Scholars Journal of Engineering and Technology (SJET) **ISSN 2321-435X (Online)**
Sch. J. Eng. Tech., 2015; 3(7):667-671 **ISSN 2347-9523 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Effects of phytic acid on nutrients and antioxidant activities of apple juice

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

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

Abstract: Phytic acid was added into apple juice at the concentration of 0.1mmol/L, and the effects of phytic acid on antioxidant activities and the contents of vitamin C and polyphenols and antioxidant activities of apple juice were investigated. The result showed as followed. Vitamin C and polyphenols of apple juice decreased during storage time. Adding phytic acid with concentration of 0.1mmol/L might reduce the decrease of vitamin C and polyphenols of apple juice. As for apple juice with added phytic acid, its DPPH free radical scavenging activity decreased, its reducing power slightly decreased and its Fe^{2+} chelating activity increased with storage time extension.
Keywords: Apple juice, phytic acid, nutrients, antioxidant activity.

INTRODUCTION

Apple is native to the central and southeastern of Europe, Central Asia and Xinjiang Uygur Autonomous Region of China. It is one of four fruits in the world, and was cultivated in many places of the world [1]. Apple is rich in nutrients, and its fruit juice is a delicious, cheap and healthy drink [2]. In China, concentrated apple juice had become most important processing and exporting products. In world trade, the trade volume of concentrated apple juice increased every year [3].

Phytic acid, named inositol hexaphosphate, widely exists in plant community, especially in the seeds [4]. It has chelating and antioxidant effect and its toxic is less than salt, belonging to natural nutrient. It was widely used in food, medicine, chemical and other industries [5]. In food industry, phytic acid was commonly used in oil food, alcoholic drinks, beverage cans, aquatic products, and fresh fruits and vegetables [6]. Research found that the suitable addition of phytic acid might effectively prevent apple juice browning [7]. In addition, phytic acid as a chelating agent could improve drink stability [8]. But the effects of phytic acid on nutrients and antioxidant activities of apple juice were not reported.

In this experiment, phytic acid was added into apple juice at 0.1mmol/L and the effects of phytic on nutrients and antioxidant activities of apple juice were investigated. This research might provide some references for apple juice processing.

MATERIALS AND METHODS

Materials and Reagents

Apples (*Malus pumila* Mill. cv. Red Fuji) at the physiologically mature stage with no insect pest and mechanical damages were purchased from an orchard in the outskirts of Linfen city. After harvest, they were quickly transported in open cartons to the laboratory of fruit and vegetable processing, and were prepared into apple juice in 24 hours.

Methanol, alcohol, 2,6-dichlorpphenolindophenol, oxalic acid, gallic acid, sodium carbonate, diphenyl-picryl hydrazyl (DPPH), potassium ferricyanide, ferric chloride and ferrozine (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin China). Pectinase and amylase (biochemical reagent) were purchased from Ruji Bio-technical Co., Ltd. (Shanghai China). Alfa Aesar Company (Tianjin, China) supplied other reagents.

Equipments and instruments

DS–1 triturator, Shanghai Jingke Instrument Company, China; SHA-C Thermostatic water bath oscillators, Jincheng Guosheng Experiment Instrument Factory, China; GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., China;

Preparation and treatment of apple juice

Apples were cleaned and cut into small cubes with the length of approximately 1.5 cm. The raw apple materials were smashed with triturator and filtered with

a 200-mesh sieve to obtain a cloudy apple juice. The juice was filled with 500 mL beaker, heated at 98 °C for 60s and then promptly cooled to 51 °C. Appropriate amounts of amylase and pectinase were added into the juice. The juice was left stationary for approximately 2 h at 50∼53 °C until starch and pectin were completely decomposed. Subsequently, the juice was filtered with a 0.22 μm membrane and diluted to 10.0 Brix with deionised water. The apple juice was divided into two parts. One served as control sample, and phytic acid with the concentration of 0.1mmol/L was added into the other apple juice. All apple juices were sealed in bottle and placed in constant temperature incubator at 50℃ for 6 days. The related nutrients and antioxidant activities of apple juice were determined during storage time.

