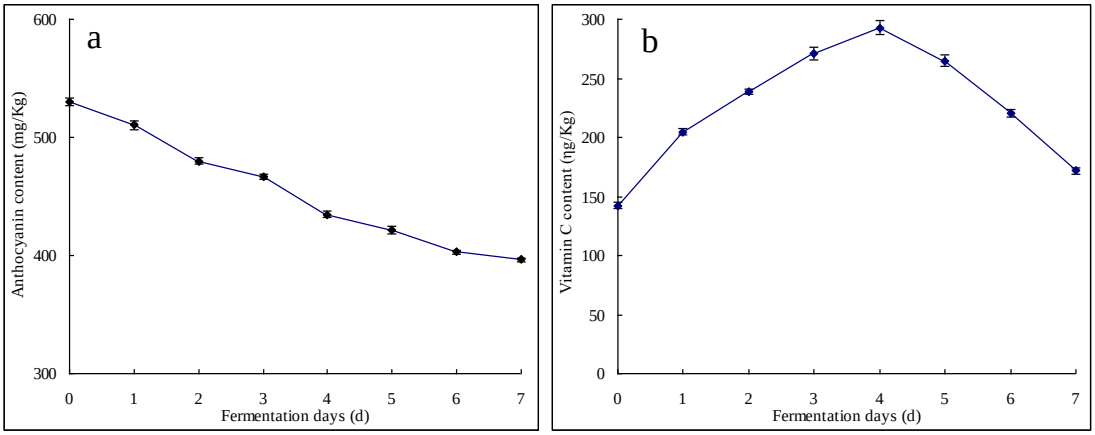

Fig-1: **The changes of vitamin C and anthocyanin content during fermentation process of purple cabbage pickle.**

As shown in Fig.1a, the anthocyanin content of purple cabbage pickle decreased with the extension of fermentation time. At 7 day, its anthocyanin content was 396.3 mg/kg, which was 25.2% lower than that of 0 day. Anthocyanin usually had higher stability under acidic condition, but physiological activity of lactic acid bacteria might result in the loss of the anthocyanins content during fermentation [11]. As described in Fig.1b, the vitamin C content of purple cabbage pickle firstly increased and then decreased with fermentation time extension. From the beginning to the 4th day, vitamin C content always increased, the maximum 293.0 ηg/kg appeared at 4th day, which was about 2 times of the beginning. Subsequently, with further extension of fermentation time, vitamin C content decreased, and the value in 7th day was 41.4% lower than that of 4th day.