Qualitative determination of pectin and starch

Pectin and starch were qualitatively detected as followed [9]. Briefly, acidulated alcohol was prepared through adding 1 mL of 37% hydrochloric acid into 99 mL of anhydrous alcohol. 100 mL of apple juice undergoing pectinase enzymolysis was filtered with a 0.22 μm membrane. Acidulated alcohol (20 mL) was added into 10 mL of filtered juice. The mixture was slightly inverted three times and was left stationary for 15∼30 min. The formation of gelatin or floccule suggested the presence of pectin in the apple juice, whereas the absence of gelatin or floccule indicated the decomposition of pectin.

Starch was qualitatively detected according to the following methods. Iodine (0.065 g) and potassium iodide (1.75 g) were dissolved with a small amount of deionised water. The solution was diluted to 500 mL and placed in a brown volumetric flask to yield a 0.005 mol/L iodine solution. Apple juice (20 mL) through amylase enzymolysis was filtered with a 0.22 μm membrane. Subsequently, 1 mL of 0.005 mol/L iodine solution was added into the juice. A colour reaction between the juice and iodine was observed. Yellow indicated the absence of starch, brown suggested the presence of a small amount of starch, and blue indicated the presence of a large amount of starch in apple juice.

Determination of nutrients

Determination of vitamin C

The vitamin C content was measured by 2, 6-dichloriondophenol titration [10]. Briefly, 2 mL of apple juice was titrated to a permanent pink colour using 0.1% of 2,6-dichlorpphenolindophenol solution dissolved with 1% oxalic acid. The vitamin C concentration was calculated according to the titration volume of 2, 6-dichloriondophenol, and expressed as mg per 100 mL apple juice.

Determination of total polyphenols

Total polyphenols content was determined using Folin–Ciocalteu's phenol reagent via spectrophotometric analysis [11]. An aliquot (1 mL) of a standard solution of gallic acid of concentration including 0, 10, 20, 30, 40, and 50 mg L^{-1} aqueous methanol, or appropriately diluted apple juice was added to a 25 mL volumetric flask containing 9 mL of water. About 1 mL of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 8 min, 2 mL of 7.5% aqueous Na_2CO_3 solution was added. The solution was then immediately diluted to a final volume of 25 mL with water and thoroughly mixed. After incubation for 30 min at 25 °C, the absorbance versus the prepared blanks was read at 765 nm. Total polyphenols content was expressed as μg gallic acid equivalents per 100 mL apple juice.

Determination of antioxidant activities

Determination of DPPH free radical scavenging activity

1.0mL of appropriately apple 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 [12]. 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 apple 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 ℃. 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 mL of distilled water and 1 mL of 0.1% ferricchloride. The reaction lasted for 10 min. Afterward, the absorbance was measured at 700nm [13].

Determination of Fe^{2+} chelating activity

1.0 mL of apple juice was mixed with 2.0 mL of 0.2% ferrous sulfate. After 30 min of incubation at 37℃, 0.5 ml of 0.3% ferrozine was added and reacted for 10 min at 37℃. 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 [14].

RESULT AND ANALYSIS

Effect of phytic acid on vitamin C content of apple juice

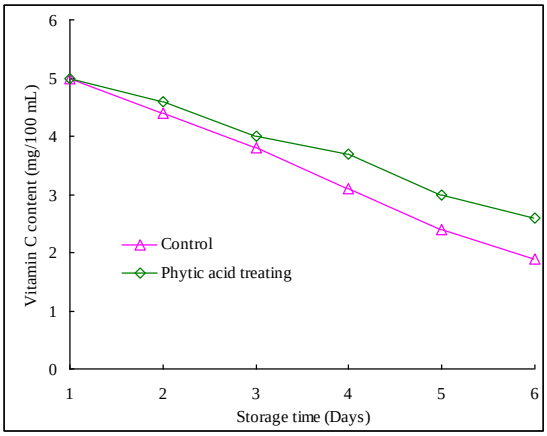

Fig-1: The vitamin C content variation of apple juice during storage time

Vitamin C is one of important nutrients of fruit juice. The fewer vitamins C reduced in fruit juice storage, the better fruit juice preservation effect was [15]. In living organisms, vitamin C is an antioxidant that protects the body from free radicals. In addition, it is also a coenzyme. As shown in Figure 1, vitamin C content of apple juice decreased with storage time expansion. During the whole storage time, the vitamin C content of apple juice with added phytic acid was higher than that of control sample. And at 6 day, it was 2.6 mg/100mL, which was 36.8% higher than that of control sample.

Effect of phytic acid on polyphenol content of apple juice

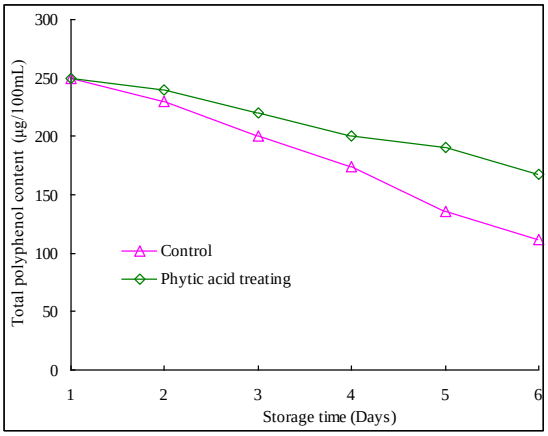

Fig-2: The total polyphenols content variation of apple juice during storage time

Apple polyphenols are a general term for kinds of polyphenol substance apple contains, and they are secondary metabolites [16]. Research shows that apple polyphenols have multiple functions such as scavenging free radicals, preventing cardiovascular disease and cancer, and resisting radiation. So apple polyphenols has great value of development and utilization. As described in Figure 2, the total polyphenols content of apple juice decreased during storage time. Apple juice with added phytic acid contained more total polythenols compared to control sample. At 6 day, the total polyphenol content of apple juice with added phytic acid was 168.1 μg/100mL, which was 50.1% higher than that of control sample.

Effect of phytic acid on DPPH free radical scavenging activity of apple juice

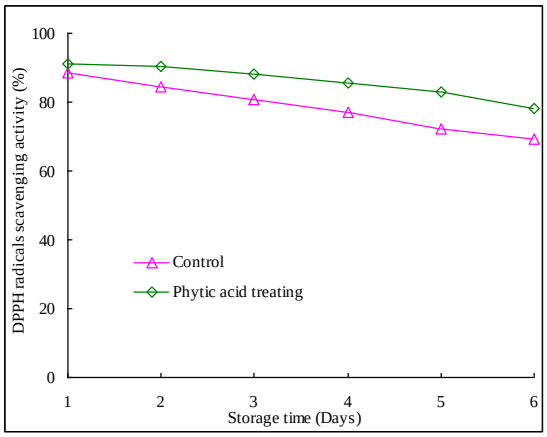

Fig-3: The variation of DPPH free radical scavenging activity of apple juice during storage time

DPPH radical scavenging activity indicates the ability of providing hydrogen atom originating from apple juice substance [17]. As shown in Figure 3, the DPPH radical scavenging activity of apple juice decreased with storage time extension. Apple juice with added phytic acid showed higher DPPH radical scavenging activity than that of control sample during the whole storage time. And at 6 day, its DPPH radical scavenging activity was 78.0%, which was 12.5% higher than that of control sample.