Change of Antioxidant activities

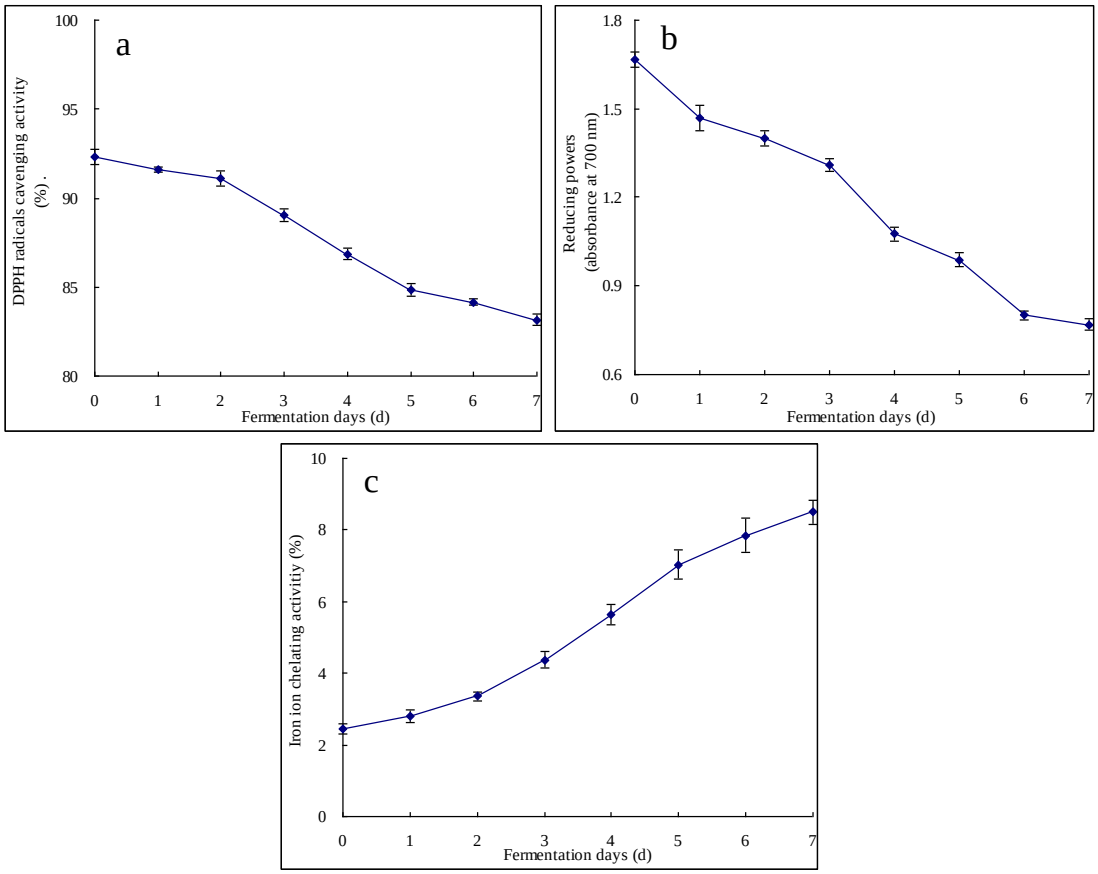

Fig-2: Changes of DPPH free radical scavenging activity, reducing power and Fe^{2+} chelating activity during the fermentation process of purple cabbage pickle

DPPH free radical scavenging activity could show the ability to provide hydrogen atoms. When purple cabbage was fermented by lactic acid bacteria, the ability to remove DPPH free radicals gradually decreased with time (Fig. 2a). At 7 day, the scavenging rate was 9.9% lower than that of 0 day. The reducing power characterized the ability to provide electron. As shown in fig. 2b, during fermentation, the reducing power of purple cabbage pickle gradually declined, which was similar to the change of DPPH free radical scavenging activity. Reducing power of 7th day was 53.9 lower than 0 day and the difference was significant ($P<0.01$). Contrary to the changes of DPPH free radical scavenging activity and reducing power, Fe^{2+} chelating activity of purple cabbage pickle increased with the extension of fermentation time Fig 2c. Fe^{2+} chelating activity of fresh purple cabbage had the minimum, but through fermentation of 7 days, the chelating rate was about 3.5 times of 0 day.

Correlation analysis of nutrients and antioxidant activity

Table-1: Correlation coefficient of nutrients and antioxidant activity of purple cabbage pickle

Correlation coefficient	anthocyanin	vitamin C	DPPH scavenging activity
vitamin C	-0.32		
DPPH scavenging activity	0.98**	-0.21	
reducing power	0.99**	-0.24	0.98**
Fe^{2+} chelating activity	-0.98**	0.15	-1.00**

* represents $p<0.05$, ** represents $p<0.01$

As shown in table 1, DPPH radical scavenging activity and reducing power of purple cabbage pickle were highly positive correlated with the content of anthocyanins, but Fe^{2+} chelating activity had strong negative correlation with anthocyanin content ($p<0.01$). DPPH radical scavenging activity, reducing power and Fe^{2+} chelating ability had little to do with vitamin C content. The reason may be as followed. Anthocyanin, as an important antioxidant, involved DPPH radical scavenging and reducing process, namely provided hydrogen atoms or electrons. Chelating ability characterized substance to provide coordination electron pair. A large number of lactic acid was generated during fermentation process of purple cabbage pickle, participating in the coordination of Fe^{2+}. Thus, Fe^{2+} chelating ability increased [12].

CONCLUSION

After purple cabbage was fermented by lactic acid bacteria to form pickle, compared with fresh purple cabbage, anthocyanin content decreased, the vitamin C content showed a trend of rising firstly and then falling. In terms of DPPH radical scavenging ability and reducing power, antioxidant activities ability of purple cabbage also decreased after fermentation. Although after fermentation, flavor and taste of purple cabbage were improved, from nutrition point of view, we suggested fresh purple cabbage should be eaten. Besides, in order to make full use of its nutritional value, purple cabbage pickle also should be eaten as early as possible.

Acknowledgments

This work was supported by Program for the Innovative Talents of Higher Learning Institutions of Shanxi (2012), by project of Natural Science Foundation of Shanxi under grant no. 2012021025-3, and by project for the 131 Leading Talent of Higher Learning Institutions of Shanxi no. 447(2013).

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