Effect of phytic acid on reducing power of apple juice

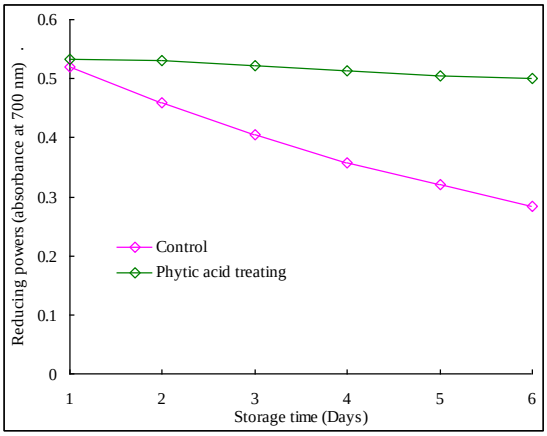

Fig-4: The variation of reducing power of apple juice during storage time

Reducing power characterizes the ability of providing electrons, usually closely associated with the antioxidant activity of substance [18]. As shown in Figure 4, the reducing power of control sample decreased rapidly during apple juice storage. At 6 day, it was 45.5% lower than that of 1 day. And the reducing power of apple juice with added phytic acid decreased more slowly during storage time. At 6 day, it was 6.0% lower than that of 1 day. Moreover, the reducing power of apple juice with added phytic acid was 76.7% higher than that of control sample.

Effect of phytic acid on Fe^{2+} chelating activity of apple juice

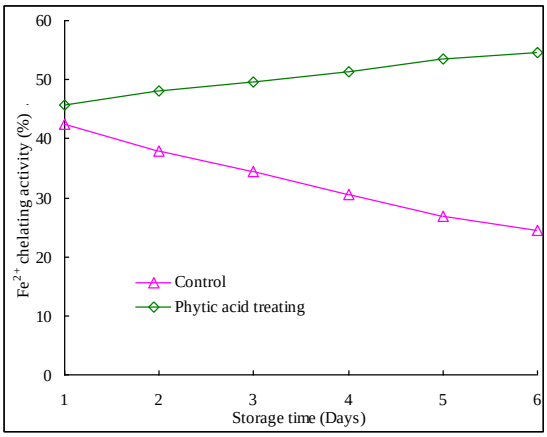

Fig-5: The variation of phytic acid of apple juice during storage time

As shown in Figure 5, the Fe^{2+} chelating ability of control sample decreased during apple juice storage. And at 6 day, it was 42.5% lower than that of 1 day. Contrarily, the Fe^{2+} chelating ability of apple juice with added phytic acid increased during storage. Phytic acid itself had chelating effect. After it was added into apple juice, it probably interacted with some nutrients of apple juice, which enhanced the chelating power of apple juice. Additionally, owing to the addition of phytic acid into apple juice, the production of Maillard reaction during apple juice storage might also enhance the Fe^{2+} chelating ability of apple juice [19].

Correlation analysis of nutrients and antioxidant activities

Table-1: Correlation coefficent of nutrients and antioxidant activities after adding phytic acid into apple juice

Correlation coefficient	Vitamin C	Total polyphenols
DPPH scavenging activity	0.97**	0.98**
Reducing power	0.99**	0.98**
Fe^{2+} chelating activity	-0.99**	-0.98**

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

As shown in Table 1, after adding phytic acid into apple juice, the contents of vitamin C and total polyphenols of apple juice showed significant positive correlation with DPPH scavenging activity or reducing power. This suggested that DPPH scavenging activity or reducing power of apple juice was probably mainly contributed by vitamin C and polyphenols. And Fe^{2+} chelating activity was negative with the content vitamin C or total polyphenols, which indicated that vitamin C and total polyphenols did not serve as leading role in Fe^{2+} chelating activity.

CONCLUSION

Vitamin C and total polyphenols of apple juice decreased during storage time. Adding phytic acid with concentration of 0.1mmol/L might reduce the decrease of vitamin C and total polyphenols of apple juice. As for apple juice with added phytic acid, its DPPH free radical scavenging activity decreased, its reducing power slightly decreased and its Fe^{2+} chelating activity increased with storage time extension.

Acknowledgments

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

REFERENCES

1. Martynenko A, Zheng WW; Electrohydrodynamic drying of apple slices: Energy and quality aspects. Journal of Food Engineering, 2016; 168: 215-222.
2. Alberti A, Santos TPM, Zielinski A AF, Santos CME, Braga CM, Demiate IM, Nogueira A; Impact on chemical profile in apple juice and cider made from unripe, ripe and senescent dessert varieties. LWT - Food Science and Technology, 2016; 65:436-443.
3. Guo YD, Zhou ZK, Yuan YH, Yue TL; Survey of patulin in apple juice concentrates in Shaanxi (China) and its dietary intake. Food Control, 2013; 34(2): 570-573.
4. Pande R, Mishra HN; Fourier Transform Near-Infrared Spectroscopy for rapid and simple determination of phytic acid content in green gram seeds (Vigna radiata). Food Chemistry, 2015; 172: 880-884.
5. Kim NH, Jang SH, Kim SH, Lee HJ, Kim Y, Ryu JH, Rhee MS; Use of phytic acid and hyper-salting to eliminate Escherichia coli O157:H7 from napa cabbage for kimchi production in a commercial plant. International Journal of Food Microbiology, 2015; 214: 24-30.
6. Stodolak B, Starzyńska A, CzyszczońM, Żyła K; The effect of phytic acid on oxidative stability of raw and cooked meat. Food Chemistry, 2007; 101(3): 1041-1045.
7. Du YJ, Dou SQ, Wu SJ; Efficacy of phytic acid as an inhibitor of enzymatic and non-enzymatic

browning in apple juice. Food Chemistry, 2012; 135(2): 580-582.
8. Zhong ZS, Wang YJ, Zhang LH; Multi- Functional Introduction of Food Additive-Phytic Acid. China Food Additives, 2003; (2): 74-77.
9. Chopda CA, Barrett DM; Optimization of guava juice and powder production. Journal of Food Processing and Preservation, 2001; 25(6): 411-430.
10. Bessey OA, King CG; The distribution of vitamin C in plant and animal tissues, and its determination. Journal of Biological Chemistry, 1933; 103, 687-698.
11. Xiao CL, Zhu LW, Luo W, Song XY, Deng Y; Combined action of pure oxygen pretreatment and chitosan coating incorporated with rosemary extracts on the quality of fresh-cut pears. Food Chemistry, 2010; 121: 1003-1009.
12. Yang ZF, Zheng YH, Cao SF; Effect of high oxygen atmosphere storage on quality, antioxidant enzymes, and DPPH-radical scavenging activity of Chinese bayberry fruit. Journal of Agriculture and Food Chemistry, 2009; 57: 176-181.
13. Jayaprakasha GK, Singh RP, Sakariah KK; Antioxidant activity of grape seed (Vitis Vinifera) extracts on peroxidation models in vitro. Food chemistry, 2001; 73: 285-290.
14. Sánchez-Vioque R, Polissiou M, Astraka K, Mozos-Pascual M, Tarantilis P, Herraiz-Peñalver D, Santana-Méridas O; Polyphenol composition and antioxidant and metal chelating activities of the solid residues from the essential oil industry. Industrial Crops and Products, 2013; 49: 150-159.
15. Dong YJ, Zhang YG, Dai HY.; Thermal Degradation Kinetics of Vitamin C in Apple Juice .Journal of Chinese Institute of Food Science and Technology, 2012;12(4):84-89.
16. Kebe M, Renard CMCG, Maâtaoui ME, Amani GNG, Maingonnat JF; Leaching of polyphenols from apple parenchyma tissue as influenced by thermal treatments. Journal of Food Engineering, 2015; 166: 237-246.
17. Dawidowicz AL, Wianowska D, Olszowy M; On practical problems in estimation of antioxidant activity of compounds by DPPH· method (Problems in estimation of antioxidant activity). Food Chemistry, 2012; 131(3): 1037-1043.
18. Wong CC, Li HB, Cheng KW, Chen F; A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry, 2006; 97(4): 705-711.
19. Limsuwanmanee J, Chaijan M, Manurakchinakorn S, Panpipat W, Klomklao S, Benjakul S; Antioxidant activity of Maillard reaction products derived from stingray (Himantura signifier) non-protein nitrogenous fraction and sugar model systems. LWT - Food Science and Technology, 2014; 57(2):718-724.